Growth, infectious morbidity, and neurodevelopment of HIV-exposed and HIV-unexposed infants in the context of lifelong maternal antiretroviral therapy and breastfeeding: a prospective cohort study

by

Stanzi Maria le Roux

LRXSTA002

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Primary supervisor: Professor Landon Myer

Co-supervisor: Professor Kirsten A Donald

Abstract

Background

In 2019, 1 million *in utero*-HIV-exposed but -uninfected children (CHEU) were born in sub-Saharan Africa. In the absence of breastfeeding and maternal antiretroviral therapy (ART), HIV-exposed uninfected children (CHEU) have higher risks of mortality, growth faltering, infectious morbidity, and developmental delay than the general population, but data are limited on these outcomes among breastfed CHEU born to women who received universal (not restricted by disease severity) ART in pregnancy. This thesis addresses these knowledge gaps by comparing health outcomes of breastfed CHEU to breastfed CHU in the first year of life.

Methods

This research incorporates data from two prospective, linked cohort studies of pregnant women living with HIV (Maternal Child Health Antiretroviral, MCH-ART study, 2013-2016) and without HIV (HIV-unexposed uninfected, HU2 study; 2014-2017) in Gugulethu, Cape Town, South Africa. Enrolled at first antenatal visit at a primary care clinic, women were followed-up during pregnancy and postpartum with their breastfed infants, through 12 months with ~3-monthly study visits. Study staff administered questionnaires addressing maternal and child health, infant feeding, psycho-social and behavioural factors; and measured maternal-infant anthropometry [expressed as Z-scores for age: weight-for-age, WAZ; length-for-age, LAZ; head circumference-for-age, HCAZ]; WLHIV provided blood for repeated HIV viral load. At 12 months, the Bayley Scales of Infant Development, 3rd edition (BSID-III) was used to assess neurodevelopment.

Findings

WLHIV (100% antenatal ART) reported worse living conditions and higher risks of alcohol use and intimate partner violence than HIV-negative women. Similar proportions of CHEU and CHU were born preterm (11%) or small-for-gestational-age (10%). Exclusive breastfeeding was more common among CHEU than CHU, but overall duration of breastfeeding was shorter among CHEU. However, unless otherwise reported, adjustment for confounders did not change inferences below.

In analysis of *child growth*, weight and head circumference trajectories were similar for CHEU and CHU from 6 weeks to 12 months. Both groups exhibited rapid weight gain with increasing WAZ over time; by 12 months, almost one-fifth of all children were overweight. Length trajectories for CHEU and CHU diverged after 6 months, with onset of linear growth faltering occurring earlier and more rapidly among the CHEU; by 12 months, stunting risk was doubled among CHEU vs CHU. Stratified by birth size, differences in LAZ between CHEU and CHU were magnified for those born small-for-gestational age and absent for those born appropriate-for-gestational age.

Infectious morbidity analyses revealed greater risks among CHEU than CHU in the first 6 months with not thereafter. Between 7 days and 3 months of life, CHEU (vs CHU) experienced three times more infection-related hospitalisations; rates for CHEU with healthier mothers (lower viral load, higher CD4 count, ART started early in pregnancy) approximated those of CHU, while CHEU of mothers with late ART initiation and advanced disease had four-fold more infectious-cause hospitalization. Breastfeeding and complete vaccinations were protective.

At 12 months, mean composite cognitive, motor and language scores were within normal range and similar for both groups. Overall, risks of any *developmental delays* were low but slightly higher among CHEU than CHU in cognitive and motor domains. Compared to term HU, term HEU children had similar odds of motor delay, preterm HU children had 5-fold increased odds of delay and preterm HEU children, 16-fold. In CHEU, cumulative maternal viremia was associated with lower average scores and increased risk of moderate delays in motor and language domains.

Conclusion

Subtle health outcome differences persisted between CHEU and CHU despite breastfeeding and universal maternal ART in pregnancy. Reassuringly, the magnitudes of differences were small and predominantly associated with preventable factors including late ART initiation, advanced maternal disease stage, lack of breastfeeding, and incomplete vaccination. CHEU born too soon or too small were at highest risk of adverse outcomes, suggesting fetal origins of disease in the context of maternal HIV.

Acknowledgements

This thesis is built upon an enduring passion for child health in Africa. This passion was kindled, and has been fed, tempered, tested, and protected by a host of precious people, without whom my work – and my life – would be empty.

I am deeply grateful to all the women and children who participated in this study, not only sharing details about their homes, their lives, and their families, but also teaching me about resilience and finding joy amidst hardship. Thank you also to the phenomenal women who worked with me in MCH-ART and HU2, at the Green clinic and in the Falmouth building. You made this fun.

Thank you to my supervisors. Landon, you have been so patient with me and my quirky work rhythms. Thank you for creating this opportunity for me, for your compassion, advice, inspiration, support, teaching and wicked sense of humour. Thank you also for making me think and write better, for 'getting' me, and for understanding rubber deadlines. Kirsty, your wise and gentle support carried me through times when I really did not know what I was doing.

I am also deeply grateful to Professor Elaine Abrams for trusting me to run with the child health aspects of MCH-ART and HU2, for bringing wisdom, sharp insights, and encouragement to each of the papers that were generated for this thesis. And for doing all this with kindness, grace, and a wonderful sense of humour. Thank you, Elaine.

My family is amazing. Thank you, Dave. For understanding me, for teaching me to see injustice and the blinkers in my own heart and eyes, for hundreds of coffee cups and chocolates, for making me laugh and for always encouraging me - even while trying to finish your own PhD and juggling front-line health work during a pandemic. Fifteen years later, it is still "*always* better when we're together" (Jack Johnson). Thank you, Ben and Freddie, for being the most amazing humans I have ever met. Thank you for stretching your Mamma's heart beyond its pre-child capacity, for teaching me about humility, and for bringing me never-ending joy and hope. Rosah, thank you for making me laugh so much, for keeping my home ticking over, for loving my children as your own. I could not function without you. Thank you also to my wonderful second set of parents, Annette and Harold. Your prayers and love have carried our entire household through this process. Mamma, dankie. You are an amazing woman and have been the role model for all (of the good) parts of my life. Thank you for taking me with you to the informal settlement outside dusty little Grootfontein to build, run and teach at an early childhood development centre in the 1980s, during those terrible Apartheid years. Thank you for endlessly caring for those around you, then and now. Thank you to my father Fred, for teaching me that all problems can have solutions, that tears do not solve said problems, but plans do, for taking me around the world in more than just 80 days, and for showing me how true partners look after each other. I love sharing a Kindle account with you, you find the best books.

In my journey from paediatric clinician to child health epidemiologist, I was profoundly inspired by Professors Mark Cotton, Heather Zar, Simon Schaaff and Anneke Hesseling. I will forever be grateful for your mentorship, wisdom, and the extra support when my steps faltered.

I am also grateful to the South African Medical Research Council for a generous Clinician Researcher PhD scholarship that enabled me to focus on my research full-time, as well as the Fogarty Foundation and the Office of AIDS Research for a fellowship that supported part of this work (NIH Fogarty International Center Grant #5R25TW009340).

Thank you, Lord, for bringing me to this place. May Your kingdom come, on earth as it is in heaven, with health, justice, peace, freedom and wholeness for everyone.

♦ ♦ ♦ ♦ ♦

"Is not this the kind of fasting I have chosen:
to loose the chains of injustice and untie the cords of the yoke,
to set the oppressed free and break every yoke?
Is it not to share your food with the hungry
and to provide the poor wanderer with shelter—
when you see the naked, to clothe them,
and not to turn away from your own flesh and blood?"

♦ ♦ ♦ ♦ ♦

Isaiah 58 (clue – yes, it is)

Preface

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in the Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, Faculty of Health Sciences, University of Cape Town. The work included in this thesis is original research, and has not, in whole or in part, been submitted for another degree at this or any other university. The contents of this thesis are entirely the work of the candidate or, in the case of multi-authored published papers, constitutes work for which the candidate was the lead author.

This thesis includes published manuscripts, as per general provision 6.7 in the General Rules for the Degree of Doctor of Philosophy (PhD) of the University of Cape Town. I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include these publications. The following manuscripts (all published) are included in the thesis, and are presented as self-contained chapters in the following order:

1. Clinical outcomes of HIV-exposed, -uninfected children in sub-Saharan Africa: a systematic review (Chapter 2)
2. Growth trajectories of breastfed South African HIV-exposed uninfected and HIV-unexposed children in the context of universal maternal antiretroviral therapy: a prospective study (Chapter 4)
3. Infectious morbidity of breastfed, HIV-exposed uninfected infants under conditions of universal antiretroviral therapy in South Africa: a prospective study (Chapter 6)
4. Neurodevelopment of breastfed HIV-exposed uninfected and HIV-unexposed children in South Africa: a prospective cohort (Chapter 7)
5. HIV viraemia during pregnancy and neurodevelopment of HIV-exposed uninfected children in the context of universal antiretroviral therapy and breastfeeding: a prospective study (Chapter 8)

In addition, the following two manuscripts are directly related to the thesis and are included as appendices:

6. Rethinking the HIV-exposed, uninfected child: epidemiologic perspectives
7. Tenofovir exposure *in utero* and linear growth in HIV-exposed, uninfected infants

The candidate's contribution to each of the above manuscripts is outlined at the start of the relevant chapter. For all the manuscripts, the candidate was lead and corresponding author, conducted all analyses and wrote the first drafts. All co-authors read and contributed to the manuscripts; comments were incorporated into the manuscripts prior to submission.

The data for analyses in this thesis originated from two related studies which were conducted in parallel: (1) the Maternal Child Health Antiretroviral Therapy (MCH-ART) and (2) the HIV-unexposed uninfected mother-infant (HU2) studies. For the MCH-ART study, the candidate was the lead investigator for all child health aspects of the study; for the HU2 study, the candidate was the overall principal investigator for both maternal and child health aspects. The candidate applied for and was granted funding for the projects, provided leadership and oversight for human resource procedures, staff appointment and performance reviews. The candidate developed the conceptual aspects of this doctoral research project in consultation with her supervisors. For both studies, the candidate designed the child health related research tools, provided oversight for implementation and supervised quality assurance practices. In a clinical capacity, the candidate conducted approximately 50% of all child neurodevelopmental assessments, supervised maternal and infant anthropometric measurement processes, and provided clinical support for children presenting intercurrent illness at study visits.

Student name: Stanzi Maria le Roux Student number: LRXSTA002

List of abbreviations

aβ	Adjusted Beta-coefficient
ACROBAT	Cochrane Risk of Bias Assessment Tool
AFASS	Acceptable, Feasible, Affordable, Sustainable and Safe
AGA	Appropriate-for-gestational-age
aHR	Adjusted Hazard Ratio
AIC	Akaike's Information Criterion
AIDS	Acquired Immune Deficiency Syndrome
aIRR	Adjusted Incidence Rate Ratio
ANC	Antenatal Care
ANOVA	Analysis of Variance
aOR	Adjusted Odds Ratio
aPR	Adjusted Prevalence Ratio
ART	Triple agent Antiretroviral Therapy
ARV	Antiretroviral drug
AUDIT-C	Alcohol Use Disorders Identification Test-Concise
AUDIT	Alcohol Use Disorders Identification Test
AZT	Zidovudine
β	Beta-coefficient
BCG	Bacille Calmette-Guerin
BF	Breastfeeding
BFHI	Baby-Friendly Hospital Initiative
BMI	Body Mass Index
BMIZ	Body Mass Index-for-age Z-score
BSID	Bailey Scales of Infant Development
CBCL	Child Behaviour Checklist
CCHIP	Community Childhood Hunger Identification Project Index
CD	Communicable Disease
CD4	Cluster of differentiation
CHEU	Children who are HIV-exposed *in utero*, but remain uninfected
CHU	Children who are HIV-unexposed *in utero*
CHW	Community Health Workers
CI	Confidence Interval
CMV	Cytomegalovirus
CPT	Co-trimoxazole Preventive Therapy

CUMC-IRB	Columbia University Medical Centre Institutional Review Board
DAG	Directed Acyclic Graph
DALY	Disability-adjusted life year
DBS	Dried blood spot
DHS	Demographic and Health Survey
DOHAD	Developmental Origins of Health and Disease
DUDIT	Drug Use Disorders Identification Test
EBF	Exclusive Breastfeeding
ECD	Early Childhood Development
ECDI	Early Childhood Development Index
EFV	Efavirenz
EIBF	Early Initiation of Breastfeeding
ELBW	Extremely low birth weight
EPDS	Edinburgh Postnatal Depression Survey
EPI	Expanded Programme for Immunization
FANTA	Food and Nutrition Technical Assistance Project
FAS	Fetal Alcohol Syndrome
FASD	Fetal Alcohol Spectrum Disorder
FF	Infant formula feeding
FTC	Emtricitabine
GA	Gestational age
GBD	Global burden of disease
GEE	Generalized estimating equations
GSH	Groote Schuur Hospital
HAZ	Height-for-age Z-score
HCAZ	Head circumference-for-age Z-score
HCT	HIV Counselling and Testing
HE	HIV-Exposed
HEU	HIV-Exposed *in utero*, but HIV-uninfected
HFIAS	Household Food Insecurity Access Scale
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee
HU	HIV-Unexposed *in utero*
HU2	HIV-Unexposed Uninfected mother and infant study
IG-21	INTERGROWTH-21st (International Fetal and Newborn Growth Consortium for the 21st Century)
IMCI	Integrated Management of Childhood Illness
IMR	Infant Mortality Rate

IPV	Intimate Partner Violence
IQ	Intelligence Quotient
IQR	Inter-quartile range
IRR	Incidence Rate Ratio
IUGR	Intra-uterine Growth Restriction
KABC	Kauffman Assessment Battery for Children
LAZ	Length-for-age Z-score
LBW	Low Birth Weight
LGA	Large-for-gestational age
LRTI	Lower Respiratory Tract Infection
LTFU	Lost to follow-up
LVW	Length velocity-for-age Z-score
M2M	Mothers to mothers
MCAZ	Mid-upper arm circumference-for-age Z-score
MCH	Maternal and Child Health
MCH-ART	Maternal and Child Health Antiretroviral study
MDI	Mental Development Index
MGRS	Multicentre Growth Reference Study
mIA	Maternal Immune Activation
MICS	Multiple Indicator Cluster Surveys
MMH	Mowbray Maternity Hospital
MOU	Midwife and Obstetric Unit
MSEL	Mullen Scales of Early Learning
MTCT	Mother to child transmission of HIV
MUAC	Mid-upper arm circumference
NCD	Non-Communicable Disease
NHLS	National Health Laboratory Systems
NICHD	Eunice Kennedy Shriver National Institute of Child Health and Human Development
NIH	National Institutes of Health
NOS	Newcastle-Ottowa Scale
NRSI	Non-randomized studies of intervention
NVP	Nevirapine
OR	Odds Ratio
PBF	Partial Breastfeeding
PCR	Polymerase chain reaction
PDI	Psychomotor Development Index
PEPFAR	US President’s Emergency Plan for AIDS Relief

PHDC	Provincial Health Data Centre
PLHIV	People living with HIV
PMTCT	Prevention of mother to child transmission of HIV
PR	Prevalence Ratio
PrBF	Predominant Breastfeeding
PTB	Preterm Birth
PTD	Preterm Delivery
RCT	Randomized controlled trial
RCWMCH	Red Cross War Memorial Children's Hospital
RoB	Risk of bias
RR	Risk Ratio
RTHB	Road to Health booklet
SA	South Africa
SAMRC	South African Medical Research Council
SAVA	Substance Abuse, Violence and AIDS
SD	Standard Deviation
SDG	Sustainable Development Goal
sdNVP	single-dose Nevirapine
SDOH	Social Determinants of Health
SDQ	Strengths and Difficulties Questionnaire
SGA	Small-for-gestational age
SHINE	Sanitation, Hygiene Infant Nutrition Efficacy trial
SSA	sub-Saharan Africa
TDF	Tenofovir Disoproxil Fumarate
U5MR	Under-5 Mortality Rate
UCT	University of Cape Town
UK	United Kingdom
UNICEF	United Nations Children's Fund
USA	United States of America
USD	United States Dollar
VAW	Violence against women
VCY	Viraemia Copy Years
VL	Viral Load
VLBW	Very Low Birthweight
VS	Viral suppression
WASH	Water, Sanitation and Hygiene
WAZ	Weight-for-age Z-score

WHO	World Health Organization
WLHIV	Women living with HIV
WLZ	Weight-for-length Z-score
WVZ	Weight velocity-for-age Z-score
ZAR	South African Rand
ZDV	Zidovudine
ZVITAMBO	Zimbabwe Vitamin A for Mothers and Babies trial

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Chapter 1: Introduction

1.1 Background & overview of the literature

1.1.1 The rapidly growing global population of children who are HIV-exposed but uninfected

In 2019, approximately 19.2 million women aged 15 years or older were living with HIV (WLHIV) globally, most of whom were residing in sub-Saharan Africa (SSA).[1] More than two-thirds of WLHIV live in Eastern and Southern Africa (figure 1.1).[1]

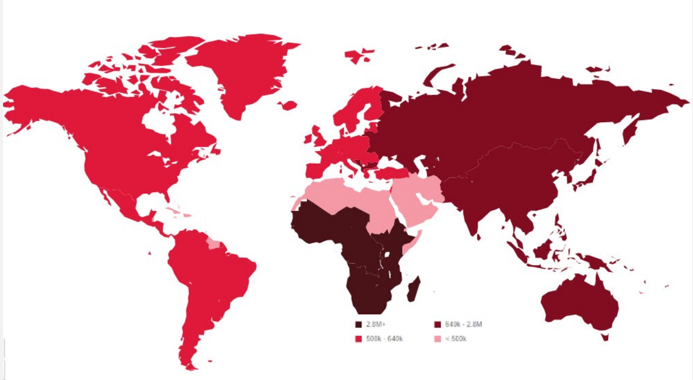


Figure 1.1. Women (15+ years) living with HIV (2019). *Source* UNAIDS data 2020

The overall prevalence of HIV in this region continues to rise, driven in part by ongoing high rates of HIV acquisition among adolescent girls and young women,[2] alongside the increased life expectancies of WLHIV receiving antiretroviral therapy (ART) (figure 1.2).[1] An estimated 720 000 new HIV infections occurred among women in 2019 globally.[1] In the same year, approximately 85% of 1.3 million pregnant WLHIV received antiretrovirals to reduce mother to child transmission of HIV (MTCT), an increase in coverage from 82% in 2017 and 45% in 2010.[1]

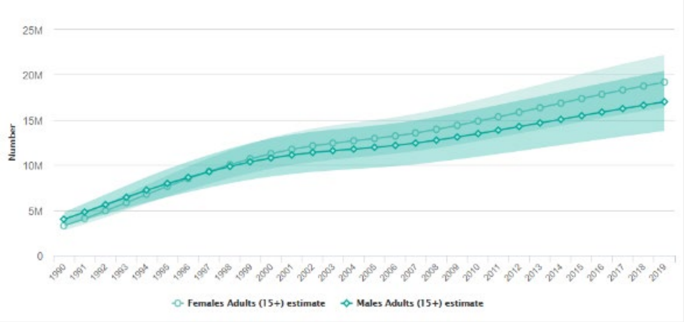


Figure 1.2. Global number of adults living with HIV, by sex (2019). *Source* UNAIDS data 2020

With effective use of ART during pregnancy and breastfeeding, MTCT can be reduced to below 1%.[3] This combination of increasing prevalence of HIV among women of child-bearing age and broadening access to highly effective prevention of MTCT (PMTCT) has resulted in an unprecedented number of children being born HIV-exposed but uninfected (HEU).[4] UNAIDS 2020 estimates suggest that over 15 million HEU children aged 14 years or younger reside in sub-Saharan Africa, with another roughly 1 million HEU children born annually.[1]

Six countries in SSA contribute over 60% of the global burden, with national prevalence of HEU children exceeding 800 000 (Kenya, Mozambique, Nigeria, South Africa, Uganda, United Republic of Tanzania, and Zimbabwe).[1] South Africa has the largest population of HEU children at 3.8 million, representing more than 20% of South Africans aged 14 years or less.[5] Therefore, at a population level, any excess risks of adverse health outcomes among HEU children are likely to have substantial impact on the success of this region generally, and South Africa specifically, in meeting the Sustainable Developmental Goals.[6]

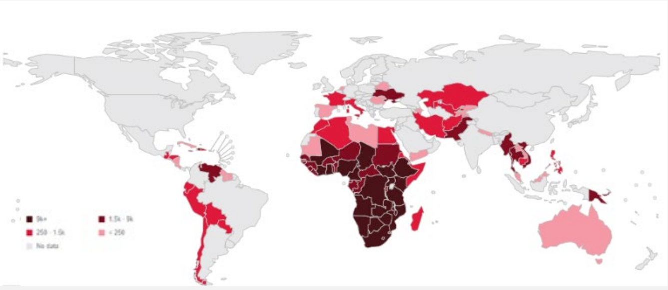
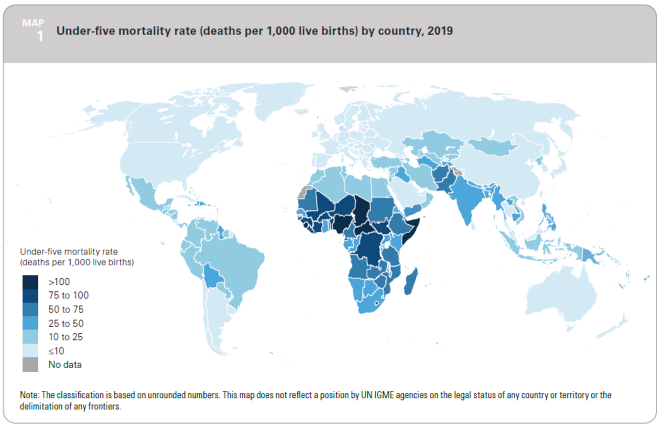


Figure 1.3. Global number of HIV-exposed uninfected children (HEU children), 2019. *Source* UNAIDS data 2020

1.1.2 Key threats to the health and survival of children under the age of 5 in sub-Saharan Africa

While child survival has improved markedly over the past decade, an estimated 5.2 million children under the age of 5 years (3.9 million in the first year of life) died in 2019. Most child deaths have preventable causes and occur in Sub-Saharan Africa. (figure 1.4).[7]

These early childhood deaths are the primary drivers of premature death globally, contributing significantly to DALYs (disability-adjusted life years) across all ages.[8] Neonatal disorders, lower respiratory tract infection (LRTI) and diarrhoeal diseases ranked first, second and third in the top ten causes of *global burden of disease* for children under 10 years of age in 2019 (figure 1.5).[8]


MAP 1 Under-five mortality rate (deaths per 1,000 live births) by country, 2019

Note: The classification is based on unrounded numbers. This map does not reflect a position by UN IGME agencies on the legal status of any country or territory or the delimitation of any frontiers.

Figure 1.4. Under-five mortality rate (deaths per 1000 live births) by country, 2019. *Source* UN-IGME Child Mortality report 2020

In sub-Saharan Africa, *LRTI and diarrhoeal disease* also contribute significantly to child morbidity. In 2016, more than 68 million episodes of LRTI and 370 million episodes of diarrhoeal disease occurred among children under 5, causing an estimated 652 572 and 290 724 deaths in this age group, respectively.[9,10]

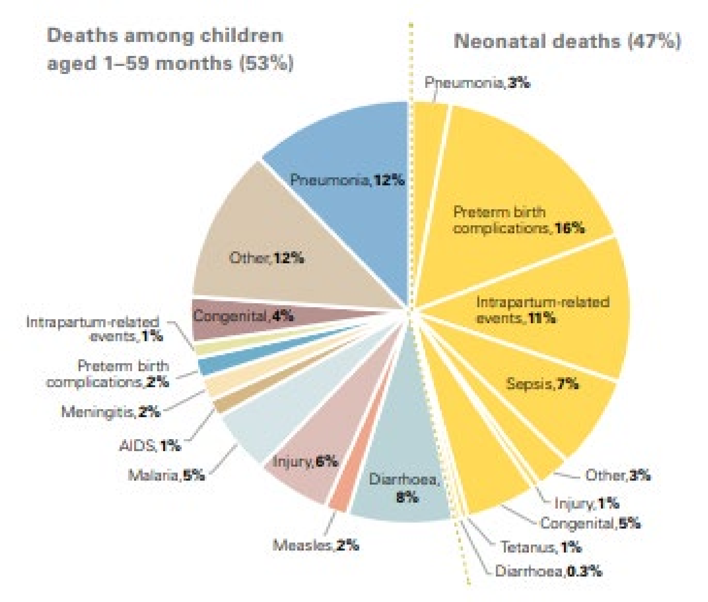


Figure 1.5. Global distribution of causes of under-5 and neonatal mortality, 2018. *Source* UN-IGME Child Mortality report 2019

Childhood wasting was the leading risk factor for mortality of both LRTI and diarrhoeal disease in 2016, responsible for 61% of LRTI deaths, and 80% of diarrheal deaths, respectively.[9,10] Malnutrition is estimated to be an underlying cause of up to 45% of all U5 child mortality.[11] Children require adequate nutrition for immune function, growth, and development; growth and development in the "first 1000 days" of life predict lifelong health and human capital.[12] In sub-Saharan Africa, almost a third of children under 5 were stunted (length-for-age Z-score, LAZ <-2SD), 7% wasted (weight-for-length Z-score, WLZ <-2SD) and 3% overweight (body mass index Z-score, BMIZ > 2SD) in 2019.[13]

Although the pathogenesis of *stunting* is still not clearly understood, chronic undernutrition, recurrent infections and micronutrient deficiencies play important roles, independently and in synchronicity.[14] A marker of chronic deprivation, it follows that stunting is strongly correlated with suboptimal *early childhood development*.[15] Using Early Childhood Development Index (ECDI) data collected between 2005 and 2015 in global surveys, a large modelling study estimated that one in three pre-school children living in lower- and middle-income countries fail to meet cognitive and/or socio-emotional developmental milestones, figure 1.6.[16]

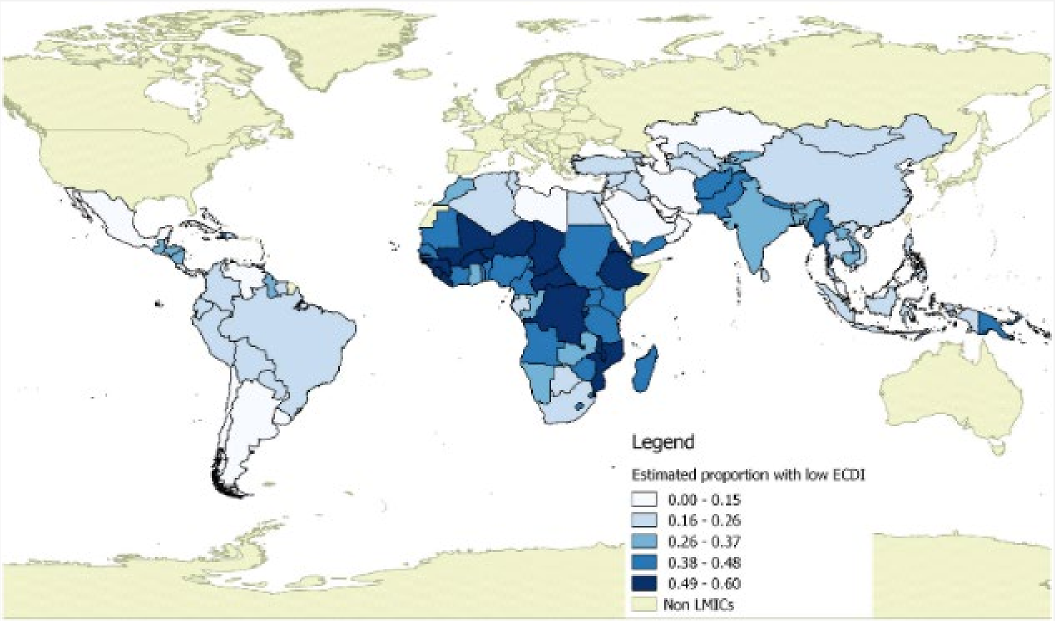


Figure 1.6. Estimated proportion of children with low development per the ECDI by country (using MICS data, 2010). *Source* PLosMED 2016, McCoy et al.

1.1.3 Previously reported health vulnerabilities of HIV-exposed uninfected children

Data accumulated from across SSA over the last twenty years have suggested that, compared to the general population, HEU children may be at increased risk of all the key threats to child health in the region discussed above, including: growth faltering, infectious morbidity and mortality (particularly related to LRTI and diarrhoeal disease), and delayed early childhood development.[17,18]

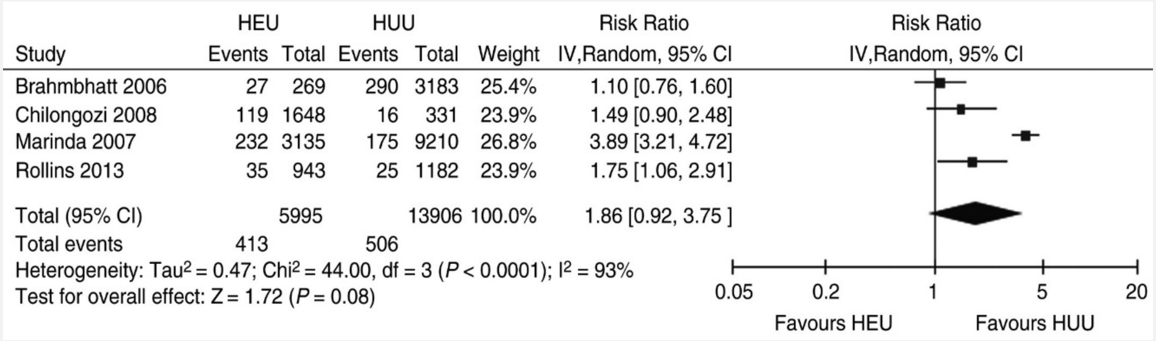
A systematic literature review focusing on clinical health outcomes of HEU children compared to HU children in sub-Saharan Africa was published in 2016 (included as Chapter 2 of this thesis).[17] Outcomes of interest included mortality, growth, LRTI, diarrhoeal disease and child development in the first 10 years of life; the final literature search was conducted in October 2015. Using the same search strategy, exclusion and inclusion criteria, an updated search on PubMed on 6 January 2021 found an additional 738 reports, 62 of which were reviewed in full text after abstract and title screening. Of these, 34 publications from 19 studies were included (providing 16 reports on growth, 11 reports on mortality or infectious morbidity, and 14 reports on developmental outcomes).[19-52] Most studies reported findings from HEU children in the first 1-2 years of life, within the context of limited ART (restricted to those with advanced HIV disease stages). An overview of publication details, study methods and key findings is shown in supplemental table 9.1.1, stratified by use of antiretrovirals for PMTCT. Notably, since 2015, only 4 studies have provided data on health outcomes of HEU children where at least 70% of mothers had received universal (no restrictions) ART in pregnancy (3 studies in addition to the research project presented in this thesis). A narrative overview of key findings from the earlier review and recent literature search is provided under subheadings below.

1.1.3.1 *Growth of HEU children*

Most studies reported lower mean weight-for-age Z-scores (WAZ), head-circumference-for-age Z-scores (HCAZ) and LAZ among HEU children than HU children over time, with up to 2-fold higher risks of underweight (WAZ <-2SD) and 3-fold higher risks of stunting (LAZ<-2SD).[36] A large Zimbabwean cohort study (no maternal ART) described 40% increased risk of microcephaly (HCAZ <-2SD) in HEU children (vs HU children) at 3 months of age but not at older ages.[21] Generally, the discrepancies in growth between HEU children and HU children were of small magnitude (around 0.1 to 0.4 of a SD), without variation by maternal use of ART.[19,26,28,30,38,44-47,51] Suboptimal infant and young child nutrition, particularly a lack of breastfeeding, emerged as an important determinant of early childhood growth faltering irrespective of maternal HIV status.[49,53-55] Low socio-economic status and food insecurity were common among the families of HEU children and predicted worse growth outcomes.[26,33,38] Two South Africa studies reported high but similar proportions of child overweight among both HEU children and HU children, with evidence of breastfeeding reducing the risk of being overweight.[38,49]

1.1.3.2 *Infectious morbidity of HEU children*

Almost all publications reported increased risks of infectious morbidity in HEU children compared to HU children.[23,29,34,36,37,39,47] Although varying definitions of infectious morbidity were used, several reports demonstrated increased incidence of hospitalisation (all-cause and infectious-cause, particularly related to LRTI and diarrhoeal disease);[17,23,29,37,39,47] non-specific 'sick child' visits;[27,32,37] and incidence or longitudinal prevalence of any diarrhoea and respiratory illness.[39,56] A few reports included details on age, severity of illness and duration of hospital stay, with indications that HEU infants tended to have more severe disease and longer admissions, especially in the first 6 months of life and in the absence of breastfeeding.[17,29,34,37,39,47] Advanced maternal disease stage, absence of maternal ART, suboptimal breastfeeding (related to both exclusivity and duration) and use of infant formula were strongly associated with increased infectious morbidity across settings.[23,24,29,37,39]



	HEU		HUU			Risk Ratio
Study	Events	Total	Events	Total	Weight	IV,Random, 95% CI
Brahmbhatt 2006	27	269	290	3183	25.4%	1.10 [0.76, 1.60]
Chilongozi 2008	119	1648	16	331	23.9%	1.49 [0.90, 2.48]
Marinda 2007	232	3135	175	9210	26.8%	3.89 [3.21, 4.72]
Rollins 2013	35	943	25	1182	23.9%	1.75 [1.06, 2.91]
Total (95% CI)		5995		13906	100.0%	1.86 [0.92, 3.75]
Total events	413		506			

Heterogeneity: Tau² = 0.47; Chi² = 44.00, df = 3 (*P* < 0.0001); I² = 93%
Test for overall effect: Z = 1.72 (*P* = 0.08)

Figure 1.7. Mortality risk ratio at 12 months between HEU and HU children
Source: TMIH, Arikawa et al, 2016

1.1.3.3 Mortality in HEU children

Prior to the widespread availability of ART, HEU children had markedly increased risks of mortality compared to HU children (figure 1.7)[17,27,30,56] The relative magnitude of increased risk differed across settings, with highest risks of death observed among young, non-breastfed infants and HEU children born to women with advanced HIV disease.[23,26,27,36,37,51,56] Under conditions of universal maternal ART (n=2 papers), the reported mortality of breastfed HEU children was low, approximating that of breastfed HU children.[32,39] Overall, common causes of death were similar for HEU children and HU children (primarily due to neonatal causes, LRTI and diarrhoea), with highest risk of death occurring in the first 6 months of life.[56] Adverse birth outcomes especially preterm birth, maternal death, suboptimal breastfeeding, and adverse living conditions were associated with higher risk of death.[23,27,36,37,39,47]

1.1.3.4 Neurodevelopment of HEU children

In 19 publications from 16 studies, approaches to the assessment of child development varied across settings and time periods.[17,20,22,25,30-32,35,40-42,48,50,52] The most commonly used assessment tools included the Bayley Scales of Infant Development® (BSID-II or BSID-III), the Kauffman assessment battery for children (KABC-II or KABC-III)® and the Mullen Scales of Early Learning (MSEL)®; behaviour was generally measured using either the Strengths and Difficulties Questionnaire (SDQ)® or the Child Behaviour Checklist (CBCL)®. Given the heterogeneity of measures and ages of application, direct comparisons over time are difficult.

A few reports indicated similar developmental scores for HEU and HU children (n=8)[20,30,32,50,57-60] However, small to moderate deficits in cognitive, motor, language, socio-emotional and behavioural domains were noted comparing HEU to HU children in at least 2 reports each.[17,22,25,31,35,40-42,48,52] A Zambian study (limited access to maternal ART) reported lower Math and English grades in primary school age HEU compared to HU children.[61] Although magnitude diminished, these differences persisted after accounting for large differences in socio-economic situation.[61] In three of the four studies reporting on developmental outcomes after at least 70% of WLHIV received universal ART in pregnancy, HEU children demonstrated deficits in cognitive,[22,31] motor,[22,31,35] and language domains,[35] even after accounting for HIV-associated differences in social determinants of health. Increased maternal disease severity in pregnancy, postpartum cessation of ART use, and adverse social circumstances were associated with the highest risks of developmental delay.[22,31,35,40,41]

1.1.4 Potential mechanisms of excess mortality and morbidity among HEU children

Designing effective public health interventions to improve child health outcomes requires a causal understanding of underlying pathological processes.

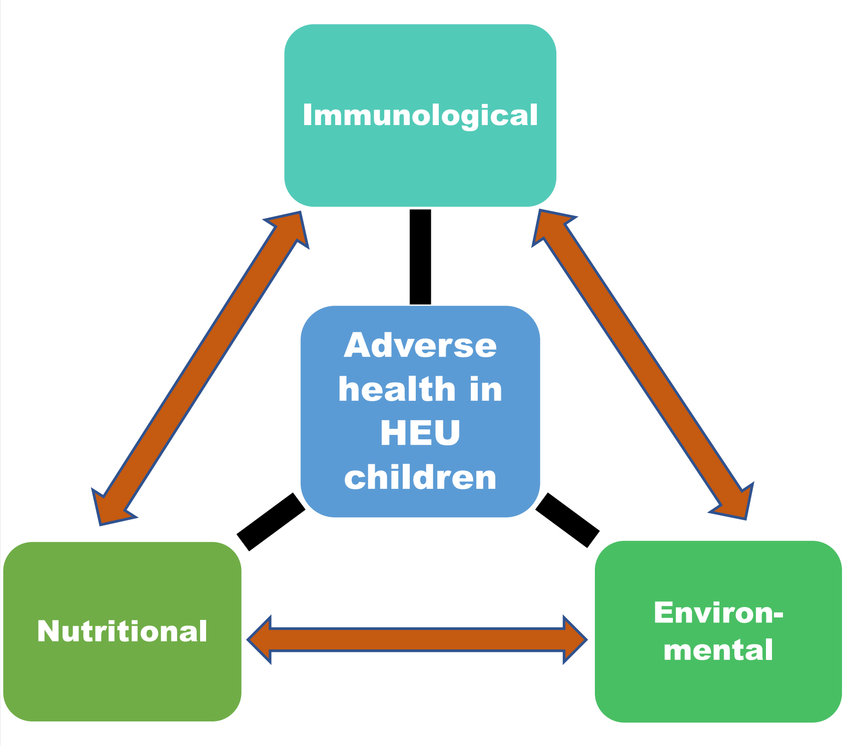


Figure 1.8. Schematic of inter-relationships between immunological, nutritional, and environmental factors related to maternal HIV and child health

While multiple co-occurring risk factors appear to work synergistically in the context of HEU child health, in broad categories, immunological, environmental, and nutritional mechanisms have been proposed to cause or contribute substantially to the excess mortality and morbidity observed in HEU children (fig 1.8).[17,18,62,63] Across mechanisms, effective maternal ART has the potential to negate or ameliorate the adverse impacts on child health.

1.1.4.1 Immunological

HIV is primarily a disease of immune dysregulation, associated with both failure of immunological defences and chronic immune activation.[64] Maternal immune depletion adversely affects the health of their uninfected offspring through several pathways, with greater risks correlating with more advanced maternal HIV disease severity, especially in the absence of ART.[17,56,65-68]

First, immune-compromised pregnant women are at an increased risk for common and opportunistic infections.[64] Tuberculosis, syphilis, and cytomegalovirus (CMV) increase systemic immune activation in pregnancy while also posing a risk for congenital infections associated with decreased neuro-development, adverse growth outcomes and higher mortality.[69-73] In the postnatal period, especially in the absence of maternal ART, recurrent common infectious illness in PLHIV such as diarrhoea and pneumonia may increase both exposure to pathogens and risk of invasive disease in household members including young children.[74,75] Maternal opportunistic infections also increase maternal mortality; maternal death is estimated to increase the risk of dying before age 5 years 4-fold, with higher relative increases in mortality risk in infancy.[76] ART dramatically reduces the risk of death in WLHIV, a reduction which is seen even when ART is initiated at very early, asymptomatic, disease stages.[64,77]

Second, reduced transplacental transfer of maternal antibodies correlates with increasing maternal immune depletion, in turn placing newborn infants at higher risk of acquiring infections such as measles.[65,66]

Third, fetal exposure to a pro-inflammatory environment adversely affects brain and immune development, with epi-genetic and potentially life-long implications.[78-82] Several transient immunological disturbances have been described in HEU children .[65,66] Although exact clinical implications are not yet clear, these changes are predominantly detectable in the first few months of life, and directly correlated with maternal disease severity in pregnancy. In general, lower levels of maternal HIV viraemia, appears to diminish the adverse impacts of maternal HIV on HEU infant immune function, but data are limited.[83-86]

Finally, preterm birth (PTB) is more common among children of WLHIV.[87] Although the exact mechanisms linking antenatal HIV to PTB remain under study, it is likely that higher likelihood of microbial translocation, vaginal infections and maternal systemic inflammation contribute.[88] Additionally, some ART regimens commonly used in pregnancy appear to increase the risk of PTB independently.[89,90] In turn, preterm infants have immature immune systems and are, irrespective of HIV exposure, at increased risks of infectious morbidity and mortality.[91]

In resource-limited settings, mutually re-enforcing pathways between poverty, recurrent infections, undernutrition, and chronic inflammation are known to have short and long-term implications for adverse birth outcomes, childhood growth and development (fig 1.9).[14,92,93] Given the known associations between untreated HIV and each of the above factors, this toxic interplay is likely to be enhanced in the context of maternal HIV, especially in the absence of maternal ART.[71]

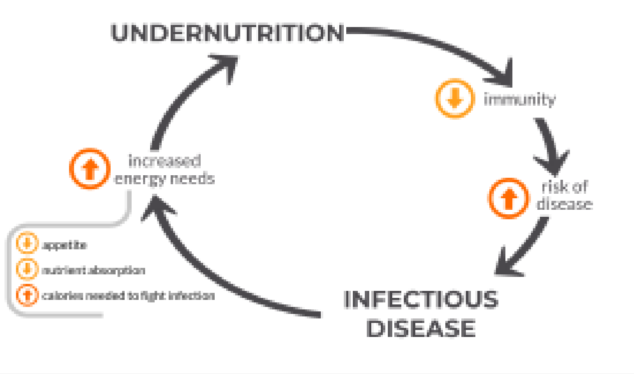


Figure 1.9. The cycle of undernutriton and infection. *Source:* The Value of Immunization Compendium of Evidence. *Accessed 19 January 2012, https://immunizationevidence.org/featured_issues/undernutrition-and-infectious-disease/*

1.1.4.2 Environmental

Several economic, psycho-social, and behavioral factors associated with HIV acquisition among women of child-bearing age are also predictive of adverse child health outcomes.[64] These include maternal education, employment, food insecurity, intimate partner violence, depression, anxiety, and substance abuse including alcohol.[94-96] Although associations between HIV incidence and household income varies across settings, crowding and poor living conditions may be more common among WLHIV, consequently increasing the exposure to household infectious burden (for example tuberculosis) and decreasing access to "WASH" (water, sanitation, and hygiene).[39,97,98] In many instances, there are bi-directional associations and interaction between these factors making causal attribution difficult. Despite these complex interrelationships, all the above factors have been recognized as contributing to the associations noted between maternal HIV status and adverse child health.[17,18,63] Central to most of these social determinants of health and disease is economic well-being. In the absence of ART, chronic HIV disease progression incapacitates PLHIV including parents and heads of households, leading to loss of income, inability to provide child-care, stigmatization by the community, and eventually, orphan-headed households.[64] Although stigmatization remains a concern, use of ART enables prevention of or restoration from such debilitating illness, enabling families to retain household income and arrange for or provide child-care. On both an individual[99] and population level[100], the use of ART has significant economic benefits alongside substantially increased life expectancy.

1.1.4.3 Nutritional

PLHIV are at a higher risk of food insecurity than the general population, a finding which extends to pregnancy.[101,102] In turn, food insecurity is associated with macro- and micro-nutrient deficiencies.[103] Infants born to women experiencing undernutrition during the peri-conception and perinatal period are more likely to be born small-for-gestational age (SGA, <10th percentile for birthweight), experience growth faltering, and fail to meet their developmental potential.[93] Furthermore, food insecurity, maternal depression and maternal anxiety independently and in combination are associated with suboptimal breastfeeding practices.[104,105] Optimal breastfeeding (early initiation, exclusivity through 5-6 months, extended alongside adequately nutritious complementary feeding from 6 months through age 2 years) is a central component of improving child survival, growth, and development globally.[93] Breastfeeding dramatically reduces risks of child mortality, morbidity, and delayed development, in both HEU and HU children.[106,107]

However, prior to the widespread availability of ART, policies aimed at reducing MTCT risk resulted in avoidance of breastfeeding in the context of maternal HIV for almost 2 decades (details provided in section 1.1.5). This absence of optimal breastfeeding among WLHIV has been a major contributor to HEU morbidity and mortality to date.[17,18] For example, a meta-analysis combining data from 21 studies (n=19 219 HEU children) demonstrated a two- to three-fold increased risk of death before 2 years of age, comparing never to ever breastfed HEU children.[56]

1.1.5 Shifts in global and national PMTCT policies over the past two decades

The last 20 years have seen a substantial shift in the focus of PMTCT strategies, reflected in the (a) use of antiretroviral agents and (b) HIV infant feeding recommendations. Overall, the shift represented a broadening of objectives for HIV-exposed children, moving from HIV-prevention to HIV-free survival and, more recently, towards "HIV-free, surviving and thriving".[108] As such, there has been significant change in the environmental and fetal milieu of HEU children over time.[109]

1.1.5.1 The role of maternal antiretroviral therapy in pregnancy and beyond

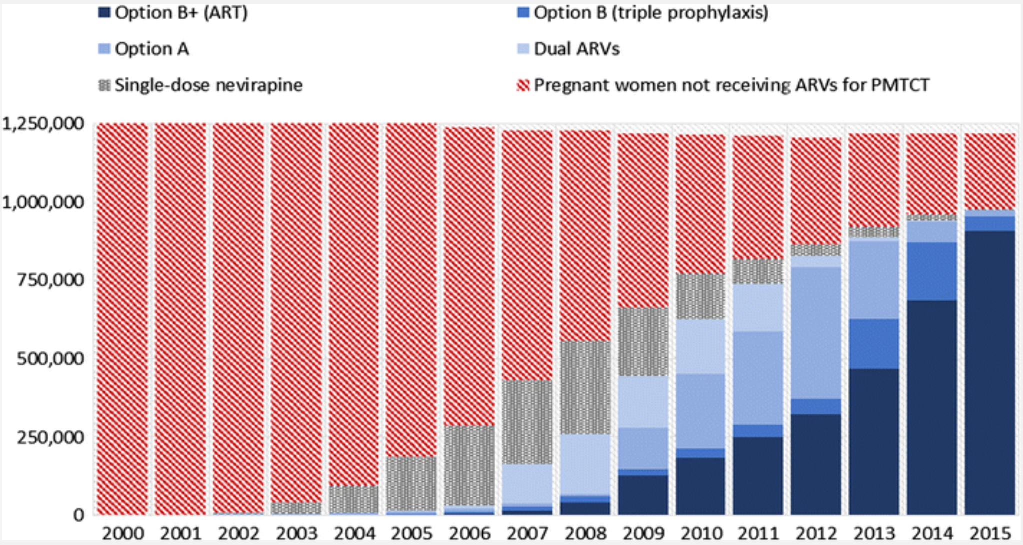


Figure 1.10. Distribution of the number of pregnant women living with HIV receiving antiretroviral medicines for PMTCT by regimen, 21 sub-Saharan African Global Plan countries, 2000–2015. *Sources:* UNAIDS/UNICEF/WHO Global AIDS Response Progress Reporting database, 2016 (from Idele et al, 2017 AIDS Behaviour)

Since the earliest reports of MTCT, antiretroviral use in pregnancy has evolved from being unavailable (≤ 2001), to one or two agents purely for prevention of vertical transmission (~2002 to 2003), to the use of triple-agent antiretroviral therapy (ART, ~2004 to 2008) in pregnancy, albeit restricted to women with advanced disease (generally, using CD4 cell count levels).[109] In the latter scenario, ART was provided for pregnant women on the same restrictions as for general HIV population; women with higher CD4 cell counts received dual prophylaxis for PMTCT ("Option A"). In 2013, recommendations expanded to include ART for all breastfeeding and pregnant women irrespective of their HIV disease staging (no CD4 restrictions, "universal ART"), termed "Option B/Option B+" (depending on whether the ART was prescribed for the duration of pregnancy and breastfeeding only, or lifelong (Figure 1.10).[109]

In 2015, the WHO released recommendations for lifelong ART for all HIV-positive individuals, irrespective of disease-staging ("test and treat all").[110]

However, little data exist on the outcomes of HEU children compared to HU children under conditions where all mothers received ART irrespective of their disease stages. Given the potential impact of universal maternal ART on almost all the mechanisms described above, it is possible that the previously observed differences between HEU and HU child health outcomes may be ameliorated when mothers receive the treatment which they need to remain alive, healthy, economically active, and able to provide childcare.

In addition to reducing vertical transmission and improving maternal health and survival, the expansion of ART access facilitated another critical transformation in PMTCT strategies towards promoting child health and survival, namely the restoration of breastfeeding as the recommended HIV infant feeding option in most resource-limited settings.

1.1.5.2 The importance of breastfeeding

In the absence of antiretroviral therapy, maternal HIV virus can be transmitted via breastfeeding, particularly in the context of non-exclusive breastfeeding. The first case of HIV transmission via breastmilk was described in 1985.[111] In resource-rich settings, an immediate move was made towards avoidance of all breastfeeding and the promotion of breastmilk substitute replacement feeding (infant formula feeding, FF). As breastfeeding has long been recognized as a key protective factor against other infectious drivers of childhood mortality, specifically diarrhea and pneumonia, there was understandable hesitancy to extend this approach to resource-limited settings.[112,113]

However, at the peak of HIV-driven child mortality in Africa (in 2002, HIV was estimated to have caused 260 000 under-5 deaths in SSA; figure 1.11), policies moved towards alternatives for breastfeeding for most infants of HIV-infected mothers.[113-115]

Promoted alternatives included infant formula, pasteurized breastmilk, and rapid weaning (exclusive breastfeeding through the first 6 months followed by a sudden and complete cessation of all breastfeeding.[113,116] In some settings, including the Western Cape region in South Africa, free infant formula was supplied to most HIV-positive mothers in the first 6 months postpartum.[113,116] Although policies aimed to limit the use of formula milk to those mother-infant pairs living in

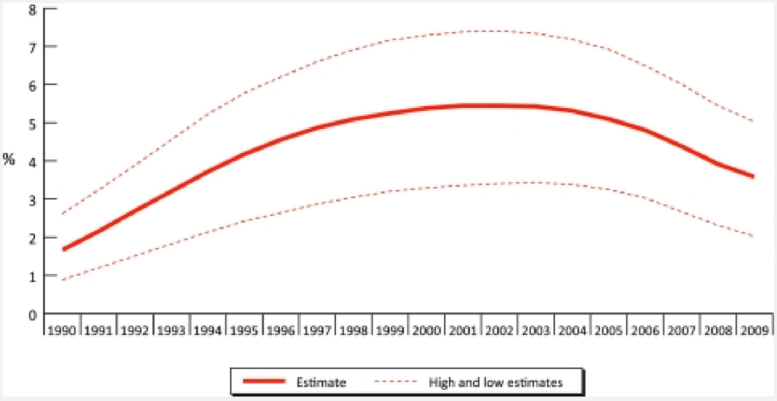
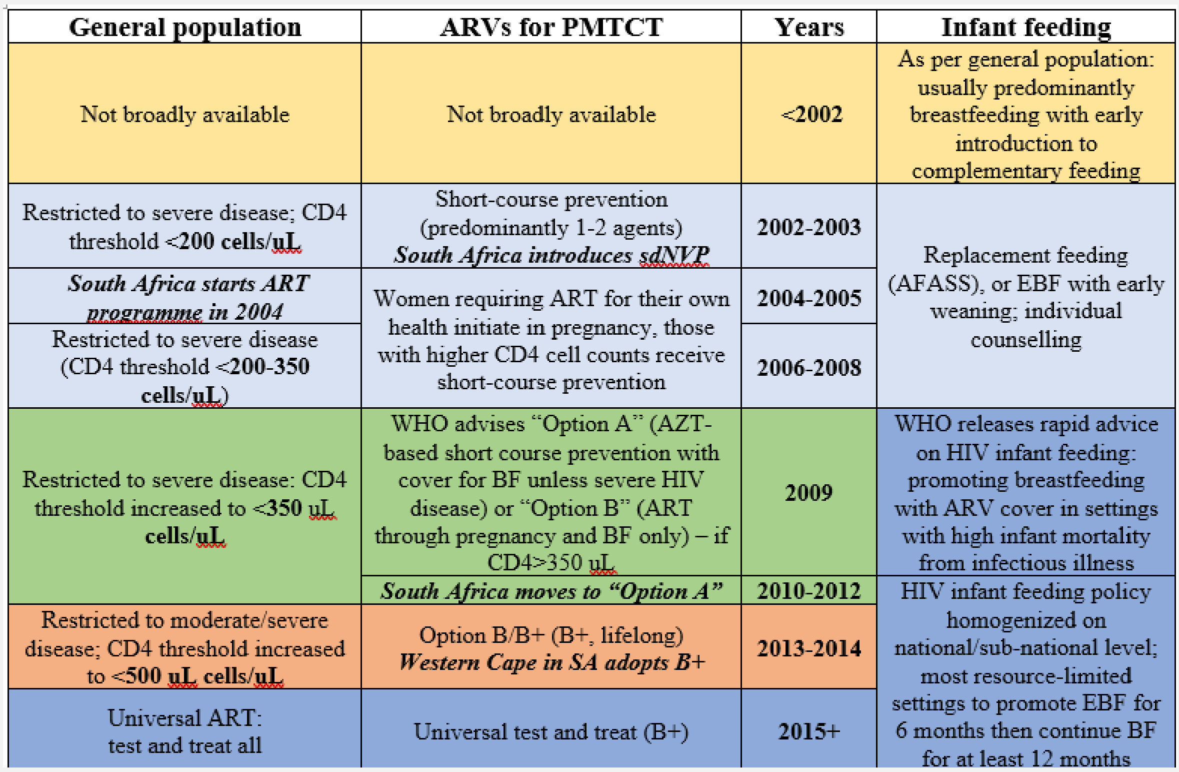


Figure 1.11. Proportion of under-5 mortality attributable to HIV in sub-Saharan Africa, 1990-2009. *Source:* Stanecki et al, Sexually Transmitted Infections, 2010 (https://dx.doi.org/10.1136%2Fsti.2010.046201)

situations where replacement feeding would be "Acceptable, Feasible, Affordable, Sustainable and Safe" ("AFASS"), successful operationalization of these criteria proved impossible.[116] Following widespread implementation of these policies, studies from across the region reported dramatic increases in child illness and death from infectious diseases.[117-122] Simultaneously, data from randomized controlled trials convincingly demonstrated improved HIV-free survival among HIV-exposed infants who were breastfed by mothers receiving antiretroviral agents to reduce maternal viral load during breastfeeding.[123-126] In 2010, the WHO changed their infant feeding policies to promote breastfeeding for at least a year as the infant feeding method of choice for virally controlled WLHIV in areas with high background rates of childhood infectious disease.[127] Table 1.2 provides an overview of policy changes regarding antiretrovirals and breastfeeding in SSA overall, and South Africa in particular.



General population	**ARVs for PMTCT**	**Years**	**Infant feeding**
Not broadly available	Not broadly available	**<2002**	As per general population: usually predominantly breastfeeding with early introduction to complementary feeding
Restricted to severe disease; CD4 threshold <**200 cells/uL**	Short-course prevention (predominantly 1-2 agents) ***South Africa introduces sdNVP***	**2002-2003**	Replacement feeding (AFASS), or EBF with early weaning; individual counselling
South Africa starts ART programme in 2004	Women requiring ART for their own health initiate in pregnancy, those with higher CD4 cell counts receive short-course prevention	**2004-2005**	
Restricted to severe disease (CD4 threshold <**200-350 cells/uL**)		**2006-2008**	
Restricted to severe disease: CD4 threshold increased to <**350 uL cells/uL**	WHO advises "Option A" (AZT-based short course prevention with cover for BF unless severe HIV disease) or "Option B" (ART through pregnancy and BF only) – if CD4>350 uL	**2009**	WHO releases rapid advice on HIV infant feeding: promoting breastfeeding with ARV cover in settings with high infant mortality from infectious illness
	South Africa moves to "Option A"	**2010-2012**	HIV infant feeding policy homogenized on national/sub-national level; most resource-limited settings to promote EBF for 6 months then continue BF for at least 12 months
Restricted to moderate/severe disease; CD4 threshold increased to <**500 uL cells/uL**	Option B/B+ (B+, lifelong) ***Western Cape in SA adopts B+***	**2013-2014**	
Universal ART: test and treat all	Universal test and treat (B+)	**2015+**	

Table 1.1. Evolution of PMTCT strategies in sub-Saharan Africa including South Africa. *Source:* Adapted from a table shared in personal communication, Dr Vundli Ramakolo, with thanks

Given these tremendous shifts in two key determinants of HEU child health, the rapidly evolving PMTCT landscape of the past 20 years needs to be accounted for and considered in interpretation of data related to HEU child health and survival, alongside the socio-environmental exposures more commonly experienced by WLHIV and their children. However, measuring and accounting for these multiple factors have posed some methodological challenges to date, raising concerns about both internal and external validity in many previous studies of HEU child health.

1.1.6 *Methodological challenges in the measurement and interpretation of HEU child health and survival data*

A complete discussion of the multiple potential threats to validity in studies of perinatal exposures and life course epidemiology are beyond the scope of this thesis. However, given the complexity of attributing causality in the relationship between maternal HIV and the health of HIV-uninfected children, an overview of common study design and implementation issues is pertinent.

1.1.6.1 Participant selection

Early reports of HEU child health outcomes were based predominantly on cohort analyses of data from children born to WLHIV participating in randomized controlled trials of PMTCT interventions, or from studies where they were used as a comparison group to assess health outcomes in HIV-infected children.[128-133] As assessment of HEU child health outcomes were not the primary aim of these studies, appropriate comparison groups of HU children were seldom included. Some studies utilized population level statistics for comparison, a comparison which is prone to selection and information bias due to differential measures used for both exposure (maternal HIV) and outcomes.[134] In the absence of randomization, having a comparable, prospectively evaluated comparator group is the optimal approach to maximise exchangeability.[135] In the context of maternal HIV, confounding by social, economic, and behavioural factors is high in the absence of having an appropriate comparison group of HU children, preferably sampled from the same community to minimize baseline differences in socio-economic factors.[136]

In perinatal epidemiology, differential left truncation is a major concern (figure 1.12).[137]

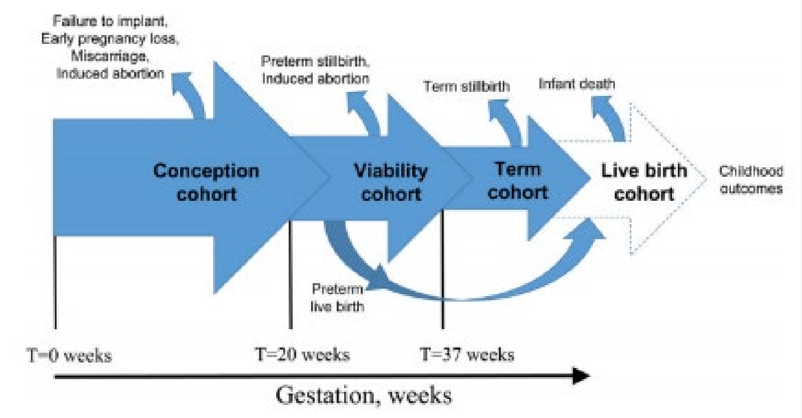


Figure 1.12. Selection and attrition processes during the course of human reproduction and gestation. *Source:* Snowden et al, Current Epidemiology Reports, 2018

In the context of maternal HIV, for example, birth before term is more common and in turn associated with higher early neonatal mortality. Enrolment of HEU and HU children after delivery, especially if enrolment is limited to term infants, increases the risk of bias from left truncation among the HEU children, and creates a situation where there is conditioning on a mediator (preterm birth) in the maternal HIV-child health outcome relationship.[137]

While not negating all risks of differential left truncation, antenatal enrolment of pregnant women with and without HIV is likely to reduce these risks and limit survival bias.

1.1.6.2 Measurement of exposure

Early in the HIV pandemic, diagnosis of HIV relied on anti-HIV antibody tests.[138] Transplacental transfer of maternal antibodies to HIV results in positive antibody-based testing even when infants are not themselves HIV-infected. Therefore, prior to the widespread availability of HIV-PCR testing, excluding HIV infection in infants was not possible in the first approximately 12-18 months of life, at which point circulating maternal antibodies start to wane.[138,139] In this pre-PCR time, diagnosis of HIV in infants was often based on clinical factors, a strategy with high false negative rates due to misclassification of asymptomatic infants during early disease stages.[140] Children infected with HIV perinatally have very high mortality in the first 12 months of life, especially in the first 2 months;[141] although lower than for HIV-infected infants, HEU children also have early, increased mortality compared to HU infants.[27,56] Therefore, prior to early PCR-based testing, many cohorts of "HIV-exposed uninfected children" were at risk both of misclassification bias in the first year of life (HIV-infected children mistakenly included in the presumed HIV-exposed but uninfected group), and survival bias (by the time HIV infection could be excluded at 12-18 months of life, only the healthiest HEU children were represented in the cohort).

1.1.6.3 Measurement of outcomes

Aside from mortality, many globally relevant child health outcomes can be difficult to measure accurately, particularly infectious morbidity and early childhood development.[142,143] Tools to measure these crucial child health indicators have developed substantially in the past 20 years, partly due to the need for standardized global tools to assess progress for global health and development goals.[143,144] Robust, valid, and standardized measurement tools are critical for the avoidance of misclassification (information) bias.[135] As such, interpretation of findings from the early HEU child health literature is complicated by inconsistent definitions and measurements of infectious morbidity and development.

1.1.6.4 Consideration and measurement of potential third variables

Given the complex interplay between multiple factors that determine and influence child health in the context of maternal HIV, an understanding of and accounting for potential third variables is critical in studies of HEU child health. Broadly, potential third variables can be divided into: (A) confounders (causally precede both primary exposure and outcome of interest independently; requires adjustment in regression modelling), for example social determinants of health that increase the risk of maternal HIV acquisition but also increase the risk of adverse child health (maternal education, poverty); (B) mediators (on the causal pathway between primary exposure and outcome of interest; unless no confounding exists between the primary exposure and the mediator, nor between the mediator and the outcome, mediators should not be adjusted for, in order to avoid collider stratification bias), for example adverse birth outcomes (can be caused by maternal HIV and ART exposure, and in turn cause vulnerability to infectious morbidity in HEU); and (C) effect modifiers (concomitant factors that change relationships between main exposure and outcome; should be looked for in analysis and if present, requires stratified presentation of results), for example breastfeeding, which appears to mitigate some of the effects of exposure to maternal HIV on child health.[135] Some factors may play more than one role in the causal pathways between maternal HIV in pregnancy and child health.

To appropriately collect and analyse data on HEU child health, these factors should be taken in consideration. This requires *a priori* planning during study design so that these factors can be measured prospectively, and with as little measurement error as possible, while ensuring an adequate sample size to allow multiple stratifications within analysis.

1.1.6.5 Analysis and interpretation of findings

Given the complex web of interrelated factors that surround the putative causal effects of maternal HIV on child health outcomes, a considered approach is required for analysis.[109] Exploring associations between the exposure, the outcome, and third variables, should be explicitly included in data exploration.

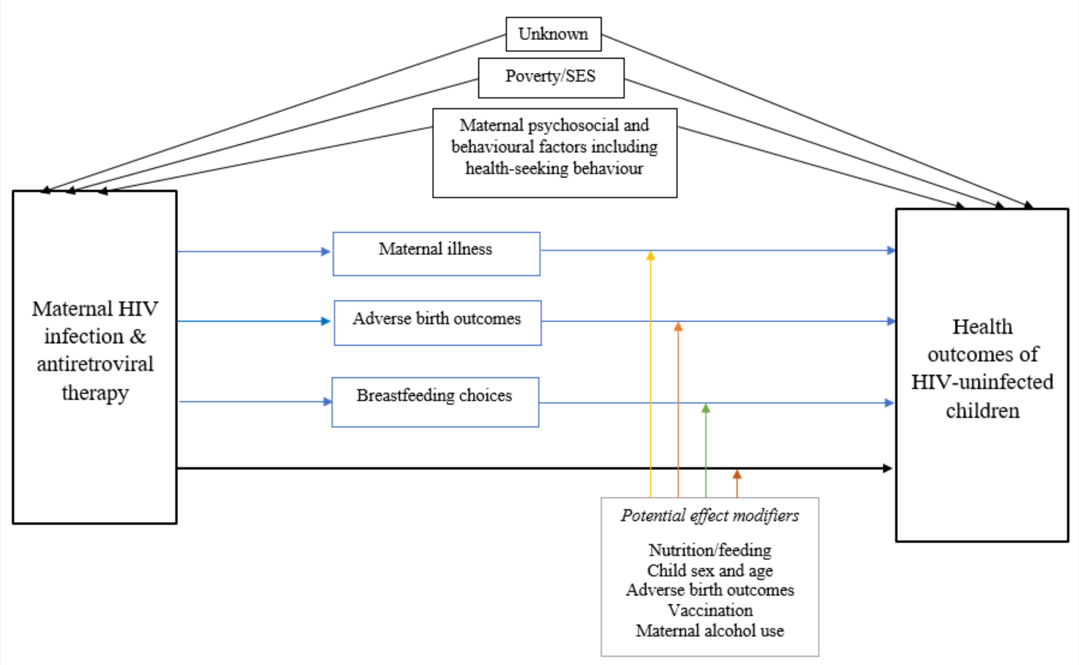


Figure 1.13. Simplified Directed Acyclic Graph demonstrating relationships between maternal HIV (exposure of interest), child health outcomes (broad category, outcome of interest) and important third variables

Using a causal framework (for example a Directed Acyclic Graphs, DAG) to define choices of third variables *a priori* is good biostatistical and epidemiological practice yet has seldom been explicitly reported in HEU child health outcome research to date.[145] Figure 1.13 shows an example of a simplified DAG demonstrating some of the crucial variables to consider in assessing relationships between maternal HIV and child health outcomes.

1.2 Problem statement and rationale

Given that HEU children form a rapidly growing proportion of the global child population, understanding the health risks and needs of these potentially vulnerable children is of significant public health importance. Previously accumulated data suggest differential risks of mortality and morbidity in HEU compared to HU children, but much of the current evidence base is at risk of issues around internal validity. In addition, previous findings may not be applicable to HEU children born under current PMTCT strategies. If HEU children do have greater risks of disease and death than HU children, it will be critical to design and implement interventions to minimize these risks urgently. Via multiple pathways and mechanisms, universal maternal ART combined with optimal breastfeeding may substantially ameliorate these previously reported risks, and evidence of such amelioration will provide additional advocacy for current PMTCT approaches in Africa. However, data from well-designed, sufficiently powered, prospective studies that directly compare breastfed HEU children whose mothers received universal ART to appropriately sampled, breastfed, HU children have been limited to date.

1.3 Aims and objectives

The overarching aim of the research underlying this thesis was to prospectively measure and compare child health outcomes during the first year of life between breastfed infants born to HIV-infected mothers who received universal ART in pregnancy (the “HEU” group) and breastfed infants born to HIV-uninfected mothers (the “HU” group), with antenatal enrolment and sampling from the same community.

The specific objectives were:

Objective 1

To critically review the literature around clinical outcomes of HEU compared to HU children in sub-Saharan Africa at the onset of this research, to provide guidance for study design, data collection and analytic choices, including the *a priori* design of a directed acyclic graph.

Objective 2

To longitudinally measure and compare postnatal growth patterns of breastfed HEU and breastfed HU children in the first year of life, and to evaluate:

(a) Crude and adjusted differences in growth trajectories and risk of malnutrition
(b) The effects of maternal HIV disease severity (antenatal CD4 cell count/HIV viral load) and quality of breastfeeding (exclusivity and duration) on growth in HEU children
(c) Other potentially modifiable early life predictors of growth in both HEU and HU children

Objective 3

To longitudinally measure and compare infectious morbidity of breastfed HEU and breastfed HU children in the first year of life, and to evaluate:

(a) Crude and adjusted differences in infectious morbidity risks, specifically
    - Longitudinal prevalence of diarrhoeal disease,
    - Longitudinal prevalence of presumed lower respiratory tract infection; and
    - Incidence of all-cause and infectious-cause hospitalization
(b) The effects of maternal HIV disease severity (antenatal CD4 cell count/HIV viral load) and quality of breastfeeding (exclusivity and duration) on infectious morbidity risk in HEU children
(c) Other potentially modifiable early life predictors of infectious morbidity risk in both HEU and HU children

Objective 4

To measure and compare childhood development in a sub-group of breastfed HEU and breastfed HU children at the age of 12 months, and to evaluate:

(a) Crude and adjusted differences in mean developmental scores, and proportion of children with developmental delay, in cognitive, motor and language domains
(b) The effects of maternal HIV disease severity (antenatal CD4 cell count/HIV viral load) and quality of breastfeeding (exclusivity and duration) on developmental outcomes of HEU children
(c) Other potentially modifiable early life predictors of developmental delay in both HEU and HU children

In investigating these specific objectives, the thesis seeks to test three related hypotheses:

Hypothesis 1: The breastfed HEU children of HIV-infected women receiving ART have clinically similar health outcomes to the breastfed HU children of HIV-uninfected women.

Hypothesis 2: Early infant feeding modifies the effect of HIV-ART exposure on child health outcomes.

Hypothesis 3: Among HEU children, health outcomes are associated with (1) maternal HIV disease severity and (2) quality of breastfeeding.

1.4 Overview and structure of the thesis

This thesis comprises an introductory chapter, a systematic literature review chapter, a methods chapter, 4 results chapters, a discussion chapter, and supporting supplemental material in an appendix. An overview of chapters is shown in table 1.2; where applicable, the corresponding objective(s) and publication details are also summarized.

This introductory chapter contextualizes the burden and health risks of HEU children in sub-Saharan Africa, introduces key child health challenges in the region and reviews the evolution of PMTCT strategies related to both maternal ART use and breastfeeding over the past 20 years. The chapter further provides insight into methodological concerns around validity of previously collected data on HEU child health and introduces the objectives of this doctoral project.

Chapters 2, and 4 to 7, include manuscripts that have been published with the candidate as first author and lead investigator. Chapter 2 is a systematic literature review and meta-analysis, conducted and published at the onset of this research (in 2015), providing insight into study rationale. Chapters 4 to 7 present data analyses focusing on the clinical outcomes of growth, infectious morbidity, and neurodevelopment.

Chapter 3 provides an overview of the two, linked, prospective cohort studies utilized to generate comparative data on HEU and HU child health outcomes for these analyses. Details are also provided on choice and measurement of important third variables, repeat measures and statistical approaches.

- Objective 1 is addressed in the systematic literature review, chapter 2.
- Objectives 2 (a) to (c) are evaluated in chapter 4, a comparative exploration of growth patterns and risk of malnutrition.
- The comparison of infectious morbidity in HEU and HU children in chapter 5 addresses objectives 3 (a) to (c).
- Chapter 6 addresses objective 4 (a) and (c), using a nested evaluation to compare the neurodevelopment of HEU and HU children at 12 months of age.
- In Chapter 7, an analysis focusing specifically on the association between maternal viremia and child development explores objective 4 (b).

- Chapter 8 provides a summary of the key findings, with interpretation and contextualization of the results to identifying necessary future actions including recommendations for policy, clinical practice, and future research.

Table 1.2. Summary of thesis chapters, objectives, and relevant manuscripts

Thesis chapter	**Summary of related objective**	**Publication details**
Chapter 1. Introduction	Not applicable	Not applicable
Chapter 2. Systematic review	**1** Critically review of the literature around clinical outcomes of HEU compared to HU children in sub-Saharan Africa (publications until October 2015)	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 Jul;21(7):829-45. doi: 10.1111/tmi.12716
Chapter 3. Methodology	Not applicable	Not applicable
Chapter 4. HEU vs HU child growth	**2a** Summarize and compare crude and adjusted differences in growth trajectories and risk of malnutrition in breastfed HEU and HU infants longitudinally **2b** Evaluate effects of maternal HIV disease severity and quality of breastfeeding on postnatal growth of HEU children **2c** Identify other potentially modifiable early life predictors of growth in HEU and HU infants	le Roux SM, Abrams EJ, Donald KA, Brittain K, Phillips TK, Nguyen KK, Zerbe A, Kroon M, Myer L. **Growth trajectories of breastfed HIV-exposed uninfected and HIV-unexposed children under conditions of universal maternal antiretroviral therapy: a prospective study.** *Lancet Child & Adolescent Health.* 2019 Feb 14; 3(4): 234-244; doi: 10.1016/S2352-4642(19)30007-0
Chapter 5. HEU vs HU child infectious morbidity	**3a** Summarize and compare crude and adjusted differences in infectious morbidity risks in breastfed HEU and HU children longitudinally **3b** Evaluate effects of maternal HIV disease severity and quality of breastfeeding on infectious morbidity risk in HEU children **3c** Identify other potentially modifiable early life predictors of infectious morbidity risk in both HEU and HU children	le Roux SM, Abrams EJ, Donald KA, Brittain K, Phillips TK, Zerbe A, le Roux DM, Kroon M, Myer L. **Infectious morbidity of breastfed, HIV-exposed uninfected infants under conditions of universal antiretroviral therapy in South Africa: a prospective cohort study.** *Lancet Child Adolesc Health*. 2020 Mar;4(3):220-231. doi: 10.1016/S2352-4642(19)30375-X
Chapter 6. HEU vs HU child neurodevelopment.	**4a** Summarize and compare crude and adjusted differences in childhood development in a sub-group of breastfed HEU and breastfed HU children at the age of 12 months **4c** Identify other potentially modifiable early life predictors of childhood development in HEU and HU children at 12 months of age	le Roux SM, Donald KA, Brittain K, Phillips TK, Zerbe A, Nguyen KK, Strandvik A, Kroon M, Abrams EJ, Myer L. **Neurodevelopment of breastfed HIV-exposed uninfected and HIV-unexposed children in South Africa.** *AIDS.* 2018 Aug; 32 (13): 1781-1791 doi: 10.1097/QAD.0000000000001872
Chapter 7. Maternal viremia and child neurodevelopment in HEU children	**4b** Evaluate effects of maternal HIV disease severity and quality of breastfeeding on child development in HEU children at 12 months of age	le Roux SM, Donald KA, Kroon M, Phillips TK, Lesosky M, Esterhuyse L, Zerbe A, Brittain K, Abrams EJ, Myer L. **HIV Viremia During Pregnancy and Neurodevelopment of HIV-Exposed Uninfected Children in the Context of Universal Antiretroviral Therapy and Breastfeeding: A Prospective Study.** *Pediatric Infectious Disease Journal.* 2019 Jan: 38 (1): 70-75 doi: 10.1097/INF.0000000000002193
Chapter 8. Discussion	Not applicable	Not applicable

Abbreviations: HEU, HIV-exposed uninfected; HU, HIV-unexposed

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Chapter 2

Clinical outcomes of HIV-exposed uninfected children in sub-Saharan Africa: a systematic review

Stanzi M le Roux, Elaine J Abrams, Kelly K Nguyen, and Landon Myer. **Clinical outcomes of HIV-exposed, HIV-uninfected children in sub-Saharan Africa.** *Trop Med Int Health*. 2016 Jul;21(7):829-45. doi: 10.1111/tmi.12716

Relevance of this paper to the thesis

This chapter provides a detailed review of the context wherein this overarching doctoral research project was conceived. Conducted in 2015, at the onset of the overall prospective study of growth, infectious morbidity, and neurodevelopment among HIV-exposed uninfected (HEU) and HIV-unexposed (HU) children, the findings from this review shaped the study design, data collection tools and approaches and quality assurance measures. Using robust criteria to judge study quality, with detailed evaluation of comparative health measures in HIV-exposed uninfected and HIV-unexposed children in sub-Saharan Africa, this paper clearly demonstrates significant knowledge gaps and issues around internal validity in much of the evidence base available at the time.

Contribution of the student and co-authors

The candidate conceived the review under the guidance of LM. Under the supervision of LM, she developed, refined, and completed the search strategies across all search engines, with iterative search updates. SLR and KN screened studies for inclusion. SLR abstracted and analyzed the data and wrote the first draft of the manuscript. EJA revised the manuscript and interpretation of findings. All authors contributed to and approved the final manuscript.

2.1 Abstract

2.1.1 Background:

HIV-exposed but uninfected (HEU) children are widely considered at increased risk of mortality and morbidity. Recent advances in prevention-of-mother-to-child-transmission of HIV (PMTCT) strategies, incorporating life-long universal maternal antiretroviral therapy (ART) with extended breastfeeding ("Option B+"), may improve HEU child health substantially.

2.1.2 Objectives:

We critically reviewed reports of mortality/morbidity among HEU and HIV-unexposed (HU) children in sub-Saharan Africa (SSA).

2.1.3 Data sources:

Medline, EMBASE, CINAHL, PsycINFO, Academic Search Premier, Global Health & Psychosocial Instruments databases, conference abstracts; reference lists

2.1.4 Study eligibility and exposure:

Longitudinal studies from SSA, reporting mortality and clinical morbidity among HIV-uninfected children aged ≤ 10 years, by maternal HIV status.

2.1.5 Study appraisal:

Newcastle-Ottawa Scale and ACROBAT-NSRI.

2.1.6 Data synthesis:

Due to substantial heterogeneity of study designs, populations, and results (I^2=75%), data synthesis was not done.

2.1.7 Results:

We included 37 reports (28 studies, 11 164 HEU children); methodological and reporting quality were variable. Most reports came from settings without universal access to maternal ART (n=35). Results were conflicting, with some studies indicating increased risk of mortality, hospitalisation and/or undernutrition among HEU children while others found no evidence of increased risk. In sub-analyses, improved maternal health, ART use and breastfeeding were strongly protective for all outcomes. Only 39% (11/28) of studies adjusted for major confounders. Reports from settings using universal maternal ART with breastfeeding (n=2) found no differences in growth or development but did not report mortality or infectious morbidity.

2.1.8 Conclusions:

The existing literature provides little insight into HEU child health under recently adopted PMTCT strategies. There is a need for robust comparative data on HEU and HU child health outcomes under Option B+; optimizing breastfeeding practices and increasing maternal use of ART should be urgent public health priorities.

2.2 Introduction

In 2014, an estimated 13.8 million women of child-bearing age were living with HIV in sub-Saharan Africa (SSA). With the rapid global expansion of effective strategies to prevent mother-to-child transmission of HIV (PMTCT), an increasing proportion of HIV-exposed children are born uninfected (HEU) annually; during 2014 more than one million HEU infants were born in the region.[1] Historically, HEU children have been reported to have higher than expected risks of mortality and morbidity.[2-6] This association is hypothesized to be driven by a combination of biologic and socio-economic risk factors, including advanced maternal HIV disease and/or death,[5,7] disruptions in family and socio-economic structures,[8] insufficient infant vaccine and other immunological responses,[9-12] altered child health care-seeking behaviour,[13] increased pathogen exposure in the home[14-16] and suboptimal infant feeding practices.[4,17-22]

However, existing insights into the health of HEU children come predominantly from a previous era of HIV and PMTCT in Africa, when access to lifelong triple drug maternal antiretroviral therapy (ART) was limited, and breastfeeding by HIV-infected mothers often controversial.[20,21] Since 2010, the World Health Organization (WHO) has recommended ART for all pregnant and breastfeeding women with HIV, maintained at least for the duration of breastfeeding ("Option B") or for life ("Option B+").[23] [24] [25] Since 2015, the WHO recommends universal ART for all HIV infected individuals, irrespective of disease stage, and in line with this, "Option B+" as the preferred PMTCT strategy.[26] Effective use of maternal ART results in viral suppression with subsequent immune restoration and protection of maternal health;[16,27,28] HEU infants born to mothers with lower HIV viral load and/or higher CD4 count have significantly lower risks of death,[7,29] hospitalisation,[7] severe infection,[7] immune dysfunction [30,31] and growth faltering [32,33] than those born to women with untreated or advanced HIV disease.[29,33]

In addition, breastfeeding is well understood to reduce child morbidity and mortality.[34-37] However in the absence of any PMTCT interventions, breastfeeding, and particularly mixed feeding, contributes to overall MTCT risk.[38] Accordingly, WHO HIV and infant feeding guidelines between 2001 and 2009 placed a strong emphasis on replacement feeding for HIV prevention; recommended feeding options included commercial infant formula, home-modified animal milk, wet-nursing by an uninfected woman, heat-treated expressed breastmilk, or exclusive breastfeeding (EBF) for 6 months followed by accelerated weaning over a short period of time ("rapid weaning").[39,40] However, by 2010 strong evidence had accumulated regarding the substantial mortality and infectious disease risk associated with both replacement feeding and rapid weaning in most of SSA [18-20,41-45] while other data illustrated reductions in postnatal MTCT with use of maternal and/or infant antiretrovirals during breastfeeding.[44,46-48] WHO HIV and infant feeding guidelines were updated accordingly, recommending exclusive breastfeeding for 6 months with continuation until at least 12 months of age in settings with high infant and child mortality, and antiretroviral prophylaxis or lifelong maternal ART to reduce the risk of postnatal transmission through breastfeeding.[40]

Understanding the impact of universal maternal ART with extended breastfeeding on HEU child health is an important step towards improving child health and survival in SSA. Given their substantial benefits it is plausible that these strategies, increasingly implemented across SSA,[49] may ameliorate the adverse effects of maternal HIV infection on child health.[19,43,50-54] To investigate this possibility, we systematically reviewed the association between maternal HIV status and child health outcomes in SSA in the absence of vertical transmission, among children aged ≤ 10 years of age. In addition to mortality, we focused on conditions that contribute significantly to under-five mortality and burden of disease on the continent, and are strongly influenced by breastfeeding; specifically, we looked at diarrhoea, pneumonia, undernutrition and developmental delay.[51-53,55,56] Our specific objectives were to (i) summarize and (ii) critically assess reports of child mortality/morbidity among HEU compared to HIV-unexposed (HU) children, and (iii) to examine variation by maternal ART status and breastfeeding practices.

2.3 Methods

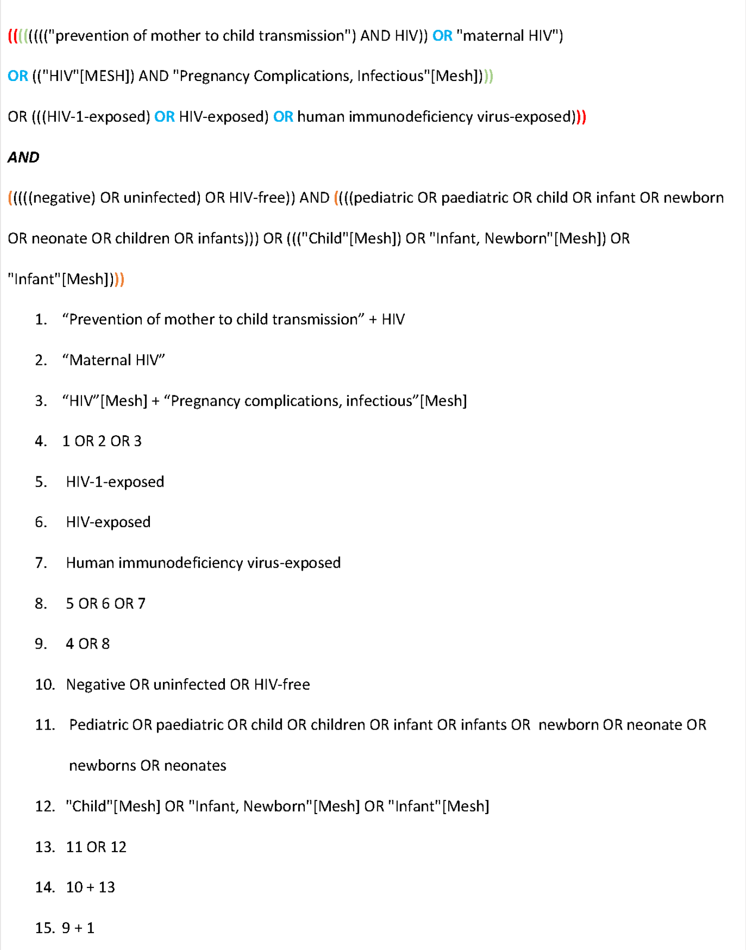
2.3.1 Criteria for study inclusion

The population of interest was pre-adolescent children (defined as age ≤10 years)[57] born to HIV-infected women, with known negative HIV status based on age-appropriate laboratory methods. Studies were excluded if child HIV status was not reported, was based only on survival and clinical criteria, or was based on antibody testing alone prior to 18 months of age. To reduce biases due to left censoring we limited our review to longitudinal study designs and excluded case-control and cross-sectional studies. We also excluded studies where participants were sampled on the basis of maternal morbidity (other than HIV infection) and/or child health status (other than HIV exposure). We included only studies where HEU children were compared to HU children from a similar setting or community.

The main exposure of interest was maternal HIV status during pregnancy and/or breastfeeding. We included studies where maternal HIV status was extrapolated from the presence of anti-HIV antibodies in children under the age of 12 months. Where data were available, we evaluated breastfeeding practices and maternal HIV disease severity as additional exposures. Primary outcomes of interest for the review were (i) mortality; (ii) diarrhoea, pneumonia and health care utilization; (iii) growth parameters focusing on weight and length; and (iv) measures of early childhood development.

2.3.2 *Search methods for identification of studies*

We built database-specific search strategies using a combination of terms for "HIV" + "mother" + "uninfected" + "child", without date limitations, and searched MEDLINE (via Pubmed), EMBASE, CINAHL Plus, PsycINFO, Academic Search Premier, Global Health and Psychosocial Instruments. For the PubMed search, we included both MeSH and free text terms, with no language restrictions (figure 2.1); the most recent search was conducted on October 28, 2015. We used the EBSCOhost research database to search for relevant conference abstracts, reviewed the reference lists of reports that met our inclusion criteria, and examined the bibliographies of editorials and review articles found in our searches.[3,4,58-69]


((((((((\"prevention of mother to child transmission") AND HIV)) **OR** "maternal HIV")

OR (("HIV"[MESH]) AND "Pregnancy Complications, Infectious"[Mesh])))

OR (((HIV-1-exposed) **OR** HIV-exposed) **OR** human immunodeficiency virus-exposed)))

AND

(((((negative) OR uninfected) OR HIV-free)) AND ((((pediatric OR paediatric OR child OR infant OR newborn OR neonate OR children OR infants))) OR ((("Child"[Mesh]) OR "Infant, Newborn"[Mesh]) OR "Infant"[Mesh])))

1. "Prevention of mother to child transmission" + HIV
2. "Maternal HIV"
3. "HIV"[Mesh] + "Pregnancy complications, infectious"[Mesh]
4. 1 OR 2 OR 3
5. HIV-1-exposed
6. HIV-exposed
7. Human immunodeficiency virus-exposed
8. 5 OR 6 OR 7
9. 4 OR 8
10. Negative OR uninfected OR HIV-free
11. Pediatric OR paediatric OR child OR children OR infant OR infants OR newborn OR neonate OR newborns OR neonates
12. "Child"[Mesh] OR "Infant, Newborn"[Mesh] OR "Infant"[Mesh]
13. 11 OR 12
14. 10 + 13
15. 9 + 1

Figure 2.1. Pubmed search strategy details

2.3.3 *Data collection and analysis*

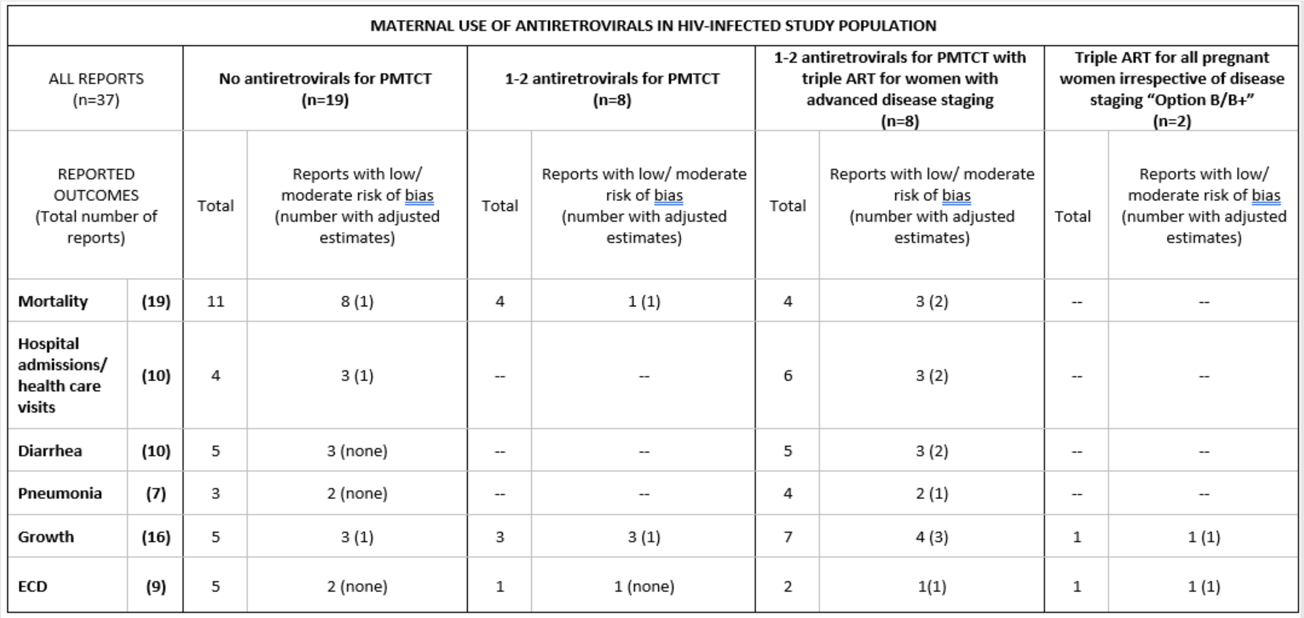
We conducted the review in accordance with the PRISMA guidelines (Supplemental table 9.2.1; PROSPERO registration number CRD42015017639). Following iterative database searches, we screened all titles and abstracts to identify reports for full-text review, and developed the data abstraction form. Two authors (SLR and KN) independently reviewed all full-text reports; disagreements were resolved through discussion with the senior author (LM).

Given the substantive risk of bias (RoB) inherent to observational studies, we decided *a priori* to utilize the Newcastle-Ottawa Scale (NOS) [70] to assess RoB, in accordance to the Cochrane Handbook.[71] Domains of potential bias were external validity, confounding, selection bias and information bias; for each domain we used questions from either the NOS or, when application of the NOS did not yield sufficient information, the Cochrane Risk of Bias Assessment Tool for Non-Randomized Studies of Interventions (ACROBAT-NRSI).[72] Following the format of the latter, we utilized 11 "signalling" questions in total (supplemental table 9.2.2), each of which could have a single answer: either yes (low RoB in the related domain), probably yes (moderate RoB), no (serious to critical RoB) or insufficient information to assess (unable to allocate RoB). Based on the answers within each domain, each study was allocated an overall RoB: “Low”, the study is comparable to a well-performed randomized trial; “Moderate”, the study is sound for a non-randomized study; “Serious”, the study has some serious problems; “Critical” the study is too problematic to provide useful evidence on the effects of exposure; or “No information”, where insufficient information was provided on which to base a overall judgement. Finally, for data synthesis purposes, we excluded all studies with insufficient information and those judged to have more than a moderate overall RoB (supplemental table 9.2.2).

In summarising study findings, we presented unadjusted mean differences, risk ratios, and incidence rate ratios, with 95% confidence intervals (CI). Adjusted measures of association were included when available. Measures of association were calculated in Stata 12.0 (StataCorp, College Station, TX, USA) using raw data presented in reports; where no raw data was presented we used the measure provided by the authors. As we anticipated substantial clinical and methodological heterogeneity we explored potential data synthesis only for mortality, limited to results from studies with low/moderate risk of bias, using a random effects meta-analytic model. We formally tested for statistical heterogeneity within sub-groups of perinatal/maternal use of ART, using the chi^2 test with 10% level of significance, presented as an I^2 statistic. [73-75] We tested for small study effects (such as potential publication bias) through the use of a funnel plot and Harbord’s modified test.[76]

2.4 Results

2.4.1 *Description of included studies*



MATERNAL USE OF ANTIRETROVIRALS IN HIV-INFECTED STUDY POPULATION									
ALL REPORTS (n=37)		**No antiretrovirals for PMTCT (n=19)**		**1-2 antiretrovirals for PMTCT (n=8)**		**1-2 antiretrovirals for PMTCT with triple ART for women with advanced disease staging (n=8)**		**Triple ART for all pregnant women irrespective of disease staging “Option B/B+” (n=2)**	
REPORTED OUTCOMES (Total number of reports)		Total	Reports with low/ moderate risk of bias (number with adjusted estimates)	Total	Reports with low/ moderate risk of bias (number with adjusted estimates)	Total	Reports with low/ moderate risk of bias (number with adjusted estimates)	Total	Reports with low/ moderate risk of bias (number with adjusted estimates)
Mortality	**(19)**	11	8 (1)	4	1 (1)	4	3 (2)	--	--
Hospital admissions/ health care visits	**(10)**	4	3 (1)	--	--	6	3 (2)	--	--
Diarrhea	**(10)**	5	3 (none)	--	--	5	3 (2)	--	--
Pneumonia	**(7)**	3	2 (none)	--	--	4	2 (1)	--	--
Growth	**(16)**	5	3 (1)	3	3 (1)	7	4 (3)	1	1 (1)
ECD	**(9)**	5	2 (none)	1	1 (none)	2	1(1)	1	1 (1)

Table 2.1. Included studies categorized by maternal ART use and risk of bias

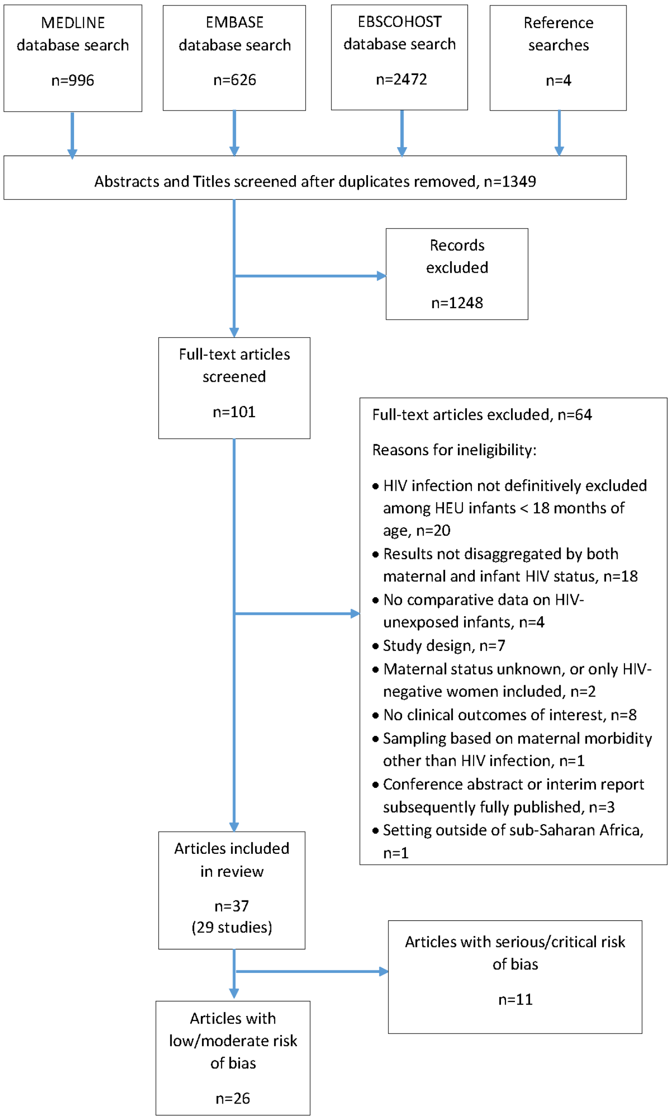


Figure 2.2. Flow of study selection

Thirty-seven reports, from 29 studies conducted in 12 African countries and involving 32 173 (11 164 HEU) children were included, as shown in figure 2.2 (characteristics of and reasons for exclusion of studies are shown in supplemental table 9.2.3; characteristics of included studies are shown in supplemental table 9.2.4).

Three studies contributed data on the same outcome at different time points (mortality,[77,78] neurodevelopment[79,80] and growth,[81,82] respectively), whilst 5 studies contributed two reports each, on different outcomes.[5,32,33,83-89] Overall, twenty-eight prospective studies were included,[77,80,81,84-87,89-109] of which 11 were randomized controlled trials (13 reports),[5,81,82,84,90,94-96,98-101,110] and 2 interventional cohort studies (4 reports) focusing on PMTCT.[33,83,85,86] The majority of studies (72%, 21/29) enrolled participants during the antenatal period or soon after birth.[33,77,80,84,88,89,91-94,96-99,101-105,107,108] Most reports (31/37, 84%) focused on health outcomes among infants and/or children ≤36 months old; there were only 6 reports on health outcomes in HEU children older than 3 years.[78,82,87,105,109,111].

2.4.1.1 Maternal treatment and health status

An overview of studies by maternal antiretroviral use is shown in table 2.1. There were 2 reports on child outcomes under universal maternal ART; in both studies, HEU children were studied either during or immediately after participation in clinical trials.[100,101]

The majority of reports were on HEU children whose mothers received no perinatal antiretroviral drugs (19/37, 51%).[5,77,78,80,81,83-85,91-93,95,103,105-109,111] Sixteen (43% of 37) reports focused on infants born to women receiving 1-2 antiretroviral drugs (such as single-dose nevirapine, sdNVP) for

PMTCT;[32,33,82,86-90,94,96-99,102,104,112] 8 of these included a small proportion of women receiving ART for advanced maternal HIV disease.[82,86,96-98,102,104,112] Information regarding maternal mortality and/or HIV disease severity (symptoms, CD4 cell counts and/or viral loads) was available for 24 of 37 reports (65%), in either the index report or a related publication from the same study.[5,32,33,77,78,80,83-89,91,93,94,97-99,102,104,105,107,112] However, infant outcome results were stratified or adjusted accordingly in only 11 of these.[5,32,33,77,84,85,89,94,98,102,107]

2.4.1.2 Infant feeding

Eighteen reports (49%) provided limited to no information on early infant feeding.[77,78,80,83,85,87,88,91,92,94,95,98-100,106,107,109,111] Available infant feeding details were summarized by study according to WHO infant feeding (supplemental table 9.2.5) [113] Most reports predated the 2001 WHO HIV and infant feeding guidelines (15 reports from 11 studies),[5,77,78,80,83-85,91-93,103,105,107,108,111] or were from the period between 2001 and 2009, when exclusive breastfeeding with rapid weaning was recommended if replacement feeding was not possible (20 reports from 17 studies).[32,33,81,82,86-90,94,95,97-99,101,102,104,106,109,112]

Two reports from post-2009 study populations provided limited infant feeding data.[96,100] Both indicated breastfeeding durations for HEU children beyond 6 months, without reporting rapid weaning; however, the majority of HEU children discontinued breastfeeding before 12 months of age, while HU children were breastfed for longer. Overall, infant feeding reporting was suboptimal, and adjustment or stratification of results according to early infant feeding modality was presented in less than a third of reports (10/37, 27% of total). [32,81,86,89,90,96,98,102,107,114]

2.4.2 Methodological quality of included studies

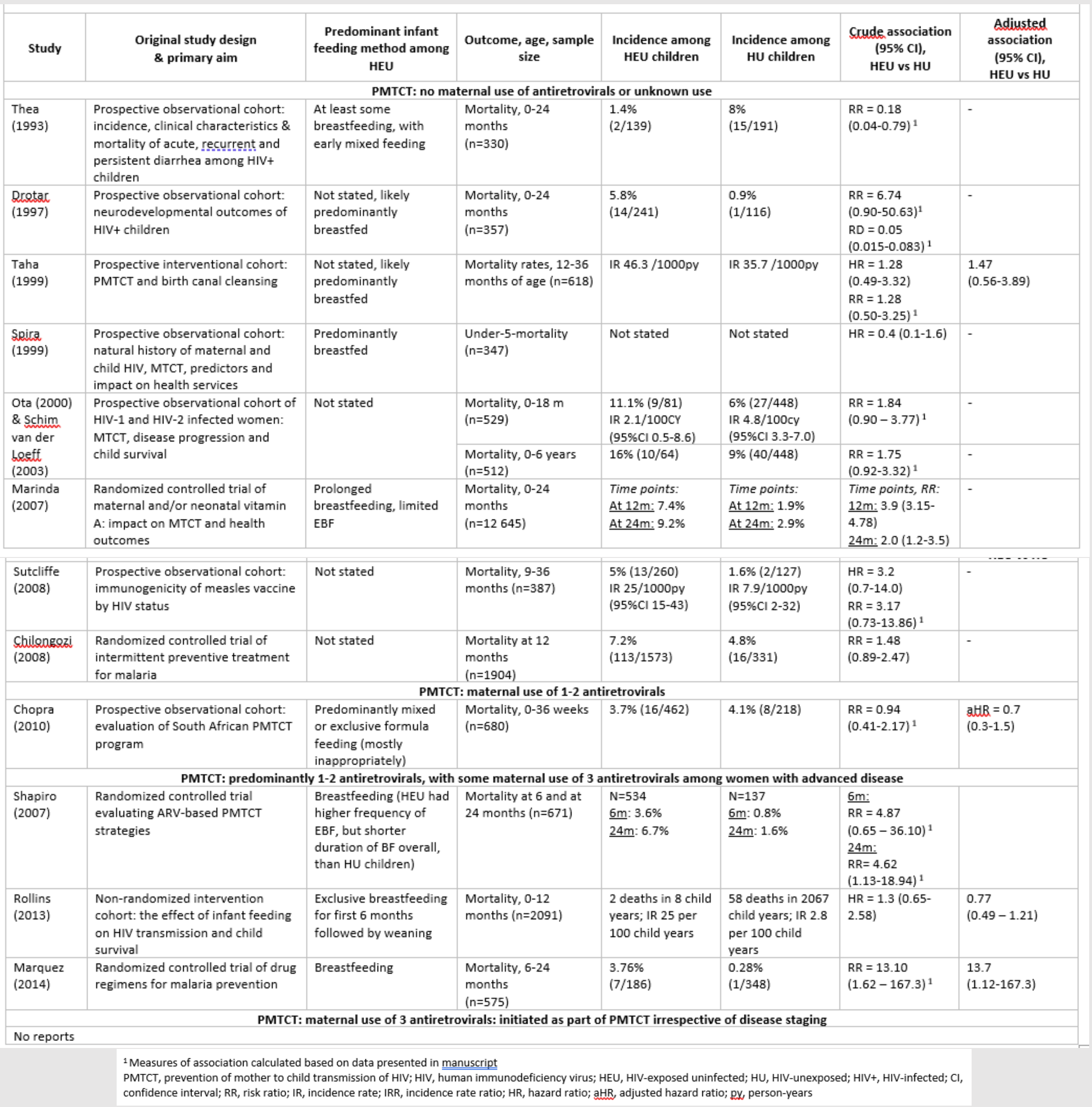
Study-level risk of bias varied widely (supplemental table 9.2.2). Six reports were considered subject to serious overall risk of bias, predominantly selection bias resulting from participant sampling or differential follow-up.[87,92,95,97,103,112] Four reports provided insufficient information on which to base a risk of bias assessment;[93,99,104,108] one study was at critical risk of selection bias.[109] Two studies (four reports) were considered to be comparable to well-performed randomized trials (low risk of bias);[5,33,84,86] the remaining studies were considered sound for non-randomized trials (moderate risk of bias). In 20 studies (26 reports), [32,33,77,80-83,85-90,92,94,96-99,101,102,104,106-108,111] post-partum HIV seroconversion was not excluded among initially HIV-negative breastfeeding women, potentially increasing the risk of exposure misclassification bias.[115]

Only 32% (12/37) of reports considered potential confounders in examining the association between HIV-exposure and child health outcomes.[5,32,33,81,82,85,86,89,96,98,100,101] Confounder selection was commonly based on statistical significance testing and seldom defined *a priori*; varying between analyses, these included social/economic measures,[32,81,82,86,89,96,98,100] early infant feeding,[32,33,81,86,89,96] child age,[5,32,96-98,100] low birth weight[32,85,100] and maternal health/survival.[5,85,89] The majority of studies reported group-averaged associations between maternal HIV status and child health outcomes; only four reports examined effect modification by other clinical or demographic factors.

2.4.3 *Association between HEU status and child health outcomes*

2.4.3.1 Mortality

There were 19 reports of unadjusted estimates of child mortality, 13 of which were considered low/moderate risk of bias (Tables 2.1, 2.2). [77,80,84-86,89,94,96,102,105-107,116]



Study	Original study design & primary aim	Predominant infant feeding method among HEU	Outcome, age, sample size	Incidence among HEU children	Incidence among HU children	Crude association (95% CI), HEU vs HU	Adjusted association (95% CI), HEU vs HU
PMTCT: no maternal use of antiretrovirals or unknown use							
Thea (1993)	Prospective observational cohort: incidence, clinical characteristics & mortality of acute, recurrent and persistent diarrhea among HIV+ children	At least some breastfeeding, with early mixed feeding	Mortality, 0-24 months (n=330)	1.4% (2/139)	8% (15/191)	RR = 0.18 (0.04-0.79)[1]	-
Drotar (1997)	Prospective observational cohort: neurodevelopmental outcomes of HIV+ children	Not stated, likely predominantly breastfed	Mortality, 0-24 months (n=357)	5.8% (14/241)	0.9% (1/116)	RR = 6.74 (0.90-50.63)[1] RD = 0.05 (0.015-0.083)[1]	-
Taha (1999)	Prospective interventional cohort: PMTCT and birth canal cleansing	Not stated, likely predominantly breastfed	Mortality rates, 12-36 months of age (n=618)	IR 46.3 /1000py	IR 35.7 /1000py	HR = 1.28 (0.49-3.32) RR = 1.28 (0.50-3.25)[1]	1.47 (0.56-3.89)
Spira (1999)	Prospective observational cohort: natural history of maternal and child HIV, MTCT, predictors and impact on health services	Predominantly breastfed	Under-5-mortality (n=347)	Not stated	Not stated	HR = 0.4 (0.1-1.6)	-
Ota (2000) & Schim van der Loeff (2003)	Prospective observational cohort of HIV-1 and HIV-2 infected women: MTCT, disease progression and child survival	Not stated	Mortality, 0-18 m (n=529)	11.1% (9/81) IR 2.1/100CY (95%CI 0.5-8.6)	6% (27/448) IR 4.8/100cy (95%CI 3.3-7.0)	RR = 1.84 (0.90 – 3.77)[1]	-
			Mortality, 0-6 years (n=512)	16% (10/64)	9% (40/448)	RR = 1.75 (0.92-3.32)[1]	-
Marinda (2007)	Randomized controlled trial of maternal and/or neonatal vitamin A: impact on MTCT and health outcomes	Prolonged breastfeeding, limited EBF	Mortality, 0-24 months (n=12 645)	*Time points:* At 12m: 7.4% At 24m: 9.2%	*Time points:* At 12m: 1.9% At 24m: 2.9%	*Time points, RR:* 12m: 3.9 (3.15-4.78) 24m: 2.0 (1.2-3.5)	-
Sutcliffe (2008)	Prospective observational cohort: immunogenicity of measles vaccine by HIV status	Not stated	Mortality, 9-36 months (n=387)	5% (13/260) IR 25/1000py (95%CI 15-43)	1.6% (2/127) IR 7.9/1000py (95%CI 2-32)	HR = 3.2 (0.7-14.0) RR = 3.17 (0.73-13.86)[1]	-
Chilongozi (2008)	Randomized controlled trial of intermittent preventive treatment for malaria	Not stated	Mortality at 12 months (n=1904)	7.2% (113/1573)	4.8% (16/331)	RR = 1.48 (0.89-2.47)	-
PMTCT: maternal use of 1-2 antiretrovirals							
Chopra (2010)	Prospective observational cohort: evaluation of South African PMTCT program	Predominantly mixed or exclusive formula feeding (mostly inappropriately)	Mortality, 0-36 weeks (n=680)	3.7% (16/462)	4.1% (8/218)	RR = 0.94 (0.41-2.17)[1]	aHR = 0.7 (0.3-1.5)
PMTCT: predominantly 1-2 antiretrovirals, with some maternal use of 3 antiretrovirals among women with advanced disease							
Shapiro (2007)	Randomized controlled trial evaluating ARV-based PMTCT strategies	Breastfeeding (HEU had higher frequency of EBF, but shorter duration of BF overall, than HU children)	Mortality at 6 and at 24 months (n=671)	N=534 6m: 3.6% 24m: 6.7%	N=137 6m: 0.8% 24m: 1.6%	6m: RR = 4.87 (0.65 – 36.10)[1] 24m: RR= 4.62 (1.13-18.94)[1]	
Rollins (2013)	Non-randomized intervention cohort: the effect of infant feeding on HIV transmission and child survival	Exclusive breastfeeding for first 6 months followed by weaning	Mortality, 0-12 months (n=2091)	2 deaths in 8 child years; IR 25 per 100 child years	58 deaths in 2067 child years; IR 2.8 per 100 child years	HR = 1.3 (0.65-2.58)	0.77 (0.49 – 1.21)
Marquez (2014)	Randomized controlled trial of drug regimens for malaria prevention	Breastfeeding	Mortality, 6-24 months (n=575)	3.76% (7/186)	0.28% (1/348)	RR = 13.10 (1.62 – 167.3)[1]	13.7 (1.12-167.3)
PMTCT: maternal use of 3 antiretrovirals: initiated as part of PMTCT irrespective of disease staging							
No reports							

[1] Measures of association calculated based on data presented in manuscript

PMTCT, prevention of mother to child transmission of HIV; HIV, human immunodeficiency virus; HEU, HIV-exposed uninfected; HU, HIV-unexposed; HIV+, HIV-infected; CI, confidence interval; RR, risk ratio; IR, incidence rate; IRR, incidence rate ratio; HR, hazard ratio; aHR, adjusted hazard ratio; py, person-years

Table 2.2. Effect of maternal HIV status on mortality among HIV-uninfected children in sub-Saharan Africa

Raw data was available for calculation of risk ratios in 11 reports; two reports are presented only with hazard ratios as provided in the original manuscript (Table 2.2 and Figure 2.3).[86,105] There was substantial evidence of between-study heterogeneity overall (I^2, 75.1%, p<0.001) and within subgroups of maternal use of antiretroviral drugs (figure 2.3).

Furthermore, the funnel plot (figure 2.4) provided evidence of small study effect, most likely due to publication bias (p=0.04, Harbord's modified test), with a paucity of small studies reporting no increased or very small decreased risk of mortality among HEU. Nonetheless, there was a clear trend towards increased mortality among HEU children versus HU comparators, particularly among non-breastfeeding HEU populations without universal maternal access to ART, although the majority of studies had small sample sizes and in turn, imprecise findings.[77,78,80,84,85,96,106] The two largest studies, both considered to be at relatively low risk of bias, provide particular insights relevant to current PMTCT strategies. In an early PMTCT trial, antiretroviral-naive Zimbabwean women and their newborns were randomly allocated to receive either vitamin A or placebo in a factorial (2X2) design. Although randomization was not stratified by HIV status, clear mortality differences were noted in these subgroups. Compared to HU infants, HEU infants experienced a significantly higher 12-month mortality risk: unadjusted risk ratio (RR), 3.71 (95% CI 0.73-13.86).[84] However, the effect of maternal HIV exposure on infant mortality was modified by maternal disease severity: HEU children born to mothers with CD4 cell counts <200 cells/µL were at substantially higher risk of death than those born to mothers with CD4 ≥ 400 cells/µL (aHR 2.62, 95% CI 1.8-3.8); maternal death predicted infant death among both HEU (aHR 2.68, 95% CI 1.86-3.87) and HU children.

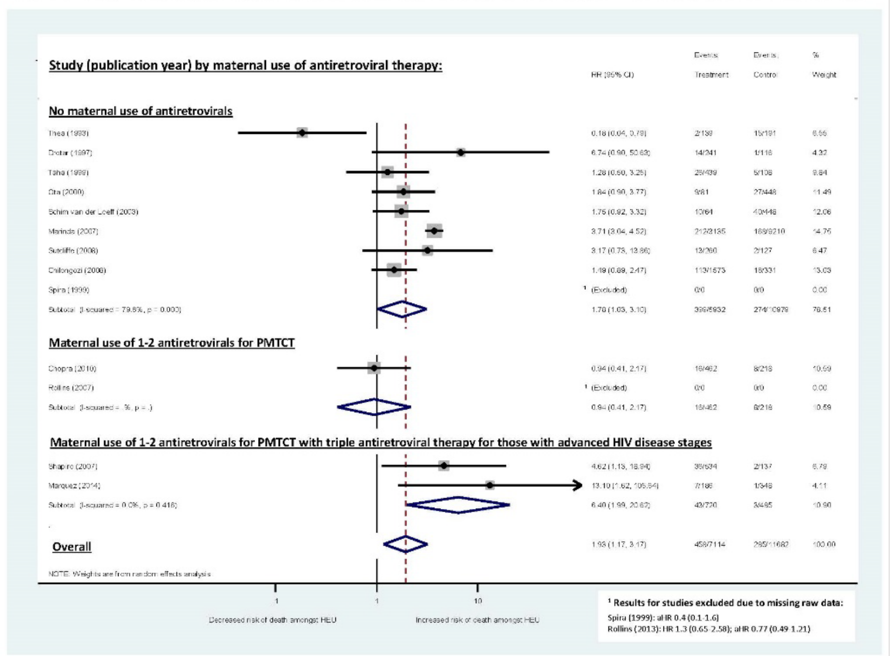


Figure 2.3. Forest plot of mortality among HIV-exposed uninfected children compared to HIV -unexposed children, by maternal antiretroviral use

In a later South African study, predominantly breastfeeding women received single dose nevirapine (sdNVP) during pregnancy, with some access to triple ART for women with severe disease. After adjusting for breastfeeding practices and socio-economic factors, there was no significant difference in the hazard of child death comparing HEU to HU children (aHR 0.77, 95% CI 0.49-1.21). Exclusive breastfeeding (EBF) was achieved by a substantial number of both HIV-infected (81.4% by 6-8 weeks) and HIV-uninfected women (92.9% by 6-8 weeks).

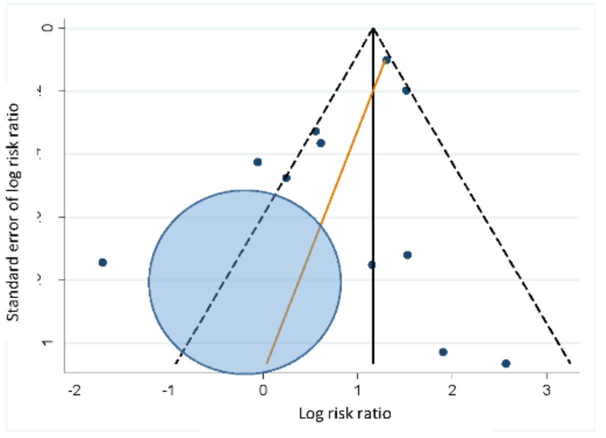


Figure 2.4. Funnel plot for meta-analysis of child mortality estimates comparing HIV exposed uninfected to HIV-unexposed children in sub-Saharan Africa

Compared to children who were EBF, both partially breastfed and never breastfed children were at substantially higher risk of death before 12 months, even after adjusting for child HIV status and socio-economic variables: aHR 2.6 (95% CI 1.9-3.8) and aHR 3.6 (95% CI 2.5-5.2), respectively.[33] Of note, there were no published reports comparing mortality between HEU and HU children in the context of universal maternal ART (tables 2.1, 2.2 and supplemental table 9.24).

2.4.3.2 Hospitalisation/sick child visits

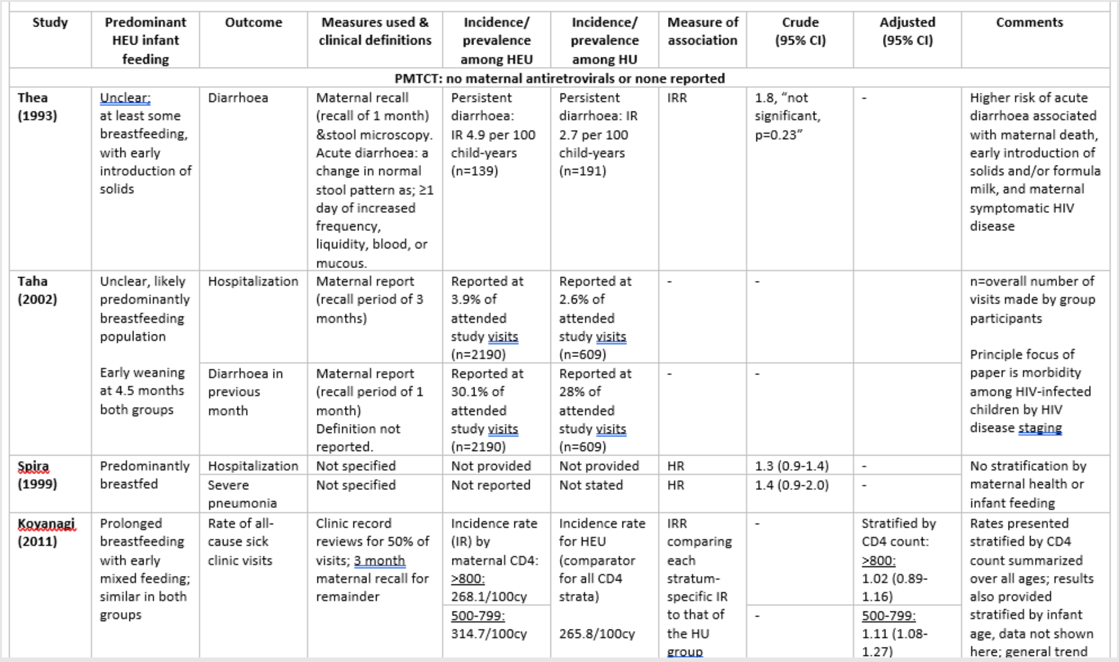
Hospitalisation and "sick-child" visits appeared to occur more commonly among HEU than HU children. There were ten reports of unadjusted hospitalisation and/or "sick-child" clinic visit risk estimates.[5,82,83,95-97,102,104,105,112] Overall, four reports were from populations without access to antiretroviral drugs;[5,83,95,105] six from populations using predominantly 1-2 antiretroviral drugs for PMTCT;[82,96,97,102,104,112] and there were no reports on populations receiving ART during pregnancy and breast feeding (table 2.1).

Estimates from the six reports with low/moderate risk of bias are shown in table 2.3.[82,96,97,102,104,112] Two reports identified factors modifying the relationship between maternal HIV status and childhood hospitalisation/clinic visitation risk. In a Zimbabwean RCT (effect of vitamin A on MTCT), where access to ART was limited, the association between maternal HIV status and child risk differed significantly by levels of maternal HIV disease severity: compared to predominantly breastfed HU infants, predominantly breastfed HEU infants born to mothers with baseline CD4 counts of >800cells/mm3 had no increased risk of sick clinic visits (IRR 1.02, 95% CI 0.89 – 1.16) whereas those born to mothers with lower CD4 cell counts showed incrementally increasing risks, with the highest risk occurring among infants born to mothers with CD4<200 cells/mm3 (IRR 1.33, 95% CI 1.17 – 1.50).[5] More recent data from an Ugandan anti-malarial RCT demonstrated effect modification by infant feeding modality: while non-breastfed HEU infants born to women receiving ART had a remarkably higher risk of hospitalisation than their breastfed HU counterparts (adjusted IRR 19.6, 95% CI 4.95-77.3), breastfed HEU and breastfed HU children had approximately similar risks, although precision for this estimate was low (adjusted IRR 1.50, 95% CI 0.14 – 16.4).[96]

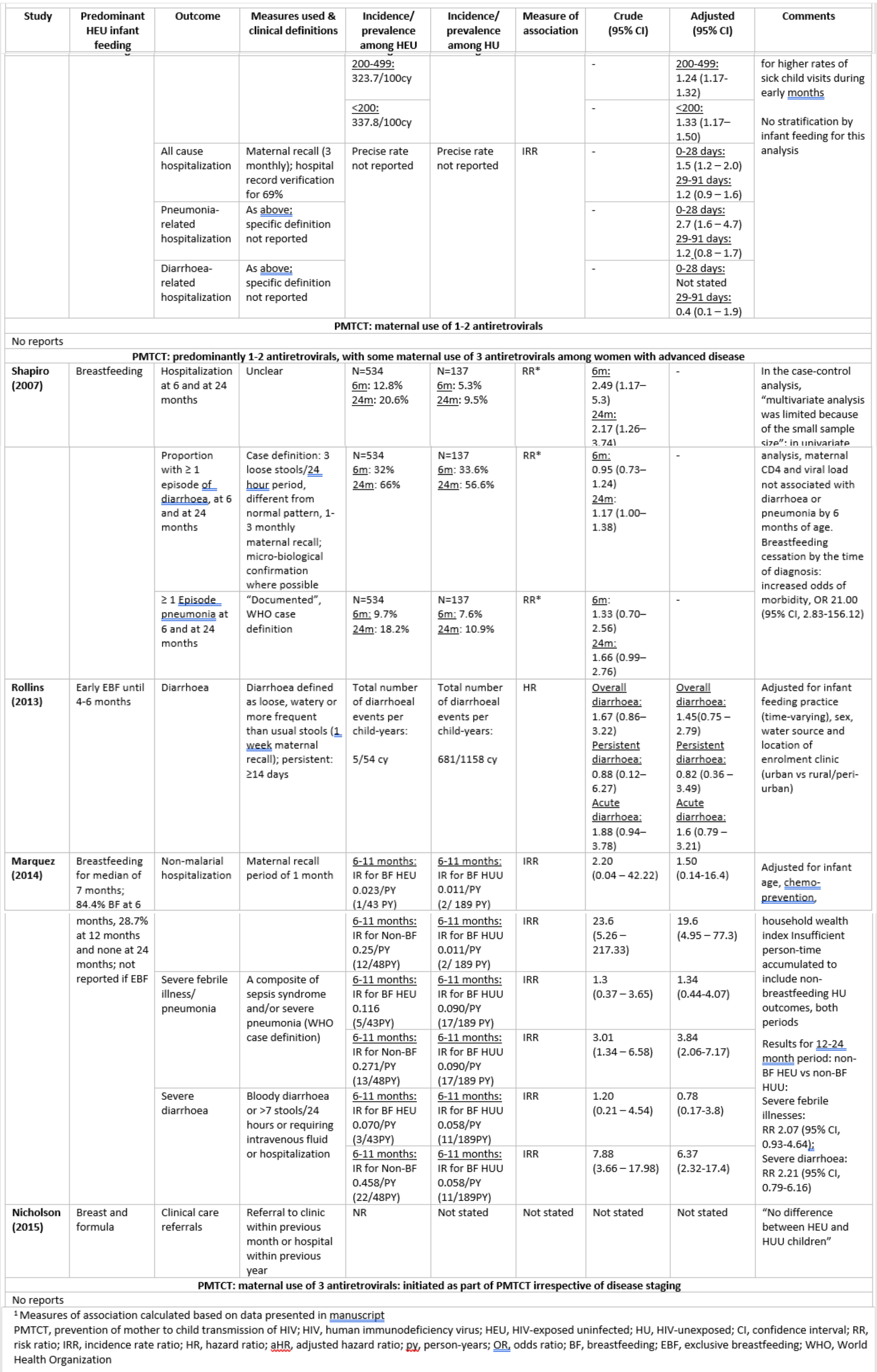
2.4.3.3 Diarrhoeal disease

Ten reports provided unadjusted estimates of risk for diarrhoeal disease.[5,83,86,95-97,102,107,108,112] Six reports were assessed to be at low/moderate risk of bias;[5,83,86,96,102,107] three included stratified/adjusted estimates[5,86,96] (tables 2.1 and 2.3). Between-study comparison of diarrhoea estimates was limited by varying definitions and measurement of diarrhoeal disease. Five of the 10 reports were on populations without access to antiretroviral drugs.[5,83,95,107,108] Four of these populations were predominantly mixed fed, combining breastmilk with early introduction of complementary feeds;[5,83,107,108] one report provided no details on infant feeding.[95] A single study with no use of antiretroviral drugs (moderate risk of bias) found an increased risk of persistent diarrhoea among HEU (IR 4.9 per 100 child-years) compared to HU infants (IR 2.7 per 100 child-years); those with earlier introduction to solids and/or formula milk were at highest risk irrespective of maternal HIV status.[107]

Table 2.3. Effect of maternal HIV status on infectious morbidity (hospitalisation, diarrhoeal disease and pneumonia) among HIV-uninfected children in sub-Saharan Africa, by maternal use of antiretrovirals



Study	Predominant HEU infant feeding	Outcome	Measures used & clinical definitions	Incidence/ prevalence among HEU	Incidence/ prevalence among HU	Measure of association	Crude (95% CI)	Adjusted (95% CI)	Comments
PMTCT: no maternal antiretrovirals or none reported									
Thea (1993)	Unclear; at least some breastfeeding, with early introduction of solids	Diarrhoea	Maternal recall (recall of 1 month) &stool microscopy. Acute diarrhoea: a change in normal stool pattern as; ≥1 day of increased frequency, liquidity, blood, or mucous.	Persistent diarrhoea: IR 4.9 per 100 child-years (n=139)	Persistent diarrhoea: IR 2.7 per 100 child-years (n=191)	IRR	1.8, "not significant, p=0.23"	-	Higher risk of acute diarrhoea associated with maternal death, early introduction of solids and/or formula milk, and maternal symptomatic HIV disease
Taha (2002)	Unclear, likely predominantly breastfeeding population	Hospitalization	Maternal report (recall period of 3 months)	Reported at 3.9% of attended study visits (n=2190)	Reported at 2.6% of attended study visits (n=609)	-	-		n=overall number of visits made by group participants Principle focus of paper is morbidity among HIV-infected children by HIV disease staging
	Early weaning at 4.5 months both groups	Diarrhoea in previous month	Maternal report (recall period of 1 month) Definition not reported.	Reported at 30.1% of attended study visits (n=2190)	Reported at 28% of attended study visits (n=609)	-	-		
Spira (1999)	Predominantly breastfed	Hospitalization	Not specified	Not provided	Not provided	HR	1.3 (0.9-1.4)	-	No stratification by maternal health or infant feeding
		Severe pneumonia	Not specified	Not reported	Not stated	HR	1.4 (0.9-2.0)	-	
Koyanagi (2011)	Prolonged breastfeeding with early mixed feeding; similar in both groups	Rate of all-cause sick clinic visits	Clinic record reviews for 50% of visits; 3 month maternal recall for remainder	Incidence rate (IR) by maternal CD4: >800: 268.1/100cy	Incidence rate for HEU (comparator for all CD4 strata)	IRR comparing each stratum-specific IR to that of the HU group	-	Stratified by CD4 count: >800: 1.02 (0.89-1.16)	Rates presented stratified by CD4 count summarized over all ages; results also provided stratified by infant age, data not shown here; general trend
				500-799: 314.7/100cy	265.8/100cy		-	500-799: 1.11 (1.08-1.27)	



Study	Predominant HEU infant feeding	Outcome	Measures used & clinical definitions	Incidence/ prevalence among HEU	Incidence/ prevalence among HU	Measure of association	Crude (95% CI)	Adjusted (95% CI)	Comments
				200-499: 323.7/100cy			-	200-499: 1.24 (1.17-1.32)	for higher rates of sick child visits during early months No stratification by infant feeding for this analysis
				<200: 337.8/100cy			-	<200: 1.33 (1.17–1.50)	
		All cause hospitalization	Maternal recall (3 monthly); hospital record verification for 69%	Precise rate not reported	Precise rate not reported	IRR	-	0-28 days: 1.5 (1.2 – 2.0) 29-91 days: 1.2 (0.9 – 1.6)	
		Pneumonia-related hospitalization	As above; specific definition not reported				-	0-28 days: 2.7 (1.6 – 4.7) 29-91 days: 1.2 (0.8 – 1.7)	
		Diarrhoea-related hospitalization	As above; specific definition not reported				-	0-28 days: Not stated 29-91 days: 0.4 (0.1 – 1.9)	
PMTCT: maternal use of 1-2 antiretrovirals									
No reports									
PMTCT: predominantly 1-2 antiretrovirals, with some maternal use of 3 antiretrovirals among women with advanced disease									
Shapiro (2007)	Breastfeeding	Hospitalization at 6 and at 24 months	Unclear	N=534 6m: 12.8% 24m: 20.6%	N=137 6m: 5.3% 24m: 9.5%	RR*	6m: 2.49 (1.17–5.3) 24m: 2.17 (1.26–3.74)	-	In the case-control analysis, "multivariate analysis was limited because of the small sample size"; in univariate analysis, maternal CD4 and viral load not associated with diarrhoea or pneumonia by 6 months of age. Breastfeeding cessation by the time of diagnosis: increased odds of morbidity, OR 21.00 (95% CI, 2.83-156.12)
		Proportion with ≥ 1 episode of diarrhoea, at 6 and at 24 months	Case definition: 3 loose stools/24 hour period, different from normal pattern, 1-3 monthly maternal recall; micro-biological confirmation where possible	N=534 6m: 32% 24m: 66%	N=137 6m: 33.6% 24m: 56.6%	RR*	6m: 0.95 (0.73–1.24) 24m: 1.17 (1.00–1.38)	-	
		≥ 1 Episode pneumonia at 6 and at 24 months	"Documented", WHO case definition	N=534 6m: 9.7% 24m: 18.2%	N=137 6m: 7.6% 24m: 10.9%	RR*	6m: 1.33 (0.70–2.56) 24m: 1.66 (0.99–2.76)	-	
Rollins (2013)	Early EBF until 4-6 months	Diarrhoea	Diarrhoea defined as loose, watery or more frequent than usual stools (1 week maternal recall); persistent: ≥14 days	Total number of diarrhoeal events per child-years: 5/54 cy	Total number of diarrhoeal events per child-years: 681/1158 cy	HR	Overall diarrhoea: 1.67 (0.86–3.22) Persistent diarrhoea: 0.88 (0.12–6.27) Acute diarrhoea: 1.88 (0.94–3.78)	Overall diarrhoea: 1.45(0.75 – 2.79) Persistent diarrhoea: 0.82 (0.36 – 3.49) Acute diarrhoea: 1.6 (0.79 – 3.21)	Adjusted for infant feeding practice (time-varying), sex, water source and location of enrolment clinic (urban vs rural/peri-urban)
Marquez (2014)	Breastfeeding for median of 7 months; 84.4% BF at 6 months, 28.7% at 12 months and none at 24 months; not reported if EBF	Non-malarial hospitalization	Maternal recall period of 1 month	6-11 months: IR for BF HEU 0.023/PY (1/43 PY)	6-11 months: IR for BF HUU 0.011/PY (2/ 189 PY)	IRR	2.20 (0.04 – 42.22)	1.50 (0.14-16.4)	Adjusted for infant age, chemo-prevention, household wealth index Insufficient person-time accumulated to include non-breastfeeding HU outcomes, both periods Results for 12-24 month period: non-BF HEU vs non-BF HUU: Severe febrile illnesses: RR 2.07 (95% CI, 0.93-4.64); Severe diarrhoea: RR 2.21 (95% CI, 0.79-6.16)
				6-11 months: IR for Non-BF 0.25/PY (12/48PY)	6-11 months: IR for BF HUU 0.011/PY (2/ 189 PY)	IRR	23.6 (5.26 – 217.33)	19.6 (4.95 – 77.3)	
		Severe febrile illness/ pneumonia	A composite of sepsis syndrome and/or severe pneumonia (WHO case definition)	6-11 months: IR for BF HEU 0.116 (5/43PY)	6-11 months: IR for BF HUU 0.090/PY (17/189 PY)	IRR	1.3 (0.37 – 3.65)	1.34 (0.44-4.07)	
				6-11 months: IR for Non-BF 0.271/PY (13/48PY)	6-11 months: IR for BF HUU 0.090/PY (17/189 PY)	IRR	3.01 (1.34 – 6.58)	3.84 (2.06-7.17)	
		Severe diarrhoea	Bloody diarrhoea or >7 stools/24 hours or requiring intravenous fluid or hospitalization	6-11 months: IR for BF HEU 0.070/PY (3/43PY)	6-11 months: IR for BF HUU 0.058/PY (11/189PY)	IRR	1.20 (0.21 – 4.54)	0.78 (0.17-3.8)	
				6-11 months: IR for Non-BF 0.458/PY (22/48PY)	6-11 months: IR for BF HUU 0.058/PY (11/189PY)	IRR	7.88 (3.66 – 17.98)	6.37 (2.32-17.4)	
Nicholson (2015)	Breast and formula	Clinical care referrals	Referral to clinic within previous month or hospital within previous year	NR	Not stated	Not stated	Not stated	Not stated	"No difference between HEU and HUU children"
PMTCT: maternal use of 3 antiretrovirals: initiated as part of PMTCT irrespective of disease staging									
No reports									

[1] Measures of association calculated based on data presented in manuscript

PMTCT, prevention of mother to child transmission of HIV; HIV, human immunodeficiency virus; HEU, HIV-exposed uninfected; HU, HIV-unexposed; CI, confidence interval; RR, risk ratio; IRR, incidence rate ratio; HR, hazard ratio; aHR, adjusted hazard ratio; py, person-years; OR, odds ratio; BF, breastfeeding; EBF, exclusive breastfeeding; WHO, World Health Organization

Table 2.3 (continued). Effect of maternal HIV status on infectious morbidity (hospitalisation, diarrhoeal disease and pneumonia) among HIV-uninfected children in sub-Saharan Africa, by maternal use of antiretrovirals

No significant increases in diarrhoeal risk were observed in the other studies reporting on populations without access to antiretroviral drugs. There were 5 reports of diarrhoeal risk among infants born to mothers receiving predominantly 1-2 antiretroviral drugs for PMTCT with limited access to ART; three were assessed as having a low/moderate risk of bias.[86,96,102] Two of these reports indicated no significant association between maternal HIV and child diarrhoeal risk, while the third found an appreciable association which was explained entirely by the absence of breastfeeding among HEU infants.[86,96,102] There were no reports on childhood diarrhoeal risk among breastfed HEU children born to women receiving universal ART (tables 2.1 and 2.3).

2.4.3.4 Pneumonia

There were seven reports on pneumonia risk overall,[5,95-97,102,105] with three reports from settings where women had no access to antiretroviral drugs,[5,95,105] and four from populations using predominantly 1-2 antiretroviral drugs for PMTCT with a limited number of women with advanced HIV disease receiving ART;[96,97,102,112] there were no reports on populations with universal maternal use of ART (table 2.1). Varying case definitions were used, and most estimates were unadjusted for potential confounders. Four reports were judged as having a low/moderate risk of bias.[5,96,102,105] Only one study reported the use of co-trimoxazole preventive therapy among HEU infants.[96] Generally the reports yielded low precision estimates indicating a somewhat increased risk of pneumonia among HEU children (table 2.3).[5,96,102,105]

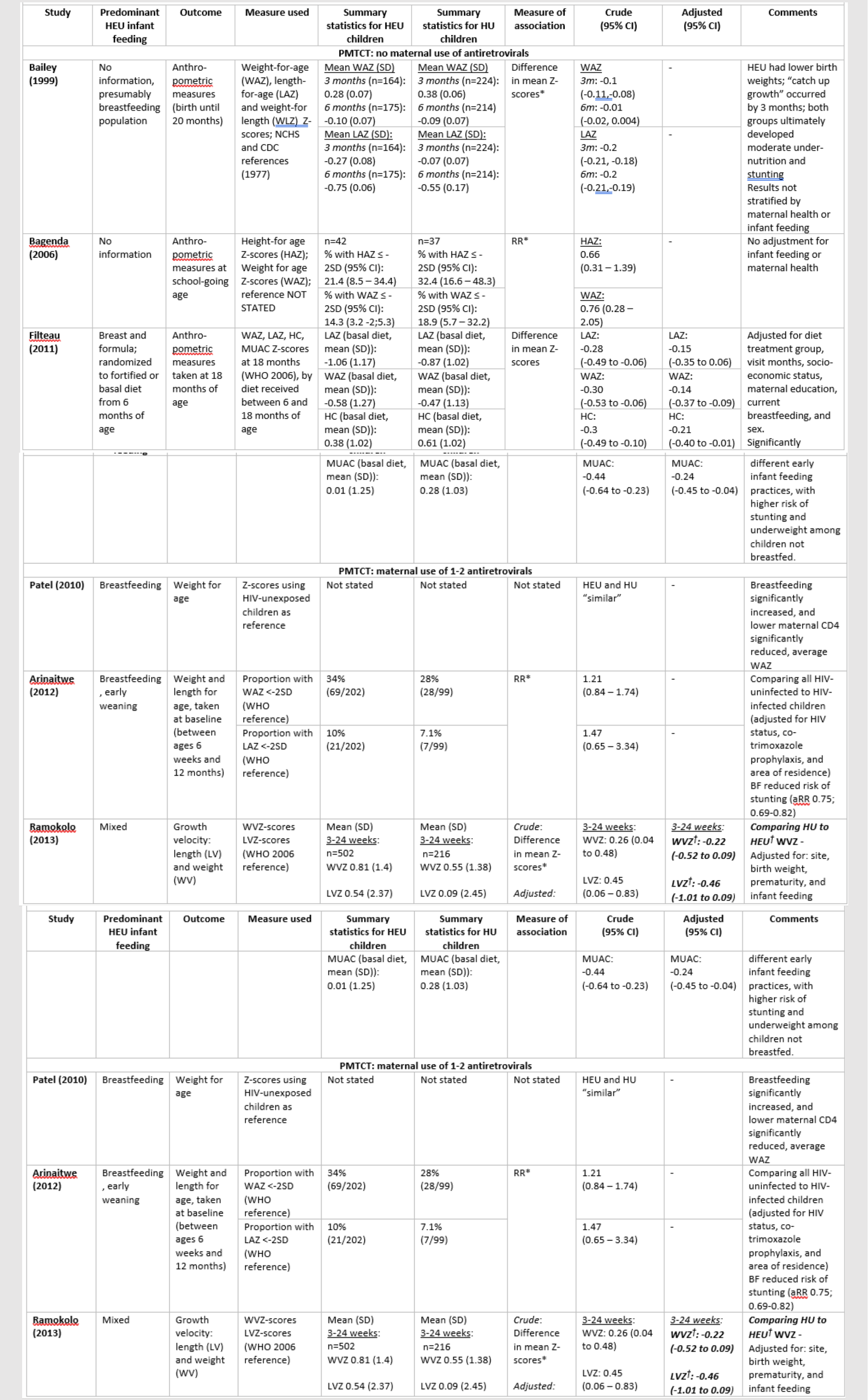
The effect of HIV exposure on childhood pneumonia was influenced by maternal use of ART and breastfeeding. In the Zimbabwean vitamin A trial, where predominantly breastfeeding women had no access to ART, the incidence of pneumonia-related hospitalisation was almost three-fold higher (IRR 2.7, 95% CI 1.6 -4.7) among HEU compared to HU infants.[5] By comparison, in a Ugandan study where some HIV-infected women used ART, breastfed HEU and breastfed HU children experienced a similar risk of pneumonia (IRR 1.34, 95% CI 0.44-4.07), whereas non-breastfed HEU children had a 4-fold higher incidence than their breastfed HU counterparts (IRR 3.84, 95% CI 2.06-7.17) (table 2.3).

2.4.3.5 Growth

Overall, 15 studies reported weight estimates adjusted for age. [32,33,81,82,90,91,95-98,101-104,111,112] [109] Of the 7 reports providing weight-for-age Z-score (WAZ) estimates with low/moderate risk of bias,[81,82,90,91,98,101,111] 3 were from populations without access to antiretroviral drugs,[81,91,111] 4 from populations using predominantly 1-2 antiretroviral drugs for PMTCT[33,82,90,98], and one from a study providing universal ART.[101] Two studies reported weight-velocity-for-age Z-scores (WVZ), one from a population using a single antiretroviral drug for PMTCT [32] and one from a population with universal maternal ART use[101] (tables 2.1 and 2.4). Most point estimates were small with low precision and unclear clinical significance; HEU children tended to have similar long term weight gain compared to HU children after adjusting for low birth weight, feeding practices and socio-economic factors.[32,90,91] The largest study reported Ugandan HEU infants to have somewhat lower mean WAZ at 12 months than HU infants from the same setting (adjusted difference in mean WAZ -0.13, 95% -0.35 to 0.00).The estimate was not adjusted for maternal HIV disease stage or infant feeding; although the majority of children received some breastmilk, no information was provided regarding exclusivity or subsequent complementary feeding practices.[98] By contrast, a randomized trial investigating the effect of a fortified diet among 18-month old Zambian children reported significantly lower WAZ scores among the HEU children, even after adjusting for infant feeding and socio-economic markers (adjusted difference in mean WAZ, -0.14; 95% CI -0.37 to -0.09). HEU children had been significantly less likely to receive breastfeeding in early infancy than HU children.[81] However, in a later report on those children still in follow-up at school-going age, the observed differences between HEU and HU children were negated after adjusting for socio-economic confounders. [82]

Overall, there were 13 reports of linear growth. Of the 8 reports at low/moderate risk of bias, 3 were from populations without access to antiretroviral drugs,[81,91,111] and 4 from populations using predominantly 1-2 antiretroviral drugs for PMTCT.[32,82,90,98] While most estimates were based on length- or height-for-age Z-scores (LAZ or HAZ), two studies reported length-velocity-for-age Z-scores (LVZ).[32,101] . Overall, sample sizes were small, with low precision estimates. Adjusted estimates of differences in mean LAZ/HAZ demonstrated no significant differences between HEU and HU children;[32,81,82,98] maternal HIV disease severity predicted poor linear growth.[32,98] Similarly, the risk of stunting (defined as % children with LAZ/HAZ-scores <-2SD from the expected mean) did not differ between HEU and HU children.[90,98,101,111]. A single report from a breastfeeding population with universal maternal ART also suggested no difference in risk of stunting between HEU and HU children at 17-18 months (RR 1.00, 95% CI 0.51-1.95), although there was some indication of effect modification by gender, with HEU girls demonstrating a slightly lower HVZ than HU girls of the same age (-0.82, 95% CI -1.52 to -0.12). [101]

Table 2.4. Effect of maternal HIV status on growth among HIV-uninfected children in sub-Saharan Africa, by maternal use of antiretrovirals



Study	Predominant HEU infant feeding	Outcome	Measure used	Summary statistics for HEU children	Summary statistics for HU children	Measure of association	Crude (95% CI)	Adjusted (95% CI)	Comments
PMTCT: no maternal use of antiretrovirals									
Bailey (1999)	No information, presumably breastfeeding population	Anthropometric measures (birth until 20 months)	Weight-for-age (WAZ), length-for-age (LAZ) and weight-for length (WLZ) Z-scores; NCHS and CDC references (1977)	Mean WAZ (SD) *3 months* (n=164): 0.28 (0.07) *6 months* (n=175): -0.10 (0.07)	Mean WAZ (SD) *3 months* (n=224): 0.38 (0.06) *6 months* (n=214) -0.09 (0.07)	Difference in mean Z-scores*	WAZ *3m*: -0.1 (-0.11,-0.08) *6m*: -0.01 (-0.02, 0.004)	-	HEU had lower birth weights; “catch up growth” occurred by 3 months; both groups ultimately developed moderate under-nutrition and stunting Results not stratified by maternal health or infant feeding
				Mean LAZ (SD): *3 months* (n=164): -0.27 (0.08) *6 months* (n=175): -0.75 (0.06)	Mean LAZ (SD): *3 months* (n=224): -0.07 (0.07) *6 months* (n=214): -0.55 (0.17)		LAZ *3m*: -0.2 (-0.21, -0.18) *6m*: -0.2 (-0.21,-0.19)	-	
Bagenda (2006)	No information	Anthropometric measures at school-going age	Height-for age Z-scores (HAZ); Weight for age Z-scores (WAZ); reference NOT STATED	n=42 % with HAZ ≤ -2SD (95% CI): 21.4 (8.5 – 34.4)	n=37 % with HAZ ≤ -2SD (95% CI): 32.4 (16.6 – 48.3)	RR*	HAZ: 0.66 (0.31 – 1.39)	-	No adjustment for infant feeding or maternal health
				% with WAZ ≤ -2SD (95% CI): 14.3 (3.2 -2;5.3)	% with WAZ ≤ -2SD (95% CI): 18.9 (5.7 – 32.2)		WAZ: 0.76 (0.28 – 2.05)		
Filteau (2011)	Breast and formula; randomized to fortified or basal diet from 6 months of age	Anthropometric measures taken at 18 months of age	WAZ, LAZ, HC, MUAC Z-scores at 18 months (WHO 2006), by diet received between 6 and 18 months of age	LAZ (basal diet, mean (SD)): -1.06 (1.17)	LAZ (basal diet, mean (SD)): -0.87 (1.02)	Difference in mean Z-scores	LAZ: -0.28 (-0.49 to -0.06)	LAZ: -0.15 (-0.35 to 0.06)	Adjusted for diet treatment group, visit months, socio-economic status, maternal education, current breastfeeding, and sex. Significantly different early infant feeding practices, with higher risk of stunting and underweight among children not breastfed.
				WAZ (basal diet, mean (SD)): -0.58 (1.27)	WAZ (basal diet, mean (SD)): -0.47 (1.13)		WAZ: -0.30 (-0.53 to -0.06)	WAZ: -0.14 (-0.37 to -0.09)	
				HC (basal diet, mean (SD)): 0.38 (1.02)	HC (basal diet, mean (SD)): 0.61 (1.02)		HC: -0.3 (-0.49 to -0.10)	HC: -0.21 (-0.40 to -0.01)	
				MUAC (basal diet, mean (SD)): 0.01 (1.25)	MUAC (basal diet, mean (SD)): 0.28 (1.03)		MUAC: -0.44 (-0.64 to -0.23)	MUAC: -0.24 (-0.45 to -0.04)	
PMTCT: maternal use of 1-2 antiretrovirals									
Patel (2010)	Breastfeeding	Weight for age	Z-scores using HIV-unexposed children as reference	Not stated	Not stated	Not stated	HEU and HU “similar”	-	Breastfeeding significantly increased, and lower maternal CD4 significantly reduced, average WAZ
Arinaitwe (2012)	Breastfeeding, early weaning	Weight and length for age, taken at baseline (between ages 6 weeks and 12 months)	Proportion with WAZ <-2SD (WHO reference)	34% (69/202)	28% (28/99)	RR*	1.21 (0.84 – 1.74)	-	Comparing all HIV-uninfected to HIV-infected children (adjusted for HIV status, co-trimoxazole prophylaxis, and area of residence) BF reduced risk of stunting (aRR 0.75; 0.69-0.82)
			Proportion with LAZ <-2SD (WHO reference)	10% (21/202)	7.1% (7/99)		1.47 (0.65 – 3.34)	-	
Ramokolo (2013)	Mixed	Growth velocity: length (LV) and weight (WV)	WVZ-scores LVZ-scores (WHO 2006 reference)	Mean (SD) 3-24 weeks: n=502 WVZ 0.81 (1.4) LVZ 0.54 (2.37)	Mean (SD) 3-24 weeks: n=216 WVZ 0.55 (1.38) LVZ 0.09 (2.45)	*Crude*: Difference in mean Z-scores* *Adjusted*:	3-24 weeks: WVZ: 0.26 (0.04 to 0.48) LVZ: 0.45 (0.06 – 0.83)	*3-24 weeks*: ***WVZ***†***: -0.22 (-0.52 to 0.09)*** ***LVZ***†***: -0.46 (-1.01 to 0.09)***	***Comparing HU to HEU***† **WVZ -** Adjusted for: site, birth weight, prematurity, and infant feeding

Study	Predominant HEU infant feeding	Outcome	Measure used	Summary statistics for HEU children	Summary statistics for HU children	Measure of association	Crude (95% CI)	Adjusted (95% CI)	Comments
				MUAC (basal diet, mean (SD)): 0.01 (1.25)	MUAC (basal diet, mean (SD)): 0.28 (1.03)		MUAC: -0.44 (-0.64 to -0.23)	MUAC: -0.24 (-0.45 to -0.04)	different early infant feeding practices, with higher risk of stunting and underweight among children not breastfed.
PMTCT: maternal use of 1-2 antiretrovirals									
Patel (2010)	Breastfeeding	Weight for age	Z-scores using HIV-unexposed children as reference	Not stated	Not stated	Not stated	HEU and HU “similar”	-	Breastfeeding significantly increased, and lower maternal CD4 significantly reduced, average WAZ
Arinaitwe (2012)	Breastfeeding, early weaning	Weight and length for age, taken at baseline (between ages 6 weeks and 12 months)	Proportion with WAZ <-2SD (WHO reference)	34% (69/202)	28% (28/99)	RR*	1.21 (0.84 – 1.74)	-	Comparing all HIV-uninfected to HIV-infected children (adjusted for HIV status, co-trimoxazole prophylaxis, and area of residence) BF reduced risk of stunting (aRR 0.75; 0.69-0.82)
			Proportion with LAZ <-2SD (WHO reference)	10% (21/202)	7.1% (7/99)		1.47 (0.65 – 3.34)	-	
Ramokolo (2013)	Mixed	Growth velocity: length (LV) and weight (WV)	WVZ-scores LVZ-scores (WHO 2006 reference)	Mean (SD) 3-24 weeks: n=502 WVZ 0.81 (1.4) LVZ 0.54 (2.37)	Mean (SD) 3-24 weeks: n=216 WVZ 0.55 (1.38) LVZ 0.09 (2.45)	*Crude*: Difference in mean Z-scores* *Adjusted*:	3-24 weeks: WVZ: 0.26 (0.04 to 0.48) LVZ: 0.45 (0.06 – 0.83)	*3-24 weeks*: ***WVZ***†***: -0.22 (-0.52 to 0.09)*** ***LVZ***†***: -0.46 (-1.01 to 0.09)***	***Comparing HU to HEU***† **WVZ -** Adjusted for: site, birth weight, prematurity, and infant feeding

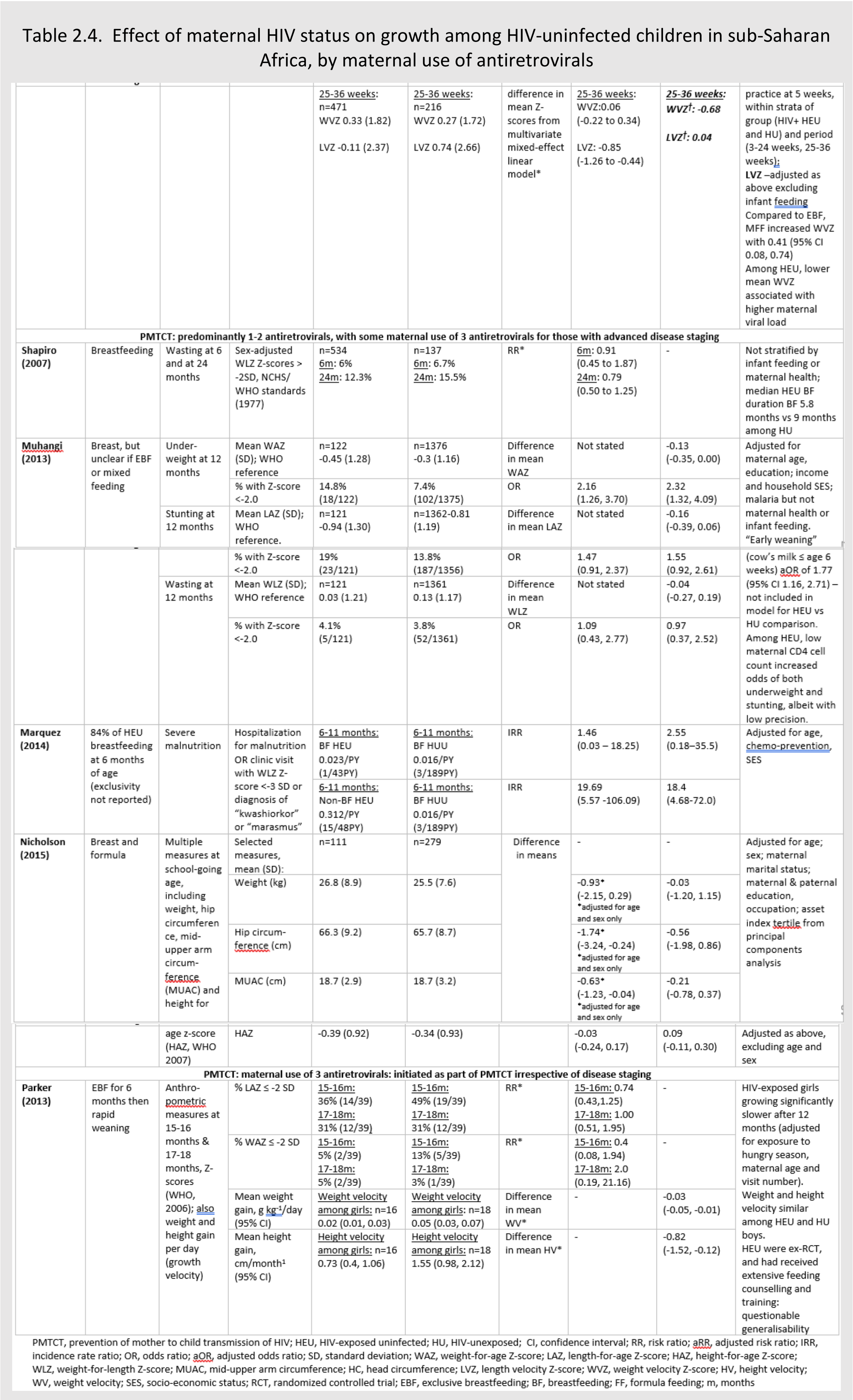


Table 2.4. Effect of maternal HIV status on growth among HIV-uninfected children in sub-Saharan Africa, by maternal use of antiretrovirals

				25-36 weeks: n=471 WVZ 0.33 (1.82) LVZ -0.11 (2.37)	25-36 weeks: n=216 WVZ 0.27 (1.72) LVZ 0.74 (2.66)	difference in mean Z-scores from multivariate mixed-effect linear model*	25-36 weeks: WVZ:0.06 (-0.22 to 0.34) LVZ: -0.85 (-1.26 to -0.44)	***25-36 weeks:*** ***$WVZ^{\dagger}$: -0.68*** ***$LVZ^{\dagger}$: 0.04***	practice at 5 weeks, within strata of group (HIV+ HEU and HU) and period (3-24 weeks, 25-36 weeks); **LVZ** –adjusted as above excluding infant feeding Compared to EBF, MFF increased WVZ with 0.41 (95% CI 0.08, 0.74) Among HEU, lower mean WVZ associated with higher maternal viral load
PMTCT: predominantly 1-2 antiretrovirals, with some maternal use of 3 antiretrovirals for those with advanced disease staging									
Shapiro (2007)	Breastfeeding	Wasting at 6 and at 24 months	Sex-adjusted WLZ Z-scores > -2SD, NCHS/ WHO standards (1977)	n=534 6m: 6% 24m: 12.3%	n=137 6m: 6.7% 24m: 15.5%	RR*	6m: 0.91 (0.45 to 1.87) 24m: 0.79 (0.50 to 1.25)	-	Not stratified by infant feeding or maternal health; median HEU BF duration BF 5.8 months vs 9 months among HU
Muhangi (2013)	Breast, but unclear if EBF or mixed feeding	Under-weight at 12 months	Mean WAZ (SD); WHO reference	n=122 -0.45 (1.28)	n=1376 -0.3 (1.16)	Difference in mean WAZ	Not stated	-0.13 (-0.35, 0.00)	Adjusted for maternal age, education; income and household SES; malaria but not maternal health or infant feeding. "Early weaning"
			% with Z-score <-2.0	14.8% (18/122)	7.4% (102/1375)	OR	2.16 (1.26, 3.70)	2.32 (1.32, 4.09)	
		Stunting at 12 months	Mean LAZ (SD); WHO reference.	n=121 -0.94 (1.30)	n=1362-0.81 (1.19)	Difference in mean LAZ	Not stated	-0.16 (-0.39, 0.06)	
			% with Z-score <-2.0	19% (23/121)	13.8% (187/1356)	OR	1.47 (0.91, 2.37)	1.55 (0.92, 2.61)	(cow's milk ≤ age 6 weeks) aOR of 1.77 (95% CI 1.16, 2.71) – not included in model for HEU vs HU comparison. Among HEU, low maternal CD4 cell count increased odds of both underweight and stunting, albeit with low precision.
		Wasting at 12 months	Mean WLZ (SD); WHO reference	n=121 0.03 (1.21)	n=1361 0.13 (1.17)	Difference in mean WLZ	Not stated	-0.04 (-0.27, 0.19)	
			% with Z-score <-2.0	4.1% (5/121)	3.8% (52/1361)	OR	1.09 (0.43, 2.77)	0.97 (0.37, 2.52)	
Marquez (2014)	84% of HEU breastfeeding at 6 months of age (exclusivity not reported)	Severe malnutrition	Hospitalization for malnutrition OR clinic visit with WLZ Z-score <-3 SD or diagnosis of "kwashiorkor" or "marasmus"	6-11 months: BF HEU 0.023/PY (1/43PY)	6-11 months: BF HUU 0.016/PY (3/189PY)	IRR	1.46 (0.03 – 18.25)	2.55 (0.18–35.5)	Adjusted for age, chemo-prevention, SES
				6-11 months: Non-BF HEU 0.312/PY (15/48PY)	6-11 months: BF HUU 0.016/PY (3/189PY)	IRR	19.69 (5.57 -106.09)	18.4 (4.68-72.0)	
Nicholson (2015)	Breast and formula	Multiple measures at school-going age, including weight, hip circumference, mid-upper arm circum-ference (MUAC) and height for age z-score (HAZ, WHO 2007)	Selected measures, mean (SD):	n=111	n=279	Difference in means	-	-	Adjusted for age; sex; maternal marital status; maternal & paternal education, occupation; asset index tertile from principal components analysis
			Weight (kg)	26.8 (8.9)	25.5 (7.6)		-0.93* (-2.15, 0.29) *adjusted for age and sex only	-0.03 (-1.20, 1.15)	
			Hip circum-ference (cm)	66.3 (9.2)	65.7 (8.7)		-1.74* (-3.24, -0.24) *adjusted for age and sex only	-0.56 (-1.98, 0.86)	
			MUAC (cm)	18.7 (2.9)	18.7 (3.2)		-0.63* (-1.23, -0.04) *adjusted for age and sex only	-0.21 (-0.78, 0.37)	
			HAZ	-0.39 (0.92)	-0.34 (0.93)		-0.03 (-0.24, 0.17)	0.09 (-0.11, 0.30)	Adjusted as above, excluding age and sex
PMTCT: maternal use of 3 antiretrovirals: initiated as part of PMTCT irrespective of disease staging									
Parker (2013)	EBF for 6 months then rapid weaning	Anthro-pometric measures at 15-16 months & 17-18 months, Z-scores (WHO, 2006); also weight and height gain per day (growth velocity)	% LAZ ≤ -2 SD	15-16m: 36% (14/39) 17-18m: 31% (12/39)	15-16m: 49% (19/39) 17-18m: 31% (12/39)	RR*	15-16m: 0.74 (0.43,1.25) 17-18m: 1.00 (0.51, 1.95)	-	HIV-exposed girls growing significantly slower after 12 months (adjusted for exposure to hungry season, maternal age and visit number). Weight and height velocity similar among HEU and HU boys. HEU were ex-RCT, and had received extensive feeding counselling and training: questionable generalisability
			% WAZ ≤ -2 SD	15-16m: 5% (2/39) 17-18m: 5% (2/39)	15-16m: 13% (5/39) 17-18m: 3% (1/39)	RR*	15-16m: 0.4 (0.08, 1.94) 17-18m: 2.0 (0.19, 21.16)	-	
			Mean weight gain, g kg^{-1}/day (95% CI)	Weight velocity among girls: n=16 0.02 (0.01, 0.03)	Weight velocity among girls: n=18 0.05 (0.03, 0.07)	Difference in mean WV*	-	-0.03 (-0.05, -0.01)	
			Mean height gain, $cm/month^1$ (95% CI)	Height velocity among girls: n=16 0.73 (0.4, 1.06)	Height velocity among girls: n=18 1.55 (0.98, 2.12)	Difference in mean HV*	-	-0.82 (-1.52, -0.12)	

PMTCT, prevention of mother to child transmission of HIV; HEU, HIV-exposed uninfected; HU, HIV-unexposed; CI, confidence interval; RR, risk ratio; aRR, adjusted risk ratio; IRR, incidence rate ratio; OR, odds ratio; aOR, adjusted odds ratio; SD, standard deviation; WAZ, weight-for-age Z-score; LAZ, length-for-age Z-score; HAZ, height-for-age Z-score; WLZ, weight-for-length Z-score; MUAC, mid-upper arm circumference; HC, head circumference; LVZ, length velocity Z-score; WVZ, weight velocity Z-score; HV, height velocity; WV, weight velocity; SES, socio-economic status; RCT, randomized controlled trial; EBF, exclusive breastfeeding; BF, breastfeeding; FF, formula feeding; m, months

2.4.3.6 Early childhood development

Eight studies contributed nine reports of early childhood development.[80,82,83,88,92,100,109,111,112] Two studies utilized unvalidated or unspecified measures of neurodevelopment,[83,112] and two other studies were considered at substantial risk of selection bias.[92,109]

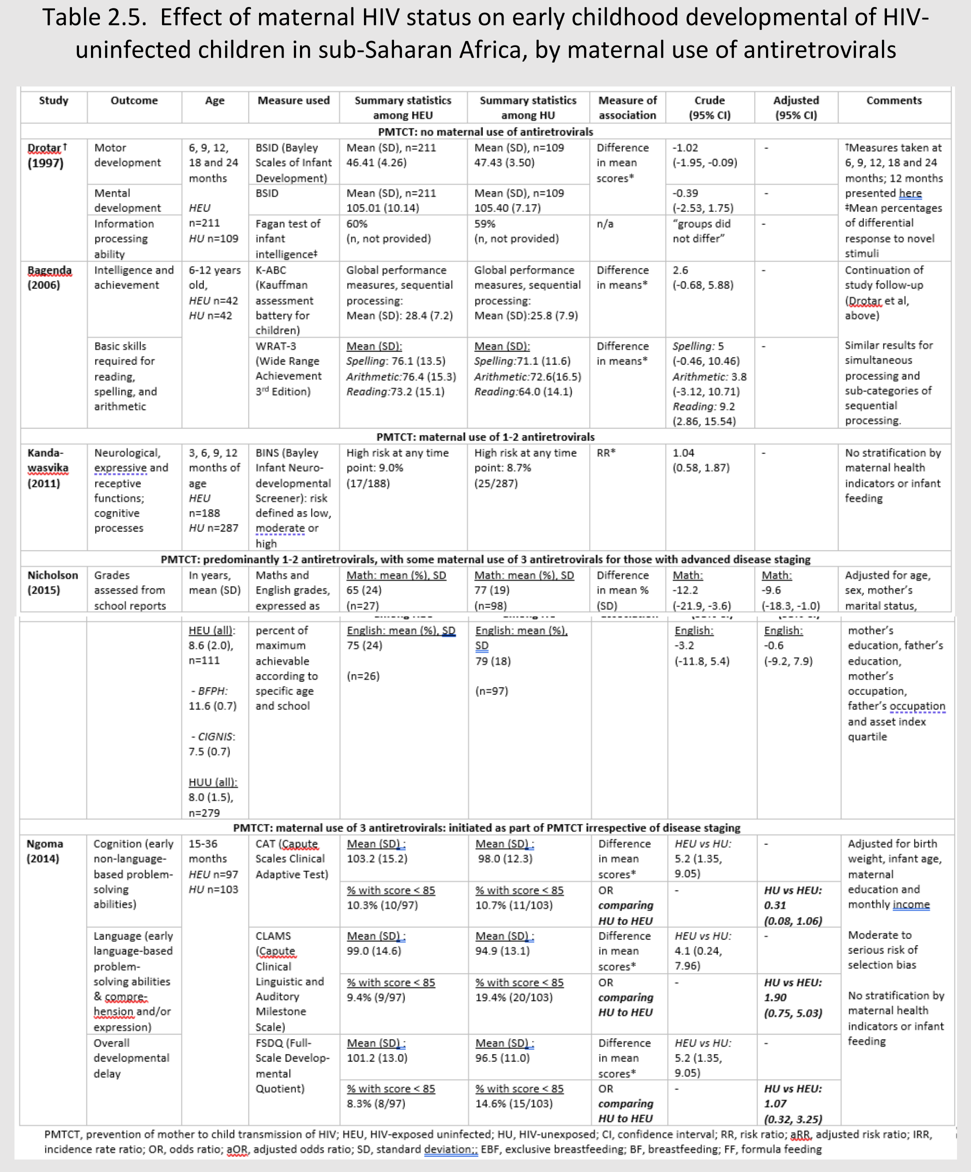


Table 2.5. Effect of maternal HIV status on early childhood developmental of HIV-uninfected children in sub-Saharan Africa, by maternal use of antiretrovirals

Study	Outcome	Age	Measure used	Summary statistics among HEU	Summary statistics among HU	Measure of association	Crude (95% CI)	Adjusted (95% CI)	Comments
PMTCT: no maternal use of antiretrovirals									
Drotar † (1997)	Motor development	6, 9, 12, 18 and 24 months *HEU* n=211 *HU* n=109	BSID (Bayley Scales of Infant Development)	Mean (SD), n=211 46.41 (4.26)	Mean (SD), n=109 47.43 (3.50)	Difference in mean scores*	-1.02 (-1.95, -0.09)	-	†Measures taken at 6, 9, 12, 18 and 24 months; 12 months presented here ‡Mean percentages of differential response to novel stimuli
	Mental development		BSID	Mean (SD), n=211 105.01 (10.14)	Mean (SD), n=109 105.40 (7.17)		-0.39 (-2.53, 1.75)	-	
	Information processing ability		Fagan test of infant intelligence‡	60% (n, not provided)	59% (n, not provided)	n/a	"groups did not differ"	-	
Bagenda (2006)	Intelligence and achievement	6-12 years old, *HEU* n=42 *HU* n=42	K-ABC (Kauffman assessment battery for children)	Global performance measures, sequential processing: Mean (SD): 28.4 (7.2)	Global performance measures, sequential processing: Mean (SD):25.8 (7.9)	Difference in means*	2.6 (-0.68, 5.88)	-	Continuation of study follow-up (Drotar et al, above)
	Basic skills required for reading, spelling, and arithmetic		WRAT-3 (Wide Range Achievement 3rd Edition)	Mean (SD): *Spelling*: 76.1 (13.5) *Arithmetic*:76.4 (15.3) *Reading*:73.2 (15.1)	Mean (SD): *Spelling*:71.1 (11.6) *Arithmetic*:72.6(16.5) *Reading*:64.0 (14.1)	Difference in means*	*Spelling*: 5 (-0.46, 10.46) *Arithmetic*: 3.8 (-3.12, 10.71) *Reading*: 9.2 (2.86, 15.54)	-	Similar results for simultaneous processing and sub-categories of sequential processing.
PMTCT: maternal use of 1-2 antiretrovirals									
Kanda-wasvika (2011)	Neurological, expressive and receptive functions; cognitive processes	3, 6, 9, 12 months of age *HEU* n=188 *HU* n=287	BINS (Bayley Infant Neuro-developmental Screener): risk defined as low, moderate or high	High risk at any time point: 9.0% (17/188)	High risk at any time point: 8.7% (25/287)	RR*	1.04 (0.58, 1.87)	-	No stratification by maternal health indicators or infant feeding
PMTCT: predominantly 1-2 antiretrovirals, with some maternal use of 3 antiretrovirals for those with advanced disease staging									
Nicholson (2015)	Grades assessed from school reports	In years, mean (SD) HEU (all): 8.6 (2.0), n=111 - *BFPH*: 11.6 (0.7) - *CIGNIS*: 7.5 (0.7) HUU (all): 8.0 (1.5), n=279	Maths and English grades, expressed as percent of maximum achievable according to specific age and school	Math: mean (%), SD 65 (24) (n=27) English: mean (%), SD 75 (24) (n=26)	Math: mean (%), SD 77 (19) (n=98) English: mean (%), SD 79 (18) (n=97)	Difference in mean % (SD)	Math: -12.2 (-21.9, -3.6) English: -3.2 (-11.8, 5.4)	Math: -9.6 (-18.3, -1.0) English: -0.6 (-9.2, 7.9)	Adjusted for age, sex, mother's marital status, mother's education, father's education, mother's occupation, father's occupation and asset index quartile
PMTCT: maternal use of 3 antiretrovirals: initiated as part of PMTCT irrespective of disease staging									
Ngoma (2014)	Cognition (early non-language-based problem-solving abilities)	15-36 months *HEU* n=97 *HU* n=103	CAT (Capute Scales Clinical Adaptive Test)	Mean (SD) : 103.2 (15.2)	Mean (SD) : 98.0 (12.3)	Difference in mean scores*	*HEU vs HU*: 5.2 (1.35, 9.05)	-	Adjusted for birth weight, infant age, maternal education and monthly income Moderate to serious risk of selection bias No stratification by maternal health indicators or infant feeding
				% with score < 85 10.3% (10/97)	% with score < 85 10.7% (11/103)	OR ***comparing HU to HEU***	-	***HU vs HEU:* *0.31* *(0.08, 1.06)***	
	Language (early language-based problem-solving abilities & compre-hension and/or expression)		CLAMS (Capute Clinical Linguistic and Auditory Milestone Scale)	Mean (SD) : 99.0 (14.6)	Mean (SD) : 94.9 (13.1)	Difference in mean scores*	*HEU vs HU*: 4.1 (0.24, 7.96)	-	
				% with score < 85 9.4% (9/97)	% with score < 85 19.4% (20/103)	OR ***comparing HU to HEU***	-	***HU vs HEU:* *1.90* *(0.75, 5.03)***	
	Overall developmental delay		FSDQ (Full-Scale Develop-mental Quotient)	Mean (SD) : 101.2 (13.0)	Mean (SD) : 96.5 (11.0)	Difference in mean scores*	*HEU vs HU*: 5.2 (1.35, 9.05)	-	
				% with score < 85 8.3% (8/97)	% with score < 85 14.6% (15/103)	OR ***comparing HU to HEU***	-	***HU vs HEU:* *1.07* *(0.32, 3.25)***	

PMTCT, prevention of mother to child transmission of HIV; HEU, HIV-exposed uninfected; HU, HIV-unexposed; CI, confidence interval; RR, risk ratio; aRR, adjusted risk ratio; IRR, incidence rate ratio; OR, odds ratio; aOR, adjusted odds ratio; SD, standard deviation;; EBF, exclusive breastfeeding; BF, breastfeeding; FF, formula feeding

The remaining 4 studies contributed 5 reports (table 2.5), 3 of which focused on children under 3 years of age.[80,88,100] Different developmental measurement tools were used in each, limiting meaningful between-study comparisons. Two reports (from a single study at different time points) were for children born to mothers without access to antiretroviral drugs;[80,111] two reports were for children born to mothers who received 1-2 antiretroviral drugs for PMTCT[82,88] and a single report was for preschool children born to women receiving universal ART (table 2.1).[100] No study reported significant differences in developmental outcomes between preschool HEU and HU children. A single report on 6-12 year old HEU and HU Congolese children found no significant differences in intelligence and scholastic achievement and skills as measured by standardised tests; the children had been mostly breastfed, and no antiretroviral drugs had been used by their mothers.[111] By contrast, HEU Zambian school-age children had significantly lower maths grades than their HU counterparts; differences remained after adjusting for several potential confounders including socio-economic measures and parental education. The HIV-infected mothers had largely received sdNVP as prophylaxis, with some women accessing ART for advanced HIV disease; HEU children were breastfed for shorter durations than HU children.[82] Of note, no reports stratified results by either early infant feeding modality or maternal health measures.

2.5 Discussion

This review highlights a highly heterogeneous literature examining the impact of maternal HIV status on major child health outcomes among HEU children in SSA, with marked diversity across studies in maternal ART use, infant feeding, and treatment of major confounding variables. Despite these limitations some general themes emerge. Reports from populations with limited or no access to maternal ART generally indicated increased risk of mortality and some morbidity among HEU compared to HU children. However, the observed associations appear to be strongly influenced by maternal HIV disease severity, ART use and infant feeding, with worse outcomes associated with advanced maternal disease, limited ART use, and restricted or no breastfeeding. We found no reports comparing mortality, diarrhoea, pneumonia, or hospitalisation between HU and breastfed HEU children born to women receiving universal ART, and very sparse data on child growth and neurodevelopment under these conditions. In this light, the available evidence regarding the effect of maternal HIV status on child health may have very limited application to the approaches of universal maternal ART and breastfeeding that are promoted across much of sub-Saharan Africa today.

Interpretation of the evidence on infectious morbidity (diarrhoea and pneumonia) was limited by varying case definitions and a reliance on maternal recall. Still there appeared to be a trend towards increased risk of pneumonia among HEU children, with some evidence of amelioration of risk by breastfeeding.[96,102] Diarrhoeal risk among HEU and HU appeared similar when infant feeding practices were accounted for, with highest risk occurring among non-breastfed children and those with premature introduction to solid foods; these findings are wholly in keeping with evidence from HU populations.[96,102,107] In addition, across studies undernutrition and stunting were prevalent in both HEU and HU populations, with growth most strongly influenced by early infant feeding[32,33,81,90] and maternal health.[32,33] Among HEU children, optimal growth was observed in breastfed children whose mothers were at an earlier stage of HIV disease.

There were few data on associations between maternal HIV status and early childhood development, and notable methodological limitations. Although there was a reassuring lack of substantial differences between HEU and HU children with regards to early neurodevelopmental outcomes in preschool populations, there was some indication of lower educational achievement among older HEU children born to women with limited access to ART.[82] The diverse tools used to assess neurodevelopment make cross-study comparisons challenging, however.

Overall, insights from the currently available literature are sharply limited around two factors that are likely to modify the putative associations between maternal HIV infection and child health outcomes. First, advanced maternal HIV disease during pregnancy and postpartum has been consistently associated with a range of adverse child health outcomes. Improving maternal health through ART use has the potential to mitigate many – but perhaps not all - of these effects. Second, poor infant nutrition, and limited breastfeeding in particular, is a well understood cause of morbidity and mortality across resource-limited settings.[35-37] The most commonly promoted intervention to address this, exclusive breastfeeding with adequate early complementary feeding strategies (including continuation of breastfeeding rather than rapid weaning), has been shown to improve each of the child health outcomes considered here regardless of HIV exposure.[52,117-119]

Thus, the existing literature provides little insight into contexts where universal maternal ART is being implemented with high levels of breastfeeding, per current policies and programmes in sub-Saharan Africa.[49] In fact, while studies providing direct comparisons are few, the currently available literature suggests that major child health outcomes in optimally-breastfed children born to mothers using ART may be the same as those of HU children from similar settings.

2.5.1 Methodological concerns

We included only longitudinal studies where HEU and HU children were sampled independent of child health status (other than HIV exposure) and were from the same communities. This approach was used to help reduce the potential for selection biases and/or confounding that may influence our assessment of the impact of maternal HIV status on child health. First, although case-based sampling approaches can play an important role in describing HEU child health status and understanding disease features, they are prone to selection bias. While there exists a larger body of evidence employing case-control methods (where children are sampled based on both their HIV exposure and a potentially related child health outcome) the potential for differential sampling of cases is profound. In addition, we limited studies to those including an HU comparator group drawn from the same community as the HEU group. While international standards for measuring a variety of child health outcomes are widely used (eg. WHO tables for anthropometry), local 'normal' child health standards may vary substantially across communities due to socio-economic and environmental parameters. In turn, any attempt to isolate the association between maternal HIV status and subsequent child health must clearly include comparators selected from the local populations that gave rise to the HEU children.[71,72]

While use of longitudinal study design and local comparators may help to address some forms of bias, the putative associations between maternal HIV exposure and child health is still likely to be confounded by the adverse economic and psychosocial circumstances associated with maternal HIV across many parts of sub-Saharan Africa. Of the 25 reports considered to be at moderate risk of bias, only 11 presented estimates adjusted for confounding; moreover, only two of the studies in this review were considered well conducted with complete reporting, contributing four of the 37 reports – approximately 10% of the evidence base on HEU child health – highlighting a substantial shortcoming in the existing literature on this issue.

2.5.2 *Limitations*

This review is subject to several limitations. First, generalizability of the available data may be limited. Only one of the included studies was designed specifically to evaluate the outcomes of HEU compared to HU children;[104] the majority of studies included in this review contributed secondary data analyses, often based on cohorts of participants drawn from intervention trials.[5,33,81-86,90,94-96,98,99,101,102] Trial participants are not necessarily representative of the general population due to specific eligibility criteria, and may receive improved medical care through trial participation.[120] For example the estimated mortality risk among HU children enrolled in the Zimbabwean Vitamin A trial was substantially lower than the expected for the general population during the time of the study.[121] This may colour the relative increase in mortality risk observed in comparing HEU children to HU counterparts in this analysis.[84] In addition, we excluded evidence on HEU children from outside SSA as the nature of the HIV epidemics, as well as the distribution of major child health outcomes, varies dramatically across continents.[1] Yet reports from Europe, Asia, South America and the United States have reported findings similar to those included in this review.[65,122-126] Second, while we attempted to minimize the risk of publication bias by including conference abstracts and searching several databases, the possibility of studies meeting our eligibility criteria appearing outside these sources remains unclear. In addition, we cannot account for changes in the sensitivity and specificity of laboratory methods to exclude infant HIV infection over the past 25 years; some misclassification of HIV-infected children as being HEU may have biased earlier reports, although it is unclear whether such misclassification could substantively alter the findings of studies.

2.5.3 *Implications for research and policy*

Taken together, these data suggest that the combination of universal, early maternal ART with optimal breastfeeding has the potential to substantially improve child health across SSA, and in turn, there is an urgent need for additional research on HEU versus HU child outcomes in this context. Critical questions that remain unanswered include (a) whether increased mortality and/or morbidity exists even among optimally breastfed HEU children born to mothers receiving ART; (b) if yes, what additional factors exist that may be amenable to intervention; and (c) which operational interventions are most effective at optimizing breastfeeding practices and maximizing ART use among HIV-infected women. Meeting these needs will require robust data from methodologically sound, prospective studies from a variety of settings, with the inclusion of otherwise similar HU comparison groups.

2.6 Conclusion

In summary, HEU children comprise a large and growing proportion of the African continent's population yet there are relatively few rigorous studies investigating their health outcomes as compared to those of HU children. Limited evidence suggests an increased risk of mortality and infectious morbidity among HEU children born to mothers without access to ART, with the highest risks occurring in the absence of breastfeeding. Insufficient comparative data exist on HEU health outcomes under the practices of universal maternal ART with extended breastfeeding, with some indication that HEU child health outcomes may be comparable to those of HU children under these conditions. Providing HIV-infected mothers with ART and supporting them to optimally feed their infants has the potential to substantially improve the health of HEU children across SSA. At the same time, it is critical to keep in mind that in many parts of SSA high rates of infant mortality and morbidity are the norm among HU populations and much work remains to be done to improve health outcomes for all children on the continent.

2.7 Acknowledgements

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2.7.1 Contribution of authors

SLR and LM conceived the review. SLR developed, refined, and completed the search strategies under the supervision of LM. SLR and KN screened studies for inclusion. SLR abstracted and analyzed the data; and wrote the first draft of the manuscript. EJA revised the manuscript and interpretation of findings. All authors contributed to and approved the final manuscript.

2.7.2 Declarations of interest

The authors declare no conflicts of interest.

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Chapter 3

Overview of research study methodology: the Maternal and Child Health Antiretroviral Therapy (MCH-ART) and HIV-Unexposed Uninfected mother-infant (HU2) studies

This chapter provides an overview of study methodology for the two linked, prospective cohort studies from whence the data for this research project was generated.

Contribution of the candidate to study methodology overall

For both studies, the candidate provided training, with clinical and managerial oversight, for anthropometric measures and child developmental assessments. She was also involved in data security and management, and collated and cleaned all infant-related health data.

The candidate was responsible for clinical research form design and piloting for all infant health aspects of the MCH-ART (HIV-exposed uninfected) cohort.

The candidate was a principal investigator on the HU2 (HIV-unexposed uninfected) mother infant study. As such, she was primarily responsible for obtaining funding and ethical approval; overseeing human resource procedures including appointments and performance reviews for staff; designing and piloting clinical research forms; and providing supervision for study implementation and quality control.

3.1 Study overview

This research draws on data from two large, prospective mother-infant cohorts enrolled and follow-up in parallel at a single research site in Gugulethu township, peri-urban Cape Town, South Africa (figures 3.1 and 3.2).

The MCH-ART (**M**aternal and **C**hild **H**ealth **A**nti-**R**etroviral **T**herapy) study enrolled pregnant women living with HIV (WLHIV) at first antenatal clinic visit to the Gugulethu Midwife Obstetric Unit (MOU); enrolment occurred from June 2013 to December 2014. Women initiating ART in pregnancy were followed longitudinally during pregnancy and through delivery (2-3 antenatal study visits). After delivery, breastfeeding WLHIV were eligible for postpartum follow-up with their breastfed infants following additional informed consent. The primary aim of MCH-ART was to assess postpartum interventions to optimize retention in care (randomized allocation to integrated maternal-child clinics vs standard of care, NCT01933477). Women were followed with their infants at 6 weeks, and 3-monthly from 3 to 12 months postpartum. A subset of mother-infant dyads was also seen at 18 months.

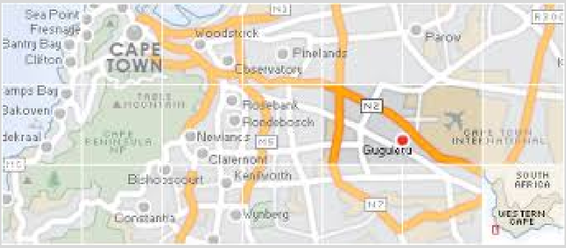


Figure 3.1. Map of Cape Town, South Africa indicating location of Gugulethu. *Source*: https://aidsoversixty.wordpress.com

The HU2 (**H**IV-**u**nexposed **u**ninfected) mother and infant cohort study was specifically designed to provide a comparison cohort of mothers and infants to complement the MCH-ART study. This study enrolled pregnant women testing HIV-negative at their first antenatal visit to Gugulethu MOU (enrolment from October 2014 to April 2016). Women were followed during pregnancy (2-3 antenatal visits) through delivery. Women who had remained HIV-negative during pregnancy were enrolled into the postpartum phase of HU2 and followed until 12 months postpartum with their breastfed infants. Apart from HIV-specific measures, the HU2 study utilized the same study site, team, measures, and procedures, with the same study visit intervals. The HU2 study enrolment was staggered with that of MCH-ART for logistical purposes. Nonetheless, care was taken for enrolment and follow-up periods to span all four seasons in both studies. In analyses of outcomes that have known seasonal variation in the study setting, season was explored as a potential confounder.

Figure 3.2. Activists of the Movement for Change and Social Justice canvassing the streets of Gugulethu in Cape Town, South Africa (2020). *Source*. Bizcommunity.com

Mother-infant dyads who participated in the postpartum phase of MCH-ART formed the HEU study group; those who participated in the postpartum phase of HU2 comprised the HU comparison group. Follow-up was completed for both studies by March 2017.

3.1.1 Study setting

Gugulethu is an impoverished urban township situated in the Klipfontein sub-district of Cape Town, Western Cape Province, South Africa (Figures 3.1 and 3.2). The Gugulethu MOU provides antenatal care to approximately 4800 women annually; antenatal HIV prevalence was 29% in 2012, and universal ART to all HIV-infected pregnant women was introduced in 2013.[1] The Gugulethu MOU is an accredited Baby Friendly Hospital (BFHI); an estimated 80-90% of women initiate breastfeeding as infant feeding of choice. A community-wide department of health-initiated breastfeeding restoration programme was introduced in 2015, with the aim to promote exclusive breastfeeding for at least 6 months irrespective of HIV status, and ensure the availability of trained breastfeeding support personnel on site and in the community. Postnatal infant care is provided at a number of local clinics, including weight checks, immunizations and management of common childhood illnesses. The paediatric tertiary referral center for the area is primarily the Red Cross War Memorial Children's Hospital (RCWMCH). Despite reasonable coverage of key child health services, the area has a high infant mortality rate (IMR, 15.6 per 1000 live births in 2012); the main causes of death in the under-5 population are pneumonia, diarrhoea and premature delivery.[2]

3.1.2 *Study population*

3.1.2.1 Recruitment and informed consent

Table 3.1. Inclusion and exclusion criteria for the MCH-ART and HU2 studies

	Applied to	Inclusion criteria	Exclusion criteria
ANTENATAL	Both studies	• 1st booking visit for index pregnancy • ≥ 18 years of age • Confirmed intra-uterine pregnancy • Able to provide informed consent	• Not currently pregnant • Intention to relocate out of Cape Town before delivery • Any medical, psychiatric, or social condition which could affect the ability to consent and/or participate in the study
	Only MCH-ART	• HIV-positive test in pregnancy • Initiation of ART during pregnancy	• Women who conceived on ART
	Only HU2	• HIV-negative test in index pregnancy	• None
POSTNATAL	Both studies	• Attended ≥1 antenatal study visit • Any breastfeeding during 1st 7 days postpartum • Willing to return for postnatal study visits	• Pregnancy loss or neonatal death before attending the screening visit • Intention to relocate out of Cape Town in the first year • Condition which would affect the ability to consent and/or participate in the study
	Only MCH-ART	• Willing to allow minimally invasive infant blood sampling at 12 months	• None
	Only HU2	• All HIV-tests in pregnancy were negative	• HIV seroconversion in pregnancy

Abbreviations: MCH-ART, maternal child health antiretroviral study; HU2, HIV-unexposed uninfected mother and infant study; ART, antiretroviral therapy

Women making their first ("booking") antenatal visit at the Gugulethu MOU were informed about the studies through the MOU staff (100% of HIV-positive women; the first 5 HIV-negative women per day, Monday to Friday). Interested women were screened based on the inclusion/exclusion criteria listed in table 3.1. Eligible women providing informed written consent (including consent to be approached for future research) were enrolled in the relevant study and phase.

3.1.2.2 Participant retention

At all study visits, participants provided up-to-date information for full names (including infant name changes), address(es) and at least two contact numbers. Follow-up appointment dates were scheduled to coincide with routine antenatal and postnatal care visits when feasible. Where employment or educational commitments precluded study visitation during the week, women were provided the opportunity to attend a study visit on the weekend. At each study visit, participants received up to 120 ZAR (~ 8 USD in 2015): 20 ZAR in cash to cover transport to their next scheduled visit, grocery vouchers worth 80 ZAR, and refreshments up to 20 ZAR. Mothers also received a small gift at the first and last post-partum visits, to show appreciate for their time and commitment. Infants received small gifts up to the value of 50 ZAR (~ 3 USD in 2015) each at the neonatal visit (usually items of baby clothing), the 12 months' visit (usually items of toddler clothing), and, for those participating in developmental assessments, another developmentally appropriate gift at completion of the assessment (usually a small book, ball, or shape-sorting toy).

Participants who did not attend scheduled visits were traced telephonically, with home visitation where telephonic contact was not possible. Throughout out-of-clinic patient contact, reasons for visitation were not provided to anyone other than the consented mother, with utmost care to maintain confidentiality. At study completion, hospital records and death registrations were checked to ascertain the risk of informative censoring among those who had not returned for their most recent study visit for more than 6 months. Women were declared lost to follow-up (LTFU) if at least 3 efforts of home tracing had failed, with no neighbours or family members contactable and no indication of vital status at the time of study closure.

3.2 Clinical care

3.2.1 General antenatal and obstetric care

All pregnant women received routine antenatal care (ANC) at the Gugulethu MOU (figure 3.3), with history and examination performed by a nurse-midwife. At first antenatal visit, all women received rapid HIV testing (with pre/post-testing counselling) and provided routine blood samples (including tests for ABO blood group, syphilis screening and haemoglobin), which were sent to the National Health Laboratory Services (NHLS) laboratory. Nurse-midwives referred complicated pregnancies for tertiary level care, at either the Mowbray Maternity Hospital (MMH) or Groote Schuur Hospital (GSH), Cape Town.

The MOU provided breastfeeding counselling, which continued throughout pregnancy and early postpartum visits, promoting early, exclusive breastfeeding. Labour and deliveries were managed at either the Gugulethu MOU or nearby obstetric facilities, including MMH and GSH. All discharged women were required to make a routine postnatal visit to the Gugulethu MOU within 7 days postnatal for "cord care".

Figure 3.3. The Gugulethu Community Health Centre. *Source*: Image taken by Stanzi le Roux, October 2015.

3.2.2 HIV-related care

At first antenatal visit, women known to be HIV-infected (diagnosed prior to current pregnancy) were initiated or maintained on lifelong ART (regimens according to Western Cape PMTCT guidelines at the time).[3,4]

Newly diagnosed women (either at booking or subsequently during pregnancy or postpartum) were fast-tracked for lifelong ART, which was initiated and monitored at the MOU during pregnancy, or the co-located Hannah Crusade HIV treatment centre for non-pregnant women. Women testing HIV negative at first ANC booking were scheduled for repeat testing per local protocols: late in third trimester (32-34 weeks) if first booking was early, during labor, at 6 weeks postpartum and 3-monthly through 2 weeks after breastfeeding cessation. At the time of the study, clinics offering contraceptive care were responsible for regular postpartum HIV testing. In addition, study-specific voluntary HIV counselling and testing were provided to all previously HIV-negative women at study exit. Throughout follow-up, women who seroconverted were post-test counselled and referred to the Gugulethu MOU PMTCT service for urgent infant testing and initiation of maternal ART.

HIV-exposed infant testing was conducted at local baby clinics in accordance to prevalent provincial guidelines (at the time of the study, testing at 6 weeks postpartum using HIV-PCR; then antibody-based testing at 9 months with confirmatory HIV-PCR test if positive; and antibody-based testing for previously negative children whenever ill, routinely at 18 months, and at 2 weeks post breastfeeding-cessation). [1,5] HIV-exposed infant prophylaxis was provided by the routine health services, following the concurrent PMTCT guidelines. This included nevirapine (NVP), given daily according to infant weight, for a minimum of 6-12 weeks depending on the risk stratification and infant feeding modality, continued until maternal viral load was <1000 copies/ml if breastfeeding; oral zidovudine (AZT) as an adjunct for infants considered at high risk of transmission (preterm, short duration of ART in pregnancy); and co-trimoxazole preventive therapy (CPT) from 6 weeks of age for all HIV-PCR positive infants and for breastfeeding, HIV-exposed but PCR negative infants. For HEU infants, CPT was discontinued after breastfeeding cessation, provisional on a negative post-breastfeeding HIV-PCR test. HIV-infected infants were referred to the nearest paediatric ART clinic, where they were fast-tracked for ART per national guidelines. All HIV-infected infants remained in study follow-up, but their clinical outcomes are not included in this thesis.

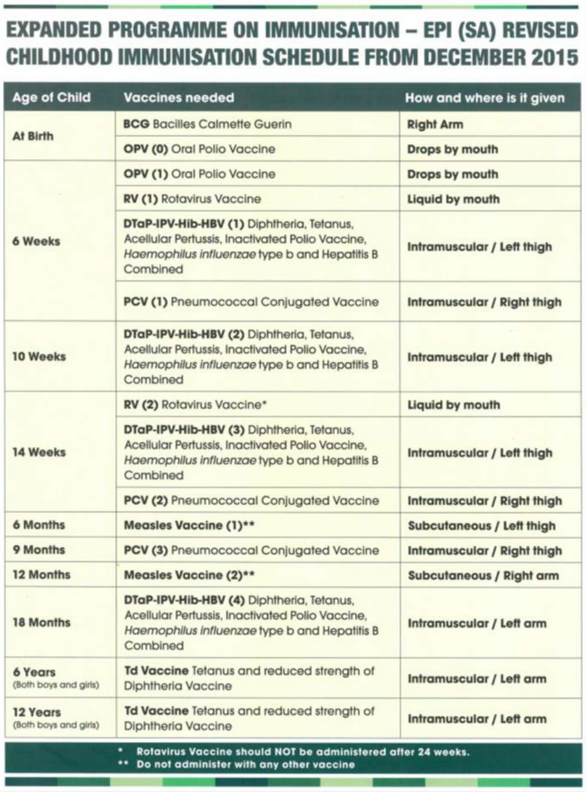
3.2.3 Postnatal maternal and infant care

With some exceptions in the MCH-ART study participants (HEU cohort, outlined below), infants received routine care at their closest clinics, according to local standard of care.

3.2.3.1 Local standard of care

The local standard of child health care at clinics includes:

- "Well baby" visits, scheduled around the national expanded programme of immunization (EPI) schedule (during the first year, this occurs at 6 weeks, 10 weeks, 14 weeks, and 9 months of age, fig 3.4).[6]
- Additional well baby checks are scheduled monthly, including growth monitoring, updating of HIV-infection status, 6 monthly deworming from the age of 6 months, and provision of vitamin A supplementation.
- Recording of relevant clinic and hospital health care findings in the infants "Road to Health" booklet (RTHB), which functions as a patient-held health record.
- An integrated management of childhood illness (IMCI) approach for assessing acute or chronically ill children, monitored by the district health services (figure 3.5).[2,7]



EXPANDED PROGRAMME ON IMMUNISATION – EPI (SA) REVISED CHILDHOOD IMMUNISATION SCHEDULE FROM DECEMBER 2015

Age of Child	Vaccines needed	How and where is it given
At Birth	**BCG** Bacilles Calmette Guerin	**Right Arm**
	OPV (0) Oral Polio Vaccine	**Drops by mouth**
6 Weeks	**OPV (1)** Oral Polio Vaccine	**Drops by mouth**
	RV (1) Rotavirus Vaccine	**Liquid by mouth**
	DTaP-IPV-Hib-HBV (1) Diphtheria, Tetanus, Acellular Pertussis, Inactivated Polio Vaccine, *Haemophilus influenzae* type b and Hepatitis B Combined	**Intramuscular / Left thigh**
	PCV (1) Pneumococcal Conjugated Vaccine	**Intramuscular / Right thigh**
10 Weeks	**DTaP-IPV-Hib-HBV (2)** Diphtheria, Tetanus, Acellular Pertussis, Inactivated Polio Vaccine, *Haemophilus influenzae* type b and Hepatitis B Combined	**Intramuscular / Left thigh**
14 Weeks	**RV (2)** Rotavirus Vaccine*	**Liquid by mouth**
	DTaP-IPV-Hib-HBV (3) Diphtheria, Tetanus, Acellular Pertussis, Inactivated Polio Vaccine, *Haemophilus influenzae* type b and Hepatitis B Combined	**Intramuscular / Left thigh**
	PCV (2) Pneumococcal Conjugated Vaccine	**Intramuscular / Right thigh**
6 Months	**Measles Vaccine (1)****	**Subcutaneous / Left thigh**
9 Months	**PCV (3)** Pneumococcal Conjugated Vaccine	**Intramuscular / Right thigh**
12 Months	**Measles Vaccine (2)****	**Subcutaneous / Right arm**
18 Months	**DTaP-IPV-Hib-HBV (4)** Diphtheria, Tetanus, Acellular Pertussis, Inactivated Polio Vaccine, *Haemophilus influenzae* type b and Hepatitis B Combined	**Intramuscular / Left arm**
6 Years (Both boys and girls)	**Td Vaccine** Tetanus and reduced strength of Diphtheria Vaccine	**Intramuscular / Left arm**
12 Years (Both boys and girls)	**Td Vaccine** Tetanus and reduced strength of Diphtheria Vaccine	**Intramuscular / Left arm**

* Rotavirus Vaccine should NOT be administered after 24 weeks.
** Do not administer with any other vaccine

Figure 3.4. South African National Immunization schedule. *Source:* https://www.westerncape.gov.za/service/immunisation

Referral to the patients' closest secondary or tertiary level hospital as indicated in emergencies or for specialist care. Following provincial health guidelines, most families attending the Gugulethu MOU are referred to the Red Cross War Memorial Children's hospital, a tertiary level institution with intensive care facilities and several sub-specialist outpatient clinics and services.

Figure 3.5. IMCI 2019 booklet cover, used in South African clinics. *Source*: https://www.knowledgehub.org.za/elibrary/integrated-management-childhood-illness-imci-2019

3.2.3.2 Considerations for the HEU group:

At the first postnatal visit, all MCH-ART mother-infant pairs enrolling in the postnatal phase of the study were randomly assigned (1:1) to one of two postpartum care models.[8]

The *intervention* model, an integrated mother and child follow-up service provided throughout breastfeeding duration.[9] In this model, a nurse practitioner trained in both IMCI and antiretroviral therapy provided maternal (including ART) and infant care (including well-baby visits, vaccinations, treatment of intercurrent illness and referral to secondary or tertiary centres as indicated), at the Gugulethu MOU where antepartum follow-up occurred. Mothers and infants were transferred from the intervention service to local standard of care following the cessation of breastfeeding.

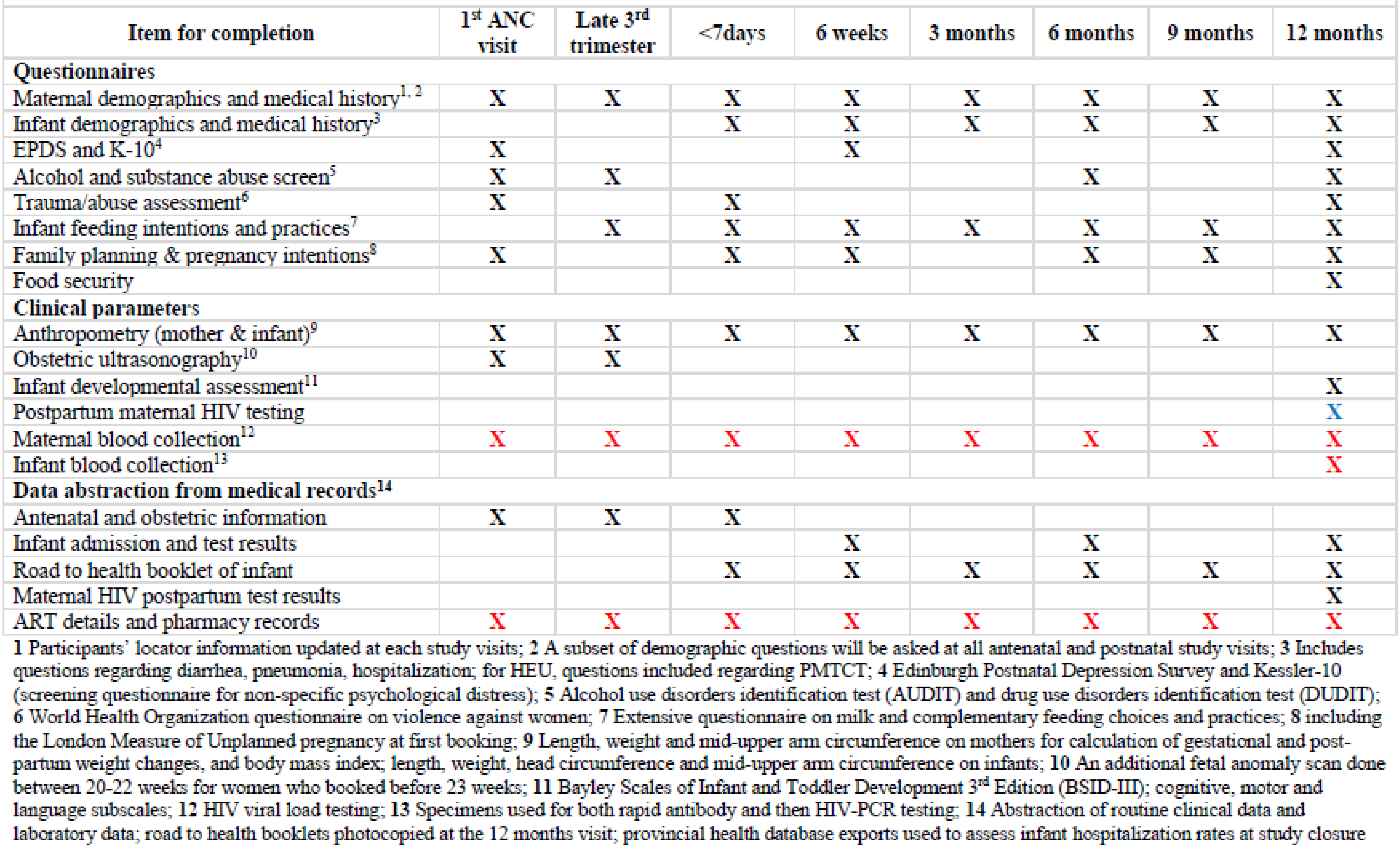
The *control* model is the local standard of care, as set out above: mothers and infants receive segregated care, with infants followed at well-baby clinics separate from the maternal health services.

3.3 Study measures

3.3.1 Visit schedule

The visit schedule including measurements utilized per visit is shown for both the MCH-ART and the HU2 studies in table 3.2, below. In the *antenatal study phases*, women were scheduled for 2-3 study visits: on the day of enrolment (1st antenatal visit); during the late third trimester, at 34+ weeks gestational age; and within 7 days post-partum (third visit of the antenatal phase, "neonatal study visit"), which coincided with the routine postnatal "cord care" clinic visit. Those who booked after 34 weeks gestational age completed 2 visits only (enrolment and neonatal visits. Ultrasound scans were done at enrolment and at >34 weeks' gestation; those who booked prior to 22 weeks gestational age (GA) also received a fetal anomaly scan at 20-22 weeks GA. At the "neonatal" study visit, mother-infant pairs were screened for enrolment in the postnatal phases. *Postnatal study visits* were scheduled at 6 weeks, and then 3-monthly from 3 to 12 months of age. A subset of HEU children also attended an 18 months' visit.

Table 3.2. Schedule of measurement visits for the MCH-ART (X), HU2 (X) or both (X) studies



Item for completion	1st ANC visit	Late 3rd trimester	<7days	6 weeks	3 months	6 months	9 months	12 months
Questionnaires								
Maternal demographics and medical history[1, 2]	X	X	X	X	X	X	X	X
Infant demographics and medical history[3]			X	X	X	X	X	X
EPDS and K-10[4]	X			X				X
Alcohol and substance abuse screen[5]	X	X				X		X
Trauma/abuse assessment[6]	X		X					X
Infant feeding intentions and practices[7]		X	X	X	X	X	X	X
Family planning & pregnancy intentions[8]	X		X	X		X	X	X
Food security								X
Clinical parameters								
Anthropometry (mother & infant)[9]	X	X	X	X	X	X	X	X
Obstetric ultrasonography[10]	X	X						
Infant developmental assessment[11]								X
Postpartum maternal HIV testing								X
Maternal blood collection[12]	X	X	X	X	X	X	X	X
Infant blood collection[13]								X
Data abstraction from medical records[14]								
Antenatal and obstetric information	X	X	X					
Infant admission and test results				X		X		X
Road to health booklet of infant			X	X	X	X	X	X
Maternal HIV postpartum test results								X
ART details and pharmacy records	X	X	X	X	X	X	X	X

1 Participants' locator information updated at each study visits; 2 A subset of demographic questions will be asked at all antenatal and postnatal study visits; 3 Includes questions regarding diarrhea, pneumonia, hospitalization; for HEU, questions included regarding PMTCT; 4 Edinburgh Postnatal Depression Survey and Kessler-10 (screening questionnaire for non-specific psychological distress); 5 Alcohol use disorders identification test (AUDIT) and drug use disorders identification test (DUDIT); 6 World Health Organization questionnaire on violence against women; 7 Extensive questionnaire on milk and complementary feeding choices and practices; 8 including the London Measure of Unplanned pregnancy at first booking; 9 Length, weight and mid-upper arm circumference on mothers for calculation of gestational and post-partum weight changes, and body mass index; length, weight, head circumference and mid-upper arm circumference on infants; 10 An additional fetal anomaly scan done between 20-22 weeks for women who booked before 23 weeks; 11 Bayley Scales of Infant and Toddler Development 3rd Edition (BSID-III); cognitive, motor and language subscales; 12 HIV viral load testing; 13 Specimens used for both rapid antibody and then HIV-PCR testing; 14 Abstraction of routine clinical data and laboratory data; road to health booklets photocopied at the 12 months visit; provincial health database exports used to assess infant hospitalization rates at study closure

3.4 Measurement tools

3.4.1 *Interview-based measures*

Questionnaires were administered in isiXhosa or English, per participant preference, by experienced staff at each study visit. All questionnaires were piloted and refined, with certified translation into isiXhosa, the most common local language. Overall, topics addressed in questionnaires varied between study visits, as shown in table 3.2. These included the Alcohol Use Disorders Toolkit (AUDIT),[10] Edinburgh Postnatal Depression Score (EPDS)[11], World Health Organization Violence Against Women questionnaire (VAW).[12] Additional details are provided below regarding questionnaires most relevant to the comparison of HEU and HU child health outcomes. A summary of all interview-based questionnaires utilized in this research is shown in supplemental table 9.3.1.

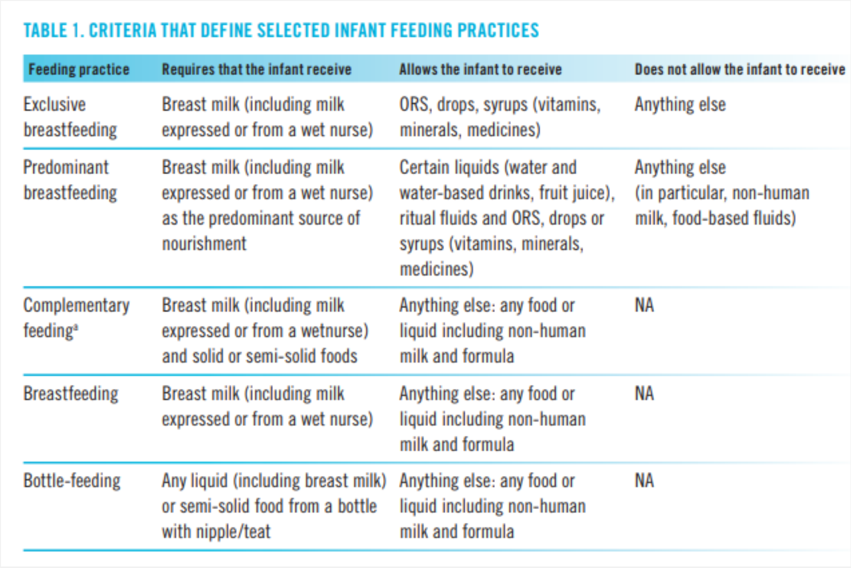


TABLE 1. CRITERIA THAT DEFINE SELECTED INFANT FEEDING PRACTICES

Feeding practice	Requires that the infant receive	Allows the infant to receive	Does not allow the infant to receive
Exclusive breastfeeding	Breast milk (including milk expressed or from a wet nurse)	ORS, drops, syrups (vitamins, minerals, medicines)	Anything else
Predominant breastfeeding	Breast milk (including milk expressed or from a wet nurse) as the predominant source of nourishment	Certain liquids (water and water-based drinks, fruit juice), ritual fluids and ORS, drops or syrups (vitamins, minerals, medicines)	Anything else (in particular, non-human milk, food-based fluids)
Complementary feeding[a]	Breast milk (including milk expressed or from a wetnurse) and solid or semi-solid foods	Anything else: any food or liquid including non-human milk and formula	NA
Breastfeeding	Breast milk (including milk expressed or from a wet nurse)	Anything else: any food or liquid including non-human milk and formula	NA
Bottle-feeding	Any liquid (including breast milk) or semi-solid food from a bottle with nipple/teat	Anything else: any food or liquid including non-human milk and formula	NA

Figure 3.6. Criteria that define selected infant feeding practices. *Source*: Indicators for assessing infant and young child feeding practices, World Health Organization

After delivery, the *infant feeding questionnaire* was used to ascertain duration and type of breastfeeding to enable classification of infant feeding according to WHO guidelines (fig 3.6).[13] Details of how these definitions were operationalized in data analysis are provided in chapter 9 (supplemental table 9.3.2)

The feeding questionnaire specifically addressed:

- Timing of breastfeeding initiation following delivery ("early initiation" defined as within 1 hour of birth)
- Prevalence and duration of exclusive breastfeeding at each study visit, based on 24-hour recall and time since either delivery or previous postnatal study visit
- Introduction to and frequency of infant formula feeding, and non-milk liquids, semi-solid or solid food, per study visit.

Part of the *infant demographics and health questionnaire* assessed the period prevalence of diarrhoeal illness and presumed lower respiratory tract infections (LRTI) at each postnatal study visit, using questions adapted from the Demographic Health Surveys (DHS) questionnaires.[14]

- Diarrhoeal disease was defined as maternal report of "any diarrhoea" in the preceding 2 weeks
- Presumed LRTI was defined as maternal report of (1) any coughing with concurrent (2) fever and (3) difficulty in breathing, in the preceding 2 weeks

3.4.2 Laboratory measures

Laboratory specimens were restricted to the MCH-ART study and included maternal viral load testing and postnatal infant testing, as shown in table 3.2. Previously HIV-negative infants enrolled in MCH-ART received HIV-PCR testing at 12 months using EDTA tubes. Results were analyzed at NHLS using the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 qualitative assay. Study staff immediately delivered HIV-negative test results to mothers telephonically. Infant found to be HIV-infected at any time during the study were urgently recalled via telephone or by home visit, for post-test counselling and immediate referral to care.

3.4.3 Clinical assessments

3.4.3.1 Fetal and child growth

During the *antenatal study phases*, fetal growth was assessed using ultrasound in both studies. Depending on gestational age at booking, measures included biparietal diameter, humerus and femur lengths and abdominal circumference; fetal weight was estimated during the third trimester.

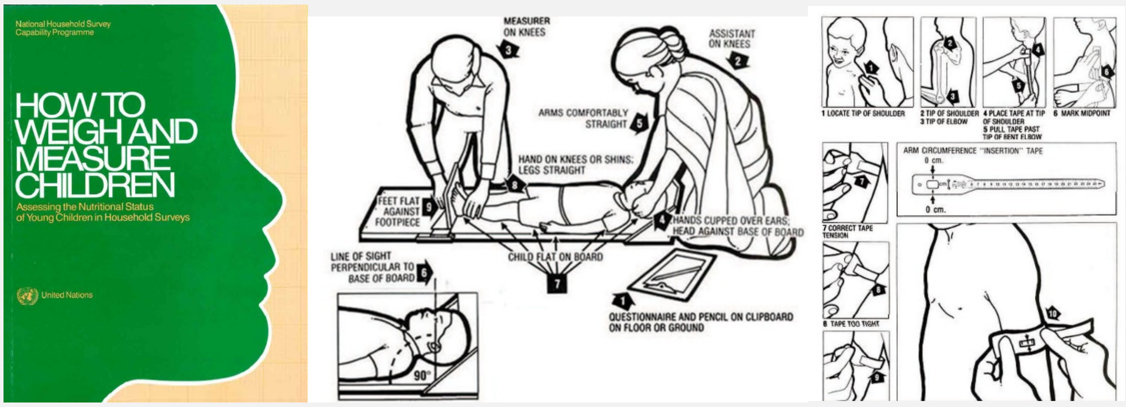


Figure 3.7.Images from "How to Weigh and Measure Children", UNICEF (1986). *Source:* https://unstats.un.org/unsd/publication/unint/dp_un_int_81_041_6E.pdf

In the *postnatal phase, clinical anthropometry* was conducted on both the mother and infant at 6 weeks postnatal and thereafter at each study measurement visit. Anthropometrical assessments followed standard operating procedures, based on WHO and UNICEF guidelines (figure 3.7).[15]

Infant length, weight, head circumference and mid-upper arm circumference (MUAC) were measured by trained research assistants, supervised by the candidate; the same study staff conducted anthropometric measurements on MCH-ART and HU2 study participants.

Infants were weighed (to the nearest 10g) on a calibrated, digital infant scale (MTB 20 Baby Scale, Adam Equipment, Milton Keynes, UK), figure 3.8. Infant lengths were measured to the nearest 0.25cm using a recumbent length board mounted horizontally on a flat, firm surface, with fixed head piece and firm foot piece perpendicular to the board (ADE MZ 1013 Baby Length Measuring Rod, ADE, Hamburg, Germany).

Figure 3.8. MTB Baby scale.
Source: www.scaletec.co.za

MUAC and head circumferences were measured using a non-stretchable tape marked, and recorded, to the nearest 1mm.

Maternal weight, length and mid-upper arm circumference were measured at 6 weeks post-partum, whereafter only weight and MUAC were measured. Maternal weight was recorded to the nearest 0.1 kg on a digital standing scale; height was measured to the nearest 2mm using a mechanical stadiometer; and MUAC was measured as for infants. Details of quality assurance measures, data cleaning approaches and transformation of measurements into age-, gestation- and sex-appropriate Z-scores are provided in the relevant manuscript (Chapter 4).

3.4.3.2 Neurodevelopmental assessment

Eligible children from both studies (11-18 months old, HIV-uninfected, gestational age at birth ≥ 28 weeks, no known congenital abnormalities or severe cerebral palsy, maternal informed consent for developmental assessment) using the Bayley Scales of Infant and Toddler Development®, Third Edition (BSID-III®, figure 3.9).

Figure 3.9. Image of the BSID-III equipment.
Source: www.pearsonassessments.com

The BSID-III® scales were chosen for this purpose as they (1) have been validated in infants, (2) are widely used internationally, and (3) do not require application by a psychologist.[16,17] The scales have been used successfully to evaluate developmental delay in the South African context.[18] Four experienced child health practitioners evaluated neurodevelopment following training in the use of BSID-III, under the supervision of a senior developmental paediatrician from the at Red Cross War Memorial Children's Hospital. Where necessary, assessments were assisted by a trained isi-Xhosa speaking counsellor present, to assist parent-assessor interaction.

The BSID-III® tests five developmental domains through application of five sub-scales. For logistic reasons, only the three scales which are administered with child interaction were used in this research study, namely the cognitive, motor and language sub-scales. Measures of quality assurance and approaches to data handling are detailed in the relevant publications (Chapters 6 and 7).

3.4.3.3 Infant mortality: ascertainment of cause of death, both groups

A combination of medical record review, death certification and verbal autopsy (WHO Standard verbal autopsy[19]) were used to ascertain the cause(s) of death in both HEU and HU infants.

Verbal autopsies were conducted at the mother's convenience, as soon as possible after the event. Interviews were scheduled and completed with sensitivity, by trained counsellors, in a private and quiet room.

3.4.4 Data abstraction from clinical records

Permission to review clinical records of participants was included in informed consent documents for all participants.

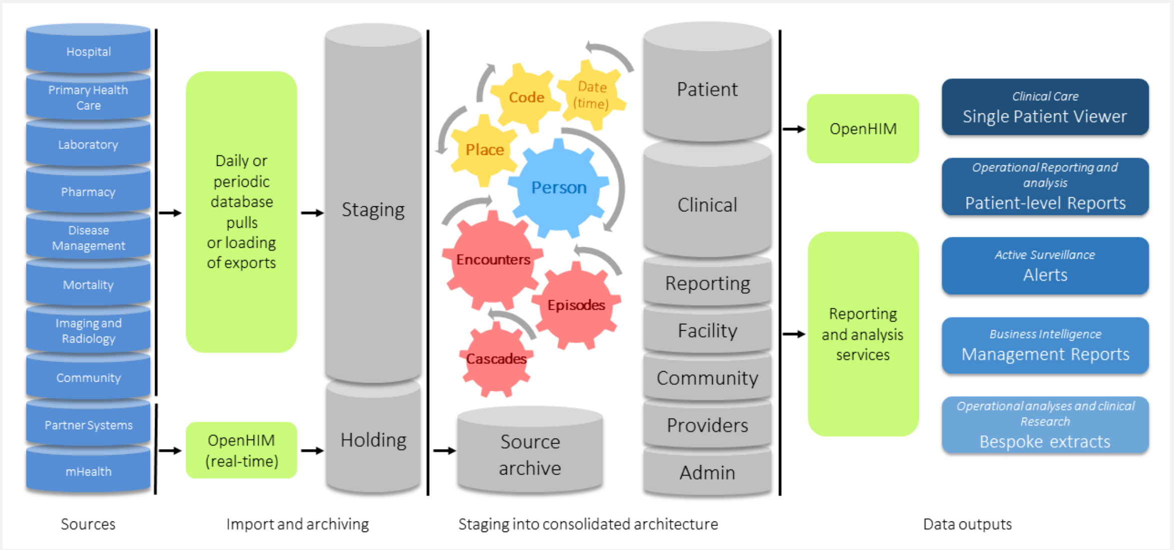


Figure 3.10. Western Cape Provincial Health Data Centre: high level architecture. *Source:* Boulle et al (2019), IJPDS Special Issue on Data Centre Profiles https://doi.org/10.23889/ijpds.v4i2.1143

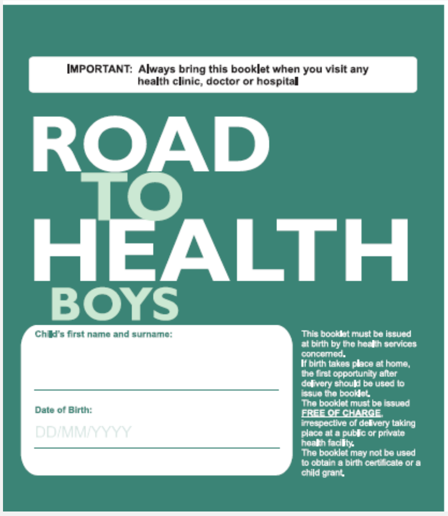
A combination of record reviews (predominantly patient-held records including the road to health booklet and the obstetric care folder) and provincial health data were used. The Western Cape Provincial Health Data Centre (PHDC) consolidates person-level health data in the province centrally. The process is governed by the principles of the Protection of Personal Information Act and uses multiple levels of harmonization and triangulation through a unique personal identifier. Details regarding the PHDC processes have been published.[20] An overview of the structure is shown in figure 3.10.

Antenatal information was obtained from MOU clinical records, including the results of routine bloods (syphilis screening, ABO blood group, and haemoglobin), the number and timing of ANC visits and any evidence of antenatal complications including episodes requiring referral to specialist obstetric care. Maternal blood results were also cross-checked using the national laboratory health system database.

Obstetric data were abstracted from the labour ward register (both studies) and individual patient records at the MOU (both studies) or nearby obstetric hospitals (MCH-ART only). In addition, obstetric information captured on the RTHB was abstracted (birth anthropometry, gestation at birth, delivery method and perinatal complications.

Maternal HIV-related information was abstracted from antenatal and obstetric records, including CD4 enumeration, dates and types of antiretroviral drugs received. Details regarding ART initiation and follow-up data were obtained from routine clinical care records as well as data exports from the PHDC.

Infant data regarding hospitalization (including ICD-10 coding, duration of admission, between-hospital transfer, medication as well as laboratory results for HIV testing, tuberculosis testing, in-hospital chemical and haematological tests) were obtained from the PHDC. The RTHB was primarily used to abstract obstetric data and completeness of immunization. To this end, the RTHB was photocopied (without personal identifiers) at each postnatal study visit (figure 3.11).



IMPORTANT: Always bring this booklet when you visit any health clinic, doctor or hospital

ROAD
TO
HEALTH
BOYS

Child's first name and surname:

Date of Birth:
DD/MM/YYYY

This booklet must be issued at birth by the health services concerned.
If birth takes place at home, the first opportunity after delivery should be used to issue the booklet.
The booklet must be issued **FREE OF CHARGE**, irrespective of delivery taking place at a public or private health facility.
The booklet may not be used to obtain a birth certificate or a child grant.

Figure 3.11. Road to Health Booklet for boys.
Source: Provincial health, 2015.

3.5 Approaches to data management and analysis

3.5.1 Data management and safety

Data capturing occurred at the Gugulethu research site of the UCT School of Public Health & Family Medicine. Data management followed procedures established for multiple previous operational studies at Gugulethu MOU. Briefly, data are collected on paper forms and entered in a customized, password-protected Microsoft Access database designed and maintained by a senior data manager, which is maintained in a fire-wall protected UCT server with daily backups. Data queries and responses are logged; data editing is based on reference to the form and source document in question and implemented through separate program files.

3.5.2 Analytic considerations

Summaries of the final analytic approaches are reported in each relevant results chapter (Chapters 4 to 7). Overall, analytic decisions were guided by basic biostatistical and epidemiological principles. Detailed exploratory data analysis preceded all modelling and hypothesis testing. This process included several rounds of data cleaning with evaluation for missing data, outliers, and implausible values, followed by cross-referencing of original data sources for corrections. Further exploratory analyses were guided by the *a priori* designed directed acyclic graph shown in the introductory chapter, which was adjusted for each outcome as indicated. Data were described and summarized according to distributional properties, using means or medians as appropriate, with indicators of dispersion. Bivariate associations were explored through the use of graphs (for example, e.g. box-and-whisker plots, scatterplots and histograms), correlation coefficients and basic statistical tests, including to the t-test, Wilcoxon rank-sum, analysis of variance (ANOVA), Kruskal-Wallis and Chi2 tests, the choice of which depended on both the nature and distribution of the covariates of interest.

Further details on sample size determination, model choices and approaches to third variables are provided in the relevant results chapters.

3.6 Human subjects' protection

The main MCH-ART study protocol, informed consent forms, data collection tools and other requested documents were reviewed and approved by the Columbia University Medical Centre Institutional Review Board (CUMC-IRB) and the University of Cape Town's Faculty of Health Sciences Research Ethics Committee (UCT-HREC). CUMC-IRB and UCT-HREC also reviewed the progress of the parent study annually (supplemental figure 9.3.1)

The HU2 study protocol, informed consent forms, data collection tools and other requested documents were reviewed and approved by the UCT-HREC; the progress of the study has been reviewed annually (supplemental figure 9.3.2).

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Chapter 4

Growth trajectories of breastfed South African HIV-exposed uninfected and HIV-unexposed children in the context of universal maternal antiretroviral therapy: a prospective cohort study

Stanzi M le Roux, Elaine J Abrams, Kirsten A Donald, Kirsty Brittain, Tamsin K Phillips, Kelly K Nguyen, Allison Zerbe, Max Kroon, and Landon Myer. **Growth trajectories of breastfed HIV-exposed uninfected and HIV-unexposed children under conditions of universal maternal antiretroviral therapy: a retrospective study.** *Lancet Child Adolesc Health* 2019; **3**(4): 234-44. DOI: 10.1016/s2352-4642(19)30007-0

Relevance of this paper to the thesis

As discussed in chapters 1 (introduction) and 2 (systematic literature review), there are scant robust data on HEU compared to HU child health outcomes in the context of universal maternal ART and breastfeeding. Key child health measures implicated in burden of disease across sub-Saharan Africa include growth faltering, infectious morbidity, and delayed early childhood development. Each of these outcomes is summarized and compared by maternal HIV status in the main results chapters (4 to 7). This chapter (4) provides an in-depth comparison of both growth patterns and risk for malnutrition during the first year of life, addressing study objectives 2(a), (b) and (c).

Contribution of the student and co-authors

SLR conceptualized this prospective infant growth comparison with the guidance of EJA and LM. KAD, AZ and MK reviewed and provided input on the proposed research approaches. SLR, TKP and KKN implemented and oversaw anthropometric measurements of mothers and infants during follow-up. KB was primarily responsible for data management; SLR conducted the final data cleaning and cross-checking. SLR conducted all data analyses, wrote the first draft of the paper and is the corresponding author. All co-authors reviewed and contributed to the paper including the final manuscript.

4.1 Abstract

4.1.1 Background

Over 1 million HIV-exposed uninfected (HEU) children are born in sub-Saharan Africa annually. Little data exist on the risk of impaired growth in this population under current policies of universal maternal antiretroviral therapy (ART) with breastfeeding. We aimed to study the growth of breastfed HEU children born to women who initiated ART during pregnancy and compare their growth with that of breastfed HIV-unexposed (HU) children drawn from the same community.

4.1.2 Methods

A prospective cohort of HIV-uninfected and HIV-infected pregnant women, who were initiating ART, were enrolled at their first antenatal care visit in a primary care centre in Gugulethu, Cape Town, South Africa. HIV infected women were participants of the Maternal Child Health Antiretroviral Therapy (MCH-ART) study, and HIV-uninfected pregnant women were participants in the HIV-Unexposed-Uninfected (HU2) study. All women were followed up during pregnancy, through delivery, to the early postnatal visit, which was scheduled for the first week after birth. At this visit, eligible breastfeeding mother–child pairs were recruited for continuation of postnatal follow-up until approximately age 12 months. Child anthropometry was measured at around 6 weeks, and every 3 months from month 3 to month 12. Weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ), head circumference-for-age, and body-mass index-for-age Z scores were compared between HEU and HU children longitudinally using mixed effects linear regression. At 12 months, proportions of HEU and HU children with moderate or severe malnutrition were compared cross-sectionally using logistic regression. MCH-ART is registered with ClinicalTrials.gov, number NCT01933477.

4.1.3 Results

Between June 2013, and April 2016, 884 breastfeeding mothers and their newborn babies (HEU, n=471; HU, n=413) were enrolled into postnatal follow-up. Excluding 12 children who tested HIV positive during follow-up, 461 HEU and 411 HU children attended 4511 anthropometric study visits in total, with a median of 6 visits (IQR 5–6) per child. Birth characteristics were similar (overall, 94 [11%] of 872 preterm [<37 weeks] and 90 [10%] small-for-gestational age [birthweight <10th percentile]). Median duration of breastfeeding was shorter among HEU than HU children (3·9 months [IQR 1·4–12·0] *vs* 9·0 months [IQR 3·0–12·0]). Although WAZ scores increased over time in both groups, HEU children had consistently lower mean WAZ scores than HU children (overall β –0·34, 95% CI –0·47 to –0·21). LAZ scores decreased in both groups after 9 months. At 12 months, HEU children had lower mean LAZ scores than HU children (β –0·43, –0·61 to –0·25), with a higher

proportion of children stunted (LAZ score <–2: 35 [10%] of 342 HEU *vs* 14 [4%] of 342 HU children; odds ratio [OR] 2·67, 95% CI 1·41 to 5·06). Simultaneously, overweight (WLZ score >2) was common in both groups of children at 12 months (54 [16%] of 342 HEU *vs* 60 [18%] of 340 HU children; OR 0·87, 95% CI 0·58 to 1·31).

4.1.4 Conclusions

Compared with HU children, HEU children had small deficits in early growth trajectories under policies of universal maternal ART and breastfeeding. Large proportions of both HEU and HU children were overweight by 12 months, indicating substantial risks for early onset obesity among South African children. Although the longer-term metabolic effects of ART exposure in the context of childhood obesity warrants further investigation, addressing childhood obesity should be an urgent public health priority in this setting.

4.1.5 Funding

Eunice Kennedy Shriver National Institute of Child Health and Human Development, Elizabeth Glaser Pediatric AIDS Foundation, South African Medical Research Council, and the Fogarty Foundation.

4.2 Introduction

Most of the one million HIV-exposed uninfected (HEU) children born annually reside in sub-Saharan Africa (SSA).[1,2] Understanding the health risks and needs of this large and growing population of children is a crucial step towards optimizing child health in the region. HEU children may be at increased risk for suboptimal growth compared to their HIV-unexposed (HU) counterparts.[3,4] However, findings from SSA have been inconsistent, and interpretation complicated by the rapidly changing landscape of HIV treatment and infant feeding recommendations over the past two decades.

Before 2010, avoidance of breastfeeding was commonly promoted as a strategy for prevention of mother-to-child-transmission of HIV (PMTCT); currently, the recommended HIV infant feeding strategy for most African countries is breastfeeding while on triple-drug antiretroviral therapy (ART).[5] Nearly contemporaneously, World Health Organization (WHO) PMTCT guidelines changed in 2013, shifting to universal ART (treatment for all, irrespective of CD4 cell thresholds) for pregnant and breastfeeding women.[6] Previously, ART was unavailable in Africa or limited to those with severe disease stages. In East and Southern Africa, antiretroviral coverage for pregnant women is now around 93%, and 66% of all people living with HIV received ART in 2017. Although reports of HEU child growth have varied over the past 20 years, two important determinants of child growth can be identified in this context. Generally, longer breastfeeding has been associated with improved growth,[7,8] while more advanced maternal HIV and absence of maternal ART were associated with suboptimal growth.[3,4,9-11]

For example, prior to the widespread availability of ART in Africa, HEU children were reported to have higher than expected risks of stunting, underweight, wasting and/or microcephaly. [3,4,7,12] Subsequently, limited data from SSA has suggested a possible increased risk of stunting among HEU children exposed to ART *in utero* compared to zidovudine alone, raising some concern about the long-term growth consequences of fetal ART exposure.[13] Per contra, higher than expected weight and length velocities have been also been reported in subgroups of formula-feeding HEU children, raising concerns for risk of childhood obesity.[9,14]

Notably, the current evidence base for HEU child growth outcomes in SSA is largely limited to data from settings where ART was not universally available in pregnancy, or from predominantly formula fed infants. There are very few data on the growth of HEU children in the context of universal maternal ART with breastfeeding, which is the currently recommended standard of care in SSA.[5]

We aimed to address this knowledge gap by studying the growth of breastfed HEU children born to women initiating ART in pregnancy and comparing this to the growth of breastfed HU children drawn from the same community. Specifically, we hypothesized that, under conditions of universal maternal

ART and breastfeeding, HEU children would have similar growth trajectories to those of HU children from the same community.

4.3 Methods

Parallel prospective cohorts of HIV-infected and HIV-uninfected women were followed from first antenatal clinic (ANC) visit through pregnancy to delivery, and with breastfed infants until ±12 months postpartum. All HIV-infected women initiated ART in pregnancy (tenofovir+emtricitabine+efavirenz) and were participants of the Maternal Child Health Antiretroviral (MCH-ART) study, a multi-phase study investigating strategies to optimize postpartum retention in care (recruitment and follow-up, 2013 – 2016).[15] HIV-uninfected women were participants of the HIV-unexposed-uninfected (HU2) cohort study (recruitment and follow-up, 2014 – 2017). Conducted in parallel, the HU2 study was designed specifically to complement MCH-ART by providing a community-control comparison group of HIV-uninfected mothers and -unexposed infants.[15] Study procedure details have been published.[15,16] In short, all women were recruited from the ANC of a large primary care centre in the peri-urban township of Gugulethu, Cape Town, South Africa. The centre serves a population of roughly 350 000 (30% antenatal HIV prevalence) with co-located antiretroviral services.[17,18] Both studies utilized the same study staff and, apart from HIV-specific measures, the same study procedures. Gestational age was estimated at first antenatal visit with ultrasound, last menstrual period, and palpation. After delivery, breastfeeding mother-infant pairs returning for a neonatal study visit within seven days postpartum (window, 28 days for MCH-ART and three months for HU2) were screened for continuation of postnatal follow-up. Exclusion criteria included intention to relocate, disinterest/inability to attend further visits and lack of breastfeeding. Study visits were scheduled at six weeks, and three-monthly from three to 12 months.

4.3.1 Ethical considerations

University of Cape Town Faculty of Health Sciences Research Ethics Committee approved the MCH-ART and HU2 studies (UCT-HREC: 567/2014, 451/2012); MCH-ART is registered on Clinicaltrials.gov (NCT01933477).

4.3.2 Measurements

4.3.2.1 Anthropometry

Pregnancy and birth anthropometry were abstracted from patient records. From six weeks onwards, mother-infant pairs were measured by trained study staff according to a standardized protocol, based on WHO guidelines.[17] Training was repeated at regular intervals, with structured, supervised competency assessments; the same staff measured both groups. Infant weight was measured to the nearest 10g using a calibrated digital infant scale (MTB 20 Baby Scale, Adam Equipment, Milton Keynes, UK); length, to the nearest 0·25 cm using a rigid recumbent length-board (ADE MZ 1013 Baby Length Measuring Rod, ADE, Hamburg, Germany); and head circumference (HC) to the nearest 0·1cm using a non-stretch tape measure.

4.3.2.2 HIV testing

Infection was excluded in HEU children at six and 48 weeks of age with HIV-PCR testing (Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 qualitative assay; Roche Molecular systems, Branchburg, NJ).[15] Routine PMTCT services repeated HIV tests on initially HIV-negative women (HU2 study) in the third trimester, and at approximately 3-monthly intervals while breastfeeding, following local guidelines.[3] In the event of maternal seroconversion, previously HIV-unexposed infants were urgently tested for HIV. Seroconverting women and their infants were immediately referred to the co-located ART clinic for further management.

4.3.2.3 Interviews

Trained field workers administered standardized questionnaires addressing maternal health, psychosocial and behavioural factors, and after delivery, infant feeding and health (table 1, footnotes).[15,16] Feeding practices were assessed with 24-hour maternal recall; last study visit with report of breastfeeding was used as date of breastfeeding cessation. Breastfeeding was classified using WHO guidelines into exclusive, predominant or partial breastfeeding.[18] Household food security was assessed at 12 months.[19]

4.3.3 Statistical methodology

Age-, sex- and gestation-adjusted Z-scores for weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ), head circumference-for-age (HCAZ) and body mass index-for-age (BMIZ) were generated using Intergrowth-21st (birth anthropometry and postnatal growth of preterm infants until 64 weeks' postmenstrual age)[20] and WHO (all other postnatal measures) growth reference standards.[21] Z-scores >3 or <-3 were reviewed and corrected in the event of data capture errors; unexplained values >5 (WAZ, HCAZ, BMIZ, WLZ), >6 (LAZ); or <-5 (HCAZ, BMIZ, WLZ), <-6 (WAZ, LAZ) were dropped as per WHO guidelines (overall, <1% of all measurements).[21] Rapid weight gain in infancy was defined as >0·67 increase in WAZ from birth to 12 months.[22]

Growth trajectories were tested using mixed effects linear regression models (absolute Z-score differences expressed as β-coefficients) including an interaction term for time. Covariates were selected using a directed acyclic graph (DAG) developed *a priori*, addressing known factors associated with maternal HIV and/or child growth (supplemental figure 9.4.1). Where multiple measures were available for a construct, variable choice was guided by improvement in model fit (Akaike's Information Criterion, AIC); when indicated, continuous/discrete variables were categorized according to published boundaries. Sensitivity analyses included repeat analysis without adjustment for birth characteristics; and restricted analyses, for children (1) with ≥ five study visits; (2) born at term, appropriate for gestational age (AGA); (3) without reports of maternal risky drinking; or (4) with similar infant feeding practices.

4.3.3.1 Sample size considerations:

Allowing for 15% early censoring and 10-15% exclusive formula feeding, we estimated *a priori* that a sample size of 1100-1150 pregnant women would provide ≥ 880 eligible infants for follow-up, achieving 84% power to detect ≥ 0.2 absolute difference in Z-scores between HEU and HU children (α=0·05, two-sided; standard deviations of 1.0).

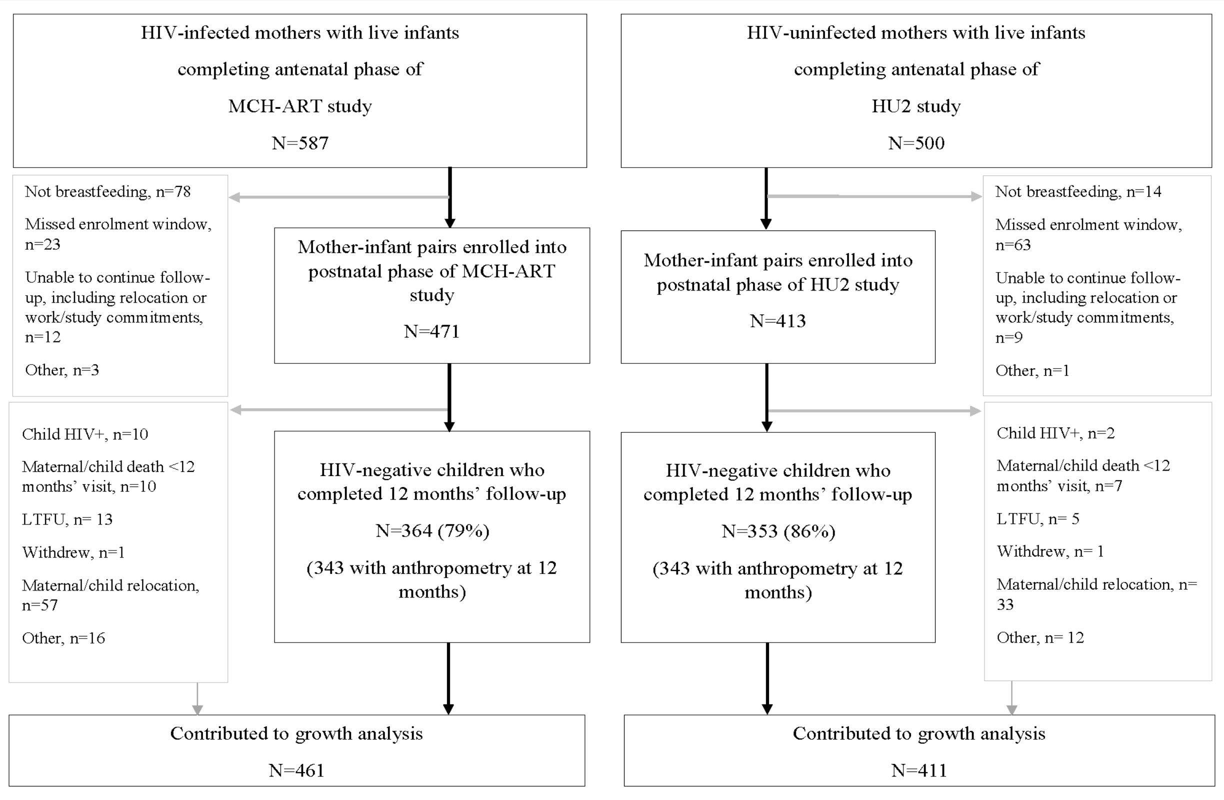
4.3.4 Role of the funding source

The study sponsors had no role in study design, data collection, analysis, interpretation, or writing of the report. The corresponding author had full access to all study data with final responsibility for the decision to submit for publication.

4.4 Results

A total of 884 breastfeeding mother-infant pairs were enrolled for postnatal follow-up (figure 4.1). Characteristics of excluded infants are shown in supplemental table 9.4.1. After enrolment, a further 12 HIV-infected children were excluded from the analysis, leaving 461 HEU [median (interquartile range, IQR) maternal pre-ART $\log_{10}$ HIV viral load, 4·0 (3·3-4·5); table 4.1] and 411 HU children, representing 4511 anthropometry visits (mean of five visits per child). Overall, 717/872 (82%) of the cohort completed study follow-up; 686/717 (96%) of these children contributed anthropometry at 12 months (figure 4.1 & supplemental table 9.4.2). Those not contributing anthropometry at 12 months had shorter breastfeeding durations, partly due to study-defined breastfeeding censoring at last attended study visit; there were no other meaningful differences (supplemental table 9.4.2). At first ANC visit, HIV-infected women reported greater socio-economic and/or psycho-social/behavioural adversity including risky drinking and intimate partner violence (IPV, table 4.1). Maternal obesity was common in both groups.

Figure 4.1. Study flow



Overall, the median birth weight was 3180g (IQR 2820-3460), with 98/872(11%) infants born low birth weight (<2500g) and 10/872(1%), very low birth weight (<1500g). Prevalence of preterm delivery (PTD) and small-for-gestational age birth weight (SGA, <10th centile) did not differ by maternal HIV status (HEU vs. HU: PTD 56/461, 12% vs. 38/411, 9%, p=0·17; SGA 51/461, 11% vs. 39/411, 10%, p=0·45); 81/90 (90%) of SGA children were born at term. HEU children were more likely to ever exclusively breastfeed than HU children but had shorter overall duration of any breastfeeding (median four vs. nine months). Complementary feeding (nutritive liquids and/or solids) had been introduced to 11% (38/363) of HEU vs 17% (64/368) of HU children by ± three months; 50% (191/382) vs 70% (244/345) by ± six; and 98% (351/360) vs 95% (314/33) by ± nine months (supplemental table 9.4.3). HIV-affected households were more likely to suffer from food insecurity at 12 months (table 4.1).

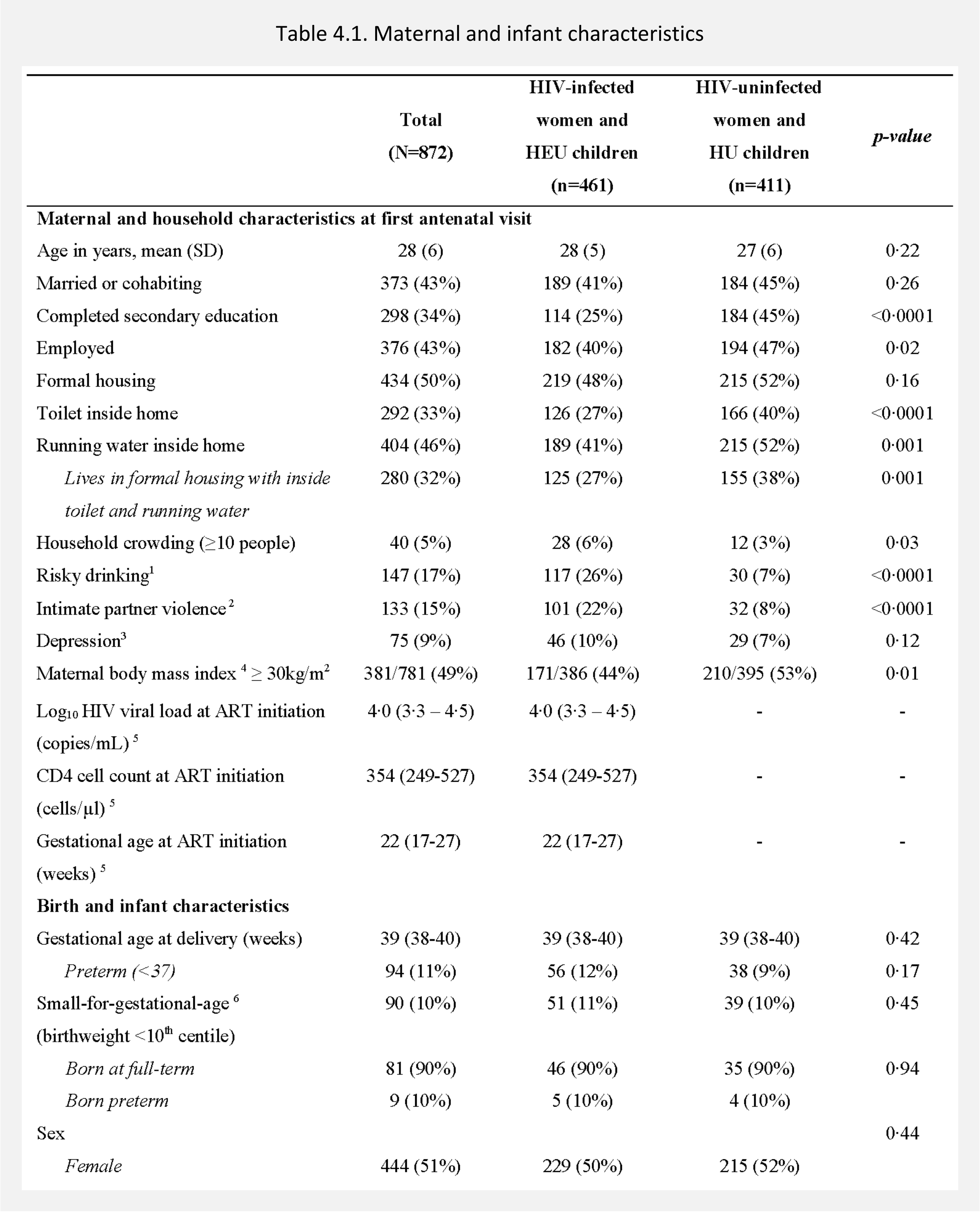


Table 4.1. Maternal and infant characteristics

	Total (N=872)	**HIV-infected women and HEU children (n=461)**	**HIV-uninfected women and HU children (n=411)**	***p-value***
Maternal and household characteristics at first antenatal visit				
Age in years, mean (SD)	28 (6)	28 (5)	27 (6)	0·22
Married or cohabiting	373 (43%)	189 (41%)	184 (45%)	0·26
Completed secondary education	298 (34%)	114 (25%)	184 (45%)	<0·0001
Employed	376 (43%)	182 (40%)	194 (47%)	0·02
Formal housing	434 (50%)	219 (48%)	215 (52%)	0·16
Toilet inside home	292 (33%)	126 (27%)	166 (40%)	<0·0001
Running water inside home	404 (46%)	189 (41%)	215 (52%)	0·001
Lives in formal housing with inside toilet and running water	280 (32%)	125 (27%)	155 (38%)	0·001
Household crowding (≥10 people)	40 (5%)	28 (6%)	12 (3%)	0·03
Risky drinking[1]	147 (17%)	117 (26%)	30 (7%)	<0·0001
Intimate partner violence[2]	133 (15%)	101 (22%)	32 (8%)	<0·0001
Depression[3]	75 (9%)	46 (10%)	29 (7%)	0·12
Maternal body mass index[4] ≥ 30kg/m^2	381/781 (49%)	171/386 (44%)	210/395 (53%)	0·01
Log$_{10}$ HIV viral load at ART initiation (copies/mL)[5]	4·0 (3·3 – 4·5)	4·0 (3·3 – 4·5)	-	-
CD4 cell count at ART initiation (cells/µl)[5]	354 (249-527)	354 (249-527)	-	-
Gestational age at ART initiation (weeks)[5]	22 (17-27)	22 (17-27)	-	-
Birth and infant characteristics				
Gestational age at delivery (weeks)	39 (38-40)	39 (38-40)	39 (38-40)	0·42
Preterm (<37)	94 (11%)	56 (12%)	38 (9%)	0·17
Small-for-gestational-age[6] (birthweight <10th centile)	90 (10%)	51 (11%)	39 (10%)	0·45
Born at full-term	81 (90%)	46 (90%)	35 (90%)	0·94
Born preterm	9 (10%)	5 (10%)	4 (10%)	
Sex				0·44
Female	444 (51%)	229 (50%)	215 (52%)	

Table 4.1. Maternal and infant characteristics (continued)

	Total (N=872)	HIV-infected women and HEU children (n=461)	HIV-uninfected women and HU children (n=411)	*p-value*
Male	428 (49%)	232 (50%)	196 (48%)	
Ever exclusively breastfed (EBF) [7]	754 (86%)	421 (91%)	333 (81%)	<0·0001
Duration of EBF (months)	1·4 (0·1-3·0)	1·4 (0·2-3·5)	1·3 (0·1-3·0)	<0·0001
Duration of any breastfeeding (months)	6·0 (1·5-12·0)	3·9 (1·4-12·0)	9·0 (3·0-12·0)	0·0001
Cumulative infant feeding from birth to 6 months [7]				<0·0001
Exclusive breastfeeding	343 (39%)	206 (45%)	137 (33%)	
Predominant breastfeeding	137 (16%)	42 (9%)	95 (23%)	
Partial breastfeeding	392 (45%)	213 (46%)	179 (44%)	
Postpartum household/ environmental factors				
Maternal smoking [8]				
At 6 months' visit	21 (3%)	19 (5%)	2 (1%)	<0·0001
At 12 months' visit	20 (3%)	20 (6%)	0	< 0·0001
Household food security at 12 months` visit [9]				<0·0001
No food insecurity	433 (63%)	190 (56%)	243 (71%)	
At risk for food insecurity	177 (26%)	88 (26%)	89 (26%)	
Has food insecurity	75 (11%)	64 (19%)	11 (3%)	

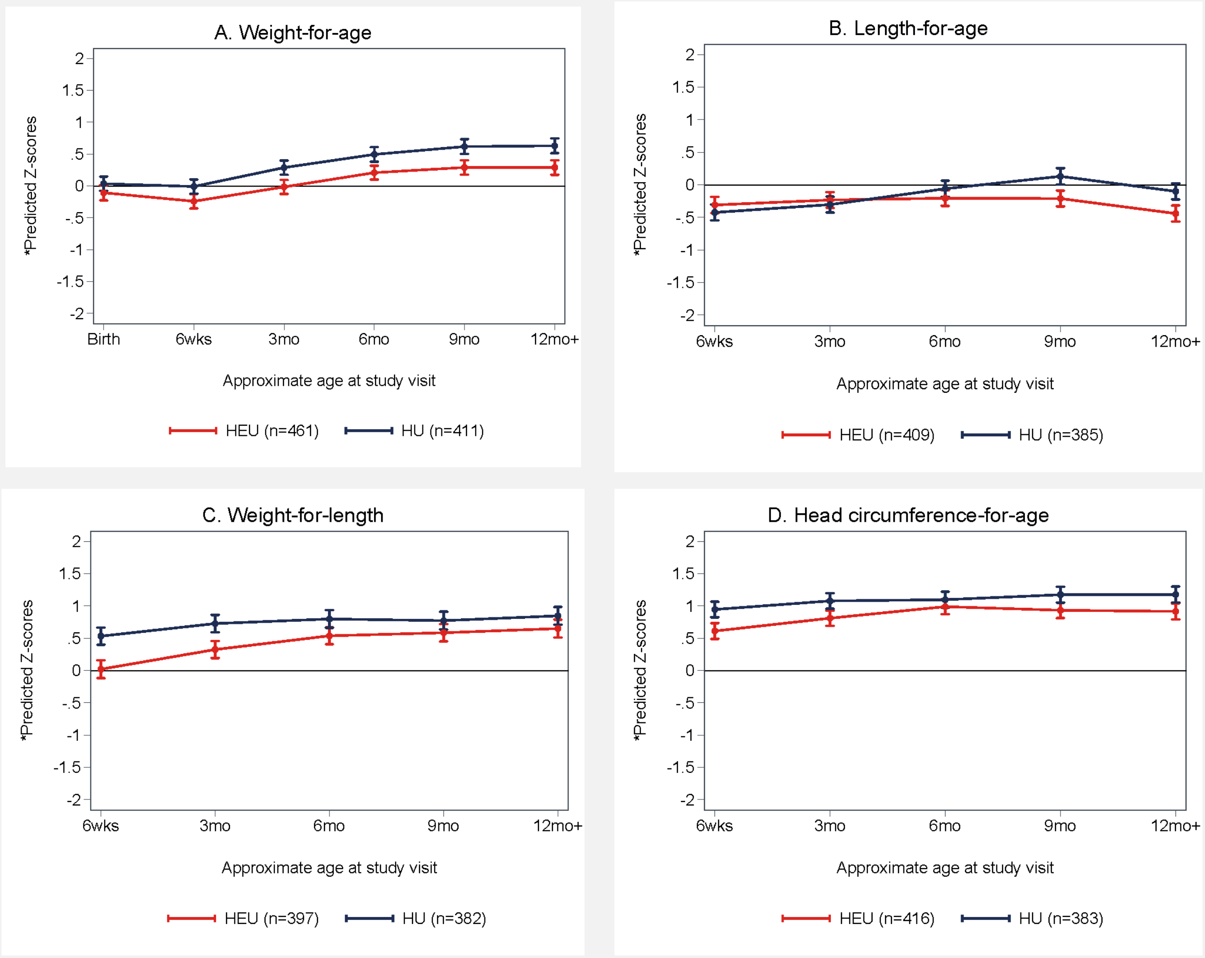
HEU, HIV-exposed uninfected; HU, HIV-unexposed uninfected; SD, standard deviation; Results are n (column %) with *p*-value from chi^2 test; mean (SD) with *p*-value from t-test for normally distributed variables; or median (interquartile range, IQR) with *p*-value from Kruskal-Wallis

[1] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 as reported at first antenatal visit (missing data, n=2); [2] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit (missing data, n=4); [3] Maternal depression, EPDS (Edinburgh postnatal depression scale) score of ≥ 13 at first antenatal visit (missing data, n=2); [4] Missing data, n=91; [5] Restricted to HIV-infected women only (N=461); [6] Birth weight percentile based on Intergrowth-21st reference standards; [7] Maternal report (24 hour recall); exclusive breastfeeding defined as only breastmilk and prescribed medicine; [8] Based on maternal recall: history of any cigarette smoking in preceding month; missing data at 6 months, n=2; missing data at 12 months, n=5; [9] Based on questionnaire adapted from the Household Food Insecurity Access Scale (HFIAS), Food and Nutrition Technical Assistance Project (FANTA) and the Community Childhood Hunger Indentification Project Index (CCHIP); limited to mother-child pairs attending 12 month visit (N=686; missing data, n=1)

4.4.1 Weight

At birth, mean (standard deviation, SD) WAZ was close to zero in both groups [HEU, -0·13(0·92) and HU children 0·12(1·00), supplemental table 9.4.3]. After six weeks, WAZ gradually increased in both groups with consistently lower values among HEU children [average crude difference in WAZ -0·34 (95% confidence interval, CI -0·47; -0·21); figure 4.2.A & table 4.2].

Figure 4.2. Adjusted Z-score growth trajectories in the first year of life comparing HIV-exposed uninfected to HIV-unexposed children: A. Weight-for-age; B. Length-for-age; C. Weight-for-length; and D. Head circumference-for-age



Data are adjusted Z-scores from mixed effects linear regression models with interaction term for time
HEU = HIV-exposed uninfected; HU = HIV-unexposed uninfected

By 12 months, mean (SD) WAZ was above zero in both groups [HEU, 0·24(1·32) and HU, 0·65(1·24); supplemental table 9.4.3). At this time point, only 2% (16/684) of the cohort were underweight [WAZ<-2: HEU vs. HU children, odds ratio (OR) 3·09 (95% CI 0·99; 9·68), table 4.3] whereas 11% (78/684) of the cohort had WAZ>2. Overall, 299/684 (44%) infants experienced rapid weight gain during the first year.

4.4.2 Length

Linear growth trajectories varied over time and by HIV-exposure status. In early infancy, HEU and HU children had similar, low-average LAZ (at six weeks, mean LAZ in HEU, -0·34 vs. HU, -0·37; supplemental table 9.4.3). Between six and nine months, HEU children maintained a low average LAZ while LAZ steadily increased in HU children (figure 4.2B). From nine months onwards, LAZ declined in both groups, signalling group-level linear growth faltering. By 12 months, HEU children had significantly lower average LAZ vs HU children [aβ -0·34 (95% CI -0·52; -0·16), table 4.2], and were twice as likely to be stunted (LAZ <-2): 10% (35/342) HEU vs. 4% (14/343) HU children, aOR 2·23 [(95% CI 1·12; 4·46) table 4.3].

Table 4.2 Differences in growth between HIV-exposed and HIV-unexposed children over time: crude and adjusted estimates from mixed-effects linear regression

Main effect: HEU vs. HU children	Weight-for-age (WAZ)[1]	Length-for-age (LAZ)[1]	Head circumference-for-age (HCAZ)[1]	Weight-for-length (WLZ)[2]	Body mass index-for-age (BMIZ)[2]
Crude regression models	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Excluding HIV-exposure-time interaction	-0·34 (-0·47; -0·21)	-0·20 (-0·34; -0·06)	-0·30 (-0·43; -0·16)	-0·35 (-0·49; -0·21)	-0·35 (-0·50; -0·21)
Including HIV-exposure-time interaction					
Birth	-0·24 (-0·40; -0·09)	-	-	-	-
6 weeks' visit	-0·30 (-0·45; -0·14)	0·01 (-0·15; 0·18)	-0·40 (-0·57; -0·23)	-0·54 (-0·72; -0·35)	-0·53 (-0·71; -0·35)
3 months' visit	-0·38 (-0·54; -0·22)	-0·02 (-0·20; 0·15)	-0·30 (-0·47; -0·13)	-0·44 (-0·62; -0·25)	-0·50 (-0·68; -0·32)
6 months' visit	-0·35 (-0·51; -0·19)	-0·23 (-0·40; -0·06)	-0·15 (-0·32; 0·02)	-0·30 (-0·48; -0·11)	-0·30 (-0·49; -0·12)
9 months' visit	-0·39 (-0·55; -0·22)	-0·43 (-0·60; -0·25)	-0·28 (-0·45; -0·11)	-0·22 (-0·38; -0·03)	-0·20 (-0·38; -0·01)
12 months' visit	-0·39 (-0·56; -0·23)	-0·43 (-0·61; -0·25)	-0·30 (-0·47; -0.13)	-0·22 (-0·39; -0·09)	-0·15 (-0·34; 0·03)
Confounder-adjusted (multivariable) models	**aβ (95% CI)**	**aβ (95% CI)**	**aβ (95% CI)**	**aβ (95% CI)**	**aβ (95% CI)**
Birth outcomes not included, no interaction terms[4]	-0·27 (-0·41; -0·13)	-0·13 (-0·27; 0·02)	-0·24 (-0·39; -0·10)	-0·32 (-0·47; -0·17)	-0·31 (-0·46; -0·16)
Birth outcomes included, no interaction terms[5]	-0·27 (-0·39; -0·14)	-0·12 (-0·26; 0·02)	-0·24 (-0·38; -0·10)	-0·32 (-0·46; -0·17)	-0·31 (-0·46; -0·16)
Birth outcomes and HIV-exposure-time interaction included[6]					
Birth	-0·14 (-0·30; 0·02)	-	-	-	-
6 weeks	-0·23 (-0·30; -0·07)	0·11 (-0·06; 0·29)	-0·33 (-0·50; -0·16)	-0·51 (-0·70; -0·32)	-0·53 (-0·72; -0·35)
3 months	-0·30 (-0·46; -0·14)	0·07 (-0·10; 0·25)	-0·27 (-0·44; -0·10)	-0·40 (-0·59; -0·21)	-0·46 (-0·64; -0·27)
6 months	-0·29 (-0·45; -0·13)	-0·14 (-0·32; 0·03)	-0·11 (-0·28; 0·06)	-0·26 (-0·45; -0·07)	-0·26 (-0·45; -0·08)
9 months	-0·33 (-0·49; -0·16)	-0·34 (-0·52; -0·17)	-0·24 (-0·42; -0·07)	-0·19 (-0·38; 0·01)	-0·16 (-0·35; 0·03)
12 months	-0·34 (-0·50; -0·18)	-0·34 (-0·52; -0·16)	-0·26 (-0·43; 0·0)	-0·20 (-0·39; 0·00)	-0·13 (-0·31; 0·06)

Abbreviations: HEU, HIV-exposed uninfected; HU, HIV-unexposed; β, regression coefficient – indicates crude absolute difference in Z-score comparing HEU to HU children; aβ, adjusted regression coefficient- indicates adjusted absolute difference in Z-score comparing HEU to HU children; CI, confidence interval; mixed effect linear regression with random intercept for child

[1] WAZ, LAZ and HCAZ-scores obtained from Intergrowth-21st and World Health Organization growth reference standards; [2] WLZ and BMIZ-scores obtained from World Health Organization growth reference standards (using gestation-adjusted age for preterm infants until 64 weeks' postmenstrual age); [3] Contrasted predictive margins based on mixed effects regression model including only HIV-exposure, study visit and an interaction term (p-values for interaction: WAZ, p=0·27; LAZ, p<0·0001; HCAZ, p=0·04; WLZ, p=0·003; BMIZ, p<0·0001)

[4] Adjusted for time (study visit), socio-economic factors [household crowding, marital status and lack of amenities (<3 of running water, flush toilet and electricity in home)], maternal alcohol use, intimate partner violence, recent history of any childhood illness and infant feeding (current milk feeding and weaning practices); [5] Further adjusted for small-for-gestational age (SGA, birth weight <10th centile) and preterm birth (delivery <37 weeks' gestation); [6] Contrasted predictive margins based on multivariable model as above (including SGA, preterm birth), with interaction term for HIV-exposure status and time (p-value for interaction: WAZ, p=0·11; LAZ, p<0·0001; HCAZ, p=0·07; WLZ, p=0·004; BMIZ, p<0·0001)

4.4.3 Proportional size

WLZ were initially much lower among HEU children [at six weeks, HEU vs. HU: aβ -0·51 (95% CI -0·70; -0·32); figure 4.2C & table 4.2]. Over time, WLZ steadily increased in both groups, with more rapid increases among HEU children, until differences became negligible (figure 4.2C). By 12 months, a large proportion of both HEU and HU children were overweight (WLZ >2: 16% (54/340) HEU vs. 18% (60/342) HU children; table 4.3; figure 4.3) or obese (WLZ>3: 5% (16/343) HEU vs. (20/343) 6 % HU; supplemental table 9.4.3).

Table 4.3 Prevalence, relative odds, and predictors of being under- or overweight, and/or stunted at study completion (approximately 12 months) among HEU compared to HU children

Maternal/child characteristics	Underweight:		Stunted:		Overweight:	
	Weight-for-age Z-score <-2		Length-for-age Z-score <-2		Weight-for-length Z-score >2	
	Total:	**2% (16/684)**	**Total:**	**7% (49/684)**	**Total:**	**17% (114/682)**
	HEU:	**3% (12/341)**	**HEU:**	**10% (35/342)**	**HEU:**	**16% (54/342)**
	HU:	**1% (4/343)**	**HU:**	**4% (14/342)**	**HU:**	**18% (60/340)**
	OR (95% CI)	**aOR (95% CI)**	**OR (95% CI)**	**aOR (95% CI)**	**OR (95% CI)**	**aOR (95% CI)**
Maternal HIV (HEU vs· HU)	3·09 (0·99; 9·68)	2·16 (0·62; 7·57)	2·67 (1·41; 5·06)	2·23 (1·12; 4·46)	0·87 (0·58; 1·31)	0·95 (0·61; 1·48)
Maternal obesity (BMI≥30 kg/m^2) [1]	0·76 (0·25-2·37)	-	0·48 (0·25-0·92)	-	1·46 (0·89-2·39)	-
Lacks amenities [2]	1·02 (0·35; 2·98)	0·97 (0·31; 3·09)	1·90 (0·93; 3·88)	1·85 (0·88; 3·89)	0·79 (0·52; 1·21)	0·80 (0·52; 1·24)
Risky drinking [3]	6·50 (2·37; 17·82)	3·99 (1·28; 12·41)	2·50 (1·32; 4·70)	1·60 (0·80; 3·19)	1·17 (0·70; 1·96)	1·51 (0·87; 2·65)
Small-for-gestational age [4]	12·57 (4·52; 34·91)	9·42 (3·19; 27·82)	5·72 (2·98; 10·96)	5·18 (2·62; 10·27)	0·13 (0·03; 0·53)	0·12 (0·03; 0·50)
Preterm birth [5]	1·91 (0·53; 6·86)	1·82 (0·45; 7·31)	2·54 (1·24; 5·21)	2·55 (1·18; 5·49)	0·57 (0·27; 1·23)	0·49 (0·22; 1·12)
No longer breastfeeding [6]	1·10 (0·40; 2·99)	0·75 (0·25; 2·23)	1·16 (0·64; 2·08)	0·94 (0·50; 1·74)	0·93 (0·62; 1·39)	0·95 (0·62; 1·44)
Introduction to complementary foods ≤ 3 months of age [7]	0·94 (0·21-4·21)	-	1·32 (0·60-2·91)	-	1·17 (0·66-2·07)	-
Household food insecurity	0·53 (0·07-4·10)	0·44 (0·05-3·67)	1·39 (0·60-3·21)	1·05 (0·43-2·57)	0·40 (0·17-0·95)	0·40 (0·17-0·97)
Recent childhood illness [9]	3·12 (1·12; 8·69)	2·89 (0·97; 8·63)	1·52 (0·85; 2·73)	1·40 (0·75; 2·60)	0·90 (0·59; 1·36)	1·04 (0·67; 1·61)

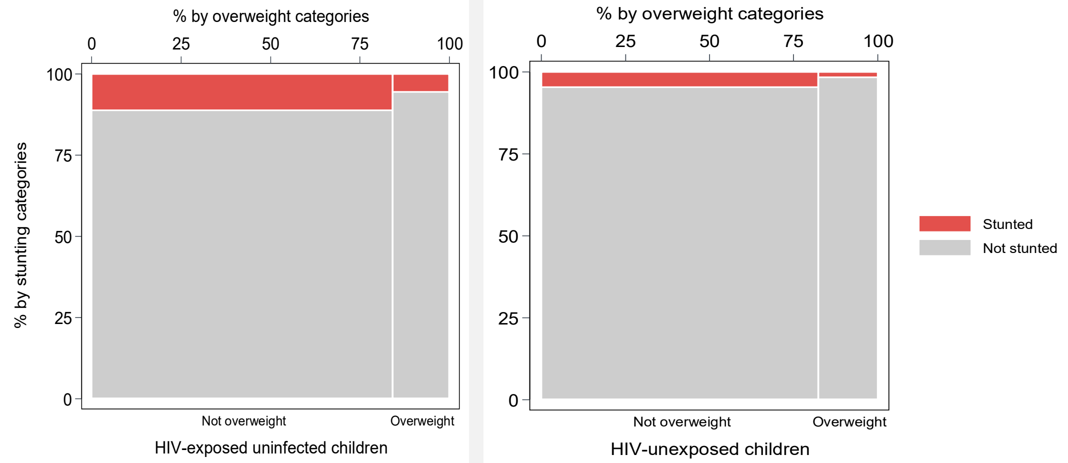
Abbreviations: HEU, HIV-exposed uninfected; HU, HIV-unexposed; OR, odds ratio from logistic regression model; CI, confidence interval; SGA, small-for-gestional age at birth; AGA, appropriate for gestational age at birth

Results are mean (sd) or n(%) from column total (per study visit); proportion of cohort attending each study visit is shown in bold in row with study number; percentages presented with no decimals to faciliate easier reading; Z-scores obtained using the Intergrowth-21st growth standards for postnatal growth of preterm infants until 64 weeks postmenstrual age; World Health Organization MSGR growth standards for term infants and preterm infants after 64 weeks' postmenstrual age; Weight-for-length from WHO standards (adjusted age for preterm infants until 64 weeks' postmenstrual age)

[1] At any time point in follow-up (ante- or postpartum) (missing data, n=114, 16%); [2] Lacks one or more of: formal (brick) housing, running water in home, toilet in home; [3] Risky drinking, defined as Alcohol Use Disorders Identification test for consumption (AUDIT-C) score ≥3 as reported at first antenatal visit; [4] Birth weight < 10th centile for gestational age; [5] < 37 completed weeks' gestation at birth; [6, 7] Maternal self-report, 24 hour recall; [8] Household food insecurity vs. at risk of/does not have food insecurity; using questionnaire adapted from the Household Food Insecurity Access Scale (HFIAS), Food and Nutrition Technical Assistance Project (FANTA) and the Community Childhood Hunger Indentification Project Index (CCHIP) ; [9] Maternal report of any diarrhoea or coughing illness in preceding 2 weeks

Only 2% (11/686) children were wasted at the end of follow-up. The proportion of children who were both overweight and stunted by 12 months was very small (4/686, 1%), with no differences between HEU and HU children (figure 4.3). Results were similar using BMIZ-scores (table 4.2; supplemental table 9.4.3).

Figure 4.3. Distribution of stunting (length-for-age Z-score <-2) within categories of overweight (weight-for-length Z-score >2) at approximately 12 months of age: HIV-exposed uninfected vs. HIV-unexposed children



4.4.4 Head circumference

Between six weeks and 12 months, HCAZ increased slightly in both groups [mean (SD) overall HCAZ 0·74 (1·29) at 6 weeks vs. 1·06 (1·12) at 12 months, supplemental table 9.4.3], with slightly lower HCAZ among HEU than HU children throughout [overall aβ -0·24 (95% CI -0·38; -0·10); figure 4.2D & table 4.2]. Only 1% (5/686) of children were microcephalic (HCAZ<-2) by 12 months (table 4.3); 19% were macrocephalic (HCAZ>2: 56/340, 16% HEU vs 76/341, 22% HU). Macrocephaly was strongly associated with WLZ>2 (p<0·0001, data not shown).

4.4.5 Modification of HIV-exposure-growth relationships by birth size and other factors

Across all measures, SGA predicted lower average Z-scores in both crude and adjusted analyses, with stronger associations noted among HEU than HU children (table 4.4; figure 4.4 A, B, C and D). In turn, associations between HIV-exposure status and child growth differed within categories of birth size (supplemental table 9.4.4). These interaction effects were most notable for linear growth.

While the average linear growth trajectory of HEU-AGA children approximated that of HU-AGA children, the trajectories of HEU-SGA and HU-SGA children differed significantly (figure 4.4B). Between six weeks and six months, LAZ steadily increased among HU-SGA – but not HEU-SGA – children.

Thereafter, all four groups experienced group-level linear growth faltering (declining LAZ), but HEU-SGA children had earlier onset and sharper decline than the other groups (figure 4.4B). By 12 months of age, HEU-AGA vs. HU-AGA differences were of relatively small magnitude [mean LAZ, -0·31 vs. -0·03; aβ -0·27 (95%CI -0·46; -0·09); supplemental table 9.4.4] whereas HEU-SGA vs. HU-SGA differences were significantly larger [mean LAZ, -1·53 vs. -0·53; aβ -1·00 (95%CI -1·53)]. In analysis restricted to those without maternal risky drinking, differences at 12 months were attenuated although trajectories were similar and inferences unchanged for both HEU-AGA vs. HU-AGA [mean LAZ, -0·26 vs. 0·02; aβ -0·28 (95% CI -0·48; -0·08)] and HEU-SGA vs. HU-SGA comparisons [mean LAZ, -1·30 vs. -0·54; aβ -0·75 (95% CI -1·39; -0·12)] after adjusting for socio-economic factors, IPV, feeding, and recent illness (data not shown).

Table 4.4. Differences in summary growth measures comparing small- vs appropriate-for-gestational age children in the first year of life, stratified by maternal HIV status: crude and adjusted mixed effects linear models

	Weight-for-age (WAZ)[1]		**Length-for-age (LAZ)[1]**		**Head circumference-for-age (HCAZ)[1]**		**Weight-for-length (WLZ)[2]**	
	β (95% CI)	**aβ[3] (95% CI)**	**β (95% CI)**	**aβ[3] (95% CI)**	**β (95% CI)**	**aβ[3] (95% CI)**	**β (95% CI)**	**aβ[3] (95% CI)**
SGA vs AGA								
Overall [4]	-1·26 (-1·45; -1·06)	-1·21 (-1·41; -1·01)	-1·04 (-1·26; -0·83)	-1·98 (-1·20; -0·77)	-0·97 (-1·18; -0·75)	-0·96 (-1·18; -0·74)	-0·48 (-0·71; -0·26)	-0·49 (-0·72; -0·26)
HEU only [4]	-1·34 (-1·60; -1·09)	-1·32 (-1·59; -1·05)	-1·24 (-1·52; -0·95)	-1·23 (-1·51; -0·94)	-1·06 (-1·35; -0·78)	-1·13 (-1·42; -0·84)	-0·48 (-0·77; -0·19)	-0·54 (-0·84; -0·23)
HU only [4]	-1·11 (-1·40; -0·82)	-1·10 (-1·40; -0·80)	-0·77 (-1·10; -0·44)	-0·75 (-1·07; -0·42)	-0·81 (-1·13; -0·48)	-0·79 (-1·12; -0·47)	-0·45 (-0·80; -0·09)	-0·45 (-0·79; -0·11)
p-value [5]	0·24		0·04		0·24		0·90	

Abbreviations: CI, confidence interval; HEU, HIV-exposed uninfected children; HU, HIV-unexposed children; SGA, small-for-gestational age (birth weight < 10th centile for gestational age based on Intergrowth-21st reference standards); AGA, appropriate for gestational age; w, weeks; m, months

[1] WAZ, LAZ and HCAZ-scores obtained from Intergrowth-21st (birth data adjusted for gestational age; postnatal data for preterm infants adjusted until 64 weeks postmenstrual age) and World Health Organization growth reference standards (postnatal growth for term infants, and for preterm infants after 64 weeks postmenstrual age)

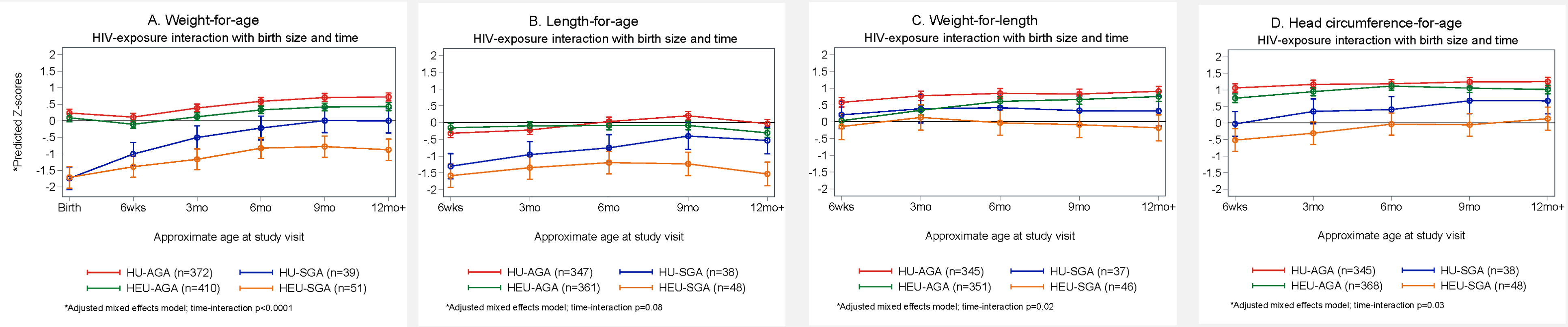
[2] WLZ-scores obtained from World Health Organization growth reference standards (using gestation-adjusted age for preterm infants until 64 weeks' postmenstrual age)

[3] Adjusted for socio-economic factors (marital status, lack of amenities, household crowding), maternal alcohol use, intimate partner violence, preterm birth, and recent history of any childhood illness (maternal report of diarrhoea and/or cough in preceding two weeks), and infant feeding (current milk feeding and weaning practices)

[4] Overall, N= 872; HEU, n=461; HU, n=411

[5] For SGA-HIV exposure status interaction term in crude model without time

Figure 4.4. Interaction between HIV exposure and birth size over time: Z-score growth trajectories in the first 12 months of life comparing HIV-exposed uninfected and HIV-unexposed children



HEU= HIV-exposed uninfected; HU = HIV-unexposed; SGA = small-for-gestational age (birthweight<10th percentile for gestation); AGA = appropriate for gestational age

Longer duration of breastfeeding (≥6 vs. <6 months) was associated with slightly lower WLZ among HEU (but not HU) children [at 12 months: WLZ β -0·31 (95% CI -0·60; -0·04); interaction *p*-value=0·06]. There was no effect of breastfeeding on prevalence of overweight (WLZ>2) and no significant differences by breastfeeding duration in other growth indices. There were also no significant interaction effects between HIV-exposure status and infant sex or preterm delivery on any of the growth indices in this cohort.

Results of sensitivity analyses approximated those of the main analysis for all measures (supplemental table 9.4.5). Maternal, birth and infant factors associated with WAZ, LAZ, WLZ, HCAZ and BMIZ trajectories among HEU children are shown in supplemental table 9.4.6.

4.5 Discussion

In the context of universal ART and breastfeeding, we found small but consistent deficits in the early growth trajectories of young, breastfed HEU compared to HU children drawn from the same peri-urban community. Prevalence of undernutrition at 12 months of age was low, with linear growth faltering more common than underweight, wasting or microcephaly. On this background, the small absolute differences in LAZ – but not WAZ or WLZ - were associated with a significantly increased proportion of HEU (vs. HU) children being stunted by the end of follow-up. Simultaneously, a high proportion of children in both groups were already overweight by 12 months of age. That is, in this cohort of young, HIV-uninfected, breastfed South African infants, we describe moderately different growth patterns by maternal HIV status and a very early onset of the double burden of malnutrition that is increasingly the norm in sub-Saharan Africa.[2]

Our findings of similar weight and proportional size among young, breastfed HEU and HU children are reassuring given the uniform exposure to maternal ART *in utero* and throughout breastfeeding. These results approximate those of other prospective, peri-urban South African studies predating universal maternal ART.[3,4,9] In comparison, research from other countries in SSA predating universal maternal ART have reported increased risks of underweight and wasting among HEU compared to HU children.[3,4,7,12] The latter reports were however largely from rural settings with high background prevalence of undernutrition, advanced maternal disease, or were based on cross-sectional studies at risk of selection bias. Nonetheless, the increased risk of stunting noted among HEU children in our study population during late infancy aligns with most other studies of HEU child growth in resource-limited settings, although the overall prevalence and severity of stunting was significantly less in our study than in previous reports.[3,4,7,11,12] Even with a 6% absolute increased risk, our observed 12 month HEU prevalence of stunting (10%) was lower than the general prevalence elsewhere in South Africa, where the most recent national estimates are 31% at this age.[23] Stunting is typically a process indicative of chronic malnutrition and closely associated with poverty.[24] In our study, both cohorts lived in socio-economically challenged situations with evidence of food insecurity, and both groups of children experienced linear growth faltering from 6-9 months, around the time of introduction to complementary foods. Although adjustment for infant feeding did not substantially change the findings, it is possible that the more marked linear growth faltering among HEU children was related to earlier discontinuation of breastfeeding, as has been reported in other studies.[11] Indeed, breastfeeding durations were concerningly short, particularly among HEU children. Our data therefore highlight an ongoing need for improved breastfeeding promotion and support in this context.

While we report largely reassuring data for comparative growth of HEU and HU children, our early childhood obesity findings suggest a looming public health crisis. Rapid weight gain in infancy, demonstrated in almost half of our cohort, has been linked to increased risk for overweight and obesity through childhood and adolescence.[22,25] In South Africa, the overweight prevalence among children under-five around 13%.[2,23] Our findings of almost 20% overweight by the age of one year in this peri-urban setting provides evidence of a worsening childhood overweight/obesity epidemic, as has been documented in older children and adolescents across SSA. While South Africa has a particularly high burden of obesity across the lifespan,[23] other countries in the region have recently shown similar obesity trends, with an estimated 11% of under-five children overweight in Botswana, 9% in Sierra Leone and 8% in Mozambique.[2] Childhood obesity predicts adult obesity, with multiple adverse metabolic health effects including dyslipidemia and insulin resistance.[26] However, HEU children may be at increased risk for dyslipidemia and altered energy metabolism, regardless of obesity.[27,28] Whether the obesity-related metabolic effects may be exacerbated by concurrent perinatal ART exposure warrants investigation, as the obesity epidemic grows across high HIV prevalence settings.[2]

Our data also highlight the critical influence of antenatal factors on child health. Specifically, we demonstrate a potential interaction effect between infant HIV-exposure and intra-uterine growth restriction (IUGR) on linear growth. Although linear growth faltering occurred across the cohort, HEU-SGA infants were at highest risk. We previously reported a similar interaction between HIV-exposure and PTD on neurodevelopment and hypothesize a potential role for cytomegalovirus (CMV) in this context.[16,29] Indeed, elucidation of underlying causal mechanisms will be critical for the design of HIV-specific interventions for this patient population going forward. Additionally, substantive evidence already exists for interventions to improve perinatal maternal-infant nutrition across settings; [30] urgent implementation of these strategies is likely to benefit both HEU and HU children.

Our findings should be interpreted in the light of several limitations and strengths. Single-site data from peri-urban South Africa may not be generalizable to rural areas or settings with different background prevalence of malnutrition. Our findings may also not extend to infants conceived on ART. Although loss to follow-up may predispose to selection bias, there were no substantial differences by completeness of follow-up, with unchanged results in complete-case analysis. Reliance on primary care nurse measures of birth anthropometry prevented assessment of IUGR symmetry, due to lack of robust estimates of length and head circumference. Unmeasured confounding is always a concern with observational research; however, potential confounding was minimized through both study design and analysis. Notably, we could account for many socio-behavioural factors, to date often neglected in HEU child research. Our cohort of breastfed HEU children universally exposed to maternal ART – with homogenous ART exposures – is one of the first to be reported since the widespread implementation of new WHO guidelines. Other study strengths include robust gestational age estimates; and repeated anthropometric measures over time, critical when assessing a dynamic process such as childhood growth.

4.6 Conclusion

In the context of universal maternal ART and breastfeeding, growth trajectories of young HEU children approximated those of otherwise similar HU. Linear growth faltering was common in both groups, particularly among HEU children with evidence of intra-uterine growth restriction. Concerningly, almost 20% of all children were already overweight by one year of age. The long-term effects of early onset overweight and stunting in children exposed to HIV and ART require further investigation.

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4.7.3 Contributors

SLR (corresponding author) was responsible for conceptualisation and implementation of the HU2 study; assisted with collection of data; conducted the analyses; wrote the first draft of the manuscript and confirms that she had full access to all the data and takes final responsibility for the decision to submit for publication. LM and EJA conceived the MCH-ART and HU2 studies, and were responsible for study design, funding, implementation, and overall leadership. KD and MK provided supervision for child health aspects of the study. KB and TKP were responsible for data management and oversight. TKP and KN were the study coordinators. AZ was the senior study manager and provided oversight of all study administration processes. All authors contributed to and approved the final manuscript.

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Chapter 5

Infectious morbidity of breastfed, HIV-exposed uninfected infants under conditions of universal antiretroviral therapy in South Africa: a prospective study

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Relevance of this paper to the thesis

This chapter is the second of the four primary results chapters, addressing one of the three main clinical outcomes of interest, as defined in chapter 1. It builds on the findings of chapter 3, further linking evidence of growth faltering and infectious disease among children in the context of adverse socio-economic settings. The paper addresses study objectives 3 (a) to (c): using multiple, complementary analytic approaches, the results from this publication demonstrate transiently increased risk of infectious morbidity among young HIV-exposed uninfected (HEU) compared to HIV-unexposed (HU) children.

Contribution of the student and co-authors

The candidate was a principal investigator for the HU2 study (source population for HU study participants), under the guidance of LM. In addition, she was co-investigator and lead researcher for all infant-related aspects of the MCH-ART study (source population for HEU study participants). In both studies, the candidate designed and implemented the infectious morbidity data capturing forms, collated, and cleaned data exports from the provincial health database, and provided oversight for quality assurance of infectious morbidity measures. In addition, she provided clinical support for children presenting to study visits with intercurrent illness. The candidate conducted all the analyses presented in this chapter and wrote the first draft of the manuscript. LM and EJA conceived the MCH-ART study, and were responsible for study design, funding, implementation, and overall leadership. KD and MK provided supervision for child health aspects of the study. DLR was responsible for blinded reviews of all verbal autopsy reports and morbidity assessments. KB and TKP were responsible for data management and oversight. TKP and KN were the study coordinators. AZ was the senior study manager and provided oversight of all study administration processes. All authors contributed to and approved the final manuscript.

5.1 Abstract

5.1.1 Background

Without breastfeeding and maternal antiretroviral therapy (ART), HIV-exposed uninfected (HEU) infants experience greater infectious morbidity than HIV-unexposed (HU) infants. We hypothesised that with the introduction of universal maternal ART, breastfed HEU and HU infants would have similar morbidity.

5.1.2 Methods

We prospectively studied a cohort of HIV-infected pregnant women initiating ART, and a parallel group of HIV-uninfected pregnant women, starting from their first antenatal care visit at the Gugulethu Midwife Obstetrics Unit in Cape Town, South Africa. All pregnant women attending their first antenatal care visit were eligible for enrolment if aged 18 years or older and planning to delivery in Cape Town, without gestational age restrictions. HIV-infected women were participants of the Maternal Child Health ART (MCH-ART) study, and HIV-uninfected women were participants of the HIV-Unexposed Uninfected (HU2) study. All enrolled women were followed up during pregnancy and through delivery. At the early neonatal visit (scheduled for the first week after birth), mother-infant pairs who practiced any breastfeeding in the first 7 days of life were eligible for further postnatal follow-up for at least 12 months postpartum. HIV infection was excluded among HEU infants at ages 6 weeks and 23 months by PCR. We evaluated the effect of HIV exposure on two primary outcomes: hospitalisation (all-cause and infection-related admission to hospital) and longitudinal prevalence of child infectious illness (diarrhoea and presumed lower respiratory tract infection [LRTI]). Hospitalisation data were abstracted from routine health records. Crude and adjusted incidence rate ratios (aIRR; with adjustment for maternal HIV disease severity, timing of ART initiation, breastfeeding, timely vaccination, and birth outcomes [gestational size and age]) for infection-related hospitalizations were calculated from Poisson regression models (with variance corrected for clustering). Prevalence of infant infectious illness was based on maternal self-report for the preceding 2 weeks of each visit, with questions based on Demographic and Health Survey (DHS) questionnaires. Infants who acquired HIV infection during follow-up were excluded from this analysis. MCH-ART is registered on ClinicalTrials.gov, NCT01933477.

5.1.3 Findings

Pregnant women were recruited between March 20, 2013, and Aug 19, 2015. Mother-infant pairs (HEU, n=459; HU, n=410) were followed up for a median of 12 months until March 24, 2017. Compared with HU infants, HEU infants had more infection-related hospitalisations between the age

of 8 days and 3 months (HEU, 34.2 admissions per 100 child-years [24.4-47.9] vs 9.8 per 100 child-years [95% CI 5.1-18.8]; IRR 3.50 [95% CI 1.68-7.30]), but rates were similar at other ages. In infants aged 8 days to 3 months, infection-related hospitalisations for HEU infants with healthier mothers (n=84; ART initiation at < 24 weeks' gestation, CD4 cell count > 350 cells/uL, HIV viral load < 4.0 $\log_{10}$ copies/mL: 15.88 admissions per 100 child-years [5.12-49.23]) approximated those of HU infants (9.77 per 100 child-years [5.08-18.78]; aIRR 1.28 [0.27-6.05]). HEU infants of mothers with late ART initiation (at ≥ 24 weeks' gestation) and advanced disease (CD4 count ≤ 350 cells/uL and HIV viral load ≥ 4.0 $\log_{10}$ copies/mL; n=44) had the highest admission rate (40.44 per 100 child-years [15.8-107.74]); aIRR 5.01 [1.50-16.71]). In this age group, reduced admissions were seen in HEU infants with optimal breastfeeding (initiated within 1 h of birth and exclusive through age 3 months) and timely vaccinations (required doses received within 2 weeks of indicated age; n=90; 9.63 admissions per 100 child-years [2.41-38.49]). Between birth and age 6 months, HEU infants had an almost five times greater prevalence of LRTIs than HU infants (aPR 4.69 [2.40-9.17]), and a three-times greater prevalence of diarrhoeal illness (aPR 2.93 [1.70-5.07]). After age 6 months, these associations were ameliorated.

5.1.4 Interpretation

Despite ART in pregnancy, breastfed HEU infants versus breastfed HU infants had transiently increased infectious morbidity risks in early infancy. However, differences were driven by factors potentially amenable to intervention, including delayed diagnosis and ART initiation in HIV-positive mothers, and suboptimal breastfeeding and vaccination of their infants.

5.1.5 Funding

US National Institute of Child Health and Human Development, Elizabeth Glaser Pediatric AIDS Foundation, South African Medical Research Council, Fogarty Foundation and the Office of AIDS research.

5.2 Introduction

An estimated one million HIV-exposed uninfected (HEU) infants are born in sub-Saharan Africa annually.[1] Historically, HEU infants have had higher risks of infectious morbidity than HIV-unexposed (HU) infants, hypothesized to be driven by a range of biological and socio-economic mechanisms.[2-5] These may include (1) an altered fetal immunological milieu, the result of maternal immune dysregulation directly caused by replicating HIV virus and/or maternal co-infections, (2) reduced transplacental transfer of maternal antibodies, (3) increased household infectious burden due to high maternal susceptibility to infectious illness, (4) reduced household socio-economic circumstances in turn associated with higher risks of childhood malnutrition and suboptimal child care, and (5) avoidance of breastfeeding to minimize perinatal HIV transmission.[2-4]

However, evidence for these associations and mechanisms are based predominantly on data pre-dating the current standard of care for HIV-infected women and their infants in sub-Saharan Africa.[5] The past decade has seen a dramatic shift towards universal maternal ART and the promotion of early, exclusive breastfeeding extended with appropriate complementary feeds through at least one year of age.[6,7] These policy changes have the potential to ameliorate several of the factors hypothesised to drive the infectious morbidity risk differential between HEU and HU infants. Specifically, maternal ART substantially improves maternal health and survival,[8] in turn addressing factors (1-4) above, while the protective benefits of breastfeeding against infectious morbidity are well established.[9] We therefore hypothesised that, under currently promoted policies of universal maternal ART with breastfeeding, the infectious morbidity risks of HEU infants may approximate those of otherwise similar HU infants. To test this hypothesis, we examined rates of infection-related hospitalisations and longitudinal prevalence of infectious illness, comparing breastfed HEU infants born to women initiating universal ART in pregnancy versus breastfed HU infants in Cape Town, South Africa.

5.3 Methods

We prospectively enrolled HIV-positive pregnant women initiating universal ART and a parallel cohort of HIV-negative pregnant women from the same community.[10] Study activities were based at the Gugulethu Midwife Obstetric Unit (MOU), which provides primary-level obstetric care (including prevention of mother-to-child HIV transmission services) to a predominantly low-income, urban population of roughly 350 000 (30% antenatal HIV prevalence).[11] Local clinics provide child health care (vaccinations include Bacille Calmette-Guerin (birth); rotavirus (6 and 14 weeks); and pneumococcal conjugate vaccines (6, 10 and 14 weeks)).[12] The area has a high infant mortality rate (15·6 per 1000 live births at the time of the study).[13]

At first antenatal care (ANC) visit, eligible pregnant women (≥18 years of age, planning to deliver in Cape Town, any gestation) were screened for enrolment into the Maternal Child Health Antiretroviral Therapy (MCH-ART; HIV-infected women, enrolment March 2013-June 2014), and HIV-Unexposed Uninfected studies (HU2; a sub-study of MCH-ART for HIV-negative women, enrolment September 2014-August 2015).[10] All HIV-infected women initiated lifelong ART at first ANC visit without CD4 or gestational age restrictions. The delivery unit and referral hospitals are certified baby-friendly; breastfeeding is promoted as infant feeding of choice for HIV-positive mothers on ART. The HU2 study was conceived and designed specifically to complement MCH-ART, by providing a control cohort of HIV-negative mothers and breastfeeding HU infants sampled from the same community. Both studies utilised the same staff, study approaches and, apart from HIV-specific measures, measurement tools and procedures, as described elsewhere.[14] Women were followed through pregnancy to delivery. At the neonatal study visit (scheduled within seven days after birth), breastfeeding mother-child pairs were eligible for further postnatal follow-up (visits at six weeks, and three-monthly from three to 12 months; a subset of MCH-ART participants returned for an additional visit at 18 months).

All women provided written informed consent. Both the MCH-ART and HU2 studies were approved by the Human Research Ethics Committee of the University of Cape Town. In addition, MCH-ART was approved by the Columbia University Medical Centre Institutional Review Board and is registered on Clinicaltrials.gov (NCT01933477).

5.3.1 Measurements

We used standardised questionnaires, administered at study visits by trained field workers, to measure maternal health, psychosocial and behavioural factors; the same questionnaires were administered to both groups of women.[14] Feeding was primarily assessed with 24-hour maternal recall; last study visit with report of breastfeeding was used as date of breastfeeding cessation.[15] Duration of exclusive breastfeeding (EBF, defined as consistently receiving only breastmilk and prescribed medicine) was calculated using (1) date of last study visit with report of EBF and (2) maternal report of the age at which non-breastmilk liquids or solids were introduced. Prevalence of infant infectious illness in the preceding two weeks was based on maternal self-report, using questions based on Demographic & Health Survey (DHS) questionnaires. Presumed lower respiratory tract infection (LRTI) was defined as maternal report of cough plus both fever and difficulty in breathing; diarrhoeal illness, as increased or loose stools. Immunisation data were abstracted from patient-held records (Road to Health booklets); delayed vaccination was defined as > two weeks after recommended age.[16] In the event of a child death, study staff compiled all available information on possible causes including, where possible, copies of death certificates, medical records and autopsy reports, and administered the World Health Organization verbal autopsy tool to willing mothers.[17]

Hospitalisation data were obtained from a centralised provincial health database, with diagnoses based on ICD-10 coding from hospital discharge summaries.[18] Admissions with same-day discharge were excluded from analysis as ambulatory events; interhospital transfers counted as a single admission. Where multiple infectious diagnoses were listed, the most life-threatening or severe infection was allocated as primary infectious cause.[19] An independent paediatrician, blinded to HIV exposure status, reviewed all hospital diagnoses to adjudicate whether the primary cause for admission was infection-related vs non-infection-related; the same pediatrician reviewed all available mortality information to allocate a final cause of death for study purposes.

HIV infection was excluded among HEU infants at six and 48 weeks of age (HIV-PCR: Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 qualitative assay; Roche Molecular systems, Branchburg, NJ.[10] Final HIV status for infants censored before 48 weeks reflected 6 weeks' results. Previously HIV-negative women received regular HIV counselling and testing throughout follow-up; where seroconversion had occurred, HIV infection was excluded in the infants. Infants who acquired HIV infection at any time during follow-up were excluded from this analysis.

5.3.2 Statistical methodology

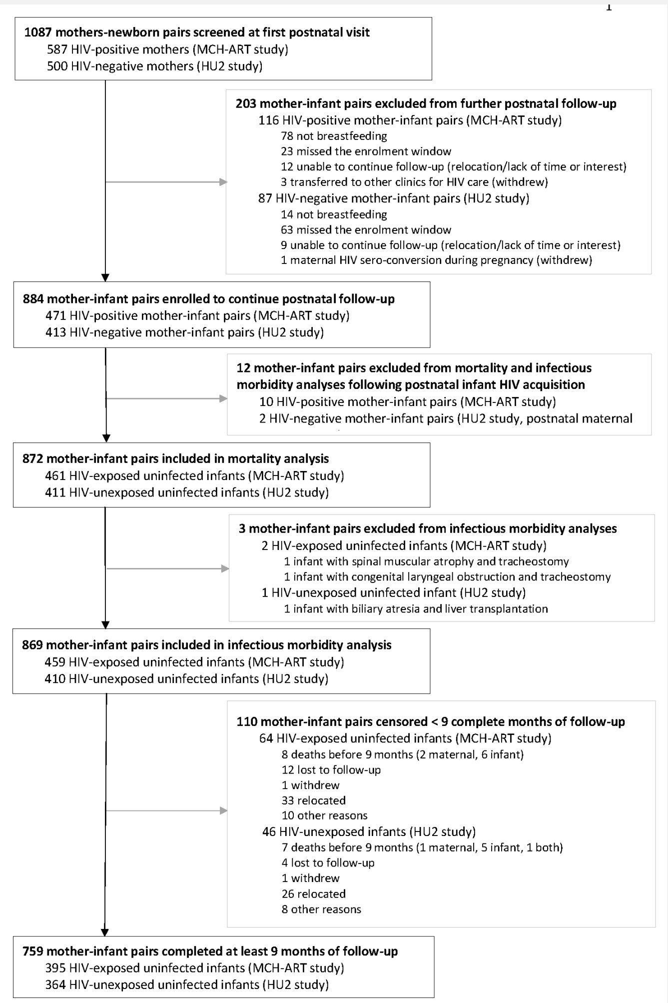
Sample size was calculated for differences in prevalence of infectious illness. Assuming an underlying risk of diarrhoea among HU infants of 0·15,[13] a sample size of 880 (440:440) would provide 84% power to detect ≥0·08 absolute difference in risk, and 80% power to detect a relative difference of at least 1·5 at α=0·05.

We evaluated the effect of HIV exposure on two primary outcomes: hospitalisation (all-cause, and infection-related) and prevalence of child infectious illness. Analysis was restricted to singleton or first-born twins. Hospitalisation rates (admissions per 100 child-years, cy) were compared with crude and adjusted incidence rate ratios (IRR and aIRR, respectively) from Poisson regression (sandwich variance estimator to account for clustering).[20] Overall person-time was from date of birth, until censoring at (1) date of death; (2) final study visit, if no hospitalisations within 3 months thereafter; or (3) day of hospital discharge if admitted within 3 months of final study visit. Following a prespecified analysis plan, we generated age-stratified rates in 3-monthly intervals. To differentiate early neonatal from later admissions, we further divided the 0-3 months' interval into 0-7 days and >7 days to 3 months. Prevalence of infectious illness was compared with crude and adjusted prevalence ratios (PR and aPR, respectively), from modified Poisson models (population-averaged models with sandwich variance estimator).[21] Third variables were chosen *a priori* based on a directed acyclic graph (supplemental figure 9.5.1). "Optimal" breastfeeding was defined has (1) early initiation of breastfeeding (< hour of birth), with (2) EBF at all attended visits before age five months and (3) breastfeeding continued throughout follow-up. Undernutrition was accounted for using time-varying weight-for-age Z-scores (underweight: WAZ<-2); lack of robust birth length measures precluded adjustment for stunting and wasting. Potential effect modifiers included maternal HIV disease severity, timing of ART initiation, breastfeeding, vaccination, and birth outcomes (gestation and size at birth). Where multiple measures were available for a single construct (e.g. socio-economic measures), variable choice followed best model fit (Akaike's Information Criterion). We conducted sensitivity analyses to assess robustness of findings within strata of child characteristics known to be associated with infectious morbidity. Missing data were assumed to be missing at random; we used a missing indicator variable for variables with >10% missing data and complete case analysis otherwise. Analyses used Stata 14·0 (Statacorp, College Station, TX, USA). Results are presented with 95% confidence intervals (CI), and *p*-values are two-sided.

5.3.3 *Role of the funding source*

The study sponsors had no role in study design, data collection, analysis, interpretation or writing of the report. The corresponding author had full access to all study data and final responsibility for the decision to submit for publication.

Figure 5.1. Study flow diagram



5.4 Results

Of 1087 mother-newborn pairs screened at the neonatal visit (median age 5 days, interquartile range, IQR 4-8), 884 (471 born to HIV-positive, 413 to HIV-negative, mothers) were enrolled for postnatal follow-up (figure 5.1) Fifteen infants were excluded from analysis, including 12 who acquired HIV and three with underlying congenital diseases predisposing to recurrent admissions and infections (figure 5.1). The median follow-up was 12 months [IQR 9-16]; 395 [86%] of 459 HEU and 364 (89%) of 410 HU infants completed ≥ nine months of follow-up. There were minor differences between these infants and those censored before nine months (supplemental table 9.5.1).

Maternal and child characteristics are shown in supplemental table 9.5.2. In general, HIV-positive women (at ART initiation, median [IQR] $\log_{10}$ HIV viral load, HIV-VL 4·0 [3·3-4·5]; CD4 cells/uL, 354 [248-528]; gestation, 22 weeks [17-28]) experienced more economic, psychosocial, and behavioural risk factors than HIV-negative women. Proportions of preterm (HEU 12% vs HU 9%, p=0·17) and small-for-gestational age (11% vs 10%, p=0·45) births were similar. Most HEU infants received co-trimoxazole preventive therapy (CPT, 238 [69%] of 347 with data on usage). HEU (vs HU) infants had shorter overall durations of breastfeeding (median 3·9 vs 9·0 months, p=0·001) but were more likely to ever have exclusively breastfed (91% vs 81%, p<0·0001). Overall, only 122 [12%] of 869 children had received optimal breastfeeding (supplemental table 9.5.2).

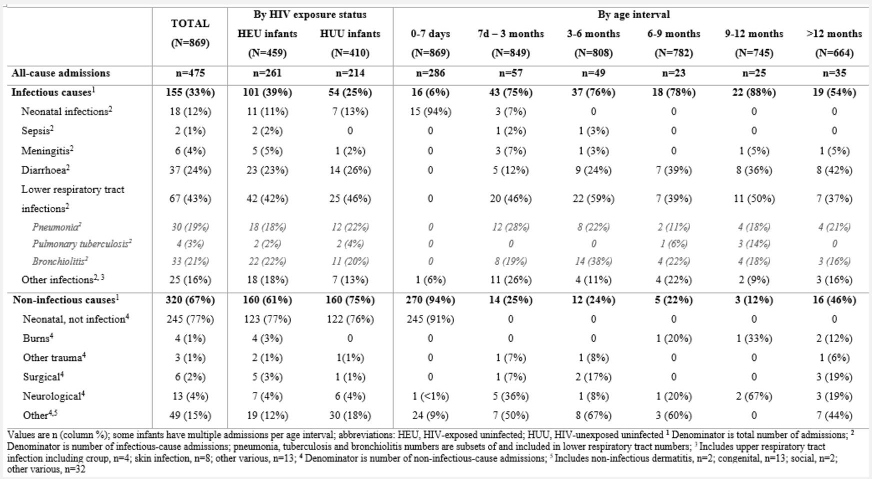
Time-varying child characteristics are shown per visit in supplemental table 9.5.3. By 12 months, only 144 (41%) of 355 HEU and 174 (50%) of 346 HU infants were still breastfeeding; 171 (52%) of 328 HEU and 107 (37%) of 286 HU infants with known vaccination status had had all vaccinations at the correct ages.

5.4.1 Mortality

Fourteen (1·6% of 872) infants died in the first 12 months (2·51 deaths per child-year), with no difference between HEU (8/461, 1·7% or 2·48 per child-year) and HU infants (6/411, 1·5% or 2·54 per child-year; mortality IRR 0·98, 95% CI 0·30-3·41). There were four deaths due to infectious causes: two were due to diarrhoea (both HEU, 5 and 10 months old respectively), one bacterial sepsis (HEU, 1 month old) and one multi-drug resistant tuberculosis (HU, 11 months old), supplemental table 9.5.4.

5.4.2 Incidence of all-cause hospitalisation

Among 869 infants (933 child-years of follow-up), 378 experienced 475 hospital admissions (table 5.1); 310 infants had one, while 68 infants had multiple admissions (HEU: 43/459, 9% vs HU: 25/410, 6%). In both

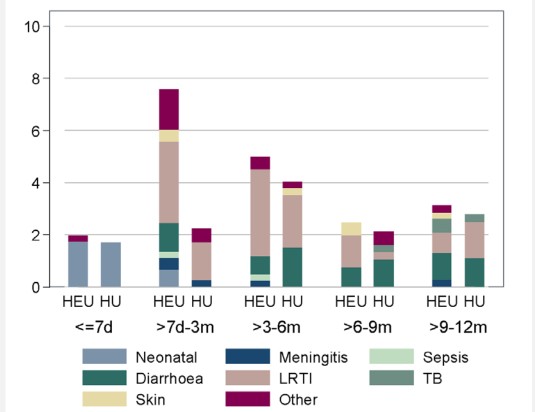


	TOTAL (N=869)	By HIV exposure status		By age interval					
		HEU infants (N=459)	HUU infants (N=410)	0-7 days (N=869)	7d – 3 months (N=849)	3-6 months (N=808)	6-9 months (N=782)	9-12 months (N=745)	>12 months (N=664)
All-cause admissions	**n=475**	**n=261**	**n=214**	**n=286**	**n=57**	**n=49**	**n=23**	**n=25**	**n=35**
Infectious causes[1]	**155 (33%)**	**101 (39%)**	**54 (25%)**	**16 (6%)**	**43 (75%)**	**37 (76%)**	**18 (78%)**	**22 (88%)**	**19 (54%)**
Neonatal infections[2]	18 (12%)	11 (11%)	7 (13%)	15 (94%)	3 (7%)	0	0	0	0
Sepsis[2]	2 (1%)	2 (2%)	0	0	1 (2%)	1 (3%)	0	0	0
Meningitis[2]	6 (4%)	5 (5%)	1 (2%)	0	3 (7%)	1 (3%)	0	1 (5%)	1 (5%)
Diarrhoea[2]	37 (24%)	23 (23%)	14 (26%)	0	5 (12%)	9 (24%)	7 (39%)	8 (36%)	8 (42%)
Lower respiratory tract infections[2]	67 (43%)	42 (42%)	25 (46%)	0	20 (46%)	22 (59%)	7 (39%)	11 (50%)	7 (37%)
Pneumonia[2]	*30 (19%)*	*18 (18%)*	*12 (22%)*	*0*	*12 (28%)*	*8 (22%)*	*2 (11%)*	*4 (18%)*	*4 (21%)*
Pulmonary tuberculosis[2]	*4 (3%)*	*2 (2%)*	*2 (4%)*	*0*	*0*	*0*	*1 (6%)*	*3 (14%)*	*0*
Bronchiolitis[2]	*33 (21%)*	*22 (22%)*	*11 (20%)*	*0*	*8 (19%)*	*14 (38%)*	*4 (22%)*	*4 (18%)*	*3 (16%)*
Other infections[2, 3]	25 (16%)	18 (18%)	7 (13%)	1 (6%)	11 (26%)	4 (11%)	4 (22%)	2 (9%)	3 (16%)
Non-infectious causes[1]	**320 (67%)**	**160 (61%)**	**160 (75%)**	**270 (94%)**	**14 (25%)**	**12 (24%)**	**5 (22%)**	**3 (12%)**	**16 (46%)**
Neonatal, not infection[4]	245 (77%)	123 (77%)	122 (76%)	245 (91%)	0	0	0	0	0
Burns[4]	4 (1%)	4 (3%)	0	0	0	0	1 (20%)	1 (33%)	2 (12%)
Other trauma[4]	3 (1%)	2 (1%)	1(1%)	0	1 (7%)	1 (8%)	0	0	1 (6%)
Surgical[4]	6 (2%)	5 (3%)	1 (1%)	0	1 (7%)	2 (17%)	0	0	3 (19%)
Neurological[4]	13 (4%)	7 (4%)	6 (4%)	1 (<1%)	5 (36%)	1 (8%)	1 (20%)	2 (67%)	3 (19%)
Other[4,5]	49 (15%)	19 (12%)	30 (18%)	24 (9%)	7 (50%)	8 (67%)	3 (60%)	0	7 (44%)

Values are n (column %); some infants have multiple admissions per age interval; abbreviations: HEU, HIV-exposed uninfected; HUU, HIV-unexposed uninfected [1] Denominator is total number of admissions; [2] Denominator is number of infectious-cause admissions; pneumonia, tuberculosis and bronchiolitis numbers are subsets of and included in lower respiratory tract numbers; [3] Includes upper respiratory tract infection including croup, n=4; skin infection, n=8; other various, n=13; [4] Denominator is number of non-infectious-cause admissions; [5] Includes non-infectious dermatitis, n=2; congenital, n=13; social, n=2; other various, n=32

Table 5.1. Distribution of primary cause of hospitalization by HIV exposure status and age interval

groups, the commonest diagnosis was non-infectious neonatal illness (245/475, 52%; figure 5.2 & table 5.1). Average all-cause hospitalisation rates were similar for HEU and HU infants (IRR 0·91, 95% CI 0·76-1·10, table 5.3). Peak all-cause hospitalisation rates were in the first 7 days of life (similar rates for HEU and HU infants). Between 7 days and 3 months of life, rates decreased more slowly among HEU than HU infants, resulting in higher incidence among the former (IRR 2·85, 95% CI 1·56-5·20). After 3 months of age, all-cause hospitalisation rates remained low for both HEU and HU infants.



Legend: HEU, HIV-exposed uninfected; HU, HIV-unexposed uninfected; d, days; m, months; LRTI, lower respiratory tract infection (includes pneumonia and bronchiolitis); TB, pulmonary tuberculosis; Percentages based on number of admissions divided by number of children in category

Figure 5.2. Infection-related causes for hospitalization: distribution by HIV exposure and age category

Sixteen (3%) of 475 all-cause hospitalisations resulted in an intensive care unit admission or in-hospital child death (supplemental table 9.5.5) This proportion was slightly higher among HEU (12 [5%] of 261 admissions) than HU infants (4 [2%] of 214; crude OR 2·53, 95% CI 0·81-7·95) but associated with similar causes (supplemental table 9.5.6).

5.4.3 Incidence of infection-related hospitalisation

Table 5.2. All-cause and infectious-cause hospitalizations comparing HIV-exposed uninfected to HIV-unexposed children: crude incidence rates and incidence rate ratios per age interval

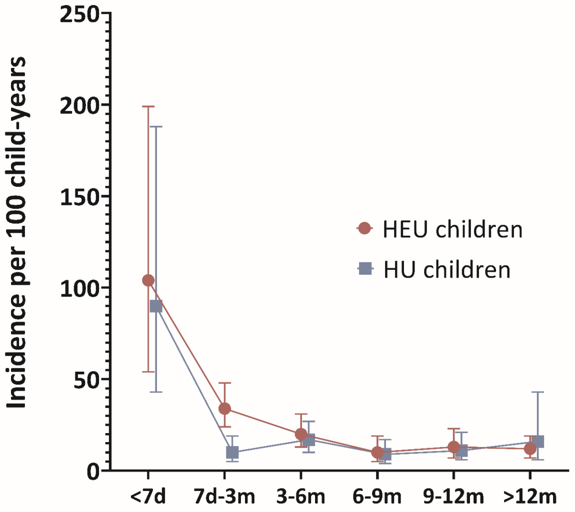
	ALL-CAUSE [1]				INFECTIOUS ILLNESS [2]			
	All children N=869	**HEU children N=459**	**HU children N=410**	**IRR (95% CI) HEU vs HU children[3]**	**All children N=869**	**HEU children N=459**	**HU children N=410**	**IRR (95% CI) HEU vs HU children[3]**
Overall, n	**869**	**459**	**410**		**869**	**459**	**410**	
Admissions	475	261	214	0·91	155	101	54	1·40
Person-time (cy)	932·6	533·2	399·4	(0·76-1·10)	932·6	533·2	399·4	(0·97-2·02)
Incidence/100 cy (95% CI)	50·9 (46·5-55·3)	48·9 (43·3-55·3)	53·6 (46·9-61·3)		16·6 (14·2-19·4)	18·9 (15·6-23·0)	13·5 (10·3-17·7)	
0 to 7 days, n	**869**	**459**	**410**		**869**	**459**	**410**	
Admissions	286	136	150	0·82	16	9	7	1·16
Person-time (cy)	16·5	8·7	7·8	(0·67-0·99)	16·5	8·7	7·8	(0·43-3·11)
Incidence/100cy (95% CI)	1734·5 (1544·7-1947·6)	1566·8 (1324·4-1853·6)	1920·8 (1636·8-2254·2)		97·0 (59·4-158·4)	103·7 (53·9-199·3)	89·6 (42·7-188·0)	
>7 days to 3 months, n	**849**	**444**	**405**		**849**	**444**	**405**	
Admissions	57	43	14	2·85	43	34	9	3·50
Person-time (cy)	191·5	99·4	92·1	(1·56-5·20)	191·5	99·4	92·1	(1·68-7·30)
Incidence/100 cy (95% CI)	29·8 (23·0-38·6)	43·4 (32·1-58·3)	15·2 (9·0-25·7)		22·4 (16·6-30·3)	34·2 (24·4-47·9)	9·8 (5·1-18·8)	
>3 to 6 months, n	**808**	**415**	**393**		**808**	**415**	**393**	
Admissions	49	29	20	1·34	37	21	16	1·21
Person-time (cy)	198·3	103·1	95·2	(0·68-2·63)	198·3	103·1	95·2	(0·63-2·32)
Incidence/100cy (95% CI)	24·7 (18·7-32·7)	28·1 (19·5-40·5)	21·0 (13·6-32·6)		18·7 (13·5-25·8)	20·4 (13·3-31·2)	16·8 (10·3-27·4)	
> 6 to 9 months, n	**782**	**405**	**377**	1·01	**782**	**405**	**377**	1·16
Admissions	23	12	11	(0·45-2·26)	18	10	8	(0·46-2·93)
Person-time (cy)	191·1	99·2	91·9		191·1	99·2	91·9	
Incidence/100cy (95% CI)	12·0 (8·0-18·1)	12·1 (6·9-21·3)	12·0 (6·6-21·6)		9·4 (5·9-15·0)	10·1 (5·4-18·7)	8·7 (4·3-17·4)	
>9 to 12 months, n	**745**	**384**	**361**		**745**	**384**	**361**	
Admissions	25	14	11	1·22	22	12	10	1·15
Person-time (cy)	179·8	91·9	87·9	(0·56-2·65)	179·8	91·9	87·9	(0·49-2·65)
Incidence/100 cy (95% CI)	13·9 (9·4-20·6)	15·2 (9·0-25·7)	12·5 (6·9-22·6)		12·2 (8·1-18·6)	13·1 (7·4-23·0)	11·4 (6·1-21·2)	
> 12 months, n	**664**	**335**	**329**		**664**	**335**	**329**	
Admissions	35	28	8	0·64	19	15	4	0·71
Person-time (cy)	155·5	130·9	24·6	(0·27-1·49)	155·5	130·9	24·6	(0·23-2·13)
Incidence/100cy (95% CI)	22·5 (16·3-31·3)	20·6 (14·1-30·1)	32·5 (16·2-64·9)		12·2 (7·8-19·1)	11·5 (6·9-19·0)	16·2 (6·1-43·3)	

Abbreviations: IRR, incidence rate ratio; CI, confidence interval; cy, child-years

[1] Hospitalized for at least 2 days, all primary diagnoses; [2] Hospitalization for at least 2 days, primary diagnosis infectious in origin; [3] Overall IRR from crude Poisson regression analysis with variance corrected for clustering by child; IRR per age category from crude regression analysis restricted by age intervals without variance correction

Approximately one-third of all hospital admissions were primarily infectious (table 5.1). The proportion of admissions due to infection was higher among HEU (101 [39%] of 261) than HU infants (54 [25%] of 214, p=0·002). The most common infectious causes were LRTI (pneumonia, pulmonary tuberculosis, or bronchiolitis, 67 [43%] of 155 infectious admissions), followed by diarrhoea (37 [24%] of 155), table 5.1 and figure 5.2.

The overall incidence of infection-related hospitalisation was 16·6/100cy (95% CI 14·2-19·4), slightly higher among HEU than HU infants (IRR 1·40, 95% CI 0·97-2·02), in crude and adjusted models (table 5.2; supplemental table 9.5.7; figure 5.3). The highest rates were in the first 7 days (overall, 97·0/100cy, 95% CI 59·4-158·4), and similar for HEU and HU infants.



	<7d	7d-3m	3-6m	6-9m	9-12m	>12m
Crude IRR	**1.16**	**3.50**	**1.21**	**1.16**	**1.15**	**0.71**
(95% CI)	(0.43-3.08)	(1.68-7.28)	(0.59-2.49)	(0.46-2.90)	(0.50-2.62)	(0.23-2.16)
Adjusted IRR	**1.14**	**3.49**	**1.12**	**1.30**	**1.45**	**0.47**
(95% CI)	(0.43-3.03)	(1.68-7.27)	(0.53-2.35)	(0.55-3.10)	(0.61-3.44)	(0.17-1.26)

Legend: HEU, HIV-exposed uninfected children; HU, HIV-unexposed children; d, days; m, months; IRR, incidence rate ratio from Poisson regression models adjusted for clustering; CI, confidence interval. Multivariable models adjusted for socio-economic factors (access to flush toilet and running water inside the home), maternal depression, preterm birth, child underweight, vaccination status, quality of breastfeeding and season at birth
Sample size per age interval: <7 days, N=869; 7 days-3 months, N=849; 3-6 months, N=808; 6-9 months, N=782; 9-12 months, N=745; >12 months, N=664

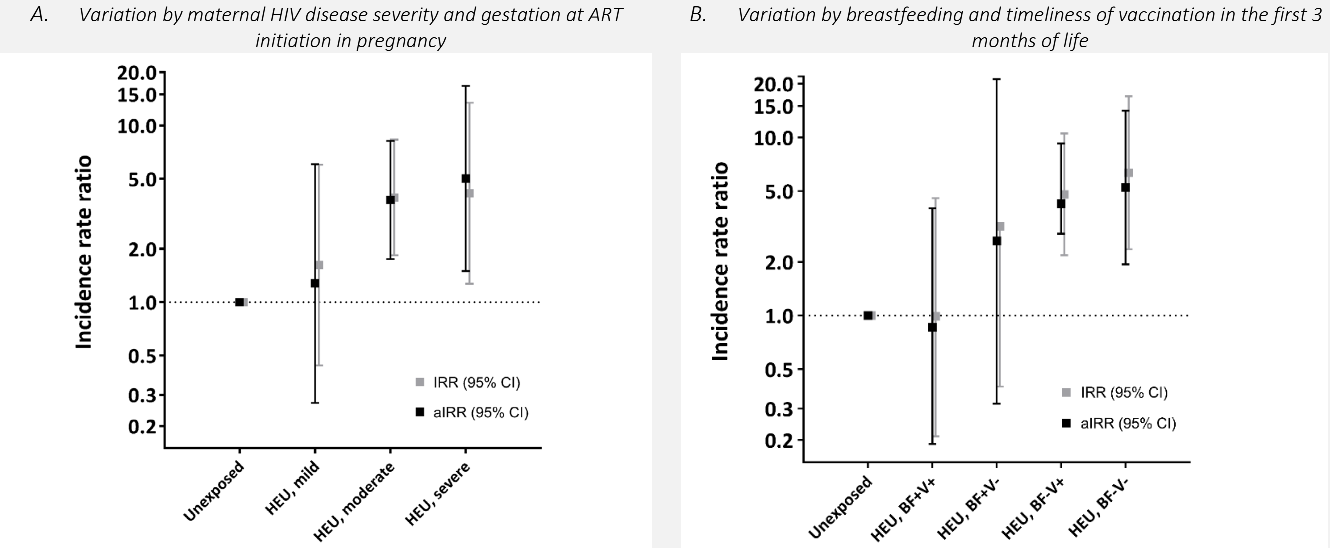
Figure 5.3. Infection-related hospitalization: incidence rates and rate ratios comparing HIV-exposed uninfected to HIV-unexposed children over time

Rates decreased in the 7 days to 3 months interval, with greater reduction among HU than HEU infants (figure 5.3). In this age group, the crude incidence was 34·2/100cy (95% CI 24·4-47·9) among HEU compared to 9·8/100cy (95% CI 5·1-18·8) among HU infants, an absolute excess of roughly 20 admissions for every 100 infants. In relative terms, HEU infants were more than 3 times as likely to experience an infection-related hospitalisation than HU infants (IRR 3·50, 95% CI 1·64-8·30), table 5.2, figure 5.3. This discrepancy disappeared after the age of 3 months, where-after incidence rates remained low, and similar for HEU and HU infants (table 5.2; figure 5.3)

We explored the effects of potentially modifiable factors on the HIV exposure-hospitalisation relationship between 7 days and 3 months of age (table 5.3). Maternal disease severity and gestation at ART initiation modified the HIV-exposure effects on infection-related hospitalisation (Fig 5.4A; table 5.3): HEU infants of women with advanced HIV disease and late ART initiation were at the highest relative risk compared to HU infants (aIRR 5·01; 95% CI 1·50-16·71). By contrast, the incidence rates among HEU infants of women with early disease stages and timely ART initiation (15·88/100cy, 95% CI 5·12-49·23) approximated those of HU infants (9·77/100cy, 95% CI 5·08-18·78; aIRR 1·28, 95% CI 0·27-6·05).

A similar dose-response relationship was seen with breastfeeding and vaccination (figure 5.4B; table 5.3). Notably, there were no infection-related hospitalisations among HEU infants (n=15) with early maternal ART initiation at less severe disease stages, who subsequently received optimal breastfeeding and timely vaccinations (table 5.3).

Figure 5.4. Incidence rate ratios of infection-related hospitalization between 7 days and 3 months of age comparing HIV-exposed uninfected to HIV-unexposed children


A. Variation by maternal HIV disease severity and gestation at ART initiation in pregnancy

B. Variation by breastfeeding and timeliness of vaccination in the first 3 months of life

Legend:

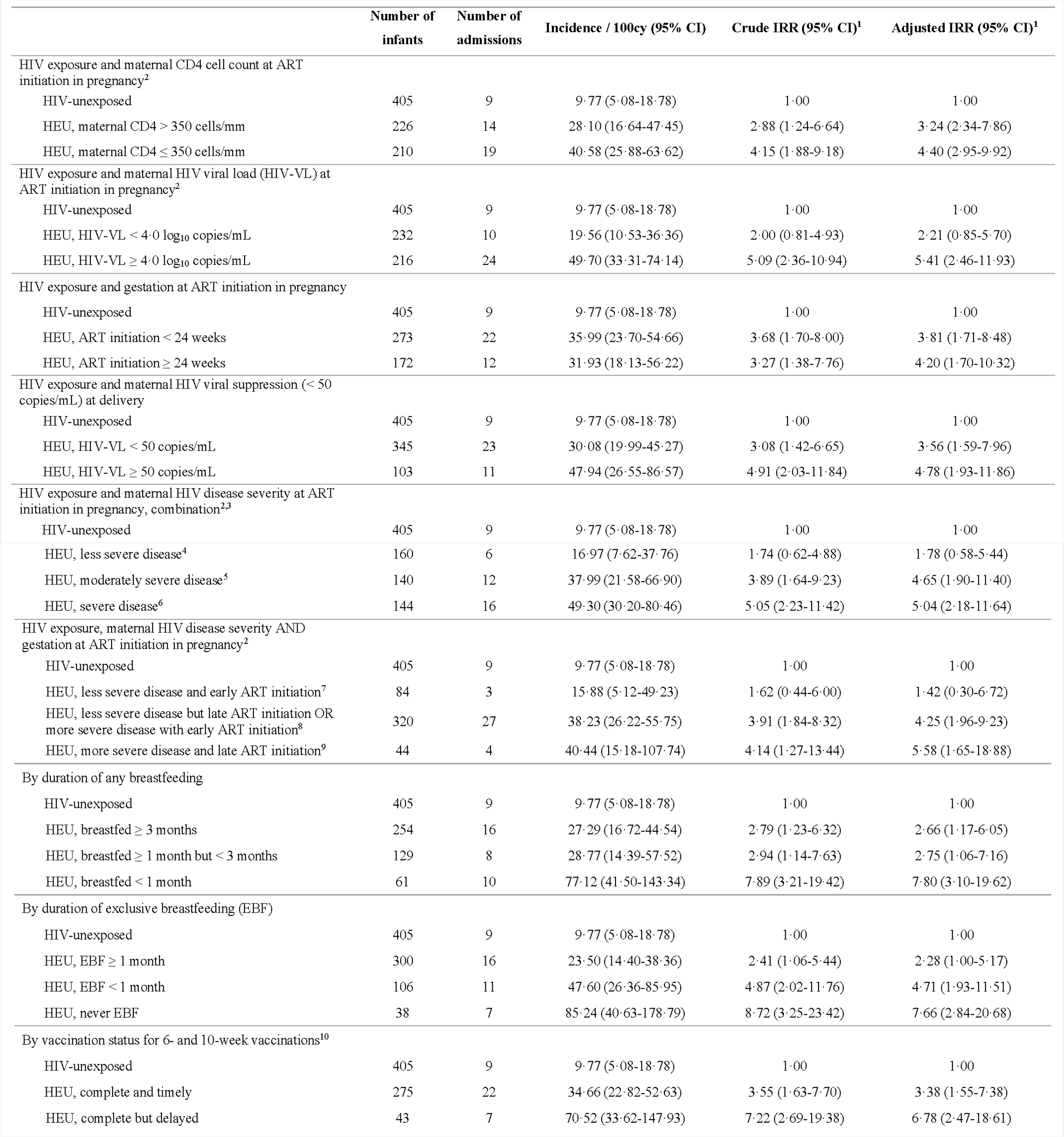
IRR, crude incidence rate ratio from Poisson regression analysis corrected for clustering; aIRR, incidence rate ratios adjusted for access to flush toilet and running water, postpartum depression, preterm birth, season at birth, being underweight in a previous age interval, quality of breastfeeding (early initiation and exclusivity in the first 3 months) and vaccination status
Reference group includes all HU children (N=405)
HEU categories (CD4 cell count and HIV viral load category boundaries set at group median value):
- "Mild" (N=84): pre-ART maternal CD4 cell count > 350 cells/mm^3 AND HIV viral load <4·0 log$_{10}$ copies/mL AND ART initiated prior to 24 weeks' gestation in pregnancy (vs HU, aIRR 1·28, 95% CI 0·27-6·05)
- "Moderate" (N=320): pre-ART maternal CD4 cell count ≤ 350 cells/mm^3 OR HIV viral load ≥4·0 log$_{10}$ copies/mL but ART initiated prior to 24 weeks' gestation in pregnancy; OR, maternal CD4 cell count > 350 cells/mm^3 OR HIV viral load <4·0 log$_{10}$ copies/mL but ART initiation at or after 24 weeks' gestation in pregnancy (vs HU, aIRR 3.79, 95% CI 1·75-8.18)
- "Severe" (N=44): pre-ART maternal CD cell count ≤350 AND HIV viral load ≥4·0 log$_{10}$ copies/mL AND ART initiation at or after 24 weeks' gestation in pregnancy (vs HU, aIRR 5·01, 95% CI 1·50-16·71)

Legend:

IRR, crude incidence rate ratio from Poisson regression analysis corrected for clustering; aIRR, incidence rate ratios adjusted for access to flush toilet and running water, postpartum depression, preterm birth, season at birth and being underweight in a previous age interval; "Optimal breastfeeding" defined as initiation within 1 hour of birth and exclusive breastfeeding through 3 months of age; vaccinations considered timely if all required doses received within 2 weeks of indicated age (based on 6 and 10 weeks' South African schedule)
Reference category includes all HU children (N=405)
HEU categories:

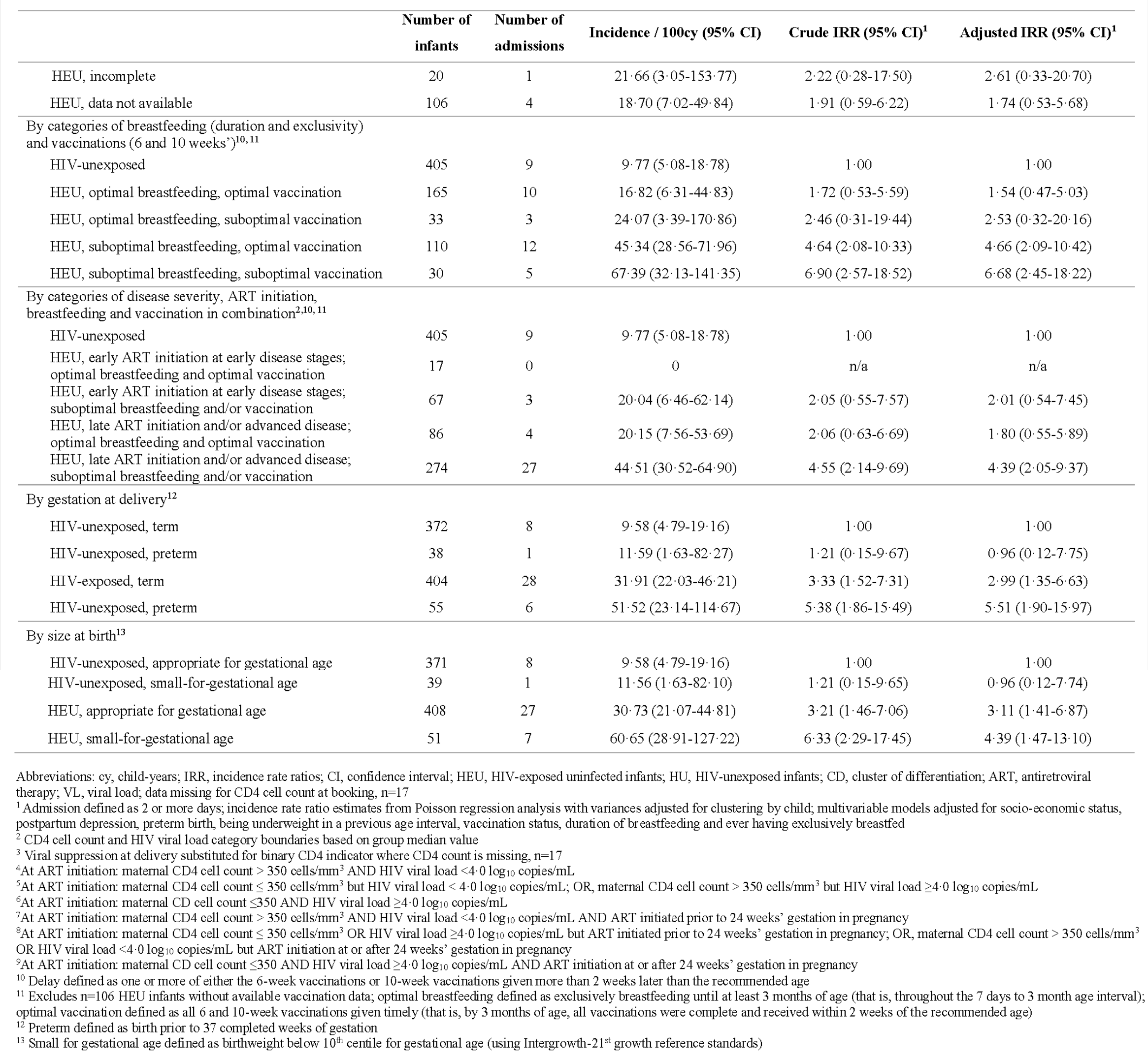
BF+ V+:	Optimal breastfeeding and timely vaccination (N=90); vs HU, aIRR 0.86, 95% CI 0.19-4.00
BF+ V-:	Optimal breastfeeding but delayed or incomplete vaccination (N=14); vs HU, aIRR 2.62, 95% CI 0.32-21.26
BF- V+:	Suboptimal breastfeeding but timely vaccination (N=187); vs HU, aIRR 4.15, 95% CI 1.88-9.15
BF- V-:	Suboptimal breastfeeding and delayed or incomplete vaccination (N=50); vs HU, aIRR 5.24, 95% CI 1.94-14.14

Compared to term HU infants, preterm HU infants had similar hospitalisation rates, whereas term HEU infants had 3-fold higher rates (aIRR 3·33, 95% CI 1·52-7·31), and preterm HEU infants, 5-fold higher rates (aIRR 5·38, 95% CI 1·86-15·49). A similar pattern was evident for HIV exposure and size at birth (table 5.3). Despite some loss of precision, inferences were unchanged in sensitivity analysis (supplemental table 9.5.8). Increased risks of infectious-cause hospitalization were seen among HEU compared to HU infants in analyses restricted to (1) term infants born appropriate-for-gestational age, (2) infants with optimal breastfeeding, (3) infants from homes with running water and flush toilet, or (4) infants born in the same season. Results were unchanged in analysis excluding HEU infants who did not receive CPT (supplemental table 9.5.8). Factors associated with infection-related hospitalisation specifically among HEU infants are shown in supplemental table 9.5.9.



	Number of infants	Number of admissions	Incidence / 100cy (95% CI)	Crude IRR (95% CI)[1]	Adjusted IRR (95% CI)[1]
HIV exposure and maternal CD4 cell count at ART initiation in pregnancy[2]					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, maternal CD4 > 350 cells/mm	226	14	28·10 (16·64-47·45)	2·88 (1·24-6·64)	3·24 (2·34-7·86)
HEU, maternal CD4 ≤ 350 cells/mm	210	19	40·58 (25·88-63·62)	4·15 (1·88-9·18)	4·40 (2·95-9·92)
HIV exposure and maternal HIV viral load (HIV-VL) at ART initiation in pregnancy[2]					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, HIV-VL < 4·0 $\log_{10}$ copies/mL	232	10	19·56 (10·53-36·36)	2·00 (0·81-4·93)	2·21 (0·85-5·70)
HEU, HIV-VL ≥ 4·0 $\log_{10}$ copies/mL	216	24	49·70 (33·31-74·14)	5·09 (2·36-10·94)	5·41 (2·46-11·93)
HIV exposure and gestation at ART initiation in pregnancy					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, ART initiation < 24 weeks	273	22	35·99 (23·70-54·66)	3·68 (1·70-8·00)	3·81 (1·71-8·48)
HEU, ART initiation ≥ 24 weeks	172	12	31·93 (18·13-56·22)	3·27 (1·38-7·76)	4·20 (1·70-10·32)
HIV exposure and maternal HIV viral suppression (< 50 copies/mL) at delivery					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, HIV-VL < 50 copies/mL	345	23	30·08 (19·99-45·27)	3·08 (1·42-6·65)	3·56 (1·59-7·96)
HEU, HIV-VL ≥ 50 copies/mL	103	11	47·94 (26·55-86·57)	4·91 (2·03-11·84)	4·78 (1·93-11·86)
HIV exposure and maternal HIV disease severity at ART initiation in pregnancy, combination[2,3]					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, less severe disease[4]	160	6	16·97 (7·62-37·76)	1·74 (0·62-4·88)	1·78 (0·58-5·44)
HEU, moderately severe disease[5]	140	12	37·99 (21·58-66·90)	3·89 (1·64-9·23)	4·65 (1·90-11·40)
HEU, severe disease[6]	144	16	49·30 (30·20-80·46)	5·05 (2·23-11·42)	5·04 (2·18-11·64)
HIV exposure, maternal HIV disease severity AND gestation at ART initiation in pregnancy[2]					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, less severe disease and early ART initiation[7]	84	3	15·88 (5·12-49·23)	1·62 (0·44-6·00)	1·42 (0·30-6·72)
HEU, less severe disease but late ART initiation OR more severe disease with early ART initiation[8]	320	27	38·23 (26·22-55·75)	3·91 (1·84-8·32)	4·25 (1·96-9·23)
HEU, more severe disease and late ART initiation[9]	44	4	40·44 (15·18-107·74)	4·14 (1·27-13·44)	5·58 (1·65-18·88)
By duration of any breastfeeding					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, breastfed ≥ 3 months	254	16	27·29 (16·72-44·54)	2·79 (1·23-6·32)	2·66 (1·17-6·05)
HEU, breastfed ≥ 1 month but < 3 months	129	8	28·77 (14·39-57·52)	2·94 (1·14-7·63)	2·75 (1·06-7·16)
HEU, breastfed < 1 month	61	10	77·12 (41·50-143·34)	7·89 (3·21-19·42)	7·80 (3·10-19·62)
By duration of exclusive breastfeeding (EBF)					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, EBF ≥ 1 month	300	16	23·50 (14·40-38·36)	2·41 (1·06-5·44)	2·28 (1·00-5·17)
HEU, EBF < 1 month	106	11	47·60 (26·36-85·95)	4·87 (2·02-11·76)	4·71 (1·93-11·51)
HEU, never EBF	38	7	85·24 (40·63-178·79)	8·72 (3·25-23·42)	7·66 (2·84-20·68)
By vaccination status for 6- and 10-week vaccinations[10]					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, complete and timely	275	22	34·66 (22·82-52·63)	3·55 (1·63-7·70)	3·38 (1·55-7·38)
HEU, complete but delayed	43	7	70·52 (33·62-147·93)	7·22 (2·69-19·38)	6·78 (2·47-18·61)

Table 5.3. Incidence rate ratios for infectious-cause hospitalization between 7 days and 3 months of age, comparing HIV-exposed to HIV-unexposed infants within sub-categories of maternal and child characteristics



	Number of infants	Number of admissions	Incidence / 100cy (95% CI)	Crude IRR (95% CI)[1]	Adjusted IRR (95% CI)[1]
HEU, incomplete	20	1	21·66 (3·05-153·77)	2·22 (0·28-17·50)	2·61 (0·33-20·70)
HEU, data not available	106	4	18·70 (7·02-49·84)	1·91 (0·59-6·22)	1·74 (0·53-5·68)
By categories of breastfeeding (duration and exclusivity) and vaccinations (6 and 10 weeks')[10, 11]					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, optimal breastfeeding, optimal vaccination	165	10	16·82 (6·31-44·83)	1·72 (0·53-5·59)	1·54 (0·47-5·03)
HEU, optimal breastfeeding, suboptimal vaccination	33	3	24·07 (3·39-170·86)	2·46 (0·31-19·44)	2·53 (0·32-20·16)
HEU, suboptimal breastfeeding, optimal vaccination	110	12	45·34 (28·56-71·96)	4·64 (2·08-10·33)	4·66 (2·09-10·42)
HEU, suboptimal breastfeeding, suboptimal vaccination	30	5	67·39 (32·13-141·35)	6·90 (2·57-18·52)	6·68 (2·45-18·22)
By categories of disease severity, ART initiation, breastfeeding and vaccination in combination[2,10, 11]					
HIV-unexposed	405	9	9·77 (5·08-18·78)	1·00	1·00
HEU, early ART initiation at early disease stages; optimal breastfeeding and optimal vaccination	17	0	0	n/a	n/a
HEU, early ART initiation at early disease stages; suboptimal breastfeeding and/or vaccination	67	3	20·04 (6·46-62·14)	2·05 (0·55-7·57)	2·01 (0·54-7·45)
HEU, late ART initiation and/or advanced disease; optimal breastfeeding and optimal vaccination	86	4	20·15 (7·56-53·69)	2·06 (0·63-6·69)	1·80 (0·55-5·89)
HEU, late ART initiation and/or advanced disease; suboptimal breastfeeding and/or vaccination	274	27	44·51 (30·52-64·90)	4·55 (2·14-9·69)	4·39 (2·05-9·37)
By gestation at delivery[12]					
HIV-unexposed, term	372	8	9·58 (4·79-19·16)	1·00	1·00
HIV-unexposed, preterm	38	1	11·59 (1·63-82·27)	1·21 (0·15-9·67)	0·96 (0·12-7·75)
HIV-exposed, term	404	28	31·91 (22·03-46·21)	3·33 (1·52-7·31)	2·99 (1·35-6·63)
HIV-unexposed, preterm	55	6	51·52 (23·14-114·67)	5·38 (1·86-15·49)	5·51 (1·90-15·97)
By size at birth[13]					
HIV-unexposed, appropriate for gestational age	371	8	9·58 (4·79-19·16)	1·00	1·00
HIV-unexposed, small-for-gestational age	39	1	11·56 (1·63-82·10)	1·21 (0·15-9·65)	0·96 (0·12-7·74)
HEU, appropriate for gestational age	408	27	30·73 (21·07-44·81)	3·21 (1·46-7·06)	3·11 (1·41-6·87)
HEU, small-for-gestational age	51	7	60·65 (28·91-127·22)	6·33 (2·29-17·45)	4·39 (1·47-13·10)

Abbreviations: cy, child-years; IRR, incidence rate ratios; CI, confidence interval; HEU, HIV-exposed uninfected infants; HU, HIV-unexposed infants; CD, cluster of differentiation; ART, antiretroviral therapy; VL, viral load; data missing for CD4 cell count at booking, n=17

[1] Admission defined as 2 or more days; incidence rate ratio estimates from Poisson regression analysis with variances adjusted for clustering by child; multivariable models adjusted for socio-economic status, postpartum depression, preterm birth, being underweight in a previous age interval, vaccination status, duration of breastfeeding and ever having exclusively breastfed

[2] CD4 cell count and HIV viral load category boundaries based on group median value

[3] Viral suppression at delivery substituted for binary CD4 indicator where CD4 count is missing, n=17

[4] At ART initiation: maternal CD4 cell count > 350 cells/mm^3 AND HIV viral load <4·0 $\log_{10}$ copies/mL

[5] At ART initiation: maternal CD4 cell count ≤ 350 cells/mm^3 but HIV viral load < 4·0 $\log_{10}$ copies/mL; OR, maternal CD4 cell count > 350 cells/mm^3 but HIV viral load ≥4·0 $\log_{10}$ copies/mL

[6] At ART initiation: maternal CD cell count ≤350 AND HIV viral load ≥4·0 $\log_{10}$ copies/mL

[7] At ART initiation: maternal CD4 cell count > 350 cells/mm^3 AND HIV viral load <4·0 $\log_{10}$ copies/mL AND ART initiated prior to 24 weeks' gestation in pregnancy

[8] At ART initiation: maternal CD4 cell count ≤ 350 cells/mm^3 OR HIV viral load ≥4·0 $\log_{10}$ copies/mL but ART initiated prior to 24 weeks' gestation in pregnancy; OR, maternal CD4 cell count > 350 cells/mm^3 OR HIV viral load <4·0 $\log_{10}$ copies/mL but ART initiation at or after 24 weeks' gestation in pregnancy

[9] At ART initiation: maternal CD cell count ≤350 AND HIV viral load ≥4·0 $\log_{10}$ copies/mL AND ART initiation at or after 24 weeks' gestation in pregnancy

[10] Delay defined as one or more of either the 6-week vaccinations or 10-week vaccinations given more than 2 weeks later than the recommended age

[11] Excludes n=106 HEU infants without available vaccination data; optimal breastfeeding defined as exclusively breastfeeding until at least 3 months of age (that is, throughout the 7 days to 3 month age interval); optimal vaccination defined as all 6 and 10-week vaccinations given timely (that is, by 3 months of age, all vaccinations were complete and received within 2 weeks of the recommended age)

[12] Preterm defined as birth prior to 37 completed weeks of gestation

[13] Small for gestational age defined as birthweight below 10th centile for gestational age (using Intergrowth-21st growth reference standards)

Table 5.3 (continued)

5.4.4 Longitudinal prevalence of infectious illness

Prevalence of LRTI and diarrhoeal illness increased over time in both HEU and HU infants (figure 5.5). On average, HEU infants had significantly higher prevalence of LRTI than HU infants (PR 3·23, 95% CI 2·20-4·74; aPR 2·33, 95% CI 1·54-3·51), and slightly higher prevalence of diarrhoeal illness in crude (PR 1·25, 95% CI 1·03-1·52) but not adjusted (aPR 1·03, 95% CI 0·83-1·28) models (supplemental table 9.5.10)

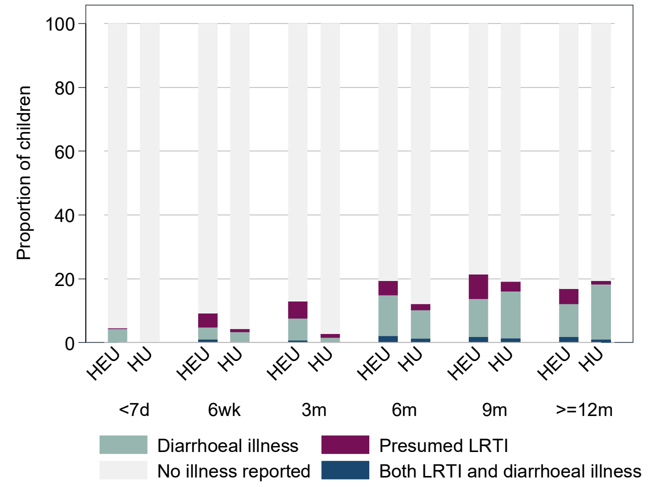
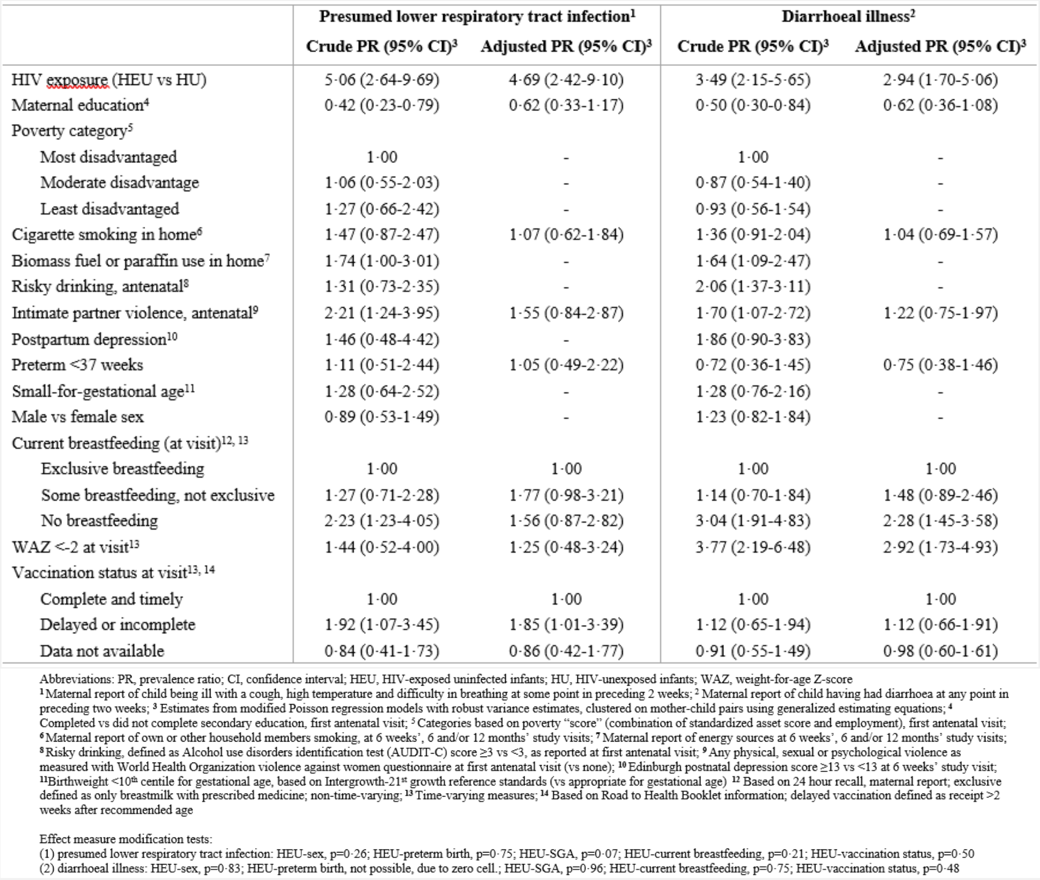


Figure 5.5. Longitudinal prevalence of self-reported child infections illness in preceding two weeks, comparing HIV-exposed uninfected (HEU) to HIV-unexposed (HU) infants at each study visit

Child age modified these associations: among infants ≤6 months, HIV-exposure was associated with an almost 5-fold increased risk of LRTI (aPR 4·69, 95% CI 2·40-9·17), and a 3-fold increased risk of diarrhoeal illness (aPR 2·93, 95% CI 1·70-5·07; table 5.4). After 6 months of age, the risk differential between HEU and HU infants was substantially smaller for LRTI, and absent for diarrhoeal illness (supplemental table 9.5.11). Variations in the HIV exposure-infectious illness relationship by maternal HIV disease severity, timing of ART initiation, breastfeeding and vaccination status approximated those of infection-related hospitalization; and were most marked in the first 6 months (supp table 9.5.12).

Table 5.4. Longitudinal prevalence of self-reported child infectious illness in preceding two weeks, comparing HIV-exposed uninfected and HIV unexposed infants in the first 6 months of life: crude and adjusted prevalence ratios from modified Poisson regression analysis



	Presumed lower respiratory tract infection[1]		Diarrhoeal illness[2]	
	Crude PR (95% CI)[3]	Adjusted PR (95% CI)[3]	Crude PR (95% CI)[3]	Adjusted PR (95% CI)[3]
HIV exposure (HEU vs HU)	5·06 (2·64-9·69)	4·69 (2·42-9·10)	3·49 (2·15-5·65)	2·94 (1·70-5·06)
Maternal education[4]	0·42 (0·23-0·79)	0·62 (0·33-1·17)	0·50 (0·30-0·84)	0·62 (0·36-1·08)
Poverty category[5]				
Most disadvantaged	1·00	-	1·00	-
Moderate disadvantage	1·06 (0·55-2·03)	-	0·87 (0·54-1·40)	-
Least disadvantaged	1·27 (0·66-2·42)	-	0·93 (0·56-1·54)	-
Cigarette smoking in home[6]	1·47 (0·87-2·47)	1·07 (0·62-1·84)	1·36 (0·91-2·04)	1·04 (0·69-1·57)
Biomass fuel or paraffin use in home[7]	1·74 (1·00-3·01)	-	1·64 (1·09-2·47)	-
Risky drinking, antenatal[8]	1·31 (0·73-2·35)	-	2·06 (1·37-3·11)	-
Intimate partner violence, antenatal[9]	2·21 (1·24-3·95)	1·55 (0·84-2·87)	1·70 (1·07-2·72)	1·22 (0·75-1·97)
Postpartum depression[10]	1·46 (0·48-4·42)	-	1·86 (0·90-3·83)	
Preterm <37 weeks	1·11 (0·51-2·44)	1·05 (0·49-2·22)	0·72 (0·36-1·45)	0·75 (0·38-1·46)
Small-for-gestational age[11]	1·28 (0·64-2·52)	-	1·28 (0·76-2·16)	-
Male vs female sex	0·89 (0·53-1·49)	-	1·23 (0·82-1·84)	-
Current breastfeeding (at visit)[12, 13]				
Exclusive breastfeeding	1·00	1·00	1·00	1·00
Some breastfeeding, not exclusive	1·27 (0·71-2·28)	1·77 (0·98-3·21)	1·14 (0·70-1·84)	1·48 (0·89-2·46)
No breastfeeding	2·23 (1·23-4·05)	1·56 (0·87-2·82)	3·04 (1·91-4·83)	2·28 (1·45-3·58)
WAZ <-2 at visit[13]	1·44 (0·52-4·00)	1·25 (0·48-3·24)	3·77 (2·19-6·48)	2·92 (1·73-4·93)
Vaccination status at visit[13, 14]				
Complete and timely	1·00	1·00	1·00	1·00
Delayed or incomplete	1·92 (1·07-3·45)	1·85 (1·01-3·39)	1·12 (0·65-1·94)	1·12 (0·66-1·91)
Data not available	0·84 (0·41-1·73)	0·86 (0·42-1·77)	0·91 (0·55-1·49)	0·98 (0·60-1·61)

Abbreviations: PR, prevalence ratio; CI, confidence interval; HEU, HIV-exposed uninfected infants; HU, HIV-unexposed infants; WAZ, weight-for-age Z-score

[1] Maternal report of child being ill with a cough, high temperature and difficulty in breathing at some point in preceding 2 weeks; [2] Maternal report of child having had diarrhoea at any point in preceding two weeks; [3] Estimates from modified Poisson regression models with robust variance estimates, clustered on mother-child pairs using generalized estimating equations; [4] Completed vs did not complete secondary education, first antenatal visit; [5] Categories based on poverty "score" (combination of standardized asset score and employment), first antenatal visit; [6] Maternal report of own or other household members smoking, at 6 weeks', 6 and/or 12 months' study visits; [7] Maternal report of energy sources at 6 weeks', 6 and/or 12 months' study visits; [8] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 vs <3, as reported at first antenatal visit; [9] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit (vs none); [10] Edinburgh postnatal depression score ≥13 vs <13 at 6 weeks' study visit; [11] Birthweight <10th centile for gestational age, based on Intergrowth-21st growth reference standards (vs appropriate for gestational age) [12] Based on 24 hour recall, maternal report; exclusive defined as only breastmilk with prescribed medicine; non-time-varying; [13] Time-varying measures; [14] Based on Road to Health Booklet information; delayed vaccination defined as receipt >2 weeks after recommended age

Effect measure modification tests:
(1) presumed lower respiratory tract infection: HEU-sex, p=0·26; HEU-preterm birth, p=0·75; HEU-SGA, p=0·07; HEU-current breastfeeding, p=0·21; HEU-vaccination status, p=0·50
(2) diarrhoeal illness: HEU-sex, p=0·83; HEU-preterm birth, not possible, due to zero cell.; HEU-SGA, p=0·96; HEU-current breastfeeding, p=0·75; HEU-vaccination status, p=0·48

5.5 Discussion

In this urban South African cohort, HIV exposure was associated with transiently increased infectious morbidity risks in early infancy, even in the context of universal maternal ART and breastfeeding. However, excess risks appeared to be predominantly driven by advanced maternal HIV disease with late ART initiation in pregnancy, alongside suboptimal vaccination, and inadequate breastfeeding practices. Mortality rates were similar for HEU and HU infants, and approximated the background risk of infant mortality in this setting.[13]

Although direct comparison with other studies is complicated by heterogeneity of morbidity measures, our overall estimates of hospitalisation and prevalence data for HIV-unexposed infants were in keeping with previous reports from South Africa.[13,22,23] Reassuringly, our estimates of HEU infectious morbidity largely approximated those of HU infants in late infancy. The transiently increased risk we observed in early infancy aligns with findings from African studies predating universal maternal ART, hypothesised to reflect the residual effects of HIV-driven maternal-fetal immune dysregulation including reduced transplacental transfer of maternal antibodies.[4] Our data suggest that this early peak in vulnerability among HEU infants may not be fully addressed by universal maternal ART when only initiated later in pregnancy, or at more advanced disease stages. In keeping with these observations, a convincing risk gradient by timing of maternal ART initiation was recently described in Europe.[24] Belgian HEU infants with pre-pregnancy maternal ART initiation experienced substantially less immune dysregulation and infection-related hospitalisation than those with maternal ART initiation in pregnancy, and in turn had hospitalisation rates which approximated those of HU controls. Taken together, the data suggest that optimising maternal HIV-related health prior to conception may have direct child health benefits extending beyond HIV-free survival. Indeed, pre-conception maternal health is increasingly recognised as a strong determinant of subsequent child health generally and deserves greater attention in the context of maternal HIV.[25]

Optimal breastfeeding and timely childhood vaccinations are foundational components of global strategies to prevent morbidity and mortality from LRTI and diarrhoea.[26] Not surprisingly, most infectious episodes in our cohort could be ascribed to these common childhood illnesses.[4] Although all mother-child pairs in our study were initially breastfeeding, delayed initiation and early cessation of both exclusive and non-exclusive breastfeeding was common, and corresponded to increasing risks of illness. Strong evidence exists for strategies to improve breastfeeding practices[27,28] but adaptation and implementation has been slow across settings including South Africa.[29] Our data also reveal concerningly high rates of delayed and incomplete vaccination among most infants, highlighting the urgent need for improvement of basic, primary health care in our setting, which would benefit both HEU and HU infants. Indeed, the reduction in infectious morbidity among those HIV-exposed infants in our cohort who were exclusively breastfed and adequately vaccinated underscores the benefits of optimising well-established, scalable interventions.

Our study has limitations. Although we aimed to address potential confounding through rigorous study design and analysis, unmeasured confounding remains a possibility. Measurement error due to maternal recall bias may have influenced our estimates of childhood illness, particularly as some of the DHS questions can be non-specific, and positive predictive value is reduced in areas with low background prevalence of disease.[30] Simultaneously, our hospitalisation rates may be underestimations, as admissions in other provinces could have been missed. Nonetheless, the alignment of our prevalence and hospitalisation findings are reassuring and suggest that a relationship does exist between HIV exposure and infectious illness in early infancy. Our study lacked detailed immunological measures, which should ideally be incorporated into future studies evaluating drivers of excess HEU child morbidity in resource-limited settings. While one of our study strengths is the homogenous use of maternal ART in pregnancy, there remained substantial variation in the severity of maternal disease at time of ART initiation. Accordingly, several of our subgroup analyses lacked precision. Our findings cannot be generalised to women who initiate treatment prior to pregnancy; however, given the persistently high incidence of HIV among young women in Africa,[1] many women will continue to receive their first diagnosis and initiate ART during rather than before pregnancy. For these HEU infants, it may be particularly important to promote breastfeeding and timely completed vaccination. Our findings may also not extend to rural areas with poor access to health care and even higher infectious disease burdens, including malaria. Despite these limitations, this study provides one of the first reports on infectious morbidity among uniformly breastfed HEU infants born under current ART policies in Africa.

5.6 Conclusion

Even with universal ART and breastfeeding, HEU infants, on average, experienced more infectious morbidity in early infancy than their HU counterparts. Reassuringly, our data suggest that these excess risks may potentially be addressed through earlier diagnosis and ART initiation for HIV-infected women, alongside optimal breastfeeding and timely vaccination of their HEU infants. Improved implementation and scaling up of available interventions to optimize breastfeeding and vaccination should be public health priorities.

5.7 Acknowledgements

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5.7.3 Contributors

SLR (corresponding author) was responsible for design, implementation, and management of the HU2 study; assisted with collection of data; conducted the analyses; wrote the first draft of the manuscript and confirms that she had full access to all the data and takes final responsibility for the decision to submit for publication. LM and EJA conceived the MCH-ART study, and were responsible for study design, funding, implementation, and overall leadership. KD and MK provided supervision for child health aspects of the study. DLR was responsible for blinded reviews of all verbal autopsy reports and morbidity assessments. KB and TKP were responsible for data management and oversight. TKP and KN were the study coordinators. AZ was the senior study manager and provided oversight of all study administration processes. All authors contributed to and approved the final manuscript.

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Chapter 6

Neurodevelopment of breastfed HIV-exposed uninfected and HIV-unexposed children in South Africa: a prospective cohort

Stanzi M le Roux, Kirsten A Donald, Kirsty Brittain, Tamsin K Phillips, Allison Zerbe, Kelly K Nguyen, Andrea Strandvik, Max Kroon, Elaine J Abrams, and Landon Myer. **Neurodevelopment of breastfed HIV-exposed uninfected and HIV-unexposed children in South Africa.** *AIDS.* 2018 Aug; 32 (13): 1781-1791. doi: 10.1097/QAD.0000000000001872

Relevance of this paper to the thesis

This paper presents data on the neurodevelopmental outcomes of a subset of HIV-exposed uninfected (HEU) and HIV-unexposed uninfected (HU) children at 12 months of age. The analysis compares the average developmental scores and risk of developmental delay in HEU vs HU children across three critical developmental domains (cognitive, motor, and language). As such, this paper addresses study objective 4(a). In addition, potentially modifiable factors associated with developmental delay among HEU children are explored and discussed, addressing study objective 4(c).

Contribution of the student and co-authors

The candidate, SLR, was responsible for implementation and supervision of all child neurodevelopmental aspects of both the MCH-ART study (source population of HEU children) and the HU2 study (source population of HU children) with guidance from KAD and LM. This included management of staff appointments, quality assurance and clinical referrals for children who were identified with developmental delay. In addition, she conducted a large proportion of the overall Bayley Scales of Infant Development ed III assessments, was responsible for data cleaning and conducted all the analyses. The candidate wrote the first draft of this paper and is the corresponding author. KAD provided training in the use of the BSID-III. KAD and MK provided supervision and support for all child health queries in both studies. KB and TKP were responsible for data management and oversight. TKP and KKN were the study coordinators for the MCH-ART and HU2 studies, respectively. AZ was the senior study manager and provided oversight of all study administration processes. AS conducted developmental assessments and assisted with training and data management. LM and EJA conceived the MCH-ART study, and were responsible for study design, funding, implementation, and overall leadership. All authors contributed to and approved the final manuscript.

6.1 Abstract

6.1.1 Objectives

To assess neurodevelopment of breastfed HIV-exposed uninfected (HEU) and breastfed HIV-unexposed (HU) children in the context of universal maternal antiretroviral therapy (ART).

6.1.2 Design

Prospective study with antenatal enrolment and follow-up of breastfeeding HEU and HU mother-infant pairs through 12-18 months postpartum.

6.1.3 Setting

Peri-urban community, Cape Town, South Africa.

6.1.4 Subjects

HEU (n=215) and HU (n=306) children.

6.1.5 Main outcome measures

Cognitive, motor and language development at median 13 (IQR 12-14) months of age: continuous and dichotomous BSID-III scores (Bayley Scales of Infant and Toddler Development 3rd edition; delay defined as composite score <85)

6.1.6 Results

Incidence of preterm delivery (PTD, <37 weeks) was similar among HEU and HU children (11% vs. 9%, p=0.31; median gestation 39 weeks); 48% were boys. Median breastfeeding duration was shorter among HEU vs. HU children (6 vs. 10 months). All HIV-infected mothers initiated lifelong ART (TDF/FTC/EFV) antenatally. HEU (vs. HU) children had higher odds of cognitive delay [OR 2.28 (95%CI 1.13-4.60)] and motor delay [OR 2.10 (95% CI 1.03-4.28)], but not language delay, in crude and adjusted analysis. PTD modified this relationship for motor development: compared to term HU, term HEU children had similar odds of delay, preterm HU children had 5-fold increased odds of delay (aOR 4.73, 95% CI 1.32; 16.91) and preterm HEU children, 16-fold (aOR 16.35, 95% CI 5.19; 51.54).

6.1.7 Conclusions

Young HEU children may be at increased risk for cognitive and motor delay despite universal maternal ART and breastfeeding; those born preterm may be particularly vulnerable.

6.2 Introduction

With the rapid expansion of lifelong, triple-drug antiretroviral therapy (ART) across sub-Saharan Africa, the incidence of pediatric HIV infection is declining while a large and growing proportion of the region's children are born perinatally HIV-exposed but uninfected (HEU). In some areas, HEU newborns constitute 20-30% of births annually, and there is growing concern regarding the potential adverse health outcomes in this specific group of children.[1,2]

HEU children may be at higher risk of neurodevelopmental delays than their HIV-unexposed (HU) counterparts.[3] While findings have been inconsistent, [4-9] neurodevelopmental delays across cognitive, motor and/or language domains have been documented among preschool HEU children, [3,10-12] with grade repetition,[13] poor school grades,[14] reduced working memory profiles[15] and lower IQ scores[16] reported among school-age children. However, data come predominantly from non-breastfeeding populations in high income countries, and/or predate the widespread availability of universal ART (treatment for all, irrespective of disease stage) in resource-limited settings.[17] In addition, inferences have been limited by the scarcity of appropriately sampled HU control groups from the same communities, inadequate consideration of psycho-social and environmental confounders including alcohol and drug use in pregnancy, as well as inconsistent use of standardized, validated assessment tools.[3]

As a result, there is a clear need for comparison of early development in HEU and HU infants and young children under conditions of breastfeeding with universal maternal ART, particularly from settings with high HIV prevalence. To address this gap, we compared cognitive, motor and language development in a well-characterized, prospective cohort of young, breastfed HU, and HEU children born to women who initiated universal ART in pregnancy, in Cape Town, South Africa.

6.3 Methods

6.3.1 Study design and population

HIV-infected women and HEU children were participants of the Maternal and Child Health Antiretroviral Therapy study (MCH-ART; 2013-2016), a prospective study of strategies to improve postpartum adherence and retention in ART care.[18] HIV-uninfected women and HU children were participants of the HIV-unexposed-uninfected mother and child health study (HU2; 2014-2017), a prospective cohort study specifically designed to complement MCH-ART, using the same study structure, design, staff and measures.[18] HIV-uninfected women, and HIV-infected women initiating ART (tenofovir-emtricitabine-efavirenz, TDF/FTC/EFV) in pregnancy, were followed from first antenatal clinic visit, through pregnancy to delivery, and with their breastfed children, until 12-18 months postpartum. Study methodology has been described elsewhere.[18] Briefly, after enrolment in pregnancy, women attended 1-3 antenatal study visits and were asked to return within 7 days postpartum. Breastfeeding mother-infant pairs were eligible for continued postnatal follow-up, with visits scheduled at 6 weeks; 3, 6, 9 and 12 months. MCH-ART participants returned for a final visit at 18 months. At the final or near-final study visit, eligible children (11-18 months old, HIV-uninfected, gestational age at birth ≥ 28 weeks, without known congenital abnormalities, hearing impairment or severe cerebral palsy) of consenting mothers from both studies received a single developmental assessment.

6.3.2 Study setting

Research was based at a primary health care center in Gugulethu, a peri-urban township in Cape Town, South Africa. The facility serves a population of about 350 000, with an estimated 30% antenatal HIV seroprevalence.[19] The Gugulethu Midwife Obstetric Unit (MOU) provides antenatal and obstetric care, and universal ART to all HIV-infected pregnant women since 2013.[19,20] Newborn hearing screenings are routinely done soon after birth, and recorded on patient-held records. Study visits, including developmental assessments, were conducted at the research unit adjacent to, but separate from, routine care.

6.3.3 Measurements

Trained interviewers administered questionnaires to both groups of women. Study-specific questionnaires, identical except for HIV-related items, asked about pregnancy intentions, maternal demographic and health information, and psycho-social measures including alcohol/drug use (AUDIT, alcohol use disorders identification test; DUDIT, drug use disorders identification test)[21,22], depression (Edinburgh postnatal depression scale, EPDS)[23] and experiences of intimate partner violence (IPV; WHO Violence against women questionnaire)[24]. After delivery, additional questionnaires assessed infant feeding practices, maternal-infant health and demographics. Obstetric, child health and laboratory data were abstracted from medical records. In addition to HIV-related phlebotomy and developmental assessments, clinical measurements included antenatal ultrasound at enrolment, repeated at 20-22 weeks for fetal anomalies where possible, and during the third trimester. Maternal-infant anthropometry was measured at all postnatal visits, with gestation-adjusted Z-scores generated using the Intergrowth-21st growth reference standards.[25]

Routine PMTCT services conducted antenatal HIV counseling and testing (HCT) using a rapid finger-prick test (Alere Determine®). Positive women provided serum for CD4 cell count and HIV viral load, and all initiated ART (TDF/FTC/EFV) at the MOU.[18] HIV-exposed children received HIV-PCR testing to exclude MTCT at 6 weeks and 12 months.[18] HU2 mothers received repeat HCT via routine health services during and after pregnancy. At final study visit, all HU2 mothers had repeat HCT at the study site.

Cognitive, motor and language development was assessed using the Bayley Scales of Infant and Toddler Development®, Third Edition (BSID-III), which has been validated in South Africa.[26,27] Developmental assessments were conducted by either a paediatric occupational therapist or a child health physician; all assessors received systematic supervised training in the use of BSID-III and were assisted by a trained, isi-Xhosa-speaking counsellor. Composite cognitive, motor and language scores were generated from cognitive, fine and gross motor, and expressive language subscale scores using BSID-III normative and conversion tables, which account for gestation at delivery.[26] Receptive language testing using standardized BSID-III tools proved contextually challenging; throughout, results represent expressive language scores only. For interrater reliability, video-graphed assessment scores were compared between assessors, generating estimates for interrater variability (correlation coefficients and percent agreement) per developmental domain. Correlation coefficients for cognitive and motor scores were above 0.9; language ranged from 0.7 to 0.98. There was perfect agreement between the binary classifications of some vs. no delay in all three domains.

Several known risk factors for maternal HIV acquisition may also be independent determinants of development in early childhood.[28,29] Potential confounders identified *a priori* for this analysis included maternal age, education, relationship status, pregnancy intentions and socio-economic status (employment and housing). Psychosocial measures included alcohol use (risky drinking at enrolment and/or in late pregnancy, AUDIT-C score ≥ 3), postpartum depression (EPDS score ≥ 13 at enrolment and/or 6 weeks), and IPV (any violence reported at enrolment). We also assessed infant sex, gestational age, and anthropometry at birth, giving special consideration to the role of preterm delivery (PTD, <37 weeks' gestation) given its potential mediating role in the HIV-exposure-development relationship. Postnatal factors included breastfeeding duration and at 12 months, maternal smoking, and child attendance at a nursery.

Loss to follow-up was minimized through use of telephonic contact and household tracing. Systematic differences between those with and without developmental assessments within strata of maternal HIV status were explored and findings are interpreted accordingly (figure 1; Supplemental digital content 3, table).

6.3.4 Statistical methodology

BSID-III composite scores generally have an expected mean (standard deviation, SD) of 100 (15).[26] While these expected values are based on the US-based reference population, similar expected values have been reported in low-resourced settings including South Africa.[27] In clinical practice, a BSID-III score below 1 SD from the mean (<85) typically indicates some delay and below 2SD, severe delay.[30] We estimated that an overall sample size of 500, including 200-250 HEU children, would achieve >90% power to detect a mean difference of ≥ 5 points (0.33 of SD).

Data were analyzed using Stata 14.0 (Statacorp, College Station TX). Composite scores were analyzed in continuous and binary form (score <85 indicating "any" delay).[30] For between-group comparisons of severity, delay was further categorized into [(i) no delay (composite score ≥85); (ii) mild/moderate delay (≥70, <85); and (iii) severe delay (<70)].[30] Exposure-outcome relationships were explored graphically and tested using correlation coefficients, Kruskal-Wallis or chi^2 tests as appropriate. Categorization of continuous variables followed published boundaries where available, or locally weighted regression plots. Absolute differences in mean composite scores and relative odds of delay were obtained from linear and logistic regression, respectively. Multivariable model selection was based on improvements in Akaike's information criterion (AIC) building on a null model that included variables chosen *a priori* (maternal education, alcohol use and IPV; infant gestational age at birth, birth-size and duration of breastfeeding; directed acyclic graph, supplemental table 9.6.1). Based on *a priori* hypotheses, effect modification was assessed between HIV-exposure and (i) gestation at birth, (ii) infant sex and (iii) duration of breastfeeding; effect modification by other variables was tested as exploratory analysis.

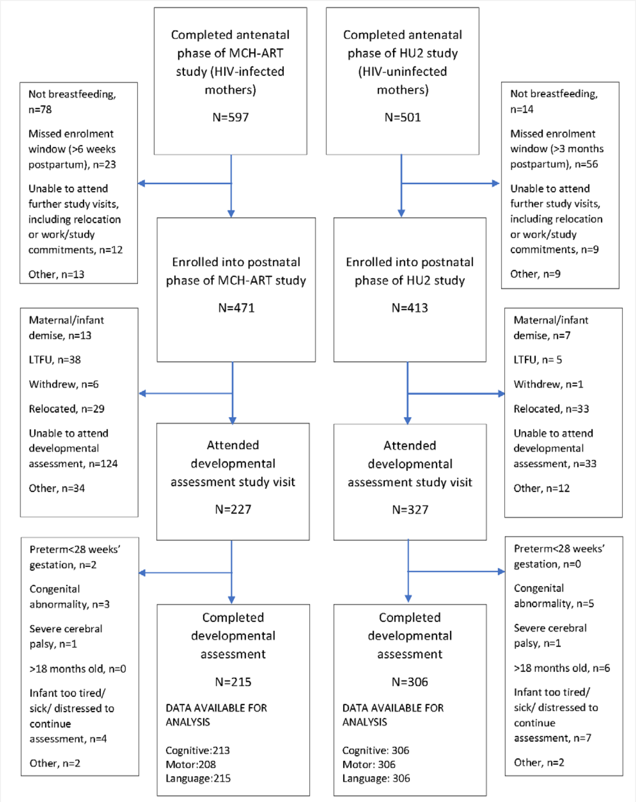
In sensitivity analyses, we examined the HIV-exposure-development relationship among relatively “healthy” children (term, appropriate-for-gestational age (AGA); no maternal IPV, risky drinking or substance use; breastfed for at least 6 months; HIV-infected maternal pre-ART CD4 ≥ 200 cells/mm^3).

6.3.5 *Ethical considerations*

Both MCH-ART and HU2 are approved by the University of Cape Town’s Faculty of Health Sciences Research Ethics Committee (UCT-HREC, 567/2014; 451/2012).

6.4 Results

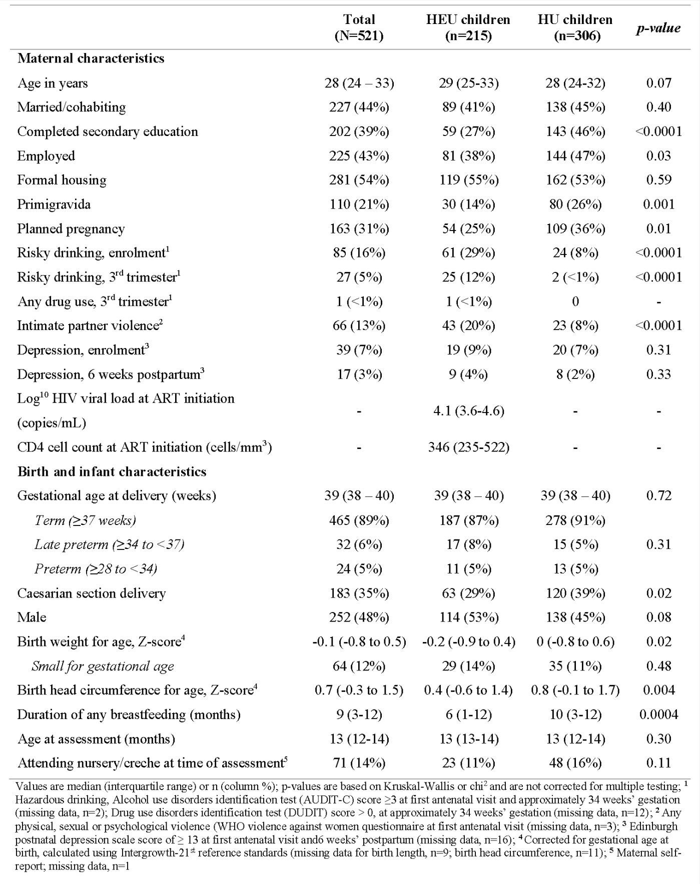
Figure 6.1. Study flow



Overall, 521 mother-infant pairs contributed to this analysis (HEU, n=215; HU, n=306; figure 1, table 1). HIV-infected women (median nadir CD4 cell count 346 cells/mm^3; 75% with HIV viral suppression < 50 copies/mL at delivery) were significantly less likely to have completed high school (27% vs. 46%, p<0.0001), and more likely to report risky drinking (29% vs. 8%, p<0.0001) and IPV (20% vs. 8%, p<0.0001) at first antenatal visit, than HIV-uninfected women. One HIV-infected mother reported drug use in pregnancy.

Comparing HEU to HU children, there were no significant differences in gestation at delivery [median 39 (IQR 39-40) weeks in both groups], the incidence (13% vs. 9%, p=0.31) or relative odds of PTD [OR 1.49 (95%CI 0.85; 2.59)]. Similar proportions of HEU and HU children were born small-for-gestational age (SGA, <10th percentile). Median duration of breastfeeding was shorter among HEU than HU children (6 vs. 10 months, p=0.0004). Differences in maternal and infant characteristics by preterm delivery-HIV exposure status reflected the overall differences between HEU and HU children (supplemental table 9.6.1). HU children contributing to these analyses were largely representative of the larger HU cohort (supplemental table 9.6.2).

Table 6.1. Maternal and infant characteristics of HIV-exposed uninfected (HEU) and HIV-unexposed (HU) children with completed neurodevelopmental assessments



	Total (N=521)	HEU children (n=215)	HU children (n=306)	*p-value*
Maternal characteristics				
Age in years	28 (24 – 33)	29 (25-33)	28 (24-32)	0.07
Married/cohabiting	227 (44%)	89 (41%)	138 (45%)	0.40
Completed secondary education	202 (39%)	59 (27%)	143 (46%)	<0.0001
Employed	225 (43%)	81 (38%)	144 (47%)	0.03
Formal housing	281 (54%)	119 (55%)	162 (53%)	0.59
Primigravida	110 (21%)	30 (14%)	80 (26%)	0.001
Planned pregnancy	163 (31%)	54 (25%)	109 (36%)	0.01
Risky drinking, enrolment[1]	85 (16%)	61 (29%)	24 (8%)	<0.0001
Risky drinking, 3rd trimester[1]	27 (5%)	25 (12%)	2 (<1%)	<0.0001
Any drug use, 3rd trimester[1]	1 (<1%)	1 (<1%)	0	-
Intimate partner violence[2]	66 (13%)	43 (20%)	23 (8%)	<0.0001
Depression, enrolment[3]	39 (7%)	19 (9%)	20 (7%)	0.31
Depression, 6 weeks postpartum[3]	17 (3%)	9 (4%)	8 (2%)	0.33
Log^{10} HIV viral load at ART initiation (copies/mL)	-	4.1 (3.6-4.6)	-	-
CD4 cell count at ART initiation (cells/mm^3)	-	346 (235-522)	-	-
Birth and infant characteristics				
Gestational age at delivery (weeks)	39 (38 – 40)	39 (38 – 40)	39 (38 – 40)	0.72
Term (≥37 weeks)	465 (89%)	187 (87%)	278 (91%)	
Late preterm (≥34 to <37)	32 (6%)	17 (8%)	15 (5%)	0.31
Preterm (≥28 to <34)	24 (5%)	11 (5%)	13 (5%)	
Caesarian section delivery	183 (35%)	63 (29%)	120 (39%)	0.02
Male	252 (48%)	114 (53%)	138 (45%)	0.08
Birth weight for age, Z-score[4]	-0.1 (-0.8 to 0.5)	-0.2 (-0.9 to 0.4)	0 (-0.8 to 0.6)	0.02
Small for gestational age	64 (12%)	29 (14%)	35 (11%)	0.48
Birth head circumference for age, Z-score[4]	0.7 (-0.3 to 1.5)	0.4 (-0.6 to 1.4)	0.8 (-0.1 to 1.7)	0.004
Duration of any breastfeeding (months)	9 (3-12)	6 (1-12)	10 (3-12)	0.0004
Age at assessment (months)	13 (12-14)	13 (13-14)	13 (12-14)	0.30
Attending nursery/creche at time of assessment[5]	71 (14%)	23 (11%)	48 (16%)	0.11

Values are median (interquartile range) or n (column %); p-values are based on Kruskal-Wallis or chi^2 and are not corrected for multiple testing; [1] Hazardous drinking, Alcohol use disorders identification test (AUDIT-C) score ≥3 at first antenatal visit and approximately 34 weeks' gestation (missing data, n=2); Drug use disorders identification test (DUDIT) score > 0, at approximately 34 weeks' gestation (missing data, n=12); [2] Any physical, sexual or psychological violence (WHO violence against women questionnaire at first antenatal visit (missing data, n=3); [3] Edinburgh postnatal depression scale score of ≥ 13 at first antenatal visit and6 weeks' postpartum (missing data, n=16); [4] Corrected for gestational age at birth, calculated using Intergrowth-21st reference standards (missing data for birth length, n=9; birth head circumference, n=11); [5] Maternal self-report; missing data, n=1

HEU children contributing to analyses had somewhat older mothers and better living conditions than those not included in the analysis (supplemental table 9.6.2). In both HEU and HU groups, children included in the analyses had longer median duration of breastfeeding than those not included, partly due to breastfeeding censoring at last attended study visit.

There were no significant differences between HEU compared to HU children in median cognitive scores [100 (IQR 95-110) vs. 100 (IQR 95-110)], motor scores [97 (IQR 89-107) vs. 97 (IQR 91-103)] or language scores [94 (IQR 89-112) vs. 100 (IQR 94-106)]. Average scores were comparable to the BSID reference standards (table 6.2). A larger proportion of HEU than HU children demonstrated any delay (composite score <85) in cognitive and motor domains [HEU vs. HU: 10% vs. 5%, relative risk (RR) 2.15 (95% CI 1.12;4.14); and 9% vs. 5% (RR 2.00, 95% CI 1.02;3.89), respectively]. Risk of language delay was similar between HEU and HU children

(RR 1.23, 95% CI 0.83; 1.83). Among children with scores <85, a very small number had severe delay (score <70), with no substantial differences noted between HEU and HU children (supplemental tables 9.6.3; 9.6.4; and 9.6.5).

Table 6.2. Summary of Bayley Scales of Infant Developmental scores at 11-18 months of age, by maternal HIV status

	Total (N=521)	HIV-exposed uninfected infants (n=215)	HIV-unexposed uninfected infants (n=306)	*p*-value[1]
Cognitive				
Cognitive composite score	100 (95-110)	100 (95-110)	100 (95-110)	0.99
Cognitive composite score <85	35 (7%)	21 (10%)	14 (5%)	0.02
Motor				
Motor composite score	97 (91-103)	97 (89-107)	97 (91-103)	0.79
Motor composite score <85	33 (6%)	19 (9%)	14 (5%)	0.04
Language*				
Language* composite score	100 (89-106)	94 (89-112)	100 (94-106)	0.46
Language* composite score <85	82 (16%)	38 (18%)	44 (14%)	0.31

* Based on expressive language scaled scores only; [1]Kruskal-Wallis and Chi²

6.4.1 *Cognitive development*

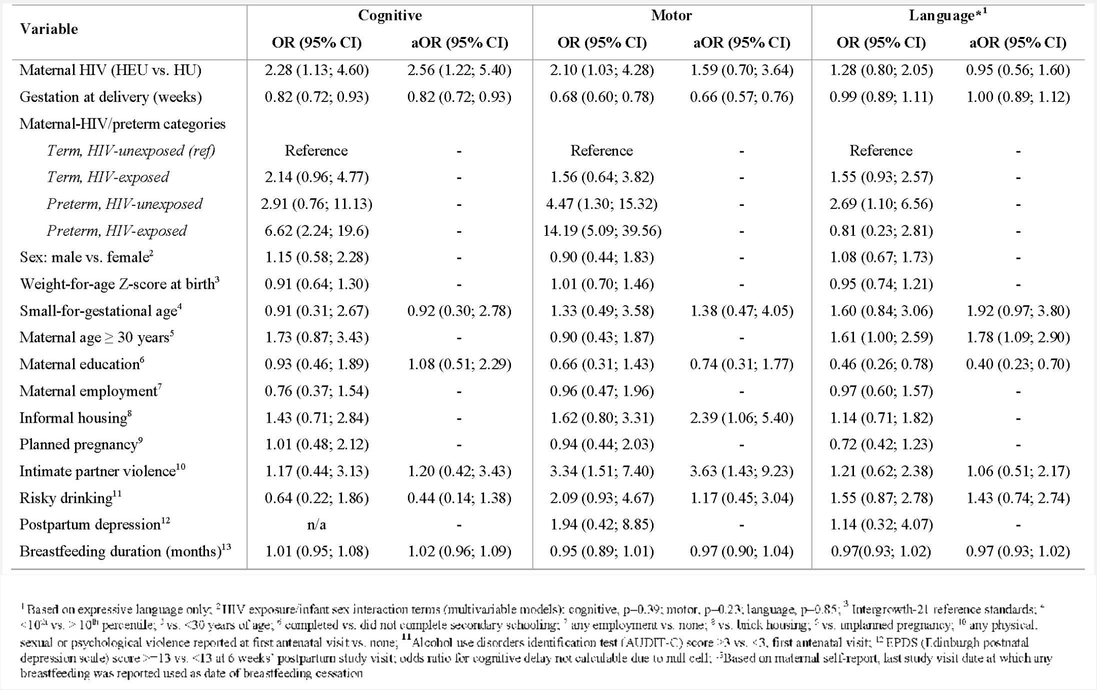
Overall, the average cognitive scores of HEU and HU children were similar (tables 6.2 and 6.3). However, in both crude and adjusted logistic regression models, HEU children were twice as likely to be diagnosed with any cognitive delay compared to HU children [adjusted odds ratio, aOR 2.56 (95% CI 1.22; 5.40), table 6.3]. Increasing gestational age at birth was protective in both linear and logistic regression (tables 6.3 and 6.4). There was some evidence for interaction between HIV exposure and preterm delivery on the odds of cognitive delay (figure 6.2a).

Table 6.3. Linear regression analysis of BSID-III composite scores comparing HIV-exposed uninfected (HEU) to HIV-unexposed (HU) children on cognitive, motor and language* development

Variable	Cognitive		Motor		Language*[1]	
	β (95% CI)	aβ (95% CI)	β (95% CI)	aβ (95% CI)	β (95% CI)	aβ (95% CI)
Maternal HIV (HEU vs. HU)[2]	0.31 (-2.07; 2.69)	0.64 (-1.89; 3.16)	0.55 (-1.84; 2.95)	1.23 (-1.28; 3.74)	1.97 (-0.6; 4.54)	2.68 (-0.08; 5.43)
Gestation at delivery (weeks)	1.19 (0.64; 1.75)	1.20 (0.63; 1.76)	1.35 (0.79; 1.91)	1.35 (0.78; 1.92)	0.50 (-0.10; 1.11)	0.51 (-0.11; 1.12)
Sex: male vs. female[2]	1.09 (-1.26; 3.43)	-	1.33 (-1.02; 3.68)	-	-0.12 (-2.65; 2.42)	-
Weight-for-age Z-score at birth[3]	0.63 (-0.58; 1.84)	-	-0.38 (-1.60; 0.83)	-	-0.43 (-1.74; 0.88)	-
Small-for-gestational age[4]	-2.83 (-6.39; 0.72)	-2.78 (-6.36; 0.81)	-0.92 (-4.53; 2.70)	-0.20 (-3.80; 3.40)	-0.86 (-4.72; 3.00)	-1.16 (-5.09; 2.77)
Maternal age ≥ 30 years[5]	1.59 (-0.81; 3.99)	-	1.76 (-0.65; 4.17)	-	-0.12 (-2.72; 2.48)	-
Maternal education[6]	0.43 (-1.98; 2.83)	0.65 (-1.82; 3.13)	-0.61 (-3.02; 1.81)	-0.73 (-3.20; 1.73)	1.78 (-0.81; 4.38)	2.50 (-0.18; 5.18)
Maternal employment[7]	0.16 (-2.21; 2.52)	-	0.07 (-2.30; 2.44)	-	-0.28 (-2.84; 2.28)	-
Informal housing[8]	-1.41 (-3.76; 0.94)	-1.69 (-4.06; 0.67)	-1.81 (-4.16; 0.55)	-2.48 (-4.83; -0.13)	-1.33 (-3.87; 1.21)	-
Planned pregnancy[9]	0.34 (-2.19; 2.87)	-	-1.15 (-3.68; 1.38)	-	-1.43 (-4.16; 1.30)	-
Intimate partner violence[10]	-0.65 (-4.20; 2.90)	-1.19 (-4.86; 2.47)	-2.45 (-5.99; 1.09)	-2.77 (-6.39; 0.86)	0.49 (-3.33; 4.30)	0.36 (-3.61; 4.34)
Risky drinking[11]	0.14 (-3.04; 3.31)	0.88 (-2.52; 4.28)	-2.17 (-5.39; 1.04)	-1.30 (-4.71; 2.11)	-0.50 (-3.93; 2.94)	-1.40 (-5.11; 2.31)
Postpartum depression[12]	3.08 (-3.54; 9.70)	-	-3.07 (-9.73; 3.59)	-	-1.94 (-9.15; 5.27)	-
Breastfeeding duration (months)[13]	0.11 (-0.11; 0.34)	0.09 (-0.13; 0.31)	0.19 (-0.04; 0.41)	0.15 (-0.08; 0.37)	0.02 (-0.22; 0.26)	0.01 (-0.24; 0.25)

[1] Based on expressive language only; [2]Test for HIV-exposure/infant sex interaction in multivariable model: cognitive, p=0.79; motor, p=0.96; language, p=0.77; [3] Intergrowth-21 reference standards; [4] <10th vs. ≥ 10th percentile; [5] vs. <30 years of age; [6] completed vs. did not complete secondary schooling; [7] any employment vs. none; [8] vs. brick housing; [9] vs. unplanned pregnancy; [10] any physical, sexual or psychological violence reported at first antenatal visit vs. none; [11]Alcohol use disorders identification test (AUDIT-C) score ≥3 vs. <3, first antenatal visit; [12] EPDS (Edinburgh postnatal depression scale) score >=13 vs. <13 at 6 weeks' postpartum study visit; [13]Based on maternal self-report, last study visit date at which any breastfeeding was reported used as date of breastfeeding cessation

Table 6.4. Logistic regression analysis of relative odds of developmental delay (BSID-III scores <85) comparing HIV-exposed uninfected (HEU) to HIV-unexposed (HU) children in cognitive, motor and language* domains



Variable	Cognitive		Motor		Language*[1]	
	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)
Maternal HIV (HEU vs. HU)	2.28 (1.13; 4.60)	2.56 (1.22; 5.40)	2.10 (1.03; 4.28)	1.59 (0.70; 3.64)	1.28 (0.80; 2.05)	0.95 (0.56; 1.60)
Gestation at delivery (weeks)	0.82 (0.72; 0.93)	0.82 (0.72; 0.93)	0.68 (0.60; 0.78)	0.66 (0.57; 0.76)	0.99 (0.89; 1.11)	1.00 (0.89; 1.12)
Maternal-HIV/preterm categories						
Term, HIV-unexposed (ref)	Reference	-	Reference	-	Reference	-
Term, HIV-exposed	2.14 (0.96; 4.77)	-	1.56 (0.64; 3.82)	-	1.55 (0.93; 2.57)	-
Preterm, HIV-unexposed	2.91 (0.76; 11.13)	-	4.47 (1.30; 15.32)	-	2.69 (1.10; 6.56)	-
Preterm, HIV-exposed	6.62 (2.24; 19.6)	-	14.19 (5.09; 39.56)	-	0.81 (0.23; 2.81)	-
Sex: male vs. female[2]	1.15 (0.58; 2.28)	-	0.90 (0.44; 1.83)	-	1.08 (0.67; 1.73)	-
Weight-for-age Z-score at birth[3]	0.91 (0.64; 1.30)	-	1.01 (0.70; 1.46)	-	0.95 (0.74; 1.21)	-
Small-for-gestational age[4]	0.91 (0.31; 2.67)	0.92 (0.30; 2.78)	1.33 (0.49; 3.58)	1.38 (0.47; 4.05)	1.60 (0.84; 3.06)	1.92 (0.97; 3.80)
Maternal age ≥ 30 years[5]	1.73 (0.87; 3.43)	-	0.90 (0.43; 1.87)	-	1.61 (1.00; 2.59)	1.78 (1.09; 2.90)
Maternal education[6]	0.93 (0.46; 1.89)	1.08 (0.51; 2.29)	0.66 (0.31; 1.43)	0.74 (0.31; 1.77)	0.46 (0.26; 0.78)	0.40 (0.23; 0.70)
Maternal employment[7]	0.76 (0.37; 1.54)	-	0.96 (0.47; 1.96)	-	0.97 (0.60; 1.57)	-
Informal housing[8]	1.43 (0.71; 2.84)	-	1.62 (0.80; 3.31)	2.39 (1.06; 5.40)	1.14 (0.71; 1.82)	-
Planned pregnancy[9]	1.01 (0.48; 2.12)	-	0.94 (0.44; 2.03)	-	0.72 (0.42; 1.23)	-
Intimate partner violence[10]	1.17 (0.44; 3.13)	1.20 (0.42; 3.43)	3.34 (1.51; 7.40)	3.63 (1.43; 9.23)	1.21 (0.62; 2.38)	1.06 (0.51; 2.17)
Risky drinking[11]	0.64 (0.22; 1.86)	0.44 (0.14; 1.38)	2.09 (0.93; 4.67)	1.17 (0.45; 3.04)	1.55 (0.87; 2.78)	1.43 (0.74; 2.74)
Postpartum depression[12]	n/a	-	1.94 (0.42; 8.85)	-	1.14 (0.32; 4.07)	-
Breastfeeding duration (months)[13]	1.01 (0.95; 1.08)	1.02 (0.96; 1.09)	0.95 (0.89; 1.01)	0.97 (0.90; 1.04)	0.97(0.93; 1.02)	0.97 (0.93; 1.02)

[1] Based on expressive language only; [2] HIV exposure/infant sex interaction terms (multivariable models): cognitive, p=0.39; motor, p=0.23; language, p=0.85; [3] Intergrowth-21 reference standards; [4] <10th vs. > 10th percentile; [5] vs. <30 years of age; [6] completed vs. did not complete secondary schooling; [7] any employment vs. none; [8] vs. brick housing; [9] vs. unplanned pregnancy; [10] any physical, sexual or psychological violence reported at first antenatal visit vs. none; [11] Alcohol use disorders identification test (AUDIT-C) score >3 vs. <3, first antenatal visit; [12] EPDS (Edinburgh postnatal depression scale) score >=13 vs. <13 at 6 weeks' postpartum study visit; odds ratio for cognitive delay not calculable due to null cell; [13] Based on maternal self-report, last study visit date at which any breastfeeding was reported used as date of breastfeeding cessation

6.4.2 Motor development

HEU and HU children had similar mean motor scores [β 0.55 (95% CI -1.84; 2.95), tables 6.2 and 6.3], but HEU children were at higher odds for any motor delay [(OR 2.10 (95% CI 1.03; 4.28), table 6.4]. The latter association was attenuated (aOR 1.59, 95% CI 0.70; 3.64) after adjusting for several other significant predictors of motor development including gestational age, informal housing and IPV (table 6.4). There was evidence for interaction between HIV-exposure and gestational age (figure 6.2b). While term HEU children had similar odds of motor delay compared to the reference group of term HU children (aOR 1.17, 95% CI 0.45; 3.07), preterm delivery increased the odds of motor delay almost 5-fold among HU children (preterm HU vs. term HU: aOR 4.73, 95% CI 1.32; 16.91) while the combination of both HIV exposure and preterm delivery increased the odds 16-fold (preterm HEU vs. term HU: aOR 16.35, 95% CI 5.19; 51.54; figure 6.2b).

6.4.3 Language development

Overall, HEU children had an average 2.8 point higher composite language score than their HU counterparts (aβ 2.8; 95% CI 0.08; 5.59; tables 6.2 and 6.3). Compared to term HU, preterm HU were at higher odds of any language delay (aOR 2.49, 95% CI 1.00; 6.29) but the odds of delay were similar comparing either term HEU or preterm HEU to term HU (figure 6.2c).

Figure 6.2. Forest plots of adjusted odds ratios for developmental delay (BSID-III composite scores < 85) by maternal HIV status and preterm delivery with term HIV-unexposed children as reference category across (a) cognitive, (b) motor, and (c) language domains

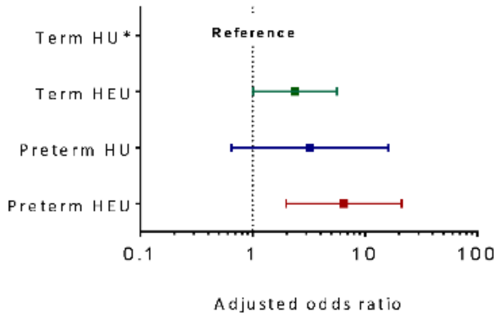


Figure 2 (a) Adjusted OR (95% CI) for cognitive delay in term HIV-exposed uninfected children, 2.52 (1.09; 5.83); preterm HIV-unexposed children, 3.30 (0.85; 12.78); and preterm HIV-exposed uninfected children, 8.25 (2.69; 25.28) [Reference group, term HIV-unexposed children; model adjusted for maternal education, intimate partner violence, risky drinking, infant size (small-for-gestational-age) and duration of breastfeeding; p-value for interaction = 0.15]

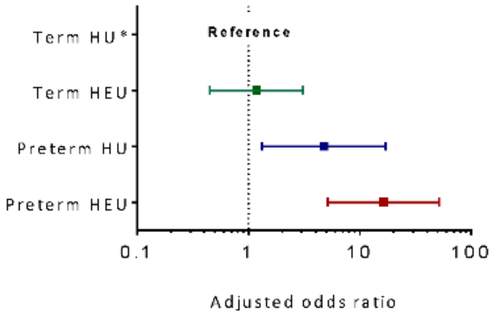


Figure 2 (b). Adjusted OR (95% CI) for motor delay in term HIV-exposed uninfected children, 1.17 (0.45; 3.07); preterm HIV-unexposed children, 4.73 (1.32; 16.91); and preterm HIV-exposed uninfected children, 16.35 (5.19; 51.54) [Reference group, term HIV-unexposed children; model adjusted for maternal education, housing, intimate partner violence, risky drinking, infant size (small-for-gestational-age) and duration of breastfeeding; *p*-value for interaction = 0.07]

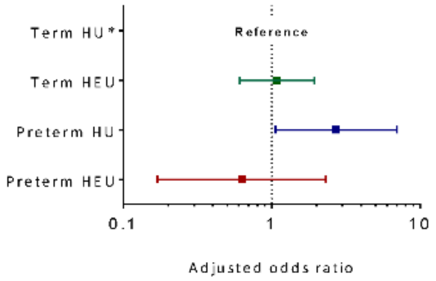
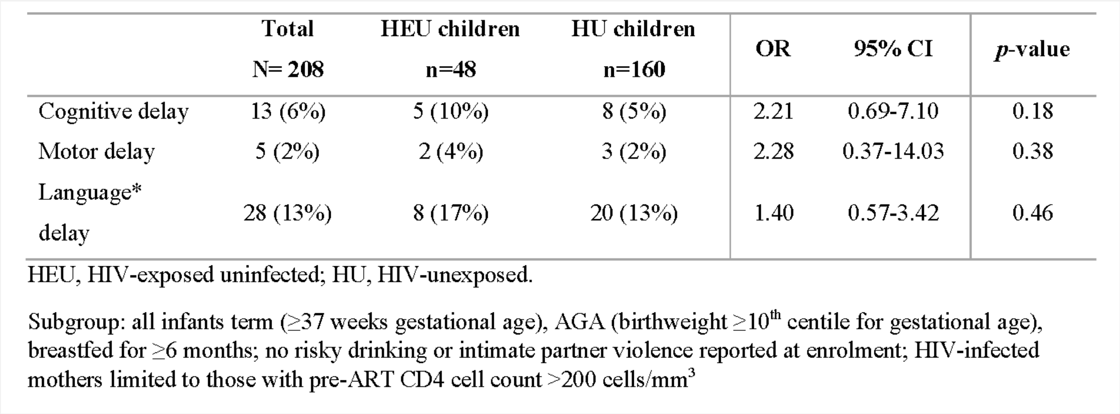


Figure 2 (c). Adjusted OR (95% CI) for language delay in term HIV-exposed uninfected children, 1.14 (0.65; 1.98); preterm HIV-unexposed children, 2.49 (1.00; 6.29); and preterm HIV-exposed uninfected children, 0.65 (0.18; 2.37). [Reference group, term HIV-unexposed children; model adjusted for maternal education, maternal age, intimate partner violence, risky drinking, infant size (small-for-gestational-age) and duration of breastfeeding; *p*-value for interaction = 0.04]

6.4.4 Sensitivity analyses

Point estimates for relative odds of any delay comparing “healthy” HEU (n=48) to similar HU (n=160) children approximated that of the full cohort for all three domains (table 6.5): OR (95% CI) for cognitive delay, 2.21 (0.69;7.10); motor delay, 2.28 (0.37;14.03); and language delay, 1.40 (0.57;3.42).

Table 6.5. Restricted logistic regression analysis comparing HIV-exposed uninfected to HIV-unexposed children: relative odds of delay across cognitive, motor, and language domains



	Total **N= 208**	**HEU children** **n=48**	**HU children** **n=160**	**OR**	**95% CI**	***p*-value**
Cognitive delay	13 (6%)	5 (10%)	8 (5%)	2.21	0.69-7.10	0.18
Motor delay	5 (2%)	2 (4%)	3 (2%)	2.28	0.37-14.03	0.38
Language* delay	28 (13%)	8 (17%)	20 (13%)	1.40	0.57-3.42	0.46

HEU, HIV-exposed uninfected; HU, HIV-unexposed.

Subgroup: all infants term (≥37 weeks gestational age), AGA (birthweight ≥10th centile for gestational age), breastfed for ≥6 months; no risky drinking or intimate partner violence reported at enrolment; HIV-infected mothers limited to those with pre-ART CD4 cell count >200 cells/mm^3

In exploratory subgroup analysis, the effects of HIV-exposure on child development varied somewhat within strata of various maternal-child characteristics (supplemental tables 9.6.6, 9.6.7, 9.6.8; and supplemental figures 9.6.2, 9.6.3 and 9.6.4)

6.5 Discussion

Compared to HIV-unexposed community controls, we observed increased odds of cognitive and motor, but not language, delay among young, breastfed HEU children born to women who initiated universal ART in pregnancy. Overall, median developmental scores of HEU children approximated those of HU children. That is, although the average scores of HEU and HU children were similar at a group level, there was an excess of minor deficits detectable among HEU children. Severe delays were scarce, and equally distributed between the groups.

Our findings are in keeping with results from several other studies, including a recent meta-analysis of developmental outcomes in young children with HIV-infected mothers.[3,10-12,14,15] Notably, this analysis included only one African study where mothers received ART during pregnancy.[3] Our findings contrast with some other recent African studies. A large cohort study in Botswana found no substantial differences between HEU and HU children at 24 months of age.[31] Maternal ART in pregnancy was restricted to women with low CD4 cell count (36% of all HIV-infected women); only 10% of HEU children were breastfed. However, living conditions were significantly better than in our cohort, and only 6% of HIV-infected women reported any prior alcohol use compared to almost 30% in ours. It may be that despite better access to ART and prolonged breastfeeding in our cohort, differences in socio-economic conditions and alcohol exposure disproportionately predisposed our HEU children towards developmental vulnerability. In a South African cohort (all mothers receiving

ART; 40% of HEU children breastfeeding by 2 weeks; similar living conditions and antenatal use of alcohol) no differences in mean BSID-III composite cognitive, motor or language scores were seen comparing HEU to HU children at 12 months of age.[9] However, a larger proportion of HEU than HU children had some evidence of developmental delay (composite score <85) in cognitive (HEU vs. HU, 9% vs. 0%) and language (HEU vs. HU, 28% vs. 18%) domains; precision was limited due to relatively small sample size.

We found no differences in language delay between HEU and HU children. However, language assessment in a multicultural setting is difficult, and the use of US-designed BSID-III language tests may not be optimal for language assessment in this setting. Reassuringly, average language scores in our cohort approximated those of the US reference group.[26] Nevertheless, assessments were conducted at a young age, when much reliance is on sounds rather than words or grammar, particularly in expressive language testing. As recently demonstrated among Kenyan HEU children, subtle differences in language development may only become detectable at an older age, underscoring the importance of repeated developmental assessments throughout childhood and adolescence.[32]

Taken together, these data indicate that breastfed HEU children born to women initiating universal ART in pregnancy may be at increased risk for some developmental delay, which is identifiable at a young age. However, delays appear to be in the mild-moderate range and associated with similar risk factors as neurodevelopmental delays in HU children.[33]

We observed a strong positive relationship between gestation at birth and neurodevelopment, reflecting findings from HIV-uninfected populations globally. [34] In our cohort, children born both preterm and HEU had the highest relative odds of motor and cognitive delay. Similar synergistic effects have been described among very preterm HIV-uninfected infants, with the highest risks of delay observed among those who were also SGA and had evidence of systemic inflammation.[35] These interaction effects can be explained by the so-called "two-hit" hypothesis, wherein intrauterine insult(s) increase vulnerability to later perinatal insults.[35,36] Our findings are particularly concerning given the known association between maternal HIV infection and preterm delivery, potentially compounded by maternal use of ART.[37,38]

There is biological plausibility for a relationship between maternal HIV infection and neurodevelopmental delay in HEU children. The immune system plays a critical role in brain development and homeostasis.[39] Neuroinflammation, including pathological microglial activation, may disrupt early brain development.[40,41] A growing body of evidence from HIV-unrelated epidemiological, preclinical and clinical studies points to antenatal maternal immune activation (mIA) as an important risk factor for offspring neurodevelopmental disorders.[40,41] Immune activation and inflammation are hallmarks of HIV infection itself; chronic inflammation can persist despite suppressive ART, particularly among those with microbial translocation and microbiome dysbiosis.[42,43] Additionally, maternal viral co-infections such as CMV typically exacerbate immune activation in both mothers and infants, while congenital CMV infection has direct effects on the developing brain. [44,45] *In utero* exposure to mIA may partly explain the pro-inflammatory immunological changes typically observed among HEU infants.[46] In animal models, perinatal neuroinflammation has consistently been associated with white matter damage.[47] Concordantly, two recent studies using diffusion tensor imaging described alterations in white matter when comparing otherwise healthy HEU and HU children.[48,49] White matter changes are also typical of perinatal brain injury in preterm infants, with the worst injuries described among those who also had *in utero* exposure to mIA.[47,50] Thus in HEU children, particularly those born to women with viral co-infections and/or altered microbiota, neuroinflammation may be a mechanism of developmental delay, and further research is required to better understand these and other related causal pathways.

To our knowledge, this is the first large study of neurodevelopment among young, breastfed HEU children who were all born to relatively healthy women initiating universal ART in pregnancy. Unlike many of the large, US-based studies, our cohort was homogenous in the use of a single WHO first-line ART regimen.[51] In addition, we were able to obtain detailed longitudinal measures of several major determinants of developmental outcomes in early childhood, with a large group of community-control HU comparators sampled and followed using the same methodology. We used a comprehensive, robust and validated measuring tool, supported by demonstration of reliability in quality assurance. Nonetheless, our findings need to be interpreted in the light of several limitations. Without measures of maternal-infant inflammation and viral co-infections we were unable to assess underlying causal mechanisms. Our inferences on language development are limited by the lack of receptive language measures. We assessed development cross-sectionally, among a subgroup of HEU children who were still in follow-up a year after birth, and whose mothers were willing to return for the assessment. All women received good perinatal care including ART for those with HIV infection, the majority of whom achieved viral suppression before delivery. Furthermore, all children in our cohort were breastfed; breastfeeding promotes neurodevelopment [52]. As such, our findings may underestimate differences between HEU and HU children in less fortunate settings. Simultaneously, our findings may not extend to populations with lower levels of antenatal alcohol use and IPV.

HEU children are vulnerable, at least partly due to social determinants of disease that cluster with maternal HIV infection, but possibly also via exposure to maternal HIV. Although our data adds significantly to the knowledge base of HEU child development at a young age, little is known about the long-term effects of *in utero* exposure to maternal HIV in the context of universal ART and breastfeeding. As such, continued follow-up and assessment throughout childhood and adolescence will be critical. Finally, our data highlight challenging environments for many families in settings such as ours, including those of HIV-uninfected women and their children. Without effectively addressing the broader social determinants of health, efforts to improve childhood developmental trajectories in resource-limited settings are unlikely to succeed.

6.6 Conclusions

Despite universal ART during pregnancy and breastfeeding, HEU children may be at increased risk of cognitive and motor delays. Early developmental screening and intervention programs are clearly warranted for this growing group of vulnerable children, prioritizing those born preterm. Data are required on neurodevelopment of HEU children born to women who initiated suppressive ART prior to conception.

6.7 Acknowledgements

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SLR was responsible for implementation and management of the HU2 study, assisted with collection of data, conducted the analyses, and wrote the first draft of the manuscript. KD provided training and supervision of developmental assessments; KD and MK provided supervision for all child health aspects of the study. KB and TKP were responsible for data management and oversight. TKP and KN were the study coordinators. AZ was the senior study manager and provided oversight of all study administration processes. AS conducted developmental assessments and assisted with training and data management. LM and EJA conceived the MCH-ART study, and were responsible for study design, funding, implementation, and overall leadership. All authors contributed to and approved the final manuscript.

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Chapter 7

HIV viraemia during pregnancy and neurodevelopment of HIV-exposed uninfected children in the context of universal antiretroviral therapy and breastfeeding: a prospective cohort study

Stanzi M le Roux, Kirsten A Donald, Max Kroon, Tamsin K Phillips, Maia Lesosky, Liza Esterhuyse, Allison Zerbe, Kirsty Brittain, Elaine J Abrams, and Landon Myer. **HIV Viremia During Pregnancy and Neurodevelopment of HIV-Exposed Uninfected Children in the Context of Universal Antiretroviral Therapy and Breastfeeding: A Prospective Study.** *Pediatr Infect Dis J* 2019; **38** (1): 70-5. DOI: 10.1097/inf.0000000000002193

Relevance of this paper to the thesis

The previous chapter (6) summarized evidence of differential risks of developmental delay comparing HIV-exposed uninfected (HEU) to HIV-unexposed uninfected (HU) children in this research study. A key HEU child health determinant is maternal HIV disease severity during pregnancy, but no prior published data exist on the impact of maternal HIV disease severity specifically on developmental outcomes. The primary focus of this paper is therefore the relationship between maternal HIV viraemia (modelled in viraemia copy-years, a measure that reflects both duration and burden of elevated HIV viral load), and developmental delay among HEU children. This chapter therefore addresses study objective 4 (b).

Contribution of the student and co-authors

SLR conceptualized and implemented this nested evaluation of developmental outcomes among HEU and HU children under guidance of KAD, EJA and LM. As the lead investigator on HEU child health outcomes in the larger MCH-ART study, SLR was responsible for quality assurance and clinical oversight; she assisted with developmental assessments in conjunction with LE. The candidate conducted the analysis with assistance from ML; wrote the first draft of the manuscript; and is the corresponding author. LM and EJA conceived the MCH-ART study, and were responsible for overall study design, funding, implementation, and primary leadership; KD and MK provided supervision for all child health aspects of the study. TKP was the study coordinator; TKP and KB were responsible for data management; and AZ was the senior study manager. All authors contributed to and approved the final manuscript.

7.1 Abstract

7.1.1 Background

Elevated HIV viral load (HIV-VL) in pregnancy has been linked to increased risk of mortality, immunological abnormalities, infectious morbidity, and restricted growth among HIV-exposed uninfected (HEU) children, but little is known about effects on child development.

7.1.2 Methods

HIV-infected women initiating lifelong ART (tenofovir+emtricitabine+efavirenz) antenatally were followed from first antenatal visit through delivery and with their breastfed infants postpartum. Cognitive, motor, and expressive language development (Bayley Scales of Infant and Toddler Development, BSID-III; delay defined as score <85) were assessed on a subset of HEU infants. HIV-VL was measured at ART initiation, in third trimester and around delivery. Cumulative viraemia in pregnancy was expressed as $\log_{10}$ VL copies x year/mL (viraemia copy-years, VCY). Relationships between VCY and development were examined after adjusting for socio-economic, behavioural and psychosocial confounders.

7.1.3 Results

Women (median pre-ART $\log_{10}$ VL 4.1, CD4 349 cells/mm^3) commonly reported adverse social circumstances (44% informal housing, 63% unemployed, 29% risky drinking). Among 214 infants (median age 13 months; 53% male; 13% born <37 weeks' gestation), viraemia predicted lower motor and expressive language, but not cognitive, scores in crude and adjusted analysis [per $\log_{10}$ VCY increase, aβ (95%CI): motor, -2.94 (-5.77; -0.11); language, -3.71 (-6.73; -0.69) and cognitive-2.19 (-5.02; 0.65)]. Increasing VCY also predicted higher relative odds of motor delay [adjusted odds ratio, aOR 3.32 (95% CI 1.36; 8.14)] and expressive language delay [aOR 2.79 (95% CI 1.57; 4.94), but not cognitive delay [aOR 1.68 (0.84; 3.34)].

7.1.4 Conclusions

Cumulative maternal HIV viraemia in pregnancy may have adverse implications for HEU child development.

7.2 Introduction

Elevated HIV viral load in pregnancy has been linked to increased risk of mortality, immunological abnormalities, infectious morbidity and restricted growth among HIV-exposed uninfected (HEU) children globally.[1,2] However, few data are available on the relationship between antepartum maternal HIV viraemia and neurodevelopment of HEU children[3,4], and little is known about this putative relationship in the context of breastfeeding with universal triple-drug antiretroviral therapy (ART).[5] In a study of HIV-infected women and their children living in Cape Town, South Africa, we investigated the relationship between antepartum HIV viraemia and neurodevelopmental outcomes of HEU children in the context of universal maternal ART initiated in pregnancy.[6]

7.3 Methods

We conducted developmental assessments in HEU children enrolled into the Maternal and Child Health Antiretroviral Therapy study (MCH-ART), a large multi-phase implementation science study evaluating strategies to optimize postpartum ART services for women and children. Study methodology for the main study has been published previously.[5,7] Briefly, pregnant HIV-infected women initiating universal ART (tenofovir+emtricitabine+efavirenz) were followed prospectively through delivery and with breastfeeding children until approximately 1 year of age. According to local guidelines at the time, all infants received daily nevirapine for a minimum of 6 weeks; those considered at high risk of vertical transmission also received zidovudine for 4 weeks.[8] Trained interviewers administered standardized instruments assessing physical health, socio-economic and psychosocial factors throughout antenatal (1-3 visits) and postnatal (up to 5 visits) study follow-up.[6] Maternal blood was collected for batched HIV viral load testing at each visit (Abbot Realtime HIV-1; Abbott Laboratories, Waltham, MA), including at ART initiation, at least once during the third trimester and within 2 weeks before or after delivery. Birth anthropometry and other clinical information were abstracted from medical records. Repeated HIV-PCR (CAP/CTM v 2.0, Roche Molecular Systems, Inc., Branchberg, NJ) testing excluded infection among children.

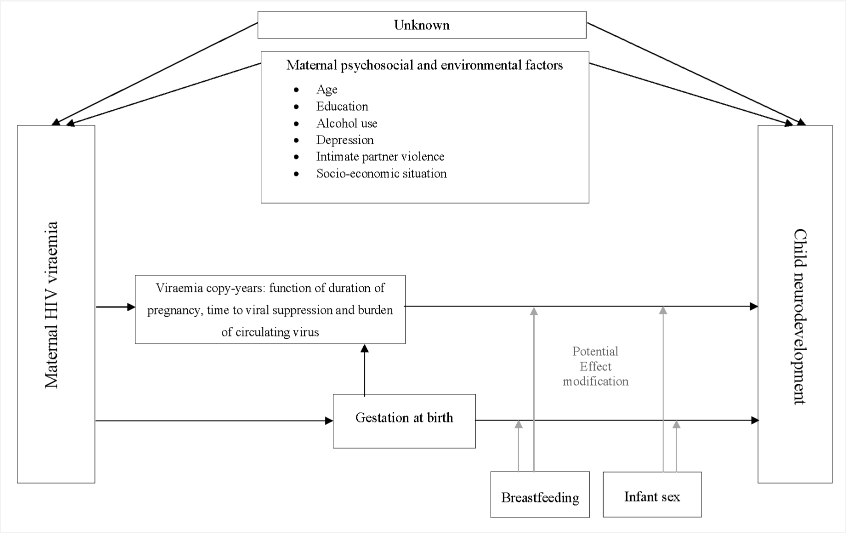
Mothers of eligible children (HIV-uninfected, ≥28 weeks' gestation at birth without known congenital abnormalities or cerebral palsy) were approached for enrolment into the neurodevelopmental sub-study when the children were approximately 13 months (window, 11-18 months) old. Following informed consent, cognitive, expressive language, fine and gross motor development were tested using the Bayley Scales of Infant and Toddler Development, 3rd edition (BSID-III, PsychCorp, 2006). The developmental assessment team, comprising three paediatric occupational therapists and one child health physician, received systematic supervised training in the use of the BSID-III by a developmental paediatrician and were assisted by a trained, isi-Xhosa-speaking counsellor. Ten assessments were video-graphed for independent blinded scoring as a quality assurance exercise. Interrater agreement was high, with correlation coefficients above 0.9 for cognitive and motor scores and ranging from 0.7 to 0.98 for expressive language scores. There was perfect agreement for “any” vs. “no delay” classifications across domains. Composite cognitive and motor scores were generated from cognitive, fine and gross motor subtest scaled scores using BSID-III normative and conversion tables, which account for gestation at delivery (adjusting for preterm birth) and child age at time of assessment.[9] Receptive language testing proved contextually challenging, necessitating a focus on expressive language only. Expressive language subtest scaled scores were transformed to have a similar distribution to composite scores (expected mean 100, standard deviation 15). Untransformed expressive language subtest scaled scores were analyzed separately in sensitivity analysis.

MCH-ART was approved by Columbia University Medical Centre Institutional Review Board and University of Cape Town’s Faculty of Health Sciences Research Ethics Committee (Clinicaltrials.gov NCT01933477)

7.3.1 Statistical methods

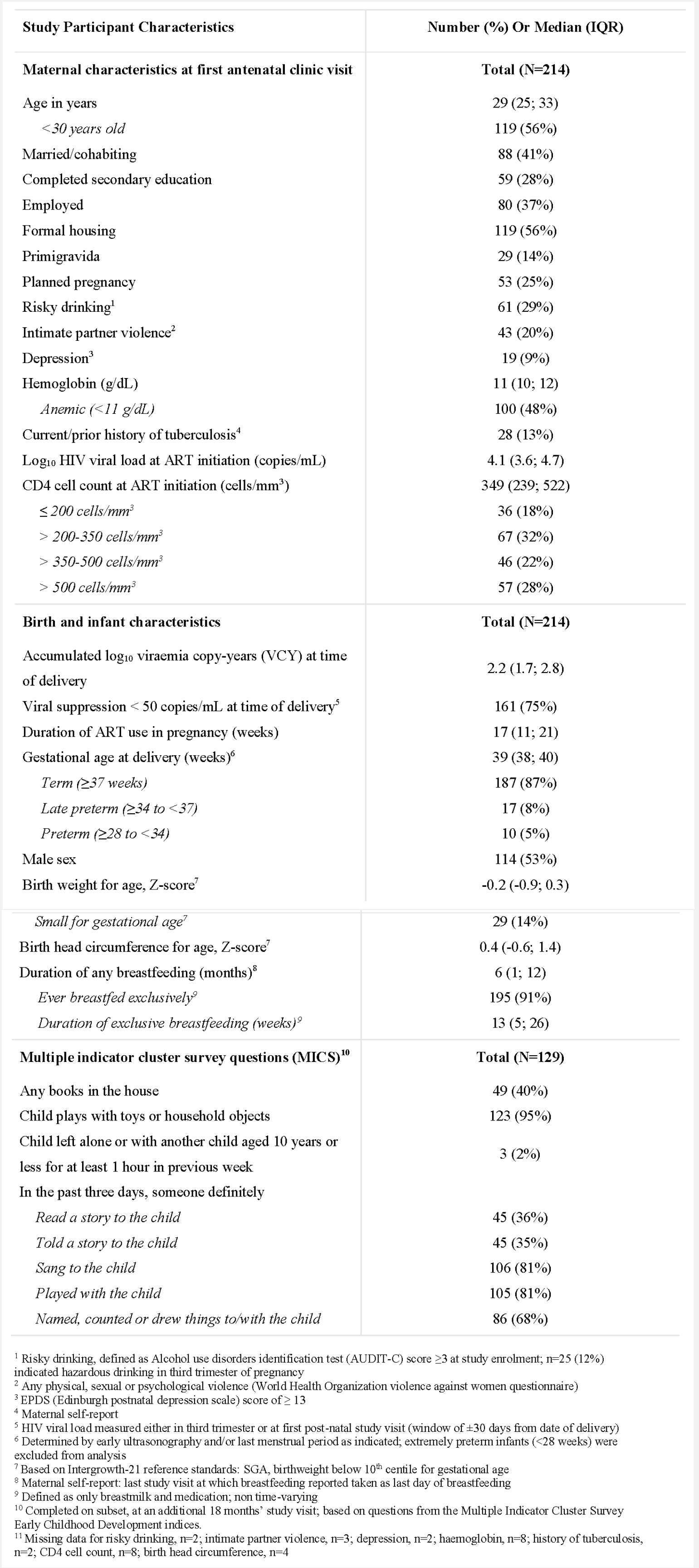
Cognitive, motor, and expressive language scores [expected mean (standard deviation, SD) of 100 (15)] were modeled separately in continuous and binary form. Based on international recommendations, “any delay” was defined *a priori* as composite score <85 (vs. ≥85, “no delay”).[10] Viraemia during pregnancy was expressed as $\log_{10}$ viral copies X year/mL (viraemia copy-years, VCY), a cumulative measure accounting for both burden and duration of viraemia.[11] For calculation of VCY, a minimum of two viral load measures was required; missing data were assumed to be missing at random. A review of childhood neurodevelopmental literature informed the choice of potential third variables, guided by a directed acyclic graph developed *a priori* (Figure 7.1).[12-14] Socio-economic, psychosocial and behavioural measures were evaluated during model building; those

Figure 7.1 Simplified directed acyclic graph: maternal HIV viraemia and neurodevelopment of HIV-exposed uninfected children



that improved model fit (based on Akaike's Information Criterion, AIC) were retained in the final model. Maternal history of current/prior tuberculosis was chosen as clinical indicator for symptomatic HIV disease, as this is the most common stage III condition in our context.[15] Preterm birth (gestation <37 completed weeks) was considered as both potential mediator (potentially on causal pathway between viraemia and development) and confounder (earlier delivery resulting in shorter duration of fetal exposure to viraemia) in separate models. Analyses (Stata 14.0, StataCorp, College Station Tx) used linear and logistic regression; point estimates are presented with 95% confidence intervals (CI).

Table 7.1 Maternal and child characteristics



Study Participant Characteristics	**Number (%) Or Median (IQR)**
Maternal characteristics at first antenatal clinic visit	**Total (N=214)**
Age in years	29 (25; 33)
<30 years old	119 (56%)
Married/cohabiting	88 (41%)
Completed secondary education	59 (28%)
Employed	80 (37%)
Formal housing	119 (56%)
Primigravida	29 (14%)
Planned pregnancy	53 (25%)
Risky drinking[1]	61 (29%)
Intimate partner violence[2]	43 (20%)
Depression[3]	19 (9%)
Hemoglobin (g/dL)	11 (10; 12)
Anemic (<11 g/dL)	100 (48%)
Current/prior history of tuberculosis[4]	28 (13%)
Log$_{10}$ HIV viral load at ART initiation (copies/mL)	4.1 (3.6; 4.7)
CD4 cell count at ART initiation (cells/mm^3)	349 (239; 522)
≤ 200 cells/mm^3	36 (18%)
> 200-350 cells/mm^3	67 (32%)
> 350-500 cells/mm^3	46 (22%)
> 500 cells/mm^3	57 (28%)
Birth and infant characteristics	**Total (N=214)**
Accumulated log$_{10}$ viraemia copy-years (VCY) at time of delivery	2.2 (1.7; 2.8)
Viral suppression < 50 copies/mL at time of delivery[5]	161 (75%)
Duration of ART use in pregnancy (weeks)	17 (11; 21)
Gestational age at delivery (weeks)[6]	39 (38; 40)
Term (≥37 weeks)	187 (87%)
Late preterm (≥34 to <37)	17 (8%)
Preterm (≥28 to <34)	10 (5%)
Male sex	114 (53%)
Birth weight for age, Z-score[7]	-0.2 (-0.9; 0.3)
Small for gestational age[7]	29 (14%)
Birth head circumference for age, Z-score[7]	0.4 (-0.6; 1.4)
Duration of any breastfeeding (months)[8]	6 (1; 12)
Ever breastfed exclusively[9]	195 (91%)
Duration of exclusive breastfeeding (weeks)[9]	13 (5; 26)
Multiple indicator cluster survey questions (MICS)[10]	**Total (N=129)**
Any books in the house	49 (40%)
Child plays with toys or household objects	123 (95%)
Child left alone or with another child aged 10 years or less for at least 1 hour in previous week	3 (2%)
In the past three days, someone definitely	
Read a story to the child	45 (36%)
Told a story to the child	45 (35%)
Sang to the child	106 (81%)
Played with the child	105 (81%)
Named, counted or drew things to/with the child	86 (68%)

[1] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 at study enrolment; n=25 (12%) indicated hazardous drinking in third trimester of pregnancy
[2] Any physical, sexual or psychological violence (World Health Organization violence against women questionnaire)
[3] EPDS (Edinburgh postnatal depression scale) score of ≥ 13
[4] Maternal self-report
[5] HIV viral load measured either in third trimester or at first post-natal study visit (window of ±30 days from date of delivery)
[6] Determined by early ultrasonography and/or last menstrual period as indicated; extremely preterm infants (<28 weeks) were excluded from analysis
[7] Based on Intergrowth-21 reference standards: SGA, birthweight below 10th centile for gestational age
[8] Maternal self-report: last study visit at which breastfeeding reported taken as last day of breastfeeding
[9] Defined as only breastmilk and medication; non time-varying
[10] Completed on subset, at an additional 18 months' study visit; based on questions from the Multiple Indicator Cluster Survey Early Childhood Development indices.
[11] Missing data for risky drinking, n=2; intimate partner violence, n=3; depression, n=2; haemoglobin, n=8; history of tuberculosis, n=2; CD4 cell count, n=8; birth head circumference, n=4

7.4 Results

At ART initiation, most mothers (n=214; median age 29 years) reported poor socio-economic status (SES) with multiple psychosocial stressors (Figure 7.2, Table 7.1): 63% were unemployed; 44% living in informal housing; 72% did not complete high school, with high prevalence of risky drinking (29%) and intimate partner violence (IPV, 20%). Median (interquartile range, IQR) pre-ART CD4 count was 349 (239-522) cells/mm^3 and log$_{10}$ HIV viral load, 4.1 (3.6-4.7). Overall, 999 viral load measures were available for calculation of antepartum VCY, with most women (82%) having five viral load measures. Six women only had 2 values. At time of delivery, the median accumulated $\log_{10}$ VCY was 2.2 (IQR 1.7-2.8); most women (75%) had achieved viral suppression <50 copies/mL (Table 1). Of 214 HEU children [median age 13 (IQR 13-14) months; 53% male], 13% were born preterm with median gestation at birth 39 (IQR 38-40) weeks. The median duration of breastfeeding was 6 (IQR 1-12) months (Table 1).

7.4.1 Differences between children with and without developmental assessments

There were limited differences by completion of developmental assessment. Overall, mother-child pairs who attended study follow-up through 12 months (vs. those who discontinued follow-up prior to 12 months) were older (mean age 29 vs. 27 years), with somewhat higher $\log_{10}$ HIV viral load at ART initiation (median, 4.0 vs. 3.8); similar proportions achieved viral suppression by delivery (77% vs. 72%). Among all mother-child pairs who completed 12 months of follow-up, those who also completed developmental assessments (vs. those who did not) had similar age and psychosocial characteristics but lived in better socio-economic circumstances. Median $\log_{10}$ HIV viral load results were similar at ART initiation (4.1 vs. 4.0); similar proportions achieved viral suppression by delivery (75% vs. 78%). There were no differences in history of tuberculosis, CD4 cell count at ART initiation or duration of breastfeeding.

7.4.2 Developmental outcomes

Overall, mean (SD) scores of HEU children approximated the BSID-III reference standards: cognitive, 101 (15); motor, 99 (15); and expressive language, 100 (17).[9] In crude linear regression, VCY [median 2.2 (IQR 1.7; 2.8)] predicted lower expressive language and motor scores [β (95%CI): -2.82 (-5.60; -0.04) and -3.71 (-6.60; -0.83), respectively], with limited effect on cognitive score [β -1.87 (95%CI: -4.59; 0.84); Table 7.2]. Estimates did not change appreciably after adjusting for multiple confounders; similar associations were seen using other indicators of maternal disease severity in pregnancy (Table 7.2). In sensitivity analysis, addition of nadir CD4 cell count and history of tuberculosis decreased precision without changing associations (Table 7.2, footnotes). Similar results were seen in analysis restricted to full-term children: per $\log_{10}$ increase in VCY, aβ (95%CI) for motor, -1.75 (-4.63; 1.13); expressive language, -3.38 (-6.59; -0.17) and cognitive scores, -1.19 (-4.14; 1.76).

Table 7.2 Relationships between maternal viraemia and other indicators of HIV disease severity in pregnancy and composite cognitive, motor and language scores: absolute differences from linear regression models

Variable	Composite cognitive score[10, 11]		Composite motor score[10, 11]		Expressive language* score[10, 11]	
	β (95% CI)	aβ (95% CI)	β (95% CI)	aβ (95% CI)	β (95% CI)	aβ (95% CI)
Maternal variables						
Log_{10} viral copies x year/mL (VCY)	-1.87 (-4.59; 0.84)	-2.19 (-5.02; 0.65)	-2.82 (-5.60; -0.04)	-2.94 (-5.77; -0.11)	-3.71 (-6.60; -0.83)	-3.71 (-6.73; -0.69)
Log_{10} HIV viral load, ART initiation	-1.96 (-4.30;0.38)	-	-2.91 (-5.30; -0.52)	-	-3.22 (-5.70; -0.73)	-
HIV VL ≥50 copies/mL vs. <50, delivery	-1.10 (-5.84; 3.64)	-	-5.14 (-9.98; -0.30)	-	-2.90 (-8.08; 2.28)	-
HIV VL ≥1000 copies/mL vs. <1000, delivery	-2.90 (-11.78; 5.98)	-	-5.89 (-14.99; 3.21)	-	0.10 (-9.64; 9.84)	-
Nadir CD4 cell count (cells/mm^3)	0.01 (0.00; 0.02)	-	0.01 (-0.004; 0.02)	-	0.01 (0.002; 0.02)	-
Nadir CD4 cell count categories:						
<200	Ref	-	Ref	-	Ref	-
200 – 349	0.92 (-5.28; 7.11)	-	1.44 (-5.10; 7.98)	-	4.85 (-1.94; 11.65)	-
350 – 499	-1.15 (-7.82; 5.53)	-	2.23 (-4.85; 9.32)	-	3.55 (-3.76; 10.87)	-
≥ 500	3.90 (-2.52; 10.33)	-	4.12 (-2.67; 10.91)	-	8.98 (1.98; 15.98)	-
Current/prior tuberculosis vs. none	-3.20 (-9.28; 2.88)	-	-5.49 (-11.81; 0.82)	-	-3.01 (-9.66; 3.64)	-
Hemoglobin (g/dL)	0.65 (-1.09; 2.39)	-	1.47 (-0.33; 3.27)	-	1.38 (-0.53; 3.30)	-
Anemic (<11 g/dL vs ≥11 g/dL)	-0.97 (-5.18; 3.24)	-	-2.19 (-6.55; 2.17)	-	-1.85 (-6.48; 2.77)	-
Maternal age (years)	0.30 (-0.05; 0.66)	-	0.24 (-0.13; 0.61)	-	0.24 (-0.15; 0.63)	-
Age ≥ 30 years (vs. <30)	2.01 (-2.11; 6.14)	1.33 (-2.94;5.61)	2.29 (-2.00; 6.57)	1.15 (-3.18; 5.48)	1.28 (-3.22; 5.79)	0.04 (-4.62; 4.69)
Poverty score [1]	1.25 (-0.63; 3.13)	1.23 (-0.76; 3.23)	1.81 (-0.14; 3.75)	2.11 (0.09; 4.13)	0.27 (-1.80; 2.33)	-0.21 (-2.38; 1.97)
Education [2]	0.57 (-4.04; 5.17)	0.02 (-4.80; 4.83)	-0.43 (-5.21; 4.35)	-1.58 (-6.46; 3.30)	3.31 (-1.69; 8.31)	3.98 (-1.23; 9.20)
Depression [3]	-2.85 (-10.07; 4.36)	-	-2.30 (-9.72; 5.13)	-	-6.64 (-14.50; 1.22)	-
Intimate partner violence [4]	-0.16 (-5.35; 5.04)	0.06 (-5.33; 5.45)	-4.21 (-9.52; 1.10)	-3.37 (-8.76; 2.02)	1.66 (-3.96; 7.27)	3.06 (-2.75; 8.89)
Risky drinking [5]	0.13 (-4.44; 4.69)	0.79 (-4.00; 5.58)	-3.63 (-8.38; 1.13)	-1.65 (-6.52; 3.23)	-2.07 (-7.06; 2.91)	-2.27 (-7.49; 2.94)
Infant variables						
Gestation at delivery (weeks) [6]	0.95 (0.05; 1.85)	1.11 (0.17; 2.04)	1.27 (0.31; 2.23)	1.46 (0.48; 2.44)	-0.08 (-1.07; 0.91)	0.10 (-0.91; 1.11)
Preterm (<37 weeks) vs. term	-2.76 (-0.92; 3.39)	-	-4.83 (-11.34; 1.68)	-	1.92 (-4.82; 8.67)	-
Infant sex: male vs. female	1.30 (-2.81; 5.41)	-	1.45 (-2.82; 5.72)	-	-0.71 (-5.20; 3.79)	-
Small-for-gestational age [7]	-2.72 (-8.69; 3.25)	-	-0.69 (-7.03; 5.64)	-	-0.28 (-6.83; 6.27)	-
Duration of any breastfeeding (months) [8]	0.16 (-0.17; 0.49)	-	0.27 (-0.08; 0.62)	-	0.17 (-0.19; 0.54)	-

*Language score based on expressive language sub-scale only; Abbreviations – BSID-III, Bayley Scales of Infant and Toddler Development Third Edition; ART, antiretroviral therapy; Hb, haemoglobin; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; VCY, viral copy-years (cumulative measure of duration and severity of HIV viraemia during pregnancy)
Except where otherwise indicated, maternal variables represent measures taken at first antenatal clinic booking, prior to ART initiation

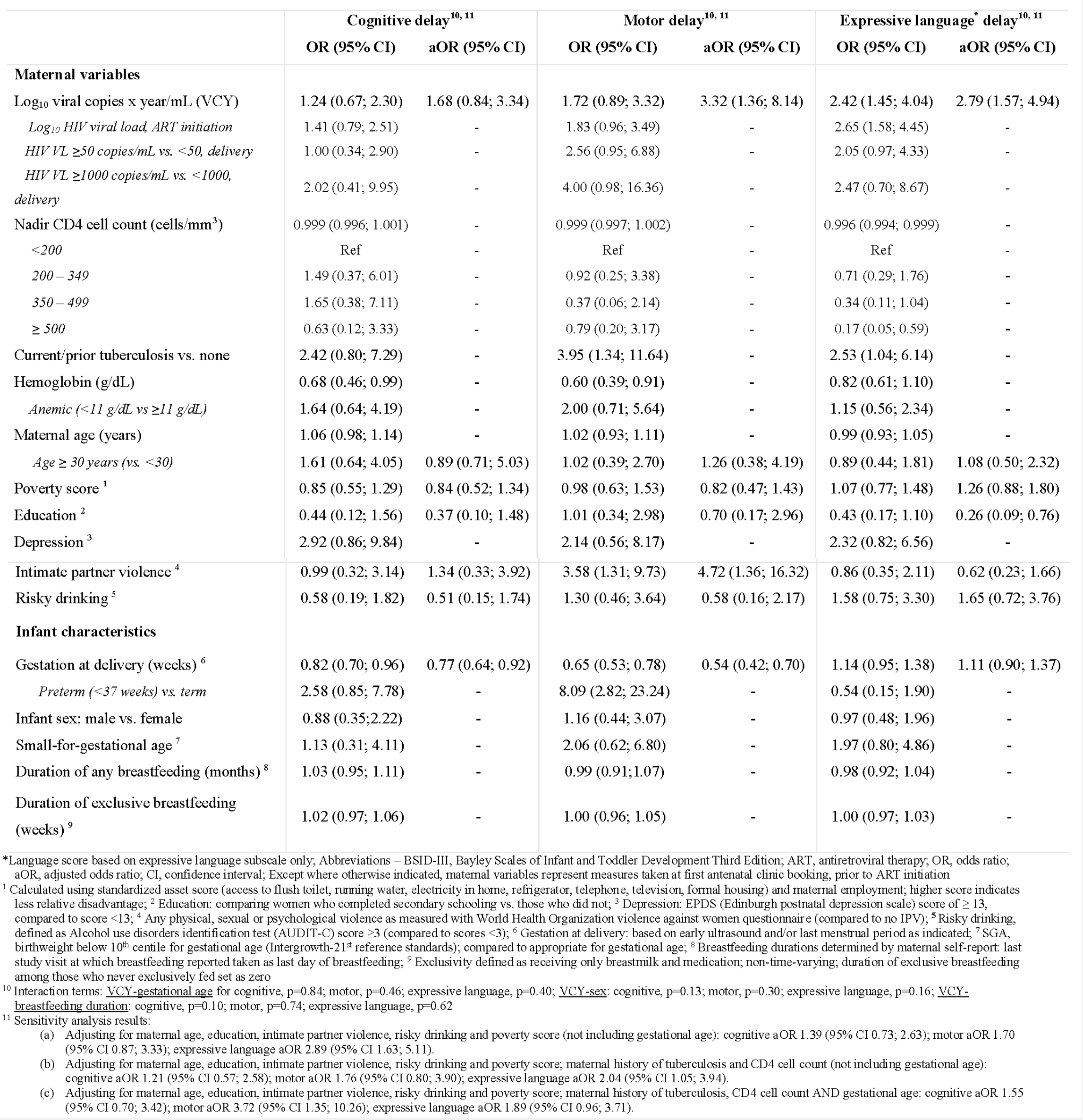
[1] Calculated using standardized asset score (access to flush toilet, running water, electricity in home, refrigerator, telephone, television, formal housing) and maternal employment; higher score indicates less relative disadvantage; [2] Education: comparing women who completed secondary schooling vs. those who did not;[3] Depression: EPDS (Edinburgh postnatal depression scale) score of ≥ 13, vs. score <13; [4] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire (compared to no reported violence); [5] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 (compared to scores <3); [6] Gestation at delivery: based on early ultrasound and/or last menstrual period as indicated; [7] SGA, birthweight below 10th centile for gestational age (Intergrowth-21st reference standards); compared to appropriate for gestational age; [8] Breastfeeding durations determined by maternal self-report: last study visit at which breastfeeding reported taken as last day of breastfeeding;

[9] Interaction terms (models adjusted for maternal age, education, intimate partner violence, risky drinking, poverty score and gestation): VCY-gestational age for cognitive, p=0.67; motor, p=0.67; expressive language, p=0.93; VCY-sex: cognitive, p=0.16; motor, p=0.40; expressive language, p=0.75; VCY-breastfeeding duration: cognitive, p=0.73; motor, p=0.13; expressive language, p=0.65

[10] Sensitivity analysis results:
(a) Adjusting for maternal age, education, intimate partner violence, risky drinking and poverty score (not including gestational age): cognitive aβ -1.63 (95% CI -4.46; 1.19); motor aβ -2.25 (95% CI -5.10; 0.60); expressive language aβ -3.66 (95% CI -6.65; -0.68).
(b) Adjusting for maternal age, education, intimate partner violence, risky drinking and poverty score; maternal history of tuberculosis and CD4 cell count (not including gestational age): cognitive aβ -0.90 (95% CI -4.17; 2.36); motor aβ -1.60 (95% CI -5.00; 1.81); expressive language aβ -2.54 (95% CI -6.09; 1.00).
(c) Adjusting for maternal age, education, intimate partner violence, risky drinking and poverty score; maternal history of tuberculosis, CD4 cell count AND gestational age: cognitive aβ -1.32 (95% CI -4.65; 2.01); motor aβ -2.36 (95% CI -5.75; 1.03); expressive language aβ -2.85 (95% CI -6.17; 1.01).

Increasing VCY was persistently associated with higher relative odds of motor and expressive language delay [aOR (95% CI): 3.32 (1.36; 8.14) and 2.79 (1.57; 4.94), respectively], but not with cognitive delay (Table 7.3). In sensitivity analysis, estimates and precision did not change significantly following further adjustment for CD4 cell count and history of tuberculosis (Table 7.3, footnotes). Increasing gestational age was protective against cognitive and motor, but not expressive language, delay (Table 7.3). Results from analysis restricted to term-born children (n=183) approximated those for the full cohort albeit with somewhat lower precision. Adjusting for maternal age, socio-economic status, IPV and maternal alcohol use, increasing VCY was association with increased relative odds of motor delay [aOR 2.55 (95% CI 0.99; 6.61)] and expressive language delay [aOR 2.62 (95% CI1.45; 4.75)], but not cognitive delay [aOR 1.46 (95% CI 0.70; 3.06)] in HEU children born at term. Inferences were unchanged in analyses using subtest scaled scores.

Table 7.3 Logistic regression analysis of odds of delay (BSID-III score <85) for cognitive, motor, and expressive language domains among HIV-exposed uninfected children, by maternal HIV viraemia in pregnancy



	Cognitive delay[10, 11]		Motor delay[10, 11]		Expressive language* delay[10, 11]	
	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)
Maternal variables						
Log$_{10}$ viral copies x year/mL (VCY)	1.24 (0.67; 2.30)	1.68 (0.84; 3.34)	1.72 (0.89; 3.32)	3.32 (1.36; 8.14)	2.42 (1.45; 4.04)	2.79 (1.57; 4.94)
Log$_{10}$ HIV viral load, ART initiation	1.41 (0.79; 2.51)	-	1.83 (0.96; 3.49)	-	2.65 (1.58; 4.45)	-
HIV VL ≥50 copies/mL vs. <50, delivery	1.00 (0.34; 2.90)	-	2.56 (0.95; 6.88)	-	2.05 (0.97; 4.33)	-
HIV VL ≥1000 copies/mL vs. <1000, delivery	2.02 (0.41; 9.95)	-	4.00 (0.98; 16.36)	-	2.47 (0.70; 8.67)	-
Nadir CD4 cell count (cells/mm^3)	0.999 (0.996; 1.001)	-	0.999 (0.997; 1.002)	-	0.996 (0.994; 0.999)	-
<200	Ref	-	Ref	-	Ref	-
200 – 349	1.49 (0.37; 6.01)	-	0.92 (0.25; 3.38)	-	0.71 (0.29; 1.76)	-
350 – 499	1.65 (0.38; 7.11)	-	0.37 (0.06; 2.14)	-	0.34 (0.11; 1.04)	-
≥ 500	0.63 (0.12; 3.33)	-	0.79 (0.20; 3.17)	-	0.17 (0.05; 0.59)	-
Current/prior tuberculosis vs. none	2.42 (0.80; 7.29)	-	3.95 (1.34; 11.64)	-	2.53 (1.04; 6.14)	-
Hemoglobin (g/dL)	0.68 (0.46; 0.99)	-	0.60 (0.39; 0.91)	-	0.82 (0.61; 1.10)	-
Anemic (<11 g/dL vs ≥11 g/dL)	1.64 (0.64; 4.19)	-	2.00 (0.71; 5.64)	-	1.15 (0.56; 2.34)	-
Maternal age (years)	1.06 (0.98; 1.14)	-	1.02 (0.93; 1.11)	-	0.99 (0.93; 1.05)	-
Age ≥ 30 years (vs. <30)	1.61 (0.64; 4.05)	0.89 (0.71; 5.03)	1.02 (0.39; 2.70)	1.26 (0.38; 4.19)	0.89 (0.44; 1.81)	1.08 (0.50; 2.32)
Poverty score [1]	0.85 (0.55; 1.29)	0.84 (0.52; 1.34)	0.98 (0.63; 1.53)	0.82 (0.47; 1.43)	1.07 (0.77; 1.48)	1.26 (0.88; 1.80)
Education [2]	0.44 (0.12; 1.56)	0.37 (0.10; 1.48)	1.01 (0.34; 2.98)	0.70 (0.17; 2.96)	0.43 (0.17; 1.10)	0.26 (0.09; 0.76)
Depression [3]	2.92 (0.86; 9.84)	-	2.14 (0.56; 8.17)	-	2.32 (0.82; 6.56)	-
Intimate partner violence [4]	0.99 (0.32; 3.14)	1.34 (0.33; 3.92)	3.58 (1.31; 9.73)	4.72 (1.36; 16.32)	0.86 (0.35; 2.11)	0.62 (0.23; 1.66)
Risky drinking [5]	0.58 (0.19; 1.82)	0.51 (0.15; 1.74)	1.30 (0.46; 3.64)	0.58 (0.16; 2.17)	1.58 (0.75; 3.30)	1.65 (0.72; 3.76)
Infant characteristics						
Gestation at delivery (weeks) [6]	0.82 (0.70; 0.96)	0.77 (0.64; 0.92)	0.65 (0.53; 0.78)	0.54 (0.42; 0.70)	1.14 (0.95; 1.38)	1.11 (0.90; 1.37)
Preterm (<37 weeks) vs. term	2.58 (0.85; 7.78)	-	8.09 (2.82; 23.24)	-	0.54 (0.15; 1.90)	-
Infant sex: male vs. female	0.88 (0.35;2.22)	-	1.16 (0.44; 3.07)	-	0.97 (0.48; 1.96)	-
Small-for-gestational age [7]	1.13 (0.31; 4.11)	-	2.06 (0.62; 6.80)	-	1.97 (0.80; 4.86)	-
Duration of any breastfeeding (months) [8]	1.03 (0.95; 1.11)	-	0.99 (0.91;1.07)	-	0.98 (0.92; 1.04)	-
Duration of exclusive breastfeeding (weeks) [9]	1.02 (0.97; 1.06)	-	1.00 (0.96; 1.05)	-	1.00 (0.97; 1.03)	-

*Language score based on expressive language subscale only; Abbreviations – BSID-III, Bayley Scales of Infant and Toddler Development Third Edition; ART, antiretroviral therapy; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; Except where otherwise indicated, maternal variables represent measures taken at first antenatal clinic booking, prior to ART initiation

[1] Calculated using standardized asset score (access to flush toilet, running water, electricity in home, refrigerator, telephone, television, formal housing) and maternal employment; higher score indicates less relative disadvantage; [2] Education: comparing women who completed secondary schooling vs. those who did not; [3] Depression: EPDS (Edinburgh postnatal depression scale) score of ≥ 13, compared to score <13; [4] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire (compared to no IPV); [5] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 (compared to scores <3); [6] Gestation at delivery: based on early ultrasound and/or last menstrual period as indicated; [7] SGA, birthweight below 10th centile for gestational age (Intergrowth-21st reference standards); compared to appropriate for gestational age; [8] Breastfeeding durations determined by maternal self-report: last study visit at which breastfeeding reported taken as last day of breastfeeding; [9] Exclusivity defined as receiving only breastmilk and medication; non-time-varying; duration of exclusive breastfeeding among those who never exclusively fed set as zero

[10] Interaction terms: VCY-gestational age for cognitive, p=0.84; motor, p=0.46; expressive language, p=0.40; VCY-sex: cognitive, p=0.13; motor, p=0.30; expressive language, p=0.16; VCY-breastfeeding duration: cognitive, p=0.10; motor, p=0.74; expressive language, p=0.62

[11] Sensitivity analysis results:

(a) Adjusting for maternal age, education, intimate partner violence, risky drinking and poverty score (not including gestational age): cognitive aOR 1.39 (95% CI 0.73; 2.63); motor aOR 1.70 (95% CI 0.87; 3.33); expressive language aOR 2.89 (95% CI 1.63; 5.11).
(b) Adjusting for maternal age, education, intimate partner violence, risky drinking and poverty score; maternal history of tuberculosis and CD4 cell count (not including gestational age): cognitive aOR 1.21 (95% CI 0.57; 2.58); motor aOR 1.76 (95% CI 0.80; 3.90); expressive language aOR 2.04 (95% CI 1.05; 3.94).
(c) Adjusting for maternal age, education, intimate partner violence, risky drinking and poverty score; maternal history of tuberculosis, CD4 cell count AND gestational age: cognitive aOR 1.55 (95% CI 0.70; 3.42); motor aOR 3.72 (95% CI 1.35; 10.26); expressive language aOR 1.89 (95% CI 0.96; 3.71).

7.5 Discussion

In this unique cohort of young, breastfed, HEU children born under conditions of universal ART, antepartum burden of HIV viraemia was associated with higher relative odds of developmental delay in motor and language domains. These associations, largely independent of preterm birth, were seen despite reasonably high median pre-ART CD4 counts and successful viral suppression at delivery among most women.

Few data are available on the relationship between antepartum HIV viraemia and neurodevelopment of breastfed HEU children in the context of universal maternal ART. Similar associations have however been described in other contexts. In a large US-based cohort of young (median age 20 months) HEU children, increasing maternal viral load in pregnancy predicted incrementally lower BSID-II mental (MDI) and psychomotor developmental indices (PDI).[3] Although not all mothers had received ART, longer duration of any antiretroviral use was associated with higher MDI scores.[3] In Tanzania, antepartum clinical HIV disease severity in the absence of antiretroviral drugs predicted lower PDI scores among 311 HEU children.[4]

There is growing evidence to support the biological plausibility of this association. Emerging evidence from animal, human and epidemiological studies point to significant alterations in brain development during *in utero* exposure to maternal immune activation.[16] In adults with chronic HIV infection, the degree and persistence of systemic immune activation and inflammation, even during suppressive ART, is directly proportional to disease severity at ART initiation.[17] Higher levels and duration of maternal HIV viraemia in pregnancy may reflect magnitude and duration of maternal immune activation. Furthermore, direct exposure to circulating HIV antigens may result in fetal immune activation; chronic activation of fetal microglia may alter synaptic plasticity. Additionally, maternal co-infections, such as with cytomegalovirus (CMV), are often associated with higher levels of HIV viraemia, and may further contribute to peripartum mother-infant immune activation, as well as having possible direct adverse effects on infant neurodevelopment.[18-20]

These data come from a well-characterized mother-infant cohort wherein all mothers received the same perinatal care and universal ART regimens, and all HEU children were breastfed.[6] For these reasons, our cohort may not be fully representative of the larger HIV-affected mother-infant population in Southern Africa. The average developmental scores of the children in our cohort were within age expectations on measures of cognition, expressive language, and motor development, while absolute differences associated with maternal viremia were small. These findings align with other recent publications on developmental outcomes of HEU children in Africa.[7,21,22]

Study strengths include prospective, repeated viral load testing and extensive measures of maternal psycho-social and economic factors. Nevertheless, our findings should be interpreted with caution given the cross-sectional nature of our developmental measure, low precision for sub-group analyses and lack of receptive language measurement. Inability to screen for maternal co-infections, such as CMV, and lack of inflammatory markers preclude in-depth evaluation of possible underlying causal mechanisms. Nonetheless, our cohort is adequately powered to detect differences in developmental outcomes using several measures of HIV disease severity in pregnancy. Included in these measures we use a novel application of VCY.[11] While VCY is increasingly used in the adult HIV literature, to our knowledge this is the first application thereof in perinatal epidemiology of HEU child health.

In conclusion, maternal HIV viraemia in pregnancy may have adverse implications for HEU child development, even in the context of universal maternal ART. Prompt identification and treatment of HIV in pregnancy is a critical aspect of not only preventing HIV transmission but also optimizing the health of HEU children generally. Data are required on developmental outcomes of children born to mothers who initiated suppressive ART prior to conception, at early disease stages.

7.6 Acknowledgements

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Chapter 8: Discussion and recommendations

8.1 Introduction

The overarching aim of this research was to evaluate whether previously observed health discrepancies between HIV-exposed uninfected (HEU) and HIV-unexposed (HU) children were maintained when women living with HIV (WLHIV) received universal antiretroviral therapy (ART) during pregnancy and all infants received breastmilk as infant feeding method of choice. Drawing on data from two linked, parallel observational studies of breastfeeding mother-infant pairs (the Maternal and Child Health Antiretroviral, MCH-ART and HIV-unexposed uninfected mother-infant, HU2 studies), three globally relevant child health outcomes were compared by maternal HIV exposure.[1-5] This chapter provides a synopsis and discussion of key findings related to the primary study objectives, framed by the three central study hypotheses as specified *a priori* (first introduced in chapter 1, and listed below):

Hypothesis 1:

The breastfed HEU children of WLHIV who received ART in pregnancy have *clinically similar health outcomes* to the breastfed HU children of HIV-uninfected women.

Hypothesis 2:

Early *infant feeding modifies* the effect of HIV-ART exposure on child health outcomes.

Hypothesis 3:

Among HEU children, health outcomes are associated with *(1) maternal HIV disease severity* and *(2)* quality of *breastfeeding*.

Additional findings, not explicitly stated in the primary hypotheses but with important implications for child health in the context of maternal HIV, are also incorporated in the summary (section 8.2). Findings are considered within the context of relevant publications on child health in sub-Saharan Africa generally, and HEU child health specifically. Important limitations and strengths of this work are reviewed in section 8.3, followed by a discussion of the potential implications of these results for policy, practice, and research in section 8.4.

8.2 Summary of key findings

A brief overview of key findings per clinical outcome is shown in table 8.1, divided into five sub-sections reflecting five important themes that emerge across this body of work. The first three themes align with the three primary hypotheses listed above. In addition, the substantial contributions of adverse birth outcomes [theme 4: preterm (PTB) and small-for-gestational age (SGA) births] to relationships between maternal HIV and child health are considered. Finally, the clear clustering and effects of social determinants of health among WLHIV are discussed (theme 5). Throughout, PTB is defined as birth prior to 37 completed weeks of gestation, and SGA as birthweight below the 10th centile for gestational age. Figures that illustrate key points are included from the relevant chapters.

8.2.1 On average, the breastfed HEU children of WLHIV who received ART in pregnancy had marginally worse clinical outcomes than the breastfed HU children of HIV-uninfected women (hypothesis 1)

In this cohort of HIV-uninfected children who all initiated breastfeeding after birth, maternal HIV exposure *in utero* was associated with slightly lower weight-for-age (WAZ), length-for-age (LAZ), head circumference-for-age (HCAZ), and weight-for-length (WLZ) Z-scores in the first 12 months of life, but the magnitude of differences were small (0.2 to 0.3 of a standard deviation, SD). In the context of longitudinal, individual-level clinical growth monitoring, an intra-individual Z-score difference of 0.67 has been proposed to broadly capture the clinical concept of "crossing" important centiles, a flag for clinically meaningful deviation from the expected growth trajectories for an individual child.[6] Although individual and population-level impacts are not interchangeable concepts, it is debatable what the clinical implications of a 0.3 SD mean difference in Z-score may be at a population level, regardless of estimate precision. Patterns of differences between HEU and HU children varied somewhat over time, with the most striking differences noted in LAZ trajectories (figure 8.1). Until 6 months of age, the mean LAZ of HEU and HU children were similar (magnitude of difference no greater than 0.1), whereafter mean LAZ gradually decreased in both groups, indicating group-level onset of linear growth faltering. This process started earlier among HEU children, resulting in a lower average LAZ than HU children by age 12

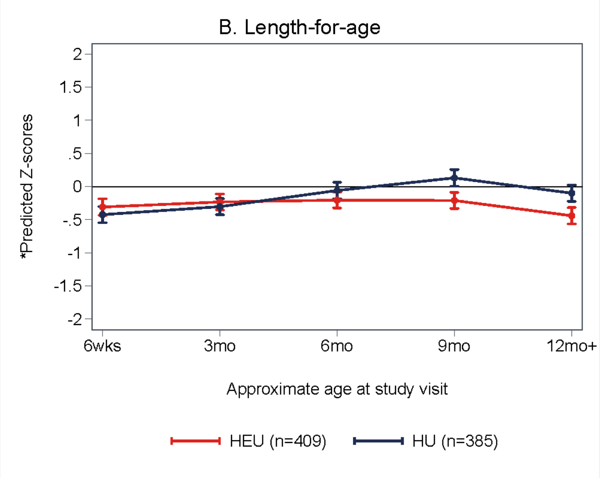


Figure 8.1. Trajectories of length-for-age Z-scores comparing mean adjusted values for HEU and HU children during the first 12 months of life (also shown in chapter 4)

months (0.4 difference in crude, and 0.3 in adjusted analysis accounting for several social determinants of health). Reflecting this, HEU children were twice as likely as HU children to be stunted (LAZ<-2) at this age, although the overall prevalence of stunting was low in both groups (10% and 4%, HEU and HU children respectively).

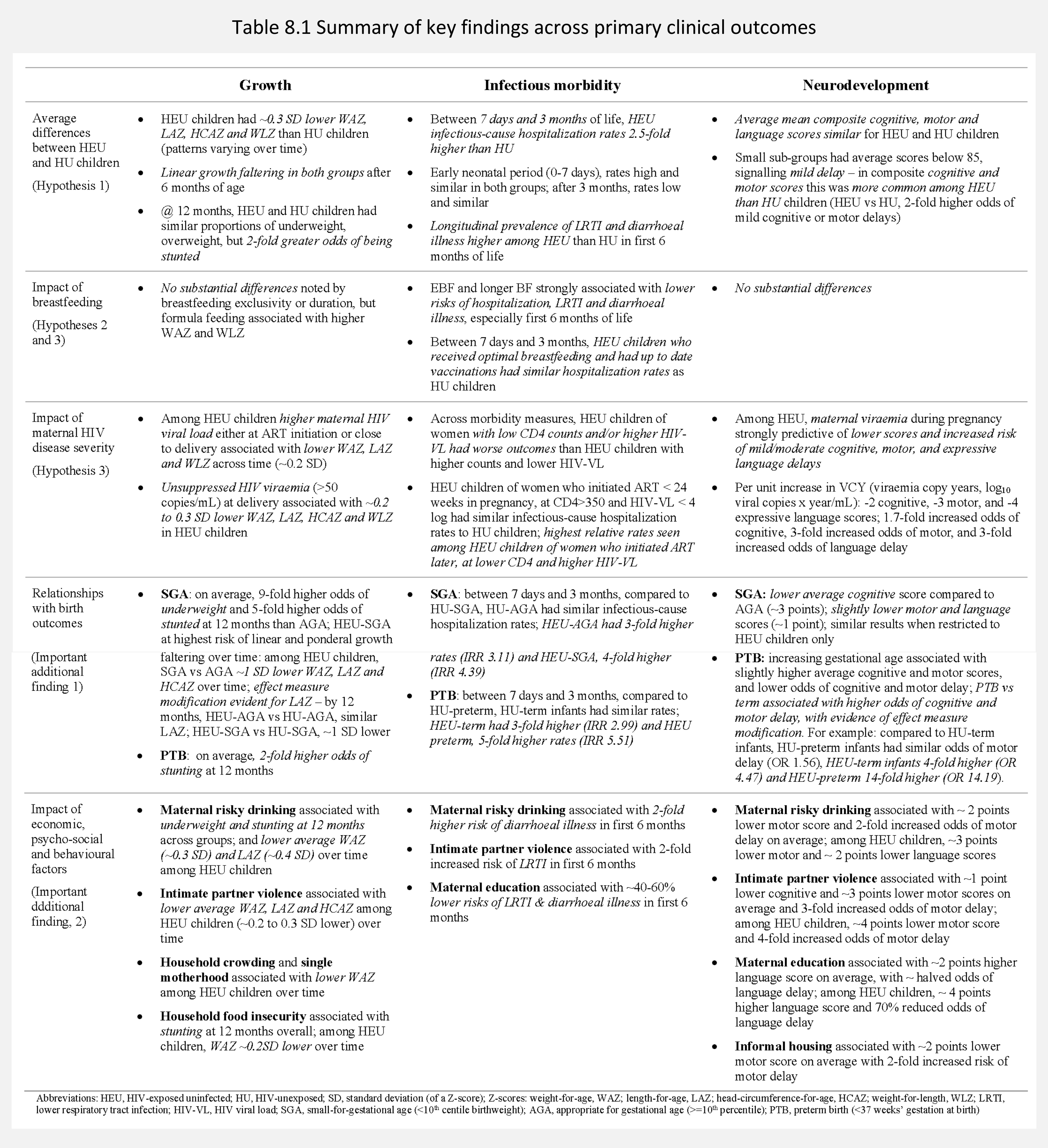


Table 8.1 Summary of key findings across primary clinical outcomes

	Growth	Infectious morbidity	Neurodevelopment
Average differences between HEU and HU children (Hypothesis 1)	• HEU children had *~0.3 SD lower WAZ, LAZ, HCAZ and WLZ* than HU children (patterns varying over time) • *Linear growth faltering in both groups* after 6 months of age • @ 12 months, HEU and HU children had similar proportions of underweight, overweight, but *2-fold greater odds of being stunted*	• Between *7 days and 3 months* of life, *HEU infectious-cause hospitalization rates 2.5-fold higher than HU* • Early neonatal period (0-7 days), rates high and similar in both groups; after 3 months, rates low and similar • *Longitudinal prevalence of LRTI and diarrhoeal illness higher among HEU* than HU in first 6 months of life	• *Average mean composite cognitive, motor and language scores similar* for HEU and HU children • Small sub-groups had average scores below 85, signalling *mild delay* – in composite *cognitive and motor scores* this was *more common among HEU than HU* children (HEU vs HU, 2-fold higher odds of mild cognitive or motor delays)
Impact of breastfeeding (Hypotheses 2 and 3)	• *No substantial differences* noted by breastfeeding exclusivity or duration, but formula feeding associated with higher WAZ and WLZ	• EBF and longer BF strongly associated with *lower risks of hospitalization, LRTI and diarrhoeal illness*, especially first 6 months of life • Between 7 days and 3 months, *HEU children who received optimal breastfeeding and had up to date vaccinations had similar hospitalization rates* as HU children	• *No substantial differences*
Impact of maternal HIV disease severity (Hypothesis 3)	• Among HEU children *higher maternal HIV viral load* either at ART initiation or close to delivery associated with *lower WAZ, LAZ and WLZ* across time (~0.2 SD) • *Unsuppressed HIV viraemia* (>50 copies/mL) at delivery associated with *~0.2 to 0.3 SD lower WAZ, LAZ, HCAZ and WLZ* in HEU children	• Across morbidity measures, HEU children of women *with low CD4 counts and/or higher HIV-VL had worse outcomes* than HEU children with higher counts and lower HIV-VL • HEU children of women who initiated ART < 24 weeks in pregnancy, at CD4>350 and HIV-VL < 4 log had similar infectious-cause hospitalization rates to HU children; *highest relative rates seen among HEU children of women who initiated ART later, at lower CD4 and higher HIV-VL*	• Among HEU, *maternal viraemia* during pregnancy strongly predictive of *lower scores and increased risk of mild/moderate cognitive, motor, and expressive language delays* • Per unit increase in VCY (viraemia copy years, log$_{10}$ viral copies x year/mL): -2 cognitive, -3 motor, and -4 expressive language scores; 1.7-fold increased odds of cognitive, 3-fold increased odds of motor, and 3-fold increased odds of language delay
Relationships with birth outcomes (Important additional finding 1)	• **SGA**: on average, 9-fold higher odds of *underweight* and 5-fold higher odds of *stunted* at 12 months than AGA; HEU-SGA at highest risk of linear and ponderal growth faltering over time: among HEU children, SGA vs AGA *~1 SD lower WAZ, LAZ and HCAZ* over time; *effect measure modification evident for LAZ* – by 12 months, HEU-AGA vs HU-AGA, similar LAZ; HEU-SGA vs HU-SGA, ~1 SD lower • **PTB**: on average, *2-fold higher odds of stunting* at 12 months	• **SGA**: between 7 days and 3 months, compared to HU-SGA, HU-AGA had similar infectious-cause hospitalization rates; *HEU-AGA had 3-fold higher rates (IRR 3.11) and HEU-SGA, 4-fold higher (IRR 4.39)* • **PTB**: between 7 days and 3 months, compared to HU-preterm, HU-term infants had similar rates; *HEU-term had 3-fold higher (IRR 2.99) and HEU preterm, 5-fold higher rates (IRR 5.51)*	• **SGA:** *lower average cognitive* score compared to AGA (~3 points); *slightly lower motor and language* scores (~1 point); similar results when restricted to HEU children only • **PTB:** increasing gestational age associated with slightly higher average cognitive and motor scores, and lower odds of cognitive and motor delay; *PTB vs term associated with higher odds of cognitive and motor delay, with evidence of effect measure modification.* For example: compared to HU-term infants, HU-preterm infants had similar odds of motor delay (OR 1.56), *HEU-term infants 4-fold higher (OR 4.47) and HEU-preterm 14-fold higher (OR 14.19*).
Impact of economic, psycho-social and behavioural factors (Important ddditional finding, 2)	• **Maternal risky drinking** associated with *underweight and stunting at 12 months* across groups; and *lower average WAZ (~0.3 SD) and LAZ (~0.4 SD)* over time among HEU children • **Intimate partner violence** associated with *lower average WAZ, LAZ and HCAZ* among HEU children (~0.2 to 0.3 SD lower) over time • **Household crowding** and **single motherhood** associated with *lower WAZ* among HEU children over time • **Household food insecurity** associated with *stunting* at 12 months overall; among HEU children, *WAZ ~0.2SD lower* over time	• **Maternal risky drinking** associated with *2-fold higher risk of diarrhoeal illness* in first 6 months • **Intimate partner violence** associated with 2-fold increased risk of *LRTI* in first 6 months • **Maternal education** associated with ~40-60% *lower risks of LRTI & diarrhoeal illness* in first 6 months	• **Maternal risky drinking** associated with ~ 2 points lower motor score and 2-fold increased odds of motor delay on average; among HEU children, ~3 points lower motor and ~ 2 points lower language scores • **Intimate partner violence** associated with ~1 point lower cognitive and ~3 points lower motor scores on average and 3-fold increased odds of motor delay; among HEU children, ~4 points lower motor score and 4-fold increased odds of motor delay • **Maternal education** associated with ~2 points higher language score on average, with ~ halved odds of language delay; among HEU children, ~ 4 points higher language score and 70% reduced odds of language delay • **Informal housing** associated with ~2 points lower motor score on average with 2-fold increased risk of motor delay

Abbreviations: HEU, HIV-exposed uninfected; HU, HIV-unexposed; SD, standard deviation (of a Z-score); Z-scores: weight-for-age, WAZ; length-for-age, LAZ; head-circumference-for-age, HCAZ; weight-for-length, WLZ; LRTI, lower respiratory tract infection; HIV-VL, HIV viral load; SGA, small-for-gestational age (<10th centile birthweight); AGA, appropriate for gestational age (>=10th percentile); PTB, preterm birth (<37 weeks' gestation at birth)

At this age, the linear growth trajectories of HEU and HU children were still declining, more steeply so among the former (figure 8.1). In the absence of intervention, it is likely that this observed process of linear growth faltering would have continued, with differences by maternal HIV status potentially worsening. In general, these findings reflect the pattern of malnutrition in urban South Africa, with stunting being the predominant form of undernutrition.[7] HEU child growth reports from other African studies and settings have similarly reflected the background patterns of malnutrition, with estimates of underweight, wasting and stunting varying across locations, and stunting being the most common form of malnutrition.[8-10] For example, in the Sanitation, Hygiene, Infant Nutrition Efficacy (SHINE) trial in rural Zimbabwe, under conditions of near universal maternal ART, predominantly breastfed HEU and HU children had high prevalence of stunting at 18 months (51% and 34%, respectively), with lower estimates of underweight (16% and 11%), wasting (4% and 3%) and microcephaly (10% vs 6%).[11] Despite variation in settings and in access to maternal ART in other studies of HEU child growth, the reported absolute between-group differences in Z-scores have generally been in the same range of magnitude (0.2 to 0.4), with lower scores in HEU than HU children and the greatest differences noted in linear growth measures.[12,13] This pervasiveness of childhood stunting, shown also in our data, has significant implications for the future economic health of a region that is already marked by poverty and income inequality.[14-16] Stunting is a complex process, and typically reflective of chronic malnutrition both during ante-and postnatal periods, including a lack of micronutrients, alongside socio-economic deprivation, and recurrent infections.[17,18]

In accordance with this, the HEU children in our study had somewhat higher risks of infectious morbidity than HU children, with differences restricted largely to early infancy. Between 7 days and 3 months of age, HEU children had more than double the risk of being hospitalized for an infectious illness than HU children. Reassuringly, infection-related hospitalization rates for both groups of children decreased substantially thereafter and continued to be low and similar through 12 months of age (figure 8.2). Similarly, maternally reported longitudinal prevalence of diarrhoeal and coughing illness was relatively increased in HEU compared to HU children in the first 6 months of life, whereafter relative differences between the groups decreased. It is worth noting that, in parallel to the

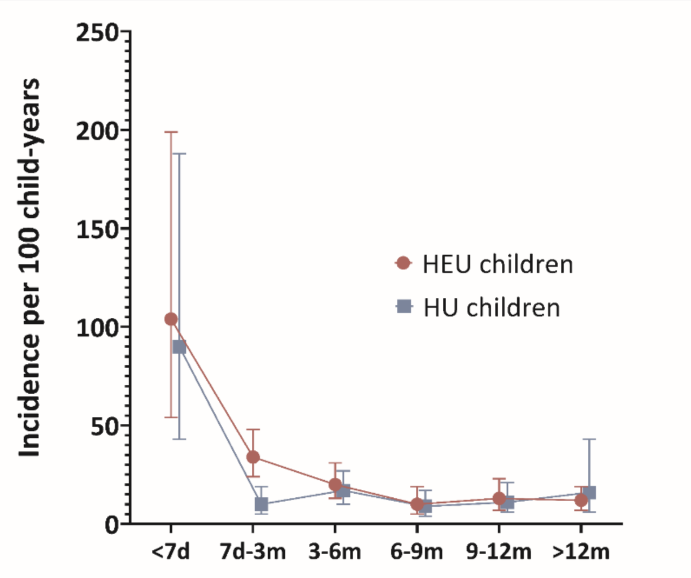


Figure 8.2. Infection-related hospitalization: incidence rates comparing HEU to HU children over time (also shown in chapter 5)

onset of linear growth stunting discussed previously, overall prevalence of diarrhoeal illness increased in both HEU and HU groups after the age of 6 months. Similarly, an increasing contribution of diarrhoeal illness to linear growth faltering towards later infancy has been described in South Africa and other settings previously.[18] Nonetheless, across infectious measures, severe illness was rare, and mortality was low and similar in both groups. Although comparisons with data from other studies are complicated by inconsistent definitions of infectious illness, and varying background infectious risks, our overall findings are in line with previous work on infectious morbidity among HEU children.[19] As has been reported in other studies, the excess infectious risks among HEU (compared to HU) children were primarily due to common childhood illnesses, and predominantly restricted to early infancy. The most striking difference between our infectious risk data and those from studies pre-dating universal ART relates to the risk of dying from an infectious illness.

As noted, mortality was low among both HEU and HU children in our study, while older studies described a steep risk gradient for death comparing HEU to HU children, largely due to infectious illness.[20,21] For example, in the ZVITAMBO vitamin A trial in rural Zimbabwe, mortality was 3-fold higher among HEU than HU infants in the first 2 months of life, and 5-fold higher between 2 and 6 months of age; two-thirds of the HEU child deaths were due to infectious illness.[21] Taken together, the data suggest that young HEU (vs HU) infants may still be at transiently increased risk of some infectious morbidity even in the context of universal maternal ART and breastfeeding, but that the severity of these episodes is ameliorated compared to those reported in earlier studies. That is, at a population level, policies of universal maternal ART and the promotion of breastfeeding may be effective strategies for reducing the overall excess risk of serious infectious illness in HEU children but are unlikely to fully address all infectious vulnerabilities.

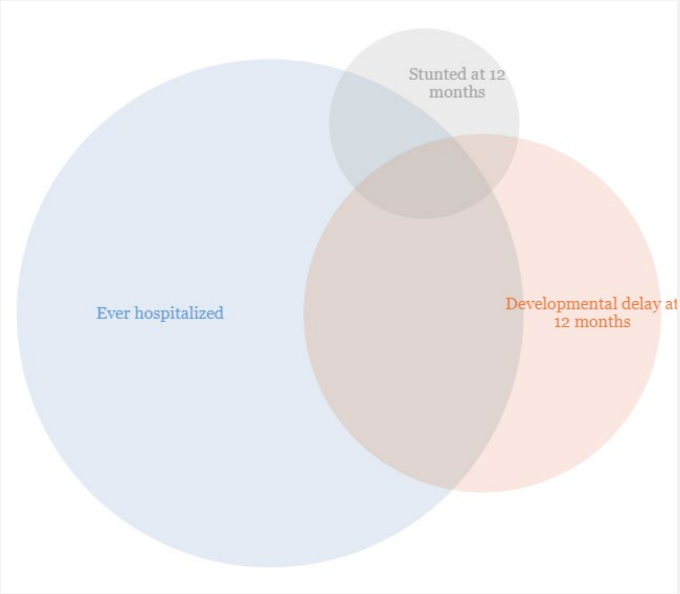


Figure 8.3. Venn diagram of interrelationships between adverse child health outcomes in the first year of life (stunting, all-cause hospitalization, and developmental delay) in the MCH-ART and HU2 cohort studies

In our cohort study, HEU children therefore had marginally increased risks of both stunting and infectious morbidity. In a cross-sectional evaluation at 12 months of age, HEU children also had marginally higher risks of developmental delay than HU children, reflecting the known associations and interrelationships between stunting, infectious illness, and loss of developmental potential in situations of poverty.[15,17] Although not explicitly evaluated in the analyses presented in Chapters 4 through 7, there was a marked overlap of risks of childhood stunting, infectious morbidity, and developmental delay in this group of young children (figure 8.3). However, as seen with both growth and infectious morbidity, the absolute differences in developmental measures were small, and generally limited to particularly vulnerable sub-groups of HEU children. A slightly larger proportion of HEU than HU children had mild to moderate delay in cognitive and motor domains, but severe delays were very rare, and similarly distributed among HEU and HU children. These results align with other studies conducted in Southern and Eastern Africa in the context of some access to maternal ART, where minimal to no differences were observed across cognitive, motor or language domains on average, although slightly larger proportions of HEU than HU children were classified as having mild/moderate delay in one or more of the tested domains.[22-25]

Collectively, the data from this doctoral research project suggest that breastfed HEU children born to WLHIV who received universal ART in pregnancy may still be at increased risks of linear growth faltering, infectious morbidity, and mild/moderate developmental delays compared to breastfed HU children, although magnitudes of differences are small and mostly restricted to vulnerable subgroups.

Policies that promote breastfeeding and enable antenatal initiation of maternal ART unquestionably improve the health outcomes of HEU children, but alone may not fully ameliorate their excess health risks. A discussion of critical contributing factors that are not fully addressed by policies of breastfeeding promotion and initiation of ART is presented under sections 8.2.2 to 8.2.4, with the specific aim to identify areas that require additional intervention.

8.2.2 Among universally breastfed children, exclusivity and longer duration of breastfeeding attenuated the associations between maternal HIV-ART exposure and infectious morbidity, but not growth or neurodevelopment (hypothesis 2, and 3.2)

Substantial evidence exists for the beneficial effects of breastfeeding on infant and child health, which ranges from shorter term protection against infectious illness and death, to longer term benefits related to intelligence, human capital, and non-communicable diseases in adulthood.[26-29] Several interrelated mechanisms underlie these positive effects, including in broad terms (i) the responsively adaptive and nutritionally replete composition of breastmilk; (ii) the abundant anti-infective and anti-inflammatory components of breastmilk, including antibodies, cytokines, growth factor and anti-oxidants; and (iii) the diverse, adaptive and beneficial microbiota of breastmilk, which in turn promote a healthy intestinal microbiome in the infant.[30,31]

The MCH-ART and HU2 study designs intentionally restricted postnatal follow-up to mother-infant pairs who had initiated breastfeeding after delivery, thereby minimizing heterogeneity in very early infant feeding data.[1] Although this restriction addressed some sources of potential confounding, it also removed the opportunity to assess and compare outcomes in infants who never breastfed. Therefore, in terms of evaluating the role of breastfeeding in any observed relationships between maternal HIV and child health outcomes in this dataset, conclusions can only be drawn on the influence of quality of breastfeeding (defined as exclusivity, overall duration, and timing of initiation).

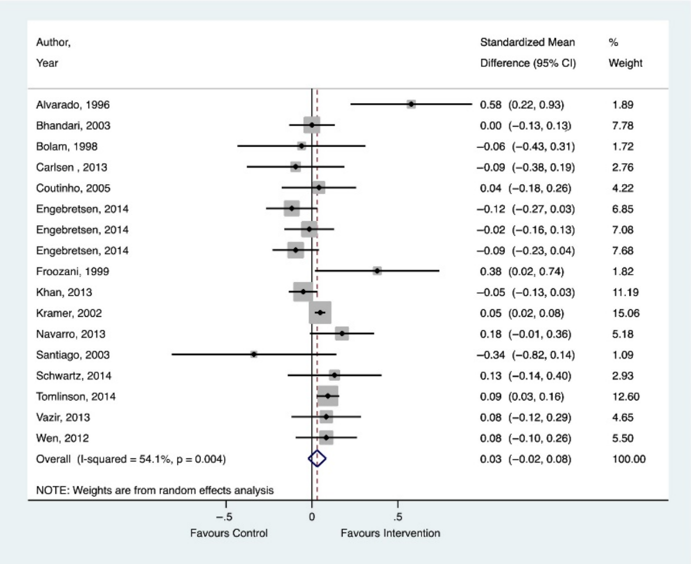
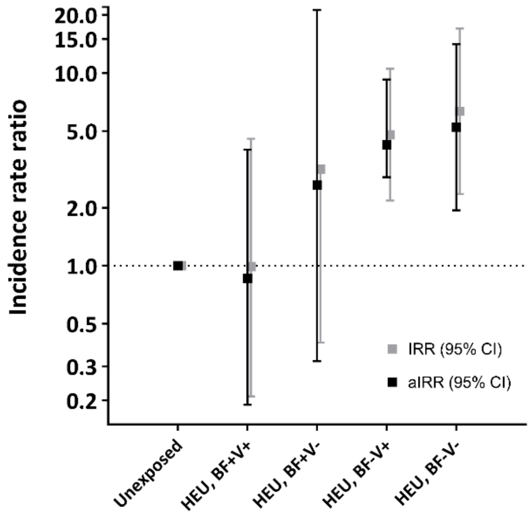


Figure 8.4. Standardized mean differences in length or height in different studies, comparing intervention vs control groups. (*Source:* Giugliani et al, Acta Paediatric 2015 https://doi.org/10.1111/apa.13160)

In this predominantly healthy cohort of children, no differences were noted in growth or developmental outcomes of HEU or HU children by exclusivity or duration of breastfeeding. This may reflect a persistence of the early benefits of breastfeeding, as even partial breastfeeding (breastmilk in addition to other sources of nutrition) may improve child health outcomes when compared to no breastfeeding,[32] and all children in this study received some breastmilk. It is also possible that the previously reported benefits of exclusive and prolonged breastfeeding on the growth and developmental outcomes of HEU children[33,34] were not yet detectable at such a young age or are less apparent in an urban setting such as Cape Town, South Africa. Conversely, a 2015 meta-analysis of the relationship between breastfeeding promotion interventions and growth also failed to demonstrate meaningful effects, which aligns with our findings in terms of breastfeeding and child growth (figure 8.4).[35] Simultaneously, our breastfeeding measurement tools were applied only at roughly 3 monthly study visits and may have lacked the sensitivity and specificity of more frequent maternal recall measures.[36] It may be that the failure to replicate previous findings of improved developmental outcomes following exclusive breastfeeding may stem partly from measurement error.

However, even with potential measurement error and only 12 months of follow-up, the quality of breastfeeding was strongly associated with infectious morbidity risks among both HEU and HU children in our study. Early initiation (EIBF, within 1 hour of birth), exclusivity (receipt of breastmilk and prescribed medicines only), and longer duration of overall breastfeeding were all associated with markedly decreased infectious morbidity risks. Indeed, between 7 days and 3 months (the only age interval where HEU children experienced excess risks of infection-related hospitalization compared to HU children), optimally breastfed HEU children had risks of infection-related hospitalization that were comparable to those of HU children. That is, the excess risks of infection-related hospitalization noted among HEU children in this cohort were restricted to those who did not receive optimal breastfeeding in the first few months of life, especially those who also had incomplete vaccinations (figure 8.5). Similar relationships were seen with longitudinal prevalence of diarrhoeal and coughing illness.



Legend:

IRR, crude incidence rate ratio from Poisson regression analysis corrected for clustering; aIRR, incidence rate ratios adjusted for access to flush toilet and running water, postpartum depression, preterm birth, season at birth and being underweight in a previous age interval; "Optimal breastfeeding" defined as initiation within 1 hour of birth and exclusive breastfeeding through 3 months of age; vaccinations considered timely if all required doses received within 2 weeks of indicated age (based on 6 and 10 weeks' South African schedule)

Reference category includes all HU children (N=405)

HEU categories:

BF+ V+:	Optimal breastfeeding and timely vaccination (N=90); vs HU, aIRR 0.86, 95% CI 0.19-4.00
BF+ V-:	Optimal breastfeeding but delayed or incomplete vaccination (N=14); vs HU, aIRR 2.62, 95% CI 0.32-21.26
BF- V+:	Suboptimal breastfeeding but timely vaccination (N=187); vs HU, aIRR 4.15, 95% CI 1.88-9.15
BF- V-:	Suboptimal breastfeeding and delayed or incomplete vaccination (N=50); vs HU, aIRR 5.24, 95% CI 1.94-14.14

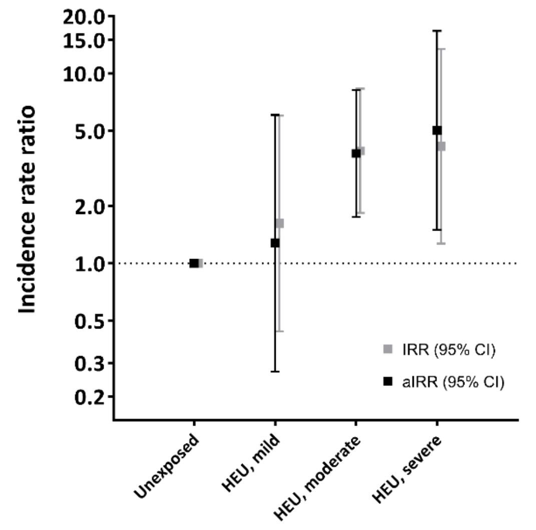
Figure 8.5. Incidence rate ratios of infection-related hospitalization between 7 days and 3 months in HEU vs HU children: variation by optimal breastfeeding and timeliness of vaccination in the first 3 months of life (also shown in Chapter 5)

Therefore, associations between maternal HIV exposure and infant infectious morbidity risks in our study were strongly attenuated by improved quality of breastfeeding.

However, a large proportion of our study's children were not exclusively breastfed, and early cessation of breastfeeding was common, especially among HEU children. This underscores the important practical difference between a policy and its successful implementation. Breastfeeding initiation alone is clearly insufficient to ameliorate the excess infectious morbidity risks of HEU children, but optimal breastfeeding is strikingly effective. Successful breastfeeding is not easy for mothers to maintain, and in the wake of great controversy around breastfeeding by WLHIV, rapidly vacillating breastfeeding policies and the distribution of free infant formula in the recent past, much work remains to be done to reinstate early, exclusive, and prolonged breastfeeding as the norm in sub-Saharan Africa in general, and South Africa in particular.[37-41] Infant feeding policy changes without focused and pragmatic implementation, training and support of breastfeeding counsellors, community mobilization and interventions to promote the support of breastfeeding mothers at work and school, are insufficient for the full benefits of breastfeeding to be experienced by either HEU or HU children.[42]

8.2.3 Among HEU children, a greater burden of maternal HIV viraemia during pregnancy was associated with greater risks of growth faltering, infectious morbidity, and developmental delays (hypothesis 3.1)

As noted for breastfeeding policies above, policy changes that promote universal ART in pregnancy are insufficient in isolation to ensure maternal viral control at delivery for all pregnant WLHIV. Although our cohort of WLHIV all initiated ART in pregnancy, those presenting for antenatal care at later gestations necessarily had less time to establish viral control prior to delivery. Achieving viral suppression by the time of delivery was also less common among women with very high HIV viral loads at ART initiation.



Legend:

IRR, crude incidence rate ratio from Poisson regression analysis corrected for clustering; aIRR, incidence rate ratios adjusted for access to flush toilet and running water, postpartum depression, preterm birth, season at birth, being underweight in a previous age interval, quality of breastfeeding (early initiation and exclusivity in the first 3 months) and vaccination status

Reference group includes all HU children (N=405)

HEU categories (CD4 cell count and HIV viral load category boundaries set at group median value):

- "Mild" (N=84): pre-ART maternal CD4 cell count > 350 cells/mm^3 AND HIV viral load <4·0 log$_{10}$ copies/mL AND ART initiated prior to 24 weeks' gestation in pregnancy (vs HU, aIRR 1·28, 95% CI 0·27-6·05)
- "Moderate" (N=320): pre-ART maternal CD4 cell count ≤ 350 cells/mm^3 OR HIV viral load ≥4·0 log$_{10}$ copies/mL but ART initiated prior to 24 weeks' gestation in pregnancy; OR, maternal CD4 cell count > 350 cells/mm^3 OR HIV viral load <4·0 log$_{10}$ copies/mL but ART initiation at or after 24 weeks' gestation in pregnancy (vs HU, aIRR 3.79, 95% CI 1·75-8.18)
- "Severe" (N=44): pre-ART maternal CD cell count ≤350 AND HIV viral load ≥4·0 log$_{10}$ copies/mL AND ART initiation at or after 24 weeks' gestation in pregnancy (vs HU, aIRR 5·01, 95% CI 1·50-16·71)

Figure 8.6 Incidence rate ratios of infection-related hospitalization between 7 days and 3 months in HEU vs HU children: variation by maternal HIV disease severity and timing of ART initiation in pregnancy (also shown in Chapter 5)

We evaluated variation in child health outcomes by maternal HIV viral control in pregnancy, using one or more of the following to define a greater antenatal burden of HIV viraemia: (a) higher maternal viral load at ART initiation, or (b) at the time of delivery; (c) lack of maternal viral suppression (>50 copies/mL) at the time of delivery; (d) maternal viraemia copy years (reflecting both duration of and level of viraemia); and (e) gestation at ART initiation. Across all three clinical outcomes, HEU children of mothers with less (vs greater) viral control in pregnancy had worse outcomes, regardless of how viraemia was expressed (table 8.1, figure 8.6). This is a key finding of this work, with substantial implications for optimizing HEU child health going forward. Before the widespread availability of ART, higher antenatal levels of maternal HIV viral load correlated with greater immunological disturbances in HEU newborns, and greater risks of growth faltering, infectious morbidity, and mortality in HEU children.[20] Whereas this correlation between increased maternal HIV disease severity in pregnancy and adverse child health was frequently reported in the absence of ART,[12,43] associations between maternal viraemia and HEU child health have been less well described in the context of universal perinatal ART.

There is accumulating evidence for the biological plausibility of these associations. Among people living with HIV (PLHIV), initiating ART at more advanced disease stages correlates with greater persistence of immune activation and inflammation, hallmarks of chronic HIV infection and strongly predictive of adverse, longer-term non-communicable diseases and death.[44] Indeed, suppressive ART initiated during chronic HIV does not fully negate all systemic inflammation and immune activation, even when viral control is established with restitution of CD4 cell counts.[45] In pregnancy, the chronic inflammation experienced by WLHIV extends to the womb, including the placenta. Generally, a pro-inflammatory fetal milieu is known to predispose infants to lifelong increased risks neurodevelopmental disorders and immune dysregulation.[46-49] While this has been most intensively studied in the fields of neuro-psychiatry and non-HIV viral illness in pregnancy, the proposed bio-physiological mechanisms can plausibly extend to other later life diseases, following the theories of Developmental Origins of Health and Disease (DoHaD, figure 8.7).[50-52] It is possible that some – but not all – of the residual effects of maternal HIV on HEU child health outcomes despite universal ART may be attributable to chronic maternal immune activation. In PLHIV, initiation of treatment during acute HIV infection appears to be the best option to minimize the life-long immune activation that characterizes HIV infection, although some degree inflammation persists.[53]

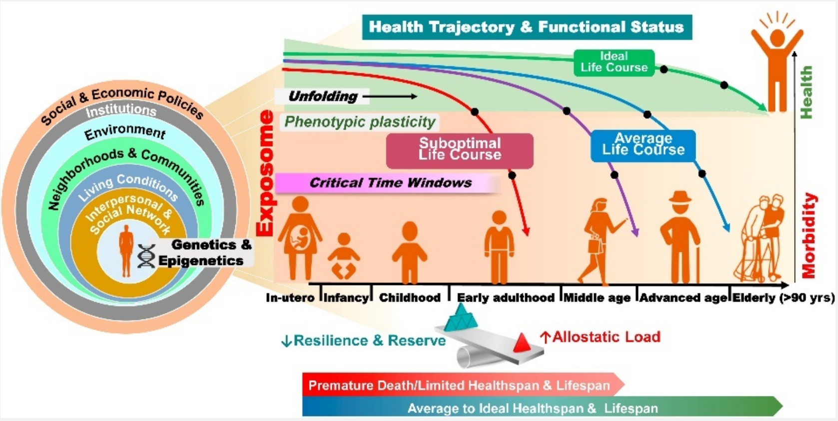


Figure 8.7. The evolution of health trajectories under the influence of macro- and micro-level factors. *Source:* Vasan, RS et al, 2020 Journal of the American College of Cardiology

Extending these findings to the impact of maternal HIV on HEU child health, maternal inflammation and immune activation during pregnancy seem likely to correlate with HIV stage and severity at maternal initiation of ART, as well as the timing of ART initiation in relation to conception.[54] Initiation of ART in pregnancy dramatically reduces maternal HIV viraemia and is the best option for prevention of mother to child transmission of HIV for WLHIV presenting for antenatal care while not on ART.[55] However, in terms of minimizing the pro-inflammatory fetal milieu characteristic of chronic HIV in pregnancy, ART initiation in pregnancy may be insufficient for several reasons. Firstly, when ART is only initiated during pregnancy, there is peri-conceptual exposure to uncontrolled HIV viraemia and maternal inflammation which extends until ART initiation, usually beyond the sensitive first trimester. Additionally, when women present for antenatal care only in late pregnancy – as was seen in many women in our study - not only is the duration of exposure to viraemia and inflammation even longer, but there may not be sufficient gestational weeks remaining for the establishment of viral suppression before delivery. Finally, given the reduced inflammation observed when ART is initiated during acute rather than chronic HIV, it can be hypothesized that ART initiated in early disease stages prior to conception may be the optimal way to control maternal inflammation during gestation.[53] In turn, the concept of optimizing maternal HIV-related health prior to conception aligns with a growing global recognition of the important role peri-conception health plays in determination of long-term health outcomes in the offspring.[56] Establishing pre-conception disease control is considered a critical aspect of optimizing maternal and child health in context of chronic diseases such as diabetes, epilepsy and hypertension.[57] Similar goals for management of chronic HIV infection in women of child-bearing age would be in line with the paradigm shift towards treating HIV as a chronic disease through the life course.[58] However, the persistence of systemic maternal inflammation despite viral control may still require additional management even for women who conceive on suppressive treatment. For adults living with HIV, there have been increasing consideration of adjunctive anti-inflammatory therapies to minimize chronic immune activation that continue despite suppressive ART.[59] When efficacious measures to this end are discovered, testing in pregnancy will clearly be an important step towards not only optimizing maternal health but also those of their HIV-exposed offspring.

Managing systemic inflammation in pregnant WLHIV may also be beneficial to minimize adverse birth outcomes. A growing body of evidence supports the role of systemic maternal inflammation as a contributor to the higher risks of adverse birth outcomes that have been reported in WLHIV, both in the presence and absence of antiretroviral therapy during pregnancy.[60]

8.2.4 Excess health risks observed in HEU children are primarily concentrated in vulnerable sub-groups: evidence of fetal origins of disease

Globally, adverse birth outcomes, particularly PTB and SGA, are the primary causes of child mortality, and contribute substantially to long-term morbidity among survivors (fig 8.8)[61,62] For example, children born SGA have increased risks of stunting and developmental impairment, with lifelong increased risks of both communicable (CD) and non-communicable diseases (NCD).[15,63] PTB in turn predisposes to, among many other, short- and long-term risks of infections, suboptimal growth and developmental disorders.[62-65] While stillbirth is an underappreciated and devastating birth outcome for both WLHIV and those without, our cohort was restricted to liveborn infants enrolled after birth. The focus of this section is therefore on the roles of PTB and SGA in the relationships between maternal HIV and adverse child health.

While not yet fully elucidated, a range of separate and shared mechanisms are known to contribute to PTB and SGA in the general population.[66] Depending on the classification of preterm birth (spontaneous vs provider-initiated), these mechanisms include inflammation, infection, and other immunologically mediated mechanisms, uteroplacental ischaemia and haemorrhage, uterine overdistension and high levels of psychological or social stress.[61,67]

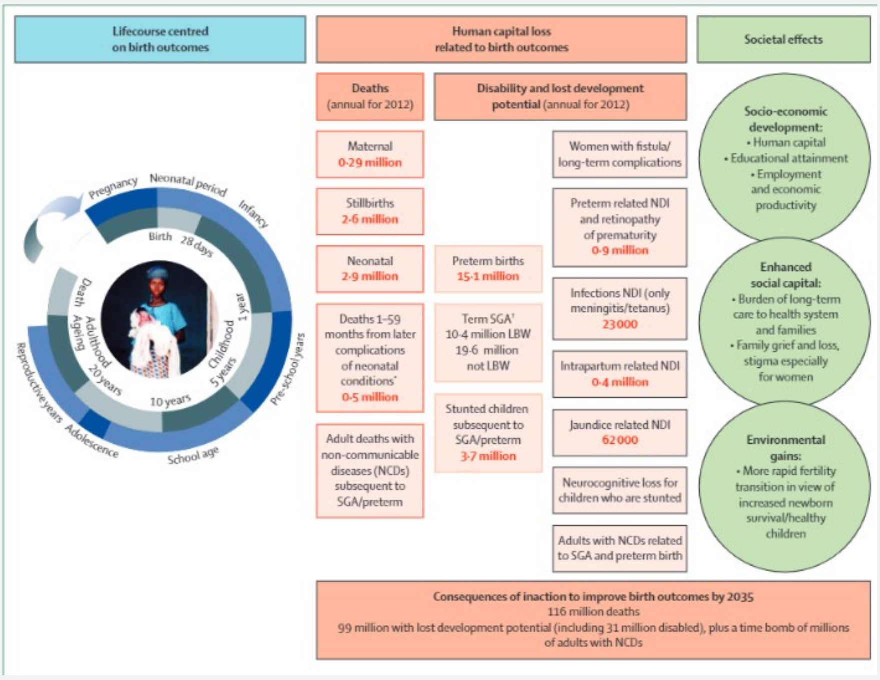


Figure 8.8. The estimated consequences of inaction to improve birth outcomes in terms of human capital loss by 2035, and wider societal effects. *Source:* Lawn, JE et al, Lancet 2014 https://doi.org/10.1016/s0140-6736(14)60496-7

In the absence of antiretroviral therapy, WLHIV are at substantially higher risk for adverse birth outcomes than HIV-uninfected women.[68] Associations between maternal HIV infection and adverse birth outcomes, particularly preterm birth, have persisted in the presence of antiretroviral therapy, with some but not all causality attributed to the use of the latter,[69] although the specifics and implications of these associations remain under debate.[70,71] Concurrently, accumulating data imply a critical role of disruptions in the vaginal and intestinal microbiome of WLHIV in both sustained immune activation and increased risks of adverse birth and child health outcomes.[72-75] Regardless of which mechanisms primarily explain the residual inflammation, it is probable that associations observed between treated maternal HIV and adverse birth outcomes may be driven in part by chronic inflammation. In support of this, inflammatory changes have been described in the placentae of WLHIV including those receiving ART,[76,77] although findings have been inconsistent.[78] In turn, both placental and

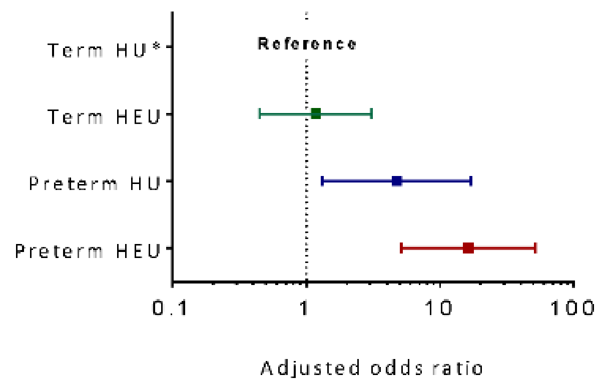


Figure 8.9. Adjusted relative odds of motor delay at age 12 months among HEU and HU children, stratified by preterm birth (also shown in chapter 6)

systemic maternal inflammation have been associated with short- and longer-term adverse health outcomes including preterm labor.[79]

As expected, both PTB and SGA were markedly associated with all child health outcomes in both mother-infant groups in our study. (figure 8.9). However, across the clinical outcomes, the increased risks associated with PTB and SGA were of greater magnitude among the HEU than the HU children. That is, our data unexpectedly suggested effect measure modification of the relationship between birth outcomes and adverse child health by maternal HIV status, and, conversely, of the relationship between maternal HIV status and adverse child health, by adverse birth outcomes (figure 8.9).

Again, support for the biological plausibility of these potential interactions can be drawn from the neuro-psychiatric, epigenetic and DoHaD fields. (figure 8.10)

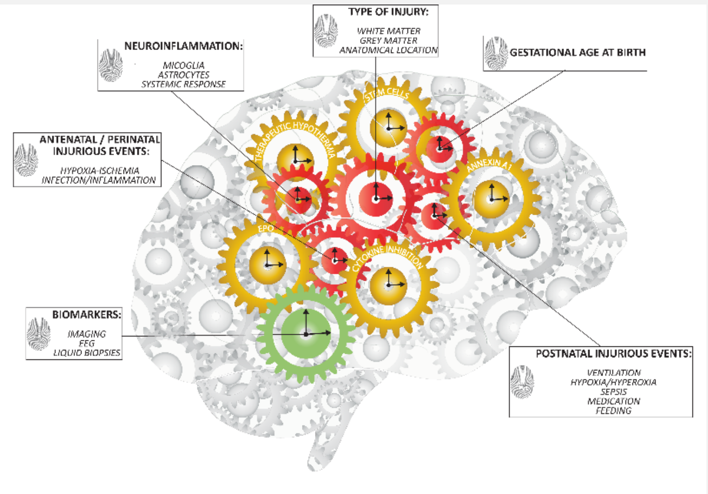


Figure 8.10 Graphical abstract of "Preterm Brain Injury, Antenatal Triggers and Therapeutics: Timing is Key" demonstrating perinatal factors that cumulatively influence adverse central nervous system outcomes in the context of preterm birth. *Source:* Ophelders D et al, Cells 2020 (https://doi.org/10.3390/cells9081871)

In research evaluating the impact of a fetal pro-inflammatory milieu on health outcomes in later life, the concept of a "dual-hit", or "multiple-hit", theory has been tested successfully in animal models.[80] This theory posits that accumulating antenatal insults are required for the clinical expression of adverse health consequences, in effect requiring a pre-disposition to secondary and subsequent insults after priming by initial exposure to maternal inflammation *in utero*.[80] These theories have been supported by observational clinical work, where adverse consequences of multiple perinatal insults appear to be additive, if not multiplicative and usually mediated through fetal and neonatal inflammation.[65,81,82]

There are two complementary causal possibilities to be considered in the light of these apparent interaction effects between adverse birth outcomes and maternal HIV on child health in our data. Firstly, it is possible that the processes driving PTB and/or SGA in the context of maternal HIV are in fact the same processes that drive the adverse health outcomes observed among HEU children. In this case, both PTB and SGA births may simply be partial mediators of the relationship between maternal HIV and adverse child health. For example, if HIV-related maternal inflammation is the primary driver of both PTB and later adverse child health, it may be that HEU children born PTB are simply representative of those HEU children exposed to the highest degrees of maternal inflammation.

However, in our dataset, associations between maternal HIV and adverse child health outcomes persisted even in analyses restricted to term children with adequate birthweight, although precision was limited for some sub-group analyses. This suggests a potential secondary pathway wherein predominantly non-HIV processes could be driving the adverse birth outcomes, while maternal HIV exposure functions as the "second hit" in accumulating fetal insults, resulting in a magnified vulnerability among those children who are both preterm and HEU. In this situation, PTB and SGA function more as effect modifiers than mediators of the effects of maternal HIV on child health. However, both these conceptual pathways may be contributing to the observed excess risks noted in those HEU children who were also born preterm or SGA. Regardless of which pathway is more likely, our analyses are hypothesis-generating, and at most raise questions that require answers in future research evaluations. Nonetheless, these findings provide a novel insight into potential mechanisms of vulnerability among HEU children. Even without acquiring a more detailed biological understanding of process, small, preterm HEU infants clearly require additional care and monitoring beyond the current norm.

8.2.5 Excess health risks observed in HEU children are primarily concentrated in vulnerable sub-groups: evidence of synergism between maternal HIV infection and social determinants of health

Across populations, PTB, SGA, growth faltering, high rates of infectious illness, and loss of developmental potential are more common, and have more severe consequences, in settings with high rates of poverty, unemployment and inequity.[15-17,61,62] Similarly, both CD and NCD are exacerbated among adults in these settings, including HIV, tuberculosis, substance abuse, obesity, cardiac disease and cancer.[83-86] Across diseases, social determinants of health (SDOH) are recognized as key driving factors, both directly and indirectly, via multiple, interrelated pathways.[87] Indeed, recognition of the importance of these "causes of causes" form the foundation of most of the Sustainable Development Goals.[88] In addition, the clustering of adverse health consequences across generations in settings of deprivation and economic strain has been well-described in both CD and NCD contexts.[87,89] The concurrent occurrence of two or more mutually reinforcing epidemics in synergism with adverse socio-economic settings has been termed a "syndemic", a concept first proposed by Dr Merrill Singer in 1994, in the context of HIV.[89,90] While the concept of syndemics has since been applied more broadly, the first published application thereof

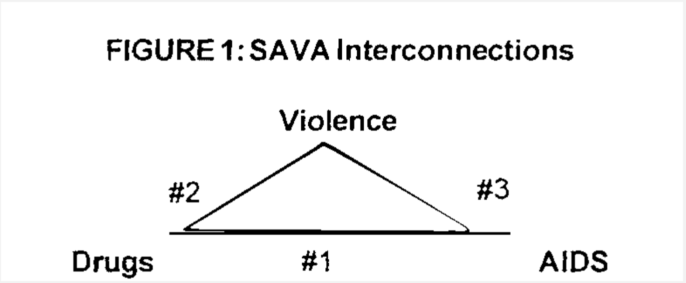


Figure 8.11. The "SAVA" syndemic (*Source: "A dose of drugs, a touch of violence, a case of AIDS: conceptualizing the SAVA syndemic" Dr Merrill Singer, Free Inquiry Special Issue on Gangs, Drugs & Violence, 2000*)

was a description of Substance Abuse, Violence and AIDS (the "SAVA" syndemic) among the disenfranchised urban poor of the United States (figure 8.10).[89,90] Among women in Africa, syndemic relationships analogous to the SAVA syndemic have been demonstrated between HIV, alcohol abuse, intimate partner violence and food insecurity, with bidirectional, mutually reinforcing relationships between all the components, in synergism with adverse socio-economic situations (fig 8.12).[91-95]

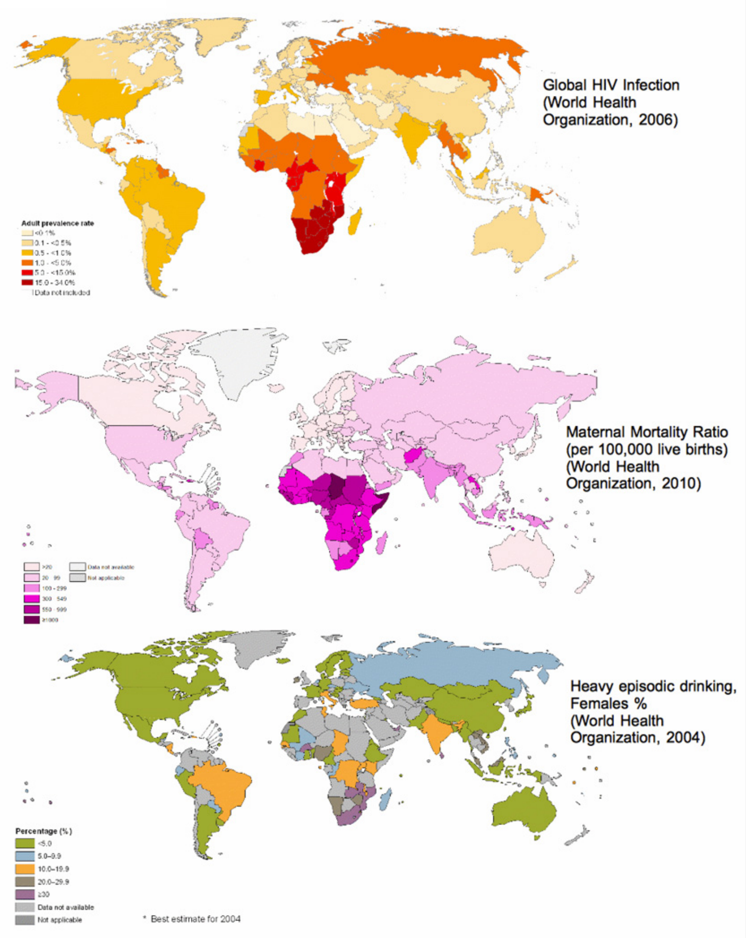


Figure 8.12. HIV prevalence, alcohol consumption and maternal morbidity.
Source: Russell, BS et al, Curr HIV/AIDS Rep (2013)

In our study design, we sampled HIV-uninfected women from the same peri-urban community as WLHIV, to minimize heterogeneity in social determinants of health and disease. The primary antenatal clinic where both groups of women were recruited, serves several, similar, impoverished communities which collectively have high rates of antenatal HIV prevalence, infant morbidity, and mortality. Importantly, the area has good access to health care, with multiple primary care clinics available, a functioning ambulance system and robust secondary and tertiary health care centres nearby. However, despite sampling from the same communities in general, there were stark differences in the prevalence and distribution of several social determinants of disease, alongside behavioural, and psycho-social risk factors, in the two cohorts of women, with substantial clustering of adverse life exposures and experiences among the WLHIV.

For example, mothers with HIV (compared to HIV-uninfected mothers) were substantially less likely to have flush toilets (27% vs 40%) and running water in the home (41% vs 52%), have completed secondary education (25% vs 45%), or be employed (39% vs 47%). They were more likely to report an unplanned pregnancy (34% vs 28%), risky drinking (25% vs 7%), and any intimate partner violence (22% vs 8%) at ART initiation in pregnancy.[70] Although we only managed to measure household food insecurity at 12 months postpartum, WLHIV were substantially more likely to be report household food insecurity (18% vs 3%, among the HIV-uninfected women).[30] Compared to HIV-uninfected women, the WLHIV were specifically more likely to report their household running out of money for food (29% vs 17%), having to cut meal sizes or skip meals because there was not enough money for food (21% vs 6%), their children having to skip meals or cut meal sizes (13% vs 2%) or their children going hungry because there was insufficient food (17% vs 2%).

Of these exposures, risky drinking, intimate partner violence and household food insecurity were the most consistently associated with adverse child health outcomes, exacerbated by single parenthood and lack of secondary education (table 8.1). In our dataset, these factors were also interrelated with significant overlap among the WLHIV, as shown in the Venn diagram in figure 8.13. The bidirectional relationships between all three of these factors with HIV have been well-described in women living in sub-Saharan Africa previously,[92,94,96] but there has been scant research done on the cumulative effects this devastating syndemic has on child health in our context (figure 8.14).

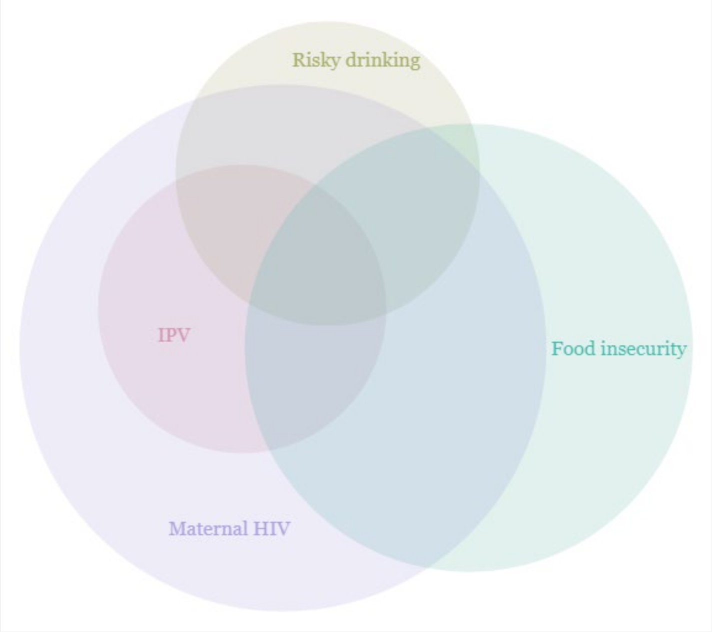


Figure 8.13 Venn diagram of relationships and overlap between maternal HIV, intimate partner violence, risky drinking, and food insecurity in the MCH-ART and HU2 studies

Each of these factors have also been directly linked to one or more of the adverse child health outcomes evaluated in this study (figure 8.14). Exposure to violence in the home has been associated with growth faltering and developmental delays in children, alongside significant lifelong mental health effects.[97,98] The lack of adequate nutrition in children of households suffering from food insecurity has directly measurable effects on health across the life course, including negative impacts on growth, development, and infectious risks in early childhood.[99,100] Alcohol exposure in the peri-conception period and during pregnancy also has multiple adverse implications for fetal and postnatal growth and development.[101] Fetal Alcohol Spectrum Disorder (FASD), which encompasses the more severe Fetal Alcohol Syndrome (FAS) with its classic craniofacial dysmorphology, is highly prevalent in several settings with high co-occurrence of HIV.[102] For example, in South Africa, the estimated prevalence of FASD is 11%, in line with the high national frequency of binge drinking.[101] Largely underdiagnosed, FASD includes a range of symptoms including mild dysmorphology, growth restriction, birth defects and developmental disorders.[101] Given the high rates of alcohol use in pregnancy among WLHIV in Southern Africa, and the difficulty of diagnosing the milder forms of FASD, it seems likely that many HEU children with FASD have not been identified to date. In turn, optimizing HEU child health will require significant efforts at addressing perinatal alcohol use and abuse.

From a population health perspective, the direct effects of circulating maternal HIV virus and chronic immune activation clearly cannot be disentangled from the complex interactions between HIV acquisition, psycho-social vulnerability, and socio-economic strain. In this, HEU children represent the second generation of a large and devastating syndemic among WLHIV (figure 8.14).

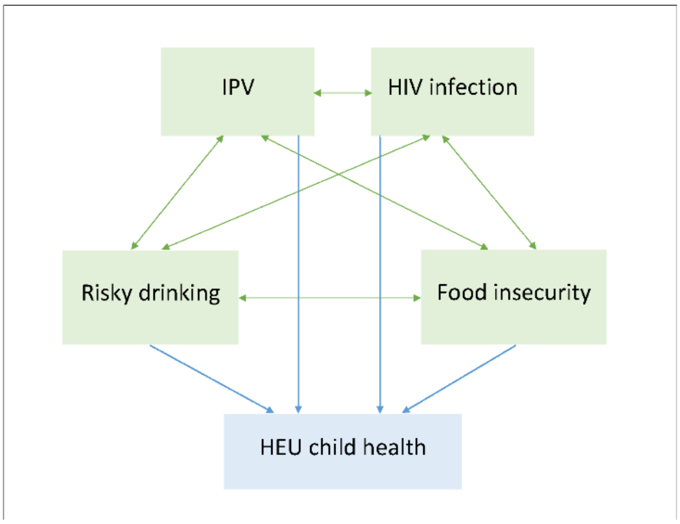
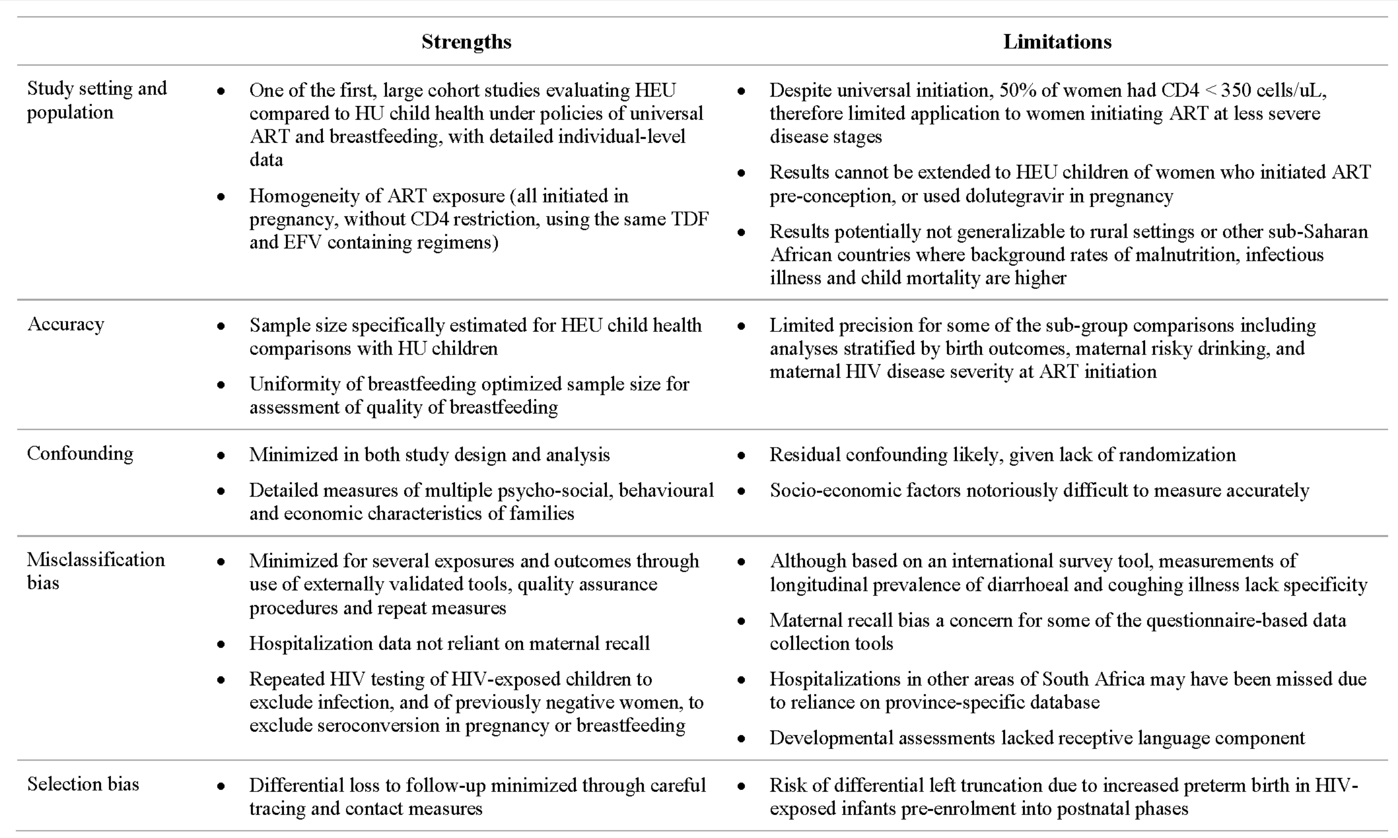


Figure 8.14. Children of a syndemic: interrelationships between intimate partner violence, risky drinking, household food insecurity and maternal health, with implications for HEU child health

Therefore, while understanding the exact immunological pathways of vulnerability in HEU children is an important step towards optimizing management of maternal HIV in the perinatal period, addressing the immediate virological and immunological effects of fetal exposure to HIV in isolation will clearly never be enough to alleviate the true vulnerabilities of these children. Truly optimizing the health of HEU children will require concerted efforts at achieving the more politic and social aspects of the SDGs across settings with high prevalence of HIV.

8.3 Strengths & limitations

This research project has contributed significantly to an improved understanding of the vulnerabilities of HEU children under current policies of universal maternal ART and the promotion of breastfeeding. Nonetheless, all findings generated by the project should be considered in the light of several strengths, and important limitations. Each results chapter has included a short discussion of strengths and limitations related to the specific analyses presented therein. To follow is a discussion of the overarching strengths and limitations of this body of work, with an overview provided in table 8.2.



	Strengths	Limitations
Study setting and population	• One of the first, large cohort studies evaluating HEU compared to HU child health under policies of universal ART and breastfeeding, with detailed individual-level data • Homogeneity of ART exposure (all initiated in pregnancy, without CD4 restriction, using the same TDF and EFV containing regimens)	• Despite universal initiation, 50% of women had CD4 < 350 cells/uL, therefore limited application to women initiating ART at less severe disease stages • Results cannot be extended to HEU children of women who initiated ART pre-conception, or used dolutegravir in pregnancy • Results potentially not generalizable to rural settings or other sub-Saharan African countries where background rates of malnutrition, infectious illness and child mortality are higher
Accuracy	• Sample size specifically estimated for HEU child health comparisons with HU children • Uniformity of breastfeeding optimized sample size for assessment of quality of breastfeeding	• Limited precision for some of the sub-group comparisons including analyses stratified by birth outcomes, maternal risky drinking, and maternal HIV disease severity at ART initiation
Confounding	• Minimized in both study design and analysis • Detailed measures of multiple psycho-social, behavioural and economic characteristics of families	• Residual confounding likely, given lack of randomization • Socio-economic factors notoriously difficult to measure accurately
Misclassification bias	• Minimized for several exposures and outcomes through use of externally validated tools, quality assurance procedures and repeat measures • Hospitalization data not reliant on maternal recall • Repeated HIV testing of HIV-exposed children to exclude infection, and of previously negative women, to exclude seroconversion in pregnancy or breastfeeding	• Although based on an international survey tool, measurements of longitudinal prevalence of diarrhoeal and coughing illness lack specificity • Maternal recall bias a concern for some of the questionnaire-based data collection tools • Hospitalizations in other areas of South Africa may have been missed due to reliance on province-specific database • Developmental assessments lacked receptive language component
Selection bias	• Differential loss to follow-up minimized through careful tracing and contact measures	• Risk of differential left truncation due to increased preterm birth in HIV-exposed infants pre-enrolment into postnatal phases

Table 8.2. Overview of key study strengths and limitations

The primary strength of this project is that it presents rigorously collected, individual data on one of the first cohorts of mothers and infants studied under universal ART initiated in pregnancy. Simultaneously, the data originate from one of the largest South African cohorts of mothers and infants in this context to date, with homogenous ART exposure and uniformly breastfed infants. Several design aspects were incorporated in an aim to address some of the methodological limitations apparent in previous research of HEU children.[103] These included a sample size specifically calculated for the outcomes measured, detailed data collection on multiple psycho-social, behavioural and economic factors, with sampling of HIV-uninfected mothers from the same communities, and followed parallel to the WLHIV. Together, these measures aimed to minimize confounding. In addition, we aimed to minimize measurement errors by using validated tools for both outcomes and third variables, with repeated quality assurance practices. The gestational age assessment tools, including early ultrasound, minimized the risks of misclassification of gestational age and SGA at birth.[104] Misclassification of exposures are a concern in both cross-sectional and longitudinal studies, an issue we further aimed to address by repeated HIV testing of previously negative HIV-exposed infants, and previously negative mothers.[103] Finally, selection bias from differential loss to follow-up was minimized by extensive measures to trace and contact mother-infant pairs not returning for study visits, as well as detailed searches of provincial databases to detect all hospitalizations and determine vital status at study completion. In study analyses, extensive exploratory analyses were utilized to identify optimal forms and expressions of third variables, which in turn were chosen *a priori* using an epidemiological approach based on prior knowledge of causal relationships in this field. Finally, recognizing the complexity of interrelationships between the primary exposures, outcomes, and a multitude of third variables, multiple sensitivity analyses were conducted and presented transparently.

Despite these strengths, several important limitations of this research may influence interpretation of our findings and serve to guide improved study methodology for future evaluations of maternal HIV and child health.

Generalisability to other settings in sub-Saharan Africa may be limited, especially where different background rates of malnutrition, mortality, and infectious morbidity, including malaria, may present unique challenges to HEU children and their mothers. Additionally, these results cannot be extended to situations where women have initiated ART pre-conception, especially those who initiated at higher nadir CD4 cell counts or during acute HIV infection. While the uniform ART regimen exposure is a strength of this study, it also limits the applicability of these findings to settings where women received dolutegravir, which is increasingly prevalent and may have specific implications for child growth among other outcomes.[105] Nonetheless, many of the important contributors to HEU child adverse health are universal, and therefore most of these findings are still relevant to other settings in Southern Africa. Maternal HIV acquisition and vulnerability is a complex web of causes and consequences. Despite the study design, multiple measures of potential confounders, and detailed analyses focused on adjusting, restricting, and stratifying, residual confounding remains likely. Interpretation of several of the stratified analyses was also complicated by a lack of precision due to small sub-group sizes. Although most of the measurement tools utilized were previously validated, several relied on maternal recall, increasing the risk for information bias. This includes the 2-week recall required for longitudinal prevalence of diarrhoeal and coughing illness, which in addition may have limited specificity.[106,107] In the postnatal cohorts of HEU and HU children, prevalence of preterm birth and SGA were similar. However, in the overall cohorts there were greater proportions of HIV-exposed (HE) than HU children born preterm. Several extremely preterm HE infants required extensive hospitalization, died, or initiated formula feeding, and were therefore not eligible for enrolment in the postnatal phase of follow-up.[3] This increased the probability of differential left truncation, which may have led to bias toward the null in our comparison of HEU to HU infants.[108] However, late preterm infants, which are the majority in our setting, are well represented in our cohort, for both groups. Finally, in the assessments of neurodevelopment at approximately 12 months, assessors were not blinded to the child's HIV exposure status.

8.4 Recommendations for policy, practice, and further research

8.4.1 Recommendations for policy

This work highlights multiple issues that adversely affect HEU children and their families, which will require improved or novel policy changes that can broadly be classified into HIV-specific policies and policies addressing general maternal and child health.

8.4.1.1 Prevention of mother to child transmission of HIV (PMTCT) policies

Current policies of universal antiretroviral therapy for pregnant women, with the support and promotion of breastfeeding for their infants, provide tremendous benefits for their children's health. Across outcomes evaluated in this research, the suppression of maternal viraemia – achievable only through ART use – alongside optimal breastfeeding dramatically decreased the discrepancies between HEU and HU children in terms of both survival and health. This research therefore supports and can provide advocacy for the tremendous child health benefits of current PMTCT policies beyond HIV-free survival.

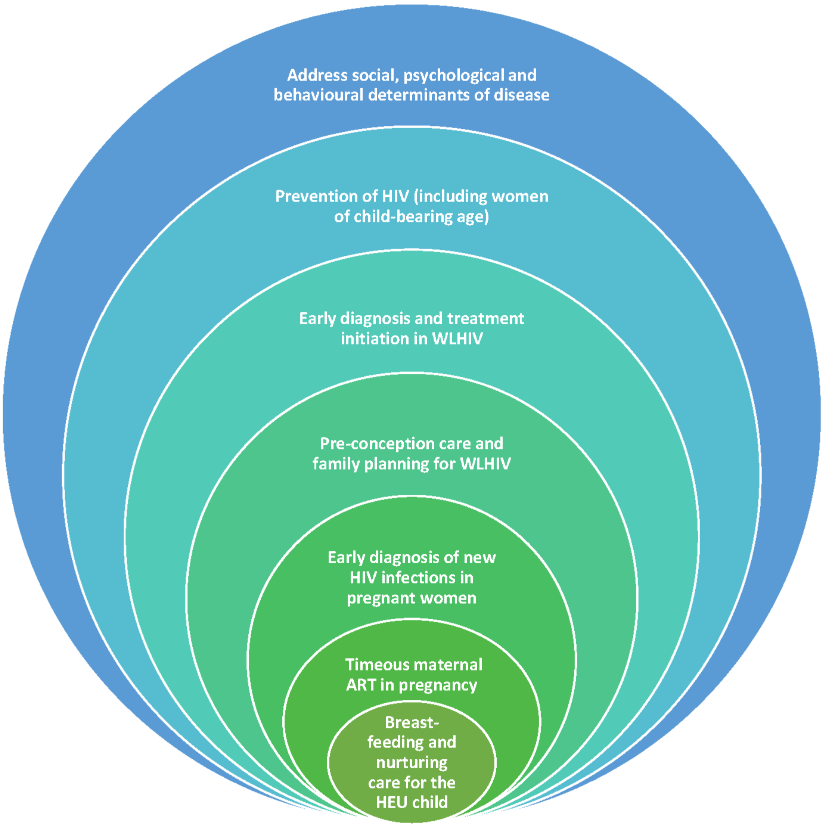


Figure 8.15 Layers of influence and opportunity for interventions to optimize the health of HIV-exposed uninfected children

Simultaneously, our data highlight areas where implementation of these policies can and should be improved.

A lower burden of maternal viraemia in pregnancy was strongly associated with improved outcomes in HEU children in our study, but not all women achieved viral suppression by the end of their pregnancies. In addition to measures that support ART adherence among pregnant WLHIV, the earlier identification and prompt treatment of HIV in pregnancy is critical, not only to optimize maternal health and reduce vertical transmission risk, but also to optimize the health of HEU children. A large proportion of WLHIV in this study presented for a first antenatal visit at a late gestation. To improve earlier initiation rates of ART in pregnancy would require earlier presentations for antenatal care, which in turn requires enhanced implementation of policies that promote early presentation for pregnancy care generally. Early presentation for antenatal care also has important maternal-infant health benefits that extend beyond HIV care.[109] Moreover, several challenges to optimal pregnancy care relate to pregnancy timing; strategies to enhance family planning should therefore also be critical components of PMTCT services. Extending this argument, optimal PMTCT policies should ideally address all of the following (figure 8.15): (a) prevention of HIV infection among all women of child-bearing age, (b) early detection of infection and initiation of treatment in WLHIV of child-bearing age, and (c) optimizing family planning in the setting of maternal HIV, including the prevention of unplanned pregnancies. The importance of pre-conceptual maternal and paternal health for optimal life-time health in offspring is increasingly recognized.[56] Optimizing pre-conceptual maternal health in the context of PMTCT includes providing WLHIV with the opportunities and means to control their fertility and minimize adverse exposures such as the risky drinking during an unplanned pregnancy, both of which are highly prevalent in the South African setting.

Finally, the adverse child health outcomes in our study were strongly correlated with maternal experiences of intimate partner violence, incomplete secondary education, and food insecurity. In the context of syndemics such as those experienced by WLHIV in settings such as ours, HIV care alone cannot be sufficient to optimize maternal or child health without additional policies and interventions that address gender-based violence, education of women and food security. Although there are clearly multiple additional factors at play, it is increasingly recognized in the global HIV community that these aspects urgently require attention to optimize health for all PLHIV.[110] Indeed, the global call for "Health in All Policies" is perhaps nowhere more applicable that in the context of vulnerable HIV-affected families.[111]

8.4.1.2 General policies that influence maternal and child health

Our data supports previous work demonstrating a wide heterogeneity in exposures and health risks among HEU children. The highest risks of adverse child health outcomes appear to cluster in subgroups of the most vulnerable HEU children, mirroring the clustering of social determinants of health and disease among WLHIV in a syndemic setting. In turn, these clustered exposures and adverse outcomes (including preterm birth, malnutrition, diarrhoeal illness, pneumonia and loss of developmental potential) reflect the most critical issues affecting child health globally. That is, maternal HIV could be viewed as a marker for increased risks of multiple threats to child health, threats which are endemic to most impoverished settings regardless of maternal HIV status. Consequently, addressing the most urgent threats to global child health generally would also address much of the driving force behind residual excess morbidity among the breastfed HEU children of WLHIV who receive universal ART, specifically.

Figure 8.16. The Sustainable Development Goals (SDGs). *Source:* https://sdgs.un.org/goals

Moreover, the realization of Sustainable Development Goals SDG 1 (No Poverty), 2 (Zero Hunger), 3 (Good Health and Well-being), 4 (Quality Education), 5 (Gender Equality), 6 (Clean Water and Sanitation), 8 (Decent Work and Economic Growth) and 10 (Reduced Inequalities) are likely to disproportionately benefit HIV-affected families across resource-limited settings (figure 8.16).[88,112] While the realisability of these ambitious goals may be debatable, potentially impossible in the wake of a world-altering pandemic,[112] the global identification of these goals with sustained efforts to improve life for the most vulnerable certainly align with addressing the greatest threats for HEU child health.

Several large reviews evaluating optimal individual and integrated interventions to promote child growth and development have demonstrated the value of a life-course perspective, promoting cross-sectoral, integrated policies for cumulative benefits extending to both children and their communities (figures 8.17 and 8.18).[42,113-117] Most of the proposed "intervention packages" discussed in these reviews firmly align with multiple SDGs and can be provided in primary health care settings, often deliverable by Community Health Workers (CHW).

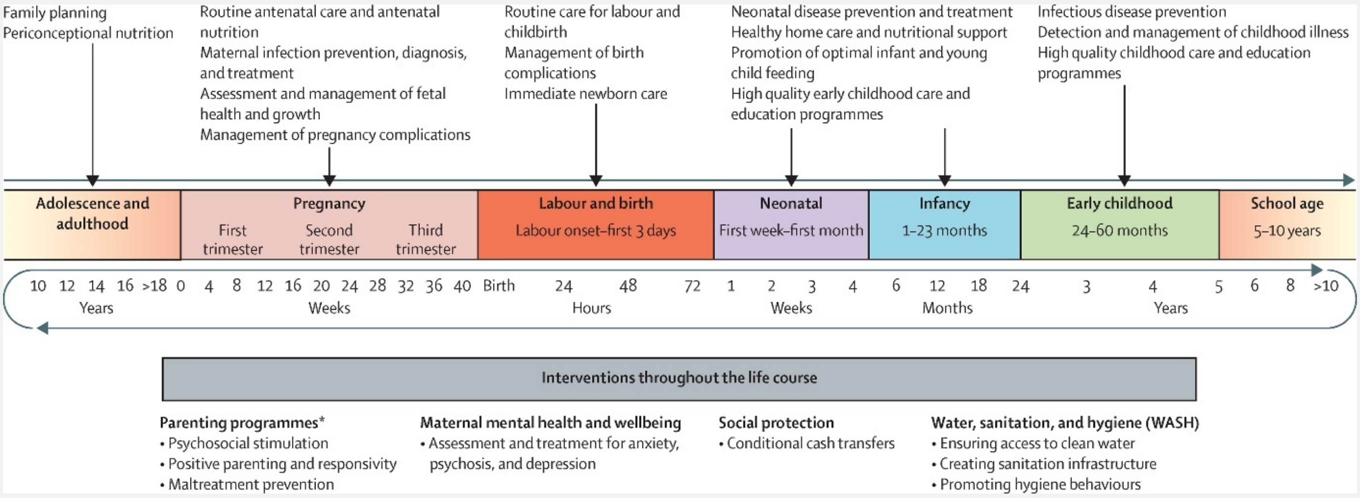


Figure 8.17 Evidence-based interventions that affect aspects of nurturing care. *Source*: Britto, PR et al. Lancet series on Advancing Early Childhood Development: from Science to Scale, 2017. *Nurturing care: promoting early childhood development.*

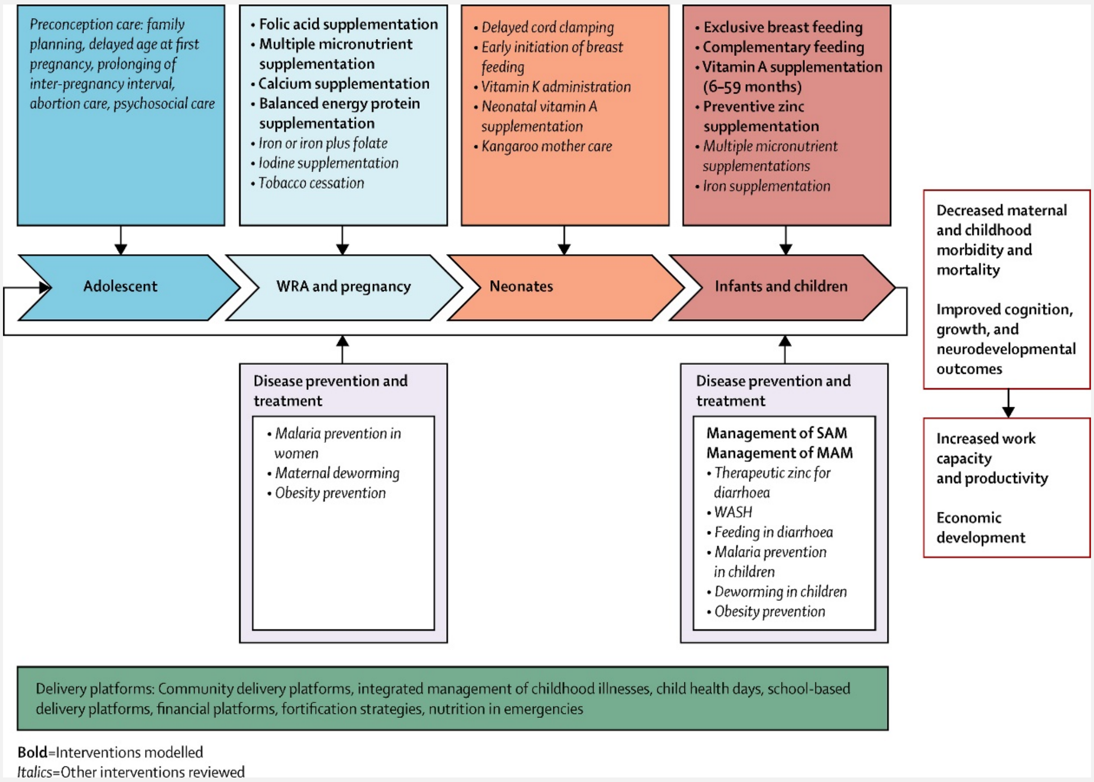


Figure 8.18 Conceptual framework: evidence-based interventions for improvement of maternal and child nutrition. *Source:* Bhutta et al, Lancet, 2013. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? https://doi.org/10.1016/S0140-6736(13)60996-4

Additionally, multiple global guidelines relevant to these suggested “intervention packages” already exist, with evidence-based recommendations to guide policy makers. The World Health Organization (WHO) guidelines are particularly applicable to settings with high HIV prevalence, and address antenatal care, preterm birth, nutrition, infectious diseases, and early childhood development (ECD).[118]

For example, the WHO have published guidelines to address antenatal nutritional supplementation (iron folic acid, calcium, energy protein supplements, small-quantity lipid-based nutrient supplements); nutritional and behavioural counselling; treatment of genito-urinary tract infections including syphilis; identification and management of substance use, substance use disorders and intimate partner violence; and identification and management of anaemia, helminthic infestations, preeclampsia, preterm labour and postpartum haemorrhage.[118] Extending further into the life course, guidelines also exist for the implementation of several crucial childhood-focused interventions. These include Kangaroo mother care; optimal breastfeeding; nutritional supplementation (vitamin K, vitamin A, zinc, fortification of foods with micronutrients, food supplementation after 6 months); vaccination (Expanded Programme for Immunization including pneumococcal and rotavirus vaccines); and prevention, diagnosis and treatment of childhood pneumonia and diarrhea including the use of an Integrated Management of Childhood Illness (IMCI).[118] In 2020, the WHO released guidelines specifically focused on promoting ECD.[119] Recommendations include responsive caregiving; promotion of early learning; integration of caregiving and nutritional interventions; and the support of maternal mental health. In addition, population-level guidelines exist that address water, sanitation, hygiene (WASH), and housing.[118]

However, implementation of most of these guidelines has been suboptimal, poorly regulated, and often constrained by supply chain issues, budgetary constraints, and other bottlenecks.[112,120,121]

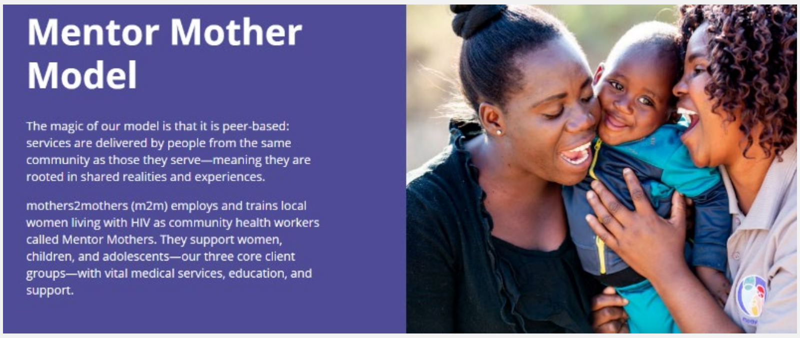
Although not applicable to every individual HEU child, the children of WLHIV represent the face of vulnerable children everywhere. In turn, addressing vulnerabilities around preterm birth, malnutrition, infectious morbidity, and loss of developmental potential broadly, through improved implementation and regulation of existing guidelines, will significantly benefit HEU children, specifically. Focusing on already existing policies and finding more effective and efficient ways of ensuring implementation, including integration within PMTCT care, may therefore more relevant and important for HEU child health than the design and implementation of new, HEU child-specific policies.

8.4.2 Recommendations for practice

Successful implementation of policies such as those discussed above require integrated service delivery that is accessible to all. Ideally, at the clinical interface between health services and the caregivers of HEU children, particular care should be provided for specific HEU child vulnerabilities. For example, while all children should receive regular growth monitoring, increasing the frequency for HEU children may be beneficial for identifying nutritional and chronic health issues early, especially among those who were also born preterm or SGA. Following exclusion of concurrent infectious diseases such as tuberculosis, intervention packages for growth faltering among HEU children could for example include protein, lipid and micronutrient supplementation, deworming, increased monitoring, a screening and referral pathway for maternal health and behavior, and social interventions that address WASH, housing, job creation and access to Child Care grants. Similarly, regular developmental screenings for HEU children may need to be somewhat more detailed than those for the general population of children, with additional support provided to assist WLHIV to provide nurturing care to their young children.[122] In addition, maternal risky behaviour and mental health screenings should ideally be standard for WLHIV across the life course, incorporating clear referral pathways for intervention when required.

The critical role of CHW in delivering primary care interventions such as those discussed above has long been acknowledged,[123] with excellent results observed in settings such as Bangladesh, Ethiopia,[124] and Brazil.[125] Optimal approaches to the selection, training, and monitoring of CHW services appear to differ by setting and programme objectives, but in general, a community-sensitive structure with adequate renumeration, support and career advancement for the CHW has been associated with improved outcomes in the context of maternal and child health.[123,126-128] In South Africa, CHW employed by the National Department of Health have faced multiple challenges around appropriate renumeration, training and support in the past,[129-131] but several successful programmes centred on CHW have been implemented and maintained by local, non-governmental organizations.[132] In addition, there is a growing evidence base demonstrating the positive effects of female CHW, identified after their own pregnancies, delivering integrated packages of maternal-child care within their own communities.[133,134] These include the "Mentor Mother" models utilized by the "Mothers2Mothers" (fig 8.19) and the Philani Maternal, Child Health and Nutrition Trust programmes, as well as the "Goodstart" study, a South African, cluster-randomized effectiveness trial which evaluated an integrated, community-based package for maternal and newborn care in the context of HIV.[132,134-136]

In addition, the South African Department of Health has recently engaged with a new mobile health application across several provinces, with positive early results.[137] Developed in South Africa in cooperation with academics, government officials, local



Mentor Mother Model

The magic of our model is that it is peer-based: services are delivered by people from the same community as those they serve—meaning they are rooted in shared realities and experiences.

mothers2mothers (m2m) employs and trains local women living with HIV as community health workers called Mentor Mothers. They support women, children, and adolescents—our three core client groups—with vital medical services, education, and support.

Figure 8.19. Image and caption from the "Mothers2mothers" website. *Source:* https://m2m.org/what-we-do/model/

businesses and communication experts, the AitaHealth™ system utilizes mHealth platforms to register and monitor families requiring primary health care services at home (https://edition.cnn.com/2017/05/02/africa/alf-aitahealth-app-healthcare/index.html).[138] Through CHW, data are transferred real-time to a central database for monitoring and intervention purposes. Of relevance to the syndemic threats to HEU child health, the system utilizes a "flag" approach, whereby a household with any pre-listed health risks are identified (for example multi-drug resistant tuberculosis) and scheduled for increased intensity of CHW visitation and monitoring, including the broader screening of other household members for related diseases. The households of HIV-affected children could potentially be flagged in this, or a similar, system, providing ethically approved, confidential methodology can be ensured. To this end, ethnographic research involving community members and leaders alongside national government institutions would be required, in addition to implementation research with objective, longitudinal measurement of outcomes. While there remains much work to be done to optimize government-driven implementation models and ensure productivity in the South African setting, a clear need exists for improved utilization of CHW in the move towards Universal Health Care. Incorporating a specific focus on HIV-affected families in the delivery of primary care intervention packages has substantial potential to improve HEU child health.

At a facility level, early evaluations of integrated general health and PMTCT services have shown benefit for maternal ART adherence and improving breastfeeding practices, with high rates of acceptability.[139,140] Similar integrations of maternal and child health services within the setting of primary HIV care may allow a more intense application of general child policies to HEU children, while simultaneously providing family planning and general health care for the parents and siblings.[132] The inclusion of specific care packages that enhance the monitoring and support of HIV-affected families within the broader health packages provided through CHW in linkage to primary care clinics have the potential to address multiple levels of HEU child health threats simultaneously and cost-effectively.[129,130,140,141]

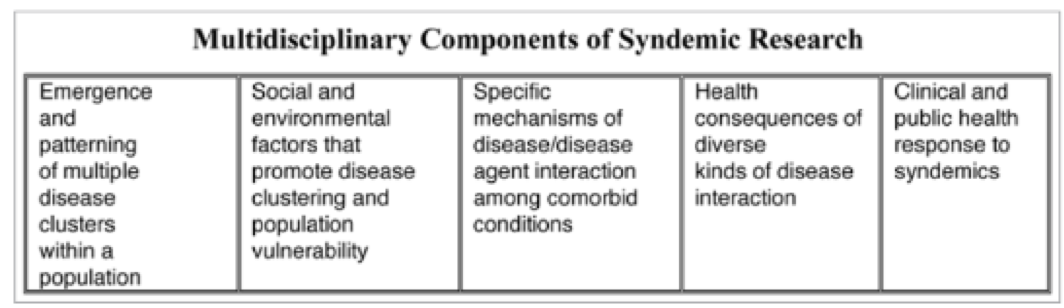
8.4.3 Recommendations for further research

To understand the health vulnerabilities of HEU children under conditions of universal maternal ART and breastfeeding, and to design interventions towards optimizing the health and survival of these children, will require further data from both observational and interventional studies.

First, this doctoral research project provides data on one of the first HEU and HU comparative infant cohorts described under these conditions. Additional observational studies are required to (a) assess the reproducibility of these findings; (b) extend generalizability by providing further data on these outcomes under different conditions, for example in malaria endemic areas or rural settings with higher rates of malnutrition, under other ART regimens, and for HEU children whose mothers initiate ART during acute infection, pre-conception; and (c) provide long-term outcome data on HEU children. To my knowledge, there are currently no published data available on the health of HEU adolescents who were breastfed and whose mothers received universal ART in pregnancy. Similarly, data on pre-school and school-aged HEU children born under these conditions are limited. Given the increasing population risks of obesity in children generally, obtaining more clinical and laboratory data on the metabolic effects of in utero exposure dolutegravir-containing regimens should be an additional research priority across the lifespan. Data addressing these knowledge gaps should ideally come from both smaller studies with detailed, individual-level data, and larger collaborative studies that utilize routinely collected data.

Second, a clearer understanding of the immuno-pathological pathways involved between maternal, fetal, and infant inflammation in this context will be critical to facilitate the design of interventions that minimize the adverse effects of chronic immune activation, on both maternal and child health.

Third, syndemic threats to HEU child health require closer evaluation with the view to design multi-faceted interventions. Syndemic research ideally requires a mixed-methods, multi-disciplinary approach (figure 8.18), with anthropologists, economists and community activists working alongside epidemiologists, clinicians, and statisticians.[142] To date, this aspect of HEU child health has been largely neglected in HIV research agendas.



Multidisciplinary Components of Syndemic Research

Emergence and patterning of multiple disease clusters within a population	Social and environmental factors that promote disease clustering and population vulnerability	Specific mechanisms of disease/disease agent interaction among comorbid conditions	Health consequences of diverse kinds of disease interaction	Clinical and public health response to syndemics

Figure 8.20. Components of syndemics and conjunctures of multidisciplinary interaction. *Source:* Singer, M. Virulence (2010) https://doi.org/10.4161/viru.1.1.9933

Finally, implementation research is urgently required to assess the most cost-effective, scale-able ways to implement "intervention packages" that include the integration of nutritional and developmental interventions for promoting nurturing care, reduces stunting, and improves the socio-economic conditions of HIV-affected households. Most of the interventions currently recommended in global guidelines have tremendous potential to be implemented at low cost with rapid scale-up, and are deliverable predominantly through primary health care strategies, which in turn align HEU child health care goals with those of the SDGs.[143]

8.5 Conclusions

In urban South Africa, previously observed excess health risks in HEU compared to HU children are reduced, but may not be fully ameliorated, under policies of universal maternal ART and breastfeeding. These residual differences are predominantly driven by a lack of HIV viral control in pregnancy despite ART, adverse socio-economic environments and exacerbated by suboptimal breastfeeding practices. Moreover, our findings suggest a potential interaction between maternal HIV exposure and adverse birth outcomes, with the highest relative risks of adverse child health outcomes observed among HEU children who were also born preterm or SGA. Concurrently, the HEU children in our setting are exposed to an ongoing syndemic of maternal HIV, risky drinking, intimate partner violence and household food insecurity. Therefore, addressing the residual discrepancies in HEU and HU child health will require earlier diagnosis and treatment of HIV in women of child-bearing age, early antenatal care incorporating strategies to optimize birth outcomes, with effective measures to support optimal breastfeeding, and multi-sectoral interventions to address the syndemic conditions under which many HEU children live. Additional research is required on the immunological pathways through which chronic maternal HIV influences the fetal environment, with the view to design treatment adjuncts that may benefit both mother and fetus, and in turn, the life-course health trajectories of HEU children. Furthermore, implementation research will be critical to identify optimal ways to implement multi-faceted packages of care that address both maternal and child health in the context of HIV. To this end, an expanded utilization of primary care interventions, delivered by community health workers and incorporating close monitoring of growth and development of preterm and SGA HEU, should be considered as a cost-effective option for improved service delivery to this vulnerable population.

8.6 References

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Chapter 9: Appendices

9.1 Supplemental material for Chapter 1 (Introduction)

Supplemental Table 9.1.1. Growth, infectious morbidity, mortality, and neurodevelopment of HIV-exposed uninfected compared to HIV-unexposed children in sub-Saharan Africa: summary of longitudinal studies published between October 2015 and January 2021

Original study details	1st author, year, journal	Enrolment age, follow-up, demographics	Perinatal exclusion criteria	Infant feeding	Primary clinical outcome (s)	Main findings (HEU vs HU)	Effect of ART/disease severity/BF	Comment
No maternal use of antiretroviral agents								
ZVITAMBO (1996-2000): RCT of vitamin A Harare, Zimbabwe	Evans (2016), AIDS	< 96 hours of birth Followed to age 2 years In this analysis: HEU, n=4029; HU, n=9133	BW < 1500g	Most initiated breastfeeding @ 3 months HEU: EBF, 5%: no BF, 40% HU: EBF, 4%; no BF, 40%	Growth (HCAZ longitudinally, odds of microcephaly)	@ 3 months: HEU 4% vs HU 2% (OR 1.39, 95% CI 1.05-1.84); no difference ito microcephaly at other ages but continuous HCAZ lower throughout follow-up	Not reported	None
	Omoni (2017), PIDJ	< 96 hours of birth Followed to age 2 years In this analysis: HEU, n=3120; HU, n=9210			Growth (LAZ and WLZ longitudinally, odds of stunting and wasting	High background rates of stunting HEU greater % and odds of stunting throughout 1st year (eg at 6 months, 17% vs 14%; OR 1.23, 95% CI 1.08-1.41)	Not reported for HEU children; EBF protective for HIV+ children	None
Predominantly 1-2 antiretrovirals peri-delivery, no use of ART (prophylaxis only, prior to "Option A")								
VTS (Vertical Transmission Study), 2001-2006: NRSI, supporting EBF	Rochat (2016) PLOSMed	VTS: antenatal enrolment Nested cross-sectional evaluation for 7-11 years old HIV-negative	Not reported	In overall VTS cohort: Mostly breastfed, high uptake of EBF both groups;	Cognition (KABC-II), executive function (NEPSY-II) and emotional-	No meaningful differences between HEU and HU across any of the measures	EBF not associated with cognition or executive function globally, but in sex-stratified	None

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among HIV+ and HIV- mothers Rural South Africa This sub-study, Siyakhula cohort: 2012-2014		VTS children (N=332 HEU, N=574 HU), plus additional N=629 HIV-negative children from local demographic platform (matched on age and HIV-exposure, not exposed to VTS intervention)		rapid weaning at 5m for HEU In this sub-study: 30% of both HEU and HU EBF between 2-5 months	behavioural function (CBCL)		analyses, EBF associated with better cognition in boys EBF associated with fewer conduct disorders	
	Houle (2019) PLOS Med	In this analysis, n=477 HEU, n=1057 HU Mean age 9.7 years	Not reported	See above HIV-unexposed children more likely to BF for > 12 months	Growth: body mass index, weight, fat, and blood pressure	No differences between HEU and HU children in risk of overfat, overweight or prehypertension (undernutrition risks not disaggregated by maternal HIV status)	Longer BF duration (between 6-11 months of age) associated with lower odds of overfat overweight	Maternal overweight predicted child overweight
BFPH and CIGNIS cohorts followed into school-age Lusaka, Zambia BFPH (2001-2003): observational, BF decisions and early postpartum health CIGNIS (2005-2009): Nutritional RCT for micronutrient	Rosala-Halles (2017) BMC Pediatrics	Original studies: BFPH: antenatal enrolment, followed through 16 weeks CIGNIS: enrolled at 6 months, followed through18 months Current analysis: repeated measures from enrolment through ~11.6 years (BFPH)	Not reported	Predominantly breastfed, varying durations BFPH: 69% of HU and 46% of HEU BF > 18 months CIGNIS: 74% of HU and 16%	Growth: WAZ, LAZ and BMIZ longitudinally	HEU lower WAZ, LAZ and BMIZ in first few years (around 0.4 to 0.7 of a SD especially WAZ and BMIZ), even after adjusting for SES	Not reported	None

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enhanced complementary feeding		and ~7.8 years (CIGNIS) HEU, n=200 and 165 HU, n=207 and 590		of HEU BF > 12 months				
Prospective, community-based cohort (2008-2011) Blantyre, Malawi	Struyf (2019), European Journal of Paediatric Neurology	Enrolled ~12 weeks, followed ~ 3 monthly with repeated measures until 2 years of age HEU, n=289 HU, n=170	Not reported	Not reported	(1) Cognitive development (2) Mortality (3) Growth	(1) No difference in BSID scores at 15 weeks or 2 years (2) 27/289 (9%) vs 6/170 (4%) (3) Stunting: 15% vs 8%; underweight: 15.2% vs 8.5%	Not reported	None
Option A: predominantly 1-2 antiretrovirals, ART restricted by CD4 cell counts (all study participants on ART initiated due to low CD4)								
RCT of malaria prophylaxis, stratified by HIV exposure Tororo District Hospital, Uganda (2010-2013)	Boivin (2016) Malaria Journal	Original study: enrolled 4-5 months of age This sub-study: co-enrolled at 2 years of age with cognitive assessments at 2 and 3 years HEU: n=143 and 122 HU: n=325 and 331 ART % NR here; linked publication, ~78% ART, initiation timing NR	Not reported	Breastfeeding was enrolment criteria for original study	Cognitive development (MSEL) measured at 2 and 3 years	Composite cognitive total score lower, reflecting lower scores for both receptive and expressive language No meaningful differences noted in motor or visual reception scores	Not evaluated	none

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Mother Infant Health Study (enrolment 2012-2013) Prospective cohort, Cape Town South Africa	Slogrove (2017) PIDJ	Enrolled < 72 hours from delivery Seen @2 weeks then 2-monthly through 6 months HEU: n=94 HU: n=82 Groups frequency matched on smoking and alcohol use 50% of women received ART, of whom 43% initiated pre-conception (overall, pre-conception ART in 21% of 94)	≤ 2000g < 34 weeks	HEU: 37% BF (free formula provided) HU: 99% BF	(1) Sick child visits (2) Infectious-cause hospitalization or death (3) Growth	(1) No difference (2) Admission risk similar for LRTI; higher for diarrhoea (HEU 8.5% vs HU 1.2%) Any infectious admission or death: OR 1.6 (95% CI 0.69-3.82) Severe infection admission or death: OR 1.83 (95% CI 0.62-6.11) (3) WAZ: similar for HEU and HU at birth and at 6 months LAZ: at birth, 0.75 SD lower in HEU than LAZ: at 6m, 0.4 SD lower in HEU than HU	Difference in odds of severe infection remained when stratified by BF	Most hospitalizations occurred in first 3 months; HEU slightly longer duration of hospitalization
	Springer (2017) TMIH	Nested cross-sectional assessment in above cohort, at 12 months HEU, n=58; HU, n=38	≤ 2000g < 34 weeks	In this sub-group, 40% of HEU and 100% of HUU were BF at age 2 weeks	Neuro-development (BSID-III) Social withdrawal (ADBB)	Composite motor, cognitive and language scores similar Decreased vocalization on ADBB more common among HEU than HU (26% vs 10%)	Not reported	None

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Original study details	1st author, year, journal	Enrolment age, follow-up, demographics	Perinatal exclusion criteria	Infant feeding	Primary clinical outcome (s)	Main findings (HEU vs HU)	Effect of ART/disease severity/BF	Comment
	Springer (2020) AIDS Care	Nested cross-sectional assessment in above cohort, at age ~ 2 years HEU, n=32; HU=27	≤ 2000g < 34 weeks	As above	Neuro-development (BSID-III) Behaviour (SDQ)	Composite motor, cognitive and language scores similar HU (vs HEU) children greater total difficulties, externalizing symptoms and conduct problems	Not reported	Declines in BSID scores compared to at 12 months; Lower BSID scores associated with stunting
2 RCT of micronutrients, Dar es Salaam, Tanzania Among HEU (2004-2008) Among HUU (2007-2010)	Locks (2017) J Pediatrics	Maternal enrolment in early pregnancy, infant enrolment and randomization at 6 weeks HEU, n=2088 HU, n=1202 No denominators on how many received ART, nor whether pre- or post-conception initiation	Multiples, congenital abnormalities or difficulty swallowing	Predominantly breastfed HEU vs HU longer duration of EBF (4 vs 2 months) but shorter overall duration of BF (4 vs 13 months)	(1) Infectious morbidity (2) Mortality (3) Growth	(1) Common childhood illness 7-45% higher in HEU than HU Unscheduled outpatient visits, RR 1.87; Hospitalizations, RR 4.32 (2) 4.6 vs 1.2 deaths per 100 PY (HR 3.76) (3) No notable differences in stunting, wasting or underweight	aHR for mortality: compared to HUU, HEU of mothers with early disease stage, no ART, 1.55; mothers with later disease but access to ART, 1.92; mothers w later disease and no ART, 4.96 (95% CI 1.76-14.04)	None
Tshipidi Prospective observational study at 2 sites, Gabarone and a nearby village,	Chaudhury (2017) Pediatrics	Enrolment antenatal or withing 7 days of birth, followed through 24 months This analysis, nested cross-sectional HEU, n=337 HU, n=387	None	HEU, 9% BF (free infant formula) HUU 99.5% BF	Neuro-development at ~24 months (BSID-III and DMC)	Generally, clinically similar scores with some small differences in language Higher risk of expressive language delay (53% vs	Not reported	None

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Botswana (2010-2012)		36% of WLHIV received ART (no detail re initiation timing), <1% received none				44%, aOR 1.44, 95% CI 1.01-2.06) Slightly lower personal-social scores on DMC		
	Ajibola (2018) J Pediatrics	This analysis, longitudinal (visits at 1 and 6 months then 6-monthly) HEU, n=431 HU, n=457 ~ 30% of WLHIV received ART (no detail on timing of initiation)	None	HEU, 8% BF HUU, 99.5% BF	(1) Risk of mortality by 24 months (2) Time to hospitalization or death	(1) 5% vs 1.8% (aHR 3.27, 95% CI 1.44-7.40) (2) Shorter among HEU, logrank p=0.001 (leading causes, gastroenteritis pneumonia and other respiratory illness)	Low baseline CD4 not associated with mortality risk among HEU	Mortality highest in 1st month of life (55% of HEU and 38% of HU deaths); associated with preterm birth
	Smith (2020) JID	This analysis, HEU, n=226; HU, n=88 ~ 16% ART (no detail on initiation timing)	None	HEU, 6% BF HU, 99% BF	All-cause hospitalization	HEU, 17.3% vs HU, 6.8% OR 2.85 (95% CI 1.17-6.57) for hospitalization in first 24 months Among HEU, 41% of admissions in 1st 6 months (31% between 12 and 24) vs HU, 16% and 50%	Not evaluated for hospitalization	Infectious cause for hospitalization, 83% of HEU admissions vs 66% of HU admissions
ANRS-Pediacam (2007-2011) Prospective cohort study, recruitment from three referral hospitals in Cameroon	Debeaudrap (2018) AIDS	Enrolment 1st week of life, followed through early childhood This analysis, nested cross-sectional assessments for 4-9 years old children who are still in follow-up	None, but assessments not done on children with sickle cell or CNS disease	Not reported	Neuro-development (KABC-II and SDQ)	HEU scores were lower than HUU across KABC indices in crude but not adjusted analyses (after accounting for household income; maternal education, mental health, and vital status)	Not reported	None

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		HEU, n=101 HU, n=110 Maternal ART use NR				SDQ scores were similar for HEU and HU children		
	Ledoux Sofeu (2019) PLOS One	See above This analysis, longitudinal through 5 years of follow-up HEU, n=205 (99 by 5 years); HU, n=196 (101 by 5 years) In a linked publication - ~38% ART for WLHIV (initiation time NR)	None	Not reported	Growth: WAZ, WLZ, LAZ	HEU vs HU, at least one measure during follow-up classified as Underweight: 20% vs 8%, RR 2.61 (95%CI 1.50-4.57) Stunted: 60% vs 42%, RR 1.43 (95% CI 1.17-1.74) Wasted: 44% vs 33%, RR 1.34 (95% CI 1.04-1.72)	Not reported	2.9% of HEU vs 1.5% of HU died Median age at death younger in HEU than HU (16.6 vs 30.6 months)
Mixed antenatal ART exposure, both Option A and some Option B/B+: < 70% of HIV+ study participants receiving <u>universal</u> ART in pregnancy (no CD4 restriction)								
Prospective cohort from 5 geographically diverse hospital postpartum wards (2012-2013) Botswana	Zash (2016), BMC pediatrics	Enrolment within 48 hours of delivery, followed telephonically 1-3 monthly until 24 months of age HEU, n=1515 HU, n=1518 No details on %pre- vs post-conception ART	None	HEU, 16% BF for at least 1 month (1.4% for more than 6 months) HU, 99% (75% for more than 6 months)	Mortality	Cumulative probability of death, HEU vs HU: 4.7% (24 per 1000 PY) vs 1.6% (8 per 1000 PY) [aHR 2.7 (95% CI 1.5-3.6)] 30% of deaths occurred within first 42 days (34% of HEU deaths, 17% of HU deaths); commonest cause was diarrhoea for both groups	Mortality risk similar for binary CD4 cut at 250 cells/uL	HEU more likely to be born preterm and/or LBW HEU vs HU ever admitted, 16% vs 11% Maternal death aHR for infant death 8.9

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DCLHS Birth cohort outside Cape Town South Africa (enrolment 2012-2015) Peri-urban, 2 communities	Tran (2016) Medicine	Enrolment antenatal, followed through 2 years of age – this analysis nested cross-sectional evaluation of neonates (median age 21 days) HEU, n=15 HU, n=22 (matched)	None	Not reported in this publication	Neonatal neuro-behavioural outcomes with diffusion tensor imaging to evaluate white matter microstructural integrity	White matter changes (higher fractional anisotropy) in middle cerebellar peduncles of HEU (vs HU), in turn correlated with greater abnormality in neurological subscale of Dubowitz	Not evaluated	None
	le Roux (2019) CID	Analysis censored at 2 years, HEU, n=247 HU, n=894 72% WLHIV received ART during pregnancy (% Option A vs Option NR); Timing of initiation NR	None	BF initiation, HEU 56%, HU 98%; Overall, only 14% still BF after 6 months	Infectious morbidity (LRTI): hospitalized, ambulatory, overall	In 1st 6 months of life, HEU vs HU risk of hospitalized LRTI IRR 1.99 (95% CI 1.11-3.54); similar risks thereafter	Maternal antenatal ART (vs dual prophylaxis) protective Among HU, BF protective (IRR 7.46) Among HEU, BF did not change risk (IRR 1.19)	None
	Wedderburn (2019) TLCAH	Random subset of infants assessed at 6 months: HEU, n=61; HU=199 Larger group assessed at 24 months: HEU, n=168; HU, n=564 In this analysis, 88% WLHIV received ART in pregnancy, 43% of whom initiated pre-	None	HEU, EBF mean duration ~1 month HU, EBF mean duration ~2 months	Neuro-development at 6 and 24 months (BSID-III)	At 6 months: similar scores At 24 months: similar scores and % of delay for fine motor, gross motor and cognitive HEU vs HU raw expressive and raw receptive scores lower, with higher relative odds of delay	OR (95% CI) compared to HU: Expressive: HEU w mothers CD4>500, OR 1.5; CD4<= 500, OR 2.59 Receptive: OR 1.64 and 2.51 respectively	Developmental results also reported in a paper linked to inflammatory markers, but the same overall HEU vs HU results

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Original study details	1st author, year, journal	Enrolment age, follow-up, demographics	Perinatal exclusion criteria	Infant feeding	Primary clinical outcome (s)	Main findings (HEU vs HU)	Effect of ART/disease severity/BF	Comment
		conception (CD4-restricted)						
Prospective cohort analysis from RCT setting (malaria prevention), along with data from surveillance Manica District Hospital, Mozambique (2010-2013)	Ruperez (2017) AIDS	Newborn infants followed at 1, 9, 12 and 18 months Data on hospitalization and mortality obtained from MDH and demographic surveillance systems HEU, n=966 HU, n=909 % ART and initiation timing NR	None reported	Implies that cohort predominantly breastfed, but no detailed information available	(1) Mortality (2) Infectious morbidity: outpatient visits and admissions (3) Growth: severe and moderate malnutrition	(1) 2.2 vs 1.6 deaths per 100PY overall, HR 1.87 Stronger difference in 1-6 months, 4.94 vs 1.67 deaths/100PY, HR ~3 (2) Outpatient visits, lower among HEU: IRR 0.77 (95% CI 0.71-0.83) Hospitalization higher among HEU: 16.8 vs 11.8/100PY, IRR 1.45 (1.15-1.83) (3) Moderate: IRR 2.14 (1.39-3.30) Severe: IRR 2.62 (1.45-4.75)	Risk for outpatient visit increased with every category of less BF (EBF, partial BF, weaned and never BF, latter highest risk, IRR 1.46 vs EBF) Risk for admission highest among children recently weaned (aIRR 6.74 vs EBF) Maternal ART associated with marginally increased IM risks	Highest mortality rate for HU neonatal period; highest mortality rate for HEU in 1-6m period
SAPMTCT Nationally representative survey to evaluate MTCT risk (2012-2013)	Ramakolo (2017) Open Forum Infectious Diseases	Enrolment between 4-8 weeks of age at immunization clinics HEU, n=2599 HU, n=6179 pre-conception ART (CD4-restricted) 24%; post-conception ART (mostly CD4	Children who were sick were excluded, and any <4 or >8 weeks	HEU, any BF ranged 58-73% HU, any BF 88%	Growth: weight for age at birth and 6 weeks (perinatal outcomes also evaluated)	WAZ<-2 @ 6 weeks 11% vs 8%, aOR 1.5 (95% CI 1.2-1.8)	HEU infants with no ART experience were at highest risk of being UFA (compared to any ART or ZDV)	

Supplemental Table 9.1.1. Growth, infectious morbidity, mortality, and neurodevelopment of HIV-exposed uninfected compared to HIV-unexposed children in sub-Saharan Africa: summary of longitudinal studies published between October 2015 and January 2021

Original study details	1st author, year, journal	Enrolment age, follow-up, demographics	Perinatal exclusion criteria	Infant feeding	Primary clinical outcome (s)	Main findings (HEU vs HU)	Effect of ART/disease severity/BF	Comment
		restricted), 30%; ZDV, 34%; none, 13%						
INFANT study Prospective observational cohorts in Jos, Nigeria and Cape Town, South Africa (2013-2017 enrolment) Conducted during era of B/B+ but also enrolled pregnant women who had initiated ART pre-conception under Option A (ie definitely had low CD4 at initiation)	Toukam Tchakoute (2018) AIDS	Enrolled antenatal and screened again at birth ~Monthly visits through 12 months (40% LTFU) HEU, n=537 HU, n=212 At time of delivery, 99% mothers on ART; includes pre- and post-conception ART initiation (see below)	BW ≤ 2400g Gestation ≤ 36 weeks Pregnancy/ delivery complications	EBF at birth: HEU 92% HU 99%	(1) Mortality (2) Infectious morbidity “infectious events” includes hospitalization and intercurrent ill visits; severity NR	(1) 11/537 HEU, 2% vs 2/212 HU, 1% HEU vs U, HR 1.08 (95% CI 0.82-1.45)	EBF reduced infectious morbidity risk No BF vs EBF, aHR 1.64 MBF vs EBF, aHR 1.18 EBF>=6m vs < 6m, aHR 0.8	BF analysis adjusted for, but not stratified by HIV exposure status
	Jumare (2019) PIDJ	This analysis limited to Nigerian cohort, followed to 18 months HEU, n=307 HU, n=108 222/307 (98) received ART in pregnancy; 74% pre- and 26% post-conception initiation	BW ≤ 2400g Gestation ≤ 36 weeks	Any BF at birth, HEU 97% HU 99% BF at 12 months, HEU 64% HU 94%	Growth: WAZ, LAZ, WLZ, BMIZ, HCAZ Trajectories and odds of <-2SD	In both crude and adjusted analysis, HEU had lower Z-scores for all measures than HU, ranging from ~0.3 to ~0.5 of a Z-score Stunting: aOR 1.55 (95% CI 1.12-2.15) Wasting: 1.46 (0.90-2.38) Underwt: 2.4 (1.41-4.09)	Similar results for EBF HEU vs HU with HEU restricted to mothers with viral suppression	
PROMISE-BF (“PROMISE-NEURO-DEV”) Uganda and Malawi sites Prospective cohort of HEU co-enrolled from PROMISE RCT	Boivin (2019) Lancet HIV	Both HEU and HU children enrolled into this sub-study at 6-24 months of age, followed through 60 months (12, 24, 48, and 60 months’ visits)	≥ 2000g BW Only mothers with high CD4 enrolled	HEU, 100% BF initiation; 73-83% still BF at 12m HUU, 95% still BF at 12m	Neuro-development (MSEL at 12, 24 and 48 months; KABC-II at 48 and 60 months) “HOME” tool for scoring home	HEU of mothers who received both antepartum and postpartum ART had similar MSEL scores to HU children; other HEU had slightly lower scores	Lower maternal VL associated with better KABC MPI (Malawi only) In Uganda, BF at 12 months associated with	HOME score (higher quality and quantity of home stimulation) strongly associated

Supplemental Table 9.1.1. Growth, infectious morbidity, mortality, and neurodevelopment of HIV-exposed uninfected compared to HIV-unexposed children in sub-Saharan Africa: summary of longitudinal studies published between October 2015 and January 2021

Original study details	1st author, year, journal	Enrolment age, follow-up, demographics	Perinatal exclusion criteria	Infant feeding	Primary clinical outcome (s)	Main findings (HEU vs HU)	Effect of ART/disease severity/BF	Comment
(2011-2016: 4 arms, antenatal ZDV vs ART, postnatal maternal ART vs infant sdNVP; women with high CD4) with age- and sex-matched HUU from well-baby clinics (enrolled 2012-2013)		HEU, n=405 total (~25% per study arm, ie ~50% with antenatal initiation of universal ART) HU, n=456			environment stimulation	KABC-II scores similar across groups	better MSEL scores for HU children	with MSEL and KABC-II in both countries and all groups
	Aizire (2020) AIDS	In this analysis, growth compared HEU vs HU at 12 and 24 months, without disaggregation by randomization arms HEU, n=471 HU, n=462 Not reported, but would be as for above	≥ 2000g BW Only mothers with high CD4 enrolled	HEU, all BF early infancy, 80% still BF at 12m, 7% at 24m HEU, 97% and 30% at 12 and 24 months	Growth at 12 and 24 months: WAZ, LAZ, WLZ, HCAZ Cross-sectional and longitudinal	*Uganda:* WAZ: mean lower at both 12 and 24 months LAZ, stunting at 24m: aRR 1.67 (95% CI 1.16-2.41) HCAZ, Microcephaly at 24m: 1.35 (1.02-1.79) *Malawi:* WAZ slightly lower at 12 and 24 months LAZ, stunting at 24m: aRR 1.32 (95% CI 1.10-1.66) HCAZ, microcephaly at 24m: 1.35 (0.91-2.02)	Not assessed	None
Predominantly Option B/B+: At least 70% of HIV+ study population receiving universal ART in pregnancy (no CD4 cell count restrictions)								
MCH-ART and HU2 studies (2014-2017): HEU from RCT of postnatal maternal retention strategies under B/B+, HU	le Roux (2018) AIDS	Antenatal enrolment, postnatal screening for continued follow-up 12m; Visits@ 6wk, 3-monthly from 3 to 12 months	Infants not BF in the 1st week of life HEU infants of mothers who did not initiate	Initiated of BF: HEU, 100% HU, 100% In this sub-set Median BF HEU 6 months	Neuro-development (BSID-III) at ~12 months	Mean scores similar for composite cognitive and motor scores, and expressive language (receptive language not included in report)	Maternal viremia (measured as VCY) predictive of worse expressive language and motor scores	Highest risk of motor delay in preterm HEU; no difference

Supplemental Table 9.1.1. Growth, infectious morbidity, mortality, and neurodevelopment of HIV-exposed uninfected compared to HIV-unexposed children in sub-Saharan Africa: summary of longitudinal studies published between October 2015 and January 2021

Original study details	1st author, year, journal	Enrolment age, follow-up, demographics	Perinatal exclusion criteria	Infant feeding	Primary clinical outcome (s)	Main findings (HEU vs HU)	Effect of ART/disease severity/BF	Comment
from linked, parallel cohort study Cape Town, South Africa		100% ART, all initiated post-conception This analysis (nested cross-sectional assessment at 24m) HEU, n=215 HU, n=306	universal ART in pregnancy For this assessment, gestational age<28 weeks, congenital abnormalities	HU 10 months		Using <-2SD for cut-off, HEU higher odds of cognitive delay (aOR 2.56) and motor delay (aOR 2.10); no difference in expressive language delay	(separate publication)	term HEU vs term HU
	le Roux (2019) TLCAH	As above This analysis HEU, n=461 HU, n=411	Infants not BF in the 1st week of life HEU infants of mothers who did not initiate universal ART in pregnancy	Ever EBF HEU HU Median BF HEU 4 months HU 9 months	Growth: trajectories and % <-2SD WAZ, LAZ, WLZ, BMIZ, HCAZ	*WAZ*: Increased in both groups over time ~0.3 Z-score lower in HEU. Overall, 44% had rapid weight gain *LAZ:* Decreased in both groups after 6 months: by 12 months, HEU lower ~0.4 Z-score and 10% vs 4% stunted (OR 2.67) *WLZ/BMIZ:* HEU lower at birth, rapid catch up until similar; 16% and 18% overweight by 12 months *HCAZ:* Slight increase over time, similar in HEU and HU	Higher maternal VL associated with lower WAZ among HEU Breastfeeding associated with lower odds of overweight at 12 months	Stunting stratified: Mean difference in LAZ at 12 months between HEU-AGA vs HU-AGA, -0.3; HEU-SGA vs HU-SGA, -0.5
	le Roux (2020) TLCAH	As above This analysis HEU, n=395	Infants not BF in the 1st week of life	Ever EBF HEU, 91% HU, 81%	(1) Infectious morbidity: all-cause and infectious-cause	(1) All-cause Hospitalization: overall, IRR 0.91; stratified by age:	In 1st 6 months, Infectious cause hospitalization risk & LRTI/diarrhoea	Hospitalization data from government hospital

Supplemental Table 9.1.1. Growth, infectious morbidity, mortality, and neurodevelopment of HIV-exposed uninfected compared to HIV-unexposed children in sub-Saharan Africa: summary of longitudinal studies published between October 2015 and January 2021

Original study details	1st author, year, journal	Enrolment age, follow-up, demographics	Perinatal exclusion criteria	Infant feeding	Primary clinical outcome (s)	Main findings (HEU vs HU)	Effect of ART/disease severity/BF	Comment
		HU, n=410	HEU infants of mothers who did not initiate universal ART in pregnancy	Median duration BF HEU, 4 months HU, 9 months	hospitalization; intercurrent diarrhoea/ respiratory infection (2) Mortality	Similar across ages except for 1-3 months, IRR 2.85 Infectious-cause hospitalization overall IRR 1.4; stratified by age - similar across ages except for 1-3 months, IRR 3.5 Both LRTI and diarrhoea more common in HEU than HU in 1st 6 months (2) 1.7% vs 1.5%	greatest among HEU born to women who initiated ART late in pregnancy/ advanced HIV disease/shorter durations of EBF/incomplete vaccination	records with ICD10-codes
PostNAPS (2013-2015), Gulu Uganda: a perinatal prospective cohort study focused on food security (antenatal component, "PreNAPS", postnatal component, "PostNAPS")	Familiar (2018) JAIDS	Enrolled antenatal, screened at delivery; follow-up to 12 months 100% ART in pregnancy ~41% initiated pre-conception ~60% post-conception (B+); In this analysis, HEU=75 (24% of mothers no longer on ART@ 6m pp) HU, n=140	Multiples	NR in this publication; elsewhere, cohort data: EBF at birth: HEU 83% HU 72% EBF at 3m: HEU 59% HU 42%	Neuro-development (MSEL at 6 and 12 months of age) [Caregiver quality & quantity of stimulation using HOME tool]	On average, HU higher mean scores than HEU across composite and subscales especially at 6 months; scores declined over time for both groups HEU lower composite and gross motor scores in regression models	Lowest MSEL scores seen in HEU of mothers no longer on ART at 6 months	HOME score similar for HEU and HU, and predicted Composite, Receptive and Expressive language scores positively for both groups
	Lane (2020) JAIDS	As above In this analysis, HEU, n=86 HU, n=152	Multiples	As above	Growth: LAZ, WLZ, MUACZ, skin folds and arm fat over time	Latent class growth mixture modelling: HEU more likely to be in shortest 2 LAZ trajectory classes [OR 3.8 for class 3 vs 2; 8.72 for class 4 vs 2,	% EBF similar across classes (ie not associated w growth in this analysis)	The same HEU infants tended to be in lowest classes for

Supplemental Table 9.1.1. Growth, infectious morbidity, mortality, and neurodevelopment of HIV-exposed uninfected compared to HIV-unexposed children in sub-Saharan Africa: summary of longitudinal studies published between October 2015 and January 2021

Original study details	1st author, year, journal	Enrolment age, follow-up, demographics	Perinatal exclusion criteria	Infant feeding	Primary clinical outcome (s)	Main findings (HEU vs HU)	Effect of ART/disease severity/BF	Comment
						respectively) but not WLZ, MUACZ or arm fat Food insecurity associated with lower skinfolds classes among HEU	Maternal VL and CD4 not evaluated	LAZ and skin folds
SHINE trial (enrolment 2012-2015) Rural Zimbabwe Cluster RCT 2X2 with WASH and ICYN interventions	Evans (2020) CID	Antenatal enrolment, home visit f/up 1, 3, 6, 12, 18m pp All encouraged to initiate ART, only 81% received ART in pregnancy (~2/3 likely post-conception) Growth analysis excluded those randomized to IYCN and those with unknown HIV status HEU, n=297 HU, n=1771	None	>88% initiated EBF across both groups (exact nr NR, similar for HEU and HU) BF duration longer for HU (HEU vs HU, 15 vs 16 months) All study arms received BF support intervention	Growth: trajectories and % <-2SD for WAZ, LAZ, WLZ, HCAZ	LAZ, WAZ and HCAZ trajectories lower for HEU than HU across time points *@18 months, HEU vs HU Mean differences (95% CI)* LAZ -0.38 (-0.51; -0.24) WAZ -0.17 (-0.32; -0.03) HCAZ -0.27 (-0.40; -0.14) WLZ -0.04 (-0.19; 0.11) MUACZ -0.18 (-0.28; -0.07) Stunted: 51% vs 34%, RR 1.46; Severely stunted: 14% vs 9%, RR 1.60; Underweight:16% vs 11%, RR 1.51; Wasted: 4% vs 3% Microcephalic: 10% vs 6%, RR 1.74	Not examined in this publication	Mortality analysis no denominator for HEU only for all HE infants (90% of HE deaths < 4-6wks PCR test) ART-exposed HE and HU similar mortality ART-non exposed HEU 3-fold higher risk Overall HE vs HU, HR 1.4
	Ntozini (2020) JIAS	As above Nested cross-sectional assessment at 24 months In this sub-study,	None Other exclusion for this sub-study:	As above	Neuro-development at 24 months (MDAT, MacArthur-Bates	HEU lower mean MDAT scores overall, crude and adjusted; particularly evident in gross motor and language (~0.15 of a SD);	Not examined in this publication	None

Supplemental Table 9.1.1. Growth, infectious morbidity, mortality, and neurodevelopment of HIV-exposed uninfected compared to HIV-unexposed children in sub-Saharan Africa: summary of longitudinal studies published between October 2015 and January 2021

Original study details	1st author, year, journal	Enrolment age, follow-up, demographics	Perinatal exclusion criteria	Infant feeding	Primary clinical outcome (s)	Main findings (HEU vs HU)	Effect of ART/disease severity/BF	Comment
		HEU, n=205 HU, n=1175 86% ART ~ 70% post-conception (B+)	children w moderate to severe disability		CDI, A-not-B test, self-control)	some in fine motor but social similar HEU ~3-4 words less vocabulary than HU Similar object permanency and self-control		
Prospective cohort study (2014-2016) Blantyre and Lilongwe, Malawi	Kapito-Tembo (2021) JAIDS	Postnatal enrolment just after delivery, followed through 18 months (visits birth, 6 weeks, 3, 6,12 and 18 months) 100% ART in pregnancy Initiation timing NR (all received TDF-EFC-3TC, likely B+ initiation in pregnancy)	BW < 2500g Gestation < 37 weeks Congenital abnormalities	HEU and HU 100%, duration through end of study Exclusivity NR	(1) Growth (mean weight, length, MUAC, HC over time) (2) Neuro-development (BSID-III) (3) Infectious morbidity: reported illness, hospitalization (4) Mortality	(1) Mean WAZ, LAZ, WLZ tended to be ~0.1 to 1.0 SD lower in HEU than HU (only lower LAZ at 6wks "statistical significance", but likely underpowered) (2) Similar developmental trajectories for HEU and HU across sub-scales and times (decreasing scores over time) (3) More "illness" among HU, hospitalizations similar (4) Mortality similar (1.6% vs 1.5%)	Not reported	High LTFU, higher among HU (57% vs HEU, 35%)

9.2 Supplemental material for Chapter 2 (Systematic literature review)

Supplemental Table 9.2.1. PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	✓1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	✓2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	✓3	Describe the rationale for the review in the context of what is already known.	4
Objectives	✓4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	✓5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	✓6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7
Information sources	✓7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	✓8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7, Appendix
Study selection	✓9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	✓10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	✓11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Appendix

Section/topic	#	Checklist item	Reported on page #
Risk of bias in individual studies	✓12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7, Appendix
Summary measures	✓13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	✓14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8
Risk of bias across studies	✓15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	11
Additional analyses	×16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Not done
RESULTS			
Study selection	✓17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8 and figure 1
Study characteristics	✓18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Appendix
Risk of bias within studies	✓19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Appendix
Results of individual studies	✓20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Appendix
Synthesis of results	✓21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Figure 2
Risk of bias across studies	✓22	Present results of any assessment of risk of bias across studies (see Item 15).	Appendix
Additional analysis	×23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Not done
DISCUSSION			
Summary of evidence	✓24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	16
Limitations	✓25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	19
Conclusions	✓26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	20
FUNDING			

Section/topic	#	Checklist item	Reported on page #
Funding	✓27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	21

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6)

Supplemental Table 9.2.2. Risk of bias assessment for longitudinal studies presenting comparative data for HIV-unexposed and HIV-exposed uninfected children

	Signalling questions used to assess risk of bias in different domains										
First author and publication year	[1]Exposed cohort reasonably representa-tive?	[2]Selection into study unrelated to outcome?	[3]Start of follow-up and exposure coincide for most subjects?	[4]Unexposed cohort chosen from the same community?	[5]Reasonably comparable groups? (design and analysis)	[6]Adequate and accountable follow-up information?	[7]Valid measures of exposure used?	[8]Valid measures of outcome used?	[9]Objective assessment of outcomes?	[10]Most important confounders adjusted for?	[11]Valid measures of confounders used?	**[12]Overall risk of bias in comparison of HEU vs HU outcomes**
Thea (1993)	✓	✓	✓	✓	✓	±	✓	✓	±	✗	n/a	[b]Moderate
Boivin (1995)	✓	?	✓	✓	✓	✗	✓	✓	✓	✗	n/a	[c]Serious
Drotar (1997) Bagenda (2006)	✓	✓	✓	✓	✓	±	✓	✓	✓	✗	n/a	[b]Moderate
Taha (1999 & 2002)	✓	±	±	✓	✓	✓	✓	±	±	✓	±	[b]Moderate for mortality and morbidity; [d]critical for development
Spira (1999)	✓	✓	✓	✓	✓	✓	✓	?	±	✗	n/a	[b]Moderate
Bailey (1999)	✓	✓	✓	✓	✓	±	✓	✓	±	✗	n/a	[b]Moderate for outcomes up to 12 months of age
Sherry (2000)	±	✗	✗	✓	±	✗	✓	✓	?	✗	n/a	[c]Serious
Ota (2000) & Schim van der Loeff (2003)	±	±	✓	✓	✓	±	✓	✓	±	✗	n/a	[b]Moderate
Brahmbatt (2006)	✓	?	✓	✓	✓	?	✓	✓	✓	✗	n/a	[e]Insufficient information

Supplemental Table 9.2.2. Risk of bias assessment for longitudinal studies presenting comparative data for HIV-unexposed and HIV-exposed uninfected children

Signalling questions used to assess risk of bias in different domains											
First author and publication year	[1]Exposed cohort reasonably representative?	[2]Selection into study unrelated to outcome?	[3]Start of follow-up and exposure coincide for most subjects?	[4]Unexposed cohort chosen from the same community?	[5]Reasonably comparable groups? (design and analysis)	[6]Adequate and accountable follow-up information?	[7]Valid measures of exposure used?	[8]Valid measures of outcome used?	[9]Objective assessment of outcomes?	[10]Most important confounders adjusted for?	[11]Valid measures of confounders used?	**[12]Overall risk of bias in comparison of HEU vs HU outcomes**
Shapiro (2007)	✓	✓	✓	✓	±	✓	✓	±	±	±	?	[b]Moderate
Marinda (2007) & Koyanagi (2011)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	±	[a]Low
Luabeya (2007)	±	✗	✗	✓	±	✓	✓	✓	±	✗	n/a	[c]Serious
Sutcliffe (2008)	✓	✓	±	✓	✓	±	±	✓	✓	✗	n/a	[b]Moderate
Chilongozi (2008)	✓	✓	✓	✓	✓	✓	±	±	±	✗	n/a	[b]Moderate
Van Rie (2009)	✗	✗	✗	✗	✗	✓	?	✓	✓	✗	n/a	[d]Critical
Naniche (2009)	✓	?	✓	✓	✓	?	±	✓	✓	✗	n/a	[e]Insufficient information
Chopra (2010) & Ramokolo (2013)	✓	✓	✓	✓	✓	±	✓	✓	±	✓	±	[b]Moderate
Patel (2010) & Rollins (2013)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	±	[a]Low
Van Eijk (2010)	✓	?	✓	✓	?	±	✓	✓	±	✗	n/a	[e]Insufficient information
Filteau (2011)	±	±	±	✓	±	±	✓	✓	✓	✓	±	[b]Moderate

Supplemental Table 9.2.2. Risk of bias assessment for longitudinal studies presenting comparative data for HIV-unexposed and HIV-exposed uninfected children

Signalling questions used to assess risk of bias in different domains												
First author and publication year	[1]Exposed cohort reasonably representative?	[2]Selection into study unrelated to outcome?	[3]Start of follow-up and exposure coincide for most subjects?	[4]Unexposed cohort chosen from the same community?	[5]Reasonably comparable groups? (design and analysis)	[6]Adequate and accountable follow-up information?	[7]Valid measures of exposure used?	[8]Valid measures of outcome used?	[9]Objective assessment of outcomes?	[10]Most important confounders adjusted for?	[11]Valid measures of confounders used?	**[12]Overall risk of bias in comparison of HEU vs HU outcomes**
Kandawasvika (2011)	±	±	✓	✓	±	±	✓	✓	✓	±	±	[b]Moderate
Kurewa (2011)	±	±	✓	✓	±	✕	✓	✓	✓	✕	n/a	[c]Serious
Landes (2012)	✓	✕	±	✓	✓	±	±	±	±	✕	n/a	[c]Serious
Slogrove (2012)	✓	✓	✓	?	?	✓	✓	✓	±	✕	n/a	[e]Insufficient information
Arinaitwe (2012)	±	✓	±	✓	✓	✓	✓	✓	✓	✕	n/a	[b]Moderate
Parker (2013)	✕	±	✓	✓	±	±	±	✓	✓	±	±	[b]Moderate
Muhangi (2013)	±	±	✓	✓	✓	±	±	✓	✓	✓	±	[b]Moderate
Marquez (2014)	±	±	±	✓	✓	✓	±	✓	✓	✓	±	[b]Moderate
Moraleda (2014)	✓	±	±	✓	✓	✕	±	✓	✓	✕	n/a	[c]Serious
Ngoma (2014)	✕	?	✕	✓	✕	±	✓	✓	✓	✓	±	[c]Serious
Nicholson (2015)	✓	±	±	✓	±	±	±	✓	±	✓	✓	[b]Moderate

Abbreviations: HEU – HIV-exposed uninfected children; HU – HIV-unexposed children; n/a = not applicable

Signalling questions for domain-level bias: [1]external validity; [2,3,6] selection bias; [4, 5, 10, 11] confounding (of relationship between maternal HIV and health of HIV-uninfected children); [7, 8, 9] information bias: Answers: ✓= yes (low risk of bias in this domain); ± = probably yes (moderate risk of bias in this domain); ✕= no (serious to critical risk of bias in this domain); ? = insufficient information to assess risk of bias; [10] Through design or in analysis: specifically, indicators of socio-economic situation; [12]Overall risk of bias (in the comparison of HEU to HU children): [a]study is comparable to a well-performed randomized trial; [b]study is sound for a non-randomized study; [c]the study has some important problems; [d]the study is too problematic to provide useful evidence; [e] insufficient information on which to base an overall judgement

Supplemental Table 9.2.3. Characteristics of studies excluded following full-text review

First author	Year	Title	Journal	Reason for exclusion
Anigilaje, EA	2015	HIV-free survival according to the early infant-feeding practices; a retrospective study in an anti-retroviral therapy programme in Makurdi, Nigeria	BMC Infectious Diseases	No comparative data for HIV-unexposed children
Belman, A	1996	Neurologic status of human immunodeficiency virus 1-infected infants and their controls: a prospective study from birth to 2 years	Pediatrics	Study setting outside sub-Saharan Africa (US-based)
Bloland, P	1995	Maternal HIV infection and infant mortality in Malawi: evidence for increased mortality due to placental malaria infection	AIDS	HIV infection not definitively excluded among HIV-exposed children
Braddick, M	1990	Impact of maternal HIV infection on obstetrical and early neonatal outcome	AIDS	Cross-sectional study design
Brahmbatt, H	2002	Polygyny, maternal HIV status and child survival: Rakai, Uganda	Social Science & Medicine	HIV infection not definitively excluded among HIV-exposed children
Chaggan, M	2009	Effect of micronutrient supplementation on diarrhoeal disease among stunted children in rural South Africa	European Journal of Clinical Nutrition	Results not disaggregated by both maternal and infant HIV status
Chi, B	2014	Universal combination antiretroviral regimens to prevent mother-to-child transmission of HIV in rural Zambia: a two-round cross-sectional study	Bulletin of the World Health Organization	Cross-sectional study design
Chihana, M	2015	Maternal HIV status associated with under-five mortality in rural northern Malawi: a prospective cohort study	JAIDS	HIV infection not excluded among HIV-exposed children
Chisenga, M	2005	Factors affecting the duration of exclusive breastfeeding among HIV-infected and –uninfected women in Lusaka, Zambia	Journal of Human Lactation	HIV infection not excluded among HIV-exposed children
Coovadia, H	2007	Mother-to-child transmission of HIV-1 during exclusive breastfeeding in the first 6 months of life: an intervention cohort study	The Lancet	No comparative data for HIV-unexposed children
Crampin, A	2003	The long-term impact of HIV and orphanhood on the mortality and physical well-being of children in rural Malawi	AIDS	HIV infection not definitively excluded among HIV-exposed children
De Beaudrap, P	2011	Impact of malaria in pregnancy on gestational age, birth weight and infant growth: a cohort study in Uganda	Tropical Medicine & International Health (Conference)	Results not disaggregated by both maternal and infant HIV status
De Cock, K	1994	Retrospective study of maternal HIV-1 and HIV-2 infections and child survival in Abidjan, Cote d’Ivoire	The British Medical Journal	HIV infection not definitively excluded among HIV-exposed children
de Deus, N	2015	Impact of elevated maternal HIV viral load at delivery on T-cell populations in HIV exposed uninfected infants in Mozambique	BMC Infectious Diseases	No data on clinical outcomes
Embree, J	2000	Risk factors for postnatal mother-child transmission of HIV-1	AIDS	No data on clinical outcomes in children
Goga, A	2012	Infant feeding practices at routine PMTCT sites, South Africa: results of a prospective observational study amongst HIV exposed and unexposed infants – birth to 9 months	International breastfeeding journal	Infant mortality results not disaggregated by both maternal and infant HIV status, outcome of interest combined “HIV-free survival”

Supplemental Table 9.2.3. Characteristics of studies excluded following full-text review

First author	Year	Title	Journal	Reason for exclusion
Gomo, E	2003	Subclinical mastitis among HIV-infected and uninfected Zimbabwean women participating in a multimicronutrient supplementation trial	Transactions of the Royal Society of Tropical Medicine and Hygiene	Results not disaggregated by both maternal and infant HIV status
Gompels, U	2012	Human Cytomegalovirus infant infection adversely affects growth and development in maternally HIV-exposed and unexposed infants in Zambia	Clinical Infectious Diseases	Results not clearly disaggregated by both maternal and infant HIV status (no denominator for HEU infants)
Gutman, J	2012	Impact of intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine on placental infection and infant birth outcomes in Malawi	American society of tropical medicine & hygiene (Conference)	Only HIV-negative women included; no infant outcomes presented
Henderson, R	1996	Longitudinal growth during the first 2 years of life in children born to HIV-infected mothers in Malawi, Africa	Pediatric AIDS and HIV infection	HIV infection not definitively excluded among HIV-exposed children
Hira, S	1989	Perinatal transmission of HIV-1 in Zambia	The British Medical Journal	HIV infection not definitively excluded among HIV-exposed children
Humphrey, J	2010	Mother to child transmission of HIV among Zimbabwean women who seroconverted postnatally: prospective cohort study	The British Medical Journal	No clinical outcome data for children other than HIV transmission risk
Kafulafula, G	2009	Intrapartum antibiotic exposure and early neonatal morbidity and mortality in Africa	Pediatrics	Results not disaggregated by both maternal and infant HIV status
Kaguthi, G	2012	Maternal HIV exposure or mother to child transmission of HIV and risk of tuberculosis in infants	American society of tropical medicine & hygiene (Conference)	Results not disaggregated by both maternal and infant HIV status
Kennedy, D	2012	The effect of maternal HIV status on perinatal outcome at Mowbray Maternity Hospital and referring midwife obstetric units, Cape Town	South African Journal of Obstetrics and Gynaecology	HIV infection not definitively excluded among HIV-exposed children
Keusch, GT	1992	Persistent diarrhea associated with AIDS	Acta Paediatrica	Interim report on findings subsequently fully published (Thea et al, 1993)
Kindra, G	2012	Breastfeeding in HIV exposed infants significantly improves child health: a prospective study	Maternal Child Health Journal	No comparative data for HIV-unexposed children
Kristensen, I	2006	Determinants of acute respiratory infections in Soweto – a population-based birth cohort	South African Medical Journal	HIV infection not definitively excluded among HIV-exposed children
Kurewa, E	2010	Effect of maternal HIV status on infant mortality: evidence from a 9-month follow-up of mothers and their infants in Zimbabwe	Journal of Perinatology	Results not clearly disaggregated by both maternal and infant HIV status
Lartey, A	2011	Maternal HIV is associated with reduced growth in the first year of life among infants in the Eastern region of Ghana	The FASEB journal (Conference Abstract)	Conference abstract; data subsequently published
Lartey, A	2012	Maternal HIV is associated with reduced growth in the first year of life among infants in the Eastern region of Ghana: the Research to Improve Infant Nutrition and Growth (RIING) Project	Maternal and Child Nutrition	HIV infection not definitively excluded among HIV-exposed children

Supplemental Table 9.2.3. Characteristics of studies excluded following full-text review

First author	Year	Title	Journal	Reason for exclusion
Lepage, P	1991	Perinatal transmission of HIV-1: lack of impact of maternal HIV infection on characteristics of livebirths and on neonatal mortality in Kigali, Rwanda	AIDS	HIV infection not definitively excluded among HIV-exposed children
Lepage, P	1996	Growth of human immunodeficiency type 1-infected and uninfected children: a prospective cohort study in Kigali, Rwanda, 1988 to 1993	The Pediatric Infectious Disease Journal	HIV infection not definitively excluded among HIV-exposed children
Longwe, H	2015	Proportions of CD4+, CD8+ and B cell subsets are not affected by exposure to HIV or to Co-trimoxazole prophylaxis in Malawian HIV-uninfected but exposed children	BMC Immunology	No data on clinical outcomes
Longwe, H	2015	The effect of daily co-trimoxazole prophylaxis on natural development of antibody-mediated immunity against P.falciparum malaria infection in HIV-exposed uninfected Malawian children	PLoS One	No data on clinical outcomes of interest
Makasa, M	2007	Early growth of infants of HIV-infected and uninfected Zambian women	Tropical Medicine and International Health	HIV infection not definitively excluded among HIV-exposed children
Manno, D	2012	Rich micronutrient fortification of locally produced infant food does not improve mental and motor development of Zambian infants: a randomised controlled trial	British Journal of Nutrition	Results not clearly disaggregated by both maternal and infant HIV status (no denominator for HEU children)
Marquis	2009	Maternal postpartum depression modifies the association between maternal HIV infection and infant diarrhoea in Ghana's Eastern region	The FASEB Journal	Conference abstract; data subsequently published
Mbeye, N	2014	Cessation of co-trimoxazole prophylaxis in HIV exposed children does not increase the incidence of malaria and other morbidities	American society of tropical medicine & hygiene (Conference)	Clinical results of interest not reported
Msellati, P	1993	Neurodevelopmental Testing of Children Born to Human Immunodeficiency Virus Type 1 Seropositive and Seronegative Mothers: A Prospective Cohort Study in Kigali, Rwanda	Pediatrics	HIV infection not definitively excluded among HIV-exposed children
Mugwaneza, P	2011	Under-two child mortality according to maternal HIV status in Rwanda: assessing outcomes within the National PMTCT program	Pan African Medical Journal	HIV infection not definitively excluded among HIV-exposed children
Musana, J	2009	Pregnancy outcomes in mothers with advanced human immunodeficiency virus disease	East African Medical Journal	Results not disaggregated by both maternal and infant HIV status
Nalwoga, A	2010	Nutritional status of children living in a community with high HIV prevalence in rural Uganda: a cross-sectional population-based survey	Tropical Medicine and International Health	Cross-sectional study design
Nathoo, K	2004	Survival pattern among infants born to Human Immunodeficiency Virus type-1 infected mothers and uninfected mothers in Harare, Zimbabwe	The Central African Journal of Medicine	HIV infection not definitively excluded among HIV-exposed children
Nunes, MC	2015	Kinetics of Hemagglutination-Inhibiting Antibodies Following Maternal Influenza Vaccination Among Mothers With and Those Without HIV Infection and their Infants	Journal of Infectious Diseases	No data on clinical outcomes
Okronipa, H	2012	Postnatal depression symptoms are associated with increased diarrhoea among infants of HIV-positive Ghanaian mothers	AIDS Behaviour	Results not disaggregated by both maternal and infant HIV status

Supplemental Table 9.2.3. Characteristics of studies excluded following full-text review

First author	Year	Title	Journal	Reason for exclusion
Okronipa, H	2013	Acute respiratory infections in children living in HIV-affected communities in Ghana	Annals of Nutrition & Metabolism	Results not disaggregated by both maternal and infant HIV status
Omari, A	2003	Infant-feeding practices of mothers of known HIV status in Lusaka, Zambia	Health Policy and Planning	Cross-sectional study design
Onono, M	2015	The role of maternal, health system, and psychosocial factors in prevention of mother-to-child transmission failure in the era of programmatic scale up in western Kenya: a case control study	AIDS Patient Care STDS	Case-control design
Pillay, T	2004	Vertical transmission of Mycobacterium tuberculosis in Kwa-Zulu Natal: impact of HIV-1 co-infection	International Journal of Tuberculosis and Lung Disease	Women selected for study participation based on morbidity other than HIV infection (tuberculosis disease)
Ryder, R	1991	Evidence from Zaire that breast-feeding by HIV-1-seropositive mothers is not a major route for perinatal HIV-1 transmission but does decrease morbidity	AIDS	HIV infection not definitively excluded among HIV-exposed children
Sidze, LK	2015	Different factors associated with loss to follow-up of infants born to HIV-infected or uninfected mothers: observations from the ANRS12140-PEDIACAM study in Cameroon	BMC Public Health	Clinical outcomes of children not reported
Simpore, J	2006	Reduction of Mother-to-Child Transmission of HIV at Saint Camille Medical Centre in Burkina Faso	Journal of Medical Virology	No comparative data for HIV-unexposed children
Slogrove, A	2010	Severe infections in HIV-exposed uninfected infants: clinical evidence of immunodeficiency	Journal of Tropical Pediatrics	Cross-sectional study design
Taha, T	2010	The effect of human immunodeficiency virus and breastfeeding on the nutritional status of African children	The Pediatric Infectious Disease Journal	Results not disaggregated by both maternal and infant HIV status
Taha, ET	2012	Child mortality levels and trends by HIV status in Blantyre, Malawi: 1989 - 2009	JAIDS	Results not clearly disaggregated by both maternal and infant HIV status (no denominator for HEU infants)
CIGNIS study team	2010	Micronutrient fortification to improve growth and health of maternally HIV-unexposed and exposed infants: a randomised controlled trial	PLoS ONE	Results not clearly disaggregated by both maternal and infant HIV status (no denominator for HEU infants)
Van Lettow, M	2011	Uptake and outcomes of a prevention-of mother-to-child transmission (PMTCT) program in Zomba district, Malawi	BMC Public Health	Results not clearly disaggregated by both maternal and infant HIV status (no denominator for HEU infants)
Villamor, E	2004	Human immunodeficiency virus infection, diarrheal disease and sociodemographic predictors of child growth	Acta Paediatrica	Maternal HIV status unknown
Von Mollendorf, C	2015	Increased risk for and mortality from invasive pneumococcal diseases in HIV-exposed but uninfected infants aged < 1 year in South Africa, 2009-2013	Clinical Infectious Diseases	Cross-sectional study design
Webb, E	2011	Effect of single-dose anthelmintic treatment during pregnancy on an infant’s response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial	The Lancet	Results not disaggregated by both maternal and infant HIV status

Supplemental Table 9.2.3. Characteristics of studies excluded following full-text review

First author	Year	Title	Journal	Reason for exclusion
Wojcicki, J	2008	Mortality among HIV-1- and Human Herpesvirus Type 8-affectd mother-infant pairs in Zambia	Cancer Epidemiology, Biomarkers & Prevention	HIV infection not definitively excluded among HIV-exposed children
Zaba, B	2005	HIV and mortality of mothers and children: evidence from cohort studies in Uganda, Tanzania and Malawi	Epidemiology	HIV infection not definitively excluded among HIV-exposed children
Zijenah, L	1998	Mortality in the first 2 years among infants born to human immunodeficiency virus-infected women in Harare, Zimbabwe	The Journal of Infectious Diseases	Results not disaggregated by both maternal and infant HIV status

HIV, human immunodeficiency virus; HEU, HIV-exposed uninfected

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
Thea, D (1993)	DRC, peri-urban Incidence, clinical characteristics & mortality of acute, recurrent and persistent diarrhoea	Prospective cohort, antenatal enrolment	1989-1990 Followed until 21 months of age	None	None	None	139	% initiating BF not stated % EBF not stated Early introduction of FF (mean 3.2 months) and/or complementary food (mean age 5.1 months) among children with diarrhoea	9% maternal deaths, 7 cases of AIDS.	191	% initiating BF not stated % EBF not stated Early introduction of FF (mean 3.2 months) and/or complementary food (mean age 5.1 months) among children with diarrhoea	Not stated No post-partum HIV testing	Diarrhoea & early childhood mortality
Boivin, M (1995)	DRC, peri-urban Cognitive and motor effects of paediatric HIV infection	Prospective cohort, enrolment at birth.	Not stated; Children followed from birth to 2 years	None	None	None	20	Not stated, presumably breastfed	Not stated	16	Not stated, presumably breastfed	Not stated No post-partum HIV testing.	Early childhood development
Drotar, D (1997)	Uganda, urban Neuro-developmental outcomes of HIV infected children	Prospective cohort, antenatal enrolment	1989-1993; children seen at 6, 9, 12, 18 and 24 months	None	None	None	241	Not stated, presumably breastfed	11.5% maternal deaths.	116	Not stated, presumably breastfed	Not stated No post-partum HIV testing	Early childhood development & mortality
Bagenda, D (2006)		Prospective cohort, extended follow-up of above	± 1999+ The children were	None	None	None	42	Not stated, presumably breastfed	Not stated	37	Not stated, presumably breastfed	Not stated	Growth and development

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
			between 6 and 12 years old										
Taha, T (1999 & 2002) The "wash study"	Malawi, urban/ peri-urban Birth canal cleansing and PMTCT	Prospective cohort, enrolled from surviving cohort of perinatal interventional PMTCT study	1995, children followed from ages 1-3 years	None	None	None	499	% initiating BF not stated % EBF not stated Duration BF not stated Complementary foods introduced at median of 4.4 months.	4.6% maternal deaths.	119	% initiating BF not stated % EBF not stated Duration BF not stated Complementary foods introduced at median of 4.4 months.	Not stated No post-partum HIV testing	Mortality (12-36 months), infectious morbidity & early childhood development
Spira, R (1999)	Rwanda, urban Natural history of maternal and child HIV, MTCT rates, predictors and impact on health services	Prospective observational cohort, enrolment at birth	1989 – followed until 5 years of age	None	None	None	138	>95% BF initiation; % EBF not stated; median duration 18 months Complementary feeding practice not stated	81% asympto-matic; 1 case of AIDS Median baseline CD4 757/ mm^3	209	>95% BF initiation; % EBF not stated; median duration 18 months; Complementary feeding practice not stated	Not stated Had post-partum HIV testing	Under-5 mortality
Bailey, R (1999)	DRC, urban Natural history of maternal and child HIV,	Prospective observational cohort, antenatal enrolment	1989-1992 Children followed median age of 18m	None	None	None	191	Not stated, presumably breastfed.	92% of women had CD4>200 at delivery.	258	Not stated, presumably breastfed	Not stated Had post-partum HIV testing	Growth

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
	MTCT rates & predictors												
Sherry, B (2000) “The Nairobi Study”	Kenya, peri-urban Mother-to-child HIV-1 transmission study	Prospective observational cohort, most enrolled at birth from larger PMTCT cohort; some recruited through clinic	1991-1994 Followed until 18-21 months of age	None	None	Not stated	155	100% BF initiation; By two weeks, 66% EBF; By 3.5 months, 7% EBF Duration of BF not stated; By 2.5 months, 61% were receiving complementary foods	Not stated	139	100% BF initiation By two weeks, 66% EBF; By 3.5 months, 5% EBF; Duration of BF not stated; By 2.5 months, 52% were receiving complementary foods	Not stated Had post-partum HIV testing	Growth
Ota, M (2000)	The Gambia, 8 health centres; mixed rural and peri-urban Dynamics of MTCT, HIV-1 & HIV-2,effects on disease progression & child survival	Prospective observational cohort, antenatal enrolment	1993-1995, followed until 18 months of age	None	None	Not stated	81	Not stated, presumably breastfed.	Mean postnatal CD4: 32%; 3/101 (3%) deaths	448	Not stated, presumably breastfed	1/468 (0.2%) deaths. No early post-partum HIV testing	Mortality at 18 months
Schim van der Loeff, M (2003)		Prospective observational cohort, continuation of follow-up of above	1993-1995; 2001 Median follow-up of 6.6 years (since birth)	None	None	Not stated	64	Not stated, presumably breastfed.	Mean postnatal CD4: 32%; 3/101 (3%) of HIV+ women died.	448	Not stated, presumably breastfed	1/468 (0.2%) of HIV- women died. Post-partum HIV testing done for mothers still in follow-up at 18 months	Mortality over 6 years

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
Brahmbatt, H (2006) Rakai Community Cohort Study (RCCS)	Uganda, rural Broad aims, long term open cohort study with multiple sub-studies	Prospective observational cohort, antenatal enrolment	1994-1998, children followed from birth to 2 years	None	None	Not stated	269	98% initiated BF % EBF not stated Median duration BF presumed 20 months; Complementary feeding practice not stated	61% of HIV+ women had CD4 count > 500.	3183	98% initiated BF % EBF not stated Median duration BF presumed 20 months; Complementary feeding practice not stated	Not recorded Had post-partum HIV testing	Mortality
Shapiro, R (2007) [1] “The Mashi study”	Botswana, mixed rural and urban RCT with factorial design, evaluating PMTCT strategies relating to BF and antiretroviral drugs	Prospective cohort, antenatal enrolment; HIV+ women and infants were participants of RCT of interventions to reduce MTCT	2001-2004; infants followed to 2 years of age	AZT ± sdNVP for all; Triple ART in 30% (advanced HIV disease stage)	AZT until BF weaned Most infants also had sdNVP	Not for HEU	534	[1] Only BF infants followed for this analysis; 31% EBF at 3 and 17.5% at 5 months; median duration of BF 5.8 months; rapid weaning Complementary feeding practice not stated	Median baseline CD4 count 372 cells/ mm^3	137	[1] 100% BF; 9.5% were EBF by 5 months; median duration of BF 9 months; Complementary feeding practice not stated	Not recorded. Post-partum HIV testing not reported.	Mortality & Infectious morbidity
Marinda, E (2007) [1] & Koyanagi, A (2011) [1]	Zimbabwe, urban Impact of single-dose postpartum maternal	Prospective cohort analysis of Vitamin A RCT; enrolled within 96	1996-2000, children followed to age 2 years	None	None	None	3135	[1] 100% initial BF; 10% EBF at 3 months; Duration: 99% still BF at 6	37% of mothers had CD4 ≥ 500 cells/mm^3; 2.6% deaths by 12 months	9210	[1] Presume 100% initial BF; 9% EBF at 3 months; Duration of BF not stated;	0.2% deaths by 12 months post-partum. Had post-partum HIV testing	Mortality; & infectious morbidity &

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
ZVITAMBO	and/or neonatal Vitamin A supplement on MTCT and several health outcomes	hours of delivery (Factorial design, not stratified by maternal HIV status)						months, 94% at 12 months; Majority received complementary food before 3 months	postpartum; high prevalence of maternal anaemia.		Majority received complementary food before 3 months		hospital admission & clinic visits
Luabeya, K (2007)	South Africa, rural Effect of micronutrient supplements on diarrhoea & respiratory disease prevalence	Prospective RCT of micro-nutrients; enrolment from 6 months of age	2003-2006; followed from 6-24 months HEU recruited from PMTCT cohort; HU recruited from community	Not stated	Not stated	Not stated	142	Not stated	Not stated	165	Not stated	Not stated	Mortality & infectious morbidity & hospital admission
Van Rie, A (2009)	DRC, urban Effect of HIV care on neuro-development of HIV+ children	Prospective observational cohort; children ≥18months enrolled	2004-2005; children followed for 12 months	None	None	Not stated	35	Not stated	All mothers had either died or had AIDS.	90	Not stated	Healthy	Early childhood development
Sutcliffe, C (2008)	Zambia, urban clinic	Prospective observational cohort,	2000-2002; followed until 36	Not stated	Not stated	Not stated	260	Not stated	94% alive at baseline, no other information.	127	Not stated	98% alive at baseline, no other information.	Mortality

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
	Immunogenicity of measles vaccine according to HIV status	enrolment at 9 months	months of age									No post-partum HIV testing	
Chilongozi (2008) HIVNET 024	Malawi, Tanzania, Zambia; hospitals and clinics RCT of antibiotics to reduce MTCT	Cohort analysis of RCT participants: antenatal enrolment	2001-2004, followed until 12 months of age	sdNVP	sdNVP	Yes	1573	Not stated	7.2% had "serious adverse event", commonest stillbirth. 1.8% mortality	331	Not stated	3.3% had "serious adverse event", commonest stillbirth No maternal deaths	Mortality by 12 months
Naniche, D (2009)	Mozambique, rural hospital RCT of intermittent preventive treatment for malaria	Prospective cohort recruited from RCT participants; antenatal enrolment	2003-2006, followed until 12 months of age	sdNVP	sdNVP	Not for HEU	134	">90% of children breastfed in the area"; % EBF, BF duration, complementary feeding not stated	Overall, HIV+ women had med 492 (IQR 329-634) CD4 cell count; also more anaemia and mal-nutrition.	640	">90% of children breastfed in the area": % EBF, BF duration, complementary feeding not stated	Some anaemia and malnutrition, less than HIV+. No post-partum HIV testing	Mortality
Chopra, M (2010) [1]	South Africa, 3 pilot PMTCT sites, rural,	Prospective observational cohort, antenatal enrolment	2002-2004, followed until 36 weeks of age	sdNVP	sdNVP	Yes	462	[1] 47% antenatal "intention" to EFF (37% not "AFASS") *At 12 weeks*:	Median viral load at 3 weeks postpartum	218	[1] 9% antenatal "intention" to EFF (7% not "AFASS"); *At 12 weeks*:	Not stated. No post-partum HIV testing	Mortality

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
	peri-urban and urban Observational cohort to evaluate South African PMTCT program							27% EBF/PBF with rapid weaning, 54% EFF; 18% MBF; BF duration and complementary feeding not stated	3.74 log copies/ml.		22% EBF/PBF;, 9% EFF; 68% MBF; BF duration, weaning and complementary feeding practices not stated		
Ramokolo, V(2013)[1]		Prospective observational cohort, multi-site (3 sites), antenatal enrolment, followed to 36 weeks	2002 – 2004; infants followed from 3 to 36 weeks of age	sdNVP	sdNVP	Yes	502	[1] Differed by research site: 70%, 32%, 25% initiated BF; At 3 wks: 53% still BF, of which 42% were EBF Duration of BF not stated Solids introduced from 3 wks age Standard local guidelines promoted EBF for 3-4m & abrupt weaning or free formula	Mean (SD) maternal viral load (log copies/ml) per site: A. 3.98 (0.7) B. 3.59 (0.68) C. 3.77 (0.78).	216	[1] Differed by research site: 94%, 92%, 96% initiated BF; At 3 wks: 93% still BF, of which 17% were EBF; Duration: 67% still BF by 9 m Solids introduced from 3 wks age Standard local guidelines promoted EBF for 6 months with continuation to	Not stated No post-partum HIV testing reported	Growth

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
								if "AFASS" criteria met.			2 years thereafter		
Patel, D (2010) [1] Vertical transmission study; VTS	South Africa, 9 primary health clinics, rural and urban Non-randomized intervention cohort study to determine effect of infant feeding on HIV transmission and child survival	Prospective (intervention) cohort, antenatal enrolment	2001-2004, followed until 24 months of age (note, subset of Rollins cohort, below)	sdNVP	sdNVP	Not stated	902	[1] % initiating BF not stated %EBF, 3m: 83% %EBF, 5m: 76% Median EBF duration, 177 days Complementary feeding not stated	3% of HIV+ mothers died (39/1261).	1061	[1] % initiating BF not stated %EBF,3m: 73% %EBF,5m: 67% Median EBF duration, 175 days Complementary feeding not stated	<1% of mothers died (7/1064). No post-partum HIV testing	Growth
Rollins, N (2013) VTS		Prospective (intervention) cohort, antenatal enrolment	2001-2005 Children followed until 12 months (this analysis)	Mostly sdNVP; "some" triple ART after 2004 (by disease staging)	sdNVP	Not stated	936	73% initiated BF: 81% EBF at 6-8 weeks 62% EBF at 3-4 months. Duration of BF not stated Complementary feeding not stated	CD4 > 500 in 40% of women CD4 200-500 in 44% of women.	1155	80% initiated BF: 93% EBF at 6-8 weeks; 73% EBF at 3-4 m Duration of BF not stated Complementary feeding not stated	Not stated in this publication No post-partum HIV testing reported	Mortality & Diarrhoea
Van Eijk, A (2010)	Kenya, hospital outpatient clinic (urban/peri-urban)	Prospective (nested) observational cohort study, antenatal screening,	1997-2001; followed from birth to 2 years	None	None	None	407	"BF was common" % initiated BF and % EBF not stated	Not stated	182	"BF was common" % initiated BF and % EBF not stated	Not stated No post-partum HIV testing reported	Diarrhoea

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
	Relationship between placental malaria and MTCT	enrolled at birth						Median age of introduction to water or food, 6 weeks; Median duration of BF, 21 months			Median age of introduction to water or food, 6 weeks; Median duration of BF, 21 months		
Filteau, S (2011) [1] CIGNIS	Zambia, urban clinic at teaching hospital Micronutrient fortification to improve growth and health of HU and HE infants (RCT)	Cohort analysis of RCT; enrolment at 6 months of age; only survivors at 12m included for this analysis	2005-2009; followed from 6 months to 18 months of age	None	None	Not stated	125	78% initiated BF %EBF not stated Median duration of BF not stated; 42% still BF by 6 months; Nutritional complementary foods inherent to study intervention	Not stated	382	99% initiated BF % EBF not stated; Median duration BF not stated; 97% still BF by 6 months; Nutritional complementary foods inherent to study intervention	Not stated No post-partum HIV testing reported.	Growth
Nicholson (2015) CIGNIS & BFPH	Zambia, urban clinic at teaching hospital CIGNIS- as above BFPH – observational cohort evaluating	Cross-sectional assessment of children previously enrolled in prospective studies: BFPH and CIGNIS	March-May 2014, evaluating outcomes among children aged 6 years and older	sdNVP for majority; some ART for those with advanced disease stage	sdNVP	Not stated	111	BFPH: EBF median duration 6 weeks, any BF median duration 17 months. CIGNIS: 42% BF at enrolment; 25% never BF (75%	Not stated	279	BFPH: EBF median duration 6 weeks, any BF median duration 17 months. CIGNIS: 97% BF at enrolment; <1%	Not stated No post-partum HIV testing reported.	Growth & School reports & Morbidity

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
	breastfeeding and postpartum health in the context of HIV		(BFPH, 2001-2004) (CIGNIS, 2005-2007)					initiated); 34% BF < 6 months			never BF(>99% initiated); 3% BF < 6 months		
Kandawasvika, G (2011) & Kurewa, E (2011) BHAMC ("Better health for the African mother & child)	Zimbabwe 3 peri-urban clinics The role of sexually transmitted infections in MTCT of HIV-1 in Zimbabwe	Prospective observational cohort, antenatal enrolment	2002-2004, 5 years of follow-up	sdNVP	sdNVP	Not stated	188	88% initiated; no other information.	At 1 year, 3% of mothers had died; By 5 years, maternal mortality 34/100py.	287	99% initiated; no other information	At 1 year, 0.5% of mothers had died; by 5 years, maternal mortality 7/100py. No post-partum HIV testing reported	Early childhood development & Mortality
Landes, M (2012)[1]	Malawi, rural health facilities Quality and uptake of PMTCT services	Retrospective cohort study; potential participants identified through registers then traced in the community	August – December 2009; data collected retrospect-tively for first 18-20 months	sdNVP for majority; 10% received triple ART during pregnancy based on	sdNVP	Not stated	128	[1] 98% initiated BF 20% *intended to* EBF for 6 months. "The majority" practiced MBF from 3 months Mean duration of BF 12 months.	6.4% maternal deaths	200	[1] 99% initiated BF 1% *intended to* EBF for 6 months. "The majority" practiced MBF from 3 months Mean duration of BF 18 months.	No maternal deaths. Post-partum HIV testing done	Early childhood development & Growth

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
				disease staging				Complementary feeding practices not stated			Complementary feeding practices not stated		
Slogrove, A (2012)	South Africa, peri-urban Infection-related outcomes of HEU and HU infants in the first year of life	Prospective observational pilot cohort study; enrolment at birth	2009, children followed for 52 weeks	15% on triple ART; 11% None; 70% sdNVP + AZT	sdNVP + AZT	Not stated	27	4% initiated BF; % EBF, median duration, complementary feeding practices not stated	Median antenatal CD4 cell count 337 (range, 131-673).	28	100% initiated BF; % EBF not stated; Median EBF duration 12wks; Median duration of any BF; and complementary feeding practices not stated	Not stated No post-partum HIV testing reported	Infectious morbidity & Growth
Arinaitwe, E (2012) [1]	Uganda, rural RCT comparing artemether-lumefantrine and dihydroartemesinin-piperaquine for falciparum malaria	Prospective cohort analysis of RCT study participants; children aged 6 weeks to 1 year enrolled (convenience sampling)	2007-2008 Children followed until 2.5 years of age	Not stated; presume at least 1-2 drugs for PMTCT	Not stated; presume at least 1-2 drugs for PMTCT	Yes	203	100% BF initiation (part of eligibility criteria) % EBF not stated Median duration of BF 7 months; abrupt weaning practiced Complementary feeding practices not stated	Not stated	100	% BF initiation not stated; presume majority % EBF not stated Median duration of BF 20 months; Complementary feeding practices not stated	Not stated No post-partum HIV testing reported	Growth

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
Parker, M (2013)[1] Breast-feeding, ARVs and Nutrition Study (BAN)	Malawi, peri-urban HIV prevention trial with factorial design, focusing on maternal ART, extended BF and nutritional support	Prospective cohort using ex-RCT participants and community controls aged >12 months	2008-2009 HEU followed from birth to 18 months; HU followed from 15-18 months	sdNVP + AZT + 3TC for at least 7 days after birth for all; some received triple ART for 6 months postnatal	sdNVP + AZT + 3TC for at least 7 days after birth; some also received NVP for first 6m whilst BF	Not stated	39	100% initiated BF (eligibility criteria); 98% EBF by 3 months Rapid weaning at 6 months 100% stopped all BF by 7 months; Complementary foods (supplement) provided from 6 months	Not stated for this sub-group	39	>95% initiation of BF; %EBF by 3 months not known; Majority had EBF<6 months; weaning method not stated; 95% were still BF by 15 months; Complementary feeding practices not stated	Not stated No post-partum HIV testing reported	Growth
Muhangi, L (2013)[1]	Uganda, peri-urban and rural antenatal clinic at hospital Anti-helminthics in pregnancy: immune responses and disease susceptibility in offspring	Cohort analysis of RCT participants; antenatal enrolment	2003-2005, infants followed until 1 year	sdNVP; some maternal ART (by disease staging)	sdNVP	Yes	122	% Initiation, % EBF, weaning, complementary feeding not stated Mean (±SD) BF duration: 7.8 (±6.07) months.	CD4 median (IQR): 538 (345-710) cells/mm3.	1362	% Initiation, % EBF, weaning, complementary feeding not stated Mean (±SD) BF duration: 16.73 (±5.48) months	Not stated No post-partum HIV testing reported	Growth

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
Marquez, C (2014) Prevention of Malaria and HIV disease chemo-prevention trial (“PROMOTE”)	Uganda, rural clinics RCT comparing three drug regimens for malaria prevention among Ugandan children	Cohort analysis of anti-malaria RCT; enrolment at 4-5 months of age	2010-2011, infants followed until 24 months of age	Not stated, presume sdNVP; At baseline: 35% of mothers reported using ART; 78% report receiving perinatal ART	Not stated, presume sdNVP	Yes	186	100% were BF at enrolment; 84.4% BF at 6 months; 28.7% BF at 12 months No BF at 24 months. Not stated: %EBF, weaning practices, complementary feeding	All mothers alive at study enrolment; no other maternal health indicators reported.	389	100% BF at enrolment; 99.7% BF at 6 months; 98.9% BF at 12 months 24.4% BF at 24 months. Not stated: %EBF, weaning practices, complementary feeding	All mothers alive at study enrolment; no other maternal health indicators reported. No post-partum maternal HIV testing reported.	Mortality, Infectious morbidity & Growth
Moraleda, C (2014)	Mozambique, district hospital; peri-urban and rural Data from the Manhiça Health Research Centre demographic surveillance system	Prospective observational cohort; enrolled at birth, excluding infants needing urgent medical attention	2008 – 2009, infants followed until 12 months of age	sdNVP intra-partum AZT + 3TC (peri-partum only). 13% triple ART for advanced disease	sdNVP + 4 weeks of AZT	Yes	158	% initiated BF not stated % EBF not stated; 10% MBF at 1 month 43% MBF at 3 months Duration: 18% weaned by 9 months, 47% by 12 months. Weaning method and complementary	66% of mothers had CD4 ≥ 500 cells/mm3; 5% had CD4<200 at delivery.	160	% initiated BF not stated % EBF not stated; % MBF not stated Duration: 100% BF at 9 months, and at 12 months Weaning method and complementary feeding not stated	Not stated No post-partum maternal HIV testing	Infectious morbidity & growth

Supplemental Table 9.2.4. Characteristics of included studies

First author & study name	Setting & primary aim of original study	Design	Duration and infant age	ARVs for mother	ARVs for infant	HEU CTX use	HEU (n)	HEU Infant feeding	HEU Maternal health	HU (n)	HU Infant feeding	HU Maternal health	Outcomes
								feeding not stated					
Ngoma, M (2014) Aluvia Study	Zambia, peri-urban clinic in Lusaka HIV transmission rates using Lopinavir/ Ritonavir (Aluvia) based regiment for PMTCT	"Double cohort": HEU: RCT trial participants still in follow-up at 15m; HU: healthy children recruited from local clinics	2011 – 2013; children enrolled if aged 15-36 months, seen for a single develop-mental assessment	Option B+: Initiated ART during pregnancy, ZDV + 3TC + LPV/r PLUS sdNVP in labour	sdNVP + 7 days ZDV	Not stated	97	% initiated BF, % EBF, weaning and complementary feeding practices not stated. Mean (SD) duration BF: 50 (9.0) weeks. Advised to EBF for 6 months and wean at 12-13 months.	Not stated	103	% initiated BF, % EBF, weaning and complementary feeding practices not stated. Mean (SD) duration BF: 71.6 (24.6) weeks No details on amount of EBF vs mixed feeding	Not stated Presumed to have remained HIV negative if children were seronegative at 15-30 months	Early childhood development

[1] Some data obtained from different publication on the same population, same study; BFPH – the breastfeeding and postpartum health study; CIGNIS – the Chilenje Infant Growth, Nutrition & Infection Study

Abbreviations: ARVs, antiretroviral drugs; ART, triple antiretroviral therapy; HIV, human immunodeficiency virus; HEU, HIV-exposed uninfected; HU, HIV-unexposed; CTX, co-trimoxazole preventive therapy; DRC, Democratic Republic of Congo, formally Zaire; AIDS, acquired immunodeficiency syndrome; AZT, zidovudine; sdNVP, single dose nevirapine; RCT, randomized controlled trial; PMTCT, prevention of mother to child transmission of HIV; feeding definitions (WHO, 2001): BF, any breastfeeding; EBF, exclusive breastfeeding; PBF, predominant BF; FF, predominant formula feeding; EFF, exclusive formula feeding; MBF, mixed breastfeeding (breastmilk with most nutrition obtained from other liquids or food, includes partial breastfeeding); MFF, mixed formula feeding (formula with other liquids or solids, no BF): AFASS, formula feeding choice considered "acceptable, feasible, affordable, sustainable and safe

Supplemental Table 9.2.5. Infant feeding choices and practices reported among HIV-exposed and HIV-unexposed children under different World Health Organization HIV and national infant feeding guidelines in sub-Saharan Africa

Author[1]	Study start	Study end	Study Period[2]	HIV-exposed uninfected children					HIV-unexposed children			
				% initiated breast-feeding[3]	By *x* months: % EBF (exclusive breast-feeding)	Average breast-feeding duration (months)	Weaning approach[4]	Age of introduction to non-milk liquids/ solids (months) [5]	% initiated breast-feeding[3]	By *x* months: % EBF (exclusive breast-feeding)	Average breast-feeding duration (months)	Age of introduction to non-milk liquids/solids (months) [5]
Thea (1993)	1989	1990	0	-	-	-	-	-	-	-	-	-
Drotar (1997) & Bagenda (2006)	1989	1993	0	-	-	-	-	-	-	-	-	-
Spira (1999)	1989	1994	0	95%	-	18	-	-	95%	-	18	-
Bailey (1999)	1989	1992	0	-	-	-	-	-	-	-	-	-
Sherry (2000)	1991	1994	0	100%	4: 7%			< 3	100%	4: 5%	-	-
Ota (2000) & Schim van der Loeff (2003)	1993	1995	0	-	-	-	-	-	-	-	-	-
Brahmbatt (2006)	1994	1998	0	98%	-	20	-	-	98%		20	
Boivin (1995)	1995[5]	1995	0	-	-	-	-	-	-	-	-	-
Taha (1999) & Taha (2002)	1995	1998	0	-	-	-	-	4.4	-	-	-	4.4
Marinda (2007) & Koyanagi (2011)	1996	2000	0	100%	3: 10%	By 12 months: 94% still breastfed	-	< 3	100%	3: 9%	-	< 3
Van Eijk (2010)	1997	2001	0	“common”		21m		< 3	“common”	-	21	-
Sutcliffe (2008)	2000	2002	1	-	-	-	-	-	-	-	-	-
Shapiro[6] (2007)	2001	2004	1	100%[6]	5: 18%	5.8	Rapid		100%	5: 10%	9	-
Chilongozi (2008)	2001	2004	1	-	-	-	-	-	-	-	-	-
Patel (2010)	2001	2004	1	-	5: 17.5%	5.8	-	-		5: 67%	5.7	-
Rollins (2013)	2001	2005	1	73%	3: 62%		-	-	80%	3: 73%		-
Nicholson[7] (2015) (BFPH aspect)	2001	2004	1	-	1.5: 50%	17	-	-		1.5: 50%	17	-

Supplemental Table 9.2.5. Infant feeding choices and practices reported among HIV-exposed and HIV-unexposed children under different World Health Organization HIV and national infant feeding guidelines in sub-Saharan Africa

Author[1]	Study start	Study end	Study Period[2]	HIV-exposed uninfected children					HIV-unexposed children			
				% initiated breast-feeding[3]	By *x* months: % EBF (exclusive breast-feeding)	Average breast-feeding duration (months)	Weaning approach[4]	Age of introduction to non-milk liquids/ solids (months) [5]	% initiated breast-feeding[3]	By *x* months: % EBF (exclusive breast-feeding)	Average breast-feeding duration (months)	Age of introduction to non-milk liquids/solids (months) [5]
Chopra (2010) & Ramakolo[8] (2013)	2002	2004	1	70/32/25 %	1: 42%	By 3 weeks: 53% still breastfed	Rapid	< 3	94/92/96 %	1: 17%	By 3wks: 93% still breastfed	< 3
Kandawasvika (2011) &Kurewa (2011)	2002	2004	1	88%	-	-	-	-	99%	-	-	-
Luabeya (2007)	2003	2006	1	-	-	-	-	-	-	-	-	-
Naniche (2009)	2003	2006	1	>90%	-	-	-	-	>90%	-	-	-
Muhangi (2013)	2003	2005	1	-	-	7.8	-	-	-	-	17m	-
Van Rie (2009)	2004	2005	1	-	-	-	-	-	-	-	-	-
Filteau (2011) (& Nicholson, CIGNIS aspect) [7]	2005	2009 (2007)	1	78%	-	By 6 months, ± 42% still breastfed	-	-	99%	-	By 6 months, ± 97% still breastfed	-
Landes (2012)	2007	2009	1	98%	Antenatal choice for 20%	12	-	< 3	99%	Antenatal choice for 1%	18m	< 3
Arinaitwe[9] (2012)	2007	2008	1	100% [8]	-	7	Rapid	-	-	-	20	-
Parker[6] (2013)	2008	2009	1	100% [6]	3: 98%	By 7 months, none	Rapid	-	>95%	"Less than 6 months"	By 15 months, 95%	-
Moraleda (2014)	2008	2009	1	-	-	By 12 months, 53%	-	< 3		-	By 12 months, 100%	-
Slogrove (2012)	2009	2009	1	4%	-	-	-	-	100%	Median 3 weeks	-	-

Supplemental Table 9.2.5. Infant feeding choices and practices reported among HIV-exposed and HIV-unexposed children under different World Health Organization HIV and national infant feeding guidelines in sub-Saharan Africa

Author[1]	**Study start**	**Study end**	**Study Period**[2]	**HIV-exposed uninfected children**					**HIV-unexposed children**			
				% initiated breast-feeding[3]	**By *x* months: % EBF (exclusive breast-feeding)**	**Average breast-feeding duration (months)**	**Weaning approach**[4]	**Age of introduction to non-milk liquids/ solids (months)** [5]	**% initiated breast-feeding**[3]	**By *x* months: % EBF (exclusive breast-feeding)**	**Average breast-feeding duration (months)**	**Age of introduction to non-milk liquids/solids (months)** [5]
Marquez[8] (2014)	2010	2011	2	100%[8]	-	By 6 months, 85%; by 12, 29%; by 18, none	-	-	100%	-	By 6 months, 100%; by 12, 99%; by 18, 24%	-
Ngoma (2014)	2011	2013	2	-	-	11.5	-	-	-	-	16	-

[1] Some data obtained from different publication on the same population, same study;

[2] Study periods defined by prominent changes in World Health Organization (WHO) HIV infant feeding guidelines:
0, study conducted prior to 2001 WHO guidelines; 1, study conducted between 2001 and 2010; 2, study conducted following or at the time of 2010 guidelines

[3] As indicated by "ever breastfed" in study report

[4] Indication of length of period over which breastfeeding cessation occurs

[5] Varying definitions used

[6] Randomized controlled trials of antiretroviral strategies to reduce mother to child transmission of HIV; only breastfeeding mothers were eligible, hence 100% "initiation" is a function of sampling

[7] A single report combining data from two studies conducted at different time periods; BFPH – the breastfeeding and postpartum health study; CIGNIS – the Chilenje Infant Growth, Nutrition & Infection Study

[8] Multi-site study: some infant feeding indicators reported by site

[9] Randomized controlled trials of antimalarial strategies; only breastfeeding mothers eligible, hence 100% "initiation" is a function of samplin

9.3 Supplemental material for Chapter 3 (Methodology)

Supplemental Table 9.3.1. Maternal and infant measurement visit schedule: Both studies, black X; MCH-ART, red X; HU2, blue X

Item for completion	1st ANC visit	Late 3rd trimester	<7days	6 weeks	3 months	6 months	9 months	12 months
Questionnaires								
Maternal demographics and medical history[1, 2]	X	X	X	X	X	X	X	X
Infant demographics and medical history[3]			X	X	X	X	X	X
EPDS and K-10[4]	X			X				X
Alcohol and substance abuse screen[5]	X	X				X		X
Trauma/abuse assessment[6]	X		X					X
Infant feeding intentions and practices[7]		X	X	X	X	X	X	X
Family planning & pregnancy intentions[8]	X		X	X		X	X	X
Food security								X
Clinical parameters								
Anthropometry (mother & infant)[9]	X	X	X	X	X	X	X	X
Obstetric ultrasonography[10]	X	X						
Infant developmental assessment[11]								X
Postpartum maternal HIV testing								X
Maternal blood collection[12]	X	X	X	X	X	X	X	X
Infant blood collection[13]								X
Data abstraction from medical records[14]								
Antenatal and obstetric information	X	X	X					
Infant admission and test results				X		X		X
Road to health booklet of infant			X	X	X	X	X	X
Maternal HIV postpartum test results								X
ART details and pharmacy records	X	X	X	X	X	X	X	X

1 Participants' locator information updated at each study visits; **2** A subset of demographic questions will be asked at all antenatal and postnatal study visits; **3** Includes questions regarding diarrhea, pneumonia, hospitalization; for HEU, questions included regarding PMTCT; **4** Edinburgh Postnatal Depression Survey and Kessler-10 (screening questionnaire for non-specific psychological distress); **5** Alcohol use disorders identification test (AUDIT) and drug use disorders identification test (DUDIT); **6** World Health Organization questionnaire on violence against women; **7** Extensive questionnaire on milk and complementary feeding choices and practices; **8** including the London Measure of Unplanned pregnancy at first booking; **9** Length, weight and mid-upper arm circumference on mothers for calculation of gestational and post-partum weight changes, and body mass index; length, weight, head circumference and mid-upper arm circumference on infants; **10** An additional fetal anomaly scan done between 20-22 weeks for women who booked before 23 weeks; **11** Bayley Scales of Infant and Toddler Development 3rd Edition (BSID-III); cognitive, motor and language subscales; **12** HIV viral load testing; **13** Specimens used for both rapid antibody and then HIV-PCR testing; **14** Abstraction of routine clinical data and laboratory data; road to health booklets photocopied at the 12 months visit; provincial health database exports used to assess infant hospitalization rates at study closure

Supplemental Table 9.3.2. Operationalizing breastfeeding classifications in the MCH-ART and HU2 studies

Feeding practice	Definition	Allocation primarily based on	Adaptation in MCH-ART and HU2 studies per study visit	Primary application in MCH-ART and HU2 studies for use in analysis
Basic classifications of infant feeding				
Early initiation of breastfeeding (EIBF)	Any breastmilk within 1 hour of birth	Single question at postnatal enrolment visit ("neonatal visit")	6-category question about timing of initiation; one option is ≤ 1 hour of birth, used to generate binary variable	Binary indicator for "EIBF" yes vs no (within 1 hour or not)
Breastfeeding (BF)	Any breastmilk, either directly through breastfeeding or as expressed breastmilk	Two questions: 1. Is your infant currently breastfeeding? 2. How many times did your baby receive breastmilk in the last 24 hours?	Infant categorized as receiving some breastfeeding if either question indicated any breastfeeding	Binary indicator for "BF", yes vs no at each attended study visit throughout postnatal follow-up. *Duration of BF*: starting from date of birth and censored at the most recent study visit where mother reported any BF. Therefore, for women who were breastfeeding at their last attended study visit (prior to censoring of follow-up), calculated breastfeeding duration is artificially shortened as breastfeeding likely continued after their last attended study visit.
Formula feeding (FF)	Commercial cow's milk or soya-based infant replacement feeds	Three questions: 1. Is your infant currently formula feeding? 2. How many times did your baby receive formula milk in the last 24 hours? 3. 24-hour single item list recall including formula milk	Infant categorized as receiving formula feeding if any of the three questions indicated use of formula milk	Binary indicator for "FF", yes vs no at each attended study visit throughout postnatal follow-up
Milk feeds	Breastmilk, formula milk or a combination	Combining questions for binary indicators of FF and BF as above	Infant categorized as receiving any milk feeds if either BF and/or FF per indicators above	*Time-varying* binary indicator
Clear fluids	Water-based liquids that do not contain protein or fat	24-hour recall requiring "yes vs no" answers for each of 22-item checklist of commonly used liquids and solids in South African communities	Tea or Water ± sugar or salt but without milk (not oral rehydration solution – ORS considered prescribed medication and therefore not considered in this categorization)	Binary indicator for "clear liquids", yes vs no at each attended study visit throughout postnatal follow-up

Supplemental Table 9.3.2. Operationalizing breastfeeding classifications in the MCH-ART and HU2 studies

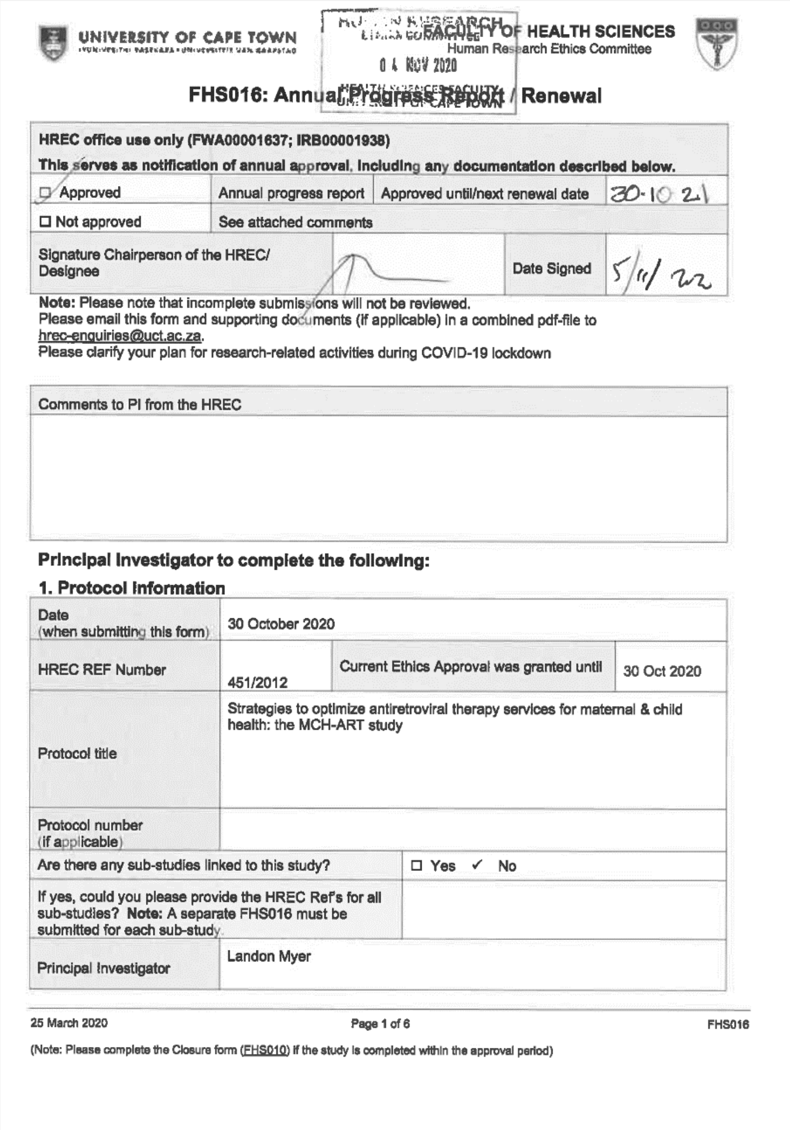
Feeding practice	Definition	Allocation primarily based on	Adaptation in MCH-ART and HU2 studies per study visit	Primary application in MCH-ART and HU2 studies for use in analysis
Nutritional liquids	Watery liquids containing protein or fat (can be provided via a bottle)	24-hour recall as above	Non-human milk, including infant formula, cow's milk, goat's milk, soya milk, buttermilk, sour milk ("Amasi"), thin porridge or gruel.	Binary indicator for "Nutritious liquids", yes vs no at each attended study visit throughout postnatal follow-up
Semi-solid and solid food	Any items of food that cannot be bottle-fed	24-hour recall as above	Food items such as mashed or sliced fruit/vegetables; meat or fish; grain-based products; solid diary products such as cheese; eggs; legumes; and commercial baby food	Binary indicator for "solid food", yes vs no at each attended study visit throughout postnatal follow-up
Complementary feeds	Solid food or nutritious liquids other than infant formula or breastmilk	24-hour recall as above	Any of the above nutritious liquids, semi-solid or solid foods, except formula milk	Binary indicator for "complementary food", yes vs no at each attended study visit
Applied definitions of feeding categories per study visit				
Exclusive breastfeeding (EBF)	Only BF and medication (allowing gripe water, oral rehydration solution, antibiotics, antivirals, multivitamins, anti-pyrectics and other prescribed medication)	24-hour recall as above	EBF vs not EBF allocated at each study visit in the first 6 months of follow-up. Non-reversible once anything else had been introduced. A baseline binary indicator for "ever EBF" was also generated according to whether the baby was exclusively breastfed in the first few days after birth	*Time-varying* indicator by study visit in the first 6 months of life. *Duration of EBF*: among infants who "ever EBF", onset of EBF duration was allocated as date of birth; EBF duration was permanently censored at the study visit date with last maternal report of exclusive feeding until 6 months of age. Therefore, for some women, EBF was never initiated, for some, EBF was censored at the postnatal enrolment visit; for others, EBF was censored at one of the postnatal visits.
Predominant breastfeeding (PrBF)	Breast milk (breast or bottle-fed) as the predominant source of nutrition	24-hour recall as above	Mother reports breastfeeding PLUS any clear fluids but no infant formula, other nutritious fluids, semi-solid or solid food	*Time-varying* indicator per study visit in the first 6 months of life (4-category variable: EBF, PrBF, PBF, or no BF)
Partial breastfeeding (PBF)	Some breast milk provided (breast or bottle-fed) but not predominant source of nutrition	24-hour recall as above	Mother reports breastfeeding (± clear fluids) PLUS one or more of infant formula or nutritious fluids (example	*Time-varying* indicator per study visit in the first 6 months of life (4-category variable: EBF, PrBF, PBF, or no BF)

Supplemental Table 9.3.2. Operationalizing breastfeeding classifications in the MCH-ART and HU2 studies

Feeding practice	Definition	Allocation primarily based on	Adaptation in MCH-ART and HU2 studies per study visit	Primary application in MCH-ART and HU2 studies for use in analysis
			cow's milk, buttermilk, yoghurt, soup), semi-solid or solid food	
Complementary feeding	Nutritious liquids or solid food	24-hour recall as above	Mother reports any nutritious fluids, semi-solid or solid food	*Time-varying* indicator per study visit in the first 6 months of life. "Early introduction to complementary feeds" created as binary variable: where complementary feeds were started prior to the age of 3 months (vs after)
Summary indicator for infant feeding in the first 6 months of life				
3-category variable summarizing early infant feeding	Combines indicators for EBF, PrBF and PBF across study visits occurring between birth and 6 months	Visit-specific binary indicators for (1) EBF vs (2) predominant breastfeeding vs (3) partial breastfeeding. (Underlying assumption, all women BF at least during the first few days of life as per enrolment criteria therefore no category for "never BF")	*EBF :* EBF reported at all attended study visits during the first 6 months; *Predominant BF:* EBF or PrBF reported at all attended study visits during the first 6 months; PrBF reported for at least 1 visit *Partial BF:* FF or complementary foods reported for at least one study visit during the first 6 months	As a single cross-sectional, summary measure

Abbreviations: MCH-ART, Maternal and Child Health Antiretroviral study; HU2, HIV-unexposed uninfected mother and child study

Supplemental figure 9.3.1. University of Cape Town Human Research Ethics Council approvals



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FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

HUMAN RESEARCH ETHICS COMMITTEE
04 NOV 2020
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) **This serves as notification of annual approval, including any documentation described below.**			
☑ Approved	Annual progress report	Approved until/next renewal date	30-10-21
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	5/11/20

Note: Please note that incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	30 October 2020		
HREC REF Number	451/2012	Current Ethics Approval was granted until	30 Oct 2020
Protocol title	Strategies to optimize antiretroviral therapy services for maternal & child health: the MCH-ART study		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes ✓ No		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Landon Myer		

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(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) **This serves as notification of annual approval, including any documentation described below.**			
☑ Approved	Annual progress report	Approved until/next renewal date	30/11/2020
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	4/11/2019

Comments to PI from the HREC

Thank you for the deviation document

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	22 May 2019		
HREC REF Number	567/2014	Current Ethics Approval was granted until	30 Dec 2018
Protocol title	Growth morbidity and development of HIV-unexposed infants: a prospective cohort study		
Protocol number (if applicable)	Version 1.1		
Are there any sub-studies linked to this study?	☒ Yes ☐ No		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Professor Landon Myer		
Department / Office Internal Mail Address	Landon.Myer@uct.ac.za		

1.1 Does this protocol receive US Federal funding?	☐ Yes	☒ No

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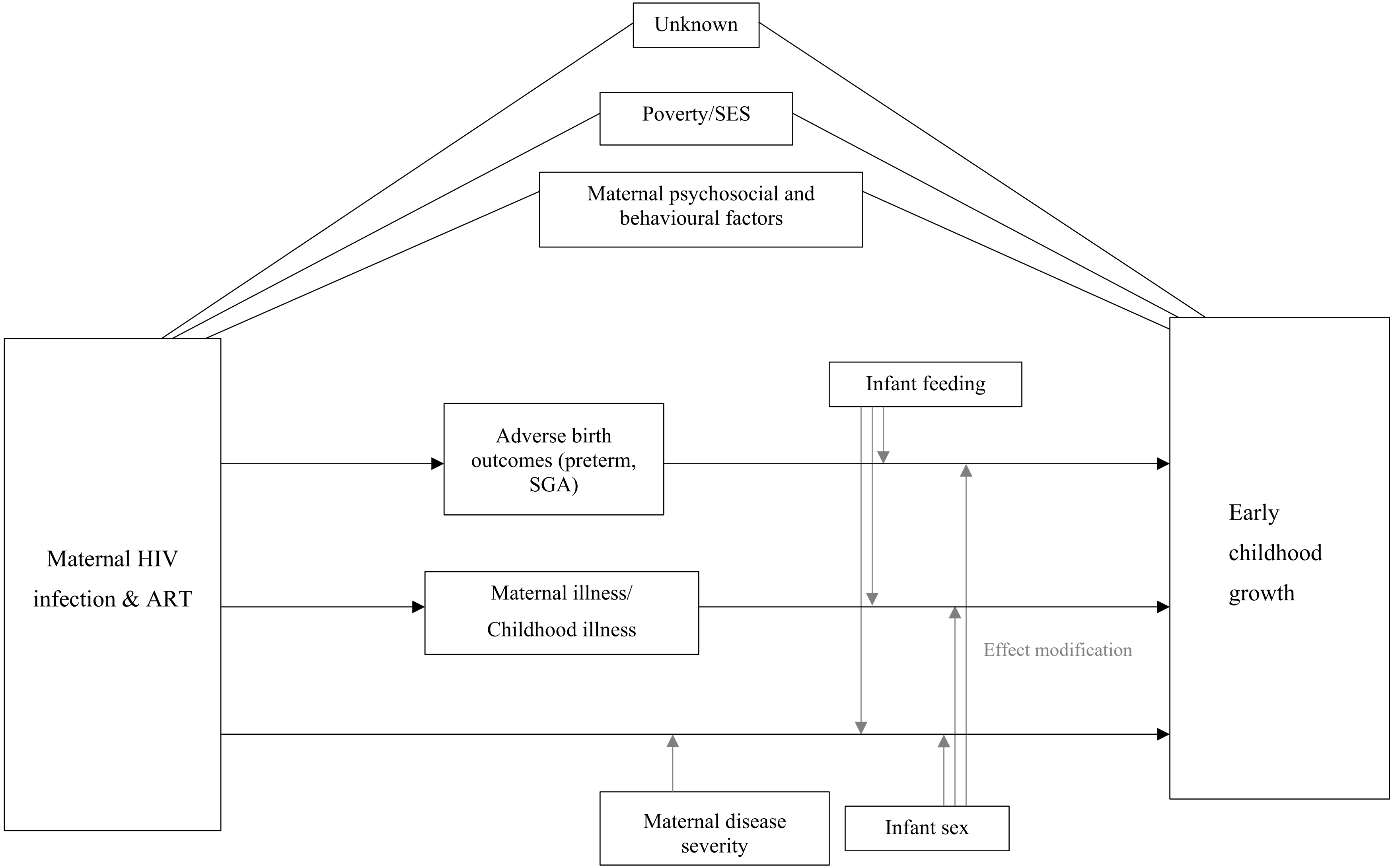
21 February 2019 Page 1 of 5 FHS016

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

9.4 Supplemental material for Chapter 4 (Infant growth)

Supplemental Figure 9.4.1. Simplified directed acyclic graph



Supplemental Table 9.4.1.

Maternal HIV status and birth outcomes of infants screened for but not enrolled into postnatal follow-up: overall and by reason for ineligibility

	Total number of children (N=203)	**Ineligible due to lack of breastfeeding (n=92)**	**Ineligible for other reasons[1] (n=111)**	***p*-value**
Birth outcomes				
Preterm (<37 weeks' completed gestational age)[2]	37 (18%)	22 (24%)	15 (14%)	0·06
Neonatal hospitalization > 5 days[3]	3 (1%)	0	3 (3%)	0·11
Small-for-gestational age (<10th centile for birthweight)[4]	24 (13%)	13 (14%)	11 (11%)	0·46
Low birth weight (<2500g)[4]	37 (18%)	19 (21%)	18 (17%)	0·45
Maternal HIV status at first antenatal clinic visit				
Positive	116 (57%)	78 (85%)	38 (34%)	<0·0001
Negative	87 (43%)	14 (15%)	73 (66%)	

Abbreviations: HEU, HIV-exposed uninfected; HU, HIV-unexposed
Results are n (column %) with p-value from chi^2 test

[1] Including: missed enrolment window (n=86), unable to continue follow-up, including relocation or work/study commitments (n=21); other (n=4)

[2] Using combination of early ultrasound, palpation and last menstrual period

[3] Based on maternal self-report with independent verification by hospital records

[4] Based on Intergrowth-21st newborn growth reference standards

Supplemental Table 9.4.2

Comparison of children who completed follow-up and contributed anthropometric measures at 12 months' study visit vs those who did not: HIV-exposed uninfected and HIV-unexposed children

	Total number of children N=872			HEU children N=461			HU children N=411		
Characteristic	**Had anthropometry at 12 months (n=686, 79%)**	**No anthropometry at 12 months (n=186, 21%)**	***p***	**Had anthropometry at 12 months (n=343, 74%)**	**No anthropometry at 12 months (n=118, 26%)**	***p***	**Had anthropometry at 12 months (n=343, 83%)**	**No anthropometry at 12 months (n=68, 16%)**	***p***
Maternal characteristics at first antenatal visit									
Age in years	28·7 (5·8)	27·1 (5·4)	0·001	29·0 (5·5)	27·4 (5·2)	0·004	28·4 (6·1)	26·5 (5·7)	0·02
Married/cohabiting	303 (44%)	70 (38%)	0·11	144 (42%)	45 (38%)	0·46	159 (46%)	25 (37%)	0·15
Completed secondary schooling	233 (34%)	65 (35%)	0·80	82 (24%)	32 (27%)	0·49	151 (44%)	33 (49%)	0·50
Employed	294 (43%)	82 (44%)	0·76	133 (39%)	49 (41%)	0·60	161 (47%)	33 (48%)	0·81
Lives in formal housing with inside toilet and running water	218 (32%)	62 (33%)	0·69	91 (27%)	34 (29%)	0·63	127 (37%)	28 (41%)	0·52
Household crowding (≥10 people)	33 (5%)	7 (4%)	0·54	21 (6%)	7 (6%)	0·94	12 (3%)	0	0·12
Risky drinking[1]	119 (17%)	28 (15%)	0·45	92 (27%)	25 (21%)	0·21	27 (8%)	3 (4%)	0·32
Intimate partner violence[2]	101 (15%)	32 (17%)	0·42	75 (22%)	26 (22%)	0·98	26 (8%)	6 (9%)	0·72
Body mass index ≥ 30 kg/m^2	300/616 (49%)	81/165 (49%)	0·93	127/285 (45%)	44/101 (44%)	0·86	173/331 (52%)	37/64 (58%)	0·42
CD4 cell count at ART initiation (cells/mm^3)	-	-	-	349 (245-502)	389 (259-555)	0·14	-	-	-
Log$_{10}$ HIV viral load at ART initiation (copies/mL)	-	-	-	4·0 (3·5-4·6)	3·7 (3·0-4·3)	0·0004	-	-	-
Delivery and infant characteristics									

	Total number of children N=872			HEU children N=461			HU children N=411		
Characteristic	**Had anthropometry at 12 months (n=686, 79%)**	**No anthropometry at 12 months (n=186, 21%)**	***p***	**Had anthropometry at 12 months (n=343, 74%)**	**No anthropometry at 12 months (n=118, 26%)**	***p***	**Had anthropometry at 12 months (n=343, 83%)**	**No anthropometry at 12 months (n=68, 16%)**	***p***
Viral load >50 copies/mL at delivery ("unsuppressed")	-	-	-	83 (24%)	26 (22%)	0·63	-	-	-
Preterm birth (<37 weeks' gestational age)	76 (11%)	18 (10%)	0·59	43 (12%)	13 (11%)	0·66	33 (10%)	5 (7%)	0·55
Small-for-gestational-age (birthweight <10th centile)	71 (10%)	19 (10%)	0·96	41 (12%)	10 (9%)	0·30	30 (9%)	9 (13%)	0·25
Male sex	330 (48%)	98 (52%)	0·28	173 (50%)	59 (50%)	0·93	157 (46%)	39 (57%)	0·08
Cumulative infant feeding[3] (0-6 months)									
Exclusive breastfeeding	269 (39%)	74 (40%)	0·96	154 (45%)	52 (44%)	0·93	115 (33%)	22 (32%)	0·77
Predominant breastfeeding	109 (16%)	28 (15%)		32 (9%)	10 (9%)		77 (22%)	18 (26%)	
Partial breastfeeding	308 (45%)	84 (45%)		157 (46%)	56 (47%)		151 (44%)	28 (41%)	
Ever exclusively breastfed[3]	602 (88%)	152 (82%)	0·03	318 (93%)	103 (87%)	0·07	284 (83%)	49 (72%)	0·04
Duration of EBF (months)	1·4 (0·2-3·1)	0·3 (0·1-2·0)	0·0001	1·5 (0·3-6·0)	0·5 (0·1-3·0)	0·0001	1·4 (0·1-3·0)	0·2 (0; 1·7)	0·004
Duration of any breastfeeding[3] (months)	9·0 (3·0-12·0)	2·5 (0·5-6·0)	0·0001	6·0 (1·4-12·0)	1·4 (0·2-6·0)	0·0001	11·4 (3·1-12·1)	3·0 (1·4-6·0)	<0·0001
Early weaning [4]	91 (13%)	27 (14%)	0·65	36 (10%)	11 (9%)	0·72	55 (26%)	16 (24%)	0·13

Abbreviations: HEU, HIV-exposed uninfected; HU, HIV-unexposed; EBF, exclusive breastfeeding; Results are n(column %) with p-value from chi^2 test; mean (sd) with p-value from ttest for normally distributed variables; or median (interquartile range, IQR) with p-value from Kruskal-Wallis for non-normally distributed variables; [1] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3, first antenatal visit; [2] Any physical, sexual or psychological violence, World Health Organization violence against women questionnaire, first antenatal visit; [3] Maternal self-report, 24 hour recall; exclusive defined as nothing other than breastmilk and prescribed medicine; [4] Introduction of semi-solid or solid foods before 3 months of age

Supplemental Table 9.4.3.

Anthropometric measures and time-varying characteristics of HIV-exposed uninfected and HIV-unexposed children at birth and per study visit: cross-sectional, fixed effects linear and logistic regression results

Time-varying mother-infant characteristics		TOTAL	HEU children	HU children	Crude β (95% CI)	Crude OR (95% CI)	Missing[4]
BIRTH		**872**	**461**	**411**	**-**	**-**	**-**
Infant feeding[1]	Any breastfeeding	872 (100%)	461 (100%)	411 (100%)	-	-	0
	Exclusive breastfeeding[2]	754 (86%)	421 (91%)	333 (81%)	-	2·47 (1·64; 3·71)	
Weight	Weight (kg)	3·11 (0·55)	3·05 (0·55)	3·19 (0·55)	-0·14 (-0·21; -0·07)	-	0
	SGA, weight < 10th centile	90 (10%)	51 (11%)	39 (9%)	-	1·19 (0·76; 1·84)	
	Weight-for-age Z-score (WAZ)	-0·01 (0·96)	-0·13 (0·92)	0·12 (1·00)	-0·24 (-0·37; -0·12)	-	
	Underweight (WAZ<-2)	20 (2%)	13 (3%)	7 (2%)	-	1·68 (0·66; 4·24)	
STUDY VISIT 1: ± 6 WEEKS		**805 (92%)**	**419 (91%)**	**386 (94%)**	**-**	**-**	-
Infant age	Age (months)	1·54 (0·43)	1·45 (0·33)	1·63 (0·50)	-0·17 (-0·23; -0·12)	-	0
Infant feeding [1]	Any breastfeeding	730 (91%)	365 (87%)	365 (95%)	-	0·39 (0·23; 0·66)	0
	Exclusive breastfeeding[2]	486/730 (67%)	288/365 (79%)	198/365 (54%)	-	3·15 (2·28; 4·37)	1 (<1%)
	Any infant formula	141 (18%)	69 (17%)	73 (19%)	-	0·86 (0·60; 1·23)	
	Receives nutritive liquids/solid food[3]	26 (3%)	10 (2%)	16 (4%)	-	0·57 (0·25; 1·26)	
	Optimal weaning practices[4]	485 (60%)	287 (69%)	198 (51%)	-	2·06 (1·55; 2·75)	
Infant health	Recent illness[5]	125 (17%)	84 (24%)	41 (11%)	-	2·72 (1·81; 4·09)	77 (10%)
Maternal	Smoking[7]	15/729 (2%)	13/343 (4%)	2/386 (<1%)	-	n/a	76 (9%)
Weight	Weight (kg)	4·70 (0·92)	4·52 (0·87)	4·88 (0·93)	-0·36 (-0·48; -0·23)	-	8 (1%)
	Weight-for-age Z-score (WAZ)	-0·12 (1·13)	-0·26 (1·15)	0·04 (1·08)	-0·30 (-0·46; -0·15)	-	
	Underweight (WAZ<-2)	48 (6%)	33 (8%)	15 (4%)	-	2·13 (1·14; 4·00)	
Length	Length (cm)	54·82 (3·18)	54·51 (3·33)	55·15 (2·98)	-0·64 (-1·08; -0·20)	-	10 (1%)
	Length-for-age Z-score (LAZ)	-0·36 (1·25)	-0·34 (1·36)	-0·37 (1·12)	0·03 (-0·15; 0·20)	-	
	Stunted (LAZ<-2)	71 (9 %)	44 (11%)	27 (7%)	-	1·60 (0·97; 2·64)	
Head circumference	Head circumference (cm), HC	38·42 (1·95)	38·03 (1·95)	38·85 (1·86)	-0·82 (-1·08; -0·55)	-	4 (<1%)
	HC-for-age Z-score (HCAZ)	0·74 (1·29)	0·55 (1·33)	0·95 (1·22)	-0·41 (-0·59; -0·23)	-	

Time-varying mother-infant characteristics		TOTAL	HEU children	HU children	Crude β (95% CI)	Crude OR (95% CI)	Missing[4]
	Microcephaly (HCAZ<-2)	18 (2%)	11 (3%)	7 (2%)	-	1·46 (0·56; 3·80)	
Proportional size	Weight-for-length Z-score (WLZ)	0·29 (1·38)	0·03 (1·35)	0·55 (1·37)	-0·53 (-0·72; -0·34)	-	26 (3%)
	Wasted (WLZ<-2)	40 (5%)	27 (7%)	13 (3%)	-	2·07 (1·05; 4·08)	
	Overweight (WLZ>2)	71 (9%)	20 (5%)	51 (13%)	-	0·34 (0·20; 0·59)	
	Obese (WLZ>3)	19 (2%)	8 (2%)	11 (3%)	-	0·69 (0·28; 1·74)	
	Body mass index (kg/m^2), BMI	15·53 (2·16)	15·14 (2·25)	15·95 (1·98)	-0·80 (-1·10; -0·51)	-	16 (2%)
	BMI-for age Z-score (BMIZ)	0·06 (1·27)	-0·20 (1·25)	0·33 (1·25)	--0·53 (-0·70; -0·35)	-	
	Overweight (BMIZ>2)	44 (6%)	10 (3%)	34 (9%)	-	0·26 (0·13; 0·54)	
	Obese (BMIZ>3)	6 (1%)	4 (1%)	2 (<1%)	-	1·92 (0·35; 10·54)	
Combined malnutrition	Wasted & stunted (WLZ<-2 and LAZ<-2)	1 (<1%)	0	1 (<1%)	-	n/a	
	Overweight & stunted (WLZ>2 and LAZ<-2)	18 (2%)	10 (2%)	8 (2%)	-	1·20 (0·47; 3·06)	
STUDY VISIT 2: ± 3 MONTHS		**731 (84%)**	**363 (79%)**	**368 (90%)**			
Infant age	Age (months)	3·18 (0·57)	3·18 (0·67)	3·18 (0·45)	0·00 (-0·09; 0·08)	-	0
Infant feeding [1]	Any breastfeeding	576 (79%)	254 (70%)	322 (87%)	-	0·33 (0·23; 0·49)	0
	Exclusive breastfeeding[2]	352/576 (61%)	204/254 (80%)	148/322 (46%)	-	4·80 (3·28; 7·01)	
	Any formula milk	235 (32%)	118 (33%)	117 (32%)	-	1·03 (0·76; 1·41)	
	Receives nutritive liquids/solid food[3]	102 (14%)	38 (11%)	64 (17%)	-	0·55 (0·36; 0·85)	
	Optimal weaning practices[4]	352 (48%)	204 (56%)	148 (40%)	-	1·91 (1·42; 2·56)	
Infant health	Recent illness[5]	177 (24%)	132 (36%)	45 (12%)	-	4·08 (2·79; 5·95)	2 (<1%)
Weight	Weight (kg)	6·19 (1·06)	6·02 (1·06)	6·35 (1·04)	-0·33 (-0·49; -0·18)	-	1 (<1%)
	Weight-for-age Z-score (WAZ)	0·13 (1·20)	-0·06 (1·19)	0·31 (1·18)	-0·37 (-0·54; -0·20)	-	
	Underweight (WAZ<-2)	35 (5%)	25 (7%)	10 (3%)	-	2·64 (1·25; 5·58)	
Length	Length (cm)	59·87 (3·27)	59·74 (3·65)	59·98 (2·84)	-0·24 (-0·71; 0·24)	-	6 (<1%)
	Length-for-age Z-score (LAZ)	-0·27 (1·23)	--0·29 (1·34)	-0·26 (1·11)	-0·03 (-0·21; 0·15)		
	Stunted (LAZ<-2)	52 (7%)	34 (9%)	18 (5%)	-	2·03 (1·13; 3·67)	
Head circumference	Head circumference (cm), HC	41·04 (1·85)	40·79 (1·87)	41·28 (1·80)	-0·49 (-0·75; -0·22)	-	1 (<1%)
	HC-for-age Z-score (HCAZ)	0·94 (1·27)	0·78 (1·25)	1·09 (1·28)	-0·30 (-0·48; -0·12)	-	

Time-varying mother-infant characteristics		**TOTAL**	**HEU children**	**HU children**	**Crude β (95% CI)**	**Crude OR (95% CI)**	**Missing[4]**
	Microcephaly (HCAZ<-2)	7 (1%)	4 (1%)	3 (1%)	-	n/a	
Proportional size	Weight-for-length Z-score (WLZ)	0·54 (1·35)	0·33 (1·27)	0·73 (1·40)	-0·40 (-0·59; -0·20)	-	11 (1%)
	Wasted (WLZ<-2)	15 (2%)	8 (2%)	7 (2%)	-	1·18 (0·42; 3·29)	
	Overweight (WLZ>2)	94 (13%)	28 (8%)	66 (18%)	-	0·39 (0·24; 0·62)	
	Obese (WLZ>3)	33 (5%)	8 (2%)	25 (7%)	-	0·31 (0·14; 0·70)	
	Body mass index (kg/m^2), BMI	17·22 (2·15)	16·81 (1·99)	17·61 (2·23)	-0·81 (-1·12; -0·50)	-	7 (1%)
	BMI-for age Z-score (BMIZ)	0·36 (1·35)	0·12 (1·27)	0·60 (1·38)	-0·48 (-0·67; -0·28)	-	
	Overweight (BMIZ>2)	79 (11%)	20 (6%)	59 (16%)	-	0·31 (0·18; 0·52)	
	Obese (BMIZ>3)	23 (3%)	4 (1%)	19 (5%)	-	0·21 (0·07; 0·61)	
Combined malnutrition	Wasted & stunted (WLZ<-2 and LAZ<-2)	1 (<1%)	1 (<1%)	0	-	n/a	
	Overweight & stunted (WLZ>2 and LAZ<-2)	13 (2%)	8 (2%)	5 (1%)	-	1·65 (0·54; 5·11)	
STUDY VISIT 3: ± 6 MONTHS		**727 (83%)**	**382 (83%)**	**345 (84%)**			
Infant age	Age (months)	6·23 (0·62)	6·21 (0·66)	6·26 (0·57)	-0·05 (-0·14; 0·04)	-	0
Infant feeding	Any breastfeeding	458 (63%)	218 (57%)	240 (70%)	-	0·58 (0·43; 0·79)	0
	Exclusive breastfeeding[2]	153/458 (33%)	117/218 (54%)	36/240 (15%)	-	6·56 (4·21; 10·22)	
	Any formula milk	384 (53%)	188 (49%)	196 (57%)	-	0·74 (0·55; 0·99)	
	No milk feeds in previous 24 hours	2 (<1%)	2 (<1%)	0	-	n/a	
	Any nutritive liquids/solid food[3]	434 (60%)	191 (50%)	244 (70%)	-	0·42 (0·31; 0·57)	
	≥3 food groups in previous 24 hours[6]	61 (8%)	20 (5%)	41 (12%)	-	0·41 (0·23; 0·71)	
	Optimal weaning practices[4]	141 (19%)	90 (24%)	51 (15%)	-	1·78 (1·22; 2·60)	
Infant health	Recent illness[5]	254 (35%)	169 (44%)	85 (25%)	-	2·46 (1·78; 3·37)	2 (<1%)
Maternal	Smoking[7]	21 (3%)	19 (5%)	2 (1%)	-	n/a	3 (<1%)
Weight	Weight (kg)	7·95 (1·24)	7·79 (1·23)	8·12 (1·22)	-0·33 (-0·51; -0·15)		5 (<1%)
	Weight-for-age Z-score (WAZ)	0·32 (1·20)	0·16 (1·23)	0·49 (1·15)	-0·33 (-0·50; -0·16)	-	
	Underweight (WAZ<-2)	17 (2%)	13 (3%)	4 (1%)	-	n/a	
Length	Length (cm)	66·32 (3·22)	66·04 (3·26)	66·63 (3·14)	-0·60 (-1·06; -0·13)	-	1 (<1%)
	Length-for-age Z-score (LAZ)	-0·12 (1·26)	-0·22 (1·26)	-0·01 (1·25)	-0·21 (-0·39; -0·03)	-	

Time-varying mother-infant characteristics		**TOTAL**	**HEU children**	**HU children**	**Crude β (95% CI)**	**Crude OR (95% CI)**	**Missing**[4]
	Stunted (LAZ<-2)	60 (8%)	38 (10%)	22 (6%)	-	1·63 (0·94; 2·81)	
Head circumference	Head circumference (cm), HC	44·05 (1·68)	43·92 (1·74)	44·19 (1·60)	-0·28 (-0·52; -0·03)	-	
	HC-for-age Z-score (HCAZ)	1·05 (1·17)	0·98 (1·20)	1·14 (1·13)	-0·17 (-0·34; 0·01)	-	2 (<1%)
	Microcephaly (HCAZ<-2)	3 (<1%)	3 (<1%)	0	-	n/a	
Proportional size	Weight-for-length Z-score (WLZ)	0·64 (1·24)	0·51 (1·24)	0·78 (1·23)	-0·27 (-0·45; -0·09)		6 (<1%)
	Wasted (WLZ<-2)	9 (1%)	8 (2%)	1 (<1%)	-	n/a	
	Overweight (WLZ>2)	101 (14%)	46 (12%)	55 (16%)	-	0·72 (0·47; 1·09)	
	Obese (WLZ>3)	24 (3%)	7 (2%)	17 (5%)	-	0·36 (0·15; 0·87)	
	Body mass index (kg/m^2), BMI	18·01 (2·02)	17·79 (1·98)	18·25 (2·04)	-0·45 (-0·75; -0·16)	-	6 (<1%)
	BMI-for age Z-score (BMIZ)	0·53 (1·26)	0·39 (1·26)	0·68 (1·25)	-0·29 (-0·47; -0·10)	-	
	Overweight (BMIZ>2)	92 (13%)	41 (11%)	51 (15%)	-	0·69 (0·44; 1·07)	
	Obese (BMIZ>3)	23 (3%)	6 (2%)	17 (5%)	-	0·31 (0·12; 0·78)	
Combined malnutrition	Wasted & stunted (WLZ<-2 and LAZ<-2)	1 (<1%)	1 (<1%)	0	-	n/a	
	Overweight & stunted (WLZ>2 and LAZ<-2)	8 (1%)	4 (1%)	4 (1%)	-	n/a	
STUDY VISIT 4: ± 9 MONTHS		**690 (79%)**	**360 (78%)**	**330 (80%)**	**-**	**-**	
Infant age	Age (months)	9·20 (0·58)	9·14 (0·57)	9·27 (0·58)	-0·13 (-0·22; -0·05)	-	0
Infant feeding [1]	Any breastfeeding	380 (55%)	172 (48%)	208 (63%)	-	0·54 (0·40; 0·73)	0
	Any infant formula	430 (62%)	217 (60%)	213 (64%)	-	0·83 (0·61; 1·13)	
	No milk feeds in previous 24 hours	13 (2%)	9 (3%)	4 (1%)	-	n/a	
	Any nutritive liquids/solid food[3]	665 (96%)	351 (98%)	314 (95%)	-	1·99 (0·87; 4·56)	
	≥3 food groups in previous 24 hours[6]	251 (36%)	145 (40%)	106 (32%)	-	1·43 (1·04; 1·95)	
	Optimal weaning practices[4]	246 (36%)	143 (40%)	103 (31%)	-	1·45 (1·06; 1·99)	
Infant health	Recent illness[5]	254 (37%)	152 (42%)	102 (31%)	-	1·63 (1·19; 2·23)	0
Weight	Weight (kg)	9·04 (1·42)	8·84 (1·42)	9·27 (1·40)	-0·43 (-0·64; -0·22)	-	
	Weight-for-age Z-score (WAZ)	0·42 (1·28)	0·23 (1·31)	0·62 (1·22)	-0·39 (-0·58; -0·20)	-	7 (1%)
	Underweight (WAZ<-2)	19 (3%)	15 (4%)	4 (1%)	-	n/a	
Length	Length (cm)	70·84 (3·19)	70·26 (3·27)	71·47 (2·97)	-1·21 (-1·68; -0·74)	-	3 (<1%)

Time-varying mother-infant characteristics		**TOTAL**	**HEU children**	**HU children**	**Crude β (95% CI)**	**Crude OR (95% CI)**	**Missing[4]**
	Length-for-age Z-score (LAZ)	-0·03 (1·22)	-0·24 (1·24)	0·19 (1·17)	-0·43 (-0·61; -0·25)	-	
	Stunted (LAZ<-2)	34 (5%)	28 (8%)	6 (2%)	-	4·60 (1·88; 11·25)	
Head circumference	Head circumference (cm), HC	45·72 (1·69)	45·50 (1·76)	45·95 (1·59)	-0·45 (-0·70; -0·20)	-	0
	HC-for-age Z-score (HCAZ)	1·07 (1·18)	0·93 (1·21)	1·22 (1·13)	-0·29 (-0·47; -0·12)	-	
	Microcephaly (HCAZ<-2)	6 (1%)	5 (1%)	1 (<1%)	-	n/a	
Proportional size	Weight-for-length Z-score (WLZ)	0·65 (1·26)	0·55 (1·28)	0·76 (1·23)	-0·21 (-0·40; -0·02)	-	12 (2%)
	Wasted (WLZ<-2)	10 (1%)	6 (2%)	4 (1%)	-	n/a	
	Overweight (WLZ>2)	99 (15%)	47 (13%)	52 (16%)	-	0·80 (0·52; 1·22)	
	Obese (WLZ>3)	24 (4%)	13 (4%)	11 (3%)	-	1·08 (0·48; 2·44)	
	Body mass index (kg/m^2), BMI	17·97 (2·04)	17·85 (2·07)	18·11 (2·01)	-0·26 (-0·57; 0·04)	-	10 (1%)
	BMI-for age Z-score (BMIZ)	0·59 (1·27)	0·49 (1·29)	0·69 (1·23)	-0·19 (-0·39; 0·00)	-	
	Overweight (BMIZ>2)	89 (13%)	43 (12%)	46 (14%)	-	0·83 (0·53; 1·30)	
	Obese (BMIZ>3)	21 (3%)	12 (3%)	9 (3%)	-	1·22 (0·51; 2·94)	
Combined malnutrition	Wasted & stunted (WLZ<-2 and LAZ<-2)	2 (<1%)	2 (<1%)	0	-	n/a	
	Overweight & stunted (WLZ>2 and LAZ<-2)	1 (<1%)	1 (<1%)	0	-	n/a	
STUDY VISIT 5: ± 12 MONTHS		**686 (79%)**	**343 (74%)**	**343 (84%)**			
Infant age	Age (months)	12·44 (1·22)	12·26 (0·95)	12·62 (1·43)	-0·36 (-0·54; -0·18)	-	0
Infant feeding [1]	Any breastfeeding	316 (46%)	141 (41%)	175 (51%)	-	0·67 (0·50; 0·91)	0
	Any infant formula	426 (62%)	209 (61%)	217 (63%)	-	0·91 (0·67; 1·23)	
	No milk feeds in previous 24 hours	39 (6%)	20 (6%)	19 (5%)	-	0·95 (0·50; 1·81)	
	Any nutritive liquids/solid food[3]	659 (96%)	340 (99%)	319 (93%)	-	8·53 (2·54; 28·59)	
	≥3 food groups in previous 24 hours[6]	356 (52%)	192 (56%)	164 (48%)	-	1·39 (1·03; 1·87)	
	Optimal weaning practices[4]	333 (49%)	177 (52%)	156 (45%)	-	1·28 (0·95; 1·72)	
Infant health	Recent illness[5]	243 (36%)	126 (37%)	117 (34%)	-	1·14 (0·83; 1·57)	3 (<1%)
Maternal	Smoking[7]	20 (3%)	20 (6%)	0	-	n/a	5 (1%)
Weight	Weight (kg)	9·93 (1·60)	9·66 (1·57)	10·19 (1·60)	-0·53 (-0·77; -0·29)	-	1 (<1%)
	Weight-for-age Z-score (WAZ)	0·45 (1·29)	0·24 (1·32)	0·66 (1·24)	-0·41 (-0·60; -0·22)	-	

Time-varying mother-infant characteristics		TOTAL	HEU children	HU children	Crude β (95% CI)	Crude OR (95% CI)	Missing[4]
	Underweight (WAZ<-2)	16 (2%)	12 (4%)	4 (1%)	-	n/a	
Length	Length (cm)	74·27 (3·44)	73·52 (3·41)	75·01 (3·32)	-1·50 (-2·00; -0·99)	-	
	Length-for-age Z-score (LAZ)	-0·30 (1·22)	-0·51 (1·27)	-0·08 (1·13)	-0·43 (-0·61; -0·25)	-	0
	Stunted (LAZ<-2)	49 (7%)	35 (10%)	14 (4%)	-	2·67 (1·41; 5·06)	
Head circumference	Head circumference (cm), HC	46·90 (1·68)	46·64 (1·72)	47·16 (1·60)	-0·52 (-0·77; -0·27)	-	
	HC-for-age Z-score (HCAZ)	1·06 (1·12)	0·91 (1·20)	1·22 (1·01)	-0·31 (-0·48; -0·14)	-	3 (<1%)
	Microcephaly (HCAZ<-2)	5 (1%)	3 (1%)	2 (1%)	-	n/a	
Proportional size	Weight-for-length Z-score (WLZ)	0·77 (1·32)	0·67 (1·39)	0·88 (1·24)	-0·21 (-0·41; -0·01)	-	4 (<1%)
	Wasted (WLZ<-2)	11 (2%)	6 (2%)	5 (1%)	-	1·20 (0·36; 3·96)	
	Overweight (WLZ>2)	114 (17%)	54 (16%)	60 (18%)	-	0·88 (0·58; 1·31)	
	Obese (WLZ>3)	36 (5%)	16 (5%)	20 (6%)	-	0·79 (0·40; 1·54)	
	Body mass index (kg/m^2), BMI	17·94 (2·13)	17·82 (2·17)	18·06 (2·08)	-0·24 (-0·56; 0·08)	-	
	BMI-for age Z-score (BMIZ)	0·84 (1·32)	0·76 (1·38)	0·92 (1·26)	-0·16 (-0·36; 0·04)	-	1 (<1%)
	Overweight (BMIZ>2)	123 (18%)	59 (17%)	64 (19%)	-	0·91 (0·61; 1·34)	
	Obese (BMIZ>3)	40 (6%)	22 (6%)	18 (5%)	-	1·24 (0·65; 2·36)	
Combined malnutrition	Wasted & stunted (WLZ<-2 and LAZ<-2)	3 (<1%)	2 (1%)	1 (<1%)	-	n/a	
	Overweight & stunted (WLZ>2 and LAZ<-2)	4 (1%)	3 (1%)	1 (<1%)	-	n/a	

HEU, HIV-exposed uninfected; HU, HIV-unexposed;β (95% confidence interval, CI) obtained from univariable linear regression; OR, odds ratio from logistic regression model (not calculated if any cell <5); Results are mean (sd) or n(%) from column total (per study visit); proportion of cohort attending each study visit is shown in bold in row with number; percentages presented without decimals for simplicity

Z-scores obtained using the Intergrowth-21st growth standards for postnatal growth of preterm infants until 64 weeks postmenstrual age; World Health Organization growth reference standards (2006) for term infants and preterm infants after 64 weeks' postmenstrual age; weight-for-length and body mass-index Z-scores from WHO standards (adjusted age for preterm infants until 64 weeks' postmenstrual age)

[1] Maternal self-report, 24 hour recall; [2]exclusive defined as nothing other than breastmilk and prescribed medicine; [3] any liquids other than breastmilk or medicine, and/or semi-solids or solids; [3] Maternal report of any diarrhoea or coughing illness in preceding 2 weeks; [4] Defined according to age rather than study visit, based on maternal report of dietary intake in the preceding 24 hours: optimal weaning up to 5 months of age defined as exclusive breastfeeding only; for children aged 5 months and older, defined as some form of milk feeds (breastmilk and/or infant formula milk), along with some solid foods of reasonable diversity (at least 3 of the following 5 foodgroups: dairy products such as yoghurt, cheese or 'amasi'; cereal/bread; vegetables/fruit; meat; and/or eggs);

[6] At least 3 of aforementioned food groups (24 hour recall, at study visit); [7] Maternal self-report of any cigarette smoking in preceding month

Supplemental Table 9.4.4

Differences in growth measures comparing HIV-exposed uninfected to HIV-unexposed children over time, stratified by size at birth (appropriate- vs small-for gestational age): crude and adjusted mixed effects linear models

		Weight-for-age (WAZ)^{1}		**Length-for-age (LAZ)^{1}**		**Head circumference-for-age (HCAZ)^{1}**		**Weight-for-length (WLZ)^{2}**	
		β (95% CI)	**aβ^{5} (95% CI)**	**β (95% CI)**	**aβ^{5} (95% CI)**	**β (95% CI)**	**aβ^{5} (95% CI)**	**β (95% CI)**	**aβ^{5} (95% CI)**
Total	SGA	-0·52 (-0·89; -0·16)	-0·45 (-0·84; -0·07)	-0·59 (-1·01; -0·18)	-0·54 (-0·96; -0·13)	-0·51 (-0·92; -0·10)	-0·54 (-0·96; -0·13)	-0·37 (-0·80; 0·06)	-0·40 (-0·83; 0·04)
	AGA	-0·29 (-0·42; -0·17)	-0·23 (-0·37; -0·10)	-0·13 (-0·27; 0·01)	-0·06 (-0·21; 0·08)	-0·25 (-0·39; -0·11)	-0·21 (-0·35; -0·06)	-0·34 (-0·48; -0·19)	-0·31 (-0·46; -0·15)
Birth	SGA	-0·11 (-0·56;0·34)	0·03 (-0·44; 0·50)	-	-	-	-	-	-
	AGA	-0·23 (-0·38; -0·07)	-0·14 (-0·31; 0·02)	-	-	-	-	-	-
6 w	SGA	-0·40 (-0·86; 0·06)	-0·38 (-0·85; 0·09)	-0·35 (-0·85; 0·16)	-0·28 (-0·79; 0·23)	-0·49 (-0·99; 0·00)	-0·49 (-1·00; 0·01)	-0·33 (0·88; 0·23)	-0·35 (-0·91; 0·22)
	AGA	-0·26 (-0·42; -0·11)	-0·22 (-0·38; -0·05)	0·08 (-0·09; 0·25)	0·16 (-0·02; 0·35)	-0·37 (-0·54; -0·20)	-0·31 (-0·49; -0·13)	-0·56 (-0·75; -0·37)	-0·55 (-0·75; -0·34)
3 m	SGA	-0·69 (-1·15; -0·22)	-0·66 (-1·13; -0·19)	-0·47 (-0·98; 0·04)	-0·39 (-0·91; 0·12)	-0·62 (-1·12; -0·12)	-0·66 (-1·17; -0·15)	-0·23 (-0·80; 0·34)	-0·26 (-0·83; 0·31)
	AGA	-0·32 (-0·48; -0·16)	-0·27 (-0·43; -0·10)	0·05 (-0·12; 0·23)	0·12 (-0·06; 0·31)	-0·24 (-0·42; -0·07)	-0·22 (-0·40; -0·04)	-0·46 (-0·66; -0·27)	-0·43 (-0·63; -0·23)
6 m	SGA	-0·62 (-1·09; -0·15)	-0·61 (-1·08; -0·13)	-0·49 (-1·01; 0·03)	-0·44 (-0·96; 0·07)	-0·39 (-0·89; 0·12)	-0·44 (-0·95; 0·07)	-0·42 (-0·99; 0·15)	-0·45 (-1·02; 0·12)
	AGA	-0·30 (-0·47 -0·14)	-0·26 (-0·43; -0·09)	-0·18 (-0·35; 0·00)	-0·12 (-0·30; 0·07)	-0·10 (-0·28; 0·07)	-0·07 (-0·25; 0·11)	-0·26 (-0·46; -0·07)	-0·24 (-0·44; -0·04)
9 m	SGA	-0·82 (-1·30; -0·34)	-0·78 (-1·27; -0·30)	-0·86 (-1·39; -0·33)	-0·83 (-1·36; -0·30)	-0·69 (-1·20; -0·17)	-0·73 (-1·25; -0·21)	-0·42 (-1·00; 0·16)	-0·42 (-1·00; 0·16)
	AGA	-0·32 (-0·48; -0·15)	-0·28 (-0·45; -0·11)	-0·36 (-0·54; -0·18)	-0·29 (-0·47; -0·10)	-0·22 (-0·39; -0·04)	-0·19 (-0·37; -0·01)	-0·18 (-0·38; 0·02)	-0·16 (-0·37; 0·04)
12 m	SGA	-0·90 (-1·38; -0·41)	-0·88 (-1·36; -0·39)	-1·03 (-1·56; -0·50)	-1·00 (-1·53; -0·47)	-0·48 (-1·01; 0·04)	-0·54 (-1·07; -0·01)	-0·47 (-1·06; 0·12)	-0·50 (-1·09; 0·09)
	AGA	-0·32 (-0·48; -0·15)	-0·29 (-0·46; -0·12)	-0·34 (-0·52; -0·16)	-0·27 (-0·46; -0·09)	-0·27 (-0·44; -0·09)	-0·24 (-0·42; -0·05)	-0·16 (-0·36; 0·04)	-0·16 (-0·36; 0·05)

Abbreviations: CI, confidence interval; HEU, HIV-exposed uninfected children; HU, HIV-unexposed children; SGA, small-for-gestational age (birth weight < 10th centile for gestational age based on Intergrowth-21st reference standards); AGA, appropriate for gestational age; w, weeks; m, months; [1] WAZ, LAZ and HCAZ-scores obtained from Intergrowth-21st (birth data adjusted for gestational age; postnatal data for preterm infants adjusted until 64 weeks postmenstrual age) and World Health Organization growth reference standards (postnatal growth for term infants, and for preterm infants after 64 weeks postmenstrual age); [2] WLZ-scores obtained from World Health Organization growth reference standards (using gestation-adjusted age for preterm infants until 64 weeks' postmenstrual age); [3] Interaction for size at birth (SGA vs· AGA) and HIV exposure status (HEU vs HU) only: in crude model, WAZ, p=0·24; LAZ, p=0·04; HCAZ, p=0·24; WLZ, p=0·91); in adjusted model, WAZ, p=0·28; LAZ, p=0·03; HCAZ, p=0·13; WLZ, p=0·71; [4] Three-way interaction between size at birth (SGA vs· AGA), HIV exposure status (HEU vs HU) and study visit: in crude model, WAZ, p<0·0001·; LAZ, p<0·0001; HCAZ, p=0·05; WLZ, p=0·0002; in adjusted model, WAZ, p<0·0001; LAZ, p=0·08; HCAZ, p=0·03; WLZ, p=0·02; [5] Adjusted for socio-economic factors (marital status, lack of amenities, household crowding), maternal alcohol use, intimate partner violence, recent history of any childhood illness (maternal report of diarrhoea and/or cough in preceding two weeks), and infant feeding (current milk feeding and weaning practices)

Supplemental Table 9.4.5

Sensitivity analysis: differences in growth measures comparing HIV-exposed uninfected and HIV-unexposed children over time, sensitivity analyses: results from multivariable mixed-effects linear regression models for full cohort and within restricted subgroups

	Full cohort analysis	**Restricted analyses**			
	Full cohort[1] **(N=873, 53% HEU)** **aβ (95% CI)**	**Complete case[2]** **(N=686, 51% HEU)** **aβ (95% CI)**	**Term, AGA[3]** **(N=697, 51% HEU)** **aβ (95% CI)**	**No risky drinking[4]** **(N= 723, 47% HEU)** **aβ (95% CI)**	**Similar feeding patterns[5]** **(N=402, 47% HEU)** **aβ (95% CI)**
HEU vs HU children: weight-for-age Z-scores					
Birth	-0·14 (-0·30; 0·02)	-0·08 (-0·26; 0·10)	-0·18 (-0·35; 0·00)	-0·14 (-0·31; 0·04)	-0·24 (-0·48; -0·01)
± 6 weeks	-0·23 (-0·30; -0·07)	-0·20 (-0·38; -0·02)	-0·32 (-0·49; -0·14)	-0·21 (-0·39; -0·04)	-0·20 (-0·43; 0·03)
± 3 months	-0·30 (-0·46; -0·14)	-0·29 (-0·46; -0·11)	-0·32 (-0·49; -0·14)	-0·27 (-0·45; -0·10)	-0·26 (-0·49; -0·03)
± 6 months	-0·29 (-0·45; -0·13)	-0·29 (-0·46; -0·12)	-0·26 (-0·44; -0·08)	-0·29 (-0·46; -0·12)	-0·36 (-0·59; -0·13)
± 9 months	-0·33 (-0·49; -0·16)	-0·30 (-0·47; -0·13)	-0·26 (-0·44; -0·08)	-0·33 (-0·50; -0·15)	-0·35 (-0·58; -0·12)
± 12 months	-0·34 (-0·50; -0·18)	-0·31 (-0·48; -0·14)	-0·26 (-0·44; -0·08)	-0·36 (-0·54; -0·18)	-0·43 (-0·66; -0·20)
HEU vs HU children: length-for-age Z-scores					
± 6 weeks	0·11 (-0·06; 0·29)	0·13 (-0·06; 0·32)	0·13 (-0·06; 0·32)	0·12 (-0·07; 0·31)	0·09 (-0·15; 0·34)
± 3 months	0·07 (-0·10; 0·25)	0·04 (-0·14; 0·23)	0·12 (-0·07; 0·32)	0·12 (-0·07; 0·31)	0·11 (-0·13; 0·35)
± 6 months	-0·14 (-0·32; 0·03)	-0·13 (-0·32; 0·05)	-0·09 (-0·28; 0·10)	-0·12 (-0·31; 0·07)	-0·13 (-0·37; 0·11)
± 9 months	-0·34 (-0·52; -0·17)	-0·33 (-0·52; -0·15)	-0·24 (-0·44; -0·05)	-0·31 (-0·50; -0·12)	-0·43 (-0·67; -0·19)
± 12 months	-0·34 (-0·52; -0·16)	-0·30 (-0·49; -0·12)	-0·24 (-0·43; -0·04)	-0·32 (-0·51; -0·13)	-0·36 (-0·60; -0·12)
HEU vs· HU children: head circumference-for-age Z-scores					
± 6 weeks	-0·33 (-0·50; -0·16)	-0·32 (-0·51; -0·13)	-0·41 (-0·60; -0·23)	-0·36 (-0·55; -0·18)	-0·26 (-0·50; -0·02)
± 3 months	-0·27 (-0·44; -0·10)	-0·29 (-0·47; -0·10)	-0·32 (-0·51; -0·14)	-0·26 (-0·45; -0·08)	-0·22 (-0·46; 0·01)
± 6 months	-0·11 (-0·28; 0·06)	-0·17 (-0·35; 0·01)	-0·10 (-0·29; 0·08)	-0·10 (-0·28; 0·09)	-0·15 (-0·38; 0·08)
± 9 months	-0·24 (-0·42; -0·07)	-0·26 (-0·44; -0·08)	-0·21 (-0·40; -0·02)	-0·26 (-0·45; -0·07)	-0·23 (-0·47; 0·00)
± 12 months	-0·26 (-0·43; -0·0)	-0·29 (-0·47; -0·10)	-0·26 (-0·45; -0·07)	-0·29 (-0·47; -0·10)	-0·27 (-0·50; -0·03)

	Full cohort analysis	**Restricted analyses**			
	Full cohort[1]	**Complete case[2]**	**Term, AGA[3]**	**No risky drinking[4]**	**Similar feeding patterns[5]**
	(N=873, 53% HEU)	**(N=686, 51% HEU)**	**(N=697, 51% HEU)**	**(N= 723, 47% HEU)**	**(N=402, 47% HEU)**
	aβ (95% CI)	**aβ (95% CI)**	**aβ (95% CI)**	**aβ (95% CI)**	**aβ (95% CI)**
HEU vs HU children: weight-for-length Z-scores					
± 6 weeks	-0·51 (-0·70; -0·32)	-0·50 (-0·71; -0·29)	-0·60 (-0·82; -0·39)	-0·52 (-0·73; -0·31)	-0·44 (-0·72; -0·16)
± 3 months	-0·40 (-0·59; -0·21)	-0·38 (-0·59; -0·18)	-0·50 (-0·72; -0·29)	-0·43 (-0·64; -0·22)	-0·42 (-0·70; -0·15)
± 6 months	-0·26 (-0·45; -0·07)	-0·29 (-0·49; -0·09)	-0·27 (-0·48; -0·05)	-0·27 (-0·48; -0·07)	-0·39 (-0·66; -0·12)
± 9 months	-0·19 (-0·38; 0·01)	-0·19 (-0·39; 0·02)	-0·18 (-0·40; 0·04)	-0·20 (-0·41; 0·01)	-0·20 (-0·47; 0·08)
± 12 months	-0·20 (-0·39; 0·00)	-0·20 (-0·40; 0·00)	-0·16 (-0·38; 0·06)	-0·20 (-0·41; 0·01)	-0·31 (-0·59; -0·03)
HEU vs· HU children: body-mass-index for-age Z-scores					
± 6 weeks	-0·53 (-0·72; -0·35)	-0·52 (-0·72; -0·32)	-0·61 (-0·82; -0·40)	-0·51 (-0·71; -0·30)	-0·51 (-0·77; -0·23)
± 3 months	-0·46 (-0·64; -0·27)	-0·44 (-0·64; -0·24)	-0·53 (-0·74; -0·32)	-0·46 (-0·66; -0·26)	-0·48 (-0·75; -0·22)
± 6 months	-0·26 (-0·45; -0·08)	-0·29 (-0·49; -0·10)	-0·26 (-0·47; -0·05)	-0·28 (-0·48; -0·08)	-0·40 (-0·66; -0·14)
± 9 months	-0·16 (-0·35; 0·03)	-0·16 (-0·36; 0·04)	-0·16 (-0·38; 0·05)	-0·19 (-0·40; 0·01)	-0·17 (-0·43; 0·10)
± 12 months	-0·13 (-0·31; 0·06)	-0·13 (-0·33; 0·06)	-0·10 (-0·31; 0·11)	-0·15 (-0·35; 0·06)	-0·23 (-0·49; 0·04)

Abbreviations: β, regression coefficient; CI, confidence interval; aβ, adjusted regression coefficient from mixed effect linear regression with random intercept for child and adjusted for time (approximate age at study visit), socio-economic factors (marital status, lack of amenities, household crowding), maternal alcohol use, intimate partner violence, recent history of any childhood illness (maternal report of diarrhoea and/or cough in preceding two weeks), and infant feeding (current milk feeding and weaning practice AGA, appropriate-for-gestational age (birth weight percentile ≥ 10th)

WAZ, LAZ and HCAZ-scores obtained from Intergrowth-21st (birth data adjusted for gestational age; postnatal data for preterm infants adjusted until 64 weeks postmenstrual age) and World Health Organization growth reference standards (postnatal growth for term infants, and for preterm infants after 64 weeks postmenstrual age)
WLZ and BMIZ-scores obtained from World Health Organization growth reference standards (using gestation-adjusted age for preterm infants until 64 weeks' postmenstrual age)

[1] All available measures used
[2] Analysis restricted to children who completed at least 5 postnatal follow-up visits
[3] Analysis restricted to children born ≥ 37 weeks' gestational age, AGA
[4] Analysis restricted to children whose mothers without report of risky drinking (defined as Alcohol Use Disorder Test-Consumption (AUDIT-C) score ≤ 3 at first antenatal visit)
[5] Analysis restricted to children with at least 6 months of breastfeeding, with only breastmilk ± clear liquids/prescribed medicine until at least 3 months of age

Supplemental Table 9.4.6. Factors associated with growth indices (weight-for-age, length-for-age, head circumference-for-age, and weight-for-length Z-scores) among HIV-exposed uninfected children over time: results from crude mixed effects linear models

	Weight-for-age[1] β (95% CI)	Length-for-age[1] β (95% CI)	Head circumference-for-age[1] β (95% CI)	Weight-for-length[2] β (95% CI)
Maternal characteristics at ART initiation				
Completed (vs did not complete) secondary education	0.02 (-0.19; 0.22)	0.05 (-0.19; 0.29)	0.05 (-0.19; 0.28)	0.06 (-0.15; 0.28)
Unemployed (vs employed)	-0.16 (-0.34; 0.02)	-0.11 (-0.32; 0.11)	-0.02 (-0.22; 1.18)	-0.13 (-0.32; 0.06)
Single (vs married/co-habiting)	-0.22 (-0.40; -0.04)	-0.13 (-0.33; 0.08)	-0.15 (-0.36; 0.05)	-0.20 (-0.39; -0.01)
Household crowding (>10 people vs 10 or less)	-0.40 (-0.77; -0.03)	-0.35 (-0.78; 0.08)	-0.30 (-0.72; 0.12)	-0.38 (-0.77; 0.01)
Does not have all three of running water, flushing toilet and brick home (vs has all three)	-0.03 (-0.23; 0.17)	-0.04 (-0.27; 0.19)	-0.09 (-0.32; 0.13)	-0.08 (-0.29; 0.13)
Risky drinking reported at ART initiation (vs not)3	-0.33 (-0.53; -0.13)	-0.41 (-0.64; -0.18)	-0.16 (-0.39; 0.07)	-0.07 (-0.29; 0.14)
Intimate partner violence reported at ART initiation (vs not)4	-0.10 (-0.31; 0.11)	-0.13 (-0.39; 0.12)	-0.03 (-0.28; 0.21)	0.04 (-0.18; 0.27)
CD4 cell count (per 100 cells/uL increase)	0.03 (-0.01; 0.07)	0.04 (-0.01; 0.09)	0.02 (-0.02; 0.07)	0.02 (-0.02; 0.06)
Log_{10} HIV viral load	-0.11 (-0.21; -0.02)	-0.12 (-0.23; -0.01)	-0.10 (-0.20; 0.01)	-0.07 (-0.17; 0.03)
Body mass index (kg/m^2)	0.01 (0.00; 0.03)	0.00 (-0.02; 0.01)	0.00 (-0.01; 0.02)	0.02 (0.00; 0.03)
Birth, infant and postpartum characteristics				
Maternal log_{10} HIV viral load at time of delivery	-0.14 (-0.28; 0.00)	-0.13 (-0.30; 0.03)	-0.07 (-0.23; 0.08)	-0.16 (-0.31; -0.01)
Maternal HIV viral load ≥50 copies/mL at time of delivery (vs <50)	-0.26 (-0.47; -0.05)	-0.23 (-0.48; 0.01)	-0.23 (-0.47; 0.00)	-0.26 (-0.48; -0.04)
Male infant (vs female)	0.10 (-0.08; 0.28)	0.20 (0.00; 0.41)	0.00 (-0.20; 0.20)	-0.02 (-0.21; 0.16)
SGA (vs AGA)	-1.34 (-1.59; -1.09)	-1.23 (-1.53; -0.94)	-1.06 (-1.36; -0.77)	-0.48 (-0.77; -0.19)
Preterm birth (<37 weeks, vs term)	0.05 (-0.23; 0.32)	-0.11 (-0.42; 0.21)	0.13 (-0.18; 0.44)	-0.16 (-0.46; 0.13)

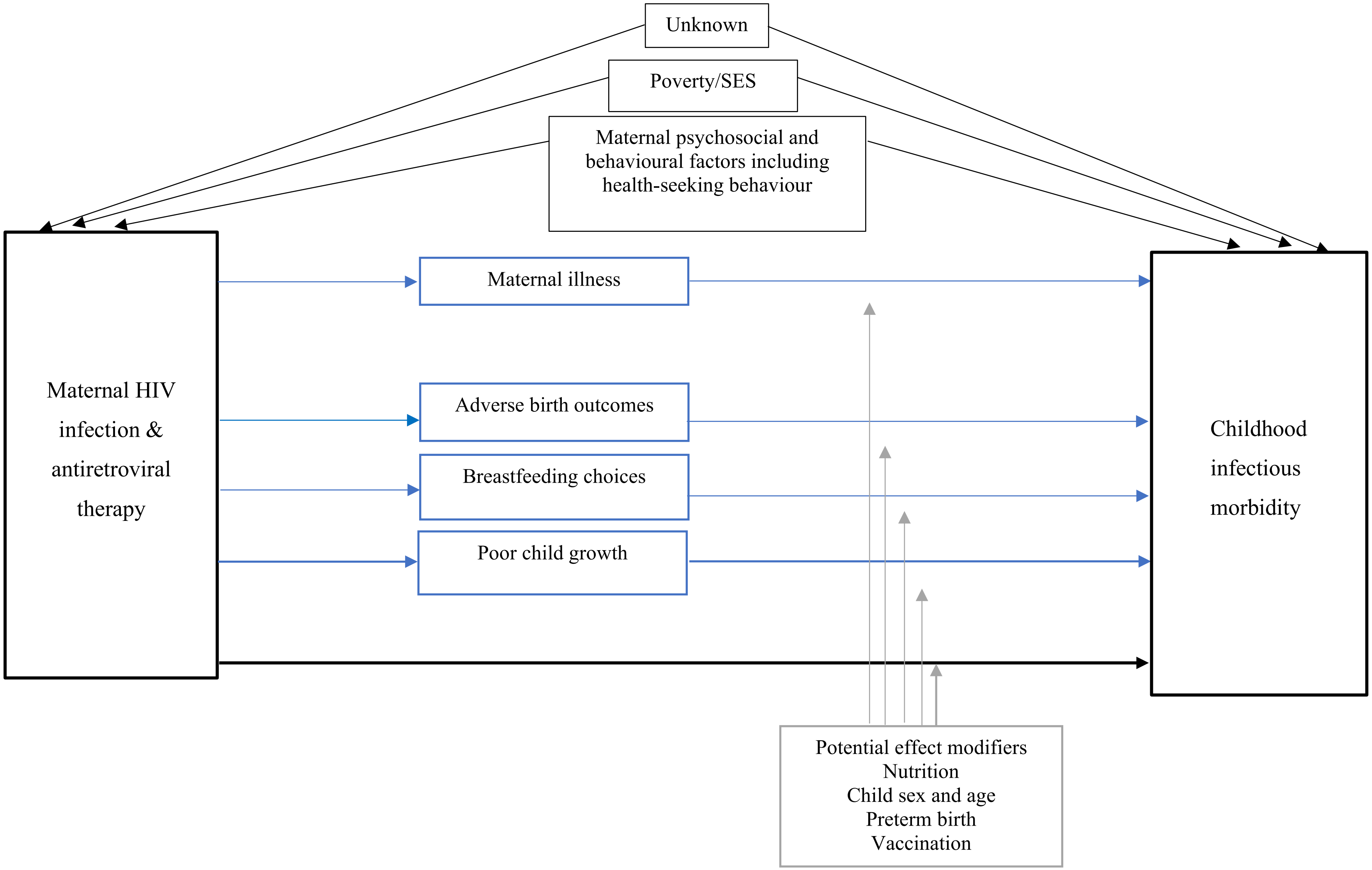
Supplemental Table 9.4.6. Factors associated with growth indices (weight-for-age, length-for-age, head circumference-for-age, and weight-for-length Z-scores) among HIV-exposed uninfected children over time: results from crude mixed effects linear models

	Weight-for-age[1] β (95% CI)	Length-for-age[1] β (95% CI)	Head circumference-for-age[1] β (95% CI)	Weight-for-length[2] β (95% CI)
Ever received exclusive breastfeeding (vs never)[5,6]	0.10 (-0.21; 0.42)	0.03 (-0.34; 0.41)	-0.09 (-0.45; 0.28)	0.13 (-0.21; 0.48)
Breastfeeding duration at least 6 months (vs < 6 months)[5]	-0.05 (-0.23; 0.13)	-0.02 (-0.22; 0.18)	0.00 (-0.20; 0.20)	-0.01 (-0.20; 0.18)
Formula feeding at time of study visit (vs not)[5]	0.23 (0.15; 0.31)	0.00 (-0.11; 0.10)	0.18 (0.08; 0.27)	0.21 (0.09; 0.33)
Early introduction to solid food (vs later)[7]	-0.17 (-0.46; 0.12)	-0.21 (-0.55; 0.12)	0.16 (-0.17; 0.49)	-0.16 (-0.47; 0.14)
Household food insecurity (at risk of/has vs does not have)[8]	-0.23 (-0.43; -0.04)	-0.06 (-0.28; 0.16)	-0.22 (-0.43; 0.00)	-0.29 (-0.49; -0.09)
Any cigarette smoking in the home (vs none/unknown)[9]	-0.14 (-0.30; 0.02)	-0.05 (-0.22; 0.11)	-0.09 (-0.24; 0.07)	-0.13 (-0.31; 0.06)
Recent childhood illness[10]	-0.01 (-0.08; 0.07)	-0.01 (-0.10; 0.08)	0.01 (-0.08; 0.09)	-0.01 (-0.12; 0.10)

Abbreviations: CI, confidence interval; SGA, small-for-gestational age (birth weight < 10th centile for gestational age based on Intergrowth-21st reference standards); AGA, appropriate for gestational age; w, weeks; m, months; [1] WAZ, LAZ and HCAZ-scores obtained from Intergrowth-21st (birth data adjusted for gestational age; postnatal data for preterm infants adjusted until 64 weeks postmenstrual age) and World Health Organization growth reference standards (postnatal growth for term infants, and for preterm infants after 64 weeks postmenstrual age); [2] WLZ-scores obtained from World Health Organization growth reference standards (using gestation-adjusted age for preterm infants until 64 weeks' postmenstrual age); [3] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3, first antenatal visit; [4] Any physical, sexual or psychological violence, World Health Organization violence against women questionnaire, first antenatal visit; [5] Maternal self-report, 24 hour recall; 6exclusive defined as nothing other than breastmilk and prescribed medicine; [7] Introduction of semi-solid or solid foods before 3 months of age; 8 **[8]** Based on questionnaire adapted from the Household Food Insecurity Access Scale (HFIAS), Food and Nutrition Technical Assistance Project (FANTA) and the Community Childhood Hunger Indentification Project Index (CCHIP), applied at 12 months ; [9] Maternal report at 6 weeks, 6 or 12 months' study visit: any household members smoking; [10] Maternal report of any diarrhoea or coughing illness in preceding 2 weeks

9.5 Supplemental material for Chapter 5 (Infectious morbidity)

Supplemental Figure 9.5.1. Simplified directed acyclic graph



Supplemental Table 9.5.1.

Characteristics of mothers and infants who completed vs. did not complete at least 9 months of follow-up

	HIV-infected women and HEU infants (N=459)			HIV-uninfected women and HU infants (N=410)		
	≥ 9 months (n=395)	< 9 months (n=64)	*p-value*	≥ 9 months (n=364)	< 9 months (n=46)	*p-value*
Maternal/household, first antenatal visit						
Age in years	28 (25-33)	26 (23-30)	0·011	28 (23-32)	26 (22-31)	0·20
Married/cohabiting	164 (41%)	24 (38%)	0·54	167 (46%)	17 (37%)	0·25
Completed secondary education	95 (24%)	18 (28%)	0·48	164 (45%)	20 (44%)	0·84
Employed	150 (38%)	32 (50%)	0·068	171 (47%)	23 (50%)	0·70
Formal housing	186 (47%)	32 (50%)	0·67	186 (51%)	28 (61%)	0·21
Household crowding (≥10)	24 (6%)	4 (6%)	0·96	12 (3%)	0	0·21
Poverty category[1]			0·53			0·73
Least disadvantaged	124 (31%)	21 (33%)		99 (27%)	15 (33%)	
Moderate disadvantage	133 (34%)	25 (39%)		158 (44%)	19 (39%)	
Most disadvantaged	138 (35%)	18 (28%)		107 (29%)	13 (28%)	
Risky drinking[2]	100 (25%)	16 (25%)	0·94	27 (7%)	3 (6%)	0·83
Intimate partner violence [3]	88 (22%)	13 (20%)	0·70	29 (8%)	3 (6%)	0·73
Depression[4]	38 (10%)	8 (12%)	0·49	24 (7%)	4 (9%)	0·59
Timing of HIV diagnosis			0·010			
Prior to current pregnancy	179 (45%)	18 (28%)		-	-	-
During current pregnancy	216 (54%)	46 (72%)		-	-	-
HIV viral load at ART initiation ($\log_{10}$ copies/mL)	4·0 (3·4-4·6)	3·8 (3·0-4·3)	0·87	-	-	-
CD4 cell count at ART initiation (cells/mm^3)[5]	350 (250-507)	415 (235-584)	0·19	-	-	-
CD4 cell count >350 at ART initiation	191 (50%)	40 (62%)	0·061	-	-	-
Gestational age at ART initiation (weeks)[6]	22 (17-27)	25 (19-31)	0·047	-	-	-
ART initiated < 24 weeks	247 (63%)	30 (47%)	0·014	-	-	-
Birth and infant characteristics						
Duration of ART use in pregnancy (weeks) [6]	17 (12-22)	14 (8-20)	0·028	-	-	-
Maternal HIV viral load <50 copies/mL at delivery	301 (76%)	50 (78%)	0·74	-	-	-
Gestational age (weeks)	39 (38; 40)	39 (37; 40)	0·45	39 (38-40)	39 (38-40)	0·71
Preterm (<37)	45 (11%)	10 (16%)	0·33	35 (10%)	3 (7%)	0·50

	HIV-infected women and HEU infants (N=459)			**HIV-uninfected women and HU infants (N=410)**		
	≥ 9 months (n=395)	**< 9 months (n=64)**	***p-value***	**≥ 9 months (n=364)**	**< 9 months (n=46)**	***p-value***
Weight at birth (kg)	3·12 (2·47-3·40)	3·18 (2·87-3·38)	0·36	3·21 (2·85-3·50)	3·28 (2·89-3·73)	0·10
Low birth weight (<2·5kg)	56 (14%)	5 (8%)	0·16	35 (10%)	1 (2%)	0·093
Weight-for-age Z-score at birth	-0·26 (-0·97; 0·36)	-0·11 (-0·64; 0·57)	0·025	-0·06 (-0·74; 0·62)	0·16 (-0·33; 0·88)	0·082
Small-for-gestational-age[7] (birthweight <10th centile)	49 (12%)	2 (3%)	0·028	34 (9%)	5 (11%)	0·74
Male sex	198 (50%)	32 (50%)	0·98	171 (47%)	25 (54%)	0·35
Ever exclusively breastfed (EBF)[8]	364 (92%)	55 (86%)	0·10	294 (81%)	38 (83%)	0·76
Duration of EBF (months)[8]	1·5 (0·2-5·5)	0·2 (0·1-1·4)	0·0001	1·4 (0·1-3·0)	0·2 (0·1-1·6)	0·083
Duration of any breastfeeding (months)	6·0 (1·4-12·0)	1·4 (0·2-2·9)	0·0001	10·1 (3·1-12·1)	3·0 (1·2-3·1)	0·0001
Postnatal characteristics						
Maternal depression at 6 weeks' postnatal visit[9]	14 (4%)	5 (10%)	0·035	9 (3%)	2 (6%)	0·27
Infant co-trimoxazole preventive therapy[10]	222 (69%)	15 (62%)	0·50	-	-	-
Any cigarette smoking in the home (maternal or others) [11]	174 (44%)	13 (33%)	0·19	103 (28%)	6 (17%)	0·16
Household use of biomass fuel or paraffin[11]	172 (44%)	8 (21%)	0·005	27 (7%)	1 (3%)	0·31

HEU, all HIV-exposed uninfected infants followed postnatally (n=2 infants excluded from further analysis due to underlying chronic disease); HU, all HIV-unexposed uninfected infants followed postnatally (n=1 child excluded from further analysis due to underlying chronic disease); SD, standard deviation; Results are n (column %) with *p*-value from chi^2 test; median (interquartile range, IQR) with *p*-value from Kruskal-Wallis
[1] Composite indicator based on standardized asset score and employment **[2]** Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 as reported at first antenatal visit (missing data, n=2); **[3]** Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit (missing data, n=4); **[4]** Maternal depression, EPDS (Edinburgh postnatal depression scale) score of ≥ 13 at first antenatal visit (missing data, n=2); **[5]** Missing data, n=12; **[6]** Missing data, n=3; **[7]** Birth weight percentile based on Intergrowth-21st reference standards; **[8]** Maternal report (24 hour recall); exclusive breastfeeding defined as only breastmilk and prescribed medicine, duration includes zero months for those who never exclusively breastfed; [9] Maternal depression, EPDS (Edinburgh postnatal depression scale) score of ≥ 13 at 6 weeks' postnatal visit (missing data, n=51); [10] Maternal report at 6 weeks' and/or 3 months' study visit: infant receipt of co-trimoxazole preventive therapy (missing data, n=114); [11] Maternal report at 6 weeks, 6 or 12 months' study visit: any household members smoking, biomass fuel defined as burning of wood or charcoal in the home (missing data, n=38)

Supplemental Table 9.5.2. Maternal and child characteristics by HIV infection and exposure status

	Total (N=869)	HIV-infected women and HEU infants (n=459)	HIV-uninfected women and HU infants (n=410)	*p-value*
Maternal/household characteristics at first antenatal visit				
Age in years	28 (24; 32)	28 (24; 32)	27 (23; 32)	0·11
Married/cohabiting	372 (43%)	188 (41%)	184 (45%)	0·24
Completed secondary education	297 (34%)	113 (25%)	184 (45%)	<0·0001
Employed	376 (43%)	182 (40%)	194 (47%)	0·023
Formal housing	432 (50%)	218 (47%)	214 (52%)	0·17
Flush toilet inside home	290 (33%)	125 (27%)	165 (40%)	<0·0001
Running water inside home	402 (46%)	188 (41%)	214 (52%)	0·001
Household crowding (≥10 people)	40 (5%)	28 (6%)	12 (3%)	0·026
Poverty category[1]				0·036
Least disadvantaged	259 (30%)	145 (32%)	114 (28%)	
Moderate disadvantage	334 (38%)	158 (34%)	176 (43%)	
Most disadvantaged	276 (32%)	156 (34%)	120 (29%)	
Risky drinking[2]	146 (17%)	116 (25%)	30 (7%)	<0·0001
Intimate partner violence [3]	133 (15%)	101 (22%)	32 (8%)	<0·0001
Depression[4]	74 (9%)	46 (10%)	28 (7%)	0·089
Timing of HIV diagnosis				-
Prior to current pregnancy	-	197 (43%)	-	
During current pregnancy	-	262 (57%)	-	
HIV viral load at ART initiation (log$_{10}$ copies/mL)	-	4·0 (3·3-4·5)	-	-
CD4 cell count at ART initiation (cells/mm^3)[5]	-	354 (248-528)	-	-
CD4 cell count >350 at ART initiation	-	231 (52%)	-	-
Gestational age at ART initiation (weeks)[6]	-	22 (17-28)	-	-
ART initiated < 24 weeks	-	277 (61%)	-	-
Birth and infant characteristics				
Duration of ART use in pregnancy (weeks) [6]	-	16·7 (11·1-21·7)	-	-
HIV viral load at time of delivery (log$_{10}$ copies/mL)	-	1·59 (1·59-1·64)	-	-
Maternal viral suppression achieved by time of delivery (HIV viral load <50 copies/mL)	-	108 (23%)	-	-

	Total **(N=869)**	**HIV-infected women and HEU infants (n=459)**	**HIV-uninfected women and HU infants (n=410)**	***p-value***
Place of delivery				0·016
Primary care	344 (39%)	181 (39%)	163 (40%)	
Hospital care	512 (59%)	266 (58%)	246 (60%)	
Born before arrival	13 (2%)	12 (3%)	1 (<1%)	
Gestational age at delivery (weeks)	39 (38-40)	39 (38-40)	39 (38-40)	0·42
Preterm (<37)	93 (11%)	55 (12%)	38 (9%)	0·20
Weight at birth (kg)	3·18 (2·82-3·46)	3·13 (2·76-3·40)	3·22 (2·86-3·51)	0·0003
Low birth weight (<2500g)	97 (11%)	61 (13%)	36 (9%)	0·035
Very low birth weight (<1500g)	9 (1%)	5 (1%)	4 (1%)	0·87
Weight-for-age Z-score at birth	-0·13 (-0·84; 0·50)	-0·21 (-0·94; 0·37)	-0·05 (-0·71; 0·64)	0·001
Small-for-gestational-age[7] (birthweight <10th centile)	90 (10%)	51 (11%)	39 (10%)	0·44
Male sex	426 (49%)	230 (50%)	196 (48%)	0·50
Ever exclusively breastfed (EBF)[8]	781 (86%)	419 (91%)	332 (81%)	<0·0001
Duration of EBF (months)[8]	1·4 (0·1-3·0)	1·4 (0·2-4·0)	1·3 (0·1-3·0)	0·0001
Duration of any breastfeeding (months)	6·0 (1·5-12·0)	3·9 (1·4-12·0)	9·0 (3·0-12·0)	0·0001
Cumulative infant feeding from birth to 6 months[8]				<0·0001
Exclusive breastfeeding	342 (39%)	205 (45%)	137 (33%)	
Predominant breastfeeding	136 (16%)	42 (9%)	94 (23%)	
Partial breastfeeding	391 (45%)	212 (46%)	179 (44%)	
Postnatal characteristics				
Maternal depression at 6 weeks' postnatal visit[9]	30 (4%)	19 (4%)	11 (3%)	0·24
Ever received co-trimoxazole preventive therapy[10]	-	237 (69%)	-	-
Any cigarette smoking in the home (maternal or others) [11]	296 (36%)	187 (43%)	109 (27%)	<0·0001
Household energy sources ever included biomass fuel or paraffin[11]	208 (25%)	180 (42%)	28 (7%)	<0·0001

HEU, all HIV-exposed uninfected infants followed postnatally (n=2 infants not included in further analysis); HU, all HIV-unexposed uninfected infants followed postnatally (n=1 child excluded from further analysis); CD, cluster of differentiation; SD, standard deviation; Results are n (column %) with *p*-value from chi^2 test; median (interquartile range, IQR) with *p*-value from Kruskal-Wallis

[1] Composite indicator based on standardized asset score and employment [2] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 as reported at first antenatal visit (missing data, n=2); [3] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit (missing data, n=4); [4] Maternal depression, EPDS (Edinburgh postnatal depression scale) score of ≥ 13 at first antenatal visit (missing data, n=2); [5] Missing data, n=12; [6] Missing data, n=3; [7] Birth weight percentile based on Intergrowth-21st reference standards; [8] Maternal report (24 hour recall); exclusive breastfeeding defined as only breastmilk and prescribed medicine; duration of EBF includes those who never exclusively breastfed as having zero months; [9] Maternal depression, EPDS (Edinburgh postnatal depression scale) score of ≥ 13 at 6 weeks' postnatal visit (missing data, n=51); [10] Maternal report at 6 weeks' and/or 3 months' study visit: infant receipt of co-trimoxazole preventive therapy (missing data, n=114); [11] Maternal report at 6 weeks, 6 or 12 months' study visit: any household members smoking, biomass fuel defined as burning of wood or charcoal in the home (missing data, n=38)

Supplemental Table 9.5.3. Time-varying child characteristics and self-reported child infectious illness in preceding two weeks, comparing HIV-exposed uninfected to HIV-unexposed infants at each study visit

Visit	Child illness and characteristics per study visit	All infants	HEU infants	HU infants	Difference in proportion of infants with illness (95% CI)	*p*-value
< 7 days	**Attended and contributed prevalence data, N**	**724**	**314**	**410**		
	Median child age in months (IQR)	0·16 (0·13; 0·26)	0·16 (0·13; 0·23)	0·20 (0·13; 0·30)	-	0·03
	Presumed lower respiratory tract infection[1], n (%)	1 (<1%)	1 (<1%)	0	0·00 (0·00; 0·01)	0·25
	Diarrhoeal illness[2], n (%)	13 (2%)	13 (4%)	0	0·04 (0·02; 0·06)	<0·0001
	Both presumed lower respiratory infection and diarrhoeal illness, n (%)	14 (2%)	14 (4%)	0	0·04 (0·02; 0·07)	<0·0001
	Breastfeeding[3], n (%)	724 (100%)	314 (100%)	410 (100%)	-	-
	Exclusive breastfeeding[3], n (% of breastfeeding)	614 (85%)	282 (90%)	332 (81%)	-	0·001
	Vaccination status known[4], n (%)	520 (72%)	227 (72%)	293 (71%)	-	0·81
	Complete and timely, n (% of known)	496 (95%)	222 (98%)	274 (93%)	-	0·02
6 weeks	**Attended and contributed prevalence data, N**	**733**	**345**	**388**		
	Median child age in months (IQR)	1·41 (1·38; 1·51)	1·38 (1·38; 1·41)	1·45 (1·38; 1·64)	-	0·0001
	Presumed lower respiratory tract infection[1], n (%)	22 (3%)	19 (6%)	3 (1%)	0·05 (0·02; 0·07)	0·0002
	Diarrhoeal illness[2], n (%)	29 (4%)	16 (5%)	13 (3%)	0·01 (-0·02; 0·04)	0·37
	Both presumed lower respiratory infection and diarrhoeal illness, n (%)	48 (6%)	32 (9%)	16 (4%)	0·05 (0·01; 0·09)	0·005
	Breastfeeding[3], n (%)	666 (91%)	299 (87%)	367 (95%)	-	<0·0001
	Exclusive breastfeeding[3], n (% of breastfeeding)	437 (66%)	238 (80%)	199 (54%)	-	<0·0001
	Vaccination status known[4], n (%)	558 (76%)	263 (76%)	295 (76%)	-	0·95
	Complete and timely, n (% of known)	478 (86%)	233 (89%)	245 (83%)	-	0·06
3 months	**Attended and contributed prevalence data, N**	**730**	**363**	**367**		

Visit	Child illness and characteristics per study visit	All infants	HEU infants	HU infants	Difference in proportion of infants with illness (95% CI)	*p*-value
	Median child age in months (IQR)	3·03 (2·99; 3·09)	2·99 (2·96; 3·06)	3·03 (2·99; 3·12)	-	0·0001
	Presumed lower respiratory tract infection[1], n (%)	27 (4%)	22 (6%)	5 (1%)	0·05 (0·02; 0·07)	0·001
	Diarrhoeal illness[2], n (%)	32 (4%)	27 (7%)	5 (1%)	0·06 (0·03; 0·09)	0·06
	Both presumed lower respiratory infection and diarrhoeal illness, n (%)	57 (8%)	47 (13%)	10 (3%)	0·10 (0·06; 0·14)	<0·0001
	Breastfeeding[3], n (%)	575 (79%)	254 (70%)	321 (87%)	-	<0·0001
	Exclusive breastfeeding[3], n (% of breastfeeding)	352 (61%)	204 (80%)	148 (46%)	-	<0·0001
	Vaccination status known[4], n (%)	575 (79%)	289 (80%)	286 (78%)	-	0·58
	Complete and timely, n (% of known)	363 (63%)	200 (69%)	163 (57%)	-	0·002
6 months	**Attended and contributed prevalence data, N**	**732**	**387**	**345**		
	Median child age in months (IQR)	6·05 (5·99; 6·15)	6·02 (5·95; 6·09)	6·05 (6·02; 6·18)	-	0·0001
	Presumed lower respiratory tract infection[1], n (%)	37 (5%)	26 (7%)	11 (3%)	0·04 (0·00; 0·07)	0·03
	Diarrhoeal illness[2], n (%)	92 (13%)	57 (15%)	35 (10%)	0·05 (0·00; 0·09)	0·06
	Both presumed lower respiratory infection and diarrhoeal illness, n (%)	117 (16%)	75 (19%)	42 (12%)	0·07 (0·02; 0·12)	0·008
	Breastfeeding[3], n (%)	459 (63%)	220 (57%)	239 (69%)	-	0·001
	Exclusive breastfeeding[3], n (% of breastfeeding)	155 (34%)	119 (54%)	36 (15%)	-	<0·0001
	Vaccination status known[4], n (%)	597 (82%)	320 (83%)	277 (80%)	-	0·40
	Complete and timely, n (% of known)	377 (63%)	215 (67%)	162 (58%)	-	0·03
9 months	**Attended and contributed prevalence data, N**	**694**	**365**	**329**		
	Median child age in months (IQR)	9·05 (8·98; 9·14)	9·01 (8·98; 9·08)	9·05 (9·01; 9·21)	-	0·0001
	Presumed lower respiratory tract infection[1], n (%)	48 (7%)	34 (9%)	14 (4%)	0·05 (0·01; 0·09)	0·009
	Diarrhoeal illness[2], n (%)	103 (15%)	50 (14%)	53 (16%)	-0·02 (-0·08; 0·03)	0·37

Visit	Child illness and characteristics per study visit	All infants	HEU infants	HU infants	Difference in proportion of infants with illness (95% CI)	*p*-value
	Both presumed lower respiratory infection and diarrhoeal illness, n (%)	141 (20%)	78 (21%)	63 (19%)	0·02 (-0·04; 0·08)	0·47
	Breastfeeding[3], n (%)	381 (55%)	174 (48%)	207 (63%)	-	<0·0001
	Vaccination status known[4], n (%)	587 (85%)	313 (86%)	274 (83%)	-	0·37
	Complete and timely, n (% of known)	364 (62%)	212 (68%)	152 (55%)	-	0·002
12 months	**Attended and contributed prevalence data, N**	**701**	**355**	**346**		
	Median child age in months (IQR)	12·04 (12·01; 12·27)	12·01 (11·97; 12·14)	12·11 (12·04; 12·43)	-	0·0001
	Presumed lower respiratory tract infection[1], n (%)	30 (4%)	23 (6%)	7 (2%)	0·04 (0·02; 0·07)	0·004
	Diarrhoeal illness[2], n (%)	106 (15%)	43 (12%)	63 (18%)	-0·06 (-0·11; -0·01)	0·02
	Both presumed lower respiratory infection and diarrhoeal illness, n (%)	127 (18%)	60 (17%)	67 (19%)	-0·02 (-0·08; 0·03)	0·40
	Breastfeeding[3], n (%)	318 (45%)	144 (41%)	174 (50%)	-	0·01
	Vaccination status known[4], n (%)	614 (88%)	328 (92%)	286 (83%)	-	<0·0001
	Complete and timely, n (% of known)	278 (45%)	171 (52%)	107 (37%)	-	<0·0001

Abbreviations: HEU, HIV-exposed uninfected; HU, HIV-unexposed uninfected; IQR, interquartile range; CI, confidence interval; n/a, not applicable
Values are n (column %); p-values from Kruskal-Wallis (continuous) or Chi2 (categorical) except where absolute differences are reported with 95% confidence intervals (two-sample test for equality of proportions); no correction for multiplicity
[1] Maternal report of illness with a cough, high temperature and difficulty in breathing; [2] Maternal report of any diarrhoeal events; [3] 24-hour maternal recall; exclusivity defined as only breastmilk and prescribed medicine; [4] based on data abstracted from Road to Health Booklet, delayed vaccination defined as any EPI (extended programme of immunization for South Africa) recommended immunization given > 2 weeks after recommended age

Supplemental Table 9.5.4. Causes and characteristics of child deaths in first 12 months of life

	HIV-exposure status	Sex	Gestation at delivery	Maternal CD4 cell count at ART initiation	Summary feeding in first 6 months of life or until date of death	Age at death (months)	Cause of death	Place of death
1	Unexposed	Female	38	-	EBF	1·3	Unknown	Home
2	Unexposed	Male	38	-	EBF	1·4	Unclassified sudden unexpected death[1]	Home
3	Unexposed	Male	38	-	EBF	2·0	Unclassified sudden unexpected death[1]	Home
4	Unexposed	Female	39	-	Partial BF	3·4	Unclassified sudden unexpected death[1]	Home
5	Unexposed	Female	37	-	EBF	8·9	Unknown	Primary care facility
6	Unexposed	Male	31	-	EBF	11·4	Multi-drug resistant tuberculosis	Unknown
7	HEU	Female	32	948	EBF	0·9	Unclassified sudden unexpected death[1]	Home
8	HEU	Male	37	516	EBF	1·0	Bacterial sepsis	Tertiary hospital
9	HEU	Female	32	173	EBF	1·5	Unclassified sudden unexpected death[1]	Home
10	HEU	Male	41	531	EBF	2·0	Severe, inoperable congenital heart defect	Home
11	HEU	Male	39	145	Partial BF	4·8	Diarrhoea	En route to care
12	HEU	Male	40	633	EBF	6·1	Presumed aspiration	Child care centre
13	HEU	Female	39	264	EBF	10·4	Diarrhoea	En route to care
14	HEU	Female	38	337	Partial BF	12·0	Accidental poisoning at home	Primary care facility

Abbreviations: HEU, HIV-exposed uninfected; EBF, exclusive breastfeeding; BF, breastfeeding

[1] Sudden unexpected death in an infant (SUDI), defined as unexpected and unexplained death in child aged< 12 months of age but allocated to "natural causes" on abridged death certificate, without clear evidence of extensive death scene investigations; to be differentiated from SIDS (Sudden infant death syndrome)

Mortality rate overall: 2·51 per child-year (cumulative mortality 14/872, 1·6%); HEU, 2·48 per child-year (cumulative 8/461, 1·7%); HIV-unexposed, 2·54 per child-year (cumulative 6/411, 1·5%); Mortality incidence rate ratio comparing HEU to HU: 0·98 (95% confidence interval 0·30-3·41)

Supplemental Table 9.5.5. Relative odds of severe (intensive care admission and/or in-hospital death), prolonged (> 5 days) or extended (>14 days) hospitalization comparing HIV-exposed uninfected to HIV-unexposed infants in the first year of life: crude and adjusted logistic regression analyses

	Severe hospitalization: [1] Death and/or intensive care	**Prolonged duration of hospitalization[1] (> 5 days)**	**Extensive duration of hospitalization[1] (> 14 days)**	**Any severe or prolonged (> 5 days) hospitalization[1]**
Cumulative risk	**Total 16/475 (3%)** **HEU 12/261 (5%)** **HU 4/214 (2%)**	**Total 94/475 (20%)** **HEU 56/261 (21%)** **HU 38/214 (18%)**	**Total 23/475 (5%)** **HEU 17/261 (6%)** **HU 6/214 (3%)**	**Total 101/475 (21%)** **HEU 63/261 (24%)** **HU 38/214 (18%)**
Crude OR[2] (95% CI)				
HIV exposure (HEU vs HU)	2·53 (0·81-7·95)	1·27 (0·75-2·12)	2·42 (0·85-6·87)	1·47 (0·88-2·47)
Infectious vs non-infectious primary diagnosis	2·12 (0·78-5·76)	2·14 (1·32-3·47)	1·11 (0·50-2·47)	2·26 (1·40-3·64)
Age at admission (months)	0·93 (0·84-1·02)	1·02 (0·98-1·07)	1·01 (0·92-1·10)	1·02 (0·98-1·06)
Maternal education[4]	0·62 (0·20-1·93)	0·85 (0·51-1·43)	0·51 (0·16-1·61)	0·79 (0·47-1·33)
Household crowding[5]	5·07 (1·60-16·02)	2·27 (0·92-5·62)	0·89 (0·12-6·53)	2·06 (0·83-5·09)
Poverty category[6]				
Most disadvantaged	1·00	1·00	1·00	1·00
Moderate disadvantage	0·89 (0·28-2·80)	1·18 (0·63-2·21)	1·04 (0·32-3·39)	1·07 (0·60-1·93)
Least disadvantaged	0·71 (0·20-2·57)	1·20 (0·63-2·31)	1·87 (0·59-5·86)	1·06 (0·56-2·00)
Cigarette smoking in home[7]	1·25 (0·44-3·54)	1·68 (1·02-2·76)	3·74 (1·45-9·63)	1·63 (1·00-2·67)
Biomass fuel or paraffin use in home[8]	0·41 (0·09-1·82)	1·19 (0·67-2·08)	0·95 (0·34-2·66)	1·21 (0·70-2·08)
Intimate partner violence, antenatal[9]	2·86 (0·99-8·27)	1·30 (0·70-2·41)	1·72 (0·54-5·46)	1·68 (0·91-3·10)
Risky drinking, antenatal[10]	2·76 (0·94-8·11)	1·37 (0·76-2·46)	1·65 (0·57-4·77)	1·74 (1·00-3·04)
Postpartum depression[11]	1·51 (0·20-11·25)	0·58 (0·19-1·73)	Unable to estimate	0·54 (0·18-1·62)
Preterm <37 weeks	1·20 (0·35-4·16)	7·41 (4·18-13·14)	18·51 (6·88-49·78)	6·89 (3·84-12·38)
Weight-for-age Z-score at birth[12]	1·03 (0·71-1·49)	0·82 (0·66-1·02)	0·91(0·67-1·24)	0·83 (0·67-1·02)
Male vs female	3·82 (1·22-11·91)	1·28 (0·78-2·09)	1·62 (0·65-4·05)	1·46 (0·90-2·37)
Ever exclusively breastfed (EBF)[13]	0·97 (0·23-4·15)	0·55 (0·28-1·08)	0·48 (0·15-1·54)	0·55 (0·27-1·12)
Duration of EBF (months)[13]	0·97 (0·75-1·25)	1·01(0·90-1·14)	1·04 (0·84-1·28)	1·01 (0·90-1·13)

	Severe hospitalization: [1] Death and/or intensive care	**Prolonged duration of hospitalization[1] (> 5 days)**	**Extensive duration of hospitalization[1] (> 14 days)**	**Any severe or prolonged (> 5 days) hospitalization[1]**
Duration of any breastfeeding (months)	0·98 (0·88-1·09)	1·01 (0·90-1·01)	0·96 (0·88-1·05)	0·97 (0·92-1·01)
WAZ <-2 in previous age interval[14]	2·12 (0·49-9·14)	2·39 (1·10-5·21)	2·27 (0·69-7·44)	2·16 (0·99-4·72)
Vaccination status, per age interval[15]				
Complete and timely	1·00	1·00	1·00	1·00
Delayed or incomplete	0·62 (0·08-4·80)	2·10 (1·03-4·30)	1·83 (0·67-4·96)	2·11 (1·03-4·33)
Data not available	3·15 (0·87-11·43)	2·06 (0·96-4·41)	1·42 (0·32-6·26)	2·17 (1·03-4·56)
Adjusted OR (95% CI)[16]				
HEU vs HU infants	2·24 (0·74-1·03)	1·07 (0·64-1·78)	2·21 (0·70-6·98)	1·38 (0·83-2·30)

11.

Abbreviations: OR, odds ratio; CI, confidence interval; HEU, HIV-exposed uninfected infants; HU, HIV-unexposed infants;
[1] Admission defined as 2 or more days; [2] Odds ratios from logistic regression analysis with variance adjusted for within-child correlation ; [4] Completed vs did not complete secondary education; [5] ≥10 people living in home, vs <10; [6] Categories based on poverty "score" (combination of standardized asset score and employment); [7] Maternal report of own or other household members smoking, at 6 weeks', 6 and/or 12 months' study visits (vs never); [8] Maternal report of energy sources at 6 weeks', 6 and/or 12 months' study visits (vs. never); [9] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit (vs none); [10] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 vs <3, as reported at first antenatal visit; [11] Edinburgh postnatal depression score ≥13 vs <13 at 6 weeks' study visit; [12] Weight-for-gestational age Z-score for birthweight, based on Intergrowth-21st growth reference standards; [13] Based on 24 hour recall, maternal report; exclusive defined as only breastmilk with prescribed medicine; non-time-varying; [14] Time-varying: weight-for-age Z-score < 2 (underweight) at any point during the preceding age interval; [15] Time varying: based on Road to Health Booklet information; delayed vaccination defined as >2 weeks after due age; [16] Adjusted for child age at admission, household crowding, intimate partner violence, duration of breastfeeding and vaccination status (death/ICU and any severe or prolonged hospitalization), as well as preterm birth (prolonged hospitalization and extended hospitalization).

Interaction effects with infant sex: (1) severe hospitalization, HEU-sex: p=0·22; (2) prolonged hospitalization, HEU-sex p=0·40; (3) extensive duration of hospitalization, HEU-sex p=0·14; (4) combination severe hospitalization, HEU-sex p=0·22

Supplemental Table 9.5.6. Distribution of primary diagnoses for severe (intensive care and/or death), prolonged (> 5 days) or extended (> 14 days) hospitalization

CAUSES	ICU/death			Prolonged (>5 days)			Extended (>14 days)		
	Total (N=869)	HEU (N=459)	HU (N=410)	Total (N=869)	HEU (N=459)	HU (N=410)	Total (N=869)	HEU (N=459)	HU (N=410)
All cause admissions	**n=16**	**n=12**	**n=4**	**n=94**	**n=56**	**n=38**	**n=23**	**n=35**	**n=65**
Infectious causes[1]	**8 (50%)**	**6 (50%)**	**2 (50%)**	**44 (47%)**	**28 (50%)**	**16 (42%)**	**8 (35%)**	**6 (35%)**	**2 (33%)**
Neonatal infections[2]	2 (25%)	1 (17%)	1 (50%)	12 (27%)	6 (21%)	6 (38%)	2 (25%)	2 (33%)	0
Sepsis[2]	2 (25%)	2 (33%)	0	2 (5%)	2 (7%)	0	0	0	0
Meningitis[2]	1 (12%)	1 (17%)	0	2 (5%)	1 (4%)	1 (6%)	1 (13%)	0	1 (50%)
Diarrhoea[2]	2 (25%)	1 (17%)	1 (50%)	11 (25%)	9 (32%)	2 (12%)	3 (38%)	3 (50%)	0
Lower respiratory tract infections[2]	0	0	0	12 (27%)	7 (25%)	5 (31%)	2 (25%)	1 (17%)	1 (50%)
Pneumonia[2]	*0*	*0*	*0*	*5 (11%)*	*2 (7%)*	*3 (19%)*	*1 (12%)*	*1 (17%)*	*0*
Tuberculosis[2]	*0*	*0*	*0*	*3 (7%)*	*2 (7%)*	*1 (6%)*	*1 (12%)*	*0*	*1 (50%)*
Bronchiolitis[2]	*0*	*0*	*0*	*4 (9%)*	*3 (11%)*	*1 (6%)*	*0*	*0*	*0*
Other infections[2]	1 (13%)	1 (17%)	0	5 (11%)	3 (11%)	2 (13%)	0	0	0
Non-infectious causes[1]	**8 (50%)**	**6 (50%)**	**2 (50%)**	**50 (53%)**	**28 (50%)**	**22 (58%)**	**15 (65%)**	**11 (65%)**	**4 (67%)**
Neonatal, not infection[3]	6 (75%)	4 (67%)	2 (100%)	28 (56%)	19 (68%)	9 (41%)	11 (73%)	8 (73%)	3 (75%)
Burns[3]	0	0	0	2 (4%)	2 (7%)	0	0	0	0
Other trauma[3]	0	0	0	1 (2%)	1 (4%)	0	1 (7%)	1 (9%)	0
Surgical[3]	0	0	0	0	0	0	0	0	0
Neurological[3]	0	0	0	7 (14%)	2 (7%)	5 (23%)	2 (13%)	1 (9%)	1 (25%)
Other[3]	2 (25%)	2 (33%)	0	12 (24%)	4 (14%)	8 (36%)	1 (7%)	1 (9%)	0

Values are n (column %); some infants have multiple admissions per age interval; abbreviations: HEU, HIV-exposed uninfected; HUU, HIV-unexposed uninfected; [1] Denominator is total number of admissions; pneumonia, tuberculosis and bronchiolitis are subsets of and included in the totals for lower respiratory tract infections [2] Denominator is number of infectious-cause admissions; [3]Denominator is number of non-infectious-cause admissions

Supplemental Table 9.5.7. Predictors and incidence of infectious-cause hospitalization comparing HIV-exposed uninfected to HIV-unexposed infants over time: crude and adjusted incidence rate ratios from Poisson regression analysis

	Crude IRR (95% CI)[1]	Adjusted IRR (95% CI)[1] Model A[2]	Adjusted IRR (95% CI)[1] Model B[2]
HIV-exposure (HEU vs HU)	1·40 (0·97-2·02)	1·30 (0·88-1·90)	1·40 (0·96-2·03)
Age interval			
0-7 days	1·00	1·00	1·00
7 days – 3 months	0·23 (0·13-4·1)	0·23 (0·13-0·41)	0·23 (0·13-0·41)
3-6 months	0·19 (0·11-0·35)	0·18 (0·10-0·34)	0·17 (0·09-0·32)
6-9 months	0·10 (0·05-0·19)	0·09 (0·05-0·18)	0·09 (0·04-0·17)
9-12 months	0·13 (0·07-0·24)	0·12 (0·06-0·24)	0·11 (0·05-0·22)
>12 months	0·13 (0·06-0·25)	0·10 (0·05-0·22)	0·09 (0·04-0·20)
Maternal education[4]	0·84 (0·57-1·24)	-	-
Household crowding[5]	1·34 (0·77-2·34)	-	-
Poverty category[6]			
Most disadvantaged	1·00	1·00	1·00
Moderate disadvantage	0·78 (0·52-1·17)	0·74 (0·49-1·12)	0·79 (0·52-1·19)
Least disadvantaged	1·03 (0·67-1·60)	0·97 (0·63-1·49)	1·02 (0·66-1·56)
Cigarette smoking in home[7]	0·78 (0·54-1·12)	-	-
Biomass fuel or paraffin use in home[8]	1·12 (0·78-1·61)	-	-
Intimate partner violence, antenatal[9]	0·96 (0·61-1·52)	-	-
Risky drinking, antenatal[10]	0·82 (0·52-1·30)	-	-
Postpartum depression[11]	1·58 (0·72-3·44)	1·46 (0·69-3·10)	1·44 (0·66-3·13)
Preterm <37 weeks	1·38 (0·85-2·26)	1·40 (0·86-2·27)	-
Small-for-gestational age[12]	1·39 (0·78-2·48)	-	-
Male vs female	0·68 (0·48-0·96)	-	-
Ever exclusively breastfed (EBF)[13]	0·79 (0·49-1·28)	0·81 (0·51-1·30)	-
Duration of EBF (months)[13]	0·95 (0·87-1·03)	-	-
Duration of any breastfeeding (months)	0·95 (0·92-0·98)	0·95 (0·92-0·98)	-
Breastfed for ≥ 6 vs. < 6 months	0·59 (0·42-0·84)	-	-
WAZ <-2 in previous age interval[14]	2·39 (1·34-4·28)	2·04 (1·17-3·58)	-
Vaccination status, per age interval[15]			
Complete and timely	1·00	1·00	1·00
Delayed or incomplete	0·94 (0·61-1·47)	1·37 (0·82-2·28)	1·50 (0·88-2·54)
Data not available	0·67 (0·43-1·05)	0·75 (0·48-1·18)	0·86 (0·54-1·35)

Abbreviations: IRR, incidence rate ratios; CI, confidence interval; HEU, HIV-exposed uninfected infants; HU, HIV-unexposed infants; [1] Admission defined as 2 or more days; estimates from Poisson regression with robust variance estimates, clustered on mother-child pairs; [2] Model A includes potential confounders and mediators of the HIV-exposure – hospitalization relationship; Model B only includes confounders; [3] Based on maternal CD4 cell count at ART initiation during pregnancy; [4] Completed vs did not complete secondary education; [5] ≥10 people living in home, vs <10; [6] Categories based on poverty "score" (combination of standardized asset score and employment); [7] Maternal report of own or other household members smoking, at 6 weeks', 6 and/or 12 months' study visits (vs never); [8] Maternal report of energy sources at 6 weeks', 6 and/or 12 months' study visits (vs. never); [9] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit (vs none); [10] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 vs <3, as reported at first antenatal visit; [11] Edinburgh postnatal depression score ≥13 vs <13 at 6 weeks' study visit; [12] Birthweight for gestational age, percentile <10th, based on Intergrowth-21st growth reference standards (vs appropriate for gestational age) [13] Based on 24 hour recall, maternal report; exclusive defined as only breastmilk with prescribed medicine; non-time-varying; [14] Time-varying: weight-for-age Z-score < 2 (underweight) at any point during the preceding age interval; [15] Time varying: based on Road to Health Booklet information; delayed vaccination defined as >2 weeks after due age;

Effect measure modification tests (interaction terms using adjusted model A): HEU status and age interval, p=0·14; HEU status and infant sex, p=0·54; HEU status and breastfeeding duration, p=0·08; HEU status and vaccination status, p=0·08; HEU status and preterm birth, p=0·73; HEU status and SGA, p=0·72; Age interval and vaccination status, p=0. 009

Supplemental Table 9.5.8. Sensitivity analysis: incidence rate ratios for infectious-cause hospitalization between 8 days and 3 months of age, comparing HIV-exposed to HIV-unexposed infants in restricted sub-groups

		RESTRICTED ANALYSES				
	Full cohort[1] (N=853)	**Restricted to HEU and HU infants born term and AGA[2] (N=681)**	**Restricted to HEU and HU infants with optimal breastfeeding[3] (N=241)**	**Restricted to HEU and HU infants with running water and flush toilet in the home[4] (N=284)**	**Excluding HEU infants who did not receive co-trimoxazole preventive therapy[5] (N=745)**	**Restricted to HEU and HU infants who were born in Winter or Autumn[6] (N=435)**
HEU vs HU infants:						
Crude IRR (95% CI)	3.50 (1.68-7.28)	2.94 (1.25-6.90)	3.74 (0.44-31.72)	3.35 (0.66-17.03)	3.29 (1.52-7.11)	2.09 (0.81-5.36)
Adjusted IRR (95% CI)	3.49 (1.68-7.27)	2.68 (1.13-6.37)	3.51 (0.41-29.76)	4.13 (0.73-23.44)	3.58 (1.68-7.61)	2.52 (0.95-6.68)

Abbreviations: IRR, incidence rate ratios from Poisson regression analysis with variances adjusted for clustering by child; CI, confidence interval; HEU, HIV-exposed uninfected infants; HU, HIV-unexposed infants; AGA, appropriate for gestational age (≥10th centile for gestation, Intergrowth-21st reference standards)

[1] Adjusted for socio-economic status (running water and flush toilet in home), postpartum depression, preterm birth, being underweight in a previous age interval, vaccination status, season of birth and optimal breastfeeding (defined as exclusive breastfeeding until at least 3 months of age or last attended visit if follow-up discontinued before 3 months, plus early initiation of breastfeeding ie within one hour of birth)

[2] Term, ≥37 weeks completed weeks gestation; adjusted for water and toilet in home, postpartum depression, underweight in previous age interval, vaccination status, optimal breastfeeding and season

[3] Defined as for full cohort model; adjusted for water and flush toilet and season of birth (other variables not included due to zero cells)

[4] Based on maternal self-report at first antenatal clinic visit; adjusted for preterm birth, postpartum depression, underweight in previous age interval, vaccination status, breastfeeding and season of birth

[5] Based on maternal self-report at either 6 week or 3 months' study visit; adjusted for water and toilet in home, postpartum depression, preterm birth, underweight in previous age interval, vaccination status, optimal breastfeeding and season

[6] Seasons defined as: Spring, 01 September to 30 November; Summer, 01 December to 28/29 February; Autumn, 01 March to 31 May; Winter, 01 June to 31 August; adjusted for water and flush toilet in home, postpartum depression, preterm birth, underweight in previous age interval, vaccination status and breastfeeding

Supplemental Table 9.5.9. Predictors and incidence of infectious-cause hospitalization among HIV-exposed uninfected infants between 7 days and 3 months of age: crude and adjusted incidence rate ratios from Poisson regression analysis

	Crude IRR (95% CI)[1]	Model A[2] Adjusted IRR (95% CI)[1]	Model B[3] Adjusted IRR (95% CI)[1]
HIV viral load **at** ART initiation ($\log_{10}$ copies/mL)	1·52 (1·02-2·27)	1·41 (0·95-2·11)	1·49 (1·01-2·21)
CD4 cell count at ART initiation (per 100 cells/mm^3)	0·85 (0·70-1·03)	-	-
CD4 cell count at ART initiation ≤350 cells/mm^3 (vs >350)	0·69 (0·35-1·38)	-	-
CD4 cell count at ART initiation, categories			
>500 cells/mm^3	1·00	-	-
>350-500 cells/mm^3	2·11 (0·71-6·30)	-	-
>200-350 cells/mm^3	2·07 (0·73-5·86)	-	-
≤200 cells/mm^3	2·42 (0·77-7·62)	-	-
HIV diagnosis during vs before index pregnancy	0·62 (0·31-1·22)	-	-
Duration of ART use in pregnancy (weeks)	0·99 (0·95-1·04)	-	-
HIV viral load at time of delivery ($\log_{10}$ copies/mL)	1·31 (0·86-1·99)	-	-
Maternal viral load ≥50 vs <50 copies/mL at time of delivery	1·59 (0·78-3·27)	-	-
Infant use of co-trimoxazole at any point	0·68 (0·30-1·51)	-	-
Maternal education[4]	0·67 (0·28-1·61)	-	-
Household crowding[5]	0·96 (0·23-4·01)	-	-
Poverty category[6]			
Most disadvantaged	1·00	1·00	1·00
Moderate disadvantage	1·04 (0·48-2·25)	0·84 (0·38-1·85)	0·86 (0·39-1·90)
Least disadvantaged	0·68 (0·28-1·63)	0·58 (0·23-1·44)	0·61 (0·25-1·48)
Risky drinking[7]	0·62 (0·26-1·50)	-	-
Intimate partner violence[8]	1·10 (0·50-2·44)	-	-
Postpartum depression[9]	2·32 (0·71-7·61)	-	-
Preterm birth, <37 weeks	1·61 (0·67-3·90)	1·00 (0·30-3·34)	-
Small-for-gestational age[10]	1·97 (0·86-4·53)	-	-
Male vs female	0·85 (0·43-1·67)	-	-
Ever exclusively breastfed (EBF)[11]	0·35 (0·15-0·80)	0·40 (0·17-0·97)	0·42 (0·17-1·02)
Duration of EBF (per month increase)[11]	0·82 (0·69-0·98)	-	-
Duration of any breastfeeding (per month increase)	0·97 (0·91-1·03)	0·95 (0·90-1·02)	0·95 (0·89-1·01)
WAZ <-2 in previous age interval[12]	3·17 (1·38-7·28)	2·76 (1·18-6·47)	-
Vaccination status, per age interval[13]			

	Crude IRR (95% CI)[1]	**Model A**[2] **Adjusted IRR (95% CI)**[1]	**Model B**[3] **Adjusted IRR (95% CI)**[1]
Complete and timely	1·00	1·00	1·00
Delayed or incomplete	7·38 (2·24-24·32)	6·04 (1·16-31·51)	6·29 (1·69-23·34)
Data not available	0·52 (0·18-1·50)	0·43 (0·15-1·25)	0·46 (0·16-1·36)

Abbreviations: IRR, incidence rate ratios; CI, confidence interval; CD, cluster of differentiation
[1] Admission defined as 2 or more days; estimates from Poisson regression with robust variance estimates, clustered on mother-child pairs; [2] Model A includes potential confounders and mediators of the maternal disease severity – HEU child hospitalization relationship; Model B only includes potential confounders; [3]Measured at ART initiation during pregnancy, or within 2 weeks of delivery date ; [4] Completed vs did not complete secondary education; [5] ≥10 people living in home, vs <10; [6] Categories based on poverty "score" (combination of standardized asset score and employment); [7] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 vs <3, as reported at first antenatal visit; [8] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit (vs none); [9]Edinburgh postnatal depression score ≥13 vs <13 at 6 weeks' study visit; [10] Birthweight for gestational age, percentile <10th, based on Intergrowth-21st growth reference standards (vs appropriate for gestational age) [11] Based on 24 hour recall, maternal report; exclusive defined as only breastmilk with prescribed medicine; non-time-varying; [12] Weight-for-age Z-score < 2 (underweight) at any point during the preceding age interval; [13] Based on Road to Health Booklet information; "late" defined as >2 weeks after due age

Supplemental Table 9.5.10. Longitudinal prevalence of self-reported child infectious illness in preceding two weeks, comparing HIV-exposed uninfected and HIV-unexposed infants over time: crude and adjusted prevalence ratios from modified Poisson regression analysis

	Presumed lower respiratory tract infection[1]		**Diarrhoeal illness[2]**	
	Crude PR (95% CI)[3]	**Adjusted PR (95% CI)[3]**	**Crude PR (95% CI)[3]**	**Adjusted PR (95% CI)[3]**
HIV exposure (HEU vs HU)	**3·23 (2·20-4·74)**	**2·33 (1·54-3·51)**	**1·25 (1·03-1·52)**	1·03 (0·83-1·28)
Maternal education[4]	**0·62 (0·42-0·91)**	0·78 (0·52-1·16)	**0·59 (0·47-0·75)**	**0·60 (0·47-0·76)**
Poverty category[5]				
Most disadvantaged	1·00	-	1·00	-
Moderate disadvantage	1·03 (0·68-1·57)	-	0·91 (0·72-1·14)	-
Least disadvantaged	1·18 (0·79-1·78)	-	0·91 (0·71-1·16)	-
Cigarette smoking in home[6]	**1·85 (1·32-2·60)**	1·34 (0·94-1·91)	**1·25 (1·02-1·52)**	1·11 (0·91-1·37)
Biomass fuel or paraffin use in home[7]	**1·60 (1·12-2·30)**	-	0·99 (0·79-1·23)	-
Risky drinking, antenatal[8]	**1·50 (1·01-2·24)**	-	**1·37 (1·09-1·72)**	-
Intimate partner violence, antenatal[9]	**1·58 (1·03-2·44)**	1·18 (0·76-1·84)	**1·60 (1·27-2·00)**	**1·53 (1·21-1·93)**
Postpartum depression[10]	1·79 (0·91-3·49)	-	**1·53 (1·06-2·22)**	-
Gestation at birth (weeks)	0·96 (0·88-1·05)	-	1·01 (0·97-1·06)	-
Preterm <37 weeks	1·32 (0·78-2·25)	1·10 (0·66-1·82)	0·90 (0·65-1·25)	0·84 (0·59-1·20)
Small-for-gestational age[11]	0·75 (0·42-1·34)	-	1·11 (0·82-1·51)	-
Male vs female sex	0·96 (0·68-1·35)	-	1·03 (0·85-1·25)	-
Ever exclusively breastfed (EBF)[12]	1·23 (0·69-2·22)	-	1·02 (0·77-1·33)	-
Duration of EBF (months)[12]	**1·08 (1·01-1·17)**	1·05 (0·96-1·14)	0·97 (0·92-1·01)	0·97 (0·92-1·02)
Duration of any breastfeeding (months)[12]	0·98 (0·95-1·01)	-	0·98 (0·81-1·20)	-
Breastfeeding vs not breastfeeding, at visit[12,13]	**0·52 (0·38-0·70)**	**0·66 (0·44-0·98)**	**0·49 (0·40-0·60)**	0·82 (0·65-1·04)
Child age >6 vs ≤ 6 months of age, at visit[13]	**1·98 (1·45-2·71)**	**1·61 (1·11-2·32)**	**3·54 (2·82-4·44)**	**3·38 (2·62-4·38)**
WAZ <-2 at visit[13]	**2·37 (1·31-4·27)**	-	1·51 (0·99-2·31)	-
Vaccination status at visit[13, 14]				
Complete and timely	1·00	1·00	1·00	1·00
Complete but delayed	1·50 (0·98-2·28)	1·17 (0·75-1·83)	1·31 (0·98-1·75)	0·94 (0·70-1·27)
Incomplete	1·00 (0·62-1·60)	0·86 (0·51-1·44)	**1·36 (1·02-1·81)**	0·99 (0·74-1·32)
Data not available	0·72 (0·44-1·16)	0·87 (0·54-1·40)	1·01 (0·78-1·32)	1·08 (0·83-1·41)

Abbreviations: PR, prevalence ratio; CI, confidence interval; HEU, HIV-exposed uninfected infants; HU, HIV-unexposed infants; WAZ, weight-for-age Z-score
[1] Maternal report of child being ill with a cough, high temperature and difficulty in breathing at some point in preceding 2 weeks; [2] Maternal report of child having had diarrhoea at any point in preceding two weeks; [3] Estimates from modified Poisson regression models with robust variance estimates, clustered on mother-child pairs using generalized estimating equations; [4] Completed vs did not complete secondary education, first antenatal visit; [5] Categories based on poverty "score" (combination of standardized asset score and employment), first antenatal visit; [6] Maternal report of own or other household members smoking, at 6 weeks', 6 and/or 12 months' study visits; [7] Maternal report of energy sources at 6 weeks', 6 and/or 12 months' study visits; [8] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 vs <3, as reported at first antenatal visit; [9] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit (vs none); [10] Edinburgh postnatal depression score ≥13 vs <13 at 6 weeks' study visit; [11]Birthweight <10th centile for gestational age, based on Intergrowth-21st growth reference standards (vs appropriate for gestational age) [12] Based on 24 hour recall, maternal report; exclusive defined as only breastmilk with prescribed medicine; non-time-varying; [13] Time-varying measures; [14] Based on Road to Health Booklet information; delayed vaccination defined as receipt >2 weeks after recommended age

Effect measure modification tests:
(1) presumed lower respiratory tract infection: HEU-sex, p=0·65; HEU-age category, p=0·04; HEU-preterm birth, p=0·91; HEU-SGA, p=0·96; HEU-current breastfeeding, p=0·29; HEU-vaccination status, p=0·95; age category-current breastfeeding, p=0·49; age category-vaccination status, p=0·07
(2) diarrhoeal illness: HEU-sex, p=0·50; HEU-age category, p<0·0001 HEU-preterm birth, p=0·20; HEU-SGA, p=0·24; HEU-current breastfeeding, p=0·21; HEU-vaccination status, p=0·47; age category-current breastfeeding, p<0·0001; age category-vaccination status, p=0·56

Supplemental Table 9.5.11. Period prevalence of self-reported child infectious illness in preceding two weeks, comparing HIV-exposed uninfected and HIV-unexposed infants after the age of 6 months: crude and adjusted prevalence ratios from modified Poisson regression analysis

	Presumed lower respiratory tract infection[1]		Diarrhoeal illness[2]	
	Crude PR (95% CI)[3]	Adjusted PR (95% CI)[3]	Crude PR (95% CI)[3]	Adjusted PR (95% CI)[3]
HIV exposure (HEU vs HU)	2·52 (1·59-3·98)	1·85 (1·15-2·96)	0·90 (0·72-1·13)	0·72 (0·57-0·92)
Maternal education[4]	0·75 (0·48-1·17)	0·90 (0·56-1·45)	0·62 (0·48-0·81)	0·60 (0·46-0·79)
Poverty category[5]				
Most disadvantaged	1·00	-	1·00	-
Moderate disadvantage	0·99 (0·60-1·63)	-	0·89 (0·68-1·15)	-
Least disadvantaged	1·08 (0·65-1·79)	-	0·88 (0·67-1·17)	-
Cigarette smoking in home[6]	2·08 (1·37-3·14)	1·57 (1·02-2·42)	1·18 (0·94-1·48)	1·15 (0·91-1·46)
Biomass fuel or paraffin use in home[7]	1·51 (0·98-2·34)	-	0·80 (0·61-1·04)	-
Risky drinking, antenatal[8]	1·60 (0·97-2·63)	-	1·16 (0·87-1·54)	-
Intimate partner violence, antenatal[9]	1·19 (0·69-2·04)	0·94 (0·55-1·60)	1·54 (1·18-2·01)	1·69 (1·29-2·21)
Postpartum depression[10]	1·83 (0·82-4·10)	-	1·42 (0·90-2·24)	-
Preterm <37 weeks	1·46 (0·76-2·79)	1·15 (0·61-2·17)	0·98 (0·67-1·43)	0·92 (0·61-1·37)
Small-for-gestational age[11]	0·44 (0·18-1·06)	-	1·08 (0·74-1·57)	-
Male vs female sex	1·02 (0·67-1·55)	-	0·99 (0·79-1·24)	-
Ever exclusively breastfed (EBF)[12]	1·41 (0·72-2·78)	-	1·00 (0·72-1·40)	-
Breastfeeding vs not breastfeeding, at visit[12,13]	0·79 (0·53-1·19)	0·75 (0·48-1·14)	0·96 (0·76-1·19)	0·91 (0·73-1·14)
WAZ <-2 at visit[13]	3·79 (1·87-7·67)	2·87 (1·47-5·57)	0·73 (0·32-1·66)	0·79 (0·61-1·37)
Vaccination status at visit[13, 14]				
Complete and timely	1·00	1·00	1·00	1·00
Delayed or incomplete	0·75 (0·47-1·19)	0·74 (0·45-1·19)	0·97 (0·75-1·25)	0·90 (0·70-1·16)
Data not available	0·67 (0·37-1·23)	0·83 (0·45-1·53)	1·17 (0·86-1·57)	1·10 (0·81-1·49)

Abbreviations: PR, prevalence ratio; CI, confidence interval; HEU, HIV-exposed uninfected infants; HU, HIV-unexposed infants; WAZ, weight-for-age Z-score

[1] Maternal report of child being ill with a cough, high temperature and difficulty in breathing at some point in preceding 2 weeks; [2] Maternal report of child having had diarrhoea at any point in preceding two weeks; [3] Estimates from modified Poisson regression models with robust variance estimates, clustered on mother-child pairs using generalized estimating equations; [4] Completed vs did not complete secondary education, first antenatal visit; [5] Categories based on poverty "score" (combination of standardized asset score and employment), first antenatal visit; [6] Maternal report of own or other household members smoking, at 6 weeks', 6 and/or 12 months' study visits; [7] Maternal report of energy sources at 6 weeks', 6 and/or 12 months' study visits; [8] Risky drinking, defined as Alcohol use

disorders identification test (AUDIT-C) score ≥3 vs <3, as reported at first antenatal visit; [9] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit (vs none); [10] Edinburgh postnatal depression score ≥13 vs <13 at 6 weeks' study visit; [11] Birthweight <10th centile for gestational age, based on Intergrowth-21st growth reference standards (vs appropriate for gestational age) [12] Based on 24 hour recall, maternal report; exclusive defined as only breastmilk with prescribed medicine; non-time-varying; [13] Time-varying measures; [14] Based on Road to Health Booklet information; delayed vaccination defined as receipt >2 weeks after recommended age

Effect measure modification tests:
(1) presumed lower respiratory tract infection: HEU-sex, p=0·18; HEU-preterm birth, p=0·70; HEU-SGA, n/a, zero cells; HEU-current breastfeeding, p=0·70; HEU-vaccination status, p=0·84
(2) diarrhoeal illness: HEU-sex, p=0·21; HEU-preterm birth, p=0·07; HEU-SGA, p=0·17; HEU-current breastfeeding, p=0·91; HEU-vaccination status, p=0·72

Supplemental Table 9.5.12. Point prevalence of self-reported child infectious illness in preceding two weeks, comparing HIV-exposed uninfected and HIV-unexposed infants in 6-month age intervals, within sub-categories of maternal HIV disease severity, timing of antiretroviral therapy initiation in pregnancy, breastfeeding and vaccination: adjusted prevalence ratios from modified Poisson regression analysis

	Presumed lower respiratory tract infection[1]		Diarrhoeal illness[2]	
	≤ 6 months old: aPR (95% CI)[3, 4]	> 6 months old: aPR (95% CI)[3, 5]	≤ 6 months old: aPR (95% CI)[3, 4]	> 6 months old: aPR (95% CI)[3, 5]
HIV exposure and maternal CD4 cell count at ART initiation in pregnancy				
HIV-unexposed	1·00	1·00	1·00	1·00
HEU, maternal CD4 > 350 cells/mm	**4·94 (2·47-9·86)**	1·29 (0·73-2·28)	**3·29 (1·84-5·87)**	0·95 (0·72-1·25)
HEU, maternal CD4 ≤ 350 cells/mm	**5·04 (2·38-10·65)**	**2·12 (1·22-3·70)**	**2·37 (1·31-4·29)**	0·78 (0·56-1·07)
HIV exposure and maternal HIV viral load (HIV-VL) at ART initiation in pregnancy				
HIV-unexposed	1·00	1·00	1·00	1·00
HEU, HIV-VL < 4·0 $\log_{10}$ copies/mL	**4·44 (2·18-9·03)**	1·38 (0·78-2·41)	**3·03 (1·70-5·42)**	0·85 (0·64-1·12)
HEU, HIV-VL ≥ 4·0 $\log_{10}$ copies/mL	**5·44 (2·62-11·29)**	**2·00 (1·15-3·47)**	**2·90 (1·64-5·14)**	0·86 (0·64-1·17)
HIV exposure and gestation at ART initiation in pregnancy				
HIV-unexposed	1·00	1·00	1·00	1·00
HEU, ART initiation < 24 weeks	**4·88 (2·43-9·79)**	**2·06 (1·22-3·49)**	**2·65 (1·50-4·68)**	0·79 (0·60-1·05)
HEU, ART initiation ≥ 24 weeks	**5·10 (2·39-10·89)**	0·98 (0·50-1·91)	**3·48 (1·93-6·27)**	0·98 (0·72-1·33)
HIV exposure and maternal HIV viral suppression (< 50 copies/mL) at delivery				
HIV-unexposed	1·00	1·00	1·00	1·00
HEU, HIV-VL < 50 copies/mL	**5·51 (2·83-10·76)**	**1·80 (1·08-3·01)**	**2·79 (1·59-4·89)**	0·91 (0·71-1·16)
HEU, HIV-VL ≥ 50 copies/mL	**3·22 (1·34-7·74)**	1·32 (0·64-2·71)	**3·57 (1·94-6·57)**	0·65 (0·39-1·09)
HIV exposure and maternal HIV disease severity at ART initiation in pregnancy, combination[6]				

	Presumed lower respiratory tract infection[1]		**Diarrhoeal illness[2]**	
	≤ 6 months old: aPR (95% CI)[3, 4]	**> 6 months old: aPR (95% CI)[3, 5]**	**≤ 6 months old: aPR (95% CI)[3, 4]**	**> 6 months old: aPR (95% CI)[3, 5]**
HIV-unexposed	1·00	1·00	1·00	1·00
HEU, less severe disease[7]	**4·47 (2·11-9·45)**	1·29 (0·67-2·48)	**3·56 (1·95-6·50)**	0·91 (0·67-1·24)
HEU, moderately severe disease[8]	**5·25 (2·48-11·10)**	1·64 (0·89-3·02)	**2·45 (1·29-6·66)**	0·78 (0·56-1·09)
HEU, severe disease[9]	**5·09 (2·25-11·54)**	**2·52 (1·47-4·32)**	**2·82 (1·53-5·18)**	0·76 (0·53-1·09)
HIV exposure, maternal HIV disease severity AND gestation at ART initiation in pregnancy				
HIV-unexposed	1·00	1·00	1·00	1·00
HEU, less severe disease and early ART initiation[10]	**4·03 (1·67-9·75)**	1·20 (0·55-2·65)	**2·96 (1·48-5·92)**	0·74 (0·47-1·18)
HEU, less severe disease but late ART initiation OR more severe disease with early ART initiation[11]	**4·97 (2·48-9·96)**	**2·11 (1·28-3·48)**	**2·99 (1·73-5·15)**	0·89 (0·69-1·15)
HEU, more severe disease and late ART initiation[12]	**6·88 (2·38-19·89)**	1·22 (0·49-3·08)	**2·83 (1·18-6·82)**	0·79 (0·39-1·56)
By breastfeeding practice at time of visit				
HIV-unexposed	1·00	1·00	1·00	1·00
HEU, exclusive breastfeeding	**3·93 (1·93-8·01)**	**-**	**2·34 (1·32-4·14)**	-
HEU, breastfeeding, not exclusively	**4·87 (1·91-12·40)**	**1·86 (1·09-3·18)**	**3·54 (1·76-7·12)**	0·80 (0·60-1·06)
HEU, not breastfeeding	**6·28 (2·82-13·98)**	**2·15 (1·29-3·58)**	**5·22 (2·85-9·59)**	0·84 (0·64-1·11)
By vaccination status at time of study visit				
HIV-unexposed	1·00	1·00	1·00	1·00
HEU, complete and timely	**4·18 (2·01-8·71)**	**2·11 (1·26-3·51)**	**3·04 (1·74-5·32)**	0·79 (0·60-1·04)
HEU, complete but delayed	**5·67 (1·80-17·83)**	**2·57 (1·37-4·80)**	**3·35 (1·38-8·12)**	1·00 (0·69-1·44)
HEU, incomplete	**8·98 (3·51-23·00)**	1·07 (0·90-3·96)	**4·36 (1·83-10·41)**	0·64 (0·37-1·13)
HEU, data not available	**3·73 (1·51-9·17)**	1·89 (0·90-3·96)	**2·74 (1·42-5·32)**	0·88 (0·53-1·47)
By categories of breastfeeding and vaccination status at study visit[13]				

	Presumed lower respiratory tract infection[1]		**Diarrhoeal illness[2]**	
	≤ 6 months old: aPR (95% CI)[3, 4]	**> 6 months old: aPR (95% CI)[3, 5]**	**≤ 6 months old: aPR (95% CI)[3, 4]**	**> 6 months old: aPR (95% CI)[3, 5]**
HIV-unexposed	1·00	1·00	1·00	1·00
HEU, optimal breastfeeding and vaccinations complete and timely	**4·40 (2·09-9·24)**	**1·95 (1·06-3·59)**	**2·65 (1·47-4·78)**	**0·69 (0·48-0·98)**
HEU, optimal breastfeeding but vaccinations delayed or incomplete	**5·73 (2·10-15·62)**	1·16 (0·44-3·11)	**2·74 (1·15-6·54)**	0·94 (0·61-1·47)
HEU, suboptimal breastfeeding but vaccinations complete and timely	**5·08 (1·75-14·72)**	**2·40 (1·33-4·33)**	**5·59 (2·75-11·38)**	0·89 (0·64-1·25)
HEU, suboptimal breastfeeding and vaccinations delayed or incomplete	**10·86 (3·80-31·04)**	**2·44 (1·29-4·63)**	**5·98 (2·53-14·09)**	0·77 (0·50-1·18)
By categories of maternal disease severity and gestation at ART initiation as well as breastfeeding and vaccination status at study visit[13]				
HIV-unexposed	1·00	1·00	1·00	1·00
HEU, early ART initiation in pregnancy, at early disease stages; optimal breastfeeding and vaccinations complete and timely	2·86 (0·85-9·57)	0·62 (0·10-4·01)	1·94 (0·69-5·45)	0·29 (0·08-1·08)
HEU, early ART initiation in pregnancy, at early disease stages; suboptimal breastfeeding OR vaccinations delayed/incomplete	2·41 (0·34-16·98)	1·98 (0·86-4·56)	**4·53 (1·83-11·21)**	0·87 (0·51-1·47)
HEU, ART initiation late in pregnancy OR at advanced disease stages; optimal breastfeeding, and vaccinations complete and timely	**4·43 (2·00-9·84)**	**2·48 (1·34-4·60)**	**2·55 (1·32-4·92)**	0·76 (0·53-1·09)
HEU, ART initiation late in pregnancy OR at advanced disease stages; suboptimal breastfeeding OR vaccinations delayed/incomplete	**7·46 (3·43-16·24)**	**2·49 (1·50-4·13)**	**4·30 (2·38-7·78)**	0·86 (0·65-1·13)
By gestation at delivery[15]				
HIV-unexposed, term	1·00	1·00	1·00	1·00
HIV-unexposed, preterm	0·91 (0·12-6·83)	1·32 (0·39-4·44)	n/a	1·27 (0·81-1·98)
HIV-exposed, term	**4·45 (2·18-9·06)**	**2·08 (1·27-3·42)**	**2·79 (1·65-4·73)**	0·88 (0·79-1·12)
HIV-unexposed, preterm	**4·66 (1·68-12·91)**	2·17 (0·96-4·87)	**2·43 (1·09-5·43)**	0·55 (0·29-1·06)
By size at birth[16]				
HIV-unexposed, appropriate for gestational age	1·00	1·00	1·00	1·00

	Presumed lower respiratory tract infection[1]		**Diarrhoeal illness**[2]	
	≤ 6 months old: **aPR (95% CI)**[3, 4]	**> 6 months old:** **aPR (95% CI)**[3, 5]	**≤ 6 months old:** **aPR (95% CI)**[3, 4]	**> 6 months old:** **aPR (95% CI)**[3, 5]
HIV-unexposed, small-for-gestational age	**3·65 (1·01-13·19)**	n/a	0·82 (0·19-3·53)	0·85 (0·44-1·64)
HEU, appropriate for gestational age	**5·69 (2·61-12·41)**	**1·96 (1·23-3·13)**	**3·09 (1·80-5·31)**	**0·76 (0·60-0·97)**
HEU, small-for-gestational age	**4·98 (1·76-14·11)**	0·84 (0·33-2·15)	**2·68 (1·22-5·89)**	1·22 (0·78-1·89)

Abbreviations: aPR, adjusted prevalence ratio; CI, confidence interval; HEU, HIV-exposed uninfected infants; HU, HIV-unexposed infants; CD, cluster of differentiation; ART, antiretroviral therapy; VL, viral load; data missing for CD4 cell count at booking, n=17

[1] Child reported as ill with a cough, high temperature and breathing difficulty in preceding 2 weeks; [2] Child reported to have had diarrhoea in preceding two weeks

[3] Modified Poisson regression models with robust variance estimates, clustered on mother-child pairs using generalized estimating equations

[4] All models adjusted for maternal education, household cigarette smoking, preterm birth and child underweight; models examining effects of maternal HIV disease severity and timing of ART initiation also adjusted for breastfeeding (time-varying, no breastfeeding vs mixed feeding vs exclusive at current visit) and vaccination status

[5] All models adjusted for maternal education, household cigarette smoking, preterm birth and child underweight; models examining effects of maternal HIV disease severity and timing of ART initiation also adjusted for breastfeeding (time-varying, no breastfeeding vs any breastfeeding at current visit) and vaccination status

[6] Binary disease severity categories allocated using median values, for CD4 cell count and HIV viral load, as measured at ART initiation in pregnancy

[7] At ART initiation: maternal CD4 cell count > 350 cells/mm^3 AND HIV viral load <4·0 log$_{10}$ copies/mL

[8] At ART initiation: maternal CD4 cell count ≤ 350 cells/mm^3 but HIV viral load < 4·0 log$_{10}$ copies/mL; OR, maternal CD4 cell count > 350 cells/mm^3 but HIV viral load ≥4·0 log$_{10}$ copies/mL

[9] At ART initiation: maternal CD cell count ≤350 AND HIV viral load ≥4·0 log$_{10}$ copies/mL

[10] At ART initiation: maternal CD4 cell count > 350 cells/mm^3 AND HIV viral load <4·0 log$_{10}$ copies/mL AND ART initiated prior to 24 weeks' gestation in pregnancy

[11] At ART initiation: maternal CD4 cell count ≤ 350 cells/mm^3 OR HIV viral load ≥4·0 log$_{10}$ copies/mL but ART initiated prior to 24 weeks' gestation in pregnancy; OR, maternal CD4 cell count > 350 cells/mm^3 OR HIV viral load <4·0 log$_{10}$ copies/mL but ART initiation at or after 24 weeks' gestation in pregnancy

[12] At ART initiation: maternal CD cell count ≤350 AND HIV viral load ≥4·0 log$_{10}$ copies/mL AND ART initiation at or after 24 weeks' gestation in pregnancy

[13] Models exclude HEU infants with unknown vaccination status at the time of the study visit (n=102); delayed vaccination defined as receipt > 2 weeks after recommended age; "optimal" breastfeeding defined as exclusive (vs non-exclusive or no) breastfeeding at study visits before age 6 months, and any (vs no) breastfeeding at study visits from 6 months onwards

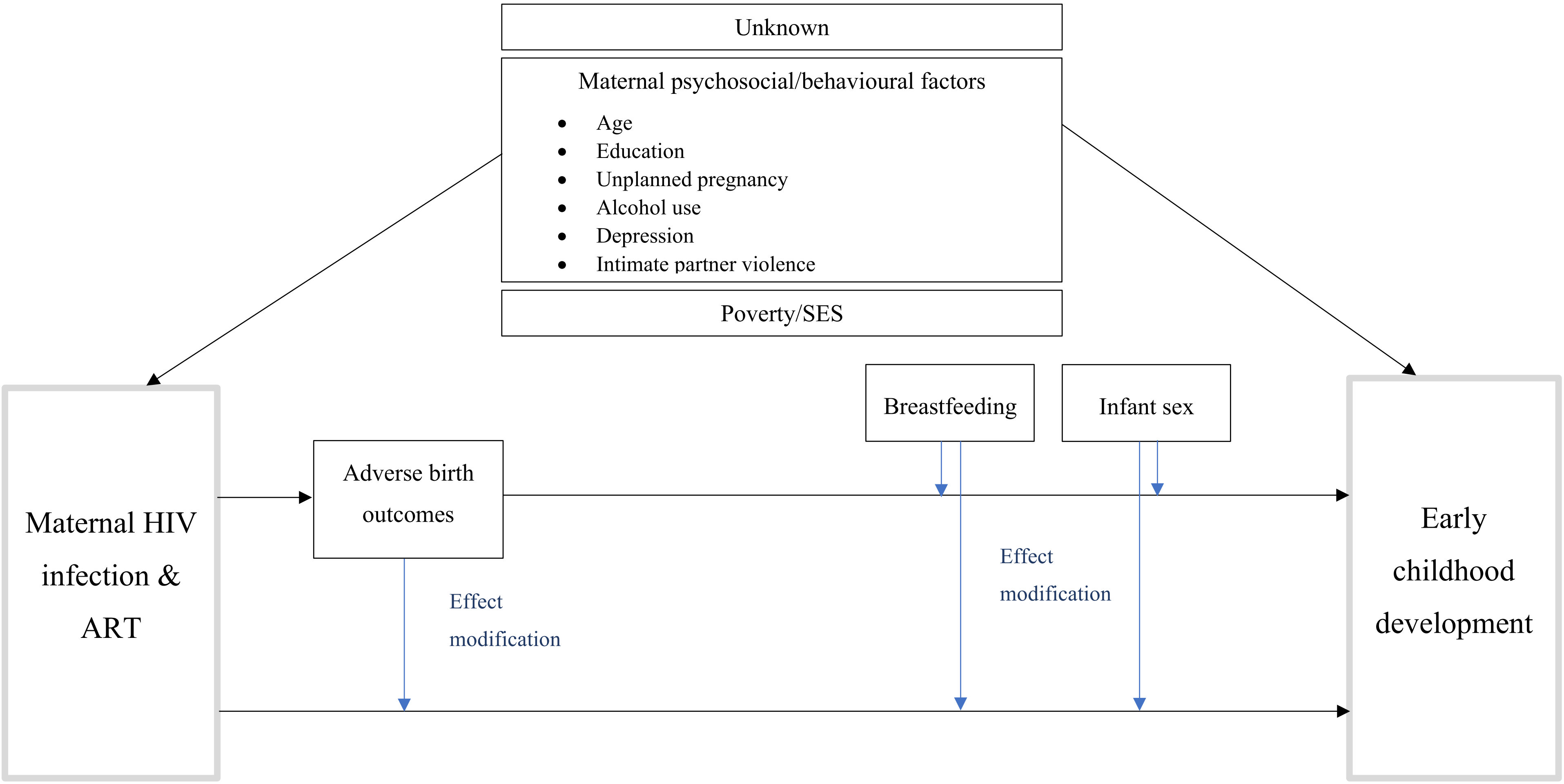
[14] Not adjusted for underweight as too few events

[15] Preterm defined as birth prior to 37 completed weeks of gestation; no diarrhoeal events occurred among HU-preterm infants in the first 6 months;

[16] Small for gestational age defined as birthweight below 10th centile for gestational age (using Intergrowth-21st growth reference standards

9.6 Supplemental material for chapter 6 (Neurodevelopment)

Supplemental Figure 9.6.1. Simplified directed acyclic graph



Supplemental Table 9.6.1. Maternal and infant characteristics by categories of preterm delivery and HIV exposure status, limited to HIV-uninfected infants with developmental assessment data

	TOTAL N=521	Term infants		Preterm infants		*p*-value
		HIV-unexposed n=278	HIV-exposed n=187	HIV-unexposed n=28	HIV-exposed n=28	
Maternal characteristics						
Age in years	28 (24-33)	28 (23-33)	29 (25-33)	29 (24-33)	29 (27-34)	0.25
Married/cohabiting	227 (44%)	125 (45%)	75 (40%)	13 (46%)	14 (50%)	0.64
Completed secondary schooling	202 (39%)	130 (47%)	48 (26%)	13 (46%)	11 (39%)	<0.0001
Employed	225 (43%)	134 (48%)	69 (37%)	10 (36%)	12 (43%)	0.09
Informal housing	240 (46%)	132 (48%)	89 (48%)	12 (43%)	7 (25%)	0.14
Unplanned pregnancy	358 (69%)	176 (63%)	142 (76%)	21 (75%)	19 (68%)	0.03
Antenatal depression	17 (3%)	6 (2%)	9 (5%)	2 (7%)	0	0.20
Risky drinking, enrolment[1]	85 (16%)	18 (6%)	53 (29%)	6 (21%)	8 (30%)	<0.0001
Risky drinking, 3rd trimester[1]	27 (5%)	2 (<1%)	22 (12%)	0	3 (11%)	<0.0001
Intimate partner violence, enrolment[2]	66 (13%)	21 (8%)	37 (20%)	2 (7%)	6 (22%)	<0.0001
Birth and infant characteristics						
Gestation at delivery (weeks)	39 (38-40)	39 (39-40)	40 (39-40)	35 (34-35)	35 (32-36)	0.0001
Male gender	252 (48%)	125 (45%)	102 (55%)	13 (46%)	12 (43%)	0.21
Weight for age Z-score at birth[3]	-0.1 (-0.8; 0.5)	0.0 (-0.7; 0.6)	-0.2 (-0.9; 0.4)	-0.2 (-1.1; 0.4)	-0.4 (-1.0; 0.3)	0.06
Small for gestational age[3]	64 (12%)	29 (10%)	24 (13%)	6 (21%)	5 (18%)	0.27

	TOTAL N=521	**Term infants**		**Preterm infants**		***p*-value**
		HIV-unexposed n=278	**HIV-exposed n=187**	**HIV-unexposed n=28**	**HIV-exposed n=28**	
Length for age Z-score at birth[3]	0.3 (-0.7; 1.5)	0.6 (-0.5; 1.9)	0.2 (-0.7; 1.3)	0.0 (-1.7; 1.1)	-0.2 (-1.3; 0.7)	0.002
Head circumference for age Z-score at birth[3]	0.7 (-0.3; 1.5)	0.9 (-0.2; 1.8)	0.5 (-0.4; 1.4)	0.1 (-0.5; 0.6)	-0.3 (-1.0; 1.5)	0.0002
Breastfeeding (months)[4]	9 (3-12)	10 (3-12)	6 (1-12)	10 (3-12)	6 (2-12)	0.005
Attending creche at time of assessment	71 (14%)	44 (16%)	18 (10%)	4 (15%)	5 (18%)	0.27

Values are median (interquartile range) or n (column %); p-values obtained from Chi^2 or Kruskal-Wallis, no correction for multiple comparisons. Antenatal depression, defined as EPDS (Edinburgh postnatal depression scale) score of ≥ 13 at enrolment study visit; **[1]** Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 as reported at first antenatal visit and at approximately 34 weeks' gestational age; **[2]** Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at study enrolment; **[3]** calculated using Intergrowth-21st reference standards; SGA defined as weight percentile at birth <10; [4] Last visit at which mother reported breastfeeding taken as breastfeeding cessation date.

Supplemental Table 9.6.2: Maternal and infant characteristics by availability of developmental outcome data

	HIV-exposed uninfected children				HIV-unexposed children			
	Total (N=471)	Completed developmental assessment (n=215)	Did not complete developmental assessment (n=256)	*p*	Total (N=414)	Completed developmental assessment (n=306)	Did not complete developmental assessment (n=108)	*p*
Maternal characteristics								
Age in years	28 (24-32)	29 (25-33)	27 (24-31)	0.01	27 (23-32)	28 (24-33)	26 (22-31)	0.01
Married/cohabiting	215 (46%)	89 (46%)	126 (45%)	0.87	306 (74%)	138 (75%)	168 (73%)	0.65
Matriculated	117 (25%)	59 (27%)	58 (23%)	0.23	185 (45%)	143 (47%)	42 (39%)	0.16
Employed	184 (39%)	81 (37%)	103 (40%)	0.57	195 (47%)	144 (47%)	51 (47%)	0.98
Informal housing	247 (52%)	96 (45%)	151 (59%)	0.002	197 (48%)	144 (47%)	53 (49%)	0.72
Unplanned pregnancy	338 (72%)	161 (75%)	177 (69%)	0.17	275 (66%)	197 (64%)	78 (72%)	0.14
Risky drinking, enrolment [1]	118 (25%)	61 (29%)	57 (22%)	0.11	30 (7%)	24 (8%)	6 (6%)	0.43
Risky drinking, 3rd trimester [1]	42 (9%)	24 (11%)	18 (7%)	0.10	3 (<1%)	2 (<1%)	1 (1%)	0.76
Intimate partner violence [2]	105 (23%)	43 (20%)	62 (24%)	0.30	33 (8%)	23 (7%)	10 (9%)	0.57
CD4 cell count at ART initiation (cells/mm^3)	354 (248-517)	346 (235-522)	360 (253-511)	0.53	-	-	-	-
Log^{10} HIV viral load at ART initiation (copies/mL)	4.0 (3.4-4.6)	4.0 (3.6-4.7)	3.9 (3.1-4.5)	0.01	-	-	-	-
Infant and postpartum maternal characteristics								

	HIV-exposed uninfected children				HIV-unexposed children			
	Total (N=471)	Completed developmental assessment (n=215)	Did not complete developmental assessment (n=256)	*p*	Total (N=414)	Completed developmental assessment (n=306)	Did not complete developmental assessment (n=108)	*p*
Gestational age at birth (weeks)	39 (38-40)	39 (38-40)	39 (38-40)	0.13	39 (38-40)	39 (38-40)	39 (38-40)	0.92
Preterm (<37 weeks)	57 (12%)	28 (13%)	29 (11%)	0.57	39 (9%)	28 (9%)	11 (10%)	0.75
Male	233 (49%)	114 (53%)	119 (47%)	0.16	213 (52%)	168 (55%)	45 (43%)	0.03
Small for gestational age [3]	70 (15%)	29 (13%)	41 (16%)	0.44	46 (11%)	35 (11%)	11 (11%)	0.79
Postpartum depression [4]	19 (4%)	9 (4%)	10 (4%)	0.99	12 (3%)	8 (3%)	4 (4%)	0.44
Breastfeeding (months) [5]	4 (1-12)	6 (1-12)	3 (1-9)	0.001	9 (3-12)	10 (3-12)	3 (2-9)	0.0001

Values are median (interquartile range) or n (column %); p-values obtained from Chi^2 or Kruskal-Wallis, no correction for multiple comparisons.

[1] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 as reported at first antenatal visit and at approximately 34 weeks' gestational age; [2] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at study enrolment; [3] Weight percentile at birth <10, calculated using Intergrowth-21st reference standards; [4] Maternal depression, EPDS (Edinburgh postnatal depression scale) score of ≥ 13 at 6 weeks' postpartum study visit; [5] Last visit at which mother reported breastfeeding taken as breastfeeding cessation date.

Supplemental Table 9.6.3. Maternal and infant characteristics by degree of COGNITIVE developmental delay as measured with BSID-III composite cognitive scores:

	TOTAL	No delay (BSID-III composite cognitive score ≥85)	Mild/moderate delay (BSID-III composite cognitive score 71-84)	Severe delay (BSID-III composite cognitive score <70)	*p-value*
HIV-uninfected women	306	292 (95%)	12 (4%)	2 (1%)	-
HIV-infected women	213	192 (90%)	19 (9%)	2 (1%)	-
Log^{10} HIV viral load at ART initiation in pregnancy	4.1 (3.6-4.6)	4.0 (3.6-4.6)	4.2 (3.6-4.7)	5.0 (4.7-5.3)	0.22
Suppressed HIV viral load (<50 copies/uL) at delivery	160 (75%)	144 (75%)	15 (79%)	1 (50%)	0.66
CD4 cell count at ART initiation in pregnancy (cells/mm^3)	345 (235-512)	349 (232 – 524)	327 (257-436)	259 (213-305)	0.58
Age in years	29 (25-33)	29 (25-33)	32 (27-34)	27 (27–28)	0.25
Married/cohabiting	88 (41%)	73 (38%)	15 (79%)	0	0.001
Completed secondary education	58 (27%)	55 (29%)	3 (16%)	0	0.33
Employed	81 (38%)	76 (40%)	4 (21%)	1 (50%)	0.27
Informal housing	95 (45%)	85 (44%)	9 (47%)	1 (50%)	0.96
Risky drinking[1]	61 (29%)	57 (30%)	3 (16%)	1 (50%)	0.34
Intimate partner violence [2]	42 (20%)	38 (20%)	2 (10%)	2 (100%)	0.01
Depression at 6 weeks postpartum[3]	19 (9%)	15 (8%)	3 (16%)	1 (50%)	0.06
Maternal self-reported smoking at time of assessment	72 (34%)	63 (33%)	7 (37%)	2 (100%)	0.13
Gestational age at delivery (weeks)	39 (38-40)	39 (38-40)	39 (35-40)	37 (35-40)	0.13
Preterm (<37)	28 (13%)	22 (12%)	5 (26%)	1 (50%)	0.06
Male	112 (53%)	102 (53%)	10 (53%)	0	0.32

	TOTAL	**No delay (BSID-III composite cognitive score ≥85)**	**Mild/moderate delay (BSID-III composite cognitive score 71-84)**	**Severe delay (BSID-III composite cognitive score <70)**	***p-value***
Birth weight for age, Z-score[4]	-0.2 (-0.9; 0.3)	-0.2 (-0.9; 0.4)	-0.9 (-0.5; 0.2)	-0.3 (0.0; 0.3)	0.65
Small for gestational age (<10th percentile)	29 (14%)	26 (14%)	3 (16%)	0	0.82
Birth head circumference for age, Z-score[4]	0.5 (-0.6; 1.4)	0.5 (-0.6; 1.4)	0.5 (-0.8; 1.4)	-0.5 (0.5; 1.5)	0.98
Duration of any breastfeeding (months)	6 (1-12)	6 (1-12)	12 (1-12)	7 (1-12)	0.86
Attends nursery/creche at time of assessment	23 (11%)	22 (12%)	1 (5%)	0	0.62

Values are median (interquartile range) or n (column %); p-values are based on Kruskal-Wallis or chi^2 and are not corrected for multiple testing

Allocation of categories of delay: (a) No delay: composite score ≥85; (b) mild/moderate delay: ≥70<85; (c) severe delay: <70

[1] Risky drinking, defined as Alcohol use disorders identification tool (AUDIT-C) score ≥3 as reported at first antenatal visit

[2] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit

[3] Maternal depression, EPDS (Edinburgh postnatal depression scale) score of ≥ 13 at 6 weeks' postpartum study visit

[4] Corrected for gestational age at birth, calculated using Intergrowth-21st reference standards

Supplemental Table 9.6.4. Maternal and infant characteristics by degree of MOTOR developmental delay as measured with BSID-III composite scores

	TOTAL	**No delay (BSID-III composite motor score ≥85)**	**Mild/moderate delay (BSID-III composite motor score 71-84)**	**Severe delay (BSID-III composite motor score <70)**	***p-value***
HIV-uninfected women (HIV-unexposed children)	306	292 (95%)	13 (4%)	1 (<1%)	-
HIV-infected women (HIV-exposed uninfected children)	208	189 (91%)	15 (7%)	4 (2%)	-
Log^{10} HIV viral load at ART initiation in pregnancy	4.1 (3.6-4.6)	4.0 (3.6-4.6)	4.4 (3.7-4.7)	4.9 (4.3-5.2)	0.10
Suppressed HIV viral load (<50 copies/uL) at delivery	155 (75%)	144 (76%)	10 (67%)	1 (25%)	0.05
CD4 cell count at ART initiation in pregnancy (cells/mm^3)	344 (231-515)	352 (239-517)	306 (223-502)	254 (126-550)	0.69
Age in years	29 (25-33)	29 (25-33)	29 (28-33)	28 (27-33)	0.82
Married/cohabiting	87 (42%)	79 (42%)	7 (47%)	1 (25%)	0.73
Completed secondary education	57 (27%)	52 (28%)	4 (27%)	1 (25%)	0.99
Employed	79 (38%)	72 (38%)	4 (27%)	3 (75%)	0.21
Informal housing	94 (45%)	87 (46%)	6 (40%)	1 (25%)	0.65
Risky drinking [1]	58 (28%)	52 (28%)	4 (27%)	2 (50%)	0.62
Intimate partner violence [2]	42 (20%)	34 (18%)	6 (40%)	2 (50%)	0.04
Depression at 6 weeks postpartum[3]	19 (9%)	16 (8%)	3 (20%)	0	0.27
Maternal self-reported smoking at time of assessment	68 (33%)	60 (32%)	5 (33%)	3 (75%)	0.19
Gestational age at delivery (weeks)	39 (38-40)	39 (38-40)	37 (31-39)	36 (34-39)	<0.001
Preterm (<37)	26 (13%)	17 (9%)	7 (47%)	2 (50%)	<0.001
Male	108 (52%)	98 (52%)	8 (53%)	2 (50%)	0.99

	TOTAL	**No delay (BSID-III composite motor score ≥85)**	**Mild/moderate delay (BSID-III composite motor score 71-84)**	**Severe delay (BSID-III composite motor score <70)**	***p-value***
Birth weight for age, Z-score[4]	-0.2 (-0.9; 0.3)	-0.3 (-0.9; 0.3)	-0.1 (-1.1; 0.6)	-0.4 (-0.9; 0.5)	0.91
Small for gestational age (<10th percentile)	27 (13%)	23 (12%)	3 (20%)	1 (25%)	0.52
Birth head circumference for age, Z-score[4]	0.5 (-0.5; 1.4)	0.4 (-0.4; 1.4)	1.4 (-0.7; 1.9)	-0.2 (-0.7; 0.7)	0.22
Duration of any breastfeeding (months)	6 (1-12)	7 (1-12)	4 (1-12)	3 (2-7)	0.61
Attends nursery/creche at time of assessment	23 (11%)	21 (11%)	1 (7%)	1 (25%)	0.58

Values are median (interquartile range) or n (column %); p-values are based on Kruskal-Wallis or chi^2 and are not corrected for multiple testing

Allocation of categories of delay: (a) No delay: composite score ≥85; (b) mild/moderate delay: ≥70<85; (c) severe delay: <70

[1] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 as reported at first antenatal visit

[2] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit

[3] Maternal depression, EPDS (Edinburgh postnatal depression scale) score of ≥ 13 at 6 weeks' postpartum study visit

[4] Corrected for gestational age at birth, calculated using Intergrowth-21st reference standards

Supplemental Table 9.6.5. Maternal and infant characteristics by degree of LANGUAGE* developmental delay as measured with BSID-III composite scores

	TOTAL	No delay (BSID-III composite language* score ≥85)	Mild/moderate delay (BSID-III composite language* score 71-84)	Severe delay (BSID-III composite language* score <70)	*p-value*
HIV-uninfected women (HIV-unexposed children)	306	262 (86%)	41 (13%)	3 (1%)	-
HIV-infected women (HIV-exposed uninfected children)	215	177 (82%)	36 (17%)	2 (1%)	-
Log^{10} HIV viral load at ART initiation in pregnancy	4.1 (3.6-4.6)	3.9 (3.5-4.5)	4.5 (4.2-5.0)	4.5 (3.7-5.3)	0.0001
Suppressed HIV viral load (<50 copies/uL) at delivery	162 (75%)	138 (78%)	22 (61%)	2 (100%)	0.07
CD4 cell count at ART initiation in pregnancy (cells/mm^3)	346 (235-522)	358 (261-552)	291 (197-399)	292 (148-436)	0.01
Age in years	29 (25-33)	29 (25-33)	28 (24-32)	30 (26-34)	0.87
Married/cohabiting	89 (41%)	75 (42%)	13 (36%)	1 (50%)	0.76
Completed secondary education	59 (27%)	53 (30%)	6 (17%)	0	0.18
Employed	81 (38%)	70 (40%)	11 (30%)	0	0.32
Informal housing	96 (45%)	82 (46%)	13 (36%)	1 (50)	0.53
Risky drinking [1]	61 (29%)	47 (27%)	13 (36%)	1 (50%)	0.43
Intimate partner violence [2]	43 (20%)	36 (21%)	1 (19%)	0	0.76
Depression at 6 weeks postpartum[3]	19 (9%)	13 (7%)	6 (17%)	0	0.19
Maternal self-reported smoking at time of assessment	73 (34%)	56 (32%)	15 (42%)	2 (100%)	0.08
Gestational age at delivery (weeks)	39 (38-40)	39 (38-40)	40 (39-41)	40 (40-41)	0.08
Preterm (<37)	28 (13%)	25 (14%)	3 (8%)	0	0.55
Male	114 (53%)	94 (53%)	18 (50%)	2 (100%)	0.39
Birth weight for age, Z-score[4]	-0.2 (-0.9; 0.4)	-0.3 (-0.8; 0.3)	-0.1 (-1.0; 0.5)	-0.4 (-1.4; 0.5)	0.95

	TOTAL	**No delay (BSID-III composite language* score ≥85)**	**Mild/moderate delay (BSID-III composite language* score 71-84)**	**Severe delay (BSID-III composite language* score <70)**	***p-value***
Small for gestational age (<10th percentile)	29 (14%)	21 (12%)	7 (19%)	1 (50%)	0.15
Birth head circumference for age, Z-score[4]	0.4 (-0.6; 1.4)	0.3 (-0.5; 1.4)	1.0 (-0.7; 1.6)	0.4 (-1.1; 2.0)	0.63
Duration of any breastfeeding (months)	6 (1-12)	7 (1-12)	5 (1-12)	10 (3-18)	0.62
Attends nursery/creche at time of assessment	23 (11%)	19 (11%)	4 (11%)	0	0.88

Values are median (interquartile range) or n (column %); p-values are based on Kruskal-Wallis or chi^2 and are not corrected for multiple testing

Allocation of categories of delay: (a) No delay: composite score ≥85; (b) mild/moderate delay: ≥70<85; (c) severe delay: <70

[1] Risky drinking, defined as Alcohol use disorders identification test (AUDIT-C) score ≥3 as reported at first antenatal visit

[2] Any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at first antenatal visit

[3] Maternal depression, EPDS (Edinburgh postnatal depression scale) score of ≥ 13 at 6 weeks' postpartum study visit

[4] Corrected for gestational age at birth, calculated using Intergrowth-21st reference standards

Supplemental Table 9.6.6. Relative odds for developmental delay comparing HIV-exposed uninfected (HEU) to HIV-unexposed (HU) children, stratified by maternal/infant characteristics

Maternal/infant characteristics	Strata	Cognitive delay		Motor delay		Language delay*	
		OR (95% CI)[1]	[2] *p*-value for interaction	OR (95% CI)[1]	[2] *p*-value for interaction	OR (95% CI)[1]	[2] *p*-value for interaction
Unadjusted estimate	n/a	2.28 (1.13-4.60)	n/a	2.10 (1.03-4.28)	n/a	1.28 (0.80-2.05)	n/a
Breastfeeding duration	< 9 months	2.41 (0.80-7.26)	0.98	1.32 (0.54-3.24)	0.16	1.04 (0.55-1.97)	0.43
	≥ 9 months	2.36 (0.94-5.91)		3.93 (1.15-13.42)		1.53 (0.75-3.11)	
Pregnancy intentions	Unplanned	2.16 (0.92-5.08)	0.79	1.71 (0.73-4.02)	0.40	1.69 (0.97-2.94)	0.04
	Planned	2.66 (0.77-9.14)		3.35 (0.90-12.43)		0.43 (0.14-1.37)	
Maternal age	< 30 years	2.50 (0.93-6.76)	0.71	1.95 (0.80-4.75)	0.75	2.00 (1.04-3.84)	0.03
	≥ 30 years	1.91 (0.71-5.15)		2.51 (0.73-8.61)		0.71 (0.35-1.43)	
Relationship status	Single	1.16 (0.38-3.54)	0.11	3.26 (1.10-9.64)	0.28	1.48 (0.79-2.77)	0.47
	Married	3.85 (1.50-9.86)		1.45 (0.54-3.92)		1.04 (0.50-2.17)	
Employment	Unemployed	3.59 (1.36-9.45)	0.13	2.27 (0.87-5.95)	0.81	1.79 (0.95-3.36)	0.11
	Employed	1.12 (0.35-3.54)		1.90 (0.64-5.63)		0.79 (0.36-1.70)	
Education	No matric	5.22 (1.73-15.80)	0.03	1.75 (0.73-4.17)	0.60	1.14 (0.66-1.99)	0.88
	Matriculated	0.73 (0.19-2.74)		2.65 (0.74-9.54)		1.04 (0.38-2.86)	
Housing	Formal	3.29 (1.09-9.56)	0.41	9.41 (2.06-42.92)	0.01	2.02 (1.04-3.92)	0.05
	Informal	1.76 (0.69-4.52)		0.89 (0.34-2.34)		0.77 (0.38-1.57)	
	No	2.33 (1.11-4.95)	0.99	1.63 (0.70-3.79)	0.66	1.41 (0.84-2.35)	0.32

Maternal/infant characteristics	**Strata**	**Cognitive delay**		**Motor delay**		**Language delay***	
		OR (95% CI)[1]	**[2] *p*-value for interaction**	**OR (95% CI)[1]**	**[2] *p*-value for interaction**	**OR (95% CI)[1]**	**[2] *p*-value for interaction**
Intimate partner violence[3]	Yes	2.32 (0.24-22.04)		2.47 (0.48-12.76)		0.70 (0.19-2.52)	
Birth size	AGA	2.15 (1.03-4.51)	0.63	1.79 (0.83-3.86)	0.33	1.18 (0.70-1.98)	0.50
	SGA	3.92 (0.39-39.92)		5.92 (0.62-56.34)		0.84 (0.56-6.10)	
Gender	Female	3.3 (1.18-9.22)	0.31	1.75 (0.67-4.56)	0.55	1.37 (0.70-2.68)	0.77
	Male	1.59 (0.61-4.18)		2.71 (0.90-8.19)		1.19 (0.61-2.32)	
Risky drinking[4]	Yes	- [5]	0.99	0.81 (0.18-3.52)	0.21	1.49 (0.44-5.09)	0.69
	No	2.45 (1.17-5.11)		2.37 (1.04-5.43)		1.13 (0.65-1.97)	

Abbreviations: OR, odds ratio; AGA, appropriate for gestational age; SGA, small for gestational age (birthweight < 10th centile); * Based on expressive language only

[1] Obtained from logistic regression analysis: stratum-specific estimates; [2] obtained from logistic regression analysis with interaction term

[3] any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at study enrolment; [4] Alcohol use disorders identification test (AUDIT-C) score ≥3 as reported at first antenatal visit; [5] Unable to determine due to null cell in sub-group (no HU children with cognitive delay and maternal risky drinking)

Supplemental Table 9.6.7. Relative odds for developmental delay comparing children in categories of study population characteristics, stratified by maternal HIV status

Maternal/infant characteristics	**Strata**	**Cognitive delay OR (95% CI)[1]**	**Motor delay OR (95% CI)[1]**	**Language[2] delay OR (95% CI)[1]**
Planned pregnancy	HEU	1.23 (0.45-3.36)	1.39 (0.50-3.87)	1.16 (0.60-2.25)
	HU	1.00 (0.33-3.08)	0.71 (0.22-2.32)	0.30 (0.10-0.89)
Maternal age ≥ 30 years	HEU	1.44 (0.59 – 3.56)	0.93 (0.36-2.41)	0.90 (0.44-1.83)
	HU	1.89 (0.64 – 5.54)	0.72 (0.22-2.36)	2.54 (1.33-4.86)
Married	HEU	4.08 (1.51-10.97)	1.01 (0.39-2.63)	0.79 (0.38-1.64)
	HU	1.23 (0.42-3.59)	2.27 (0.74-6.95)	1.13 (0.60-2.14)
Employed	HEU	0.48 (0.17-1.36)	0.95 (0.36-2.52)	0.62 (0.29-1.33)
	HU	1.53 (0.52-4.52)	0.95 (0.36-2.52)	1.42 (0.75 – 2.70)
Matriculated	HEU	0.42 (0.12-1.47)	0.94 (0.32-2.74)	0.44 (0.17-1.11)
	HU	2.99 (0.92-9.75)	0.62 (0.20-1.89)	0.48 (0.24-0.95)
Informal housing	HEU	1.14 (0.46-2.82)	0.68 (0.26-1.81)	0.67 (0.33-1.39)
	HU	2.09 (0.68-6.40)	7.27 (1.60-33.07)	1.76 (0.92-3.37)
Intimate partner violence [3]	HEU	0.93 (0.30-2.94)	3.25 (1.22-8.70)	0.86 (0.35-2.12)
	HU	0.94 (0.12-7.56)	2.15 (0.45-10.25)	1.74 (0.61-4.95)
Risky drinking [4]	HEU	0.55 (0.18-1.70)	1.20 (0.43-3.32)	1.59 (0.76-3.33)
	HU	n/a [5]	3.52 (0.91-13.60)	1.21 (0.39-3.72)
SGA	HEU	1.06 (0.29-3.87)	1.92 (0.59-6.30)	1.98 (0.80-4.89)
	HU	0.58 (0.07-4.60)	0.58 (0.07-4.60)	1.27 (0.49-3.26)

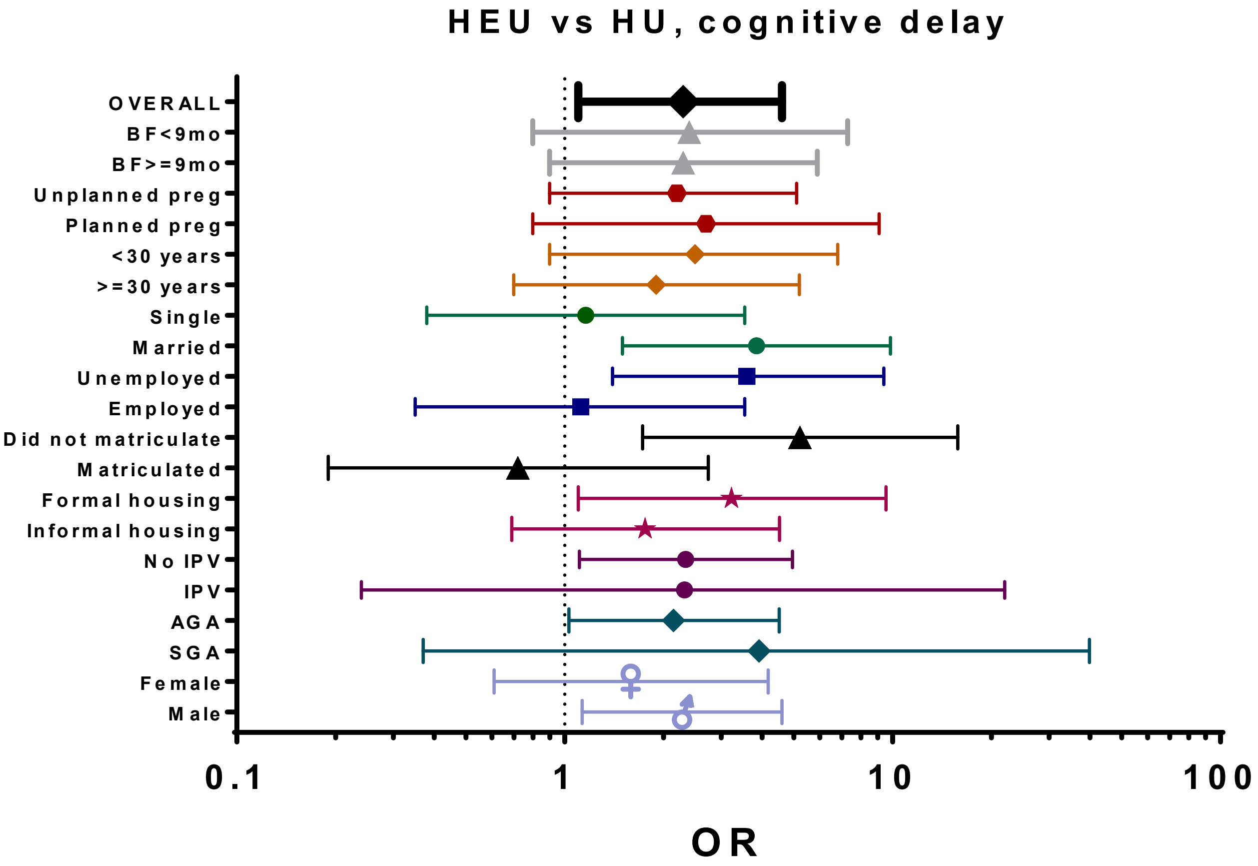
Maternal/infant characteristics	Strata	Cognitive delay OR (95% CI)[1]	Motor delay OR (95% CI)[1]	Language[2] delay OR (95% CI)[1]
Male	HEU	0.80 (0.33-1.98)	1.03 (0.40-2.65)	0.98 (0.49-1.98)
	HU	1.66 (0.56-4.91)	0.66 (0.22-2.03)	1.13 (0.60-2.14)
Breastfed ≥ 9 months	HEU	1.41 (0.57-3.49)	0.89 (0.34-2.31)	0.90 (0.44-1.83)
	HU	1.44 (0.47-4.42)	0.30 (0.09-0.98)	0.61 (0.32-1.16)
Preterm birth (<37 weeks)	HEU	3.09 (1.09-8.80)	9.11 (3.25-25.49)	0.52 (0.15-1.82)
	HU	2.91 (0.76-11.13)	4.47 (1.30-15.32)	2.69 (1.10-6.56)
Gestational age at delivery (weeks)	HEU	0.80 (0.68-0.93	0.63 (0.53-0.76)	1.15 (0.96-1.38)
	HU	0.89 (0.70-1.14)	0.77 (0.62-0.95)	0.87 (0.75-1.01)

Abbreviations: HEU, HIV-exposed uninfected children; HU, HIV-unexposed children; OR, odds ratio; AGA, appropriate for gestational age; SGA, small for gestational age (birthweight < 10th centile);

[1] Obtained from logistic regression analysis: stratum-specific estimates; [2] Based on expressive language only

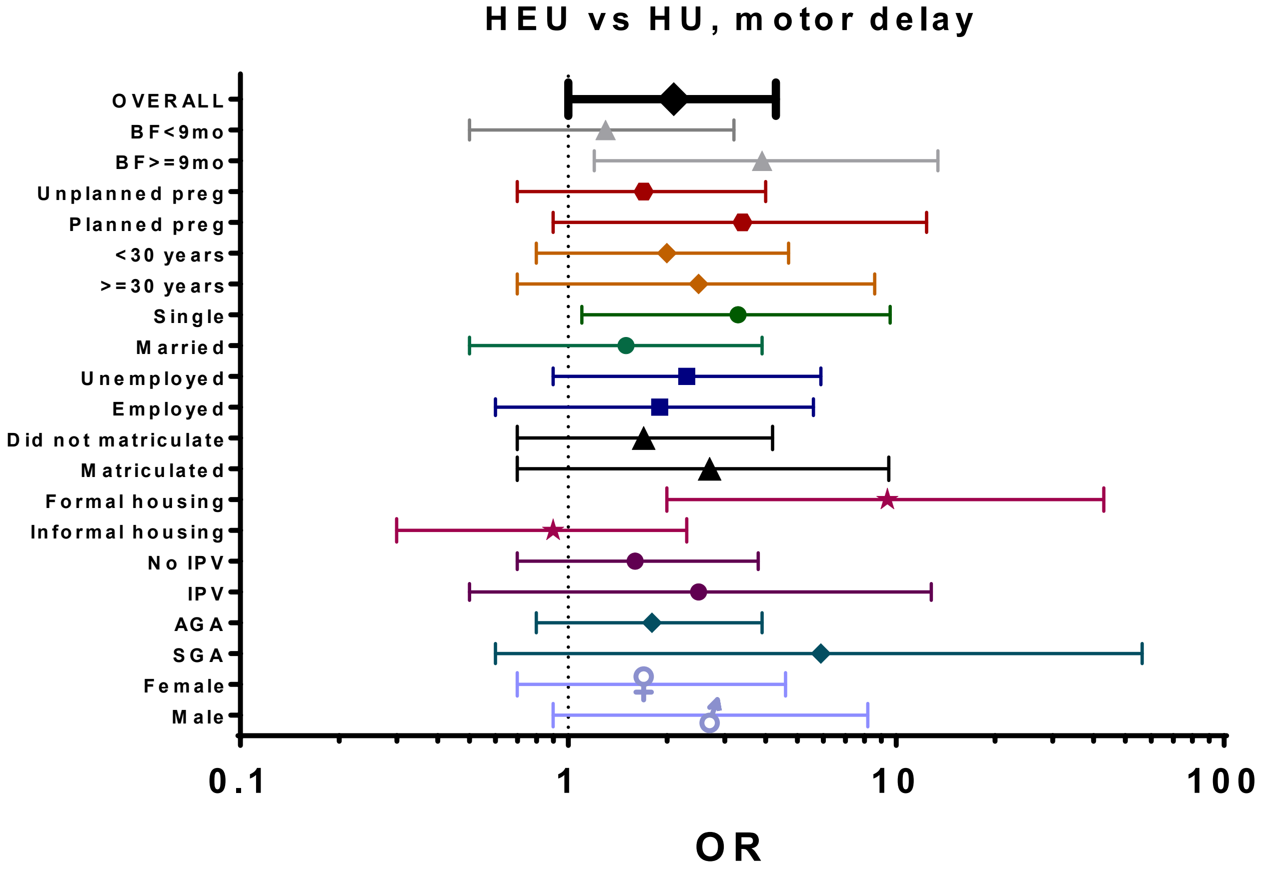
[3] any physical, sexual or psychological violence as measured with World Health Organization violence against women questionnaire at study enrolment; [4]Alcohol use disorders identification test (AUDIT-C) score ≥3 as reported at first antenatal visit; [5] Unable to determine due to null cell in sub-group (no HU children with cognitive delay and maternal risky drinking

Supplemental Figure 9.6.2. Interaction forest plot of odds ratios for COGNITIVE delay comparing HIV-exposed uninfected to HIV-unexposed infants within strata of maternal and infant characteristics



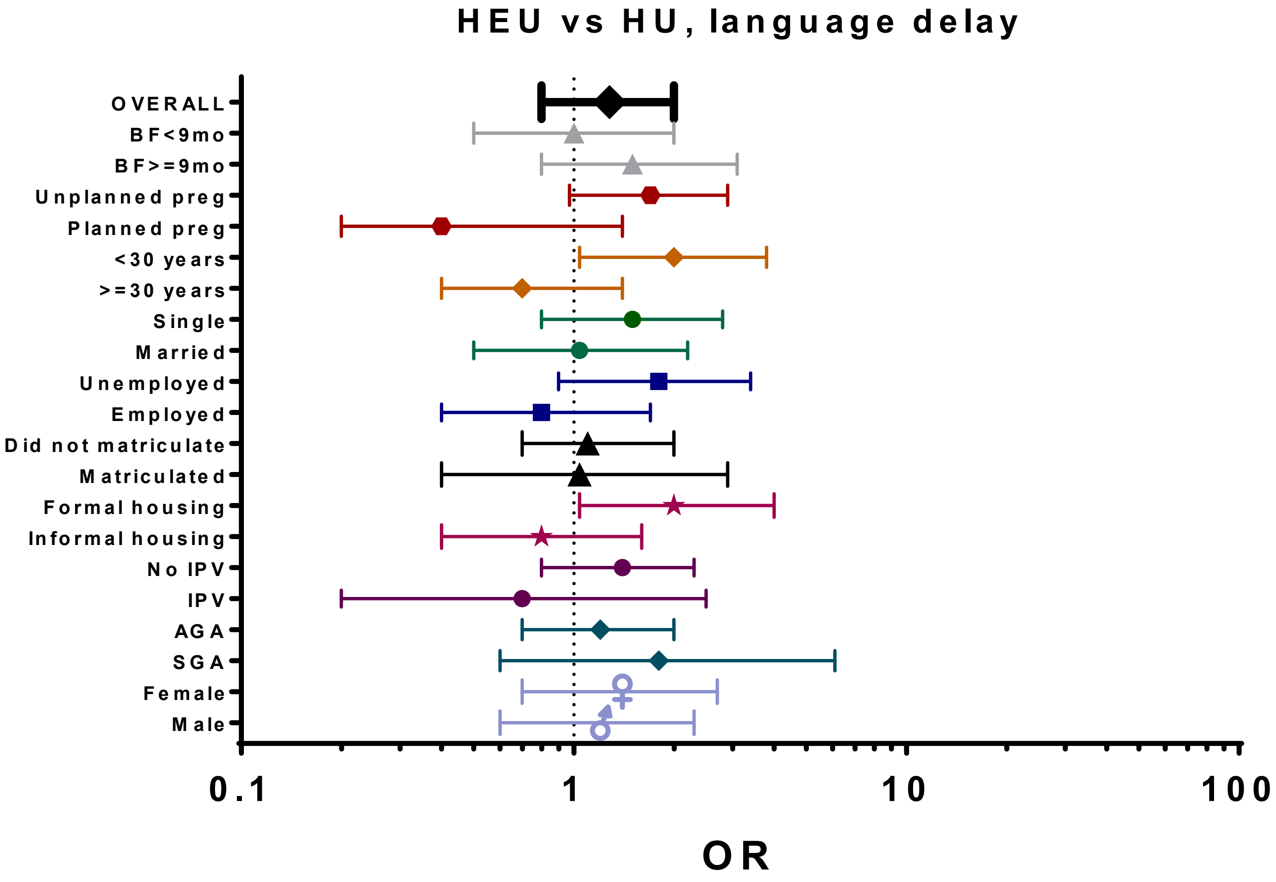
Abbreviations: HEU, HIV-exposed uninfected children; HU, HIV-unexposed children; OR, odds ratio; BF, Breastfeeding; preg, pregnancy; IPV, intimate partner violence; AGA, appropriate for gestational age; SGA, small for gestational age (birthweight < 10th centile)

Supplemental Figure 9.6.3. Interaction forest plot of odds ratios for MOTOR delay comparing HIV-exposed uninfected to HIV-unexposed infants within strata of maternal and infant characteristics



Abbreviations: HEU, HIV-exposed uninfected children; HU, HIV-unexposed children; OR, odds ratio; BF, Breastfeeding; preg, pregnancy; IPV, intimate partner violence; AGA, appropriate for gestational age; SGA, small for gestational age (birthweight < 10th centile)

Supplemental Figure 9.6.4. Interaction forest plot of odds ratios for LANGUAGE delay comparing HIV-exposed uninfected to HIV-unexposed infants within strata of maternal and infant characteristics



Abbreviations: HEU, HIV-exposed uninfected children; HU, HIV-unexposed children; OR, odds ratio; BF, Breastfeeding; preg, pregnancy; IPV, intimate partner violence; AGA, appropriate for gestational age; SGA, small for gestational age (birthweight < 10th centile

9.7 Rethinking the HIV-exposed, uninfected child: epidemiologic perspectives

Stanzi M le Roux, Elaine J Abrams, Landon Myer. **Rethinking the HIV-exposed, uninfected child: epidemiologic perspectives.** *Future Microbiol*. 2016 Jun;11:717-20. doi: 10.2217/fmb-2016-0055. Epub 2016 May 25. PubMed PMID: 27224215.

Relevance of this paper to the thesis

This opinion piece was written and published at the onset of this research project and summarizes the broader epidemiological context wherein the research was designed and conducted. In particular, the paper addresses several methodological issues around previous epidemiological work related to HIV-exposed uninfected children.

Contribution of the student and co-authors

The student conceptualized the opinion piece with LM, wrote the first draft and was the corresponding author. All co-authors reviewed the manuscript, providing conceptual and intellectual comment. All authors were involved in the final manuscript draft.

9.7.1 Background

Globally, more than 1 million HIV-exposed but uninfected (HEU) children were born in 2014.[1] The number of HEU children born annually continues to rise, the predictable result of an ongoing adult HIV epidemic alongside new and effective strategies to prevent mother to child transmission of HIV (PMTCT).[1] Since 2013, the World Health Organization has recommended lifelong universal triple antiretroviral therapy (ART) for HIV-infected pregnant and breastfeeding women ("Option B+"), with a strong emphasis on exclusive and extended breastfeeding, in most resource-limited settings including sub-Saharan Africa (SSA).[2]

Historically, breastfeeding by HIV-infected women was discouraged in PMTCT programs globally, including those in most resource-limited settings.[3] At the same time, access to ART was sharply limited, largely to those with advanced HIV disease. In this context, a substantial body of evidence accumulated over time and across countries demonstrating that HEU children were at increased risk of childhood mortality and morbidity. For example, a 2009 review encapsulated this thinking in providing an overview of these vulnerabilities,[4] drawing on publications reporting HEU children to be at higher-than-expected risk of death, hospitalization, and infectious diseases including pneumonia and diarrhea.[4,5] Similarly, a range of immunological abnormalities have been reported among HEU children born during this time, including altered cytokine profiles, changes in lymphocyte subsets, and low levels of protective maternal IgG antibodies in early infancy.[4,6]

9.7.2 Why should we rethink the "HIV-exposed, uninfected child"?

HEU children born under previous PMTCT strategies differed substantially from the "average", healthy, HIV-unexposed child by more than *in utero* exposure to maternal HIV. In particular, there are three critical child health concerns that require careful consideration.

9.7.2.1 Infant feeding

In the past, HEU and HU children have typically had vastly different infant feeding experiences. In an attempt to reduce the risk of vertical HIV transmission, previous PMTCT strategies included an emphasis on minimizing the HEU infant's exposure to maternal breastmilk, incorporating policies promoting exclusive formula feeding or restricted breastfeeding with abrupt, early weaning.[3] Given the known and substantial benefits of exclusive and prolonged breastfeeding,[7] it is not surprising that these infant feeding approaches were associated with significant increases in the risk of HEU child death,[8] hospitalization[9] and infectious diseases including both diarrhoea[8] and pneumonia.[9] The current promotion of early, exclusive and prolonged breastfeeding for HEU children, in line with global infant feeding recommendations across populations, is likely to substantially ameliorate at least some of the observed risk increases among HEU, particularly where maternal viral suppression through the use of ART minimizes the risk of breastfeeding-associated HIV transmission.[2,7]

9.7.2.2 Maternal well-being

Maternal health is a central determinant of child health globally, regardless of HIV status. For HIV-infected mothers, the risks of maternal morbidity and mortality in the absence of early and effective ART are dramatically higher than those of uninfected mothers.[10] Maternal death increases the risk of child death roughly 25-fold, even in the absence of maternal HIV infection;[11] correspondingly, a 16-fold increased risk of death has been reported among HEU children who lost their mothers, compared to those whose mothers survived.[12] In addition, maternal HIV disease severity also predicts the mortality and morbidity risks of uninfected children. A clear gradient exists linking maternal HIV disease severity and increased risk of child death, hospitalization, poor growth, and pneumonia, even in the absence of maternal death.[5,12] In keeping with this, immunological abnormalities are more common among HEU children born to women with higher HIV viral loads and/or lower CD4 cell counts, than among HEU children born to healthier women.[13]

9.7.2.3 Socio-economic determinants of child health

Across the world, the distribution of HIV is heavily influenced by social and economic conditions within countries and communities.[14] In turn, HEU and HU children may have differential exposures to broader social determinants of health, due to the uneven distribution of these factors among HIV-affected and HIV-unaffected households.[14] Children born into conditions of higher social and economic position, on average, survive longer and thrive compared to children born into poorer conditions.[15] The drivers of these differences are myriad and include household crowding, sanitation, and food security, among other factors commonly reported among HIV-affected families.[16] These differences are well known in investigations of HEU child health, demonstrating that even within the same community, variations in socio-economic situation can impact the health outcomes of HEU children compared to their HU counterparts, confounding the association between maternal HIV status and adverse HEU child health.[8]

Based on these considerations, four alternate explanations deserve consideration to understand the evidence regarding the health of the HEU child and the differences observed over the past two decades in morbidity and mortality comparing HEU with HU children: (i) the direct effect of *in utero* exposure to the virus; (ii) maternal morbidity or mortality; (iii) the quality and duration of breastfeeding; and (iv) the broader social and economic determinants of child health. Much of the current thinking around the health of HEU children has been focused on the first of these four points, with inconsistent attention to the other factors, particularly all four factors in combination. However, it is plausible that the differences observed are more highly attributable to the latter three issues.

9.7.3 Key considerations for epidemiologic studies of HIV-exposed, uninfected children

Global policies seeking to optimize the health of all HIV-infected mothers and their children through lifelong use of ART (Option B+) with promotion of breastfeeding are being implemented across resource-limited settings including SSA.[2] With these policies, we are only now reaching a point when these alternate explanations may be distinguishable. While it is widely recognized that many questions remain unanswered regarding the impact of Option B+ on maternal health and HIV-free child survival,[2,17] we argue that the potential impact of Option B+ policies on HEU child health beyond survival is a critical but perhaps neglected component of the research agenda. The central research questions going forward need to be focused on (a) whether HEU children are at increased risk of mortality and morbidity even under conditions of universal maternal ART with optimal breastfeeding; (b) if yes, what further modifiable factors can be identified; and lastly, (c) what the best strategies are to address these factors and optimize the health of HEU children going forward.

To address these questions and obtain meaningful estimates of HEU child health risks under Option B+, three salient factors - maternal HIV disease severity, infant feeding, and social determinants of health – require careful consideration in the design, analysis and reporting of both observational and interventional studies investigating the health of HEU children. From this, we identify three key issues in the design, conduct and analysis of these studies which, given the shortcomings of the existing literature, require special consideration going forward.

First, appropriate measurements of maternal HIV disease severity are essential towards determining the true effects of universal maternal ART on HEU child health outcomes. Recognition of the course of HIV disease, and how we measure disease progression, may help elucidate causal mechanisms driving HEU health. Outside of pregnancy, it is well understood that adults who initiate treatment at very low CD4 cell counts never fully recover functional immunity, even when virally suppressed.[18] Therefore even with maternal viral suppression and adequate CD4 cell counts, functional immune dysfunction, which has the potential to influence HEU outcomes, may still be present in women who had been at advanced HIV disease stages when they initiated treatment prior to pregnancy. In turn, classifying maternal ART experience into simply receiving vs. not receiving ART may lead to confounding by indication.[19] On a related point, while binary categorization of CD4 counts at historical treatment thresholds (such as 350 or 200 cells/mm^3) is commonplace, under policies of universal ART under “Option B+” it will be increasingly important to understand how maternal HIV disease at higher CD4 counts may impact on both maternal and child outcomes over time.[20]

Second, both maternal HIV disease severity and breastfeeding are likely to be major modifiers of the effect of maternal HIV infection on the health of HEU children.[9,12] Analyses of HEU child health outcomes should therefore ideally take place within strata of maternal disease severity and infant feeding. In addition to feeding modality, the quality of breastfeeding – at the minimum, distinguishing exclusivity and duration of breastfeeding – requires careful consideration.[8] Where significant effect modification is evident, as may be expected, stratum-specific rather than pooled estimates should be presented.[20] In turn, sample size calculations should ideally take into account the need for adequate power to obtain stratum-specific estimates with precision.[20]

Third, estimating and predicting any excess health risks in HEU children requires appropriate comparison groups to understand local levels of child mortality and morbidity. In the ideal scenario, study designs should include HIV-unexposed comparator groups selected from the same source communities as HIV-exposed children, with detailed measurements of multiple social determinants of disease. Often HEU child health data are obtained from PMTCT trial cohorts (with all children under study HIV-exposed) and comparative estimates of risks among HIV-unexposed children are unavailable. In some contexts, population-based child health statistics such as those generated by the Demographic and Healthy Surveys (DHS) program are available for comparison.[21] However, as survey data collection methods differ substantially from clinical trial data collection methods, such comparisons are at substantial risk of bias.[20] Moreover, HEU children are often concentrated in conditions of poverty, carrying with it multiple causes of child morbidity and mortality in addition to HIV exposure.[16] These social determinants of disease can be complex to measure, and attempts to minimize confounding through reliance on analysis alone can provide misleading results.

9.7.4 Conclusion

In summary, much of the evidence to date on the health of the HEU child – and in turn the public health discourse in this area – is rooted in an anachronistic view of the HIV epidemic and maternal and child health, particularly in sub-Saharan Africa. Given the rapid recent changes to policies regarding maternal ART and breastfeeding,[2] it is time for greater recognition that the health outcomes of HEU children are strongly related to maternal health, infant feeding, and background social and economic conditions. That is, under policies of universal and lifelong maternal ART, ultimately HEU child health and survival is likely to depend on the same conditions child health advocates have been promoting through the last 50 years, for all children: ensuring maternal health and well-being, optimal nutrition including exclusive and extended breastfeeding, and improved living conditions for families.[15] Future research in this area must recognize these crucial factors, and include design elements and analytic approaches to address each specifically, as we seek to understand and optimize HEU child health going forward.

9.7.5 *Acknowledgements*

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9.7.6 *Declarations of interest*

The authors declare no conflicts of interest.

9.7.7 *References*

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9.8 Tenofovir exposure *in utero* and linear growth in HIV-exposed, uninfected infants

Stanzi M le Roux, Jennifer Jao, Kirsty Brittain, Tamsin K Phillips, Seun Olatunbosun, Agnes Ronan, Allison Zerbe, Elaine J Abrams, Landon Myer. **Tenofovir exposure in utero and linear growth in HIV-exposed, uninfected infants.** *AIDS.* 2017 Jan 2;31(1):97-104. doi: 10.1097/QAD.0000000000001302. PubMed PMID: 27898591; PubMed Central PMCID: PMC5814299.

Relevance of this paper to the thesis

This manuscript provides details on a preliminary analysis of the early growth data presented in chapter 4. At the time of this analysis, there was concern that *in utero* exposure to tenofovir may adversely impact linear growth of children. As such, this analysis aimed to contribute to a growing body of knowledge around this issue. However, as the final analysis on a full dataset is presented in chapter 4, this paper is included only as a supplementary item.

Contribution of the student and co-authors

The student conceptualized this paper under the guidance of her primary supervisor, LM. The student conducted all analyses, presented the data in poster-format at the Conference for Retroviruses and Opportunistic Infections, drafted the first manuscript and was corresponding author. All co-authors contributed to and approved of the final manuscript, providing intellectual and conceptual input.

9.8.1 Abstract

9.8.1.1 Objective

Tenofovir (TDF) affects bone health and is widely used in pregnancy but data are limited on the effects of TDF exposure in utero. We examined the association between duration of in utero TDF exposure and linear growth in HIV-exposed, uninfected (HEU) infants.

9.8.1.2 Design

A prospective cohort of pregnant women initiating TDF-containing regimens at primary care services in Cape Town, South Africa were enrolled and followed with their breastfeeding infants through 12 months postpartum.

9.8.1.3 Methods

Length-for-age z-scores (LAZ) were calculated from infant lengths reported at birth and measured at 6, 12, 24, 36 and 48 weeks, using Fenton and World Health Organization standards. Linear mixed effects models were used to examine the association between duration of TDF exposure and LAZ over time.

9.8.1.4 Results

In 464 singleton mother-infant pairs (median CD4 at ART initiation, 346 cells/µL; viral load (VL), 4.0 $\log_{10}$ copies/ml), the median duration of *in utero* TDF exposure was 16.7 weeks (interquartile range, IQR 11.0-22.0) with 31%, 44% and 25% of infants exposed to <12, 12-22 and >22 weeks of TDF, respectively. Overall, 12% of children were stunted (LAZ<-2) at 48 weeks. Duration of exposure was not associated with LAZ: adjusted mean difference for >22 vs <12 wks, -0.12 (95% CI: -0.47; 0.23); 12-22 vs <12 wks, -0.06 (95% CI: -0.35; 0.24). Mean LAZ was 0.15 lower per log increase in maternal VL at ART initiation (95% CI: -0.29; -0.0001).

9.8.1.5 Conclusions

These data suggest no association between duration of TDF exposure *in utero* and early linear growth.

9.8.2 Introduction

In 2014, 66% of the estimated 1.5 million pregnant women living with HIV globally received efficacious antiretroviral regimens for their own health.[1] This number is set to increase substantially over the next decade with the expansion of access to triple-drug antiretroviral therapy (ART) for prevention of mother-to-child HIV transmission (PMTCT) and the promotion of universal treatment for all individuals with HIV infection.[1]

Tenofovir disoproxil fumarate (TDF) is widely used as part of first-line ART during pregnancy. Although generally safe and well-tolerated, TDF has been associated with decreased bone mineral density (BMD) and increased bone turnover in HIV-infected adults and children.[2] Data on the effect of *in utero* TDF exposure on bone health and growth of HIV-exposed uninfected (HEU) children have been mixed. In a large US cohort, the average length-for-age Z-score (LAZ) for 12-month old HEU infants exposed to TDF-containing ART regimens *in utero* was 0.14 lower than for those without TDF exposure.[3] In a similar but smaller cohort, TDF-exposed neonates had demonstrably lower bone mineral content (BMC) than their unexposed counterparts. [4] By contrast, preliminary data from the randomized IMPAACT- PROMISE trial conducted in Malawi, Zimbabwe, Uganda and South Africa showed no adverse association between TDF exposure and either birth length or neonatal BMC, although triple ART-exposed infants as a group had lower BMC than those exposed only to short course antiretrovirals for PMTCT.[5] Notably, in this study both ART regimens (with and without TDF) contained the protease inhibitor (PI) lopinavir-ritonavir (LPVr), which has also been associated with bone loss.[6]

Given the increasing number of children exposed to TDF-containing regimens *in utero,* more longitudinal data are needed on the potential growth effects of early exposure to ART, especially from resource-limited settings where childhood stunting is common and in the absence of concurrent PI use. Previously we found no association between TDF-exposure *in utero* and fetal long bone growth in a cohort of HIV-infected pregnant women using TDF-containing ART without concurrent PI use in Cape Town, South Africa. [7] Here we report on the postnatal growth of breastfed, HEU infants from the same cohort, specifically examining the association between duration of *in utero* TDF exposure and linear growth in the first year of life.

9.8.3 Methods

As part of the Maternal-Child Health-Antiretroviral (MCH-ART) study, consecutive HIV-infected, pregnant women initiating TDF-containing ART were followed during pregnancy and with their breastfeeding infants through 12 months (ClinicalTrials.gov NCT01933477).[8] The study was approved by the ethics review committees of the University of Cape Town Faculty of Health Sciences and Columbia University Medical Center.

9.8.3.1 Antiretroviral exposure

Our analysis is limited to women who initiated lifelong ART during pregnancy, on a first-line regimen of TDF with efavirenz (EFV) and either emtricitabine (FTC) or lamivudine (3TC). As per provincial guidelines, infants were prescribed nevirapine (NVP) prophylaxis within 72 hours after birth, continued through 4-12 weeks depending on MTCT risk assessment.[9]

9.8.3.2 Measures

Maternal interviews and serum collection for batched HIV viral load (VL) testing were completed at all study visits. Gestational age was based on research ultrasound at first antenatal visit, maternal recall of last menstrual period (LMP) or fundal height (from clinical records). At the first postnatal visit (within 28 days after birth), breastfeeding mother-infant pairs were recruited for additional study visits at approximately 6 (range, 0-8), 12 (9-20), 24 (21-32), 36 (33-44) and 48 (>44) weeks of age. HIV infection was excluded at 6 weeks and 48 weeks with HIV-PCR testing (Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 qualitative assay; Roche Molecular systems, Branchburg, NJ). Birth length was abstracted from clinical records. Trained research staff measured maternal height at enrolment; and infant length to the nearest 0.5cm using a firm recumbent stadiometer at all subsequent study visits.

We calculated infant length-for-age Z-scores (LAZ) based on Fenton and World Health Organization growth reference standards, using a corrected age for infants born prior to 37 completed weeks of gestation.[10,11] Stunting was defined as LAZ <-2. Duration of *in utero* TDF-exposure was expressed in weeks, calculated from number of days between date of ART initiation and date of delivery; for analysis, duration was categorized based on the interquartile range (<12, 12-22 and > 22 weeks). Potential third variables included maternal and infant factors known to affect child growth in general populations (maternal height, haemoglobin, substance abuse and socio-economic factors including education; infant prematurity and feeding) as well as HIV-specific factors (maternal HIV VL and CD4 cell count at ART initiation).[12,13] A composite socio-economic score was used to categorize participants into one of three groups according to relative levels of disadvantage, as previously described.[14] Maternal smoking was assessed by self-report and the Alcohol Use Disorders

Identification Test (AUDIT-C) was used to identify hazardous drinking (score ≥ 3 on questions 1-3) during pregnancy and within the first 6 months postpartum.[15]

9.8.3.3 Analytic methods

Exploratory data analysis assessed relationships between potential third variables and both the exposure of interest (duration of TDF exposure during pregnancy) and outcome of interest (LAZ over time) using visual plots, basic statistical tests, and simple linear regression. Relationships between influential and other third variables were evaluated for descriptive purposes. The proportion of stunted children was compared cross-sectionally by TDF exposure categories using chi-square tests. Mixed-effects linear regression models were used to examine the association between duration of *in utero* TDF exposure and infant LAZ over time, using a random intercept and slope for categories of TDF exposure duration. Model building included interaction terms where indicated; final selection was based on Akaike's Information Criterion. Analyses were conducted in Stata 12 (StataCorp College Station, TX).

Supplemental table 9.8.1. Characteristics of women at ART initiation and infants from birth, by duration of tenofovir exposure *in utero*

	Duration of TDF exposure (weeks)			**TOTAL (N=464)**
	< 12 (n=144)	**12-22 (n=203)**	**> 22 (n=117)**	
MATERNAL				
Age (years)	27 (24-32)	28 (24 – 32)	27 (25 – 31)	28 (24-32)
Height (cm)	159 (154 – 163)	156 (153-160)	157 (153 – 161)	157 (153.5 – 161)
Anemia prior to ART initiation (hemoglobin < 10 g/dL)	29.6% (40)	20.5% (41)	8.8% (10)	20.3% (91)
HIV viral load (log10 copies/mL) prior to ART initiation	3.9 (3.3 – 4.5)	4.2 (3.7 – 4.7)	4.0 (3.5 – 4.3)	4.0 (3.5 – 4.6)
HIV viral load <50 copies/mL at delivery	59.0% (85)	78.8% (160)	90.6% (106)	75.7% (361)
CD4 cell count (cells/mm³)	356 (255 – 539)	345 (220 – 492)	337 (251 – 487)	346 (235 – 502)
SES categories:				
Lowest	34.7% (50)	28.5% (58)	23% (27)	29% (135)
Moderate	31.3% (45)	38% (77)	35% (41)	35% (163)
Highest	34% (49)	33.5% (68)	42% (49)	36% (166)
AUDIT-C: Above threshold for hazardous drinking during the first 6 months postpartum	11.9% (14)	10.1% (19)	8.2% (8)	10.2% (41)
INFANT				
Duration of TDF exposure (weeks)	7.3 (4.2-10.4)	17.1 (15.1 – 19.1)	25.3 (23.1 – 26.5)	16.7 (11.0-21.9)
Male sex	53% (77)	45% (92)	53% (62)	50% (231)
Gestational age at birth (weeks)	39 (37–40)	39 (38-40)	39 (38-40)	39 (38-40)
Premature delivery				
34-37 completed weeks	9.0% (13)	6.9% (14)	7.7% (9)	7.8% (36)
< 34 completed weeks	9.0% (13)	2.5% (5)	0.0% (0)	3.9% (18)
Duration of any breastfeeding (months)	7.6 (2.2 – 12.1)	7.0 (2.2 – 12.2)	5.3 (2.1 – 12.1)	6.6 (2.2 – 12.2)
Length at birth (cm)	49 (47 – 51)	49 (48 – 52)	49.5 (48 – 51)	49 (48 – 51)
LAZ[1]	0.23 (-0.78 to 1.32)	0.44 (-0.78 to 1.47)	0.30 (-0.60 to 1.47)	0.32 (-0.77 to 1.46)
Stunted (LAZ<-2SD)	5.8% (7)	4.9% (9)	9.2% (10)	6.3% (26)
Weight at birth (kg)	3.08 (2.7 – 3.4)	3.12 (2.8 – 3.4)	3.2 (2.9 – 3.4)	3.15 (2.76 – 3.4)
WAZ[1]	0.03 (-0.84 to 0.52)	-0.26 (-0.80 to 0.43)	-0.02 (-0.59 to 0.48)	-0.10 (-0.80 to 0.48)
Underweight (WAZ<-2SD)	3.8% (5)	2.6% (5)	2.6% (3)	2.9% (13)

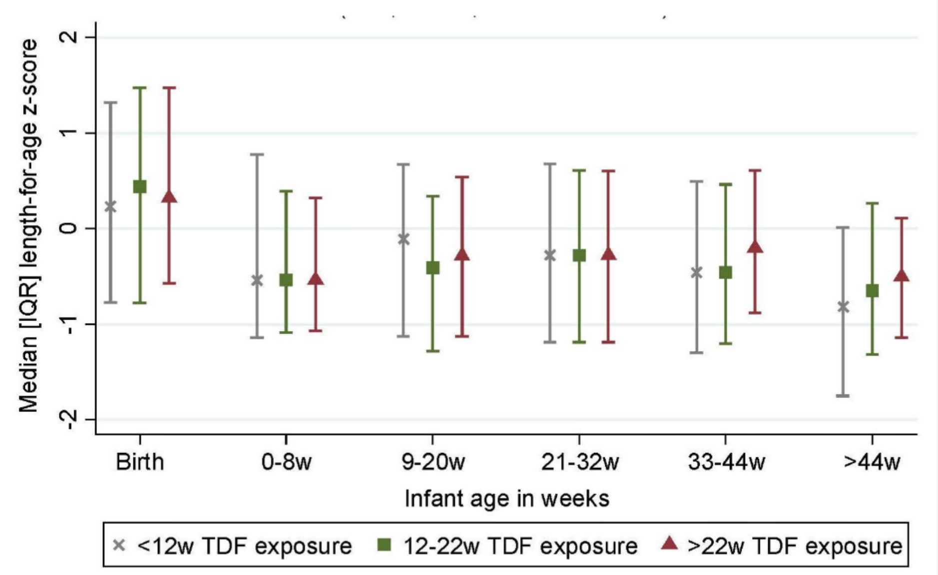
Values are median (IQR) or column % (n); TDF, tenofovir (exposure calculated from day of antiretroviral therapy initiation until day of birth); ART, triple antiretroviral therapy; AUDIT-C, Alcohol Use Disorders Identification Test for hazardous drinking (questions 1-3); LAZ – length-for-age Z-score: SD – standard deviation; WAZ – weight-for-age Z-score; [1] Calculated using Fenton growth charts (adjusted for gestational age and sex)

9.8.4 Results

In 464 singleton mother-infant pairs (median CD4 at enrolment, 346 cells/µL; VL, 4.0 $\log_{10}$ copies/ml), the median duration of *in utero* TDF-exposure was 16.7 wks (interquartile range, IQR 11.0-22.0) with 31%, 44% and 25% of infants exposed to <12, 12-22 and >22 weeks of TDF respectively (supplemental table 9.8.1). Mothers and infants were followed for a median of 76.0 (IQR 52.6-79.0) weeks postpartum. Fifty-eight (12.5%) did not complete study follow-up, predominantly due to relocation (n=29), maternal or infant demise (n=9) or work/school commitments (n=8). Fifteen mother-infant pairs were considered lost to follow-up (median follow-up time 13.0 weeks, IQR 6.0-26.4), with no difference by TDF exposure (5, 7 and 3 in the <12, 12-22 and > 22 weeks' groups respectively).

Log VL at enrolment was similar between TDF exposure groups but a larger proportion of mothers from the >22 weeks' group had achieved viral suppression (≤ 50 copies/mL) by delivery compared to those from the 12-22 and <12 week groups (91% vs. 79% and 59%, respectively). Compared to those with 12-22 weeks and <12 weeks of TDF use, mothers in the >22 weeks' group were also less likely to have anaemia before initiating ART, report hazardous drinking, or be in the lowest SES category (supplemental table 9.8.1). Median gestational age at birth did not differ between groups although a larger proportion of infants with <12 weeks of TDF exposure were born prematurely (supplemental table 9.9.1). Median duration of breastfeeding did not vary substantially between the groups (supplemental table 9.8.1). Exclusive breastfeeding (EBF) was initiated by 93% (432/464) of mothers; the median duration of EBF was 3.0 months (IQR, 1.3 – 5.7 months).

Supplemental figure 9.8.1. Length-for-age Z-scores of HIV-exposed uninfected infants at birth and during the first 12 months of life, by duration of *in utero* tenofovir exposure



IQR, inter-quartile range; w, weeks; TDF, tenofovir disoproxil fumarate

The median LAZ in the cohort was slightly above zero at birth (0.32, IQR -0.77 to 1.46), and declined thereafter, but did not vary by duration of TDF exposure at any time point (supplemental figure 9.8.1).

The lowest median LAZ was observed at 48 weeks (-0.65, IQR -1.36 to 0.11). Overall, 12% of children were stunted at 48 weeks (supplemental table 9.9.2). The prevalence of stunting did not vary between categories of TDF exposure at early study visits (supplemental table 9.8.2). At 48 weeks of age, a somewhat higher prevalence of stunting was observed among infants exposed to <12 weeks of TDF compared to those exposed to 12-22 and > 22 weeks (18%, compared to 9% and 10% respectively, p=0.12).

Supplemental table 9.8.2. Postnatal linear growth by duration of *in utero* exposure to tenofovir

	Duration of TDF exposure (weeks)				**TOTAL (N=464)**
	< 12 (n=144)	**12-22 (n=203)**	**> 22 (n=117)**	***p*-value**	
1st Postnatal visit (< 2 months, 0-8 wks)					
Length-for-age Z-score	-0.54 (-1.14 to 0.77)	-0.54 (-1.09 to 0.39)	-0.55 (-1.07 to 0.32)	-	-0.54 (-1.14 to 0.39)
Stunted (LAZ<-2SD), (n)[1]	12.4% (14/113)	14.7% (27/184)	9.3% (10/107)	0.42	12.6% (51/404)
2nd Postnatal visit (2-4 months, 9-20 wks)					
Length-for-age Z-score	-0.11 (-1.13 to 0.67)	-0.41 (-1.28 to 0.34)	-0.28 (-1.13 to 0.54)	-	-0.29 (-1.21 to 0.52)
Stunted (LAZ<-2SD), (n)[1]	11.7% (11/94)	9.1% (15/165)	8.8% (9/102)	0.74	9.7% (35/361)
3rd Postnatal visit (5-7 months, 21-32 weeks)					
Length-for-age Z-score	-0.28 (-1.19 to 0.68)	-0.28 (-1.19 to 0.61)	-0.28 (-1.19 to 0.60)	-	-0.28 (-1.19 to 0.66)
Stunted (LAZ<-2SD), (n)[1]	9.5% (10/105)	11.8% (21/178)	6.5% (6/93)	0.37	9.8% (37/376)
4th Postnatal visit (8-10 months, 33-44 weeks)					
Length-for-age Z-score	-0.46 (-1.3 to 0.49)	-0.46 (-1.2 to 0.46)	-0.21 (-0.88 to 0.67)	-	-0.44 (-1.2 to 0.48)
Stunted (LAZ<-2SD), (n)[1]	10.3% (10/97)	9.0% (15/166)	4.6% (4/88)	0.32	8.3% (29/351)
5th Postnatal visit (≥11 months, > 44 weeks)					
Length-for-age Z-score	-0.82 (-1.75 to 0.01)	-0.65 (-1.32 to 0.26)	-0.51 (-1.14 to 0.11)	-	-0.65 (-1.36 to 0.11)
Stunted (LAZ<-2SD), (n)[1]	17.6% (16/91)	9.15% (14/153)	9.7% (6/62)	0.12	11.8% (36/306)

Values are median (IQR) or column % (n); p-values calculated using Chi2 statistic

TDF, tenofovir (exposure calculated from day of antiretroviral initiation until day of birth); LAZ – length-for-age Z-score; calculated using World Health Organization MSGR (adjusted for gestational age and sex)

[1] Missing data for lengths: group denominator (n) for length measures as follows: 1st visit = 404; 2nd visit = 361; 3rd visit = 376: 4th visit =351; 5th visit =306

There was no association between duration of *in utero* TDF-exposure and LAZ in either univariable or multivariable analysis (supplemental table 9.8.3): mean adjusted difference in LAZ (aβ) for >22 vs <12 wks, -0.12 (95% CI: -0.47;0.23); aβ for 12-22 vs <12 wks, -0.06 (95% CI: -0.35; 0.24). The model was adjusted for maternal VL at ART initiation, height, SES, preterm delivery, hazardous drinking in the early postpartum period, and current breastfeeding as a time-varying covariate (supplemental table 9.9.3).

Maternal height (aβ=0.03 per cm increment, 95% CI: 0.01;0.05), log VL at ART initiation (aβ= -0.15 per log increase, 95% CI: -0.29; -0.0001) and infant gestational age [aβ=-0.73 (95% CI -1.3; -0.12) for <34 weeks and aβ=-0.1 (95% CI -0.5;0.32) for 34-37 weeks vs. ≥37 weeks] were strongly associated with infant LAZ.

Supplemental table 9.8.3. Regression analyses of infant length-for-age Z-scores by duration of *in utero* exposure to tenofovir

	Univariable mixed-effects regression (N=461)		**Multivariable mixed-effects regression[1] (N=323)**	
Duration of *in utero* exposure to tenofovir	Difference in LAZ (95% CI)	*p*-value	Difference in LAZ (95% CI)	*p*-value
> 22 weeks	0.05 (-0.22 to 0.31)	0.72	-0.12 (-0.47 to 0.23)	0.49
12- 22 weeks	-0.02 (-0.25 to 0.21)	0.87	-0.06 (-0.35 to 0.24)	0.71
< 12 weeks (reference)	0	-	0	-

95% CI – 95% confidence interval; LAZ – length-for-age Z-scores, calculated using Fenton (birth) and World Health Organization (postnatal) growth reference standards (adjusted for sex and gestational age);

[1] Adjusted for maternal characteristics at initiation of antiretroviral treatment (height, log10 HIV viral load, hemoglobin, cd4 cell count, socio-economic situation), preterm delivery, hazardous drinking in the early postpartum period, and time-varying, current breastfeeding

9.8.5 Discussion

Our findings demonstrate a reassuring lack of association between duration of TDF exposure *in utero* and linear growth in the first year of life among breastfed HEU infants, and patterns of early linear growth that are broadly comparable to those of the general population. We further demonstrate two independent risk factors for suboptimal linear growth among HEU children, namely high maternal VL at initiation of treatment in pregnancy, and premature delivery.

The lack of a significant effect of TDF-exposure on infant linear growth is consistent with findings from other African data on this association. A recent study in Malawi reported no negative growth effects among breastfed HEU children exposed to TDF vs. non-TDF-containing ART regimens in a setting of universal maternal ART during pregnancy.[16] A reflection of the evolving Malawian national PMTCT guidelines at the time, mothers with infants exposed to TDF-containing regimens were encouraged to breastfeed for 12 months compared to the recommended weaning age of 6 months for the earlier group who comprised all non-TDF-exposed infants. The authors postulate that the higher attained 12-month LAZ among TDF-exposed infants (LAZ at 12 months higher by 0.48, 95% CI 0.25 to 0.71) was mediated through extended breastfeeding. TDF is a highly potent antiretroviral;[17] an additional explanation may be that maternal viral suppression was achieved more effectively and rapidly among women receiving the TDF-containing regimen, limiting fetal exposure to circulating virus and its consequences. In an earlier report from Uganda and Zimbabwe, where ART was restricted to women with advanced disease, TDF-exposed children under 2 years of age also attained higher LAZ than their TDF-unexposed counterparts (LAZ at 48 weeks, -1.13 in TDF-exposed vs. -2.22 in TDF-unexposed group; p=0.03); however, the differences were negated over time, with no residual differences by age 3 years. [18] While the recent IMPAACT-PROMISE results raise concern regarding bone effects of LPVr-containing ART-exposure, no association between TDF and either BMC or neonatal growth was observed.[5]

Reasons for the apparently differential effect of TDF on bone growth in the African compared to US cohorts remain unclear but may be due in part to differences in prescribing practices and indications for treatment. In contrast to the observational African cohorts, the US cohorts have been followed over several years and in multiple sites. Over time, substantial changes have occurred in prescribing patterns resulting in highly heterogeneous ART exposure, making it difficult to single out the effects of a single drug whilst also increasing the risk for confounding by indication.[3,4] In particular, the use of TDF with a PI has generally been more common in the US than in African settings including ours,[16,18,19] and residual confounding may explain some of the differences observed in observational studies. Differences in co-occurring risk factors for poor child growth may however also contribute to the differential results. For example, the background prevalence of childhood undernutrition – and intergenerational stunting - is generally higher in resource limited settings;[12] potential adverse growth effects resulting from TDF exposure may be difficult to distinguish in background settings of impaired child growth.

The HEU infants in our study demonstrated linear growth comparable to the general South African population; the estimated prevalence of stunting in our study (12% at 1 year) is roughly half the national South African prevalence of stunting among children <3 years.[20] In keeping with findings from other settings in sub-Saharan Africa, the average LAZ of our cohort decreased over time, highlighting the need for better complementary feeding practices in resource-limited settings where food insecurity and low dietary diversity are common.[16]

Of note, our data suggest a confounding effect of gestational age at delivery on the relationship between duration of TDF exposure and infant linear growth.[21] Although prematurity is a well described, independent predictor of childhood stunting, the association seen in our data is partly artificial as a result of how our exposure variable was defined; infants born at earlier stages of pregnancy were necessarily exposed to ART for shorter periods of time. Nonetheless, maternal HIV infection is a known risk factor for preterm delivery, which in turn substantially increases the risk of child mortality and morbidity including suboptimal growth.[22,23] As such, identifying and providing appropriate care to premature infants should be integral to strategies aimed at optimizing HEU child health.

We found higher pre-treatment maternal VL to be associated with reduced postnatal linear growth. Several possible mechanisms can be hypothesized. Firstly, higher HIV VL is strongly associated with increased immune activation.[24,25] Substantial immune activation in maternal/fetal units – commonly seen in chronic viral infections during pregnancy - has been associated with long-term risk of neurodevelopmental disorders; fetal programming for linear growth might be similarly affected. [26,27] Although severe immune reconstitution inflammatory syndrome (IRIS) is not common in pregnancy, the fetal effects of maternal immune reconstitution during pregnancy (particularly among women with advanced disease initiating potent ART) are not clear. The risk of ART-related bone resorption in HIV-infected adults appears to be highest among those initiating treatment at advanced disease stages,[6,28] and a similar relationship may exist between disease severity at ART initiation in pregnancy and subsequent infant health outcomes. HIV disease severity is also associated with increased risk of maternal cytomegalovirus (CMV) reactivation, which increases the risk for congenital CMV and in turn, growth restriction. [26,29,30] Finally, HEU children born to women with advanced HIV disease are known to be at higher risk for common childhood infections;[12] in turn, recurrent infections substantially increase the risk of childhood stunting.[21]

Our data are unique in providing longitudinal clinical growth data on a well-characterized cohort of TDF-exposed HEU children not exposed to PI-containing regimens, in a setting with universal maternal ART and breastfeeding. However, our study is limited by the lack of a non-TDF exposed comparison group, the lack of serum bone markers and radiographic measures of BMC. Moreover, these observational data could not control for possible unobserved systematic differences between TDF exposure categories, and data from randomized clinical trials comparing child growth outcomes following *in utero* exposure to different drug combinations remain critical.

9.8.6 Conclusion

As HIV-free survival continues to increase dramatically across Africa, optimizing the health of HEU children is a growing priority. Long-term monitoring of potential adverse growth effects of *in utero* ART exposure is a crucial step towards finding optimal drug regimens for pregnant and breast feeding women. We found no evidence of adverse linear growth following *in utero* TDF exposure, and demonstrate maternal HIV disease severity and premature birth as risk factors for poor infant growth. These findings provide reassuring safety data in support of currently recommended ART regimens for pregnant women, and support prevention of premature delivery with early diagnosis and treatment of HIV-infected women as crucial strategies to optimize the health of their uninfected children.

9.8.7 Acknowledgements

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SLR assisted with collection of data, conducted the analysis and wrote the first draft of the manuscript. JJ provided consultation on the concept, manuscript drafting and analysis. LM and EJA conceived the MCH-ART study, and were responsible for study design, funding, implementation and overall leadership. TKP was the study coordinator. TKP and KB were responsible for data management and oversight. KB contributed to the data analysis. SO was responsible for data cleaning processes. AR and AZ were the senior study managers and provided oversight of all study administration processes. All authors contributed to and approved the final manuscript.

9.8.8 References

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