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BRIEF REPORT

# Lymphocyte Subsets in HIV-Exposed Uninfected Infants: The Impact of Neonatal Postexposure Prophylaxis With Zidovudine

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HIV-exposed, uninfected (HEU) infants receiving neonatal postexposure prophylaxis with zidovudine showed nonsignificant trends of lower CD4 and CD8 T cells as well as CD19 B cells than those who did not, suggesting toxicity that might impact the overall health of HEU children.

**Keywords.** adjusted mean difference; HIV-exposed uninfected infants; lymphocyte subsets; neonatal postexposure prophylaxis; zidovudine.

Over the last decade, the risk of mother-to-child transmission (MTCT) of HIV has declined to virtually zero in resource-rich countries due to improved prevention strategies, including especially the widespread use of combination antiretroviral therapy (cART) during pregnancy, leading to full suppression of the maternal HIV plasma viral load (pVL) [1, 2]. In 2016, for the first time in a high-income country, applying the well-accepted principle of undetectable = untransmittable [3] to MTCT prevention measures, neonatal postexposure prophylaxis (PEP) has been abandoned in Switzerland in HIV-exposed newborns if maternal HIV plasma viral load (pVL) is fully suppressed in the last trimester of pregnancy [4].

Studies in low- [5, 6] and high-income countries [7] showed higher incidence of severe infections in HIV-exposed, uninfected (HEU) infants within their first 2 years of life compared with unexposed children. Additionally, in low-income countries, HEU infants experience higher mortality rates compared with unexposed children [8, 9]. The underlying mechanisms of these observations are not known, but HIV proteins have been shown to cross the placental barrier and to influence progenitor cells, leading to immunologic abnormalities in HIV-exposed infants, even without being HIV-infected [10]. Several studies have shown reduced numbers of CD4 T cells and increased numbers of both CD8 T and CD19 B cells among HEU infants compared with their unexposed peers [10–13]. Additionally, detectable hematological toxicity of neonatal PEP with zidovudine (AZT), mainly neutropenia and anemia, has been previously described [14].

In this study we aim to analyze the impact of AZT-based neonatal PEP on lymphocyte subsets and hematological parameters in HEU children from birth to 24 months of age.

## METHODS

### Setting, Patients, and Data Sources

HEU children born between January 2003 and January 2018 and cared for at the University Children's Hospital Zurich were included in the study if they were participating from birth in the Swiss Mother and Child HIV Cohort Study (MoCHiV) and the Swiss HIV Cohort Study (SHCS; www.shcs.ch) and written informed consent was provided. A child was considered HIV-uninfected if fourth-generation combination HIV-1/2 immunoassay testing was negative between 18 and 24 months of age. Postnatal AZT exposure was defined as administration of neonatal PEP with AZT for at least 2 weeks.

Lymphocyte subsets and hematological parameters were evaluated at 1, 2, 6, and 24 months of age during routine visits and included absolute counts and percentage of CD4 and CD8 T cells and CD19 B cells, as well as hemoglobin levels, neutrophil counts, red blood cell (RBC) counts and their mean corpuscular volume (MCV). All measurements were performed in certified laboratories at our institution. Cell populations were compared with age-dependant normal ranges. Hemoglobin and neutrophil counts outside the normal range were graded according to the Division of AIDS (DAIDS) table for grading the severity of adult and pediatric adverse events [15]. The data for the analysis were extracted from the patient records and completed with information regarding the mothers from the SHCS database.

### Statistical Analysis

Categorical variables were compared using the Fisher exact test. Continuous variables were analyzed with the Mann-Whitney *U* test. Linear multivariate mixed models, allowing for repeated measures, were constructed to determine the effect of AZT-based

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neonatal PEP on hematological and immunological parameters over the period of 2, 6, and 24 months of age adjusting for different maternal (last maternal CD4 counts, RNA viral load and CDC classification before delivery, maternal cART type, and duration during pregnancy) and neonatal (age, sex, ethnicity, and gestational age) characteristics. Parameters with statistically significant associations in the linear multivariate mixed models were additionally included as covariates in a multivariate logistic regression to identify risk factors for hematological toxicity. Two-tailed *P* values <.05 were considered statistically significant. Data analysis was performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corporation, Armonk, NY, USA).

### Ethics

MoCHiV and SHCS have both been approved by the Ethics Committee Zurich (BASEC-Nr. PB_2016-01102).

## RESULTS

Among 178 HEU infants included in the analysis, 25 (14%) did not receive any neonatal PEP after birth. Eighty-two (53.6%) neonatal PEP recipients and 1 (4%) of the infants without PEP were born to mothers with AZT-containing cART during pregnancy ($P < .001$). No deaths occured in the study population. The clinical and demographic characteristics of the infants and their mothers are shown in Supplementary Table 1.

### Lymphocyte Subsets

In the linear multivariate mixed models, lower mean CD4 T-cell counts were independently associated with older age (24 months vs 1, 2, and 6 months) and black ethnicity (at 6 and 24 months), whereas lower mean CD8 T-cell counts were independently associated with older age (24 months vs 1 and 6 months), black ethnicity (at 24 months), and last maternal CD4 T-cell counts <200 cells/mm$^3$ (at 24 months, vs >500 cells/mm$^3$). Lower mean CD19 B-cell counts were associated with older age (24 months vs 2 and 6 months) and maternal cART start in the first trimester (at 24 months, vs third trimester) (Supplementary Table 2).

Overall, there were no statistically significant differences in either absolute CD4 and CD8 T-cell counts or absolute CD19 B-cell counts comparing adjusted means between HEU infants who received neonatal PEP and those who did not (Table 1). In the graphical representation, unadjusted mean CD4 and CD8 T-cell counts were lower in neonatal PEP recipients up to 6 months and 2 years of age, respectively, compared with infants without PEP. Unadjusted mean absolute CD19 B-cell counts, on the other hand, were lower in infants receiving neonatal PEP only at the age of 1 month. For comparison, the well-known increase of MCV under AZT was only observed up to the age of 2 months in neonatal PEP recipients (Figure 1).

Based on previous reports on gender differences in lymphocyte populations in HEU children [16] and on the association of black ethnicity with lower white blood cell (WBC) count [17], we performed a subgroup analysis in HEU infants with at least 1 parent of sub-Saharan African origin as well as in male HEU infants. Children of black ethnicity who received neonatal PEP had significantly lower mean CD4 and CD8 T-cell counts during the first 24 months after birth compared with their peers not receiving neonatal PEP (CD4: adjusted mean difference, –495 cells/mm$^3$; 95% confidence interval [CI], –938 to –50

**Table 1. Adjusted[a] Mean Lymphocyte Subsets and RBC Levels Over Time Period by Postpartum AZT Exposure Status**

| | 1–2 Months of Age | | | | 1–6 Months of Age | | | | 1–24 Months of Age | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Adjusted Mean | | Adjusted | | Adjusted Mean | | Adjusted | | Adjusted Mean | | Adjusted | |
| | No[b] | NeoPep[c] | Mean | | No[b] | NeoPep[c] | Mean | | No[b] | NeoPep[c] | Mean | |
| Parameters[d] | (n = 25) | (n = 153) | Difference | *P* Value | (n = 25) | (n = 153) | Difference | *P* Value | (n = 25) | (n = 153) | Difference | *P* Value |
| CD4 counts, cells/mm$^3$ | 2737 | 2534 | 203 | .187 | 2869 | 2623 | 245 | .202 | 2685 | 2481 | 190 | .194 |
| | | | (–99 to 505) | | | | (–147 to 597) | | | | (–116 to 496) | |
| CD8 counts, cells/mm$^3$ | 860 | 834 | 26 | .697 | 914 | 880 | 33 | .684 | 958 | 934 | 24 | .744 |
| | | | (–104 to 155) | | | | (–127 to 193) | | | | (–120 to 168) | |
| CD19 counts, cells/mm$^3$ | 1177 | 1106 | 71 | .417 | 1441 | 1311 | 129 | .259 | 1369 | 1224 | 145 | .139 |
| | | | (–101 to 243) | | | | (–96 to 355) | | | | (–48 to 338) | |
| RBC counts, T/mm$^3$ | 3.213 | 3.062 | 0.151 | .026 | 3.715 | 3.582 | 0.133 | .058 | 4.060 | 3.899 | 0.161 | .024 |
| | | | (–0.02 to 0.28) | | | | (–0.005 to 0.27) | | | | (–0.02 to 0.3) | |
| MCV, fl | 91.068 | 93.947 | –2.879 | .004 | 85.327 | 86.773 | –1.145 | .142 | 81.95 | 83.45 | –1.498 | .122 |
| | | | (–4.8 to –0.94) | | | | (–3.38 to 0.49) | | | | (–3.4 to 0.4) | |

Abbreviations: AZT, zidovudine; cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention; HEU, HIV-exposed, uninfected; MCV, mean corpuscular volume; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RBC, red blood cell.

[a]Models were adjusted for age, sex, ethnicity, gestational age, last maternal CD4 counts and RNA viral load before delivery, maternal cART exposure during pregnancy (AZT, PI, NNRTI), trimester of cART start, and last maternal CDC classification.

[b]HEU infants who did not receive neonatal postexposure prophylaxis.

[c]HEU infants who received neonatal postexposure prophylaxis with AZT 2 mg/kg every 6 hours for at least 2 weeks.

[d]Age-dependant reference ranges in cells/mm$^3$ at 2 months/6 months/24 months: CD4: 2100–4900/1500–5100/1000–4600; CD8: 500–1600/600–2200/500–2300; CD19: 770–1990.

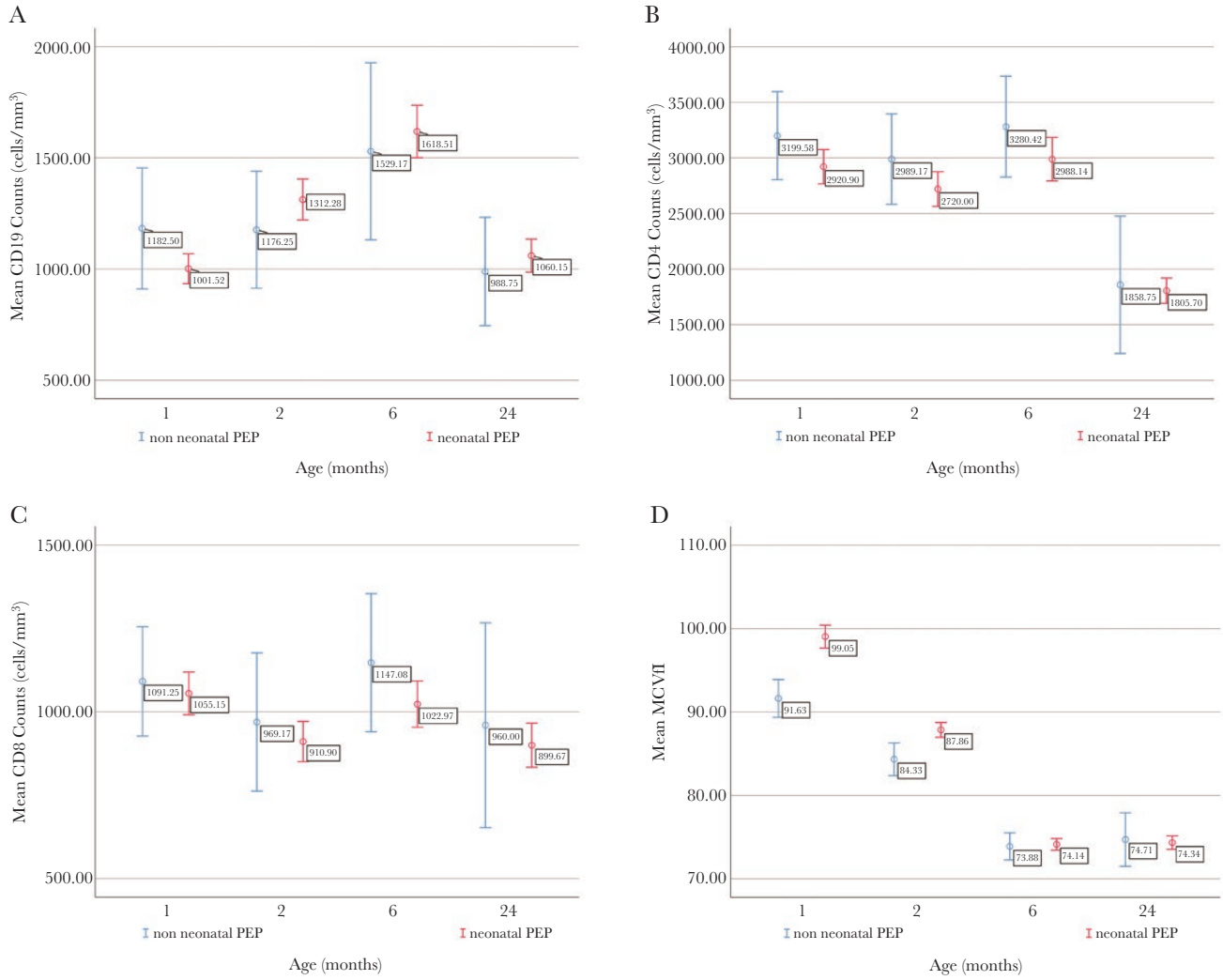


**Figure 1.** Unadjusted means (with 95% CI) for changes in distribution of CD19 B cells (*A*), CD4 (*B*) and CD8 (*C*) T cells as well as MCV (*D*) with age by neonatal PEP exposure status. MCV, mean corpuscular volume; PEP, postexposure prophylaxis.

cells/mm$^3$, $P = .029$; CD8: adjusted mean difference, −198 cells/mm$^3$; 95% CI, −386 to −10 cells/mm$^3$, $P = .039$). For male HEU children, no significant difference in lymphocyte subsets was found between the 2 groups.

#### Hematological Toxicity

Clinically relevant anemia, defined as grade 2 toxicity or higher, was seen in 23.5% of all HEU children at 1 and in 19.8% at 2 months of age. In multivariate logistic regression analysis, the use of prenatal AZT-containing cART in the mother was the only significant risk factor for anemia (odds ratio [OR], 2.81; 95% CI, 1.2 to 6.6; $P = .016$) within the first month of life (Supplementary Table 3). Neonatal PEP was not associated with clinically relevant anemia in this analysis, but in the linear multivariate mixed model RBC levels were significantly lower at 2 months (adjusted mean difference, 0.15 T/mm$^3$; 95% CI, −0.02 to 0.28 T/mm$^3$; $P = .026$) and 24 months (adjusted mean difference, 0.16 T/mm$^3$; 95% CI, −0.02 to 0.3 T/mm$^3$; $P = .024$) of life in infants receiving neonatal PEP compared with those who did not receive neonatal PEP (Table 1).

Clinically relevant neutropenia, defined as grade 2 toxicity or higher, was seen in 3.3% of HEU infants at 1 month and 6.8% at 2 months of age. No significant difference was found with respect to child ethnicity or exposure to maternal AZT or neonatal PEP (Supplementary Table 3).

## DISCUSSION

In our analysis, no statistically significant differences in mean CD4 and CD8 T-cell or CD19 B-cell levels during the first 24 months of life were observed in HEU infants receiving neonatal PEP with AZT compared with those not receiving PEP. But even if not statistically significant, a consistent trend of lower adjusted means during the first 24 months of life in HEU infants receiving neonatal PEP compared with those not receiving PEP could be observed in the performed linear multivariate mixed

models, especially for absolute CD4 T cells. The limited sample size and the resulting reduced statistical power may explain the lack of statistical significance. Although measured lymphocyte levels were consistently within the age-dependent normal ranges, which leads us to question the clinical significance of the observed differences, these findings suggest a negative influence of AZT-based neonatal PEP on T and B lymphocyte counts. Additionally, the use of prenatal AZT-containing cART in the mothers was associated with increased risk of clinically relevant anemia, corroborating the previously described toxicity of AZT in the infant [14]. Surprisingly, no association between AZT-based neonatal PEP and clinically relevant anemia was found in our analysis, and the difference in RBC count at 1 and 24 months, although statistically significant, was far from clinical relevance. We hypothesize that the small sample size and the short duration of neonatal PEP contributed to this lack of association.

The present study has several limitations. First, the relatively limited size of the study population influences the significance of statistical analysis, especially within subgroups. As an example, a possible influence of maternal cART regimens on lymphocyte subsets could not be completely ruled out, because even though no significant association with maternal cART was shown, the great diversity of cART regimes did not allow for deeper analysis. Second, this retrospective study relies only on the experience of a single center, and therefore the results may not be representative of the entire HEU population. Third, although cell counts and hemoglobin levels were compared with age-dependant normal ranges, a control group of HIV-unexposed infants is lacking. Nevertheless, because of the change of paradigm in the 2016 Swiss recommendations for prevention of MTCT [4], this study was the first opportunity to analyze the impact of AZT-based neonatal PEP on lymphocyte subsets and hematological parameters in a well-defined cohort of HEU children.

## CONCLUSIONS

Lymphocyte subsets in HEU infants receiving neonatal PEP did not differ from those of infants not receiving PEP but did show nonsignificant trends of lower adjusted means. Even though these observations are of questionable clinical significance, it remains unclear whether the effect of postnatal AZT exposure might have an impact on the overall health of HEU infants or might contribute to the previously observed higher incidence of infection in HEU infants within their first 2 years of life [5–7]. As other high-income countries may soon follow the Swiss example and revisit their neonatal PEP recommendations, further studies to investigate the impact of postnatal AZT exposure in larger cohorts of HEU infants should be pursued.

### Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.


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