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 Mnani Mpega,2 Marielle Leboueny,1 Offilia Mvoundza Ndjidjji,1 Armel Mintsa Ndong,1 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 naïve 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, lymphocyes and thrombocytes have also been reported in HEU infant.11,12 Most studies investigating hematological parameters of HEU were carried in France, Netherlands, 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), 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 breastfeed, 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 different 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 MVPs 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.

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  | Zidovudine/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 made in other studies.

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

| Parameter                        | Median | Mean   | Min   | Max   | 25% percentile | 75% percentile | P-value |
|----------------------------------|--------|--------|-------|-------|----------------|----------------|---------|
| Granulocytes (cells/mm³)         |        |        |       |       |                |                |         |
| HEU-infants (n=13)               | 2700   | 3231   | 1600  | 6900  | 2350           | 4100           | 0.5     |
| HU-infants (n=9)                 | 4000   | 3811   | 1700  | 6400  | 2150           | 5200           |         |
| Lymphocytes (cells/mm³)          |        |        |       |       |                |                | 0.34    |
| HEU-infants (n=14)               | 4900   | 5143   | 2600  | 10,000 | 3775           | 5775           |         |
| HU-infants (n=9)                 | 5400   | 5633   | 3500  | 7600  | 4660           | 6950           |         |
| Leucocytes (cells/mm³)           |        |        |       |       |                |                | 0.38    |
| HEU-infants (n=14)               | 9300   | 9979   | 6100  | 18,800 | 7950           | 11,350         |         |
| HU-infants (n=8)                 | 11,200 | 10,889 | 5900  | 15,100 | 8300           | 13,900         |         |
| Red blood cells                  |        |        |       |       |                |                |         |
| HEU-infants (n=15)               | 3.37   | 3.482  | 2.86  | 4.31  | 3.17           | 3.66           | 0.27    |
| HU-infants (n=9)                 | 3.63   | 4.159  | 3.12  | 8.36  | 3.255          | 4.245          |         |
| Platelets (x10³/mm³)             |        |        |       |       |                |                | <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           | 32.8           |         |
| 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           | 97.75          |         |
| 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           | 32.8           |         |
| 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           | 16.2           |         |
| 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           |         |

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).
described in the literature.\textsuperscript{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/mm\(^3\)), 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 pretreatment 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.\textsuperscript{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.\textsuperscript{18,19}

Others groups including Bunders and associates have also reported an average platelets count above the normal age associated range\textsuperscript{13} 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.,\textsuperscript{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/mm\(^3\). 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,\textsuperscript{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 these alterations are central (bone marrow) or only peripheral (peripheral blood) need to 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.

## References

1. Marina E, Humphrey JH, Iliff PJ, et al. Child mortality according to maternal and infant HIV status in Zimbabwe. Pediatr Infect Dis J 2007;26:519-26.
2. Newell ML, Brahmbhatt H, Ghys PD. Child mortality and HIV infection in Africa: a review. AIDS 2004;18:S27-34.
3. Landes M, van Lettow M, Bedell R, et al. Mortality and health outcomes in HIV-infected and HIV-uninfected mothers at 18-20 months postpartum in Zomba District, Malawi. PLoS One 2012;7:e44396.
4. Landes M, van Lettow M, Chan AK, et al. Mortality and health outcomes of HIV-exposed and unexposed children in a PMTCT cohort in Malawi. PLoS One 2012;7:e47337.
5. Fawzy A, Arpadi S, Kankasa C, et al. Early weaning increases diarrhea morbidity and mortality among uninfected children born to HIV-infected mothers in Zambia. J Infect Dis 2011;203:1222-30.
6. Slogrove A, Reikie B, Naidoo S, et al. HIV-exposed uninfected infants are at increased risk for severe infections in the first year of life. J Trop Pediatr 2012;58:505-8.
7. Slogrove AL, Cotton MF, Esser MM. Severe infections in HIV-exposed uninfected infants: clinical evidence of immunodeficiency. J Trop Pediatr 2010;56:75-81.
8. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet 2015;385:430-40.
9. Nielsen SD, Jeppesen DL, Kolte L, 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.
10. Abramczuk BM, Mazzola TN, Moreno YM, et al. Impaired humoral response to vaccines among HIV-exposed uninfected infants. Clin Vaccine Immunol 2011;18:1406-9.
11. Rovira N, Nogueira-Julian A, Rives S, et al. Influence of new antiretrovirals on hematologic toxicity in HIV-exposed uninfected infants. Eur J Pediatr 2016;175:1013-7.
12. Pacheco SE, McIntosh K, Lu M, et al. Effect of perinatal antiretroviral drug exposure on hematologic values in HIV-uninfected children: an analysis of the women and infants transmission study. J Infect Dis 2006;194:1089-97.
13. Bunders MJ, Bekker V, Scherpier HJ, et al. Haematological parameters of HIV-1-infected infants born to HIV-1-infected mothers. Acta Paediatr 2005;94:1571-7.
14. Read JS, Hau Y, Patel K, et al. Laboratory abnormalities among HIV-exposed, uninfected infants: IMPACT protocol P1025. J Pediatr Infect Dis Soc 2012;1:92-102.
15. Le Chenadec J, Mayaux MJ, Guihenneuc-Jouyaux C, et al. Perinatal antiretroviral treatment and hematopoiesis in HIV-uninfected infants. AIDS 2003;17:2053-61.
16. Evans C, Jones CE, Prendergast AJ. HIV-exposed, uninfected infants: new global challenges in the era of paediatric HIV elimination. Lancet Infect Dis 2016;16:e92-107.
17. Afan L, Garcia Knight M, Nduati E, et al. HIV-exposed uninfected children: a growing population with a vulnerable immune system? Clin Exp Immunol 2014;176:11-22.
18. Rose SR, Petersen NJ, Gardner TJ, et al. Etiology of thrombocytosis in a general medicine population: analysis of 801 cases with emphasis on infectious causes. J Clin Med Res 2012;4:415-23.
19. Chiarello P, Magnolia M, Rubino M, et al. Thrombocytosis in children. Minerva Pediatr 2011;63:507-13.
20. Maloupazoa Siawaya AC, Mveang-Nzoghe A, Mvoundza Ndindji O, et al. Cases of impaired oxidative burst in HIV-Exposed uninfected infants’ neutrophils-a pilot study. Front Immunol 2017;8:262.
21. Smith C, Jabbert E, de Almeida V, et al. Altered natural killer cell function in HIV-exposed uninfected infants. Front Immunol 2017;8:470.
22. Ruck C, Reikie BA, Marchant A, et al. Linking Susceptibility to infectious diseases to immune system abnormalities among HIV-exposed uninfected infants. Front Immunol 2016;7:310.
23. Abu-Raya B, Kollmann TR, Marchant A, MacGillivray DM. The immune system of HIV-exposed uninfected infants. Front Immunol 2016;7:383.