Health and survival of HIV perinatally exposed but uninfected children born to HIV-infected mothers

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Abstract: (250/250 words)

Purpose of review: the number of HIV-exposed but uninfected (HEU) infants exposed to both HIV and multiple antiretroviral drugs in utero and during prolonged breastfeeding is increasing in low-income countries where HIV prevalence is the highest. We review recent evidence on the effects of perinatal/postnatal exposure to maternal HIV and combined antiretroviral therapy (cART) on health outcomes of HEU children (mitochondrial and metabolic toxicity, adverse pregnancy outcomes, neurodevelopment, growth, infectious morbidity and mortality).

Recent findings: Several studies have reported ART-associated mitochondrial toxicity and metabolic disorders with conflicting results on adverse pregnancy outcomes, underscoring the need to conduct further investigations on these questions. Studies about congenital abnormalities report no significant differences between HEU exposed to ART and HIV-unexposed (HUU) children. Updated French data showed no significant difference in cancer incidence between HEU cART-exposed children and the general paediatric population. Furthermore, HEU children exposed to maternal cART have modest but significant impairment of development and a higher risk of growth impairment. Finally, HEU have higher risks of infections (mainly low respiratory tract infections and diarrhoea) and malaria than HUU children, particularly in children not breastfed or after early weaning. Higher mortality risk from infectious disease is reported in HEU compared to HUU children.

Summary: As we move towards the elimination of MTCT, HEU children are an emerging population whose health outcomes remain to be fully described. Future large cohorts of HEU children using careful comparison groups of HUU in the post-ART era are needed to better understand their long-term health outcomes.

Keywords: child; HIV-exposed uninfected; health; mortality; infectious morbidity.

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Introduction

Antiretroviral therapy (ART) among HIV-infected pregnant women has dramatically decreased risk of mother-child-transmission worldwide, with the number of newly HIV-infected infants decreasing from 570,000 in 1999 to 240,000 in 2013 (1). As a consequence of this success, the number of HIV-exposed but uninfected (HEU) infants exposed to both HIV and multiple antiretroviral drugs in utero and often during prolonged breastfeeding has increased in low-income countries where HIV prevalence is the highest (2). For this growing population, it is crucial to measure their short and long-term outcomes. There is some evidence that HEU African children are at increased risk of morbidity, early failure to thrive and mortality compared to HIV-unexposed uninfected (HUU) counterparts (3, 4). Appropriate comparisons of HEU and HUU were rare before the antiretroviral era. With increasing ART access, it is methodologically challenging to disentangle effects of HIV exposure from those associated with ART exposure, particularly in regions where malnutrition and co-morbidities are common and could increase the potential effects of these exposures. Few data are available so far on long-term effects in this context, and for obvious reasons there is no randomised clinical trial data to directly compare the effects of ART-exposure in HEU children. However, information from observational prospective cohorts provides useful evidence. Our aim is to summarize recent evidence on the effects of perinatal/postnatal exposure to HIV and ART on health outcomes in children.

Review: effects on health outcomes in HEU children

1. Mitochondrial and metabolic toxicity attributable to ART exposure

Multiple potential mechanisms of ART-associated mitochondrial toxicity in HEU infants have been proposed and several studies have identified toxicity markers, although findings regarding presence of clinical mitochondriopathy and mitochondrial DNA (mtDNA) levels in HEU children conflict (5).

A study of 133 HEU, ART-exposed infants and 73 healthy controls in Spain investigated mitochondrial respiratory chain (MRC) function alongside mitochondrial mass (MM) during infancy. Although groups had similar MM, an inverse correlation between MM and MRC enzymatic activity of complex IV (CIV) was seen, with the latter significantly lower in HEU infants when accounting for MM. This difference remained significant at age 12 months, while no clinical manifestation of mitochondrial dysfunction was observed in HEU children (6). In a Cameroon study (38 HEU, 118 HUU infants), HEU infants with in utero/neonatal exposure to ZDV or NVP had significantly lower pre-prandial insulin levels at age 6 weeks than HUU infants in adjusted analyses (7); ZDV-exposed infants had higher insulin levels at higher fuel consumption levels than NVP-exposed infants, suggesting altered metabolism and greater insulin sensitivity. In a study in New-York State (infants born 2005-2008), more HEU ART-exposed infants had a positive screen for inborn errors of metabolism than HUU newborns (2.2% versus 1.2%, p=0.000025), with the pattern of metabolic disorders suggesting mitochondrial dysfunction mechanisms (8). In USA, zidovudine was associated with an increased risk of metabolic toxicity (RR:1.69; 95% Confidence Interval [CI]: 1.08-2.64) (9).

These findings underscore the need for further longitudinal studies to follow-up ART-exposed HEU children to explore persistence of metabolic abnormalities, clinical significance, and potential mechanisms.

2. Adverse pregnancy outcomes, congenital abnormalities, cardiac effects and cancer

In a meta-analysis (35 studies: 20 prospective cohorts, 12 retrospective cohorts, three case-control studies), HIV infection in women not receiving ART was clearly associated with preterm birth, low birthweight, small for gestational age (SGA), and stillbirth, especially in sub-Saharan Africa (10). Recent data on prevalence of congenital abnormalities in HEU children, and associations with in utero ART exposure are presented in Table 1, which are consistent with the literature to date, indicating a similar risk of defects as in the general population (11-20).

Lack of clinically significant cardiac toxicity was reported from the US: comparison of left ventricular function and structure adjusted for age and body surface area between 417 HEU (95% cART-exposed) and 98 HUU controls aged 2-7 years showed no significant differences (21). Another study showed that prevalence and risk of ventricular septal defects and congenital heart defects among zidovudine-exposed infants is not significantly different from that in infants exposed to non-zidovudine-containing regimens (16). However, sub-clinical findings associated with in utero cART-exposure were reported, requiring further prospective investigation.

The French Perinatal Cohort has presented updated information on incidence of cancers in >15,000 HEU children exposed to ≥1 NRTI in utero with a median age of 9 years: although cancer incidence overall was not significantly different from the expected rate in the general paediatric population (standardized incidence ratio 0.8 [95%CI 0.47-1.24]), incidence among children exposed to didanosine was twice the rate expected (SIR = 2.5 [95%CI 1.01-5.19]) and associated with first trimester exposure (22). Nucleos(t)ide analogs can integrate into nuclear DNA and terminate DNA replication. A “genotoxic signature” in cord blood cells of ZDV+3TC-exposed neonates was reported in 2013 (23); in a subsequent study both ZDV and TDF exposure were associated with altered regulation of genes involved in DNA repair, DNA/RNA synthesis, cell cycle and telomere maintenance, with aneuploidy significantly increased in ZDV and TDF-exposed neonates versus controls. Aneuploidy may be a predisposing factor for cancer, but persistence of these genotoxic signatures over time and clinical implications remain unclear (24).

3. Neurodevelopment

Investigation of potential effects of in utero ART-exposure on neurodevelopment is complicated by the need to adjust for other factors including maternal substance use, socio-economic factors and other infections. Recent studies (Table 2) have adjusted for many of these confounders (25-29). In 2013, atazanavir was implicated in language delay in HEU children in the SMARTT study, but a later SMARTT study suggested that any effect may be transient as the increased risk of late language emergence at age 12 months was no longer apparent at age 24 months. Another study found no association between meconium atazanavir concentrations and late language emergence (Table 2). More recently, didanosine plus stavudine exposure was associated with an increased risk of both neurodevelopmental (RR = 12.40, 95%CI 5.29-29.08) and language (RR = 4.84, 95%CI 1.14-20.51) delays (9). A study conducted in Asia

demonstrated that HEU children have modest but significant impairment of development, particularly concerning language that needs to be investigated in terms of long-term significance (28).

4. Growth

The one study in the pre-ART era reported that anthropometric characteristics of HEU children were similar to those of HUU children over the first 18 months of life (30). Since then, there is evidence that early growth of HEU children in the first 3-6 months of life is poorer than that of their HUU counterparts without any consideration of ART exposure effects (31)(Table 3) (32-39). HIV infection can directly contribute to disturbances in both growth and weight gain in early childhood (36). At 6 months of age, anthropometric weight and height Z scores of HEU children were lower than those of HUU children in Zambian children participating in a trial of micronutrient-fortification after adjustment for treatment arm, socioeconomic factors, breastfeeding and gender (32). In Ugandan infants, underweight was more frequent in HEU versus HUU children (adjusted OR 2.32; 95%CI 1.32, 4.09; P=0.006) but there was no evidence for an association with stunting or wasting (34). In a US cohort, growth of HEU and HUU children were similar over the first two years of life, but HEU infant birth weight was lower and HEU children had a pattern of slightly accelerated growth over this period. HEU children had less subcutaneous fat and decreasing mid-upper arm circumference over time when compared with US standards in the first two years of life (35). Recently, a comparative study of cohorts of HEU and HUU children aged 6-12 years in Zambia reported that although HEU children were smaller and had lower percent fat than HUU children, this appeared to be due mainly to their poorer socioeconomic status (33) (Table 3). Few studies on reversibility of early growth faltering in HEU children have been reported. In a before/after intervention study in Haiti, early growth faltering observed in HEU improved at 12 months of age after an intervention based on lipid-based nutrient supplementation, education, promotion of existing clinical services, and social support (39).

Exposure to antiretroviral drugs perinatally could also affect growth among HEU children. However initial studies exploring these effects were reassuring. There are conflicting data about the effects of tenofovir use during pregnancy. Recently, tenofovir was reported to be associated with significantly lower neonatal bone mineral content (40), but not with increased risk of low birth weight or SGA (41, 42). By most measures, in utero tenofovir-exposure did not significantly predict infant birth weight or growth through 6 months of age (43). However, ritonavir-boosted protease inhibitors have been associated with an independent higher risk of prematurity that has a confounding effect on growth (44). In a study pooling children from the Mashi and Mma Bana prospective cohorts in Botswana, excluding multiple and preterm births, cART-exposure was associated with lower birth weight compared to zidovudine exposure, with a rapid correction of the weight over the first 6 months of life (37). However, recent findings report after their 24-month follow-up, that in-utero cART-exposed compared to zidovudine-exposed children had significantly lower Length-for-Age (LAZ) and Weight-for-Age (WAZ) z-scores (38). As poor growth impacts childhood and adult mortality, these findings raise concerns for potential lasting health impacts among HEU children with in-utero cART exposure.

5. Infectious disease morbidity and mortality

For years, cohort studies from developing countries have reported that HEU infants have increased risk

of morbidity and mortality from infectious disease compared to HUU infants (45), often correlated with advanced maternal HIV disease, and maternal death (3, 46) (Table 4), (45, 47-57). A recent review identified 13 studies published in the past decade (58), where mortality rates in HEU infants ranged from 34/1000 in Malawi between 2004-2010 to 116/1000 child-years by 18 months in rural Uganda between 2002-2010 (34, 35). The ZVITAMBO project in Zimbabwe was one of the largest studies to investigate this; the 2-year mortality rate was a three-fold higher in HEU compared to HUU (9.2% vs 2.9%) and cause of death was mostly lower respiratory tract infection (45). High incidence of infectious morbidity and mortality has been repeatedly reported in HEU infants in low-resource settings (4, 45, 47-52). Finally, in a recent systematic review, the mortality risk in HEU children compared to HUU children was about double at 24 months (pooled risk ratio:2.4 (95% CI: 1.1-5.1; I2=93%, three studies), although the association was not statistically significant at 12 months (46). Two recent publications from Europe, unfortunately without comparison to HUU group, have also reported a high incidence of severe infectious events occurring in HEU infants despite a low infectious disease burden (51, 52). The French Perinatal Cohort showed an association between serious bacterial infections occurring over the first year of life in HEU infants and maternal CD4 cell count <350 cells/µl (OR= 1.7 (95%CI 1.2-2.6 versus maternal CD4 >500) (52).

Other studies have described more severe infections, more hospitalizations for infectious diseases, infections caused by uncommon pathogens, or pneumonia failing empiric treatment in HEU compared to HUU children (49, 59-61). In Botswana, among children with pneumonia, HEU children had higher in-hospital mortality rates compared to HUU children (49). Most infections reported involved the respiratory tract, and occurred in the first year of life peaking between 2 and 6 months of age (52, 60, 62).

Moreover, invasive infections due to encapsulated bacteria like group B Streptococcus (GBS) (48, 51, 63) and Streptococcus pneumoniae (53) were also more often observed in HEU infants (61). GBS is the main cause of neonatal sepsis associated with a high mortality rate in Sub-Sahara Africa (64). Report from the South African nationwide invasive pneumococcal disease (IPD) laboratory surveillance showed that HEU infants had a three-fold greater risk of IPD than HUU infants over the first six months of life before and after implementation of pneumococcal vaccination (53).

Absence of breastfeeding is associated with increased risk for infectious disease in early childhood in HEU infants. A recent cohort study in South Africa reported similar overall mortality rates in HEU and HUU infants, but among those who had never been breastfed, HEU infants had increased risk of mortality compared to HUU infants (54). In Uganda also, among infants aged 6-11 months non-breastfed HEU had a higher risk of hospitalizations, severe febrile illness, severe diarrhoea and severe malnutrition than non-breastfed HUU, whereas there were no differences of morbidity outcomes between breastfed HEU and HUU infants (47). Similar results were also reported in KwaZulu Natal, where exclusive breastfeeding was associated with fewer acute, persistent and total diarrheal events than mixed or no breastfeeding in both HEU and HUU infants (54). The Kesho-Bora study reported a seven-fold higher risk of death within 6 months in never breastfed or early-weaned HEU children compared to breastfed children (55). Similarly, in a Zambian study an increased mortality risk in HEU infants was observed after weaning, ranging from a two-fold increase when weaning at 4-5 months of age to a 4.2 fold increase

when weaning at 12-18 months compared to weaning > 18 months (65). However, for HIV-infected mothers, it is well known that breastfeeding in the absence of cART places their HEU infant at risk of HIV infection and breastfeeding with cART will expose them to ARVs toxicities. A recent model-based analysis showed that optimal breastfeeding duration depends substantially on maternal HIV disease and availability of postnatal ARVs (66).

6. Mechanisms underlying susceptibility to infections

ART-related mechanisms may include the depressed haematopoiesis, involving several lineages and persisting for several years, associated with perinatal exposure to nucleoside reverse transcriptase inhibitors (9, 10). Maternal HIV infection may also influence infant immune development (67, 68). Several studies have demonstrated reduced trans-placental transfer of maternal antibodies, reduced thymus output and functional defects of antigen-presenting cells in HEU infants. The mechanisms of these changes are unknown but might involve inflammatory responses at the placental level or trans-placental transfer of HIV particles or soluble mediators with immunosuppressive properties. Owing to the close association of growth with immune function, an understanding of growth patterns is an important tool to ensure provision of appropriate care for HEU children. Altered immune responses to vaccination could also contribute to the higher rate of infection in HEU compared with HUU infants. In South Africa, although comparison of vaccine-specific antibody responses were equivalent in HEU and HUU infants, a large fraction of both groups had antibody titers below the protective levels over their first two years of life (69). In another study, significant, but transient, differences in cytokine production by mononuclear cells were detected between HEU and HUU infants (70). Their hypothesis was that time course of innate immune deviation early in life corresponds to the clinical window of vulnerability to infections in HEU infants and may be partially responsible for their increased morbidity and mortality from infectious disease.

Discussion

Recent studies have provided new evidence that HEU infants have poorer health outcomes than HUU infants, although some have acknowledged methodological weaknesses. Concerns remain regarding ART-associated mitochondrial toxicity and metabolic disorders, with a higher risk of metabolic disorder with zidovudine exposure (9). The data on congenital abnormalities is reassuring, but more research is needed to assess every individual drug and how ART regimens affect other perinatal outcomes. Some studies suggest neurological development impairment in HEU but longitudinal studies are warranted to examine the long-term clinical significance. HEU children exposed to maternal cART are at a higher risk of growth impairment during their first 24 months of life. Finally, HEU are at higher risk of morbidity and mortality from infectious disease compared to HUU. Evidence to date describing mortality in HEU infants is primarily from data from the pre-ART era, before the scale-up of PMTCT interventions and describes early infant mortality <2 years in breastfed populations (4). Further studies are needed to evaluate long-term health outcomes in HEU-infants in the ART era.

Many of the adverse outcomes associated with in utero HIV and cART exposure have multifactorial

aetiologies, but those related to HIV exposures could involve the environment of an HIV-affected household, including poor socio-economic conditions, increased maternal microbe carriage, and exposure to more infectious agents. Moreover, poor outcomes could simply be attributable to universal risk factors like low birthweight, prematurity, and small for gestational age that are also more prevalent in HEU not exposed to cART compared to HUU (10). Finally, maternal death, formula feeding and suboptimal breastfeeding practices clearly also reduce infant immune defences leading to a higher infectious disease morbidity (3, 46, 57). Adjustment for confounders is needed to ascertain properly the magnitude of the effects of HIV and cART exposure on health outcomes in HEU children.

Conclusion

To conclude, there is a need to better understand and assess the risks to health impairment associated with in utero and early life exposure to HIV and antiretroviral drugs in HEU children, particularly as these children become increasingly numerous. Ideally, long-term follow-up of large prospective cohorts of HEU children with careful comparative groups of HUU children should be constituted, but this poses key logistical and financial challenges.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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* of special interest

** of outstanding interest

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Table 1: Summary of recent studies investigating congenital abnormalities in HEU infants

Study	Setting	Subjects	Key results	Reference
Ford et al, 2014 Systematic review of 16 studies reporting outcomes of cotrimoxazole exposure	Global	4196 women (HIV positive and HIV negative) receiving cotrimoxazole during pregnancy	Pooled prevalence of congenital defects = 3.5% (95% CI 1.8, 5.1%). Pooled prevalence for studies including women with 1st trimester exposure = 4.8% (95% CI 0.6%, 8.9%) Quality of the evidence was assessed to be very low (GRADE)	(11)
Ford et al, 2014 Systematic review of 21 studies reporting outcomes of 1st trimester EFV exposure	Global	2026 live births among women exposed to efavirenz during the 1st trimester of pregnancy	Pooled prevalence = 1.63% (95% CI 0.78, 2.48); 0.05% prevalence for neural tube defects One of 44 congenital defects was a neural tube defect No difference in risk of defects between efavirenz and non-efavirenz-containing regimens received in 1st trimester (relative risk 0.78, 95% CI 0.56, 1.08) [12 studies]	(12)
Floridia et al, 2015 Multi-site surveillance study of ART-exposed pregnancies	Italy	1257 pregnancies with ART use ending in live birth, still birth, termination of pregnancy	Prevalence = 3.2% (95% CI 1.9, 4.5) for 1st trimester exposure to any ART, 3.4% (95% CI 1.9, 4.9) for exposure in 2nd or 3rd trimester only. No associations between 1st trimester exposure to any ART, to any ART class or to EFV and major birth defects.	(13)
Liu et al, 2014 Multi-site, pilot pregnancy ART registry	South Africa and Zambia	600 women conceiving on cART, October 2010 and April 2011	Prevalence of major congenital anomaly = 2.2% (13/583)	(14)
Vannappagari et al, 2015, Antiretroviral	Global	12 780 singleton birth outcomes with in utero ZDV exposure. Comparison exposure to ZDV-containing ARV regimens versus non-	RR for spontaneous abortions was 0.18 (95% confidence interval [95% CI] 0.14-0.22); induced abortions 0.28 (95% CI 0.22-0.36); stillbirths 0.76 (95% CI 0.51-1.12); premature births 1.00 (95% CI 0.87-1.15) and LBW 1.17 (95% CI 1.02-1.33).	(15)

Pregnancy Registry		ZDV ARV regimens		
Vannappagari et al, 2016, Antiretroviral Pregnancy Registry		15,451 live birth outcomes	The prevalence of congenital heart defects was 0.60% (95% confidence interval: 0.47, 0.74) for infants exposed to zidovudine-containing regimens and 0.50% (95% confidence interval: 0.26, 0.88) for non-zidovudine regimens. The relative risk comparing the 2 was 1.18 (95% confidence interval: 0.64, 2.17).	(16)
William et al, 2015 The Pediatric HIV/AIDS Cohort Study's Surveillance Monitoring of ART Toxicities (SMARTT) Study	US	2580 HIV-exposed uninfected children	In adjusted models, no association of first-trimester exposures with congenital abnormalities (CA) was found for any ARV, for combination ARV regimens, or for any drug class. No individual ARV in the reverse transcriptase inhibitor drug classes was associated with an increased risk of CAs. Among protease inhibitors, higher odds of CAs were observed for atazanavir sulfate (adjusted odds ratio [aOR], 1.95; 95% CI, 1.24-3.05) and for ritonavir used as a booster (aOR, 1.56; 95% CI, 1.11-2.20). With first-trimester atazanavir exposure, risks were highest for skin (aOR, 5.23) and musculoskeletal (aOR, 2.55) CAs.	(17)
Sibiude et al, 2015 Multi-site prospective French Perinatal Cohort	France	12,888 live births in 1994-2010 with in utero exposure to ART	Overall rate of congenital heart defects (CHD)= 0.93% (95% CI 0.76, 1.1%) Ventricular septal defects most common (75% of defects) 1st trimester ZDV exposure associated with CHD (adjusted odds ratio 2.2 (95% CI 1.5, 3.2) Four of 120 children with defects died due to heart failure	(18)
Sibiude et al, 2014 Multi-site prospective French Perinatal Cohort	France	>10,000 live births from 1994 to 2010	Prevalence of congenital defects in infants with 1st trimester exposure ranged from 4.4% to 7.0% depending classification used A significant association was found between exposure to EFV in the 1st trimester and neurological defects (adjusted odds ratio = 3.15), exposure to ZDV with congenital heart defects (aOR = 2.34), and	(19)

			didanosine, with head and neck birth defects (aOR = 2.89).	
Phiri et al, 2014 Health insurance record linkage study	United States	806 infants (671 exposed to ART in utero, 221 with 1st trimester exposure)	Prevalence of major birth defect = 4.0% (32/806) Prevalence in those with 1st trimester exposure = 4.1% (95% CI 1.5, 6.7%) and 2nd/3rd trimester exposure = 4.4% (95% CI 2.5, 6.3%) No association between 1st trimester exposure to any ART or to any drug class and congenital defects	(20)

Table 2: Recent studies assessing neurodevelopment in HEU children

Study	Setting	Subjects	Outcome	Key results	Reference
Sirois et al, 2013 SMARTT	USA	374 HEU infants with in utero exposure to ART and 49 HEU infants without exposure	Bayley-III (cognition, language, motor skills, socio-emotional development and adaptive behaviour) at around 12 months of age	No association between exposure to cART or any ART regimen in analyses adjusting for potential confounders; lower mean language score was reported in atazanavir-exposed infants including in sensitivity analyses without adjustment for preterm delivery or small-size-for-gestational-age.	(25)
Ngoma et al, 2014	Zambia	97 HEU with in utero and postpartum exposure (12 months) to ZDV+3TC+LPV/r and 103 HUU children	Capute Scales to assess non-verbal problem solving and language development. Developmental delay defined as Capute Full-Scale Developmental Quotient <85	Developmental delay was found in 14.6% of HUU and 8.3% of HEU children. In adjusted analyses, low birthweight was the only risk factor for developmental delay	(26)
Rice et al, 2013 SMARTT	USA	792 HEU children, 86% with in utero ART exposure	Late language emergence (LLE). 12 months: MacArthur-Bates Communicative Development Inventory (CDI). Age- and sex-adjusted percentiles in four domains with LLE defined as score ≤10th percentile in any domain. 24 months: the Communication scale of the Ages and Stages Questionnaire (ASQ), with LLE defined as total score ≥1 SD below the age-specific mean.	26% of 12 month olds and 23% of two year olds had LLE. No significant differences in summary scores for CDI or ASQ by in utero exposure to cART or any ARV class were found in adjusted analyses. Atazanavir was associated with significantly increased risk of LLE at age 12 months, but not at 24 months of age	(27)
Kerr et al, 2014	Thailand &	160 HEU 6-8 years old children and 1678 HUU age and gender	Wechsler Intelligence tests Stanford-Binet Intelligence Scales Children's Behavior Checklist	HEU children have modest but significant reductions in Verbal IQ, Full Scale IQ, and Bead Memory (assessed by Stanford Binet) compared to HUU	(28)

PREDICT trial	Cambodia	matched controls	CBCL syndrome based scales	children	
Himes et al, 2015 SMARTT	USA	175 HEU infants with in utero atazanavir exposure and a meconium sample available	LEE according to CDI as above.	LEE risk was significantly lower in infants with higher meconium concentrations of atazanavir.	(29)

Table 3: Summary of recent studies investigating growth in HEU infants

Study	Setting	Subjects	Comparisons	Key results	Reference
Filteau et al, 2011 Zambian cohorts post-trial	Zambia	Children < 18 months of age participating in a trial of micronutrient-fortified	125 HEU versus 382 HUU	In unadjusted analyses, most anthropometric Z scores of HIV-EU children were lower than those of HIV-unexposed children; after adjustment for treatment arm, socioeconomic factors, breastfeeding and sex, head and arm circumference Z scores remained lower. Subscapular skinfold Z scores were lower among HIV-EU than HIV-unexposed children at 6 months but not 18 months.	(32)
Nicholson et al, 2015 Zambian prospective cohort studies	Zambia	HEU and HUU	111 HEU versus 279 HUU children aged 6-12 years	Anthropometric measures were lower among HEU than HUU children, significantly so for hip circumference (age- and sex-adjusted difference -1.74 cm; 95% confidence interval (CI) -3.24, -0.24; P = 0.023) and mid-upper-arm circumference (adjusted difference -0.63 cm, 95% CI -1.23, -0.04; P = 0.037) and with borderline effects for body mass index, thigh circumference and subscapular skinfolds. HEU children had significantly lower total, trunk, and limb fat percentages. All anthropometric and body composition differences became non-significant after adjustment for sociodemographic variables which differed between HEU and HUU children. All anthropometric and body composition differences became non-significant after adjustment for sociodemographic variables which differed between HEU and HUU children.	(33)
Muhangi et al, 2013 Ugandan cohorts	Entebbe and Katabi sub-county,	1 year old HEU and HUU children	122 HEU versus 1380 HUU	Underweight was significantly higher in HEU versus HUU children (adjusted OR52.32; 95% CI 1.32, 4.09; P=0.006) but no evidence for an association with stunting or with wasting.	(34)

conducted before the ART era	Uganda				
Neri et al, 2013 US Cohort	USA	Children < two years old	111 HEU versus 82 HUU	No statistical differences were found in anthropometric measurements between HEU and HUU. HEU were smaller than US standards at birth with mean (SD) weight-for-age and weight-for-length z-scores of -0.39 (1.06); P = .002 and -0.35 (1.04); P = .005, respectively. Over the first 2 years of life, there was a trend toward increasing weight-for-age z-score, length-for-age z-score, and weight-for-length z-score in HEU. Subscapular and triceps skinfolds among HEU were lower than national standards and there was a trend that mid-upper arm circumference decreased over time.	(35)
Ramokolo et al, 2014 Prospective multisite cohort study in South Africa	South Africa	Children between 3 and 36 weeks post-partum	In period 1 (3-24 wk), 65 HIV-infected, 502 HIV-exposed uninfected (HEU), and 216 HIV-unexposed infants were included.	Mean weight velocity Z-scores (95% CI) was significantly (P < 0.001) lower in infected [-0.87 (-1.77, 0.04)] than HEU [0.81 (0.67, 0.94)] and unexposed [0.55 (0.33, 0.78)] infants. Length velocity Z-scores showed similar associations.	(36)
Powis et al, 2011 Follow-up of the Mashi and Mma Bana intervention trial	Botswana	full-term, HEU children breastfed until 6 months	619 exposed to maternal triple antiretrovirals (cART) versus 440 zidovudine (ZDV) monotherapy exposed	Mean birth weights were 3.01 kg for HAART and 3.15 kg for zidovudine-exposed infants (P < 0.001) with lower mean birth WAZ, length-for-age (LAZ), and weight-for-length (WLZ) among HAART-exposed infants (all P < 0.001). HAART-exposed infants had greater improvement in WAZ and weight-for-length (WLZ) from birth through 2 months (P = 0.03, P < 0.001, respectively). The WAZ did not differ between groups from 3 through 6 months (P = 0.26). Length-for-age (LAZ) remained lower in HAART-exposed infants but the incidence of wasting or stunting did not differ between exposure groups.	(37)

Powis et al, 2016 Follow-up of the Mashi and Mma Bana intervention trial	Botswana	819 singleton, full-term, HEU children breastfed until 6 months	516 exposed to in-utero triple antiretrovirals (cART) versus 303 zidovudine (ZDV) monotherapy exposed	At 24 months, mean LAZ and WAZ were significantly lower among cART-exposed children (LAZ -1.01 versus -0.74; P = 0.003) (WAZ -0.53 versus -0.30; P = 0.002) in unadjusted analyses. Adjusting for maternal CD4, viral load, enrolment site and maternal anthropometric measures, cART-exposed children had significantly lower LAZ and WAZ at 24 months (P = 0.0004 for both). At 24 months, in-utero cART-exposed children had significantly lower LAZ and WAZ.	(38)
Heidkamp et al, 2012 Before/after intervention study on nutritional support during the first year of life	Haiti	non-breast-fed infants 6-12 months of age attending GHESKIO pediatric clinic in Port-au-Prince, Haiti	73 HEU exposed to intervention versus 294 HEU not exposed (historical control	The intervention and historical control groups did not differ significantly at age 6 mo (wk 0). At age 12 mo (wk 24), the intervention group had a lower prevalence of underweight and stunting than the historical control group (weight-for-age Z-score < -2 SD: 6.8 vs. 20.8%, P = 0.007; length-for-age Z-score < -2 SD: 9.6 vs. 21.2%, P = 0.029). Wasting tended to be lower in the intervention group than the historical control (weight-for-length Z-score < -2 SD: 2.9 vs. 8.9%, P = 0.11).	(39)

Table 4: Summary of recent studies investigating infectious disease and mortality in HEU infants

Study	Setting	Subjects	Comparison group	Key results	Reference
Munyagwa M *et al,* 2012 Prospective cohort	Rural Uganda 2002 – 2010	206 HEU children recruited between 6 weeks and 13 years	89 HIV-infected infants	Mortality rate among HEU was 1.3/100 person-years Predictors of mortality were a hospital admission since the last visit, and decreasing HAZ, WAZ or BMI-for-age Z score	(47)
Koyanagi A *et al*. 2011 Prospective cohort within the ZITVAMBO trial	Zimbabwe	2849 HEU recruited between 0-96 hours of age and followed-up up to 24 months	9207 HUU	Mortality among HEU was a 4-fold higher. HEU infants made an average of 30% more sick visits and 20% more hospitalisations.	(45)
Epalza C *et al,* 2010	Belgium 2001 - 2008	397 HEU newborns	20158 HUU newborns born in the same hospital and period	Higher incidence of GBS sepsis among HEU infants, with a later onset suggesting an increased susceptibility in HEU	(48)
Kelly *et al,* 2015 Hospital-based prospective cohort study	Botswana	64 HEU children aged 1-23 months with pneumonia	153 HUU children aged 1-23 months with pneumonia	HEU children with pneumonia had higher rates of treatment failure and in-hospital mortality during the first 6 months of life.	(49)

Kourtis AP *et al*. 2013 Prospective cohort within the BAN trial	Malawi	2250 HEU recruited at birth exposed to maternal, infant, or no extended antiretroviral prophylaxis during breastfeeding	None	High rate of respiratory infections in infant early life; increases in the rate of respiratory infections, malaria and growth faltering after 6 months of life possible explained by early weaning. Infant cotrimoxazole prophylaxis was significantly associated with lower frequency of severe respiratory/other infectious and gastrointestinal morbidity, and of severe malaria	(50)
Adler et al, 2015 Retrospective cohort study	Belgium	527 HEU infants	None	Incidence rate of severe infections was 16.8/100 infant-years. Invasive pneumococcal disease was a 4-fold higher and group B streptococcus infection was 13-fold higher in these HEU compared to general population rates in Belgium	(51)
Taron-Brocard *et al,* 2014 French Perinatal Cohort	France	7638 HEU neonates	None	Association between lower maternal CD4 counts and risk of serious lower respiratory tract infections in HEU is constant over the first year of life.	(52)
Von Modlenforf *et al,* 2015	South Africa	Children aged <1 year	laboratory- based surveillance database	HEU children are twice as likely to have invasive pneumococcal disease associated hospitalisation and HEU children < 6 months are less likely to survive compared to HUU children.	(53)
Rollins *et al,* 2013 Non-	KwaZulu Natal, South Africa	HEU and HUU exclusively breastfed infants	HEU with replacement feeding.	Higher morbidity and mortality among non-breastfed infants; longer duration of breastfeeding, the better the protection.	(54)

randomised intervention cohort study				No significant difference in the frequency of diarrheal events among exclusively breastfed infants between the HEU and HUU.	
Bork et al, 2014 Kesho Bora trial	Kenya, Burkina Faso and South Africa	751 HEU infants	None	Absence of breastfeeding was associated with only slightly increased risks of maternal-reported morbidity and 4- to 6-fold greater risks of serious infections	(55)
Kuhn et al, 2010 Early weaning trial (4 months vs time of choosing) cohort	Zambia	747 HEU infants	None	The mortality rate was 9.4% by 12 months of age and 13.6% by 24 months of age. Early weaning was associated with an increased risk of mortality	(56)
Marquez C *et al,* 2014 Prospective cohort within the PROMOTE trial	Uganda	200 HIV-exposed children aged 4–5 months	400 HUU	13-fold higher risk of mortality and a higher risk of non-malarial morbidity among HEU children.	(57)

Key points

- The number of HEU infants exposed to both HIV and multiple antiretroviral drugs in utero and during prolonged breastfeeding has increased in low-income countries.
- Recent studies have provided new evidence that HEU infants have poorer health outcomes than HUU infants. There are conflicting results about ART-associated mitochondrial toxicity, metabolic disorders and adverse pregnancy outcomes. There are reassuring results about congenital abnormalities. HEU children exposed to maternal cART have modest but significant impairment of development and growth. HEU have higher risks of infections than HUU children, particularly in children not breastfed or after early weaning and higher mortality risk from infectious disease.
- Adjustment for confounders is needed to ascertain properly the magnitude of the effects of HIV and cART exposure on health outcomes in HEU children.
- There is a need to conduct further investigations on HEU health outcomes. Long-term follow-up of large prospective cohorts of HEU children with careful comparative groups of HUU children should be constituted.