Incident High-Grade Squamous Intraepithelial Lesions in Senegalese Women With and Without Human Immunodeficiency Virus Type 1 (HIV-1) and HIV-2

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Background: Women infected with human immunodeficiency virus type 1 (HIV-1) and -2 may be at higher risk of developing cervical cancer than uninfected women. We assessed the relationships among human papillomavirus (HPV) types and persistence, HIV-1 and/or HIV-2 infection, and the development of high-grade cervical squamous intraepithelial lesions (HSILs) in a prospective study. Methods: We studied 627 women with and without HIV-1 and/or HIV-2 infection and high-risk HPV infection in Senegal, West Africa, who were assessed every 4 months for HSIL and HPV DNA over a mean follow-up of 2.2 years. Cox regression modeling was used to assess risks associated with development of HSIL. Results: During follow-up, 71 (11%) of 627 women developed HSIL as detected by cytology. HIV-infected women with high-risk HPV types were at greatest risk for development of HSIL. In multivariable modeling, infection with oncogenic HPV types—both persistent (hazard ratio [HR] = 47.1, 95% confidence interval [CI] = 16.3 to 136) and transient (HR = 14.0, 95% CI = 3.7 to 54)—was strongly associated with HSIL risk. In univariate analyses, HIV-positive women infected with HIV-2 were less likely to develop HSIL (HR = 0.3, 95% CI = 0.1 to 0.9) than HIV-positive women infected with HIV-1. HIV-positive women with CD4+ cell counts between 200 and 500 cells per microliter (HR = 2.2, 95% CI = 0.8 to 6.3) or fewer than 200 cells per milliliter (HR = 5.5, 95% CI = 2.0 to 15.2) were at greater risk of HSIL than HIV-positive women with CD4 counts of more than 500 cells per milliliter. High plasma HIV RNA levels were associated with increased HSIL risk (HR for each order of magnitude increase in the level of plasma HIV RNA = 1.4, 95% CI = 1.1 to 1.7; P = .005). After adjustment for HPV types and persistence, however, HIV type, plasma HIV RNA level, and CD4 count were no longer statistically significantly associated with increased risk of HSIL. Conclusions: HIV-1 and HIV-2 are associated with increased risk for development of HSIL. This risk appears to be associated primarily with increased HIV persistence that may result from immunosuppression related to HIV-1 and/or HIV-2 infection. [J Natl Cancer Inst 2006;98:100–9]

Infection with high-risk types of human papillomavirus (HPV) is required for the development of invasive cervical cancer (1–4). HPV oncoproteins E6 and E7 set the stage for development of malignancy by disrupting normal host cell cycle controls by binding to and promoting the degradation of p53 by increasing the expression of telomerase (E6) and by binding to the retinoblastoma protein (E7). Our studies (5–7) and those of others (8–11) have shown that human immunodeficiency virus (HIV) infection is associated with an increased risk for detection of high-risk types of HPV, cervical cancer, and the cervical cancer precursor lesions low-grade and high-grade squamous intraepithelial lesions (LSILs and HSILs, respectively). In a previous...
cross-sectional study among women infected with high-risk types of HPV (7), women with HIV-1, HIV-2, and dual HIV-1 and HIV-2 infection were 2.2, 6.0, and 8.0 times, respectively, more likely to have HSIL and invasive cancer than those without HIV infection. 

The mechanism by which HIV infection increases risk of cervical neoplasia is not completely understood. One plausible explanation is that HIV-induced immunosuppression leads to an inability to control the expression of HPV and the production of HPV oncoproteins E6 and E7. Several previous longitudinal studies have assessed the effect of HIV-1 infection, HIV viral load, and HIV-associated immunosuppression on the detection of high-risk types of HPV and on the risk of cervical neoplasia (12–16). However, in most of those studies women were monitored only to the development of LSIL, a lesion that is generally transient and associated with a low risk of neoplastic progression, rather than to development of HSIL or carcinoma in situ, lesions with substantial neoplastic potential. Moreover, no data are available that describe the risk of developing HSIL or carcinoma in situ associated with HIV-2 infection. We therefore undertook this study to characterize the risk of developing HSIL/carcinoma in situ that is associated with HPV types and persistence, HIV-1 or HIV-2 infection, CD4+ cell counts, and HIV viral load.

**Materials and Methods**

**Study Population**

We conducted a prospective study of HSIL development among women with and without HIV-1 and/or HIV-2 infection and high-risk HPV infection in Senegal, West Africa. Between October 1, 1994, and January 1, 1998, all women older than 15 years presenting to the University of Dakar Infectious Disease Clinic (n = 4349) and commercial sex workers attending one of two sexually transmitted disease clinics in Dakar (n = 773) and in M’Bour (n = 270) were offered serologic testing for HIV-1 and HIV-2 and screening for cervical HPV DNA and cytology. Women returned 4 weeks later for their HIV, HPV, and cervical cytology results, and those found to be HIV seropositive were counseled according to the guidelines established by the Senegalese AIDS national committee. None of the HIV-infected women was receiving antiretroviral therapy. We invited all women with HIV or high-risk HPV infection to participate in a longitudinal study, with visits occurring every 4 months, to assess the risk of developing cervical lesions. Of the 5392 women screened, 1348 (25%) were positive for HIV-1 and/or HIV-2 or had high-risk HPV types detected in cervical swabs. Of these 1348 eligible women, 939 (70%) enrolled. Enrollment among HIV-positive and/or HPV-positive women varied by site of recruitment, with 63%, 85%, and 88% of such women from the University of Dakar Outpatient Infectious Disease Clinic, the Dakar sexually transmitted disease clinic, and the M’Bour sexually transmitted disease clinic, respectively, consenting to enroll. Enrollment rates varied somewhat by HIV status; 76% of HIV-positive women (with or without HPV infection) and 64% of HPV-positive, HIV-negative women enrolled. However, after adjusting for study site and HIV status, enrollment rates did not differ by demographic or behavioral characteristics, such as age, marital status, parity, use of contraception, or the use of alcohol or tobacco. In addition to HIV-positive and/or HPV-positive women, a subset of 177 HIV-negative women without HPV infection (i.e., 4% of the 3937 without HIV or HPV infection) were also enrolled in the study as a reference group at low risk of developing HSIL during follow-up. Written informed consent was obtained from all enrolled women. These women were screened earlier in the study, were more likely to be from one of the two sexually transmitted disease clinics, and were somewhat older than the HIV-negative, HPV-negative women who were not enrolled. They, however, did not differ by other measured demographic or behavioral factors after adjustment for study site and age. In total, the longitudinal study enrolled 1116 women. The study was conducted according to procedures approved by the institutional review boards of both the University of Washington and the University of Dakar.

**Collection of Specimens and Study Procedures**

At the screening visit, blood was collected for HIV-1 and HIV-2 serologic assays, and cervical cellular samples were obtained for cytologic and HPV screening as previously described (7). Subjects underwent a general physical examination and completed a short standardized interview, including questions pertaining to medical history and to sexual behavior. At the return visit 4 weeks later, and at each subsequent 4-month follow-up visit, enrolled subjects underwent a more detailed interview regarding sexual behavior that included questions about marital history, contraception use, age at first intercourse, and number of lifetime sexual partners. Interviewers also asked questions about medical history to obtain information about previous hospitalizations and the use of medications. At each visit, blood was obtained for qualitative and quantitative RNA and DNA assays of HIV-1 and/or HIV-2 and for lymphocyte subset analysis.

**Cytology Screening**

Pap smears were interpreted and classified according to the Bethesda System (17) as unsatisfactory, negative, atypical squamous cells of uncertain significance, LSIL, HSIL, or invasive cervical cancer. From October 1, 1994, through March 31, 1998, conventional Pap smears were used. Slides were initially read by a pathologist in Senegal and then sent to Seattle for a second reading by a cytopathologist and the study cytopathologist (N.B.K.), who were blinded to HIV and HPV results. All conventional slides classified as LSIL or worse by either pathologist were restained, recovered, and reread by the pathologist in Seattle. The final cytologic diagnosis used in our analyses was that made by the Seattle pathologist; however, if the slide was unavailable (broken or lost) for reexamination in Seattle, the diagnosis made by the Senegalese pathologist was used. Beginning April 1, 1998, the Thin Prep monolayer cell preparation system (Cytyc Corp., Boxborough, MA) was used to obtain all Pap smears, and diagnoses from these slides were made by the cytopathologist in Seattle.

**HPV DNA Detection and Typing**

Polymerase chain reaction (PCR) assays for the detection of HPV DNA were performed with HPV L1 consensus primers and HPV type-specific oligonucleotide probes specific for high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, or 56 and a generic HPV probe, as previously described (18). Initially, screening for the presence of high-risk HPV was done with a 10-probe mixture, testing for HPV DNA by PCR that used the consensus primers MY09 and MY11, which are specific for a highly conserved region in the
L1 open reading frame. Positive samples were then reamplified to assess presence of 12 HPV types in primer groups for low-risk HPV types (i.e., combined HPV 6 and 11) and for high-risk HPV types (i.e., HPV 16; HPV 18; combined HPV 31, 33, 35, and 39; combined HPV 45 and 56; and combined HPV 51 and 52). Beginning April 1, 1998, when new probes were available, HIV detection and typing analyses were performed via a PCR-based reverse-line strip test method (Roche Molecular Systems, Alameda, CA) with probes for low-risk HPV types 6, 11, 40, 42, 53, 54, 57, 66, and 84 and high-risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73, 82, and 83, as previously described (19).

**HIV Serology and Lymphocyte Subset Analysis**

Initial screening of serum samples for HIV-1 or HIV-2 antibodies was performed with a microwell plate enzyme immunoassay that detects antibodies to both HIV types in one well (HIV-1/2 EIA; Sanofi Diagnostics Pasteur, Redmond, WA). Positive samples were confirmed with a rapid, HIV peptide–based membrane immunoassay that distinguishes between antibodies to HIV-1 and HIV-2 (Multispot; Genetic Systems, Redmond, WA). Whole blood collected in EDTA tubes was analyzed by flow cytometry with a FACSpot analyzer (Becton Dickinson Biosciences, San Jose, CA) to determine the number of CD4+, CD8+, and CD3+ cells per microliter of blood. Cell counts were performed for all samples from HIV-infected women and for all baseline visit samples from HIV-negative women.

**Quantitation of HIV-1 and HIV-2 Plasma RNA**

Quantitative and qualitative assays for HIV-1 RNA were performed as previously described (20). The qualitative assay was performed on all available samples that were negative by the quantitative assay. The quantitative and qualitative HIV-1 RNA assays detected as few as 80 and 40 copies of HIV-1 RNA per milliliter, respectively, with reproducible sensitivities of 200 and 100 HIV-1 RNA copies per milliliter, respectively. The HIV-2 quantitative and qualitative assays detect as few as 40 and 20 copies of HIV-2 RNA per milliliter, respectively, with reproducible sensitivities of 200 and 100 HIV-2 RNA copies per milliliter, respectively.

**Statistical Methods**

Cervical HPV DNA types were categorized according to their known associations with invasive cervical cancer, with high-risk types including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, and 56 and with low-risk types including HPV 6 and 11. Untyped/other HPV types were those HPV types that were included in the reverse-line strip test but not the type-12 mixture used before 1998 (HPV types 26, 40, 42, 53, 54, 55, 56, 57, 58, 59, 66, 68, 73, 82, 83, and 84) or unclassified HPV types. HPV oncogenicity grouping was hierarchical, and categories were mutually exclusive. A sample was called “HPV negative” if HPV was not detected by any of the methods used. At a given study visit, persistence of a “high-risk,” “low-risk,” or “untyped/other” HPV type was defined as the detection at the current and immediately preceding study visit (that is, two consecutive visits) of an HPV type within the indicated risk group, regardless of the specific type detected. At a given study visit, a current HPV infection was considered transient if it was not detected at the immediately preceding visit (that is, if HPV was not detected at two consecutive visits).

Women with initial cytologic diagnoses of LSIL or worse were excluded from analysis. At least two visits with a satisfactory Pap smear (i.e., with a sufficient squamous component and less than 75% of epithelial cells obscured) were necessary for inclusion into the analysis. For women who had an unsatisfactory Pap smear at the enrollment visit, the first satisfactory negative or atypical Pap smear after the initial unsatisfactory Pap smear(s) was considered as the baseline visit. Incidence rates for development of HSIL, as determined by cytology, stratified by HIV status and high-risk HPV detection at the baseline visit, were estimated by Kaplan–Meier methods (21).

Cox proportional hazards regression analyses, with time-dependent covariates, were performed to evaluate the independent effects of HIV status and HPV type (independent variables) on time to development of HSIL (outcome), adjusting for the number of follow-up visits and other confounding factors. The proportional hazards assumption was tested by creating and assessing the significance of interaction terms for all predictors of interest and our time variable. All final models met the proportional hazards assumption. To investigate any potential lack of fit of our model, we conducted a formal analysis of residuals. Martingale (for continuous factors such as HIV plasma load) and deviance residuals for the proportional hazards regression analyses were plotted to look for outliers. No overly influential outliers were identified.

In analyses of the entire study population, women without detectable HPV were used as the reference group. However, in subgroup analyses among HIV-positive women, because of the lack of HSIL development in this reference group, women with transient or persistent HPV 6 or HPV 11 infection and transient untyped/other HPV infection, in addition to women with no HPV infection, were used as a reference category. Relative risks (RRs) were estimated by use of hazard ratios. Factors examined as potential confounders included employment as a commercial sex worker, age, marital status, parity, contraceptive method, smoking, and alcohol use; factors were retained in a specific model if the unadjusted and adjusted coefficients for HIV status (HPV type) differed by more than 10% (22). A priori, CD4 counts were stratified into three groups (>500 cells per microliter, 200–500 cells per microliter, and <200 cells per microliter) and plasma HIV RNA levels were evaluated per order of magnitude increase in HIV-1 or HIV-2, as in previous analyses (7,20).

Factors measured at baseline and follow-up visits (e.g., presence and persistence of types of HPV DNA, new sex partners, CD4 count, and HIV plasma RNA level) were included as time-dependent variables. In this manner, for a given subject, incident HPV infections, HPV persistence, and clearance of various HPV types were accounted for and evaluated over time. This procedure was important because the median length of follow-up in this study was longer than the estimated average time of detection of prevalent (i.e., 6–12 months) (23,24) or incident (i.e., 1–2 years) (25) HPV infections described in previous studies. Sensitivity analyses were conducted to determine whether the differences in the definition of persistence substantially altered study findings.

Among HIV-positive subjects, Cox regression analyses separately evaluating HIV type, CD4 counts, plasma HIV RNA levels, and HPV detection and persistence were performed because these factors were associated with each other and have been previously shown to be associated with cervical neoplasia (7). To determine the potential independent association of each factor...
with development of HSIL after simultaneously controlling for the effects of the other factors, a final composite Cox regression analysis was performed. Data analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC). All statistical tests were two-sided.

RESULTS

Characteristics of Study Population

Of 1116 women enrolled (i.e., 939 positive for HIV and/or HPV and 177 negative for both HIV and HPV), 137 with an initial cytologic diagnosis of LSIL or worse and 17 who never had a satisfactory Pap smear were not included in these analyses. Of the remaining 962 women, 627 (246 HIV-positive women and 381 HIV-negative women) had at least one follow-up visit for which a satisfactory Pap smear was available and were thus included in the final analyses. HIV-positive women and HIV-negative women included in our analyses were similar with regard to site of recruitment, parity status, smoking behavior, alcohol use, age at first sexual intercourse, employment as a commercial sex worker, and length of follow-up (Table 1). However, HIV-positive women, compared with HIV-negative women, were more likely to be older; to be divorced, separated, or widowed; to have been born outside of Senegal; to not use contraception; and to have had more follow-up visits. Among HIV-positive women, those infected with HIV-2 were older and more likely to be a commercial sex worker than those infected with HIV-1.

By study design, we oversampled HIV-negative women with high-risk HPV infection to enroll a group of women at high risk of developing HSIL. Consequently, at baseline, among 627 study participants, 402 (64%) were infected with high-risk HPV, including 92 (37%) of 246 HIV-infected and 310 (81%) of 381 HIV-uninfected women (P<.001, chi-squared test). Women infected with HIV-1 and women infected with HIV-2 were similar to each other with regard to HPV infection (i.e., presence of any HPV or a high-risk type). Women infected with HIV-1 had lower CD4 cell counts but higher CD8 cell counts, as well as higher plasma HIV RNA levels, than women infected with HIV-2. Among women infected with HIV-1, plasma HIV-1 RNA levels were lower among women dually infected with HIV-1 and HIV-2 than among women infected with HIV-1 alone (P<.001); however, among women infected with HIV-2, plasma HIV-2 RNA levels were similar between women dually infected with HIV-2 and HPV and women infected with HIV-2 alone. None of the HIV-infected women in this cohort was receiving antiretroviral therapy.

Table 1. Demographic and laboratory characteristics of the study population by human immunodeficiency virus (HIV) status at the enrollment visit*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV status</th>
<th>P†</th>
<th>P‡</th>
</tr>
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<tbody>
<tr>
<td>Site of recruitment, No. (%)</td>
<td></td>
<td>.3</td>
<td>.002</td>
</tr>
<tr>
<td>Dakar STD Clinic</td>
<td>108 (28)</td>
<td>36 (22)</td>
<td>15 (26)</td>
</tr>
<tr>
<td>M’Bour STD Clinic</td>
<td>39 (10)</td>
<td>18 (11)</td>
<td>14 (25)</td>
</tr>
<tr>
<td>Dakar Outpatient ID Clinic</td>
<td>234 (62)</td>
<td>111 (67)</td>
<td>28 (49)</td>
</tr>
<tr>
<td>Mean age, y (±SD)</td>
<td>30.5 ± 7.4</td>
<td>31.0 ± 7.7</td>
<td>34.2 ± 7.1</td>
</tr>
<tr>
<td>Marital status, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never married</td>
<td>81 (21)</td>
<td>23 (14)</td>
<td>7 (12)</td>
</tr>
<tr>
<td>Married—monogamous</td>
<td>128 (34)</td>
<td>47 (28)</td>
<td>17 (30)</td>
</tr>
<tr>
<td>Married—polygamous</td>
<td>52 (14)</td>
<td>19 (11)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Divorced/separated</td>
<td>113 (29)</td>
<td>48 (29)</td>
<td>24 (42)</td>
</tr>
<tr>
<td>Widowed</td>
<td>6 (2)</td>
<td>5 (9)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Country of birth—Senegal, No. (%)</td>
<td>339 (89)</td>
<td>130 (79)</td>
<td>51 (89)</td>
</tr>
<tr>
<td>Parity ≥ 1, No. (%)</td>
<td>333 (88)</td>
<td>139 (85)</td>
<td>52 (91)</td>
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<tr>
<td>Lack of contraception use, No. (%)</td>
<td>139 (37)</td>
<td>109 (66)</td>
<td>24 (43)</td>
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<tr>
<td>Current cigarette use, No. (%)</td>
<td>91 (24)</td>
<td>34 (21)</td>
<td>20 (35)</td>
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<tr>
<td>Current alcohol use, No. (%)</td>
<td>49 (13)</td>
<td>25 (15)</td>
<td>13 (23)</td>
</tr>
<tr>
<td>Mean age at first intercourse, y (±SD)</td>
<td>17.5 ± 3.6</td>
<td>17.2 ± 3.3</td>
<td>16.4 ± 2.9</td>
</tr>
<tr>
<td>Commercial sex worker, No. (%)</td>
<td>151 (40)</td>
<td>62 (38)</td>
<td>30 (53)</td>
</tr>
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<td>Cervical HPV DNA detected, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No HPV</td>
<td>45 (12)</td>
<td>75 (45)</td>
<td>25 (45)</td>
</tr>
<tr>
<td>Untyped HPV or HPV 6/11</td>
<td>23 (6)</td>
<td>23 (14)</td>
<td>14 (25)</td>
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<tr>
<td>High-risk HPV</td>
<td>310 (81)</td>
<td>67 (41)</td>
<td>17 (30)</td>
</tr>
<tr>
<td>Mean CD4 count, cells per mL (±SD)</td>
<td>910 ± 346</td>
<td>402 ± 249</td>
<td>639 ± 331</td>
</tr>
<tr>
<td>CD4 count, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;500 cells per mL</td>
<td>321 (91)</td>
<td>53 (32)</td>
<td>37 (65)</td>
</tr>
<tr>
<td>200–500 cells per mL</td>
<td>29 (8)</td>
<td>72 (43)</td>
<td>15 (26)</td>
</tr>
<tr>
<td>&lt;200 cells per mL</td>
<td>4 (1)</td>
<td>42 (25)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Mean CD8* cell count, cells per mL (±SD)</td>
<td>488 ± 236</td>
<td>952 ± 501</td>
<td>694 ± 356</td>
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</tbody>
</table>

*STD = sexually transmitted disease; SD = standard deviation; HPV = human papillomavirus; ID = infectious disease.
†P value was from a chi-squared test for difference between HIV-negative women and HIV-positive women. All statistical tests were two-sided.
‡P value was from a chi-squared test for any difference among women infected with HIV-1, with HIV-2, or dually with HIV-1 and HIV-2.
Study participants had a mean number of 4.7 follow-up visits (maximum of 18 follow-up visits) over 2.2 years (maximum follow-up of 5.8 years), and 84% of them had at least two follow-up visits. Of 627 women included in the final analysis, 70 (11.3%) developed HSIL, as detected by cytology during follow-up, and one woman developed invasive cervical cancer (a woman infected with HIV-1 with four previous negative cytologic test results). For analysis purposes, this subject was considered to have developed HSIL.

Risk of Developing HSIL in Relation to HIV and Baseline HPV Status

During follow-up, 71 (11%) of the 627 women developed HSIL as detected by cytology. Kaplan–Meier survival curves were calculated for development of HSIL during follow-up for women without cytologic abnormalities detected at baseline. HIV-positive women coinfected with high-risk HPV types were at greatest risk for development of HSIL (Fig. 1; \( P < .001 \)). The 71 HIV-negative women without high-risk HPV infection did not develop HSIL within the first 2 years, but by 3 years, 6% of high-risk women who were HPV negative, regardless of HIV status, had developed HSIL. Among the 402 women who were positive for high-risk HPV DNA at study entry, 10.3% (95% confidence interval [CI] = 6% to 14%) of 310 HIV-negative women and 34.1% (95% CI = 20% to 48%) of 92 HIV-positive women developed HSIL by 3 years of follow-up.

Risk of incident HSIL among HIV-infected women varied by HIV type \( (P = .04) \), with women infected with only HIV-1 and women infected with both HIV-1 and HIV-2 more likely to develop HSIL during follow-up than women infected with HIV-2 alone (Fig. 2). Among HIV-infected individuals, increased levels of immunosuppression were strongly associated with increased development of HSIL (Fig. 3; \( P < .001 \)). By 3 years, only 5.7% (95% CI = 0% to 11%) of 102 women with CD4 counts of more than 500 cells per microliter developed HSIL during follow-up, compared with 19.7% (95% CI = 10% to 30%) of 95 women with CD4 counts between 200 and 500 cells per microliter and 38.9% (95% CI = 14% to 64%) of 48 women with CD4 counts of less than 200 cells per microliter.

Multivariable Cox regression analyses were performed to examine the risk of developing HSIL in relation to detection of specific types of HPV and HIV status at study entry after adjusting for age, employment as a commercial sex worker, parity, and birthplace in Senegal. Among women in whom high-risk types of HPV were not detected at study entry, HIV-positive women were not statistically significantly more likely to develop HSIL (hazard ratio [HR] = 3.0, 95% CI 0.7 to 13.3) than HIV-negative women (data not shown). However, among HIV-negative women, those with high-risk types of HPV detected at baseline were 4.3 times more likely to develop HSIL during follow up \( (HR = 4.3, 95\% CI = 2.7\) to 6.8\) compared with HIV-negative women with no high-risk HPV.
CI = 1.0 to 18.1) than those who were negative for high-risk HPV at study entry; HIV-positive women with high-risk HPV detected at baseline were more than 11 times more likely to develop HSIL during follow-up (HR = 11.5, 95% CI = 2.7 to 49) than HIV-negative women without high-risk HPV at baseline. Among women with high-risk HPV detected at baseline, those infected with HIV-1 were at elevated risk for developing HSIL (HR = 3.0, 95% CI = 1.6 to 5.6) than women without HIV infection, as were women infected with HIV-1 and HIV-2 (HR = 5.9, 95% CI = 1.7 to 20.8), whereas women infected with HIV-2 alone were not at increased risk (HR = 1.2, 95% CI = 0.3 to 3.9).

**Association of HIV infection, Transient or Persistent Detection of HPV DNA, and Development of HSIL**

Of the 402 women who were infected with high-risk HPV (types 16, 18, 31, 33, 35, 39, or 45) at baseline, 164 (41%) had persistent high-risk HPV, defined as detection of any high-risk type of HPV DNA in at least two consecutive follow-up visits. HIV-infected women were statistically significantly more likely than HIV-negative women to have persistent detection of high-risk types of HPV (61% versus 35%, respectively; P < .001). Among HIV-infected women with high-risk HPV at baseline, persistent detection of high-risk HPV did not vary substantially by HIV type, because women infected with HIV-1 and with high-risk HPV at baseline were somewhat more likely than women infected with HIV-2, but less likely than women infected with both HIV-1 and HIV-2, to have persistent detection of HPV during follow-up (63% versus 44% versus 88%, respectively; P = .10). Persistent detection of untyped or other HPV types was also more common in HIV-infected women than in HIV-negative women during follow-up (38% versus 23%, respectively; P = .004).

Multivariable Cox regression analyses with time-dependent covariates were performed to examine the risk of developing HSIL in relation to HPV type and persistence (both of which were allowed to vary at each time point during follow-up) and to HIV status. In all models, risk estimates were adjusted for baseline age, parity, birthplace in Senegal or elsewhere, and commercial sex work, as well as cytology reader and type of Pap smear (conventional versus Thin Prep), which were also allowed to vary over time. Compared with women without an HPV infection at a given time, women with a persistent infection with high-risk HPV types were at highest risk for developing HSIL (HR = 47.1, 95% CI = 16.3 to 136, adjusting for HPV type in addition to the above-mentioned variables; Table 2). Women with a transient infection with high-risk HPV types (HR = 14.0, 95% CI = 3.7 to 53.5) and women with a persistent (HR = 19.1, 95% CI = 5.9 to 61.7) or transient (HR = 18.7, 95% CI = 5.9 to 59.3) infection with untyped or other HPV types were also statistically significantly more likely to develop HSIL than women who were always HPV negative. Development of HSIL was not associated with the detection or persistence low-risk HPV types (i.e., HPV 6 and HPV 11). HSIL risks estimates for transient and persistent infections with high-risk and untyped/other HPV types were similar between HIV-negative women and HIV-positive women (P_interaction between incident high-risk, persistent high-risk, incident untyped/other, or persistent untyped/other HPV and HIV infection = .7, .7, 9, and .9, respectively). Among HIV-infected women, HIV type (HIV-1, HIV-2, or both HIV-1 and HIV-2) was not independently associated with risk of developing HSIL after adjusting for HPV type and persistence. With regard to HPV persistence, there were no statistically significant differences in parameter estimates for risk for HSIL associated with transient and persistent infections with the various HPV types when analyses were conducted by defining HPV persistence as three consecutive visits instead of two consecutive visits.

**Risk of Developing HSIL in HIV-Positive Women in Relation to HIV Type and to HPV Type, Persistence, Immunosuppression, and Viral Load**

To further explore the hypothesis that HIV-associated immune suppression and induction of persistent HPV infection increase the risk of progression to HSIL, we next evaluated the risk of developing HSIL among HIV-infected women in relation to persistent and transient HPV detection and to factors related to HIV-associated immunosuppression, including HIV type, CD4 count, and the level of plasma HIV RNA. Multivariable time-dependent Cox regression analyses were performed among HIV-infected women, after adjusting for cytology reader, type of Pap smear (conventional versus Thin Prep), age, employment as a commercial sex worker, parity, and birthplace in Senegal versus elsewhere (Table 3). In model 1, women infected with HIV-2 were less likely to develop HSIL (HR = 0.3, 95% CI = 0.1 to 0.8) than women infected with HIV-1, whereas women dually infected with HIV-1 and HIV-2 and women infected with HIV-2 only were at similar risk (HR = 1.4, 95% CI = 0.5 to 4.1). Risk of developing HSIL increased with decreasing CD4 counts as assessed during follow-up (model 2); women with CD4 counts between 200 and 500 cells per microliter were 2.2 times (HR = 2.2, 95% CI = 0.8 to 6.3) more likely and women with CD4 counts of less than 200 cells per microliter were 5.5 times (HR = 5.5, 95% CI = 2.0 to 15.2) more likely to develop HSIL than women with CD4 counts more than 500 cells per microliter. Among HIV-infected women, the level of HIV plasma RNA was associated with a statistically significantly increased risk of developing HSIL (HR for

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV status</td>
<td></td>
</tr>
<tr>
<td>HIV negative</td>
<td>1.0 (ref.)</td>
</tr>
<tr>
<td>HIV-1 only</td>
<td>1.0 (0.6 to 1.7)</td>
</tr>
<tr>
<td>HIV-2 only</td>
<td>0.5 (0.2 to 1.6)</td>
</tr>
<tr>
<td>Dual HIV-1 and HIV-2 infection</td>
<td>1.6 (0.5 to 4.7)</td>
</tr>
<tr>
<td>Detection of HPV DNA</td>
<td></td>
</tr>
<tr>
<td>No HPV DNA</td>
<td>1.0 (ref.)</td>
</tr>
<tr>
<td>Transient HPV 6 or HPV 11 DNA only</td>
<td>0.0 (N/A)</td>
</tr>
<tr>
<td>Persistent HPV 6 or HPV 11 DNA only</td>
<td>0.0 (N/A)</td>
</tr>
<tr>
<td>Transient untyped/other HPV DNA only</td>
<td>18.7 (5.9 to 59.3)</td>
</tr>
<tr>
<td>Persistent untyped/other HPV DNA only</td>
<td>19.1 (5.9 to 61.7)</td>
</tr>
<tr>
<td>Transient high-risk HPV DNA†</td>
<td>14.0 (3.7 to 53.5)</td>
</tr>
<tr>
<td>Persistent high-risk HPV DNA†</td>
<td>47.1 (16.3 to 135.9)</td>
</tr>
</tbody>
</table>

*Hazard ratios (HRs) are from multivariable Cox regression analysis, with simultaneous adjustment of all factors in the table as well as age, employment as a commercial sex worker, parity status, birth in Senegal, and cytology reader. ref. = referent; CI = confidence interval; N/A = not available.

†"Untyped/other" HPV refers to HPV type 26, 40, 42, 53, 54, 55, 56, 57, 58, 59, 66, 68, 73, 82, 83, or 84 or untyped HPV, without detection of high-risk HPV type 16, 18, 31, 33, 35, 39, or 45.

‡High-risk HPV refers to HPV type 16, 18, 31, 33, 35, 39, or 45.
Each order of magnitude increase in level of HIV plasma RNA = 1.4, 95% CI = 1.1 to 1.7; model 3). This increased risk for HSIL associated with increasing levels of HIV plasma RNA was similar among those infected with HIV-1 and those infected with HIV-2 (P = .5; data not shown).

Compared with an HPV-negative status or a transient infection with untyped or other HPV, persistence of high-risk HPV types (HR = 14.3, 95% CI = 5.6 to 36.8) or persistence of untyped or other HPV types (HR = 5.1, 95% CI = 1.6 to 16.0; model 4) was associated with an increased risk of developing HSIL. Transient infection with high-risk HPV was not statistically significantly associated with increased risk for development of HSIL (HR = 3.2, 95% CI = 0.6 to 16.7). In our final multivariable analyses that modeled the potential independent effects of HIV type, CD4 counts, HIV plasma viral load, and HPV types and persistence (model 5), only persistent infection with high-risk HPV types (HR = 10.5, 95% CI = 3.7 to 30.0) and persistent infection with untyped/other HPV types (HR = 4.5, 95% CI = 1.4 to 14.6) were statistically significantly associated with risk for development of HSIL. That is, HIV type, level of CD4 immune suppression, and plasma HIV RNA levels were not statistically significantly associated with independent increased risk for developing HSIL after adjustment for HPV persistence.

**DISCUSSION**

In this study, we found that HIV-infected women were at greatest risk for development of HSIL and that transient and especially persistent infections with oncogenic HPV types were strongly associated with HSIL risk. HIV-positive women infected with HIV-2 were less likely to develop HSIL than HIV-positive women infected with HIV-1. HIV-positive women with CD4+ cell counts of less than 200 cells per microliter were at greater risk of HSIL than HIV-positive women with CD4 counts of more than 500 cells per microliter. High plasma HIV RNA levels were also associated with increased HSIL risk. After adjustment for HPV types and persistence, however, HIV type, plasma HIV RNA levels, and CD4 counts were no longer statistically significantly associated with an increased risk of HSIL. Thus, risk of HSIL appeared to be primarily associated with increased HPV persistence, and this increased persistence probably results from immunosuppression related to HIV-1 and/or HIV-2 infection.

This study represented a unique opportunity to assess the relative importance of HIV-1 and HIV-2 infection, HIV-associated immunosuppression, and plasma load, as well as detection and persistence of various HPV types. This longitudinal study of HIV and cervical neoplasia was conducted among participants who had never received antiretroviral therapy and had not been previously screened for cervical abnormalities. Consequently, this study was not complicated by these factors. HIV-2 is rarely detected in the United States; however, both HIV-1 and HIV-2 are common in many West African countries, India, Brazil, and Europe. HIV-2 infection is associated with a longer period of subclinical infection, lower plasma RNA loads, and a slower decline in the number of CD4 cells than HIV-1 infection. Although previous studies have evaluated associations between SIL and infection with both HIV-1 and HIV-2 (5–7,11,26), none had a longitudinal study design.

In this study, we found that, among women who tested negative for high-risk HPV DNA at study entry, increased risk of development of HSIL was not statistically significantly associated.
with HIV infection. However, among women who tested positive for high-risk HPV DNA at study entry, infection with HIV-1 or with both HIV-1 and HIV-2, but not HIV-2 alone, was associated with an increased risk of incident HSIL compared with no HIV infection. After adjustment for the increased levels of immunosuppression, as measured by CD4 counts over time, and the increased HPV detection and persistence experienced by women infected with HIV-1 compared with women infected with HIV-2, this difference was somewhat attenuated and no longer statistically significant.

At first glance this finding seems to contradict results of our previous case–control studies in women that showed that HIV-2 infection is associated with an increased risk for invasive cervical cancer, compared with HIV-1 infection or with an HIV-negative status (7). Given the slow decline in CD4 count that is characteristic of HIV-2 infection, women infected with HIV-2 live statistically significantly longer than those with HIV-1, especially if they do not receive antiretroviral therapy. Development of cervical cancer is a relatively slow process because, in addition to requiring a persistent HPV infection, which is associated with mild immunosuppression (27), exposure to additional cocarcinogens and acquisition of many genetic and epigenetic changes are necessary for progression to malignancy. Furthermore, our studies (1,28) and those of others (29) among HIV-negative women have shown that development of HSIL is an early event. Thus, although the increased immune suppression associated with HIV-1 infection, compared with HIV-2 infection, is associated with increased risk for incident HSIL in the relatively short length of observation in this study (2–3 years), we speculate that the longer survival experienced by women infected with HIV-2 results in a greater overall lifetime risk for development of invasive cancer compared with that experienced by women infected with HIV-1. These findings could have implications for HIV–1–positive women who are receiving highly active antiretroviral therapy as well. Women receiving highly active antiretroviral therapy will likely experience increased years of mild immunosuppression with long-term alteration of HPV control, and such women may be at substantially increased risk for HPV-associated HSIL and development of invasive cervical cancer.

Previous studies (10,26,30–32) have also reported that HIV infection was associated with an increased risk of detection and persistence of HPV, and multiple types of HPV. Some (14,33) but not other (12,13) previous studies have reported that HIV infection was not associated with an increased risk for development of HSIL after adjusting for HPV, although none of these studies was able to assess these factors in relationship to HIV-2 infection alone or to dual infection with HIV-1 and HIV-2. However, several studies (12,33) did not characterize persistence of high-risk HPV detection, which is an important predictor of development of HSIL and cervical cancer (3,4,23,34–37).

Few studies have been able to evaluate risk of HSIL in relation to HPV and HIV infection among women who were not receiving antiretroviral therapy. Previous studies of untreated women include that of Six et al. (12) and a multicenter study (13) in Europe that examined 229 HIV-infected women who were monitored for a median of 2 years. However, these studies contained few HSIL lesions (n = 6 in each study). In the large study by Delmas et al. (15), HPV and HIV-associated immunosuppression was assessed at only one visit. Likewise in the ALIVE cohort (14), HPV persistence was strongly associated with detection of SIL; in stratified analyses, repeated HPV-positive tests explained the observed association between HIV infection and SIL. However, only 11 HSILs were observed in this cohort, providing only limited power to evaluate specific risk factors for HSIL. Ellerbrock et al. (13) reported on 10 patients with incident HSIL among 328 HIV-infected and 325 HIV-uninfected women, but approximately half of the HIV-positive participants were on antiretroviral therapy. Persistent HPV infection was strongly associated with HIV infection and with development of any SIL, although surprisingly, CD4 count was not statistically significantly associated with incident SIL.

There are several possible limitations to our study. First, the study endpoint HSIL was based on cytology rather than on histology, which is generally considered the “gold standard” for classification of cervical lesions. However, cytologic endpoints have been used in many other large cohort studies of HPV and cervical disease, and recent evidence suggests that that histopathology of cervical biopsies is not more reproducible than monolayer cytology (38). Further, although the use of cytologic rather than histologic endpoints may have led to misclassification of our outcome of interest in some cases, this potential misclassification is unlikely to have differed by HIV status (39).

Another possible limitation is related to the definition of persistence. Studies that have examined the association between HPV persistence and the risk for cervical neoplasia have defined persistence in many ways (40). Prevalently detected HPV infections last an average of 7–9 months (23,24) and incident infections, at least in young women, remain HPV positive for 1–2 years (25,41). From a practical standpoint, persistence of an HPV infection can be defined as its detection two or more times over a certain period (42). However, there is no consensus as to the length of time that defines a persistent infection. In statistical analyses, HPV persistence has been defined in a variety of ways in prospective studies of the risk of cervical neoplasia. HPV persistence has often been defined as a HPV-positive status in consecutive visits (22,42), although few longitudinal studies have used follow-up visits with only 4 months in between visits as in our study. In other studies (1,13,44), HPV detection in multiple or most HPV-positive visits over a given period has been used to define persistence. Still others (45,46) have defined persistence as time from a prevalent HPV-positive status until clearing of the HPV infection (not detecting HPV at one or more visits). For ease of presentation, study subjects have usually been classified as having had persistent infection (or not) on the basis of one of the criteria above, and this classification has not been allowed to vary over time in the statistical analyses. An advantage of this study is that HPV status and persistence were evaluated at every visit, allowing a given woman to have periods of nondetection, transient HPV infection, and HPV persistence during follow-up, and the Cox regression analysis with time-dependent covariates allowed for and evaluated this variability in HPV status over time in a single woman. We defined persistence as the presence of an HPV type at two consecutive visits. However, we found no statistically significant differences in parameter estimates for risk for HSIL associated with the various transient and persistent HPV types when analyses were conducted by defining HPV persistence as three consecutive visits instead of two consecutive visits.

Another possible limitation is selection bias. As with all prospective studies, bias may have arisen because of selection of study participants and loss to follow-up, although our high participation rate and retention was high (>70%) of women approached agreed to enroll and 79% of enrolled women were monitored.
for more than 1 year) indicates that this bias is unlikely in our study. Further, the length of follow-up was similar between women with and without HIV infection, and analyses were adjusted for the number of follow-up visits in an attempt to adjust for opportunity to assess relevant exposures and HSIL outcome.

Finally, our evaluation of development of HSIL in women infected with HIV-2 and women coinfected with HIV-1 and HIV-2, compared with women infected with HIV-1, was limited by the few women with these infections enrolled and monitored for development of incident HSIL. Improved screening methodologies and increased follow-up of HPV infection in those infected with HIV-1 or HIV-2 is needed, especially in view of the increasing availability of antiretroviral treatments.

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NOTES

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