Factors Associated with Lower Respiratory Tract Infections in HIV-Exposed Uninfected Infants

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Abstract

To identify factors that predispose human immunodeficiency virus (HIV)-exposed uninfected infants (HEUs) to higher incidence of severe infections, hospitalization, and death in the first 6–24 months of life compared with HEUs with and without lower respiratory tract infection (LRTI) in the first 6 months of life. Nested case–control study of 107 LRTI+ infants enrolled in the International Site Development Initiative (NISDI) Perinatal and Longitudinal Study in Latin American Countries (LILAC) studies with and 140 LRTI– in the first 6 months, matched by date and place of birth. Infants and mothers had plasma antibodies measured against respiratory syncytial virus (RSV), parainfluenza (PIV) 1, 2, 3, influenza, and pneumococcus 1, 5, 6B, and 14. Compared with LRTI–, mothers of LRTI+ HEUs had lower years of education, lower CD4⁺ cells, and higher HIV plasma viral load at delivery, but similar use of antiretrovirals and cotrimoxazole and other sociodemographic characteristics. LRTI+ and LRTI– HEUs had similar demographic and hematological characteristics and antibody concentrations against respiratory pathogens at birth. At 6 months, the rates of seroconversions to respiratory pathogens and antibody responses to tetanus vaccine were also similar. However, antibody concentrations to RSV were significantly higher in LRTI+ compared with LRTI– HEUs and marginally higher to PIV1. Maternal factors associated with advanced HIV disease, but unrelated to the use of antiretrovirals, cotrimoxazole, or the level of maternal antibodies against respiratory pathogens, contribute to the increased risk of LRTI in HEUs. In HEUs, antiretroviral and cotrimoxazole use, exposure to respiratory pathogens and humoral immune responses were not associated with the incidence of LRTI.

Keywords: HIV-exposed infants, respiratory infection, respiratory syncytial virus, tetanus vaccine, HIV infection

Introduction

INTERRUPTION OF MOTHER-TO-CHILD transmission (MTCT) of human immunodeficiency virus (HIV) has been one of the major achievements in the area of HIV treatment and prevention. Due to the large number of women of reproductive age already infected with HIV, as well as the successes in prevention of MTCT, HIV-exposed, uninfected infants (HEUs) are a rapidly growing population. HEUs experience greater morbidity and mortality than HIV-unexposed, uninfected children (HUUs), especially due to infections. In South Africa, the hospitalization rate of HEUs was 2.7-fold higher than that of HUUs^{1–3}; in Zimbabwe, HEUs

had 2- to 4-fold higher mortality than HUUs.^{4,5} The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) International Site Development Initiative (NISDI) Perinatal Study (Perinatal) and the NISDI Longitudinal Study in Latin American Countries (LILAC) were consecutive prospective cohort studies that enrolled HIV-infected women during pregnancy in several Latin American and Caribbean countries between 2002 and 2009, and followed the mothers and their infants for ≥6 months after delivery/birth. In a prior analysis of the NISDI data, 20% of HEUs had lower respiratory tract infections (LRTIs) in the first 6 months of life, and 41% of these LRTIs required hospitalization.⁶ Overall, 18% of the HEUs enrolled in the NISDI protocols were

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hospitalized due to severe infections. LRTIs have been the most common severe infections leading to hospitalization and death of HEUs, followed by gastroenteritis, sepsis, and malaria.^{1,4,7–10} It is important to note that neither prophylactic use of cotrimoxazole nor breastfeeding decreased the incidence of LRTIs or hospitalizations in HEUs.^{11,12} Although high morbidity and mortality of HEUs have been most frequently reported in resource-limited areas, large European studies have reported similar observations.^{13–15}

Both maternal and infant factors may contribute to the increased susceptibility of HEUs to infections. Maternal HIV infection (especially untreated) compromises vigorous antibody responses to infections and vaccines and produces high concentrations of nonspecific immunoglobulin G (IgG) that compete for the placental IgG receptors. This is reflected in decreased transplacental transfer of pathogen-specific, protective antibodies in HEUs.^{16,17} In addition, HEUs may have their own functional immune defects compared with HUUs, including decreased antibody and cellular immune responses to vaccines and decreased natural killer functionality.^{18–25} HEUs have other phenotypic immune differences compared with HUUs, for which functional significance remains to be determined.

The goal of this study was to determine the contributions of common respiratory viruses and *Streptococcus pneumoniae* (PNC) to the burden of LRTI in HEUs and identify actionable maternal and infant characteristics associated with the risk of LRTI. To this end, we conducted a nested case-control study of LRTI+ and LRTI- HEU infants enrolled in the NISDI Perinatal and LILAC cohorts.

Participants and Methods

Study population

This study used archived plasma from the NISDI Perinatal and LILAC prospective cohort studies. These previously described studies aimed to characterize adverse events in pregnant women with HIV and their HIV-uninfected offspring.²⁶ The studies were approved by the local Institutional Review Boards and written informed consent was obtained from each participant. Subjects were enrolled during the years 2002–2009. Women eligible to participate had access to antiretrovirals (ARVs), and their infants had access to formula feeding.

Maternal study visits were conducted during pregnancy, at delivery, and hospital discharge, and 6–12 weeks and 6 months postpartum (Perinatal) or every 6 months up to 2 years postpartum (LILAC). Study visits for the infants were conducted at birth, 6–12 weeks and 6 months of age (Perinatal), and every 6 months thereafter (LILAC). During these visits, a medical history was obtained, a physical examination was performed, and blood samples were collected for laboratory studies and for plasma storage at -70°C in a central repository. Infants were considered HIV uninfected if they had ≥ 2 negative HIV nucleic acid tests with one test performed at ≥ 1 month and one at ≥ 4 months of age or if they had ≥ 2 negative HIV antibody test results, including ≥ 1 negative test result after 6 months of age.

NISDI Perinatal and LILAC HEUs were included in this analysis if they were born in Brazil, with gestational age >37 weeks, birth weight $>2,500$ g, and with at least 6 months of follow-up. Multiple gestation births and children with severe underlying cardiovascular or pulmonary conditions were excluded.

As shown in Figure 1, of the children who satisfied the inclusion criteria, we selected 144 children that developed LRTIs (LRTI+) and 144 controls that did not develop LRTIs in the first 6 months of life (LRTI-). An LRTI was defined as having occurred when either a definitive or a presumptive diagnosis of an LRTI was recorded. An infection was considered incident if the infant had been free of any signs and symptoms consistent with the same LRTI for at least 7 days before the incident event. Twenty-five LRTI+ HEUs, whose LRTIs were diagnosed after 6 months of age, were reclassified as LRTI-. Although LRTI+ HEUs could have up to three LRTI- HEUs matched, eight LRTI- were not included in the analysis because they did not have a matched case. In addition, 33 (12 LRTI+ and 21 LRTI-) infants were withdrawn from the analysis because they received ≥ 1 dose of pneumococcal conjugate vaccine before the 6-month study visit.

Laboratory assays

ELISA for antibodies against influenza A, respiratory syncytial virus, parainfluenza 1, 2, and 3, and tetanus. These assays were performed by technologists blinded to the group allocation of the sample using the following kits according to the manufacturer's instructions for quantitative assays: Influenza A IgG ELISA (IBL America), respiratory syncytial virus (RSV) IgG ELISA (IBL America), VIR-ELISA Anti-Parainfluenza (PIV) 1–IgG/IgA (Viro-Immun Labor-Diagnostika GmbH, Germany), VIR-ELISA Anti-PIV 2–IgG/IgA (Viro-Immun Labor-Diagnostika GmbH), VIR-ELISA Anti-PIV 3–IgG/IgA (Viro-Immun Labor-Diagnostika GmbH), and Tetanus IgG ELISA (IBL America), respectively. Laboratory-specific high- and low-positive controls were included in each run as an additional quality assurance of the quantitative aspect of the test. Assays were considered valid when all controls performed as expected. The concentration of antibodies in each plasma sample was calculated by interpolation on a standard curve using Prism 4 (GraphPad, USA) or Excel (Microsoft, USA) software.

ELISA for anti-PNC antibodies. Anticapsular IgG antibody concentrations against serotypes 1, 5, 6B, and 14 were measured using a previously described ELISA protocol that measures binding of serotype-specific IgG to antigen-coated ELISA wells.^{27,28} Plasma at a 1:10 dilution in phosphate-buffered saline containing 0.1% Tween 20% and 1% bovine serum albumin was incubated for one hour at room temperature with cell wall polysaccharide at $50\text{ }\mu\text{g/mL}$ and 22F polysaccharide at $40\text{ }\mu\text{g/mL}$ to absorb nonspecific binding antibodies. Absorbed samples from the mother and infant pairs were assayed simultaneously. The antibody concentration in test samples was calculated by interpolation in the standard curve using Prism software (GraphPad).

Statistical analyses. Continuous variables were described using mean and standard deviations or geometric mean concentrations and 95% confidence intervals. *p* Values for assessing differences between groups (LRTI+ vs. LRTI-) were investigated by linear mixed-effects models to adjust for one-to-*n* matching, with LRTI status as a predictor. In some of the models, other covariates, such as infant antibody concentration measured at birth, were also included for adjustment. Categorical variables were described by frequencies and proportions and differences by groups analyzed by

FIG. 1. Consort diagram. LRTI, lower respiratory tract infection; PCV, pneumococcal conjugate vaccine.

conditional logistic regression for one-to- n matching, as appropriate. Seroconversion by 6 months of life was defined by ≤ 2 -fold decrease or an increase in antibody concentrations compared with birth. Differences between groups in mean infant/mother antibody ratios and infant antibody concentrations at birth and 6 months were assessed by linear mixed-effects models. All analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

Results

Maternal and infant characteristics at birth

Table 1 shows the demographic and social characteristics of the study population. Maternal characteristics were similar between LRTI+ and LRTI- groups, with the exception of completed years of education, which was higher in

LRTI- compared with LRTI+ mothers. In contrast, HIV disease characteristics around the time of birth differed between groups, with LRTI+ mothers having lower CD4⁺ T-lymphocyte (CD4) cell counts and percent and higher HIV plasma viral loads compared with LRTI- mothers. There were no significant differences with respect to gestational age, intrauterine growth, and hematological parameters at birth between LRTI+ and LRTI- infants.

Maternal antibody transfer

At delivery, maternal antibody concentrations against RSV, and PNC1, 5, 6B, and 14 were similar in LRTI+ and LRTI- mothers (Fig. 2). Transplacental transfer of antibodies, defined as the mean of the infant:mother ratio at birth, tended to be lower against RSV and PNC5 in mothers of LRTI+ infants, without reaching statistical significance,

TABLE 1. MATERNAL AND INFANT CHARACTERISTICS

Variable	LRTI+N=107	LRTI-N=140	p
Maternal characteristics			
Race: <i>n</i> (%)			
Black	14 (14.7)	23 (17.7)	.24
Mestizo	6 (6.3)	19 (14.6)	
Other	3 (3.2)	5 (3.8)	
White	46 (48.4)	54 (41.5)	
Unknown	26 (27.4)	29 (22.3)	
Missing	12	10	
Substance use during pregnancy: <i>n</i> (%) exposed			
Alcohol	19 (17.8)	30 (21.7)	.52
Tobacco	21 (19.6)	37 (27.0)	.23
Marijuana	3 (2.8)	6 (4.5)	.73
Crack or cocaine	4 (3.8)	4 (3.0)	1.00
Mean (STD) maternal age at enrollment	26.3 (5.0)	27.4 (5.8)	.14
Mean (STD) years of education	6.9 (3.2)	7.7 (3.2)	.04
Mean (STD) no. of prior pregnancies	2.3 (2.2)	2.3 (1.9)	.98
Mean (STD) parity	1.7 (1.5)	1.7 (1.7)	.96
Mean (STD) no. of people in household	4.4 (3.0)	4.0 (1.9)	.14
Mean (STD) CD4 count (cells/mm ³) at hospital discharge	505.1 (239.5)	601.5 (287.1)	.01
Mean (STD) CD4% at hospital discharge	30.0 (9.4)	33.2 (11.1)	.04
Mean (STD) HIV viral load (log ₁₀ copies/mL) at hospital discharge	2.8 (0.8)	2.5 (0.8)	.03
HIV viral load <400 copies/mL at hospital discharge: <i>n</i> (%)	24 (34.3)	56 (58.3)	.002
Antiretrovirals during pregnancy: <i>n</i> (%)			
Zidovudine monotherapy	57 (53.3)	70 (50.0)	.62
Combination antiretrovirals	50 (46.7)	69 (49.3)	
Unknown	0 (0.0)	1 (0.7)	
Cotrimoxazole during pregnancy: <i>n</i> (%) Yes	14 (13.1)	15 (10.7)	.92
Infant characteristics			
Female gender: <i>n</i> (%)	38 (35.5)	62 (44.3)	.19
Small for gestational age (<10th percentile): <i>n</i> (%)			
Yes	4 (3.7)	4 (2.9)	.72
No	103 (96.3)	136 (97.1)	
Large for gestational age (>90th percentile): <i>n</i> (%)			
Yes	14 (13.1)	13 (9.3)	.41
Mean (STD) gestational age at birth (weeks)	38.6 (1.1)	38.9 (1.2)	.07
Mean (STD) birth weight (g)	3145.5 (391.7)	3169.6 (369.6)	.62
Mean (STD) percent hematocrit at birth	41.4 (5.4)	42.6 (6.4)	.12
Mean (STD) hemoglobin at birth (g/dL)	14.2 (1.8)	14.6 (2.1)	.15
Mean (STD) red blood cell count at birth (10 ⁶ /μL)	3.7 (0.5)	3.8 (0.7)	.11
Mean (STD) white blood cell count at birth (10 ³ /μL)	11.5 (7.1)	11.5 (3.8)	.97
Mean (STD) days on zidovudine	42.8 (4.7)	43.4 (6.5)	.25
Used cotrimoxazole: <i>n</i> (%) yes	100 (93.5)	131 (93.6)	.56
Mean (STD) days of cotrimoxazole use	102.7 (39.4)	98.3 (43.7)	.43

Bolded p values indicate significant and marginal differences. To adjust for one-to-*n* matching, *p* values for continuous measures were calculated by linear mixed-effects models; *p* values for categorical measures were calculated by conditional logistic regression.

LRTI, lower respiratory tract infection; HIV, human immunodeficiency virus.

STD, standard deviation.

and were similar for PNC1, 6B, and 14 (Table 2). Antibody concentrations at birth did not differ between LRTI+ and LRTI- infants (Table 2).

Infections with respiratory viruses and PNC in the first 6 months of life

The incidence of infections with specific pathogens in the first 6 months of life was measured by the incidence of seroconversions to each pathogen (Table 3). In addition to LRTI, seroconversion might have resulted from upper respiratory tract infections or asymptomatic infections. The rate of serconversions/infections did not show any differences

between LRTI+ and LRTI- infants. However, the geometric mean antibody concentrations at 6 months of life were significantly higher for RSV and marginally higher for PIV1 in LRTI+ compared with LRTI- infants. There were no differences in antibody concentrations against the other respiratory pathogens.

To determine if there were any differences in the humoral immune competence of LRTI+ and LRTI- infants, we compared their antibody responses to the tetanus vaccine according to the number of doses received. Anti-tetanus toxoid antibody concentrations, stratified by the number of vaccine doses that each infant received, did not differ between the two groups (Table 4).

FIG. 2. Maternal antibodies at delivery.

Discussion

In this study, LRTI+ and LRTI- HEUs had similar rates of seroconversions to common childhood respiratory viruses and PNC in the first 6 months of life. The corollary of this observation is that development of LRTIs is not due to increased exposure to respiratory pathogens in a segment of HEUs. Previous investigations showed that RSV and PNC were major causes of severe infection, hospitalization, and death in HEUs.^{2,29} There are two important hypotheses that result from merging these observations: (1) HEUs have various degrees of immune deficiency that determine the severity of infections; and (2) environmental and socioeconomic factors differ between HEUs with and without severe infection and death. These hypotheses are not mutually exclusive, but it is important to determine their relative contributions to devise strategies to prevent adverse outcomes in HEUs.

The only socioeconomic difference between LRTI+ and LRTI- HEUs in this study was a lower number of completed years of education in mothers of LRTI+ infants. Maternal education is a strong predictor of socioeconomic and environmental factors that are associated with incidence, morbidity, and mortality of LRTI in low and middle-income countries.^{30–32} Although it is not clear if maternal education plays a direct or indirect role in LRTI mortality in low and middle-income countries, it appears to be a modifiable factor that could be included in the strategy to decrease infant mortality in these countries.³³

There were also differences in maternal HIV disease characteristics, including lower CD4⁺ cell counts and percentages and higher HIV plasma viral load in mothers of LRTI+ infants. Previous studies in HEUs have also shown that maternal immune status correlates with infant infectious morbidity.¹⁴ As expected with their more advanced degree of

TABLE 2. TRANSPLACENTAL TRANSFER OF MATERNAL ANTIBODIES AND ANTIBODY CONCENTRATIONS IN LRTI+ AND LRTI- INFANTS AT BIRTH

Respiratory pathogen	LRTI+		LRTI−		p
	Infant/mother antibody ratio				
	N	Mean (95% CI)	N	Mean (95% CI)	
RSV	83	1.28 (1.12–1.47)	111	1.46 (1.30–1.65)	.09
PNC1	81	0.89 (0.83–0.95)	104	0.88 (0.83–0.94)	.64
PNC5	81	0.65 (0.59–0.71)	104	0.73 (0.66–0.80)	.06
PNC6B	81	0.76 (0.70–0.84)	104	0.75 (0.69–0.81)	.87
PNC14	81	0.91 (0.84–0.97)	104	0.89 (0.84–0.95)	.86
Infant antibody concentrations at birth (EU/mL)					
	N	GMC (95% CI)	N	GMC (95% CI)	
Influenza A	86	54.7 (43.5–68.8)	111	50.6 (38.9–65.8)	.46
RSV	87	70.5 (55.2–90.0)	113	83.2 (68.8–100.6)	.28
PIV1	78	3.5 (2.0–5.9)	105	4.4 (2.9–6.6)	.14
PIV2	79	6.6 (4.0–10.9)	104	8.7 (5.8–13.0)	.54
PIV3	79	11.4 (7.5–17.4)	104	11.0 (7.6–16.0)	.80
PNC1	82	0.8 (0.7–0.9)	106	0.7 (0.6–0.7)	.11
PNC5	82	0.8 (0.7–0.9)	106	0.8 (0.7–0.9)	.82
PNC6B	82	0.7 (0.6–0.8)	106	0.7 (0.6–0.7)	.53
PNC14	82	2.9 (2.6–3.3)	106	2.8 (2.5–3.1)	.78

Bolded *p* values indicate marginal differences. To adjust for one-to-*n* matching, all *p* values were calculated by linear mixed-effects models. N, no. of participants tested; GMC, geometric mean concentration; RSV, respiratory syncytial virus; PIV, parainfluenza; CI, confidence intervals. EU, ELISA units.

TABLE 3. INCIDENCE OF RESPIRATORY TRACT INFECTIONS AND ANTIBODY CONCENTRATIONS IN LRTI+ AND LRTI- INFANTS AT 6 MONTHS OF LIFE

	<i>LRTI+</i>		<i>LRTI−</i>		
	<i>Incidence of seroconversions at 6 months</i>				
<i>Respiratory pathogen</i>	N	<i>Incidence (%)</i>	N	<i>Incidence (%)</i>	p
Influenza A	69	3 (4.4)	103	5 (4.9)	.55
RSV	72	6 (8.3)	103	6 (5.8)	.53
PIV1	71	30 (42.3)	104	35 (33.7)	.11
PIV2	70	24 (34.3)	103	19 (18.5)	.14
PIV3	67	24 (35.8)	100	30 (30.0)	.55
PNC1	63	59 (93.7)	95	87 (91.6)	.68
PNC5	63	53 (84.1)	95	77 (81.1)	.41
PNC6B	63	56 (88.9)	95	79 (83.2)	.30
PNC14	63	57 (90.5)	95	83 (87.4)	.66
	<i>Infant antibody concentration at 6 months (EU/mL)</i>				
	N	<i>GMC (95% CI)</i>	N	<i>GMC (95% CI)</i>	
Influenza A	84	2.3 (1.6–3.2)	121	1.7 (1.1–2.4)	.33
RSV	86	1.5 (0.8–2.6)	119	0.5 (0.3–0.8)	.01
PIV1	84	1.1 (0.6–1.8)	120	0.5 (0.3–0.8)	.08
PIV2	84	0.2 (0.1–0.4)	120	0.1 (0.1–0.2)	.17
PIV3	82	1.3 (0.7–2.4)	118	0.7 (0.4–1.3)	.14
PNC1	78	0.7 (0.6–0.8)	110	0.6 (0.6–0.7)	.63
PNC5	78	0.7 (0.6–0.7)	110	0.7 (0.7–0.8)	.10
PNC6B	77	0.6 (0.5–0.6)	110	0.6 (0.5–0.7)	.88
PNC14	78	2.9 (2.6–3.2)	110	2.6 (2.4–2.9)	.53

Bolded p values indicate significant and marginal differences. To adjust for one-to-*n* matching, *p* values for antibody concentrations were calculated by linear mixed-effects models; *p* values for seroconversions were calculated by conditional logistic regression.

N, no. of children tested; Incidence, no. of children with seroconversion; %, no. of children with seroconversion/total no. of children.

HIV infection, mothers of LRTI+ infants tended to have lower RSV transplacental antibody transfer. However, the extent to which the transplacental transfer of maternal antibodies contributed to the risk of LRTI is uncertain, because LRTI+ and LRTI- infants had similar plasma levels of maternal antibodies at birth. There are other maternal factors associated with HIV infection that might have affected the development of infant immune competence, including transfer of immunosuppressive inflammatory or regulatory factors; transplacental passage of HIV subviral particles; and other congenital infections, such as CMV or syphilis, both of which may increase in pregnancies complicated by HIV infection.^{34–39}

It is important to note that ARV utilization did not differ between LRTI+ and LRTI- HEUs or their mothers. ARVs have toxic effects on cellular metabolism and may affect

immune responses. Zidovudine in particular was thought to be associated with anemia, neutropenia, lymphopenia, and other T cell abnormalities among HEUs compared with HUUs.^{40–43} Drugs in several ARV classes, such as nucleoside and non-nucleoside reverse transcriptase inhibitors, cross the placenta relatively well.^{44–47} Integrase inhibitors were not used by the mothers included in this analysis. Protease inhibitors are subject to a higher transplacental gradient than reverse transcriptase inhibitors, but reach measurable levels in the amniotic fluid and cord blood.^{45,48} However, our data do not support the notion that specific ARVs contribute to the risk of LRTI in HEUs.

We analyzed infant and maternal use of cotrimoxazole. Cotrimoxazole is effective in preventing *Pneumocystis jirovecii* pneumonia and malaria in HIV-infected individuals. There is limited benefit from the use of cotrimoxazole in HEUs outside of malaria endemic areas, such as in our study. However, both maternal and infant use of cotrimoxazole may alter the microbiome and indirectly affect the development of the infant immune system. The use of cotrimoxazole did not differ between LRTI+ and LRTI- HEUs or their mothers, which precluded the assessment of its potential contribution to LRTI.

The main difference between LRTI+ and LRTI- HEUs were the anti-RSV and anti-PIV1 antibody concentrations at 6 months of age. The increased antibody concentrations against these viruses in LRTI+ HEUs suggest that viruses underwent more extensive replication in LRTI+ compared with LRTI- HEUs and the higher antigenic burden generated higher antibody production. The increased viral burden probably also played an important role in the development of LRTI.

TABLE 4. ANTI-TETANUS TOXOID ANTIBODY CONCENTRATIONS (μG/mL) AT 6 MONTHS OF LIFE

No. of vaccine doses	LRTI+			LRTI-			p
	N	GMC	95% CI	N	GMC	95% CI	
0	5	0.1	0.02–0.3	15	0.1	0.03–0.3	.97
1	28	0.1	0.04–0.1	29	0.1	0.03–0.1	
2	49	0.4	0.3–0.7	73	0.5	0.4–0.8	.50
3	1	0.4	—	2	1.3	—	
4	1	1.1	—	0	—	—	—

To adjust for one-to-*n* matching, *p* values were calculated by linear mixed-effects models; *p* values for the comparison of LRTI+ to LRTI- were determined combining no. of vaccine doses of 0 and 1 and also for combining 2 and 3 doses.

Why did viruses replicate more extensively in LRTI+ infants? The most likely explanation is that immune defenses of LRTI+ HEUs were insufficient to contain viral replication. In this study, we found that humoral immunity did not differ between LRTI+ and LRTI- HEUs, suggesting that cellular immunity, adaptive and/or innate, may be the critical risk factors for severe infection in HEUs. Multiple studies have shown decreased cell-mediated immune responses to vaccines in HEUs compared with HUU. ^{19,49–51} In a previous study, we showed decreased natural killer cell function in HEUs compared with HUUs ⁵² and our unpublished observations also indicate defective antigen-presenting cell function and increased regulatory T cells in HEUs compared with HUUs. Any of these defects may increase the severity of viral infections in HEUs.

Our study has limitations due to its retrospective design. We made an effort to mitigate this problem by matching cases and controls by date of birth and geographic location to ensure that they had similar exposure to seasonal respiratory infectious agents. Additional strengths of our study included the collection of data by uniformly training abstractors, using standardized definitions and data collection instruments that were completed for predefined study visits.

Our findings significantly advance understanding of the factors that increase the risk of severe infection in HEUs by demonstrating that low maternal antibodies or defects in infant humoral immunity were not associated with HEU disease. This suggests that cell-mediated immunity of the HEU is likely to play a major role in their increased susceptibility to infections. We also showed that advanced maternal HIV disease was associated with increased risk of severe infection in the infant, but ARV or cotrimoxazole utilization were not.

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References

1. Slogrove A, Reikie B, Naidoo S, De Beer C, Ho K, Cotton M, *et al.*: HIV-exposed uninfected infants are at increased risk for severe infections in the first year of life. *J Trop Pediatr* 2012;58:505–508.
2. Slogrove AL, Goetghebuer T, Cotton MF, Singer J, Bettinger JA: Pattern of infectious morbidity in HIV-exposed uninfected infants and children. *Front Immunol* 2016;7:164.
3. Cohen C, Moyes J, Tempia S, Groome M, Walaza S, Pretorius M, *et al.*: Epidemiology of acute lower respiratory tract infection in HIV-exposed uninfected infants. *Pediatrics* 2016; 137:e1–e10.
4. Koyanagi A, Humphrey JH, Ntozini R, Nathoo K, Moulton LH, Iliff P, *et al.*: Morbidity among human immunodeficiency virus-exposed but uninfected, human immunodeficiency virus-infected, and human immunodeficiency virus-unexposed infants in Zimbabwe before availability of highly active antiretroviral therapy. *Pediatr Infect Dis J* 2011;30:45–51.

5. Marinda E, Humphrey JH, Iliff PJ, Mutasa K, Nathoo KJ, Piwoz EG, *et al.*: Child mortality according to maternal and infant HIV status in Zimbabwe. *Pediatr Infect Dis J* 2007; 26:519–526.
6. Mussi-Pinhata MM, Freimanis L, Yamamoto AY, Korelitz J, Pinto JA, Cruz ML, *et al.*: Infectious disease morbidity among young HIV-1-exposed but uninfected infants in Latin American and Caribbean countries: The National Institute of Child Health and Human Development International Site Development Initiative Perinatal Study. *Pediatrics* 2007;119:e694–e704.
7. Kourtis AP, Wiener J, Kayira D, Chasela C, Ellington SR, Hyde L, *et al.*: Health outcomes of HIV-exposed uninfected African infants. *Aids* 2013;27:749–759.
8. Kuhn L, Kasonde P, Sinkala M, Kankasa C, Semrau K, Scott N, *et al.*: Does severity of HIV disease in HIV-infected mothers affect mortality and morbidity among their uninfected infants? *Clin Infect Dis* 2005;41:1654–1661.
9. Mussi-Pinhata MM, Motta F, Freimanis-Hance L, de Souza R, Szyld E, Succi RC, *et al.*: Lower respiratory tract infections among human immunodeficiency virus-exposed, uninfected infants. *International journal of infectious diseases: IJID: Official publication of the International Society for Infectious Diseases* 2010;14 Suppl 3:e176–e182.
10. Kelly M, Wirth K, Steenhoff A, Cunningham C, Arscott-Mills T, Boiditswe S, Patel M, Shah S, Finalle R, Makone I, Feemster K: Treatment failures and excess mortality among HIV-exposed, uninfected children with pneumonia. *J Pediatr Infect Dis Soc* 2015;4:e117–e126.
11. Shapiro RL, Hughes M, Powis K, Ajibola G, Bennet K, Moyo S, Makhema J, Zwierski S, Essex M, Lockman S. *Similar Mortality with Cotrimoxazole Vs. Placebo in HIV-Exposed Uninfected Children*. Conference on Retroviruses and Opportunistic Infections, Boston, MA, 2016.
12. Coutoudis A, Kindra G, Esterhuizen T: Impact of cotrimoxazole prophylaxis on the health of breast-fed, HIV-exposed, HIV-negative infants in a resource-limited setting. *AIDS* 2011;25:1797–1799.
13. Epalza C, Goetghebuer T, Hainaut M, Prayez F, Barlow P, Dediste A, *et al.*: High incidence of invasive group B streptococcal infections in HIV-exposed uninfected infants. *Pediatrics* 2010;126:e631–e638.
14. Taron-Brocard C, Le Chenadec J, Faye A, Dollfus C, Goetghebuer T, Gajdos V, *et al.*: Increased risk of serious bacterial infections due to maternal immunosuppression in HIV-exposed uninfected infants in a European country. *Clin Infect Dis* 2014;59:1332–1345.
15. Adler C, Haelterman E, Barlow P, Marchant A, Levy J, Goetghebuer T: Severe infections in HIV-exposed uninfected infants born in a European Country. *PLoS One* 2015; 10:e0135375.
16. Simani OE, Adrian PV, Violari A, Kuwanda L, Otjombe K, Nunes MC, *et al.*: Effect of in-utero HIV exposure and antiretroviral treatment strategies on measles susceptibility and immunogenicity of measles vaccine. *AIDS* 2013;27: 1583–1591.
17. Weinberg A, Mussi-Pinhata MM, Yu Q, Cohen RA, Almeida VC, Amaral F, *et al.*: Excess respiratory viral infections and low antibody responses among HIV-exposed, uninfected infants. *AIDS* 2017;31:669–679.
18. Clerici M, Saresella M, Colombo F, Fossati S, Sala N, Bricalli D, *et al.*: T-lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV. *Blood* 2000;96:3866–3871.
19. Kidzeru EB, Hesselning AC, Passmore JA, Myer L, Gamielidien H, Tchakoute CT, *et al.*: In-utero exposure to maternal HIV infection alters T-cell immune responses to vaccination in HIV-uninfected infants. *AIDS* 2014;28: 1421–1430.
20. Borges-Almeida E, Milanez HM, Vilela MM, Cunha FG, Abramczuk BM, Reis-Alves SC, *et al.*: The impact of maternal HIV infection on cord blood lymphocyte subsets and cytokine profile in exposed non-infected newborns. *BMC Infect Dis* 2011;11:38.
21. Gesner M, Papaevangelou V, Kim M, Chen SH, Moore T, Krasinski K, *et al.*: Alteration in the proportion of CD4 T lymphocytes in a subgroup of human immunodeficiency virus-exposed-uninfected children. *Pediatrics* 1994;93:624–630.
22. Hygino J, Lima PG, Filho RG, Silva AA, Saramago CS, Andrade RM, *et al.*: Altered immunological reactivity in HIV-1-exposed uninfected neonates. *Clin Immunol* 2008; 127:340–347.
23. Hygino J, Vieira MM, Guillermo LV, Silva-Filho RG, Saramago C, Lima-Silva AA, *et al.*: Enhanced Th17 phenotype in uninfected neonates born from viremic HIV-1-infected pregnant women. *J Clin Immunol* 2011;31: 186–194.
24. Miyamoto M, Pessoa SD, Ono E, Machado DM, Salomao R, Succi RC, *et al.*: Low CD4+ T-cell levels and B-cell apoptosis in vertically HIV-exposed noninfected children and adolescents. *J Trop Pediatr* 2010;56:427–432.
25. Rich KC, Siegel JN, Jennings C, Rydman RJ, Landay AL: Function and phenotype of immature CD4+ lymphocytes in healthy infants and early lymphocyte activation in uninfected infants of human immunodeficiency virus-infected mothers. *Clin Diagn Lab Immunol* 1997;4:358–361.
26. Read JS, Duarte G, Hance LF, Pinto J, Gouveia MI, Cohen RA, *et al.*: The NICHD International site development initiative perinatal cohorts (2002–2009). *Int J Epidemiol* 2012;41:642–649.
27. Quataert S, Martin D, Anderson P, Giebink GS, Henrichsen J, Leinonen M, *et al.*: A multi-laboratory evaluation of an enzyme-linked immunoassay quantitating human antibodies to *Streptococcus pneumoniae* polysaccharides. *Immunol Invest* 2001;30:191–207.
28. Inostroza J, Villanueva S, Mason K, Leiva LE, Sorensen RU: Effects of absorption with pneumococcal type 22F polysaccharide on maternal, cord blood, and infant immunoglobulin G antipneumococcal polysaccharide antibodies. *Clin Diagn Lab Immunol* 2005;12:722–726.
29. von Mollendorf C, von Gottberg A, Tempia S, Meiring S, de Gouveia L, Quan V, *et al.*: Increased risk for and mortality from invasive pneumococcal disease in HIV-exposed but uninfected infants aged <1 year in South Africa, 2009–2013. *Clin Infect Dis* 2015;60:1346–1356.
30. Nascimento-Carvalho CM, Rocha H, Benguigui Y: Effects of socioeconomic status on presentation with acute lower respiratory tract disease in children in Salvador, Northeast Brazil. *Pediatr Pulmonol* 2002;33:244–248.
31. Cashat-Cruz M, Morales-Aguirre JJ, Mendoza-Azpiri M: Respiratory tract infections in children in developing countries. *Semin Pediatr Infect Dis* 2005;16:84–92.
32. Pinzon-Rondon AM, Aguilera-Otalvaro P, Zarate-Ardila C, Hoyos-Martinez A: Acute respiratory infection in children from developing nations: A multi-level study. *Paediatr Int Child Health* 2016;36:84–90.
33. Sonogo M, Pellegrin MC, Becker G, Lazzerini M: Risk factors for mortality from acute lower respiratory infections

- (ALRI) in children under five years of age in low and middle-income countries: A systematic review and meta-analysis of observational studies. *PLoS One* 2015;10:e0116380.
34. Frederick T, Homans J, Spencer L, Kramer F, Stek A, Opersalski E, *et al.*: The effect of prenatal highly active antiretroviral therapy on the transmission of congenital and perinatal/early postnatal cytomegalovirus among HIV-infected and HIV-exposed infants. *Clin Infect Dis* 2012;55:877–884.
 35. Gompels UA, Larke N, Sanz-Ramos M, Bates M, Musonda K, Manno D, *et al.*: Human cytomegalovirus infant infection adversely affects growth and development in maternally HIV-exposed and unexposed infants in Zambia. *Clin Infect Dis* 2012;54:434–442.
 36. Mwaanza N, Chilukutu L, Tembo J, Kabwe M, Musonda K, Kapasa M, *et al.*: High rates of congenital cytomegalovirus infection linked with maternal HIV infection among neonatal admissions at a large referral center in sub-Saharan Africa. *Clin Infect Dis* 2014;58:728–735.
 37. Temmerman M, Gichangi P, Fonck K, Apers L, Claeys P, Van Renterghem L, *et al.*: Effect of a syphilis control programme on pregnancy outcome in Nairobi, Kenya. *Sex Transm Infect* 2000;76:117–121.
 38. Adachi K, Xu J, Ank B, Watts DH, Mofenson LM, Pilotto JH, *et al.*: Cytomegalovirus urinary shedding in HIV-infected pregnant women and congenital cytomegalovirus infection. *Clin Infect Dis* 2017;65:405–413.
 39. Yeganeh N, Watts HD, Camarca M, Soares G, Joao E, Pilotto JH, *et al.*: Syphilis in HIV-infected mothers and infants: Results from the NICHD/HPTN 040 study. *Pediatr Infect Dis J* 2015;34:e52–e57.
 40. Andre-Schmutz I, Dal-Cortivo L, Six E, Kaltenbach S, Cocchiarella F, Le Chenadec J, *et al.*: Genotoxic signature in cord blood cells of newborns exposed in utero to a Zidovudine-based antiretroviral combination. *J Infect Dis* 2013;208:235–243.
 41. Enomoto L, Anderson PL, Li S, Edelstein CL, Weinberg A: Effect of nucleoside and nucleotide analog reverse transcriptase inhibitors on cell-mediated immune functions. *AIDS Res Hum Retroviruses* 2011;27:47–55.
 42. Olivero OA, Shearer GM, Chougnet CA, Kovacs AA, Landay AL, Baker R, *et al.*: Incorporation of zidovudine into leukocyte DNA from HIV-1-positive adults and pregnant women, and cord blood from infants exposed in utero. *AIDS* 1999;13:919–925.
 43. Nielsen SD, Jeppesen DL, Kolte L, Clark DR, Sorensen TU, Dreves AM, *et al.*: Impaired progenitor cell function in HIV-negative infants of HIV-positive mothers results in decreased thymic output and low CD4 counts. *Blood* 2001;98:398–404.
 44. Chappuy H, Treluyer JM, Jullien V, Dimet J, Rey E, Fouche M, *et al.*: Maternal-fetal transfer and amniotic fluid accumulation of nucleoside analogue reverse transcriptase inhibitors in human immunodeficiency virus-infected pregnant women. *Antimicrob Agents Chemother* 2004;48:4332–4336.
 45. Bennetto-Hood C, Bryson YJ, Stek A, King JR, Mirochnick M, Acosta EP: Zidovudine, lamivudine, and nelfinavir concentrations in amniotic fluid and maternal serum. *HIV Clin Trials* 2009;10:41–47.
 46. Bryson YJ, Mirochnick M, Stek A, Mofenson LM, Connor J, Capparelli E, *et al.*: Pharmacokinetics and safety of nelfinavir when used in combination with zidovudine and lamivudine in HIV-infected pregnant women: Pediatric AIDS Clinical Trials Group (PACTG) Protocol 353. *HIV Clin Trials* 2008;9:115–125.
 47. Jackson JB, Parsons T, Musoke P, Nakabiito C, Donnell D, Fleming T, *et al.*: Association of cord blood nevirapine concentration with reported timing of dose and HIV-1 transmission. *AIDS* 2006;20:217–222.
 48. McCormack SA, Best BM: Protecting the fetus against HIV infection: A systematic review of placental transfer of antiretrovirals. *Clin Pharmacokinet* 2014;53:989–1004.
 49. Mazzola TN, da Silva MT, Abramczuk BM, Moreno YM, Lima SC, Zorzeto TQ, *et al.*: Impaired *Bacillus Calmette-Guerin* cellular immune response in HIV-exposed, uninfected infants. *AIDS* 2011;25:2079–2087.
 50. Ota MO, O'Donovan D, Marchant A, Yamuah L, Harding E, Jaffar S, *et al.*: HIV-negative infants born to HIV-1 but not HIV-2-positive mothers fail to develop a *Bacillus Calmette-Guerin* scar. *AIDS* 1999;13:996–998.
 51. Van Rie A, Madhi SA, Heera JR, Meddows-Taylor S, Wendelboe AM, Anthony F, *et al.*: Gamma interferon production in response to *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* antigens in infants born to human immunodeficiency virus-infected mothers. *Clin Vaccine Immunol* 2006;13:246–252.
 52. Smith C, Jalbert E, de Almeida V, Canniff J, Lenz LL, Mussi-Pinhata MM, *et al.*: Altered natural killer cell function in HIV-exposed uninfected infants. *Front Immunol* 2017;8:470.

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