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**Variation of CD4 count and percentage among women receiving antiretroviral prophylaxis for the prevention of mother-to-child transmission of HIV:**

**Implication for HAART initiation in resource-limited settings.**

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**Composition of the ANRS 1201/1202 Ditrime-Plus Study Group**

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**Conflict of interest:** None of the authors had any conflict of interest to declare.

## ABSTRACT

**Background:** We studied whether the variation of T-lymphocyte CD4+ (CD4) absolute count and percentage prior and after delivery could affect the decision process regarding HAART initiation in African HIV-infected pregnant women.

**Methods:** Prospective cohort in Abidjan, Côte d'Ivoire before HAART was available. Participating women received a perinatal antiretroviral prophylaxis (zidovudine + single-dose of nevirapine). CD4 count and percentage were measured by flow cytometry at baseline (32 weeks of amenorrhea) and at month-1 after delivery. Signed-rank test was used to compare the distributions of the CD4 absolute count and percentage values.

**Results:** 325 HIV-1-infected pregnant women were included. At baseline, the median CD4 count was 355 cells/mm<sup>3</sup> and the median percentage was 24.8%, 17.8% of women had CD4 count <200 cells/mm<sup>3</sup>, and 14.8 % had CD4 percentage <15%. One month after delivery, the median CD4 count was 489 cells/mm<sup>3</sup> (*vs* baseline:  $p < 0.001$ ), the median CD4 percentage was 25.6% (*vs* baseline:  $p = 0.107$ ), 9.5% of women had CD4 count <200 cells/mm<sup>3</sup> (*vs* baseline:  $p < 0.001$ ), and 15.1% of women had CD4 percentage <15% (*vs* baseline:  $p = 0.823$ ). When combining the CD4 count and the WHO clinical stage, the proportion of women who met the WHO 2006 criteria for initiating HAART was 28.3% at baseline but 17.2% only at month-1 after delivery ( $p < 0.001$ ).

**Conclusion:** CD4 percentage is more stable than the absolute count for deciding on initiating HAART during pregnancy in Africa. Its use should minimize the risks associated with early prescription of HAART at high CD4 counts in low-income settings with limited monitoring capacity.

## **INTRODUCTION**

The T-CD4+ lymphocyte (CD4) absolute count is a strong predictor of HIV-1 disease progression, independently of the HIV RNA plasma viral load (1), and one of the parameters to use in association with the World Health Organization (WHO) clinical staging system to decide when to initiate an highly active antiretroviral therapy (HAART) in resource-limited settings. According to the 2003 and 2006 WHO guidelines, HIV-infected (HIV+) adults are eligible for HAART if they meet one of the following conditions: WHO clinical stage 4 irrespective of CD4 count, CD4 count  $<200/\text{mm}^3$  irrespective of WHO clinical stage, or WHO stage 3 and CD4 count between 200 and  $350/\text{mm}^3$  (2-3). These criteria apply to all adults, including pregnant women as suggested by WHO 2004 guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) (4). Pregnant women who do not meet these criteria should receive short-course antiretroviral prophylaxis for PMTCT (4). Although its efficacy for PMTCT is very high, the use of HAART during pregnancy in high-income countries has been associated with increased rates of preterm delivery (5, 6). Furthermore, nevirapine (NVP)-containing HAART regimens are no longer recommended for women with  $\text{CD4} > 250/\text{mm}^3$  considering elevated risk of hepatotoxicity (7). Thus, important treatment decisions may be taken during pregnancy, with potential consequences for the mother and her future child. CD4 and CD8 absolute count have been reported to be slightly modified during pregnancy with and without HIV infection with no clinical consequences described in high-income countries (8). In sub-Saharan Africa, few studies have described the CD4 count variation in pregnancy but none has looked concomitantly at its implications for the therapeutic decisions to initiate HAART (9-11). The present study was performed in Abidjan, Côte d'Ivoire within a cohort of HIV-infected pregnant women who benefited from PMTCT short-course antiretroviral prophylaxis and were followed up to two year post-partum before access to HAART became possible. We investigated whether changes in CD4 count and percentage between the pre-and early post-partum periods could have consequences on the decision process regarding HAART initiation.

## **PATIENTS AND METHODS**

The ANRS 1201/1202 Ditrane Plus study was a non-randomised open-labelled trial initiated in March 2001 in Abidjan to assess the field efficacy of a short course of zidovudine (ZDV) beginning at 36 weeks of gestation plus a single dose of NVP during labor for PMTCT (12). The study protocol was approved by the Ethical Committee of the National AIDS Control Programme in Côte d'Ivoire and the Institutional Review Board of the French Agence Nationale de Recherches sur le SIDA. All pregnant women attending the prenatal clinics were offered pre-test counselling and HIV testing with a serial testing algorithm if they were  $\geq 18$  years old. Women whose HIV infection were confirmed and accepted the study principles were enrolled 36 weeks of gestation. Baseline socio-economic, demographic and clinical characteristics were recorded. The CD4 count and percentage were measured in antenatal period at the screening visit carried out at 32 weeks of gestation and thereafter at months-1, 6 and 12 after delivery using a dual-platform flow-cytometry technique with an automated blood cell counter (MaxM, Beckman Coulter, Miami, FL, USA) for absolute lymphocyte count and a flow cytometer (FACScan, Becton Dickinson, San Jose, CA, USA) for measuring the percentage of CD4<sup>+</sup> T-cells (CD4%). Absolute CD4 count was then calculated multiplying the CD4% by the total lymphocyte count. The laboratory quality control procedures included: i) daily internal controls for both the automated blood cell counter (Coulter 5C cell control, Beckman Coulter, Fullerton, CA, USA) and the flow cytometer (BD multicheck control, Becton Dickinson); ii) participation in two international assurance quality programs (UK-NEKAS and QASI). The enrolled women received ZDV 300 mg tablets twice daily during the pre-partum period, completed by a single dose of two tablets (600 mg) at beginning of labour. A single tablet of NVP (200 mg) was to be taken orally at the same time as ZDV during labour. The HIV-infected women who had CD4 counts  $< 500/\text{mm}^3$  at enrolment received prophylactic cotrimoxazole for the prevention of opportunistic infections according to national guidelines. HAART was not available at that time in Abidjan and could not be prescribed to those eligible pregnant or delivering women.

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We used signed-rank test to compare the distribution of CD4 count measurements in the same patient between two individual different time points (matched pairs of observations) and McNemar's  $\chi^2$  test for the comparison of categorial variables at two different time points such as the eligibility criteria before and after pregnancy. Kruskal-Wallis one-way analysis of variance was used for the comparison of the CD4 count variation according to the absolute CD4 count class or CD4 percentage. Statistical analyses were processed using STATA<sup>TM</sup> software, version 8.0 (STATA Inc, College Station, TX, USA).



## RESULTS

Overall, 402 HIV-1-infected pregnant women were enrolled in the ANRS Ditrane Plus project during the study period. Among them 325 (81%) had two CD4 measurements available at 32 weeks of gestations and month-1 after delivery. No difference was found between 325 and the remaining 77 (19%) women for the following items: age, gestational age, WHO clinical stage, CD4 count and viral load.

At baseline, the median age of the 325 HIV-1 infected women was 26 years, (Interquartile range [IQR] 23–30 years), the median CD4 absolute count was 355 cells/mm<sup>3</sup> (IQR: 246–530 cells/mm<sup>3</sup>), the median CD4 percentage was 24.8%, (IQR: 18.5-32.0%), 17.8% of women had CD4 count <200 cells/mm<sup>3</sup>, and 14.8 % had CD4 percentage <15%. Two women (0.6%) were classified at WHO clinical stage 4, 93 (28.6%) at WHO stage 3 and 230 (70.8%) at WHO stage 1 or 2.

All women were prescribed the PMTCT antiretroviral regimen described above and none of them received HAART during the study period. The median duration of ZDV prophylaxis between enrolment and delivery was 30 days (IQR: 20-41 days), 94% of these women received the additional labour dose of ZDV and the single-dose of NVP at the beginning of labour.

Distributions of total lymphocytes count and the absolute CD4 count and percentage changed between baseline, month-1 (Table). The median progression of the CD4 count after delivery was 119 cells/mm<sup>3</sup> (IQR: 31-217) without any significant difference according to the class of CD4 count (p=0.156).

The kinetic of the absolute CD4 count in 229 HIV infected women (57%) who had four CD4 measurements available showed a significant increase in the median CD4 absolute count between baseline and month-1 post-partum (p<0.001) as well as a significant but slighter decrease between months 6 and 12 (p=0.004) but no significant difference between months 1 and 6 (p=0.354). In contrast, there was no significant variation in CD4 percentage between the pre-partum and 4 weeks

post-partum measurements ( $p=0.823$ ) and the median variation of the percentage of the CD4 was -0.4 (IQR: -1.9-2.9).

When considering the proportions of women having a CD4 count below the thresholds used for HAART initiation, 17.8% and 30.5% of women had baseline CD4 counts  $<200$  cells/mm<sup>3</sup> and 200-349 cells/mm<sup>3</sup>, respectively, and these proportions fell down to 9.5% and 19.4% one month after delivery ( $p<0.001$  when using the 200/mm<sup>3</sup> threshold). Meanwhile, the proportions of women with CD4 percentage  $<15\%$  and 15-19% remained stable between the pregnancy period (14.8% and 16.3%, respectively), and one month after delivery (15.1% and 13.2 %) ( $p=0.823$  when using the 15% threshold) (Table). When combining the CD4 count and the WHO clinical stage, the proportion of women who met the WHO 2006 criteria for initiating HAART was 28.3% at baseline and 17.2% at month-1 after delivery ( $p<0.001$ ) (Table). Also, among 92 HIV-infected pregnant women who met in prepartum, only the WHO 2006 criteria for initiating HAART, 50 (54.3%) only were still meeting these criteria one month after delivery.

## DISCUSSION

The change of the T-Lymphocytes count and its subsets during pregnancy is well known and already reported by Miotti in Malawi and Ekpini in Côte d'Ivoire (9, 10) and also in developed countries in the European Collaborative Study and the Swiss HIV Pregnancy Cohort (8) and in the US (13, 14). All these studies reported an increase of the absolute count between late pregnancy and early post-partum period in HIV-infected mother. This reported is consistent with data observed in our cohort with the absolute CD4 count increased significantly after delivery in women who had been under ZDV prophylaxis during a median of one month for the PMTCT indication. A similar observation was made in another PMTCT trial in Abidjan, where the CD4 count increased after delivery by 157 cells/mm<sup>3</sup> in HIV-infected pregnant women who had received a placebo and by 158 cells/mm<sup>3</sup> in those randomized for a short-course of ZDV and then declined gradually at the 2 and 4 week postpartum with a difference of 36 cells/mm<sup>3</sup> in the placebo group and 31 cell in the short-course of ZDV group (9). Moreover an increase of the absolute number of CD4+ cells was also noted in Malawi between late pregnancy and early post-partum period among both HIV-infected and uninfected women without any antiretroviral prophylaxis (10). However, two studies were not found any relation between the pregnancy and the variation of the CD4 absolute count (11, 15). Also in our study, when considering only the class of the CD4 count there is no statistical difference between month-1 and baseline values ( $p=0.156$ ) showing that the difference in each class of the CD4 varied between 70 cells and 129 cells/mm<sup>3</sup>.

Our study confirmed the stability of CD4 percentage values between pre-partum and post-partum periods in comparison to absolute CD4 counts, a finding consistent with the Malawi study in which CD4 and CD8 percentages remained virtually unchanged before and after delivery (10) as well as in European Collaborative Study and the Swiss HIV Pregnancy Cohort (8).

The variation of the T-lymphocytes and absolute CD4 counts before and after pregnancy related to the hemodilution is due to the progressive rise of cortisol during pregnancy and to the plasma

adrenocorticotropin (16). The post-partum changes in lymphocyte subsets most likely represent a return to baseline values due to the physiologic changes of pregnancy in the immediate post-partum period (10, 16).

The originality of this study is the operational aspect of pregnancy associated changes of absolute CD4 counts on the initiation of HAART in HIV-infected women especially in the context of access to treatment in resources limited settings. Indeed, the measurement of an absolute CD4 count does not depict well the quantitative immunological status of HIV-infected pregnant women. When we used the 2006 WHO HAART eligibility criteria, this substantially over-estimated the proportion of HIV-infected women eligible to HAART in the last trimester of pregnancy, 28% against 17% only one month after delivery. These findings have two major potential consequences. The first one is related to the initiation of HAART in HIV-infected pregnant women and risk of side effects for themselves and their child. Because of the hemodilution related to pregnancy, some pregnant women will then start HAART whilst they do not have any immediate indication yet compared to when they will return to pre-pregnancy values. In HIV-infected pregnant women, it has been reported that HAART may be associated with adverse pregnancy outcomes such as preterm delivery they initiated at the early period of the pregnancy (5, 6). Moreover, the incidence of rash and hepatic toxicity with NVP-containing HAART, the most commonly used first-line regimen in resource-limited settings, is significantly higher in female patients with higher CD4 cell counts including during pregnancy (7, 17). The second potential consequence is related to the early immunological response in HIV-infected pregnant women on HAART. Based on the above data, it appears difficult to truly appreciate the effect of HAART in the first six months of treatment initiated during pregnancy as the majority of women show an important increase in CD4+ count after delivery, even without any treatment (9). It is also likely that a decrease of the absolute CD4 count can be anticipated when women already on HAART become pregnant. Facing this situation, we suggest that the guidelines for initiation HAART among pregnant women in post-partum should

be revisited. The similar recommendation was proposed by Mulcahy et al from Ireland who demonstrated that CD4 count in pregnant women do not accurately reflect the need for long-term HAART(18).

One of the limits of this study is the absence of a control group of HIV negative pregnant women to demonstrate CD4 count variation during pregnancy. Nevertheless, others studies reported an increase of the CD4 count in HIV negative pregnant women in postpartum (10, 13).

In conclusion, it may be important to define specific conditions for HAART initiation in pregnant women in resource-limited settings to go beyond the 2006 WHO recommendations (3). Because of the stability of the CD4 percentage before and after pregnancy, we suggest using exclusively the CD4 percentage instead of CD4 absolute counts for initiating HAART in pregnant women, despite the technical limitations for its routine measurement. In addition further studies are needed to evaluate the use of CD4 percentage in assessing the immunological response to HAART initiated and continued during pregnancy in low-income countries.

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**Table. T-Lymphocytes count and T-lymphocyte CD4+ distribution (absolute count and percentage) and HAART eligibility criteria in HIV-1 infected pregnant women in prepartum and postpartum periods. ANRS 1201/1202 Ditrane Plus study, Abidjan, Côte d'Ivoire (N=325).**

	Prepartum (32 weeks of amenorrhea)	Postpartum (month-1)	Difference	P value	P value¶
<b>Lymphocytes counts</b>					
Median [IQR]	1500 [1144-1920]	2016 [1624-2400]	489 [132-815]	<0.001*	
<b>Absolute CD4+ count</b>					
Median [IQR]	355 [246-530]	489 [332-698]	119 [31-217]	<0.001*	
CD4 distribution: n (%)					
<200/mm <sup>3</sup>	58 (17.8)	31 (9.5)	70 [21-151]	<0.001#	0.156
200-349/mm <sup>3</sup>	99 (30.5)	63 (19.4)	118 [59-186]		
350-499/mm <sup>3</sup>	81 (24.9)	75 (23.1)	148 [38-267]		
≥500/mm <sup>3</sup>	87 (26.8)	156 (48.0)	129 [-12-267]		
<b>Percentage of CD4+</b>					
Median [IQR]	24.8 [18.5-32.0]	25.6 [18.7-32.1]	-0.4 [-1.9-2.9]	0.107*	
CD4 distribution: n (%)					
<15%	48 (14.8)	49 (15.1)	1.1 [-0.4-2.6]	0.823&	0.026
15-19%	53 (16.3)	43 (13.2)	0.3 [-1.3-3,1]		
20-24%	65 (20.0)	66 (20.3)	0.6 [-0.9-3.2]		
≥25%	159 (48.9)	167 (51.4)	-0.4 [-2.9-2.3]		
<b>Eligibility for HAART</b>					
WHO 2006 criteria <sup>a</sup>	92 (28.3)	56 (17.2)	-	<0.001 <sup>s</sup>	

IQR: Interquartile range

\*Signrank test: comparison of the T-lymphocytes CD4+ count and percentage distributions between prepartum and early postpartum (month-1) periods

# Mc Nemar Chi<sup>2</sup> test: comparison of the CD4 count (with the threshold <200/mm<sup>3</sup> vs >200/mm<sup>3</sup>) between prepartum and early postpartum (month-1) periods

& Mc Nemar Chi<sup>2</sup> test: comparison of CD4 percentage (with the threshold <15% vs ≥15%) between prepartum and early postpartum (month-1) periods

\$ Mc Nemar Chi<sup>2</sup> test: comparison of the qualitative variables between prepartum and early postpartum (month-1) periods

¶ Kruskal-Wallis test one way analysis of variance (comparison of the difference between month-1 and baseline according to the class of the CD4 count)

a: WHO stage 4, WHO stage 3 and CD4 count <350 cells/mm<sup>3</sup> and CD4 count <200 cells/mm<sup>3</sup> whatever the clinical stage