The impact of pregnancy and menopause on CD4 lymphocyte counts in HIV-infected women

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Objectives: To determine indirectly the effect of changes in levels of reproductive hormones on CD4 lymphocyte counts by investigating the impact of pregnancy and menopause on CD4 lymphocyte counts in HIV-infected women.

Methods: Participants were 382 women with a known interval of HIV seroconversion. Review of questionnaires or patient charts provided information on pregnancy and menopause. A linear regression model with a random intercept and slope, which adjusts for multiple CD4 lymphocyte counts per woman, was applied to estimate the CD4 decline following HIV seroconversion and to evaluate the effect of pregnancy and menopause on the CD4 path.

Results: The 382 women had a median age of 25 years at seroconversion and yielded 1428 CD4 lymphocyte counts from 3 to 10 years after seroconversion. At 3 years from seroconversion, 20 women had passed the menopause (i.e., the last menses) and five more subsequently passed this point during follow-up; 25 women had a pregnancy after study entry. Postmenopausal women had lower CD4 lymphocyte counts 3 years after seroconversion than premenopausal women (333 vs 399 $\times 10^6$ cells/l; $P = 0.09$), and pregnant women had lower counts than non-pregnant women (375 vs 399 $\times 10^6$ cells/l; $P = 0.36$). The monthly CD4 decline was not associated with pregnancy and menopause. Adjustment for age did not change the results.

Conclusions: The results suggest that CD4 lymphocyte counts differ between pre- and postmenopausal women, perhaps because of changes in the level of reproductive hormones in the menopause, but associations were not statistically significant. Pregnancy had no statistically significant effect on CD4 lymphocyte counts.

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Introduction

The two most important markers of HIV disease progression display gender differences. CD4 lymphocyte counts are higher in women than in men throughout HIV infection [1,2], whereas HIV RNA levels are initially lower in women than in men early in HIV infection [3] but seem to equalize with ongoing infection [4,5]. Since current treatment guidelines are based on results of marker studies among men, HIV-infected women may well be undertreated if these differences have a functional meaning in disease progression. In the general (HIV-uninfected) population, CD4 lymphocyte counts differ between men and women but not between those under the age of 15 years and over the age of 50 [6,7]. However, a recent study among children born to HIV-infected women showed that gender differences are present in children [8]. These findings suggest that CD4 lymphocyte counts and HIV RNA levels in both men and women could be affected by levels of reproductive hormones, which differ by gender and change in a woman during her life (e.g., in pregnancy or at the menopause).

Among HIV-uninfected women, postmenopausal women have fewer CD4 lymphocytes than fertile women [9]. Reproductive hormones decrease in the menopausal period. In the case of oestrogens, a shift in type of oestrogen from oestradiol to oestrone occurs, whereas the concentration of progesterone decreases.

Alterations in reproductive hormones also occur during pregnancy. Progesterone particularly has an important immunosuppressive function, preventing rejection of the fetus [10]. Blood levels of reproductive hormones such as progesterone and oestradiol are elevated during pregnancy, which might explain the reduced number of CD4 and CD8 lymphocytes in blood during pregnancy. Oestrogens and progesterone indeed appear to have an immunoregulatory effect through production of cytokines [11,12]. It is interesting that even the combined oral contraceptive pill, with synthetic oestrogen and progestin, was found to be associated with a trend towards a lower CD4 cell count, albeit in HIV-uninfected women [7].

Although concentrations of several reproductive hormones were not measured, the present study aimed to determine indirectly the effect of changes in levels of reproductive hormones by investigating the impact of pregnancy and menopause on CD4 lymphocyte counts in HIV-infected women.

Methods

Study population

The European Study on the Natural History of HIV Infection in Women comprised 487 HIV-infected women from 12 European countries who had a known interval of seroconversion, described in detail elsewhere [13]. The study gathered information on pregnancy and menopause, using a common standardized questionnaire administered at each woman's visit. The Swiss HIV Cohort Study comprises HIV-infected men and women who are followed through seven study centres in Switzerland [14]. Only women with a known interval of HIV seroconversion who were postmenopausal at study entry or became postmenopausal during follow-up were included in the current analysis ($n = 6$). Information on the menopause of these women was gathered by systematic review of their charts. In both studies, T cell subsets were determined by flow cytometry. All women were HIV positive at study entry; their interval of seroconversion was retrospectively determined, and the midpoint of that interval was used as the date of seroconversion. Data were collected from 1993 onwards and the cut-off date of the analysis was December 1, 2000.

Statistical analyses

CD4 lymphocyte counts were modelled using a regression analysis for repeated measurements (i.e., a random effects model with a random intercept and slope) [15] to determine the impact of pregnancy and menopause on CD4 lymphocyte count after HIV seroconversion. This random effects model corrects for dependency among multiple measurements taken for one woman [15]. Since CD4 lymphocyte counts are not normally distributed, they were modelled on the square-root scale ($\sqrt{}$CD4). This transformation appeared to be appropriate for describing CD4 marker paths [16]. The median time between HIV seroconversion and study entry was 4.5 years; consequently, few CD4 cell count values were available for the first 3 years after seroconversion. Additionally, the number of CD4 lymphocyte measurements substantially decreased after 10 years from seroconversion. Therefore, only those values obtained in the period from 3 to 10 years after seroconversion were considered; such values were available for 376 of the 487 women participating in the European Women Study. Women in the menopause were excluded from the model that determined the effect of pregnancy. The decline in CD4 cell count was also modelled for the 9 months before and after the visit at which a pregnancy was first noted and in the 2.5 years before and after the point of menopause (defined as the date of last menses). All models were adjusted for individual age, use of progestin and the use of antiretroviral therapy, which was categorized as no therapy, monotherapy, double therapy and triple therapy. Models that predicted CD4 marker paths around the point of menopause or pregnancy were adjusted for time since HIV infection.

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Results

CD4 lymphocyte counts were available for 382 women for the period from 3 and 10 years from seroconversion, including 376 from the European Women Study and six from the Swiss HIV Cohort Study. The median number of CD4 lymphocyte count measurements per woman was three [interquartile range (IQR) 2–5 measurements], and the median CD4 lymphocyte count at study entry was 360×10^6 cells/l (IQR, 200–530). The median age at HIV seroconversion was 25 years (IQR, 21–30) and 240 (63%) women reported use of oral contraceptives at at least one visit. At 30% of all visits, no treatment was reported used, and at 18, 23 and 29% of the visits monotherapy, double or triple therapy, respectively, was reported used.

Twenty women were postmenopausal at 3 years from seroconversion and five more women passed the menopause thereafter: six had had hysterectomies and 19 had a natural menopause. For the random effect model including menopause, 1428 CD4 lymphocyte count measurements were available, including 107 measurements of postmenopausal women. Therefore, 1321 CD4 lymphocyte count measurements were available to determine the effect of pregnancy (only premenopausal women), including 34 measurements obtained from 25 pregnant women. Figure 1 shows the CD4 decline in pre- and postmenopausal women and in pregnant and non-pregnant women after adjustment for the use of antiretroviral therapy and/or progestin. The model estimates for changes in CD4 cell count ($\sqrt{}$CD4) is given by [the intercept at seroconversion] − [the decline with time in months] − [the change owing to menopause/pregnancy]. For menopause (Fig. 1a) $\sqrt{}CD4 = 20.91$ [95% confidence interval (CI), 19.73–22.09] − 0.026 (95% CI, −0.044 to −0.008) − 1.72 (95% CI, −3.69 to 0.26). For pregnancy (Fig. 1b), the model estimate is $\sqrt{}CD4 = 20.81$ (95% CI, 19.57–22.05) − 0.023 (95% CI, −0.042 to −0.004) − 0.61 (95% CI, −1.91 to 0.69). Figures are shown for women who received neither antiretroviral therapy nor progestin and are based on the above-mentioned numbers. Postmenopausal women had lower CD4 lymphocyte counts 3 years after seroconversion than did premenopausal women, although this difference was only marginally significant (333 vs 399×10^6 cells/l; $P = 0.09$). Pregnant women had lower CD4 lympho-

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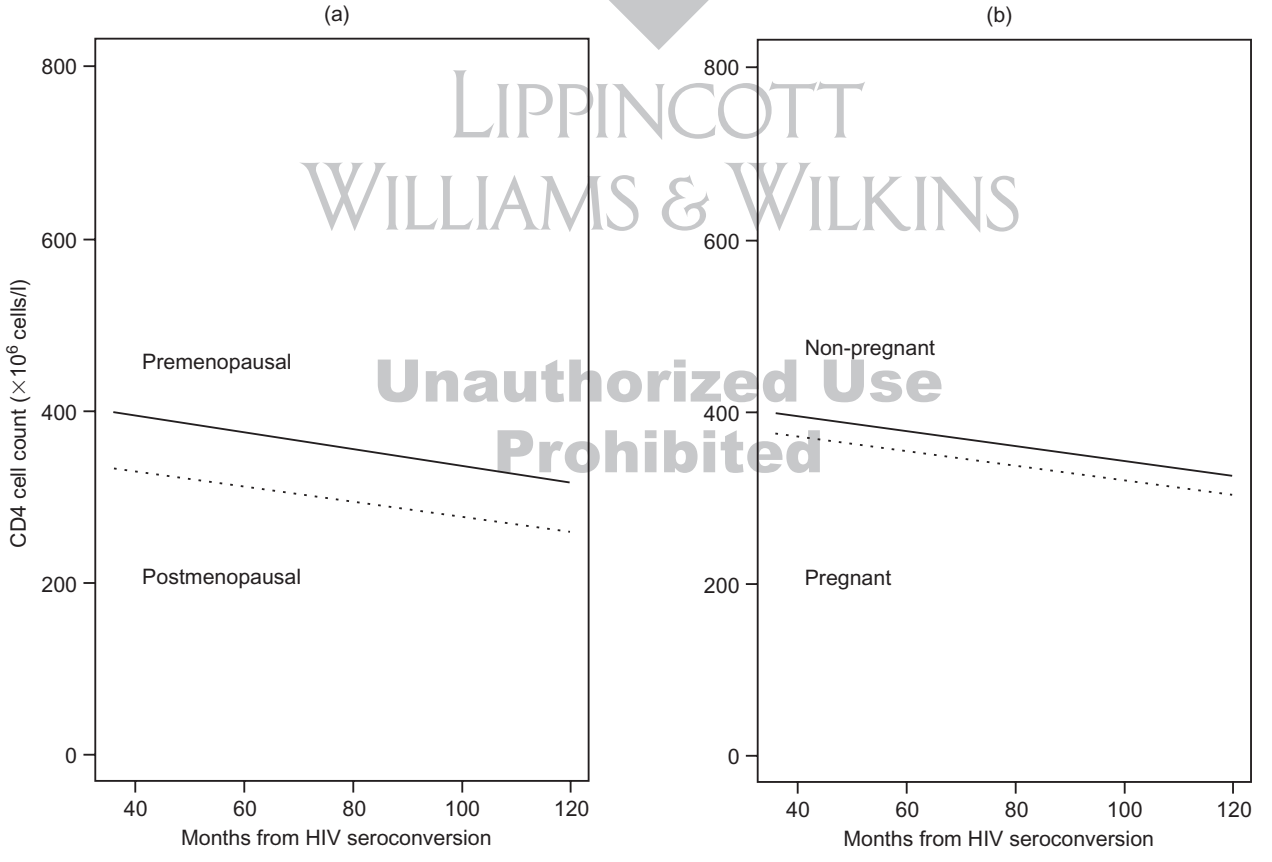

Fig. 1. Predicted absolute CD4 lymphocyte counts for (a) pre- and postmenopausal women and (b) pregnant and non-pregnant women from 3 to 10 years after seroconversion. The curves were created by back-transforming the predicted square root CD4 lymphocyte counts from a random effects linear regression model.

cyte counts than non-pregnant women 3 years after seroconversion, although again, the difference was not statistically significant, (375 vs 399×10^6 cells/l; $P = 0.36$). The monthly CD4 decline was not associated with pregnancy and menopause. Adjustment for age at seroconversion did not substantially change the results.

Figure 2a shows CD4 marker paths around the menopause and is based on 116 CD4 lymphocyte counts from 25 women (16 measurements from six women before the menopause and 100 measurements from 25 women after the menopause). Figure 2b shows CD4 marker paths around pregnancy and is based on 83 measurements from 39 pregnant women also including women who became pregnant in the first 3 years after seroconversion; 13, 39 and 31 CD4 cell count values before, at and after a pregnancy visit, respectively. The median CD4 cell count was 418×10^6 cells/l (IQR, 338–768), 398×10^6 cells/l (IQR, 278–615) and 442×10^6 cells/l (IQR, 353–659) at the prepregnancy, pregnancy and postpregnancy visit, respectively ($P > 0.05$). No significant change in CD4 decline after the menopause ($P = 0.54$) or during pregnancy ($P = 0.81$) was observed when the CD4 marker paths were modelled around menopause or pregnancy, taking into account time since infection. The model estimates for changes in CD4 cell count ($\sqrt{\text{CD4}}$) is given by [the intercept at seroconversion] − [the decline with time in months] − [the decline in months for time from menopause/pregnancy], taking time zero as the visit at which a pregnancy was first noted or the date of last menses. The model estimates for menopause (Fig. 2a) are $\sqrt{\text{CD4}} = 18.97$ (95% CI, 15.77–22.18) − 0.016 (95% CI, −0.31 to 0.28) − 0.091 (95% CI, −0.38 to 0.20). The model estimates for pregnancy (Fig. 2b) are $\sqrt{\text{CD4}} = 21.60$ (95% CI, 19.53–23.67) − 0.34 (95% CI, −0.76 to 0.09) + 0.074 (95% CI, −0.61 to 0.76).

Discussion

Although postmenopausal HIV-infected women had lower CD4 lymphocyte counts than their premenopausal counterparts, menopause *per se* did not affect

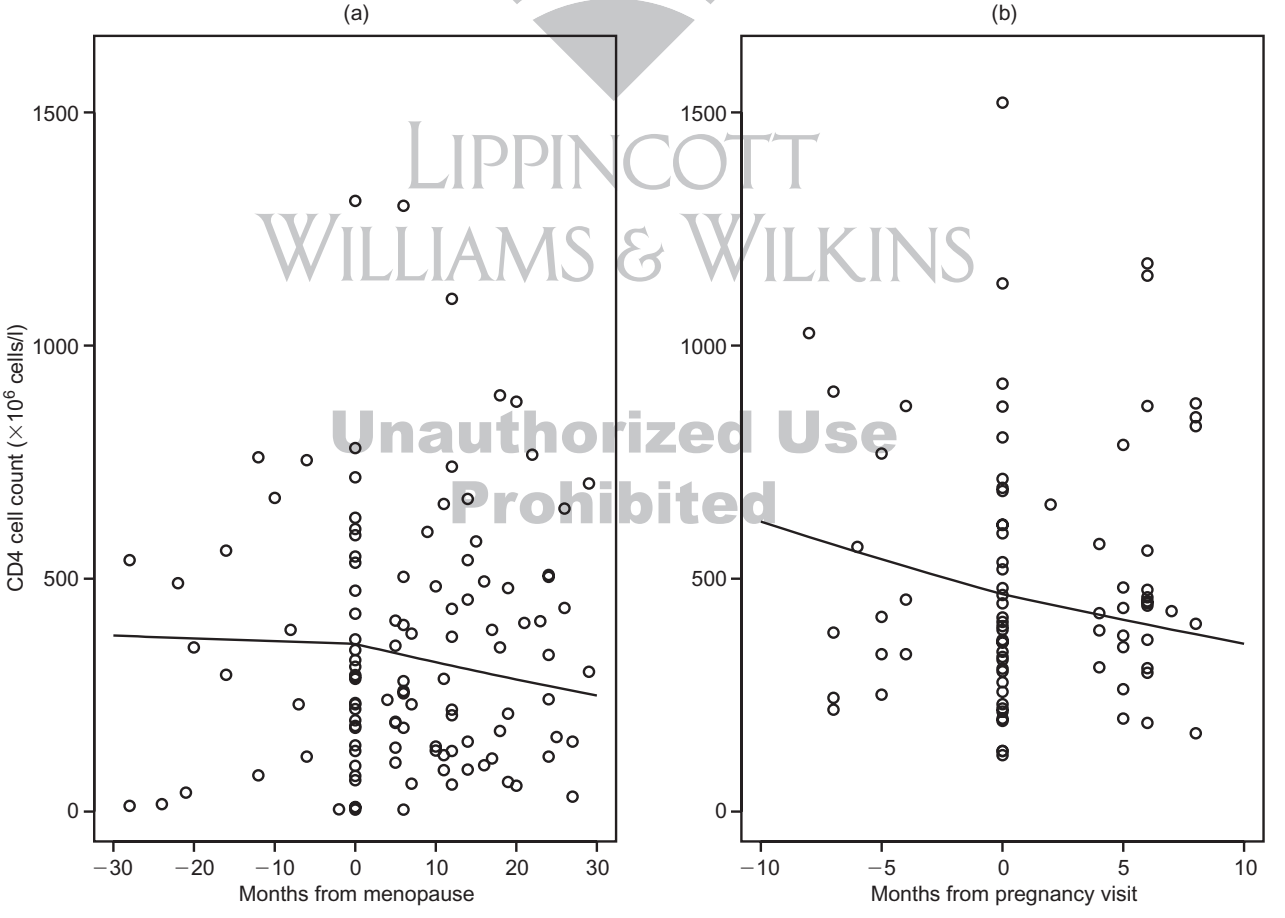

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Fig. 2. Predicted absolute CD4 lymphocyte counts for (a) 25 postmenopausal women around the menopause and (b) 26 pregnant women around a visit at which she was pregnant. The curves were created by back-transforming the predicted square root CD4 lymphocyte counts from a random effects linear regression model.

CD4 decline. The difference of 66×10^6 cells/l might instead be explained by a change in the level of reproductive hormones after the menopause. These hormones differ also between men and women and, therefore, may explain gender differences in CD4 lymphocyte counts: the CD4 lymphocyte count in women is approximately 100×10^6 cells/l higher than in men at the same stage of infection. Recently, one study showed, whereas another study did not, that CD4 lymphocyte counts as well as HIV RNA levels fluctuate during the menstrual cycle in HIV-infected women [17,18].

Whether the differing marker levels have any functional meaning for HIV disease progression is unclear. They may have implications for treatment initiation, since guidelines include CD4 lymphocyte counts and HIV RNA levels as criteria for starting therapy. Strictly speaking, postmenopausal women start treatment earlier than premenopausal women because they reach the CD4 lymphocyte count threshold earlier. This implies a delay of treatment initiation in fertile women and urges investigation of how such delay may relate to HIV disease progression in these women.

Pregnancy had no statistically significant effect on CD4 lymphocyte counts, although median levels pre- and postpregnancy suggested a temporal decrease. These results agree with some studies conducted among HIV-negative pregnant women [19,20] but conflict with others [21–23]. The drawback of our study is that we did not measure changes in reproductive hormones, whereas the drawback of the cited studies is that they did not use appropriate statistical methods (i.e., repeated measurement analyses). Nowadays, methods adjusting for the dependency of multiple measurements per person are widely available, and one of them was used in the present study. If a temporary decrease in the number of CD4 lymphocyte counts during pregnancy occurs, this has probably no functional meaning in HIV infection, because pregnancy does not accelerate HIV disease progression [24–30]. Nevertheless, diminished immunoreactivity during pregnancy is important in preventing rejection of the fetus, which from an immunological point of view is foreign tissue.

Three limitations of our study should be noted. Only six women who were or became postmenopausal could be included from the Swiss HIV Cohort Study; however, the results were comparable when analyses were repeated without these women. In the European Women Study, two visits per year are scheduled, and consequently the maximum number of visits in any pregnancy was two. Furthermore, the date of conception was not known. Finally, information on pregnancy and menopause was gathered by questionnaires and chart review and not confirmed by laboratory markers. However, if a pregnancy or menopause were missed, this could have led to bias towards the null, diminishing differences in CD4 lymphocyte counts.

Our results suggest that CD4 lymphocyte counts differ between pre- and postmenopausal women, but associations were not statistically significant, probably because of our small sample of postmenopausal women and our possibly missed evidence of menopause. Therefore, studies including a larger number of older women, preferably including laboratory markers of menopause, are needed to confirm these findings. Whether marker differences have a functional meaning in HIV disease progression is highly relevant for therapy guidelines for HIV-infected women and should be the subject of further investigations.

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Appendix

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