Initiation of Antiretroviral Therapy Before Pregnancy Reduces the Risk of Infection-related Hospitalization in Human Immunodeficiency Virus–exposed Uninfected Infants Born in a High-income Country

Initiation of anti-retroviral therapy before pregnancy reduces the risk of infection-related hospitalization in HIV-exposed uninfected infants born in a high-income country

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Summary:

HIV-exposed uninfected (HEU) infants born in Belgium have a two-fold higher risk of hospitalization for infection than HIV-unexposed infants. Infectious risk was reduced by initiation of anti-retroviral therapy before pregnancy and was predicted by immune alterations induced during fetal life.

ABSTRACT

Background: Epidemiological studies conducted in low and high-income countries showed that infants exposed to maternal HIV but not infected themselves by the virus have a high risk of severe infections. Immune alterations during fetal life have been proposed as a possible mechanism.

Methods: This prospective study was conducted to assess the relative risk of hospitalization for infection in HIV-exposed uninfected (HEU) infants as compared to HIV-unexposed (HU) infants born in a high-income country (HIC). Markers of monocyte activation and levels of vaccine and pathogen specific antibodies were measured at birth in maternal and cord blood to identify correlates of infant susceptibility.

Results: 27 of 132 HEU infants and 14 of 123 HU infants were hospitalized for infection during the first year of life (adjusted HR, 95% CI: 2.33, 1.10-4.97). Most of this increased risk was associated with time of initiation of maternal anti-retroviral therapy (ART). As compared to HU infants, risk of hospitalization for infection of HEU infants was four-fold higher (3.84, 1.69-8.71) when mothers initiated ART during pregnancy and was not significantly increased (1.42, 0.58-3.48) when ART was initiated before pregnancy. Activation of maternal and newborn monocytes and reduced transfer of maternal antibodies were most intense following ART initiation during pregnancy and predicted the risk of infant hospitalization.

Conclusions: These observations indicate that initiation of maternal ART before pregnancy reduces the susceptibility of HEU infants born in a HIC to severe infections and that this effect could be related to the prevention of immune alterations during fetal life.

Keywords: maternal HIV; anti-retroviral therapy; maternal antibodies; immune activation; infant infections

INTRODUCTION

Prevention of mother to child transmission (PMTCT) of HIV has markedly reduced the incidence of pediatric HIV infections worldwide (1). As a result, the number of infants exposed to maternal HIV but not infected themselves by the virus has increased, reaching up to 30% of the infant population in some countries in Africa (2,3). HIV-exposed uninfected (HEU) infants are at higher risk of serious clinical complications than their HIV-unexposed (HU) peers (2–5). Retrospective studies of HEU infants born in low and middle-income countries (LMIC) reported high incidence of infectious diseases related health care visits, hospitalizations and mortality (6–8). Prospective birth cohort studies in South Africa and Mozambique estimated that HEU infants have a 1.5 to 2.7 higher risk of hospitalization for infection than HU infants (9–11). A high incidence of hospitalization for infection has also been reported in HEU infants born in high-income countries (HIC) but the excess risk as compared to HU infants has not yet been quantified in these populations (12–14). In both LMIC and HIC, severe infections affecting HEU infants involve multiple pathogens and predominantly occur during the first 6 months of life (5,7,12,14–16).

The most consistently observed risk factor of infection-related morbidity and mortality in HEU infants is advanced maternal HIV disease (6,7,13,17). This could be related to an association with universal risk factors of infections in infancy including lower socio-economic conditions, maternal morbidity and mortality, prematurity and absence of breastfeeding. On the other hand, risk factors specific to maternal HIV infection could play an important role. Nucleoside reverse transcriptase inhibitors (NRTI) used for PMTCT have been proposed to reduce infant immunity to pathogens through their hematological toxicity (3,18). On the other hand, maternal HIV infection is associated with alterations of several immune parameters in HEU infants. The chronic immune activation that is typical of HIV infection is also observed in HEU infants and can be assessed by measuring the expression of activation markers by peripheral blood innate immune cells (3,18,19). In addition, HEU newborns have lower concentrations of antibodies to

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pathogens and vaccine antigens, as a result of lower concentrations in the mother and of a reduction of transplacental transfer (3,18,20,21). Although immune alterations have been proposed to contribute to the susceptibility of HEU infants to infectious pathogens, no correlation with clinical outcomes has yet been established (3).

We previously reported a retrospective study of the incidence of hospitalization for infection in HEU infants born in Belgium between 1985 and 2006 (14). This prospective study was conducted to assess the relative risk of hospitalization for infection in HEU versus HU infants. Maternal and infant characteristics were analyzed to determine whether risk factors were specific or common to HEU and HU infants. Immune parameters were measured at birth in mothers and newborns to identify correlates of susceptibility.

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METHODS

Study population

The study was conducted at the Hôpital Saint-Pierre, Brussels, a public hospital and HIV reference center taking care of a population of diverse socioeconomic and ethnic backgrounds. Between December 2010 and November 2013, HIV-infected and uninfected pregnant women were recruited from the 32nd week of gestation following informed consent at the hospital antenatal clinic. PMTCT included administration of ART in pregnant women, zidovudine prophylaxis in infants during 6 weeks and avoidance of breastfeeding, following European guidelines (22). Absence of HIV transmission was defined by at least 2 negative HIV DNA PCR during the first 3 months of life and by a negative serology after 12 months of age. HIV-uninfected pregnant women did not present chronic disorders, including autoimmune or inflammatory diseases, and did not receive immunosuppressive therapy before pregnancy. Children were examined at birth and at 1, 2, 3, 4, 6, 9 and 12 months by one of the clinical investigators (T.G. or C.A.). Data collected at each visit were entered in standardized case report forms. All children were vaccinated according to the Belgian immunization program (www.vaccine-schedule.ecdc.europa.eu). Anticipating an incidence of hospitalization for infection in HEU infants at 17% (14), a sample size of 120 infants per group was required to detect a two-fold increased risk as compared to HU infants with a 90% power, using a test of proportions for independent samples. The study was approved by the hospital Ethics Committee.

Clinical outcome

The primary clinical outcome was hospitalization for infection for more than 24 hours over the first year of life. Decision of hospitalization for infection was made following local clinical care guidelines by emergency unit clinicians who were not informed of the study objectives. Hospitalization criteria included fever before the age of 3 months, infection requiring parenteral antibiotic therapy, respiratory distress with poor feeding or hypoxemia, first episode of febrile

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seizure, gastro-enteritis with dehydration and suspicion of sepsis. Neonatal sepsis was defined as an infection occurring before the age of 28 days and requiring IV antibiotic therapy for at least 5 days. Adjudication of discharge diagnosis was made on the basis of routine hospital tests by senior ward clinicians not involved in the study.

Immune markers

Serum levels of vaccine (diphtheria toxoid, tetanus toxoid, pertussis toxin, H*aemophilus influenza* type B and Pneumococcus polysaccharides) and pathogen-specific antibodies (varicella zoster virus (VZV), cytomegalovirus (CMV)) were measured by multiplex immunoassays (23,24). Phenotype of peripheral blood monocyte subsets was analyzed by flow cytometry as described in Supplementary data. Respiratory syncytial virus (RSV) neutralizing antibodies were measured using a microneutralization assay (25). RSV antigen-specific IgG were analyzed by Western blot (25,26). Immune markers were measured at birth in maternal and cord blood. In addition, infant RSV antibodies were measured at 6 months of age to define rates of natural infections.

Statistical analyses

Description of methods used for statistical analyses of clinical and immunological data is provided in Supplementary data.

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RESULTS

130 HIV-infected and 120 HIV-uninfected mothers were recruited during pregnancy (Supplementary Figure 1). HIV-infected mothers were significantly more often of Sub-Saharan African origin, older and multiparous than HIV-uninfected mothers and were similar for education, occupation and income (Table 1). 132 HEU children, including 2 twin pairs and 5 pairs of siblings, and 123 HU children, including 3 twin pairs and 1 pair of siblings, were enrolled at birth (Supplementary Figure 1). HEU children had lower gestational age and birth weight than HU children. Ninety-five percent of HU infants were breastfed for a median duration of 6 months whereas none of the HEU infants were breastfed. ART was initiated before pregnancy in 81 women and during pregnancy in 51 women for PMTCT of HIV, including 18 women diagnosed with HIV infection during pregnancy.

Incidence and risk factors of infection-related hospitalizations

During the first year of life, 34 hospitalizations for infection occurred in 27/132 HEU infants and 18 hospitalizations occurred in 14/123 HU infants (Supplementary Table 1a). Hazard ratio of hospitalization for infection in HEU versus HU infants was 1.78 before adjustment and 2.33 after adjustment for birth weight, gestational age, maternal African origin, age, literacy, level of education, and primiparity (Table 2). Similar results were obtained when analysis excluded siblings and second twins (Supplementary Figure 2 and Supplementary Table 1b). Most infection-related hospitalizations occurred during the first 3 months of life (Figure 1A). Detected pathogens included bacteria and viruses (Supplementary Tables 1 and 2). RSV bronchiolitis was the cause of hospitalization in 10 HEU infants and in only 1 HU infant. In contrast, rate of RSV seroconversion at 6 months was similar in both groups (Supplementary Table 3 and Supplementary Figure 3). Notably, the incidence of hospitalizations for other causes than infections during the study period was not significantly different in HEU and HU infants (Supplementary Table 1).

Risk factors associated with hospitalization for infection were analyzed separately in HEU and HU infants (Table 3 and supplementary Table 4). In HEU infants, time of initiation of maternal ART was significantly associated with infant hospitalization for infection (adjusted HR, 95% CI: 2.31, 1.03-5.15). Intra-partum administration of antibiotics was associated with an increased risk of hospitalization for infection in both HEU and HU infants. Risk of hospitalization for infection was not associated with gestational age in HEU or HU infants or with duration of breastfeeding in HU infants (Table 3, Supplementary Table 4 and Supplementary Figure 4).

The characteristics of HIV-infected mothers who initiated ART before or during pregnancy are presented in Table 4. Mothers who initiated ART during pregnancy were younger and more often primiparous than mothers who conceived on ART. ART regimen initiated before pregnancy were heterogeneous whereas regimen initiated during pregnancy predominantly included protease inhibitors (PI). However, administration of PI during pregnancy was not significantly associated with clinical outcome of HEU infants (data not shown). At delivery, mothers who initiated ART during pregnancy had similar viral control and higher CD4 counts as compared to mothers who initiated ART before pregnancy (Table 4).

The role of time of initiation of maternal ART in HEU infants led us to compare the risk of infection-related hospitalization of infants born to mothers who initiated ART before or during pregnancy to that of HU infants (Figure 1B). As compared to HU infants, the adjusted hazard ratio of HEU infants was 3.84 when mothers initiated ART during pregnancy and only 1.42 when ART was initiated before pregnancy (Table 2). Notably, risk of hospitalization for infection was higher in infants born to mothers who initiated ART during the last as compared to the first two trimesters of pregnancy, although this difference did not reach statistical significance (Supplementary Figure 5). The robustness of our findings was validated using alternate modeling approaches on the entire dataset, combining HU and HEU infants (Supplementary Table 5).

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Immune activation in newborns and transfer of maternal antibodies

Next, we sought to identify potential immune correlates of susceptibility to infection-related hospitalization. Markers of immune activation and transfer of maternal antibodies to a panel of vaccine and pathogen antigens were measured at birth (Supplementary Tables 6 to 9). Markers of immune activation included the level of expression of CD40, CD86 and HLA-DR by peripheral blood monocyte subsets (classical, intermediate and non-classical) in mothers and newborns. The three subsets of monocytes are considered to play distinct roles in immune and inflammatory responses by expressing distinct profiles of cytokines and homing receptors (27).

First, we used t-SNE to visualize how markers of immune activation and transfer of maternal antibodies vary across the three groups. For each subject, t-SNE reduces the entire set of measured immune parameters to two values that best summarize the subject's immunological profile (28). This summarization, formally known as dimensionality reduction, is unsupervised, i.e. the method does not use any information regarding which clinical group (HU, HEU before or HEU during) each subject belongs to. This analysis indicated that immune parameters were very different not only between HU and HEU mother-newborn pairs, but also according to time of initiation of maternal ART during pregnancy (Figure 2A). These separations involved both a reduced transfer of maternal antibodies and a significantly higher level of monocyte activation (Figure 2B and C). The lowest maternal antibody transfer ratios were observed in HEU newborns of mothers who initiated ART during pregnancy (Figure 2B and Supplementary Table 8). HIV-infected mothers who initiated ART during pregnancy and their newborns also displayed the highest levels of monocyte activation (Figure 2C and Supplementary Table 9).

Immune parameters and risk of hospitalization for infection

To obtain a more refined understanding of immune correlates of risk, we focused on the potential link between immune alterations and clinical outcome in the highest risk group. We

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used a multivariate LASSO/PLS model to separate HEU infants born to mothers who initiated

ART during pregnancy based on whether they were hospitalized during the first year of life. The

model identifies only a minimal set of markers that are most relevant in terms of predicting

clinical outcome (29). Based on mathematical combinations of these minimal immune markers,

or composite latent variables, the model then classifies infants into the two groups –

hospitalized or not (Figure 3A). We assessed the robustness of the model in a rigorous five-fold

cross-validation framework. The model achieved high overall median classification accuracy and

had balanced performance for both groups (Figure 3B). The model included 3 immune

parameters – increased risk of hospitalization was associated with higher titers of maternal

antibodies against VZV, higher expression of CD40 by non-classical newborn monocytes, and

lower transfer ratios of maternal antibodies to Pneumococcus 18s polysaccharide (Figure 3C).

While the 3 immune parameters included in the model have the best predictive power, there

could be other correlated parameters that capture some information relevant to risk of

hospitalization. To obtain a holistic understanding of all immune parameters tracking with

hospitalization risk, we explored other features significantly correlated with the 3 minimal

markers. Maternal VZV antibody levels were correlated with newborn VZV antibody levels

(Figure 3D). The expression of activation markers by newborn and maternal monocyte subsets

and maternal antibody transfer ratios followed complex co-ordinations, suggesting the

involvement of multiple pathways in the risk of infection-related hospitalization in HEU infants

(Figure 3D).

DISCUSSION

In this prospective birth cohort study, HEU infants born in Belgium had a 2-fold increased risk of hospitalization for infection as compared to HU infants. This result is in the range of those reported from Mozambique and South Africa (9–11). In agreement with previous studies, most infection-related hospitalizations occurred during the first months of life and involved diverse bacterial and viral pathogens (3,5,7,12–16).

This study provides two novel and important insights. First, in our study population, most of the excess risk of infection-related hospitalization in HEU as compared to HU infants could be attributed to initiation of ART during rather than before pregnancy. This observation supports the notion that the activity of maternal HIV infection is a central determinant of the risk of severe infections in HEU infants (6,7,13,17). It also indicates that the increased burden of infectious diseases observed in HEU infants can be reduced by the control of HIV replication throughout pregnancy.

The second novel insight of our study is that the risk of hospitalization for infection in HEU infants was correlated with immune alterations measured at birth. Activation of monocytes and reduced transfer of maternal antibodies were observed in HEU newborns, confirming previous studies (3,18–21). This study showed that the most intense immune alterations are detected in the highest risk group of HEU infants born to mothers who initiated ART during pregnancy. Modeling demonstrated that within this group, immune alterations predicted clinical outcome. This observation has important implications for the management of this vulnerable population. Identifying HEU newborns at risk of severe infections using immune biomarkers could guide the implementation of preventive interventions, including anti-microbial prophylaxis and immunization.

Our observation that HEU infants had an increased risk of hospitalization following RSV infection with similar seroconversion rates supports the notion that this population is at increased risk of severe infections rather than infections per se (14,15,30–32). Understanding the mechanisms involved could help in the design of interventions targeting the immune system (33). Modeling indicated that several immune pathways are likely to be involved. The reduced transfer of maternal antibodies to multiple pathogens is likely to contribute and may be overcome by immunization during pregnancy (34). On the other hand, immune activation involving innate immune cells may promote pathogenic inflammatory responses (3,18). Indeed, studies suggest that severe infections in early life may be related to excessive inflammation rather than to an immaturity of the immune system and that interventions promoting immune homeostasis may reduce the burden of severe infections in infants (35). The association between the risk of hospitalization for infections with the level of maternal anti-VZV antibodies is intriguing. History of zoster infection was not available for most patients. The absence of association with anti-CMV antibodies does not support a role for the reactivation of persistent viruses resulting from immune activation.

Several characteristics of the study may limit its interpretation and its implications. The study was conducted at a single site in Belgium. This increased the homogeneity of the study population and the consistency of the study procedures but may limit the generalizability of the results. As the decision of hospitalization was made independently of the HIV status of the mother, this information is unlikely to have biased the results. The sample size was not sufficient to analyze the severity of infections or the impact of ART regimen and duration during pregnancy. The cohorts of HIV-infected and HIV-uninfected mothers and their children differed for several parameters. However, adjusting our analysis for most of these parameters did not affect the results. Gestational age was lower in HEU than in HU newborns and prematurity is a known risk factor for infectious diseases (36). However, gestational age was not significantly associated with the risk of hospitalization for infection in our study population and the increased risk of HEU infants was still observed after exclusion of prematurely born infants. As

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HEU infants were not breastfed, the analysis could not be adjusted for this parameter. However, the risk of infection-related hospitalization was similar in HEU infants born to mothers who initiated ART before pregnancy (who were not breastfed) and in HU infants (who were almost all breastfed). In addition, duration of breastfeeding was not significantly associated with the risk of hospitalization for infection in HU infants. The limited impact of breastfeeding on the risk of hospitalization for infection in our study population may not be unexpected. Indeed, while there is strong supportive evidence for a protective effect of breastfeeding on infectious morbidity in LMIC, the evidence from HIC is less consistent (37–39). Studies should be conducted in LMIC to determine the impact of pre-conception ART and breastfeeding on the risk of severe infections in HEU infants.

In conclusion, initiation of maternal ART before pregnancy is associated with a reduced risk of hospitalization for infection in HEU infants born in a HIC and this effect could be related to the prevention of immune alterations during fetal life. The lifelong administration of ART to all HIV-infected women, as recommended by the World Health Organization (40), could therefore positively impact the health of HIV-exposed infants beyond PMTCT. Confirming these observations in LMIC where the burden of HIV and pediatric infectious diseases are highest would have critical implications for the health of HEU infants worldwide.

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CONFLICT OF INTEREST

T.G. no conflict, K.K.S. no conflict, C.A. no conflict, J.D. no conflict, T.M.B. no conflict, G.S. no conflict, S.L. no conflict, E.H. no conflict, P.B. no conflict, P.A.P. no conflict, F.v.d.K. no conflict, T.R.K. reports grants from NIAID, CIHR outside the submitted work, D.A.L. no conflict. G.A. reports grants from the Bill and Melinda Gates foundation and Gilead, outside the submitted work, J.L. no conflict, A.M. reports consultancy fees from GSK Vaccines outside the submitted work.

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Table 1. Characteristics of the study population.

MOTHERS	HEU (n=132)	HU (n=123)	p-value[a]
	n (%)	n (%)	
Region of origin			
North Africa	6 (4.5%)	11 (8.9%)	
Sub-Saharan Africa	108 (81.8%)	58 (47.2%)	
Central Europe	9 (6.8%)	30 (24.4%)	
Eastern Europe	6 (4.5%)	11 (8.9%)	
South and Central America	1 (0.8%)	11 (8.9%)	
Asia	2 (1.5%)	2 (1.6%)	<0.001[b]
Missing	0	0	
Maternal level of education[e]			
Primary non achieved	6 (5.2%)	7 (6.0%)	

Primary achieved	23 (19.8%)	13 (11.1%)	
Secondary inf. achieved	29 (25.0%)	26 (22.2%)	
Secondary sup. achieved	39 (33.6%)	39 (33.3%)	
Higher education	19 (16.4%)	32 (27.4%)	0.18
Missing	16	6	
Maternal illiteracy	8 (6.3%)	12 (10.3%)	0.25
Missing	4	6	
Maternal occupation			
Workless	63 (52.1%)	53 (45.7%)	
Full time work	33 (27.3%)	37 (31.9%)	
Part time work	8 (6.6%)	11 (9.5%)	
Student/foster	8 (6.6%)	11 (9.5%)	
Others	9 (7.4%)	4 (3.4%)	0.43

Missing	11	7	
Maternal age at delivery, years (mean(SD))	31.0 (6.2)	28.8 (5.5)	0.004 [d]
Missing	0	0	
Maternal age at delivery, years			
<20	4 (3.0%)	6 (4.9%)	
20-34	88 (66.7%)	98 (79.7%)	
≥35	40 (30.3%)	19 (15.4%)	0.014[b]
PREGNANCY & DELIVERY			
Primigestity	20 (15.4%)	46 (37.4%)	<0.001

Missing	2	0	
Primiparity	40 (30.3%)	58 (47.2%)	0.006
Missing	0	0	
Smoking during pregnancy	11 (8.8%)	11 (9.6%)	0.84
Missing	7	8	
Alcohol during pregnancy	10 (8.0%)	14 (12.4%)	0.26
Missing	7	10	
GBS positive swab	40 (31.5%)	30 (24.4%)	0.21
Missing	5	0	
CMV positive serology	128 (99.2%)	105 (87.5%)	<0.001
Missing	3	3	
HBs Ag positive	12 (9.3%)	3 (2.5%)	0.023
Missing	3	2	

Mode of delivery			
Vaginal	92 (69.7%)	90 (73.2%)	
Planned caesarean section	17 (12.9%)	17 (13.8%)	
Emergency caesarean section	23 (17.4%)	16 (13.0%)	0.62
Missing	0	0	
Antibiotics at delivery	71 (54.6%)	62 (50.4%)	0.50
Missing	2	0	
ART during pregnancy			
Initiation before start of pregnancy	81 (61.4%)	-	
Initiation during pregnancy	51 (38.6%)		
Missing	0		
Onset of pregnancy VL, copies/mL			
<20	60 (52.2%)	-	

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20-1000	22 (19.1%)	
>1000	33 (28.7%)	
Missing	17	
End of pregnancy VL, copies/mL		
<20	85 (72.0%)	-
20-1000	31 (26.3%)	
>1000	2 (1.7%)	
Missing	14	
Nadir CD4 count, cells/mm^3 (median (min-max))	340 (21-888)	-
Missing	18	
Nadir CD4 count, cells/mm^3		
<250	27 (23.7%)	-
250-500	57 (50.0%)	

>500	30 (26.3%)	
Onset of pregnancy CD4 count, cells/mm^3 (median (min-max))	528 (132-1500)	-
Missing	14	
Onset of pregnancy CD4 count, cells/mm^3		
<250	12 (10.2%)	-
250-500	41 (34.7%)	
>500	65 (55.1%)	
End of pregnancy CD4 count, cells/mm^3 (median (min-max))	596 (101-1805)	-
Missing	14	
End of pregnancy CD4 count, cells/mm^3		
<250	6 (5.1%)	-
250-500	32 (27.1%)	
>500	80 (67.8%)	

INFANTS

Female gender	74 (56.1%)	61 (49.6%)	0.30
Missing	0	0	
Gestational age, weeks (median (min-max))	39 (32-42)	40 (36-42)	<0.0001[c]
Missing	0	0	
Gestational age, weeks			
32-33	4 (3.0%)	0 (0.0%)	
34-36	10 (7.6%)	7 (5.7%)	
≥ 37	118 (89.4%)	116 (94.3%)	0.15[b]
Birth weight (mean (SD))	3048 (575)	3350 (459)	<0.0001[d]
Missing	0	0	
Birth weight <2500 grams	22 (16.7%)	4 (3.3%)	<0.0001
Breast feeding	0 (0.0%)	117 (95.1%)	<0.0001[b]

Missing	0	0	-
Duration of breast feeding (median (min-max))	-	6.0 (0.0-36.0)	
Missing		16	
Duration of breast feeding			
0-3 months	-	35 (32.7%)	
>3 months		72 (67.3%)	
Day care attendance	73 (55.3%)	55 (49.1%)	0.33
Missing	0	11	

[a] Chi-square test unless indicated otherwise; [b] Exact-test; [c] Mann-Whitney U test; [d] Student *t* test; [e] primary level corresponds to age 12 years, secondary inf. level

corresponds to 14 years, secondary sup. corresponds to 18 years. SD: standard deviation. The term missing indicates the number of missing data for each variable.

Table 2. Risk of infection-related hospitalization over the first year of life in HEU and HU infants.

	n	cHR[a] (95% CI)	p-value[b]	aHR[b] (95% CI)	p-value[c]
HU	123	1		1	
HEU	132	1.78 (0.94-3.40)	0.079	2.33 (1.10-4.97)	0.028
HU	123	1		1	
HEU before	81	1.22 (0.56-2.63)		1.42 (0.58-3.48)	
HEU during	51	2.84 (1.37-5.88)	0.012	3.84 (1.69-8.71)	0.003

[a] Crude hazard ratio (95% confidence limits); [b] Hazard ratio (95% confidence limits) adjusted for gestational age, birth weight, illiteracy, upper level of education, African origin, primiparity, and maternal age at birth; [c] Wald test.

Table 3. Risk factors for infection-related hospitalization over the first year of life in HEU infants.

	n	cHR[a] (95% CI)	p-value[e]	aHR[b] (95% CI)	p-value[c]
Sub-Saharan African origin					
Yes	20	0.63 (0.27-1.50)		0.60 (0.25-1.42)	
No	7	1.00	0.30	1.00	0.24
Maternal higher education					
No	22	1.04 (0.36-3.02)		0.87 (0.28-2.65)	
Yes	4	1.00	0.94	1.00	0.80
Maternal illiteracy					
Yes	3	1.99 (0.60-6.61)		1.89 (0.56-6.38)	
No	24	1.00	0.26	1.00	0.31
Maternal unemployment					

Yes	14	1.09 (0.51-2.31)		1.04 (0.49-2.23)	
No	13	1.00	0.83	1.00	0.91
Maternal age ≥35 years					
Yes	9	1.16 (0.52-2.57)		1.15[d] (0.52-2.57)	
No	18	1.00	0.73	1.00	0.73
Primiparity					
Yes	5	0.55 (0.21-1.44)		0.44 (0.16-1.23)	
No	22	1.00	0.22	1.00	0.12
Antibiotics at delivery					
Yes	19	2.26 (0.99-5.17)		2.30 (0.99-5.34)	
No	8	1.00	0.053	1.00	0.052
Caesarean section					
Yes	11	1.72 (0.80-3.71)		1.85 (0.82-4.20)	

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No	16	1.00	0.17	1.00	0.14

Gender

Male	11	0.85 (0.40-1.84)		0.84 (0.38-1.81)	
Female	16	1.00	0.69	1.00	0.65

Gestational age, weeks

32-33	1	1.47 (0.20-10.89)		1.39[e] (0.19-10.36)	
34-36	3	1.86 (0.56-6.20)		1.81[e] (0.54-6.05)	
≥37	23	1.00	0.57	1.00	0.61

Birth weight < 2500g

Yes	4	0.88 (0.31-2.55)		0.86 (0.25-2.99)	
No	23	1.00	0.82	1.00	0.81

ART initiation

During pregnancy	15	2.34 (1.09-5.00)		2.31 (1.03-5.15)	

Before pregnancy	12	1.00	0.028	1.00	0.041
Onset of pregnancy VL, copies/mL					
<20	11	1.00		1.00	
20-1000	3	0.73 (0.21-2.63)		0.73 (0.20-2.67)	
>1000	9	1.73 (0.72-4.18)	0.32	1.80 (0.67-4.85)	0.33
End of pregnancy VL, copies/mL					
<20	16	1.00		1.00	
20-1000	5	0.85 (0.31-2.32)		0.81 (0.29-2.26)	
>1000	1	4.36 (0.58-32.97)	0.32	7.08 (0.80-62.78)	0.18
Nadir CD4 count, cells/mm^3					
<250	2	0.19 (0.04-0.84)		0.17 (0.04-0.81)	
250-500	12	0.56 (0.24-1.31)		0.55 (0.23-1.29)	
>500	10	1.00	0.07	1.00	0.07

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Onset of pregnancy CD4 count, cells/mm^3					
<250	0	-		-	
250-500	7	0.54 (0.23-1.28)		0.48 (0.20-1.20)	
>500	19	1.00	0.16	1.00	0.12
End of pregnancy CD4 count, cells/mm^3					
<250	0	-		-	
250-500	7	1.16 (0.48-2.81)		1.17 (0.48-2.84)	
>500	16	1.00	0.75	1.00	0.74

[a] Crude hazard ratio (95% confidence limits); [b] Hazard ratio (95% confidence limits) adjusted for gestational age and maternal age at birth; [c] Wald test.

[d] Hazard ratio (95% confidence limits) adjusted for gestational age; [e] Hazard ratio (95% confidence limits) adjusted for maternal age at birth.

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Table 4. Baseline characteristics of HIV-infected mothers and their infants according to

	ART initiation before pregnancy (n=81)	ART initiation during pregnancy (n=51)	p-value [a]

time of ART initiation.

ART during pregnancy			
Whole pregnancy	81 (100%)	0	
2 last trimesters	0	18 (35%)	
Last trimester	0	26 (51%)	
Last month	0	5 (10%)	
<1 month	0	2 (4%)	<0.0001 [b]
Missing	0	0	

ART regimen during pregnancy [e]			
NRTI + PI	54 (67%)	48 (94%)	
NRTI + NNRTI	17 (21%)	-	
NRTI only	7 (9%)	-	
Other regimen	3 (4%)	3 (6%)	<0.0001 [b]
Missing	0	0	

Onset of pregnancy VL, copies/mL			
<20	56 (77%)	4 (10%)	
20-1000	13 (18%)	9 (21%)	
>1000	4 (6%)	29 (69%)	<0.0001
Missing	8	9	

End of pregnancy VL, copies/mL

<20	55 (74%)	30 (68%)	
20-1000	19 (26%)	12 (27%)	
>1000	0 (0%)	2 (5%)	0.20 [b]
Missing	7	7	
Nadir CD4 count [f], cells/mm³	319 (21-868)	424 (66-888)	0.002 [e]
Missing	10	8	
Nadir CD4 count, cells/mm³			
<250	21 (30%)	6 (14%)	
250-500	36 (51%)	21 (49%)	
>500	14 (20%)	16 (37%)	0.06 [b]
Onset of pregnancy CD4 count [f], cells/mm³	520 (141-1035)	545 (132-1500)	0.46 [e]
Missing	8	6	
Onset of pregnancy CD4 count, cells/mm³			
<250	8 (11%)	4 (9%)	
250-500	25 (34%)	16 (36%)	
>500	40 (55%)	25 (56%)	1.00 [b]
End of pregnancy CD4 count [f], cells/mm³	585 (138-1118)	662 (101-1805)	0.03 [e]
	8	6	

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Missing			

End of pregnancy CD4 count, cells/mm³			
<250	4 (6%)	2 (4%)	
250-500	24 (33%)	8 (18%)	
>500	45 (62%)	35 (78%)	0.16 [b]

Region of origin			
North Africa	3 (4%)	3 (6%)	
Sub-Saharan Africa	65 (80%)	43 (84%)	
Central Europe	6 (7%)	3 (6%)	
Eastern Europe	6 (7%)	0 (0%)	
South and Central America	0 (0%)	1 (2%)	
Asia	1 (1%)	1 (2%)	0.25 [b]
Missing	0	0	

Maternal level of education [f]			
Primary non achieved	3 (4%)	3 (7%)	
Primary achieved	13 (18%)	10 (23%)	
Secondary inf. achieved	21 (29%)	8 (18%)	
Secondary sup. achieved	24 (33%)	15 (34%)	
Higher education	11 (15%)	8 (18%)	0.72

Missing	9	7	
Maternal illiteracy	3 (4%)	5 (10%)	0.26 [b]
Missing	2	2	
Unemployed	38 (51%)	25 (54%)	0.42
Missing	6	5	
Maternal age at delivery, years (mean(SD))	32.4 (6.0)	28.8 (5.9)	0.001 [c]
Missing	0	0	
Maternal age at delivery, years			
<20	2 (3%)	2 (4%)	
20-34	48 (59%)	40 (78%)	
≥35	31 (38%)	9 (18%)	0.028 [b]
Primigestity	8 (10%)	12 (25%)	0.025
Missing	0	2	
Primiparity	20 (25%)	20 (39%)	0.08
Missing	0	0	
Smoking during pregnancy	5 (6%)	6 (13%)	0.33£
Missing	2	5	
Alcohol during pregnancy	7 (9%)	3 (7%)	0.74 [b]

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Missing	2	5	
GBS positive swab	23 (29%)	17 (35%)	0.46
Missing	2	3	
CMV positive serology	80 (99%)	48 (100%)	0.44 [b]
Missing	0	3	
HBs Ag positive	8 (10%)	4 (8%)	1.00 [b]
Missing	0	3	
Mode of delivery			
Vaginal	56 (69%)	36 (71%)	
Planned caesarean section	13 (16%)	4 (8%)	
Emergency caesarean section	12 (15%)	11 (22%)	0.29
Missing	0	0	
Antibiotics at delivery	40 (50%)	31 (62%)	0.18
Missing	1	1	
Female gender	50 (62%)	24 (47%)	0.10
Missing	0	0	
Gestational age, weeks (median(min-max))	39 (32-42)	39 (33-41)	1.00 [d]
Missing	0	0	

Gestational age, weeks			
32-33	3 (4%)	1 (2%)	
34-36	4 (5%)	6 (12%)	
≥ 37	74 (91%)	44 (86%)	0.43 [b]
Birth weight, grams (mean (SD))	3089.6 (619.9)	2983.1 (495.6)	0.30 [c]
Missing	0	0	
Birth weight <2500 grams	15 (19%)	7 (14%)	0.47
Day care attendance	51 (63%)	22 (43%)	0.026
Missing	0	0	

[a] Chi-square test unless indicated otherwise; [b] Exact-test; [c] Student *t* test; [d] Mann Whitney *U*-test. [e] NRTI include tenofovir plus emtricitabine in 51% and 3TC plus AZT in 38%; NNRTI include nevirapine in 99%; PI include ritonavir-boosted lopinavir in 48% and atazanavir in 43%. [f] primary level corresponds to age 12 years, secondary inf. level corresponds to 14 years, secondary sup. corresponds to 18 years. SD: standard deviation. The term missing indicates the number of missing data for each variable.

FIGURE LEGENDS

Figure 1. Occurrence of infection-related hospitalizations during the first year of life in HIV-exposed and unexposed infants. Kaplan-Meier probability estimates of infant hospitalization for infection according to: **A.** maternal HIV status (aHR (95% CI) HEU vs HU: 2.33 (1.10-4.97)); **B.** maternal HIV status and time of initiation of ART (aHR (95% CI) HEU before vs HU: 1.42 (058-3.48); HEU during vs HU: 3.84 (1.69-8.71)). Follow-up was censored at the time of first hospitalization for infection, at the time of loss to follow-up or at 12 months of age. Numbers are infants at risk at each time point. HU: HIV-unexposed infants; HEU ART before: HIV-exposed uninfected infants born to mothers who initiated ART before pregnancy; HEU ART during: HIV-exposed uninfected infants born to mothers who initiated ART during pregnancy.

Figure 2. Immune parameters measured at birth in HIV-exposed and unexposed newborns and their mothers. A. Stratification according to immune parameters (maternal antibody transfer ratios and expression of markers of activation by peripheral blood monocyte subsets) measured at birth in newborns and mothers using t-distributed stochastic neighbor embedding (t-SNE). **B.** Median maternal antibody transfer ratios to pathogen and vaccine antigens (DTX: diphtheria toxoid: TTX: tetanus toxoid; PTX: pertussis toxin; VZV: varicella zoster virus; CMV: cytomegalovirus; RSV: respiratory syncytial virus; PPs: Pneumococcus polysaccharide; Hib: H*aemophilus influenza* type B). Antibody data were available for 71% of mothers and newborns. **C-E.** Violin plots illustrating CD40 expression (mean fluorescence intensities (MFI)) by maternal and newborn peripheral blood monocyte subsets. Monocyte phenotype data were available from 45% of mothers and 41% of newborns. +: mean and □: median MFI. HU: HIV-unexposed infants; HEU ART before: HIV-exposed uninfected infants born to mothers who initiated ART before pregnancy; HEU ART during: HIV-exposed uninfected infants born to mothers who initiated ART during pregnancy. *: P<0.01 by Mann-Whitney *U* test.

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Figure 3. Modeling the risk of hospitalization for infection in HEU infants born to mothers who initiated ART during pregnancy. A. Scores plot corresponding to LASSO/PLS model that can stratify HEU infants of mothers who initiated ART during pregnancy, by hospitalization during the first year of life. Input features comprise immune parameters (maternal antibody transfer ratios and expression of markers of activation by peripheral blood monocyte subsets) measured at birth in newborns and their mothers. LV: latent variable. **B.** Antibody data were available for 53% of mothers and newborns. Monocyte phenotype data were available from 53% of mothers and 43% of newborns. Violin plots illustrating classification accuracy (overall as well as for each of the 2 groups) of the LASSO/PLS model, as measured in a 5-fold cross-validation framework, across 50 independent runs. +: mean and □: median classification accuracy. **C.** Variable importance in the projection (VIP) scores for parameters used by the model. Length of the bars corresponds to magnitude of VIP scores, while direction is based on positive or negative association with risk (i.e., increased risk of hospitalization). **D.** Networks illustrating co-correlates of the parameters used in the model. A relationship is defined as significant if the Spearman correlation coefficient is >0.5 and the *p* value associated with the correlation coefficient, post Bonferroni multiple-testing correction, is <0.05. c-monocytes: classical monocytes; i-monocytes: intermediate monocytes; nc-monocytes: non-classical monocytes; TR: transfer ratio; PPs: Pneumococcus polysaccharide; DTX: diphtheria toxoid; VZV: varicella zoster virus; PTX: pertussis toxin; TTX: tetanus toxoid.
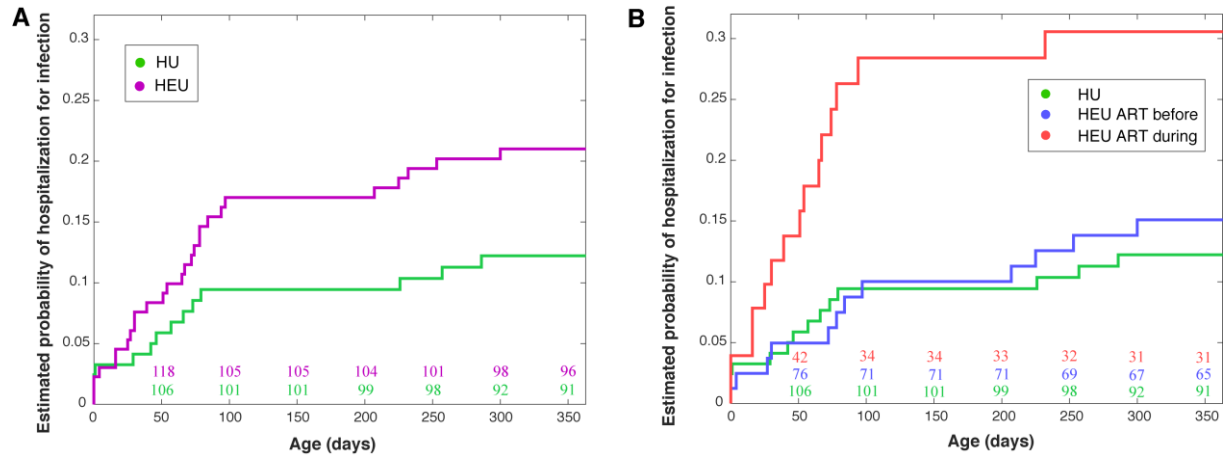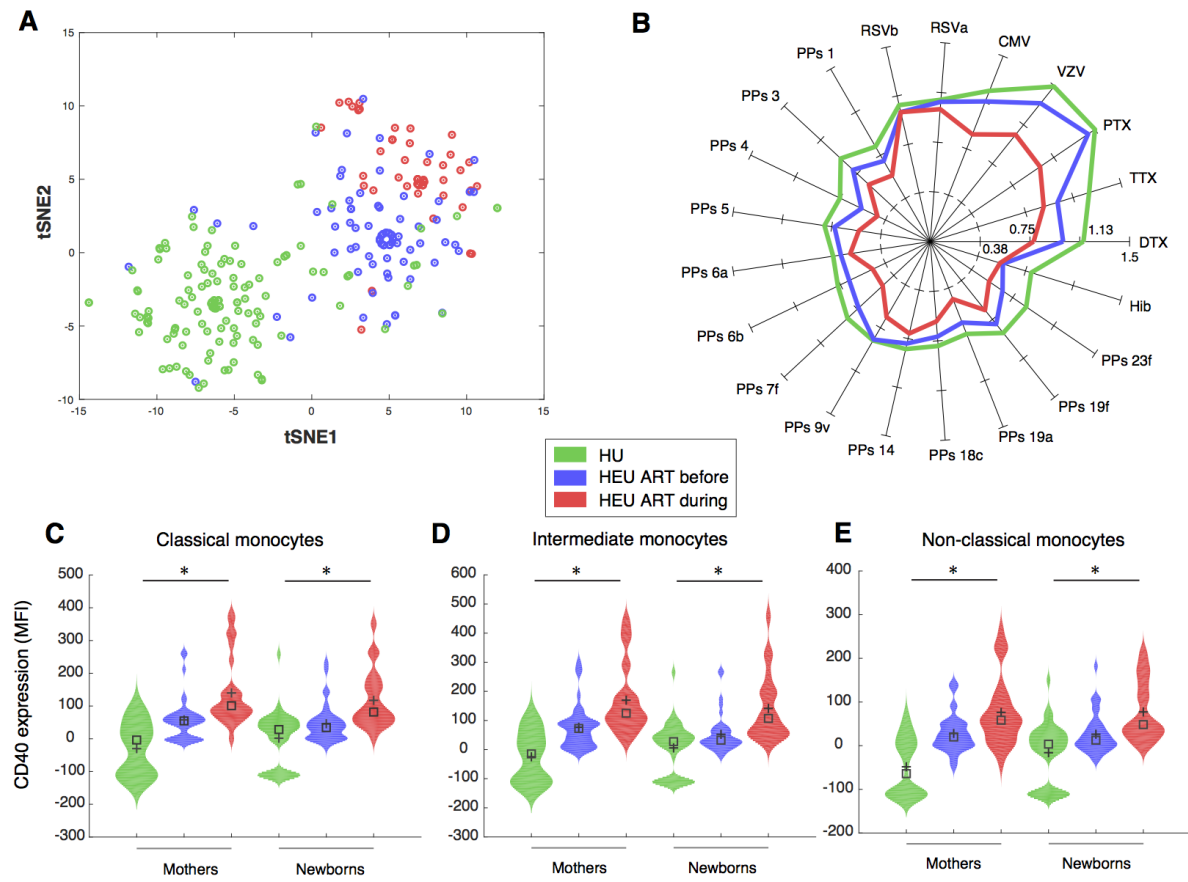
Figure 1

Figure 2

Figure 3