chronic infected patients,[1–4] but not during PHI. Chronic HIV-infected patients with CXCR4-using viruses are described as having faster disease progression compared with patients with CCR5-tropic strains. Our analysis did not support this relation, and we found a greater $CD4^+$-cell increase after initiation of ART in patients harboring CXCR4-using virus, regardless of algorithm choice. Patients with CXCR4-tropic viruses have a greater CD4-cell decrease without ART,[4] and in chronic patients HIV tropism has no impact on immunovirological response.[2] CXCR4-tropic viruses replication is lower than that of CCR5 variants in GALT, and CCR5 viruses are also more prone to infect macrophages especially during PHI.[17] Starting ART during PHI in patients with CXCR4 viruses could help in increasing plasma $CD4^+$ cells by targeting viral replication in peripheral blood, whereas ART has a limited effect on GALT $CD4^+$-cell depletion which occurs mainly due to CCR5 variants.[18] This may explain our findings of a better immunological recovery as measured by plasma $CD4^+$-cell increase. The influence of predicted coreceptor usage on GALT CD4-cell depletion remains unclear.

All of the patients had antiretroviral syndrome, and no data are available regarding the association between symptoms and coreceptor usage: this could represent a selection of more pathogenic viruses, usually CXCR4 using.[2] Further studies with phenotypic assays are needed to better evaluate the feasibility of genotypic methods to predict coreceptor usage in the setting of PHI. In conclusion our study remarks an immunological recovery in patients treated during PHI, even if harboring CXCR4-tropic virus; these results encourage treatment during this phase of infection.

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# OPEN Prevalence of Premalignant Cervical Lesions in Women With a Long-term Nonprogressor or HIV Controller Phenotype

***To the Editors:***

In 2008, cervical cancer was the third most common cancer among women worldwide, responsible for 250,000 deaths annually. This burden is primarily borne

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K.E.M. with N.A.M. wrote the article. C.T.T. and N. A.M. devised the study. T.O. oversaw the cervical smears, and N.K. coordinated the study.

The authors have no conflicts of interest to disclose.

**TABLE 1.** Baseline Characteristics of Study Participants: Overall and by HIC/LNTP Classification

| | All Eligible Women in Study (95% CI) or (IQR) n = 37 | HIC (VL < 400) (95% CI) or (IQR) n = 14 | LTNP (VL > 400) (95% CI) or (IQR) n = 23 |
|---|---|---|---|
| Baseline characteristics* | | | |
| Median age, yrs | 36 (31–42) | 34.5 (28–43) | 36 (32–41) |
| Median CD4 count, cells/mm$^3$ | 677 (608–852) | 842 (659.5–1002) | 647 (584–776) |
| Median VL, copies/mL | 975 (240–27,985) | 147.5 (19–322.5) | 13,875 (1185–74,315) |
| Median weight, kg | 77.1 (65.45–91.3) | 72.6 (52.33–88.63) | 81.6 (68.8–101) |
| BMI, kg/m$^2$ | 30.59 (24.49–35.58) | 27.86 (21.47–33.96) | 32.28 (25.27–38.01) |
| Median monthly income, ZAR | 3550 (1500–6630) | 3570 (1560–7697.5) | 3400 (1480–7000) |
| Median number of sexual partners last 6 mo | 1 (1–1) | 1 (1–1) | 1 (1–1) |
| Proportion ever smokers (%) | 13.51 (5.91–2.80) | 7.14 (1.27–31.47) | 17.39 (6.98–37.14) |
| Proportion with STI symptoms at baseline (%) | 5.41 (1.50–17.70) | 0 | 8.7 (2.68–26.80) |
| Proportion using hormonal contraception at first visit | 32.43 (19.63–48.54) | 21.43 (7.57–47–59) | 39.13 (22.16–59.21) |
| Prevalence of cervical smear results | | | |
| Normal smears (%) | 43.24 (28.67–59.09) | 35.71 (15.34–61.24) | 47.82 (29.24–67.04) |
| Premalignant Lesions (%) (all) | 56.76 (40.91–71.33) | 64.29 (38.76–83.65) | 52.17 (32.96–70.76) |
| ASCUS | 12.51 (5.91–27.98) | 14.29 (4.01–39.94) | 13.04 (4.54–32.13) |
| LSIL | 29.73 (17.49–45.78) | 35.71 (15.34–61.24) | 26.09 (12.55–46.47) |
| HSIL | 12.51 (5.91–27.98) | 14.29 (4.01–39.94) | 13.04 (4.54–32.13) |
| Cancer | 0 | 0 | 0 |

*Baseline characteristics were defined at time of enrollment into cohort.

ASCUS, atypical squamous cells of uncertain significance; HIC, HIV controllers; HSIL, high-grade squamous intraepithelial lesion; IQR, interquartile ratio; LSIL, low-grade squamous intraepithelial lesion; LTNP, long term nonprogressors.

by low- and middle-income countries.[1] Invasive cervical cancer is preceded by gradual progression of premalignant cervical squamous intraepithelial lesions (SIL),[2] allowing opportunity for screening, early intervention and cure. Among HIV-seropositive women, SIL are more prevalent,[3,4] more persistent, and have poorer treatment outcomes[5] compared with seronegative women. Increased risk of SIL has been consistently shown to be associated with lower CD4 counts,[3,6,7] whereas antiretroviral therapy seems to prevent progression and encourage regression of these lesions.[8] Studies have suggested that HIV-infected women with CD4 > 500 cells per cubic millimeter experience rates of SIL comparable with those of HIV-negative women.[3,7] Little is known about prevalence and progression of SIL occurring among infrequent HIV-infected women with the innate ability to control their HIV infection in the absence of antiretroviral therapy. Although defined variably, they are recognized by key characteristics: long-term nonprogressors (LTNP) maintain normal (>500 cells/mm$^3$) and stable CD4 count over a long period, with or without a detectable viral load (VL).[9] HIV controllers (HIC) are a less frequent phenotype able to maintain undetectable or extremely low VL.[10] This report is a cross-sectional analysis describing the prevalence of premalignant lesions among the baseline cervical smears of a study cohort of LTNPs and HICs in Soweto, South Africa.

A cohort of treatment-naive participants with sustained CD4 counts >500 cells per cubic millimeter or with VL below 2000 copies per milliliter was identified from studies with long-term follow-up and from an HIV counseling and testing clinic at Chris Hani Baragwanath Hospital.[11] This cohort recruited patients from April to November 2011. Participants were screened at entry, and annually thereafter. All cervical smear data from this period were included in our analysis.

In this cohort, CD4 counts and VL are ascertained every 6 months and conventional Pap smears offered annually. The panleukogating method[12] was used to obtain CD4 counts, and HIV VLs were estimated using the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 (Roche, Indianapolis, IN). Cervical smears are assessed at the National Health Laboratory Service (NHLS) Cytopathology Division in Johannesburg, using the 2001 Bethesda reporting system.[13] All negative cervical smears are rapidly reviewed by a second screener. In addition, all positive (atypical cells of uncertain significance and worse) smears are re-evaluated by a senior cytology screener. While there was no independent review of study slides, the cytology laboratory subscribes to an international external quality assurance program and is accredited annually by the South African National Accreditation System. Women with cytology reports of atypical squamous cells that cannot exclude a high-grade lesion, high-grade squamous intraepithelial lesion (HSIL), or worse are referred for colposcopy and further treatment.

For this analysis, HICs were defined as women with VL of <400 copies per milliliter at the time of their cervical smear and had to have a minimum of 2 VL recorded 6 months apart and no VL exceeded 850 copies per milliliter. LTNPs were defined as women who sustained a stable CD4 count >500 cells per cubic millimeter for at least 5 years. Individuals meeting both definitions were classified as HIC. Women with a CD4 count <500 cells per cubic millimeter for LTNPs or subsequent VL > 2000 copies per milliliter for HICs were terminated from the study; they were not included in this analysis. The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand.

Of 37 participants included in this study, 14 were classified as HIC and 23 as LTNP. Median age was 36 years; median CD4 count and VL at time of baseline cervical smear was 677 cells per cubic millimeter and 975 copies per milliliter, respectively (Table 1). For HICs, median time since patient's first CD4 count was 4 years and median CD4 count at time of first smear was 842 cells per cubic millimeter. Possible risk factors for SIL and cervical cancer such as age, smoking, sexually transmitted infections, hormonal contraceptive use, and number of sexual partners are documented (Table 1).

The prevalence of premalignant lesions in the group as a whole was 56.8% [95% confidence interval (CI) 40.9 to 71.3]. Low-grade squamous intraepithelial lesion (LSIL) was reported in 29.7% of smears and HSIL in 12.5%. Among HICs, 64.3% (95% CI 38.8 to 83.7) had abnormal cytology, with LSIL and HSIL prevalences of 35.7% (95% CI 15.3 to 61.2) and 14.3% (95% CI 4.0 to 39.9), respectively. Among LTNP, 52.2% (95% CI 33.0 to 70.8) had abnormal cytology; 26.1 (95% CI 12.6 to 46.5) were diagnosed as LSIL and 13.0% (95% CI 4.5 to 32.1) as HSIL. Nine (24%) of the women had had a second annual cervical smear at the time of analysis; 4/9 had progressed from an initial normal smear to LSIL.

Although participant numbers are few, to our knowledge, this is the largest group of LTNP/HIC in which the prevalence of SIL has been reported. The overall point estimate of the rate of abnormal cytology (56.8%) we report is uncommonly high for the local population (although the 95% CIs of this estimate overlap with previous studies from this region). In more than 2000 women living with HIV in Soweto, the prevalence of all premalignant lesions was 38.1% (95% CI 36.1 to 40.1) and 20.4% and 13.5% of these women had LSIL and HSIL, respectively.[8] Previous studies in South Africa have reported prevalence of abnormal cervical smears among HIV-positive women between 38% and 55%, with prevalence of LSIL and HSIL of 20% to 35% and 13% to 18%, respectively.[3,6,7] These studies recorded median CD4 counts of 231 to 356 cells per cubic millimeter, suggesting advanced HIV infection and compromised immune function. In a group of 118 HIV-infected women with CD4 counts similar to ours, (median 626 cells/mm$^3$), Firnhaber et al[6] (2010) reported abnormal cytology in 32.2% of participants (LSIL in 14.4% and HSIL in 10.7%), far lower than in our study. The median age in our study is comparable to that reported previously in prevalence studies from this region,[3,6–8] suggesting that age is not a major contributor to the higher prevalence of premalignant lesions we observed. The potentially longer duration of HIV/HPV co-infection and longer mean survival of LTNP/HIC compared with progressors may contribute to the higher SIL prevalence found. However, women on highly active antiretroviral therapy, with similar potential for prolonged co-infection and survival, do not show comparable SIL prevalence. A 7-year cohort study of women living with HIV in Soweto found those on highly active antiretroviral therapy were 38% less likely to have an incident abnormal smear than those not on therapy.[14]

There are data that suggest the involvement of HLA-C1 alleles as a possible explanation for our apparently paradoxical finding. HLA-C1 alleles (HLA-Cw alleles with an asparagine at position 80) are ligands for specific killer immunoglobulin-like receptors (KIR), namely KIR2DL2 and KIR2DL3, which have been shown to be associated with invasive cervical cancer.[15] Moreover, the possession of at least 1 HLA-C1 allele has been associated with decreased VL among black South African HIV-1 infected women.[16] Preliminary data have found a significantly higher representation of HLA-C1 allotypes among South African LTNP and HICs compared with ethnically matched healthy controls and progressors (C. Tiemessen, unpublished data, 2013). It may be that KIR2DL2 or KIR2DL3—HLA-C1 interactions, known to be more weakly inhibitory than the interaction between KIR2DL1 and HLA-C2, result in higher natural killer cell activation which could predispose to cervical cancer yet may be protective in the context of HIV-1 disease progression. However, we have no evidence to support or refute this hypothesis in this group, and further studies are needed.

Our study is limited by small sample size, and lack of data on both duration of HIV infection and on several risk factors for malignant and premalignant lesions of the cervix, particularly the presence of high-risk HPV subtypes. Moreover, because the risk of SIL increases with time since HPV infection, irrespective of immune competency, we cannot rule out that survival bias and duration of HPV infection may be the cause of our results. Additionally, no blinded review or histological confirmation of cytology was performed. Nevertheless, our data suggest that the prevalence of SIL may be higher among LTNPs and HICs despite an apparent resistance to HIV.

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