

Increased platelets count in HIV-1 uninfected infants born from HIV-1 infected mothers

Anicet Christel Maloupazoa Siawaya,¹
Amandine Mveang-Nzoghe,¹
Chérone Nancy Mbani Mpega,²
Marielle Leboueny,¹
Ofilia Mvoundza Ndjindji,¹
Armél Mintsá Ndong,³
Paulin N. Essone,^{1,4}
Joel Fleury Djoba Siawaya¹

¹Unités de Recherche et de Diagnostics Spécialisés, Laboratoire National de Santé Publique, Libreville, Gabon; ²Département de Chimie, Faculté des Sciences, Université des Sciences et Techniques de Masuku, Franceville, Gabon; ³Unité de Virologie, Laboratoire National de Santé Publique, Libreville, Gabon; ⁴Division of Immunology, Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Science, University of Cape Town, South Africa

Abstract

HIV-exposed uninfected infants (HEU) represent a growing population in developing countries including Gabon. Several studies have shown the vulnerability of these infants toward infectious diseases. The aim of the study was to contribute to the global effort to understand how HIV-exposure or anti retroviral therapy affects infants' blood elements. We assessed HEU infants' complete blood count using a blood analyzer instrument. Our investigations showed that among the observed clinically relevant hematological abnormalities events, thrombocytosis was the most prevalent clinically relevant hematological abnormality associated with HEU infants'. We showed that HEU infants had significantly higher platelets count than HU-infants. Therefore, higher level of platelets seems to characterize HEU infants when compared to HU infants.

Introduction

Mother-to-child transmission prevention programs have dramatically reduced the mother-to-child transmission rate increasing the HIV exposed uninfected infant (HEU) population. Many research groups have focused on this population and

demonstrated a high mortality and morbidity in the HEU population when compared to HIV negative unexposed (HU) infants from HIV uninfected mothers.¹⁻⁸ This increased morbidity/mortality in HEU infants has been linked to recurrent infections.^{1,4}

Research on HIV exposed infants has reported a number of immune cell characteristics of this population including lower numbers of naive CD4⁺ cells, reduced thymic output⁹ and an impaired humoral response to vaccines.¹⁰ Furthermore, a number of hematological alterations such as low levels hemoglobin Granulocytes, lymphocytes and thrombocytes have also been reported in HEU infant.^{11,12} Most studies investigating hematological parameters of HEU were carried in France, Netherland, Spain and the USA.¹¹⁻¹⁴ These studies revealed low hemoglobin concentrations, reduced neutrophil and platelet counts as well as signs of moderate-to-severe toxicity in HIV-1/ART-exposed children. Data on how HIV or HIV therapy affects African HEU infants is limited.

The present cross-sectional study investigated blood figurative elements levels in HEU infants from Gabon in Central Africa.

Materials and Methods

In the setting of The National Laboratory of Public Health in Libreville (Gabon), from January to December 2015, a total of fifteen (15) healthy HEU and nine (9) HU infants aged 6 to 12 weeks were recruited for the present study. For all infants, we collected information on age, childbirth (vaginal delivery or caesarean section and preterm or full-term birth), infant preventive therapy and breastfeeding. To establish HIV perinatal infection, peripheral blood was taken at 4, 6 and 24 weeks after delivery and tested for HIV-RNA (RT-PCR Biomerieux, France). At 18 months, an additional sample was taken to detect anti-HIV-1 antibodies by ELISA. The National Laboratory of Public Health Ethics Review Board approved this study protocol. Consent forms were obtained from parents before enrolment.

Blood count and hemogram

Blood was collected from infants in a 5 mL EDTA tubes. Homogenized samples were analyzed using the Mindray BC-3000 plus instrument (Mindray, Shenzhen, China).

The measured blood components were: white blood cells count, differential leukocytes count, red blood cells count and morphology (mean corpuscular volume (MCV),

Correspondence: Joel Fleury Djoba Siawaya, Unités de Recherche et de Diagnostics Spécialisés, Laboratoire National de Santé Publique, BP 10736, Libreville, Gabon.
E-mail: joel.djoba@gmail.com

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mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)), platelet count and sizing (mean platelet volume (MPV) included), hemoglobin rate, hematocrit and the red cell distribution width (RDW).

Statistical analysis

All statistical were done using the software GraphPad Prism version 6. Parameters levels in HU and HEU infants were comparatively analyzed using the Mann-Whitney test. Descriptive statistics (frequencies or percentages) were used to characterize the study population.

Results

Infants' information

We found that 14 of the 15 HEU infants, were born vaginally and one by C-section. Only two of the HEU-infants were breast-fed, and only one was not on preventive therapy (Table 1).

Blood count and hemogram

The leukocytes evaluation showed no significant difference in the total white blood cells, red blood cells, lymphocytes, monocytes and granulocytes counts between HEU and HU infants (Table 1). No significant difference were observed on, hemoglobin concentration, hematocrit, MCV, MCH, MCHC and RDW (Table 2).

Platelet count was significantly higher in HEU-infants than HU-infants ($P=0.01$). The difference between the groups was physiologically significant (Table 2 and Figure 1).

The mean platelet volume (MPV) was significantly lower in HEU-infants than HU-infants ($P<0.05$) (Table 2 and Figure 2), however, HU and HEU infants MPVs were within normal range (6-11.1 fl).

Number of infants with events of clinically relevant hematological abnormalities

Hemoglobin concentration: 5 (33%) of the 15 HEU infants had their hemoglobin concentration below the normal age associated ranges, whereas 2 of the 9 (22%) HU had their hemoglobin concentration below

the normal ranges (10.4-16.5 g/dL).

Thrombocytes numeration: 12 out 15 (80%) HEU infants had their thrombocytes count above the normal age associated ranges, whereas only 2 out of 8 (25%) HU had their thrombocytes count above the normal age associated ranges (150,000-400,000 cells/mm³).

Leucocytes and lymphocytes numeration: 2 (13%) and 5 (33%) HEU infants had respectively their leucocytes and lymphocytes count below the normal age associated ranges (leucocytes [4000-11,000 cells/mm³], lymphocytes [7000-17,000 cells/mm³]). All HU infants except from 1 had their lymphocytes count within the normal range.

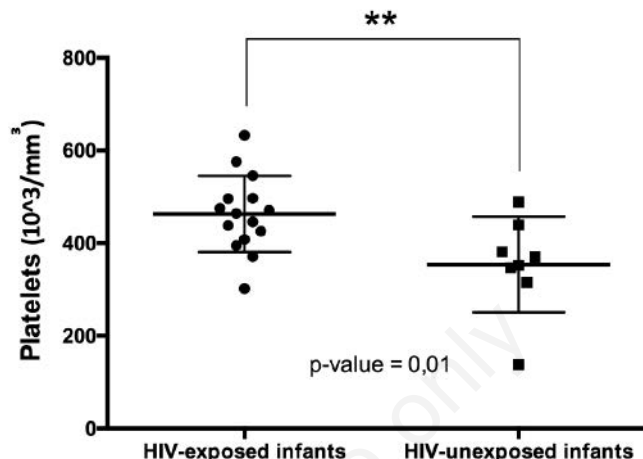


Figure 1. Platelets count in HIV-exposed uninfected (HEU) and HIV unexposed (HU) infants. The platelets count was significantly higher in HEU infants than HU infants ($P=0.01$). The observed difference was physiologically and clinically relevant.

Table 1. HIV-exposed and unexposed infants information (age, gender delivery mode, preventive therapy, etc.)

Infants code	Age, weeks	Gender	Delivery route	Delivery time	Infants' preventive therapy	Breastfed
HEU3	12	Female	Vaginally	Not informed	Zidovudine/Bactrim	No
HEU4	6	Female	Vaginally	Full-term	No information	Missing data
HEU5	8	Female	Vaginally	Full-term	Zidovudine/Bactrim	No
HEU6	6	Male	Vaginally	Not informed	NVP/Bactrim	No
HEU8	10	Male	Vaginally	Not informed	NVP/Bactrim	No
HEU9	10	Male	Vaginally	Full-term	NVP/Bactrim	No
HEU10	6	Male	Vaginally	Full-term	NVP/Bactrim	No
HEU11	6	Female	C-section	Missing data	NVP/Bactrim	No
HEU13	6	Female	Vaginally	Full-term	NVP/Bactrim	No
HEU14	12	Male	Vaginally	Not informed	NVP/Bactrim+NVP	Yes
HEU15	6	Male	Vaginally	Full-term	NO	Yes
HEU16	6	Male	Vaginally	Full-term	NVP/Bactrim	No
HEU17	8	Male	Vaginally	Not informed	NVP/Bactrim	No
HEU18	7	Male	Vaginally	Full-term	NVP/Bactrim	No
HEU19	6	Female	Vaginally	Full-term	NVP/Bactrim	No
HU1	6	Male	Vaginally	Missing data	NA	Yes
HU2	8	Male	Vaginally	Missing data	NA	Yes
HU3	12	Male	Vaginally	Missing data	NA	Yes
HU4	12	Female	Vaginally	Missing data	NA	Yes
HU5	12	Female	Vaginally	Missing data	NA	Yes
HU6	8	Female	Vaginally	Missing data	NA	Yes
HU7	8	Female	Vaginally	Missing data	NA	Yes
HU8	8	Female	Vaginally	Missing data	NA	Yes
HU9	6	Male	Vaginally	Missing data	NA	Yes

Granulocytes numeration: 2 (13%) out of 15 HEU infants had their Granulocytes count below the normal age associated ranges [2000-7000 cells/mm³]. All HU infants except from 1 had had their Granulocytes count within the normal range.

Discussion

Clinically relevant hematological abnormalities events were found to be higher in HEU infants when compared with HU infants. The rate of infants with low lymphocytes count was higher by 21% in HEU infants as compared to HU-infants. The prevalence of anemia was also higher in HEU infants (33%) compared to HU infants (22%). Similar observations have been

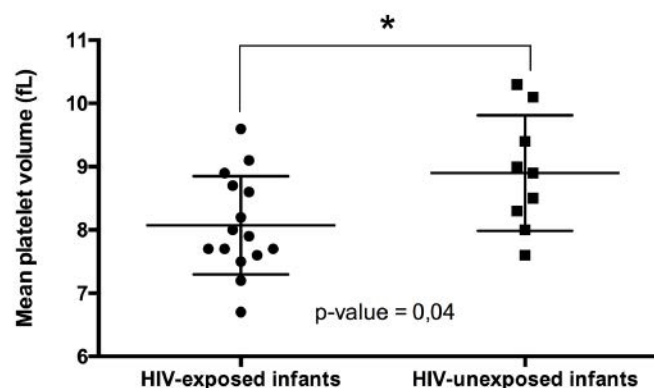


Figure 2. HIV-exposed uninfected (HEU) and HIV unexposed (HU) infants' mean platelet volume: the mean platelet volume (MPV) was significantly lower in HEU infants than HU infants ($P=0.04$). The observation has no physiological significance as HEU MVPs is within normal range (6-11.1 fl).

Table 2. Median, mean and ranges of hematological parameters measured in HIV-exposed and unexposed infants.

	Median	Mean	Min	Max	25% percentile	75% percentile	P-value
Granulocytes (cells/mm ³)							0.5
HEU-infants (n=13)	2700	3231	1600	6900	2350	4100	
HU-infants (n=9)	4000	3811	1700	6400	2150	5200	
Lymphocytes (cells/mm ³)							0.34
HEU-infants (n=14)	4900	5143	2600	10,000	3775	5775	
HU-infants (n=9)	5400	5633	3500	7600	4650	6950	
Leucocytes (cells/mm ³)							0.38
HEU-infants (n=14)	9300	9979	6100	18,800	7950	11,350	
HU-infants (n=9)	11,200	10,889	5900	15,100	8300	13,900	
Red blood cells							0.27
HEU-infants (n=15)	3.37	3.482	2.86	4.31	3.17	3.66	
HU-infants (n=9)	3.63	4.159	3.12	8.36	3.255	4.245	
Platelets (x10 ³ /mm ³)							<0.013
HEU-infants (n=15)	464	462.9	302	633	408	497	
HU-infants (n=8)	361	353.9	138	489	323	424.5	
Hemoglobin							0.25
HEU-infants (n=15)	10.5	10.45	8.4	12.5	9.5	11.5	
HU-infants (n=9)	11.3	11.31	9.1	14.2	9.95	12.45	
Hematocrit							0.42
HEU-infants (n=15)	34.1	32.78	25.4	38.6	29.3	35.8	
HU-infants (n=9)	34	38.91	29.6	77.5	31.25	38.55	
Mean platelets volume							<0.043
HEU-infants (n=15)	7.9	8.073	6.7	9.6	7.6	8.7	
HU-infants (n=9)	8.9	8.9	7.6	10.3	8.15	9.75	
Mean corpuscular hemoglobin conc					0.96		
HEU-infants (n=15)	31.8	31.88	29.1	33.7	31.4	32.8	
HU-infants (n=9)	32	30.56	18.3	34.7	29.7	33.15	
Mean corpuscular volume							0.99
HEU-infants (n=15)	94.3	94.52	80.9	114.7	85.6	102.7	
HU-infants (n=9)	94.9	94.09	84.4	98	92.75	97.75	
Mean corpuscular hemoglobin							0.91
HEU-infants (n=15)	29.5	30.07	26.3	35	27.2	32.8	
HU-infants (n=9)	30.3	28.69	16.9	33	27.4	31.1	
Red cell distribution width							0.65
HEU-infants (n=15)	16	15.98	15.5	16.6	15.8	16.2	
HU-infants (n=9)	15.9	16.12	15.7	16.8	15.75	16.6	

described in the literature.^{12,15} Our study showed that HEU infants had significantly higher platelets count than HU-infants. Moreover, the difference between the groups was physiologically or clinically relevant. Indeed, with a prevalence of 80% of HEU infants against 25% in HU infants (cut-off set at 400,000 cells/mm³), mild thrombocytosis seems to be a feature of HEU infants. In newborns, thrombocytosis can be caused by a large number of conditions: inflammation, infection, drug therapy preterm or C-section birth etc. Here, in utero exposure to a pro-inflammatory fetal environment could explain the high prevalence thrombocytosis observed in HEU-infants.^{16,17}

MVP levels were within normal range from both infant populations although HEU-infants had significantly lower MPV than HU-infants. HEU-infant's high platelet count and low MVP suggest a reactive thrombocytosis frequently observed during inflammation or infection.^{18,19}

Others groups including Bunders and associates have also reported an average platelets count above the normal age associated range¹³ in HEU infants. This is consistent with our findings. However, in the Bunders and colleague study, no statistically significant difference in platelets count was found between controls and HEU infants. Pacheco *et al.*,¹² reported that HEU infants exposed to ARV therapy have small but significant differences in hemoglobin concentrations, lymphocytes neutrophil, and platelets counts than HEU infants not exposed to ARV therapy. Although their study did not include comparison with HU infants, their data showed that all HEU infants aged 8-16 weeks had their platelets counts above the normal range of 400000 cells/mm³. Again, this is similar to what we found. Considering HEU infants in the same age range (6-16 weeks), in our setting, the average hemoglobin level and average lymphocytes count was lower than what Pacheco and colleague observed.

HUE-infants have increased mortality/morbidity due to infections. Today it is increasingly clear that aspects of their immune response are impaired,²⁰⁻²³ which might explain their susceptibility. Based on our data and previously reported data on HEU-infants, this group of infants has altered blood elements, whether those alterations are central (bone marrow) or only peripheral (peripheral blood) need to be investigated further. Also, consequences of these alterations also need to be investigated.

Our study design has its limitation, as infants were not followed up to determine the transient or long-lasting character of our observations. Another limit resides in the

fact that, in the present study, it is impossible to separate the effect of HIV-exposure from the effect of antiretroviral therapy (ART) exposure.

Conclusions

Our results show HEU have significantly higher platelets count than HU infants. Further studies are needed to separate the effect of HIV-exposure from the effect of antiretroviral therapy (ART) exposure on infant biology.

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