

Marisa M Mussi-Pinhata¹, Adriana Weinberg², Qilu Yu³, Rachel A. Cohen³, Sahera Dirajlal-Fargo⁴, Nicholas Funderburg⁵, Emily Bowman⁵, Nahida Chakhtoura⁶, Grace A. McComsey⁴

¹University of São Paulo, Ribeirão Preto, Brazil; ²University of Colorado Denver, CO, USA; ³WESTAT, Rockville, MD, USA; ⁴University Hospitals Health System and Case Western Reserve University, Cleveland, Ohio; ⁵ Ohio State University, Columbus, OH, USA;

⁶ Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, USA.

ORIGINAL ABSTRACT

Background: HIV infection is accompanied by high levels of inflammation that predict increased mortality in adults. HIV-exposed uninfected (HEU) infants also have increased infectious morbidity and mortality, but little is known about their levels of inflammation and immune activation. In this study, we assessed how inflammatory and monocyte activation markers correlated between mothers and HEUs at delivery and compared markers between HEU and HIV-unexposed (HU) infants at birth and at 6 months of life.

Methods: The study enrolled term singletons ≥ 2500 g at birth and their mothers. Samples obtained at birth and 6 months from 86 HEU mother-infant pairs enrolled in the NICHD cohorts in Brazil were analyzed and compared to 88 HU mother-infant pairs. All HIV-infected mothers received ARV during pregnancy. HEUs received neonatal zidovudine prophylaxis and formula. HUs were born to healthy mothers, and most received formula feeding by 4 weeks of age. Infants had clinical and laboratory evaluations at birth and 6 months. IL-6, TNFRI, TNFRII, sCD14, sCD163, IP-10, VCAM, OxLDL, D Dimer, and hsCRP were assayed by ELISA. Data were analyzed using two-sample t-test, correlation coefficients and linear regression models. $p < 0.005$ was used to adjust for multiple comparisons.

Results: Among HIV-infected mothers, 81.4% had HIV-RNA $< 1,000$ copies/mL prior to delivery. In addition, they were older (27 vs. 24 years), and more frequently non-white (64.4% vs. 25.0%) when compared to uninfected mothers. Compared to HU, HEU infants were born more frequently by C-section (59% vs. 32%), with a lower median gestational age (38.6 vs. 39.3wk) and weight (3.2 vs. 3.3kg); and reached lower weight (5.9 vs. 8.5kg) and height (53.5 vs. 68.8 cm) at 6 months of age. Majority of inflammatory markers were significantly higher ($p < 0.005$) in HEU compared with HU at birth, but at 6 months only TNFRI and IL-6 remained higher (Table 3). Among HU mother-infant pairs, infant IL-6, TNFRI, TNFRII, sCD14, sCD163 levels at birth were associated with maternal levels at delivery ($r \geq 0.31$; $p < 0.0004$). For HEU pairs, the only association was for IP-10 ($r = 0.34$; $p < 0.0001$) at birth.

Conclusions: HIV-exposed infants have higher inflammation and monocyte activation than HU at birth, which for some markers persisted to 6 months of life, and was not related to maternal inflammatory status. Inflammation may contribute to the increased HEU infectious morbidity and poor growth. This hypothesis warrants further investigation.

BACKGROUND

❖ HIV-exposed uninfected (HEU) infants have higher morbidity and mortality.

❖ HEU infants have higher incidence of metabolic alterations, infectious disease morbidity, impaired growth and altered immunity compared to unexposed uninfected children (HU).

❖ Inflammation and immune activation are independently associated with mortality in HIV-infected adults and children.

❖ Little is known about the long-term effects of exposure to HIV and ART and whether HEU infants have higher levels of inflammation and immune activation.

HYPOTHESIS

❖ At birth and at 6 months of life, HEU will have higher markers of inflammation when compared to HIV-unexposed (HU) infants

OBJECTIVES

1. To compare markers of inflammation and monocyte activation at birth and at 6 months of life between HEU and HU infants;

2. To correlate markers of inflammation and immune activation in HEU and HU infants to those of their mothers around the time of delivery;

METHODS

STUDY DESIGN: PROSPECTIVE COHORTS

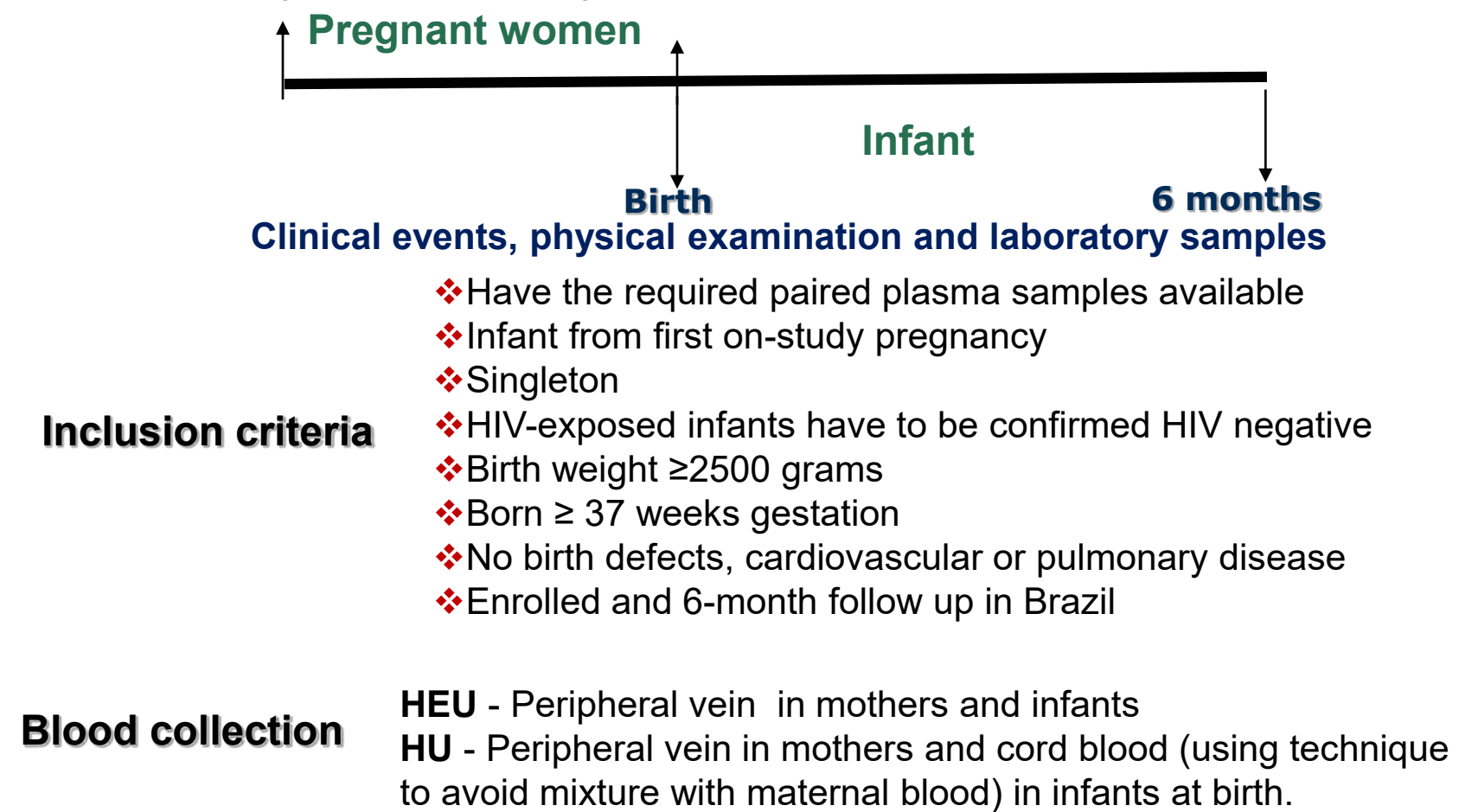
HIV-Exposed Uninfected (HEU)
NISDI and LILAC cohorts

HIV-Unexposed (HU)
CIRAI cohort

VS.

Infants born to HIV-infected women in Brazil, known to be HIV-uninfected with follow-up through six months of age

Infants born to healthy mothers and were receiving mostly artificial milk feedings by 4 weeks of age



Inflammation markers

- sTNFRI:
- IL-6
- D Dimer
- IP-10
- TNFRII
- VCAM
- hsCRP

Assays - ELISAs

Markers of monocyte activation

- sCD14
- sCD163

Marker of oxidized lipids

- Ox LDL Av

Table 1 – Characteristics of mothers and infants

MOTHERS	Total (N=175)	HEU (N=87)	HU (N=88)	p-value
Maternal age at delivery (years)				
Mean (Std. Dev)	26.6 (6.2)	27.7 (6.3)	25.5 (5.9)	0.02
Maternal race				
Black	32 (18.3)	25 (28.7)	7 (8.0)	<.0001
Mestizo	5 (2.9)	5 (5.7)	0 (0.0)	
Other	20 (11.4)	5 (5.7)	15 (17.0)	
Unknown	21 (12.0)	21 (24.1)	0 (0.0)	
White	97 (55.4)	31 (35.6)	66 (75.0)	
Alcohol use during pregnancy				
No	157 (89.7)	78 (89.7)	79 (89.8)	1.00
Yes	18 (10.3)	9 (10.3)	9 (10.2)	
Tobacco use during pregnancy				
No	152 (86.9)	76 (87.4)	76 (86.4)	1.00
Yes	23 (13.1)	11 (12.6)	12 (13.6)	
Marijuana use during pregnancy				
No	174 (99.4)	86 (98.9)	88 (100)	0.50
Yes	1 (0.6)	1 (1.1)	0 (0.0)	
Cocaine/crack use during pregnancy				
No	173 (98.9)	85 (97.7)	88 (100)	0.25
Yes	2 (1.1)	2 (2.3)	0 (0.0)	
Heroin/opiate use during pregnancy				
No	175 (100)	87 (100)	88 (100)	
Any substance use during pregnancy				
No	141 (80.6)	69 (79.3)	72 (81.8)	0.71
Yes	34 (19.4)	18 (20.7)	16 (18.2)	
Mode of delivery				
Elective C-Section	51 (29.1)	37 (42.5)	14 (15.9)	0.0002
Vaginal	96 (54.9)	36 (41.4)	60 (68.2)	

INFANTS	Total (N=175)	HEU (N=87)	HU (N=88)	p-value
Gender				
Female	93 (53.1)	50 (57.5)	43 (48.9)	0.29
Male	82 (46.9)	37 (42.5)	45 (51.1)	
Apgar scores (at 1 min)				
<7	21 (12.1)	8 (9.4)	13 (14.8)	0.35
≥ 7	152 (87.9)	77 (90.6)	75 (85.2)	
Gestational age at birth (weeks)				
Mean (Std. Dev)	39.0 (1.4)	38.6 (1.3)	39.3 (1.4)	0.001
Weight at birth (grams)				
Mean (Std. Dev)	3261 (389.4)	3137 (349.0)	3382 (390.9)	<.0001
Weight at 6 months (grams)				
Mean (Std. Dev)	7227 (2138)	5944 (2090)	8495 (1244)	<.0001
Height at enrollment (cm)				
Mean (Std. Dev)	50.4 (3.9)	48.2 (2.0)	55.5 (2.1)	<.0001
Height at 6 months (cm)				
Mean (Std. Dev)	61.2 (9.9)	53.5 (8.6)	68.8 (2.3)	<.0001
White blood count at 6 months ($10^3/mm^3$)				
Mean (Std. Dev)	10.7 (3.1)	10.5 (2.8)	10.8 (3.4)	0.57

Table 2 – Virologic variables among HIV-infected mothers

HIV-INFECTED MOTHERS	Frequency	Percent
Any ARVs used during pregnancy		
Yes	87	100
Reason for ARV use during pregnancy		
Prevention of mother-to-child transmission	44	51.16
Treatment	42	48.84
Most complex ARV regimen used ≥ 28 days during pregnancy		
2NRTIs + 1PI	55	63.22
2NRTIs + 1NNRTI	21	24.14
Dual	1	1.15
Mono	8	9.20
Other ARVs	2	2.30
Most complex ARV regimen used ≥ 28 days during 1st trimester		
2NRTIs + 1PI	4	4.60
2NRTIs + 1NNRTI	6	6.90
Dual	2	2.30
Mono	0	0
Other ARVs	2	2.30
No ARVs used ≥ 28 days	73	83.91
Most complex ARV regimen used ≥ 28 days during 2nd trimester		
2NRTIs + 1PI	35	40.23
2NRTIs + 1NNRTI	12	13.79
Dual	0	0
Mono	9	10.34
Other ARVs	1	1.15
No ARVs used ≥ 28 days	30	34.48
Most complex ARV regimen used ≥ 28 days during 3rd trimester		
2NRTIs + 1PI	54	62.07
2NRTIs + 1NNRTI	21	24.14
Dual	0	0
Mono	8	9.20
Other ARVs	2	2.30
No ARVs used ≥ 28 days	2	2.30
First viral load during pregnancy (copies/ml)		
<1000	59	68.60
1000-9999	15	17.44
$\geq 10,000$	12	13.95
Last viral load before delivery (copies/ml)		
<1000	70	81.40
1000-9999	10	11.63
$\geq 10,000$	6	6.98
Highest viral load before delivery (copies/ml)		
<1000	55	63.95
1000-9999	17	19.77
$\geq 10,000$	14	16.28
First CD4 count during pregnancy (cells/mm³)		
<200	9	10.47
200 – 499	41	47.67
≥ 500	36	41.86
Last CD4 count before delivery (cells/mm³)		
<200	8	9.30
200 – 499	39	45.35
≥ 500	39	45.35
Lowest CD4 count before delivery (cells/mm³)		
<200	9	10.47
200 – 499	44	51.16
≥ 500	33	38.37

RESULTS

Table 3 – Comparisons of all infants' inflammation markers and markers of monocyte activation according to age

Age	Marker	Mean (st.dev)		Difference (95% CI)	p-value*
		HEU	HU		
Birth	TNFRI (pg/mL)	3.32 (0.16)	3.15 (0.14)	0.17 (0.12, 0.22)	<0.0001
	IL-6 (pg/mL)	0.78(0.44)	0.42 (0.53)	0.36 (0.21, 0.51)	<0.0001
Six months	D Dimer (ng/mL)	3.17(0.47)	3.21 (0.44)	-0.04 (-0.18, 0.10)	0.56
	IP10 (pg/mL)	2.06(0.45)	1.87 (0.22)	0.19 (0.08, 0.30)	0.001
	Ox LDL Av (mU/L)	4.40(0.28)	4.14 (0.17)	0.26 (0.19, 0.33)	<0.0001
	TNFRII (pg/mL)	3.64(0.17)	3.60 (0.19)	0.04 (-0.02, 0.09)	0.21
	VCAM (ng/mL)	3.22(0.11)	3.42 (0.26)	-0.20 (-0.26, -0.14)	<0.0001
	hsCRP (ng/mL)	3.27(0.49)	2.56 (0.45)	0.71 (0.56, 0.85)	<0.0001
	sCD14 (pg/mL)	3.04(0.14)	2.80 (0.10)	0.23 (0.20, 0.27)	<0.0001
	sCD163 (ng/mL)	2.72(0.23)	2.75 (0.19)	-0.02 (-0.09, 0.04)	0.45
	TNFRI (pg/mL)	3.06(0.11)	2.96 (0.13)	0.10 (0.06, 0.13)	<0.0001
Six months	IL-6 (pg/mL)	0.36(0.57)	0.13 (0.51)	0.22 (0.06, 0.39)	<0.0001
	D Dimer (ng/mL)	2.78(0.32)	2.95 (0.27)	-0.16 (-0.25, -0.08)	0.0003
	IP10 (pg/mL)	2.01(0.40)	2.21 (0.36)	-0.19 (-0.31, 0.08)	0.001
	Ox LDL Av (mU/L)	4.49(0.16)	4.57 (0.18)	-0.08 (-0.13, -0.03)	0.003
	TNFRII (pg/mL)	3.55(0.18)	3.62 (0.18)	-0.07 (-0.12, -0.01)	0.02
	VCAM (ng/mL)	3.13(0.13)	3.23 (0.16)	-0.11 (-0.15, -0.06)	<0.0001
hsCRP (ng/mL)	3.05(0.55)	2.93 (0.57)	0.12 (-0.05, 0.29)	0.16	
sCD14 (pg/mL)	3.21(0.14)	3.26 (0.12)	-0.05 (-0.09, -0.01)	0.03	
sCD163 (ng/mL)	2.89(0.28)	2.90 (0.21)	-0.01 (-0.08, 0.07)	0.86	

* two-sample t test

Summary findings

- Inflammatory markers were significantly higher in HEU compared with HU at birth, at 6 months only TNFRI and IL-6 remained higher.

❖ With the exception of IP-10, maternal markers did not correlate with infants' in HIV-exposed (but did in HIV-unexposed)

❖ Oxidized LDL was positively correlated with TNFRI, hsCRP, sCD14, D Dimer, IL-6 ($p \leq 0.03$) and approaching significance with IP-10 ($p = 0.08$)

❖ Maternal CD4 was correlated with only infant TNFRII ($p = 0.03$).

❖ Maternal viral load did not correlate with any of the markers at baseline.

❖ Birth infant CRP was associated with birth weight ($p = 0.04$) and baseline IP10 was associated with weight at 6 mos ($p = 0.04$).

CONCLUSIONS

❖ HIV-exposed infants have higher inflammation and monocyte activation than HU at birth, which for some markers persisted to 6 months of life.

❖ Higher levels of inflammation in HEU is not merely the result of passive maternal transfer.

❖ Inflammation and immune activation may contribute to the increased infectious and metabolic complications seen in HEU. This hypothesis warrants further investigation.

REFERENCES

- Mussi-Pinhata MM, Freimanis L, Yamamoto AY, 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 Mar;119(3):e694-704.
- Reikie BA, Adams RC, Leligidowicz A, et al. Altered innate immune development in HIV-exposed uninfected infants. *J Acquir Immune Defic Syndr* (2014) 66(3):245
- Choungnet C, Kovacs A, Baker R, et al. Influence of human immunodeficiency virus-infected maternal environment on development of infant interleukin-12 production. *J Infect Dis* (2000) 181(5):1590-7. 10.1086/315458
- Borges-Almeida E, Milanez HM, Vilela MM, 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. 10.1186/1471-2334-11-38