

Increased Risk of High-Grade Cervical Squamous Intraepithelial Lesions and Invasive Cervical Cancer among African Women with Human Immunodeficiency Virus Type 1 and 2 Infections

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To assess the risk of prevalent high-grade cervical squamous intraepithelial lesions (HSILs) or invasive cervical cancer (ICC) associated with human immunodeficiency virus (HIV) type 1, HIV-2, and human papillomavirus (HPV) infections, HIV load, and CD4 cell count, we studied 4119 women attending an outpatient clinic in Senegal. HIV infection was associated with increased rates of cervical infection with high-risk HPVs. Among women infected with high-risk HPVs, those with HIV-1 (odds ratio [OR], 2.2; 95% confidence interval [CI], 1.0–4.8), HIV-2 (OR, 6.0; 95% CI, 2.1–17.1), or dual HIV infection (OR, 8.0; 95% CI, 2.0–31.5) were more likely to have HSILs or ICC diagnosed than were HIV-negative women; this association was not observed among women not infected with high-risk HPVs. Among women with HIV, higher HIV plasma RNA loads and lower CD4 cell counts were associated with high-risk HPV infection and degree of cervical abnormality. Furthermore, HIV-2–positive women were more likely to have HSILs (OR, 3.3; 95% CI, 0.9–12.4) or ICC (OR, 7.9; 95% CI, 1.1–57) than were HIV-1–positive women.

Cancer of the uterine cervix remains a major cause of morbidity and mortality among women, especially in the developing world, where routine cytologic screening is generally unavailable [1, 2]. It is well established that specific “high-risk” types of sexually transmitted human papillomaviruses (HPVs) play a central role in the development of preinvasive cervical lesions and invasive cervical cancer (ICC) [3–7]. Previous studies have shown that human immunodeficiency virus (HIV) type 1–positive women, especially those with marked HIV immunosuppression, are at increased risk for the de-

velopment of both cervical HPV and cervical intraepithelial neoplasia (CIN) [8–20]. In 1993, ICC was named an AIDS-defining disease [21]. Recent studies that linked HIV/AIDS and cancer registries in the United States and Europe showed that there is an increased risk of developing cervical cancer among HIV-1–positive women [22–26]. However, results from studies evaluating this association among African women are inconsistent [16, 27–31], with a positive association demonstrated in only one investigation [27]. Furthermore, few data are available concerning the risk of ICC associated with HIV-2 infection, which is characterized by a reduced rate of CD4 cell count decline and slower progression to AIDS and death than is HIV-1 infection [32]. One study from the Ivory Coast [16] found HIV-2 to be associated with ICC; however, this association was not significant after adjusting for age. Finally, although little is known about the relationships among HIV load, immunosuppression (i.e., decreased CD4 cell count), detection of high-risk HPV types, and ICC or its immediate precursor (high-grade cervical squamous

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intraepithelial lesions [HSILs]), elucidation of these interrelationships is important for the development of effective cervical cancer-prevention strategies among HIV-positive women.

It is thought that HIV infection increases the risk of developing CIN, as a result of HIV-induced immunosuppression and the subsequent inability to control expression of oncogenic types of HPV [12, 15, 33]. This phenomenon permits the continued generation of HPV oncoproteins E6 and E7, which are central to the pathogenesis of cervical cancer. We hypothesized that, among HIV-1-positive women, those with severe immunosuppression would be at greatest risk of prevalent HSILs or ICC. Given the slower rate of CD4 cell decline characteristic of HIV-2 infection, for a given CD4 cell count, an HIV-2-positive woman is likely to have been HIV-positive for a longer period of time than an HIV-1-positive woman. Therefore, we further hypothesized that the longer periods of immunosuppression characteristic of HIV-2 infection will result, after adjusting for CD4 cell count, in an increased risk for diagnosis of HSILs or ICC among HIV-2-positive women, compared with HIV-1-positive women. To assess the risks of detecting high-risk types of HPV and of diagnosing HSILs or ICC associated with HIV-1 infection, HIV-2 infection, HIV load, and HIV-induced immunosuppression, we conducted the present study among women presenting to an outpatient infectious-disease clinic in Dakar, Senegal (West Africa).

SUBJECTS, MATERIALS, AND METHODS

Study population. Between October 1994 and January 1998, female subjects >15 years old who presented to the University of Dakar outpatient infectious-disease clinic were offered HIV-1 and HIV-2 serologic testing and cervical HPV and Pap smear screening. Women present to this clinic for various reasons, including family planning (24%), gynecologic problem or examination (35%), respiratory symptoms (4%), HIV infection in the patient (5%) or spouse (2%), and other specified (21%) or unspecified (9%) reasons. The study was conducted according to procedures approved by the Institutional Review Boards of both the University of Washington and the University of Dakar, and women found to be HIV seropositive were counseled according to the guidelines established by the Senegalese AIDS national committee. Informed consent was obtained according to procedures approved by the Human Subjects Committee of the University of Washington and the Senegalese National AIDS Committee. Women with HIV and/or HPV infection were invited to participate in a longitudinal study assessing the risk of developing HSILs, the results of which will be presented elsewhere.

Collection of specimens and study procedures. Blood was collected for HIV-1 and HIV-2 serologic testing. Cervical cellular samples were obtained for cytologic screening and HPV

detection. Subjects underwent a physical examination and completed a standardized interview. Additional blood samples were obtained from HIV-positive subjects who were enrolled in our longitudinal study, for lymphocyte subset analysis and for HIV-1 and HIV-2 qualitative and quantitative RNA assays.

Cytologic screening. Pap smears were classified according to the Bethesda System [34] as negative, atypical cells of uncertain significance (ASCUS), low-grade squamous intraepithelial lesions (LSILs), HSILs, or ICC. Pap smears were initially read by a Senegalese pathologist and later were sent to Seattle for a second reading. All slides classified by either pathologist as "LSILs or worse" and a random subset of cytologically negative slides were subsequently restained, recovered, and reread. For data analysis, we used the cytologic diagnosis made by the Seattle pathologist. If the slide was not available for shipment to Seattle, the original Senegal diagnosis was used. Overall, 3236 (79%) of 4119 Pap smears were reread in Seattle.

Histologic methods. According to the study design, colposcopically directed cervical biopsy specimens were supposed to be obtained from women with cytologic evidence of HSILs or ICC. Representative hematoxylin-eosin-stained slides were prepared from paraffin-embedded biopsy specimens and were reviewed by the pathologist. The pathologist had no knowledge of other clinical or laboratory data. Standard gynecologic pathology criteria and terminology were used to classify all intraepithelial lesions and ICCs.

HPV DNA detection and typing. Polymerase chain reactions (PCRs) for the detection of HPV DNA were performed by use of HPV L1 consensus primers, HPV type-specific oligonucleotide probes, and a generic probe, as described elsewhere [35]. Samples were screened first for the presence of high-risk HPV types by use of a 10-probe mix, which tests for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, or 56, by use of consensus primers MY09 and MY11 to a highly conserved region in the L1 open-reading frame, to identify women eligible for enrollment in a longitudinal study assessing HSIL risk factors. Samples were tested subsequently with a generic probe, to detect the presence of any HPV DNA. Positive samples then were redotted for 12 HPV types in primer groups for low-risk (6/11) and high-risk (16, 18, 31/33/35/39, 45/56, and 51/52) HPV types. Samples that tested positive by the generic probe but negative by the type-specific probes were called "positive, untyped." Samples that tested positive by the initial 10-probe screening mix but not positive by the latter type-specific probes were classified as "unspecified high-risk" HPV types.

HIV serologic and lymphocyte subset analyses. Initial testing for HIV-1 or HIV-2 antibodies was performed using a microwell plate EIA that detects antibodies to both HIV types in 1 well (HIV 1/2 EIA; Sanofi Diagnostics Pasteur). Positive samples were confirmed using a rapid, HIV peptide-based membrane immunoassay that distinguishes between antibodies

to HIV-1 and HIV-2 (Multispot; Genetic Systems). For women who consented to enrollment in the longitudinal study, whole blood collected in EDTA was analyzed using the FACSCount analyzer (Becton Dickinson Biosciences), to determine CD4, CD8, and CD3 cell counts in blood.

Quantitation of HIV-1 and HIV-2 plasma RNA. Quantitative and qualitative assays for HIV-1 and HIV-2 RNA were performed for women also enrolled in our longitudinal study, as described elsewhere [36]. The qualitative assay was performed on all available samples that tested negative by the quantitative assay. The quantitative and qualitative HIV-1 RNA assays detect as few as 80 and 40 HIV-1 RNA copies/mL, respectively, with reproducible sensitivities of 400 and 200 HIV-1 RNA copies/mL, respectively. The HIV-2 quantitative and qualitative assays detect as few as 40 and 20 of HIV-2 RNA copies/mL, respectively, with reproducible sensitivities of 200 and 100 HIV-2 RNA copies/mL, respectively.

Statistical methods. For most analyses, HIV-1 and HIV-2 were included as 2 dichotomous variables. In other analyses, HIV antibody status was considered to be either a 4-level categorical variable (HIV negative, HIV-1 only, HIV-2 only, or dually infected) or a dichotomous variable (HIV positive versus HIV negative). The presence of cervical HPV DNA was coded as a 4-level hierarchical variable: high-risk HPV type (with or without other types), low-risk HPV type, untyped HPV, and HPV not detected. Pap smear results were classified as negative, ASCUS, LSILs, HSILs, or ICC. Subjects with unsatisfactory Pap smears were excluded from analyses on the basis of cervical cytologic test results. By study design, women with cytologic evidence of HSILs or ICC were to undergo biopsy to provide histologic confirmation of the cytologic diagnosis. Unfortunately, because of unavoidable logistical difficulties, many women never underwent biopsy, and, for some women who did undergo biopsy, the procedure was done several months after the original cytologic diagnosis. Ultimately, 27 (36%) of 75 women with a cytologic diagnosis of HSIL/CIS and 1 (8%) of 12 women with cytologic evidence of ICC subsequently underwent biopsy. Hence, because of the large quantity of missing histologic data and the length of time between cytologic and histologic diagnoses, histologic findings from biopsy specimens were not used in our analyses, except when ICC was detected. Demographic and behavioral variables were considered to be either risk factors for our outcomes of interest (detection of HPV DNA or CIN) or potential confounding variables for the association between HIV status and these outcomes.

Two-sided Mantel-Haenszel χ^2 or Fisher's exact test was used to assess univariate associations of HIV-1, HIV-2, and HIV dual infection with detection of high-risk HPV types or cytologic diagnosis. The Mantel-Haenszel test for trend was used to test associations with ordered categorical factors, and Student's *t* test or analysis of variance was used to compare groups with

respect to continuous variables. Multivariable logistic regression analyses were performed to evaluate the independent effects of HIV status and HPV detection on the risk of prevalent HSILs or ICC, after adjusting for possible confounding factors. In these analyses, women with negative cytologic test results were used as the reference group. Variables were considered to be confounders if differences between adjusted and unadjusted coefficients were >10%. The cytologic test reader (Senegal vs. Seattle) and subject age were included as covariates in all multivariable analyses. Data analyses were conducted using SAS 8.2 for Windows (SAS Institute).

RESULTS

Characteristics of the study population, by HIV serologic status.

Overall, 433 (10.5%) of the 4119 women screened were found to be HIV positive, including 335 (8.1%) infected with HIV-1 only, 69 (1.7%) infected with HIV-2 only, and 29 (0.7%) with dual HIV-1 and HIV-2 infection. HIV-positive women were older, more likely to work as a commercial sex worker (CSW), and less likely to have received any formal education or use contraception than were HIV-negative women (table 1). HIV-2-positive women were somewhat older than HIV-1-positive women. Women infected with HIV-2 only had higher CD4 cell counts than did women infected with HIV-1 only, whereas women with dual infection had levels intermediate to those of women with single HIV infection. Cervical HPV DNA was detected in 228 (69.1%) of 330 women with HIV-1 only, 42 (61.8%) of 68 women with HIV-2 only, 19 (67.8%) of 28 women with HIV dual infection, and 918 (25.3%) of 3633 HIV-negative women ($P < .001$). High-risk HPV was more often present in HIV-positive women (52.1%) than in HIV-negative women (15.0%; $P < .001$). Among HIV-positive women, high-risk HPV types were detected in 29%, 49%, and 56% of women with CD4 cell counts >500, 200–500, and <200 cells/ μ L, respectively ($P = .002$, test for trend; data not shown). In addition, high-risk HPV types were detected more frequently among women with higher plasma HIV RNA levels. Among HIV-1-positive women, high-risk HPV types were found in 55.8% of those with plasma loads $\geq 10,000$ HIV RNA copies/mL and in 28.6% of those with virus loads <10,000 HIV RNA copies/mL ($P = .002$). Similarly, among HIV-2-positive women, high-risk HPV types were found in 60.0% of those with plasma loads $\geq 10,000$ HIV RNA copies/mL and in 29.6% of those with plasma loads <1000 HIV RNA copies/mL ($P = .04$).

Association of CIN with HIV and HPV infection. Overall, 162 (3.9%) of 4119 cervical Pap smears were unsatisfactory for diagnosis. Of the remaining 3957 smears, 3241 (81.9%) were classified as negative, 498 (12.6%) were classified as ASCUS, 131 (3.3%) were classified as LSIL, 75 (1.9%) were classified as HSIL, and 12 (0.3%) were classified as ICC. Detection of

Table 1. Clinical and demographic characteristics of women presenting to an outpatient clinic in Dakar, Senegal, by human immunodeficiency virus (HIV) serologic status.

Characteristic	HIV negative (n = 3686)	HIV positive			P ^a
		HIV-1 only (n = 335)	HIV-2 only (n = 69)	Dual infection (n = 29)	
Age, mean years ± SD	31.9 ± 7.9	31.0 ± 8.1	36.1 ± 8.0	33.8 ± 7.4	<.001
Born in Senegal	3442/3654 (94)	302/333 (91)	59/69 (86)	25/27 (93)	.002
Any formal education	1473/2313 (64)	101/233 (43)	19/45 (42)	4/16 (25)	<.001
CSW	6/3686 (0.2)	21/335 (6.3)	5/69 (7.2)	2/29 (6.9)	<.001
Use of contraception	1606/3661 (44)	41/331 (12)	15/68 (22)	6/28 (21)	<.001
Parity, mean ± SD	4.0 ± 3.1	3.4 ± 2.7	5.4 ± 3.3	3.8 ± 2.4	<.001
CD4 cell count, cells/μL					<.001
>500	316/352 (90)	34/216 (16)	16/52 (31)	2/17 (12)	
200–500	32/352 (9)	78/216 (36)	17/52 (33)	8/17 (47)	
<200	4/352 (1)	104/216 (48)	19/52 (37)	7/17 (41)	
CD8 cell count, mean cells/μL ± SD	493 ± 259	957 ± 568	666 ± 588	843 ± 483	<.001
Cervical HPV detected					<.001
High-risk HPV types	545/3633 (15.0)	177/330 (53.6)	28/68 (41.2)	17/28 (60.7)	
HPV 6/11 only	13/3633 (0.4)	3/330 (0.9)	1/68 (1.5)	0/28 (0.0)	
Untyped HPV only	360/3633 (9.9)	48/330 (14.6)	13/68 (19.1)	2/28 (7.1)	
HPV not detected	2715/3633 (74.7)	102/330 (30.9)	26/68 (38.2)	9/28 (32.1)	

NOTE. Data are no. of women/total no. tested (%), except where noted. CSW, commercial sex worker; HPV, human papillomavirus.

^a HIV-positive vs. HIV-negative women.

high-risk HPV types was highly associated with HSILs or ICC, both among HIV-negative women (odds ratio [OR], 7.0; 95% confidence interval [CI], 4.0–12.2) and HIV-positive women (OR, 12.7; 95% CI, 3.6–44.5; estimates adjusted for age, marital status, employment as a CSW, and cytologic test reader). HIV was strongly associated with the presence of cervical lesions, with LSILs or worse found in 4.0% of HIV-negative women, in 17.2% of women infected with HIV-1 only, in 19.5% of women infected with HIV-2 only, and in 34% of women with dual HIV infection ($P < .001$; table 2). HSIL was detected in 1.4% of HIV-negative women, compared with 4.5% of women with HIV-1 only (OR, 3.7; 95% CI, 1.9–7.4), 10.5% of women with HIV-2 only (OR, 7.1; 95% CI, 2.9–17.4), and 13.8% of women with dual infection (OR, 14.4; 95% CI, 4.2–49.6; estimates adjusted as described above).

ICC was diagnosed by cytologic testing for 12 women and by histologic evaluation of biopsy tissue from an additional 9 women with cytologic diagnoses of either LSIL or HSIL. Overall, ICC was found in 0.3% of HIV-negative women, compared with 1.9% of HIV-1-positive women (OR, 6.7; 95% CI, 2.1–21.7), 4.5% of HIV-2-positive women (OR, 16.0; 95% CI, 3.8–67.7), and 6.9% of dually infected women (OR, 37.2; 95% CI, 6.6–210) (table 2). Similar results were seen when the analysis was limited to those with histologically confirmed ICC.

Of interest, the association between HIV infection and diagnosis of HSILs or ICC was confined to women in whom high-risk HPV types were detected. Among women with high-risk HPV, women with HIV-1 (OR, 2.2; 95% CI, 1.0–4.8), HIV-2 (OR, 6.0; 95% CI, 2.1–17.1), or HIV dual infection (OR, 8.0; 95% CI, 2.0–31.5) were at greater risk of having HSILs or ICC than were HIV-negative women (table 3); this association was not observed among women not infected with high-risk HPVs (OR, 1.4; 95% CI, 0.4–5.1).

Risk of CIN or ICC associated with CD4 cell count and HIV plasma RNA load among HIV-positive women.

Given the observed associations between CIN and HIV infection, particularly in the presence of high-risk HPV types, we next examined associations between cytologic diagnosis, CD4 cell count, and HIV load. Overall, 65% of HIV-positive women had lymphocyte subset analyses and HIV-1 and/or HIV-2 plasma RNA levels assessed as a result of their enrollment in a longitudinal study assessing the risk of developing HSILs.

The presence of LSILs or worse was strongly correlated with decreased CD4 cell counts among women with HIV-1 infection only ($P < .001$), HIV-2 infection only ($P < .001$), or dual infection ($P = .02$; table 4). Overall, HSILs were detected in 5.7% of women with CD4 cell counts <200 cells/μL and in 3.0% of women with CD4 cell counts 200–500 cells/μL, but were not

Table 2. Presence of cervical intraepithelial neoplasia, by human immunodeficiency virus (HIV) serologic status.

Screening cytologic diagnosis	HIV negative (n = 3552)	HIV positive		
		HIV-1 only (n = 309)	HIV-2 only (n = 67)	Dual infection (n = 29)
Negative	2991 (84.2)	195 (63.1)	41 (61.2)	14 (48.3)
ASCUS	419 (11.8)	61 (19.7)	13 (19.4)	5 (17.2)
LSILs	85 (2.4)	37 (12.0)	4 (6.0)	5 (17.2)
HSILs	50 (1.4)	14 (4.5)	7 (10.5)	4 (13.8)
ICC	7 (0.2)	2 (0.7)	2 (3.0)	1 (3.4)
Diagnosed by histologic testing only	3 (0.1)	4 (1.3)	1 (1.5)	1 (3.4)
Diagnosed by cytologic and/or histologic testing	10 (0.3)	6 (1.9)	3 (4.5)	2 (6.9)

NOTE. Data are no. (%) of subjects. ASCUS, atypical cells of uncertain significance; HSILs, high-grade squamous intraepithelial lesions; ICC, invasive cervical cancer; LSILs, low-grade squamous intraepithelial lesions.

^a Diagnoses were classified as described elsewhere [34].

detected in the 47 women with CD4 cell counts >500 cells/ μ L. ICC, diagnosed by cytologic or histologic testing, was found in 3.3% of 123 women with CD4 cell counts <200 cells/ μ L and in none of the 148 women with CD4 cell counts above that level. In a multivariable logistic regression analysis quantifying the risks of diagnosis with HSILs or ICC associated with CD4 cell count, we found that each 100-cell/ μ L decrease in CD4 level <500 cells/ μ L was associated with a 1.7-fold-increased risk of diagnosis with HSILs (95% CI, 1.1–2.4) and a 2.6-fold-increased risk of diagnosis with ICC (95% CI, 1.2–5.4; each estimate adjusted for age, HIV type, and cytologic test reader). Furthermore, HIV-2 infection, unlike HIV-1 infection, was independently associated with increased detection of both HSILs (OR, 3.3; 95% CI, 0.9–12.4) and ICC (OR, 7.9; 95% CI, 1.1–57; adjusted for CD4 cell count, age, and cytologic test reader).

The presence of cervical Pap smear abnormalities was also associated with higher plasma HIV RNA levels among HIV-1-positive ($P < .001$) and HIV-2-positive ($P < .001$) women (table 4). Among HIV-1-positive women, LSILs or worse was diagnosed in 21.1% of women with plasma virus levels $\geq 10,000$ copies/mL and in none of those with plasma virus levels $<10,000$ copies/mL ($P < .001$); among HIV-2-positive women, LSILs or worse was diagnosed in 42.1% of women with plasma virus levels ≥ 1000 copies/mL and in 3.7% of women with plasma virus levels <1000 copies/mL ($P = .004$) (data not shown). HSILs were detected in 3.7% of women with plasma HIV-1 RNA levels $\geq 10,000$ copies/mL and in none of the women with plasma virus levels $<10,000$ copies/mL. All 4 HIV-1-positive women with ICC diagnosed by cytologic or histologic testing had plasma virus levels $\geq 10,000$ HIV-1 RNA copies/mL. Similarly, HSILs were detected in 15.8% of women with plasma virus levels ≥ 1000 HIV-2 RNA copies/mL and in 3.7%

of women with plasma virus levels <1000 copies/mL. As with HIV-1, the only HIV-2-positive woman with ICC (with an HIV plasma load result available) had a very high HIV-2 plasma virus load (4.71 logs HIV-2 RNA copies/mL).

To test the independence of associations among HSILs or ICC, HIV type, level of immunosuppression, and HIV load, we performed multivariate logistic regression analyses for women with HIV-1 and/or HIV-2 infection. In analyses adjusted for age and cytologic test reader, HIV-2 infection (OR, 4.1; 95% CI, 1.0–16.7) and low CD4 cell count (OR, 0.66; 95% CI, 0.44–0.98 [per 100 cell/ μ L increase]), but not HIV plasma RNA level (OR, 1.2; 95% CI, 0.8–1.7 [per log₁₀ increase]), were independently associated with an increased risk of prevalent HSILs or ICC.

Table 3. Association of human immunodeficiency virus (HIV) infection with high-grade squamous intraepithelial lesions (HSILs) or invasive cervical cancer (ICC) among women with and without high-risk human papillomavirus (HPV) types detected.

HPV detected, HIV status	Women with HSILs or ICC/no. tested (%)	OR (95% CI)
No high-risk HPV		
HIV negative	31/2976 (1.0)	1.0 (reference)
HIV positive	3/192 (1.6)	1.4 (0.4–5.1)
High-risk HPV		
HIV negative	26/527 (4.9)	1.0 (reference)
HIV-1 only	14/164 (8.5)	2.2 (1.0–4.8)
HIV-2 only	8/27 (29.6)	6.0 (2.1–17.1)
HIV-1 and HIV-2 coinfection	5/17 (29.4)	8.0 (2.0–31.5)

NOTE. CI, confidence interval; OR, odds ratio.

Table 4. Association of cervical intraepithelial neoplasia with CD4 cell count and human immunodeficiency virus (HIV) plasma RNA load among HIV-infected women.

Infection status, variable	Cytologic test result ^a					ICC ^b	P ^c
	Negative	ASCUS	LSILs	HSILs	ICC		
Infection with HIV-1 only							
No. of subjects	135	46	28	7	1	4	
CD4 cell count, cells/ μ L	313 \pm 237	228 \pm 191	151 \pm 133	142 \pm 102	164 ^d	117 \pm 79	<.001
Log ₁₀ plasma HIV-1 RNA load	4.57 \pm 1.4	5.04 \pm 1.4	5.53 \pm 0.5	5.26 \pm 0.7	6.09 ^d	5.24 \pm 0.9	<.001
Infection with HIV-2 only							
No. of subjects	33	10	4	4	2	3	
CD4 cell count, cells/ μ L	400 \pm 239	436 \pm 317	191 \pm 178	207 \pm 30	37 \pm 6	37 \pm 6	<.001
Log ₁₀ HIV-2 plasma RNA load	2.05 \pm 1.9	2.10 \pm 1.7	3.87 \pm 0.4	3.71 \pm 0.8	4.71 ^d	4.71 ^d	<.001
HIV-1 and HIV-2 dual infection							
No. of subjects	11	2	4	2	1	2	
CD4 cell count, cells/ μ L	353 \pm 220	368 \pm 105	115 \pm 155	56	145 ^d	219 \pm 105	.02
Log ₁₀ plasma HIV-1 RNA	4.09 \pm 2.1	3.41 \pm 1.2	4.11 \pm 1.2	0.30	4.38 ^d	4.33 \pm 0.1	.7
Log ₁₀ plasma HIV-2 RNA load	2.22 \pm 1.6	1.36 \pm 1.5	0.30	2.67 \pm 0.4	NA	2.42 ^d	.5

NOTE. Data are mean \pm SD, except where noted. ASCUS, atypical cells of uncertain significance; HSILs, high-grade squamous intraepithelial lesions; ICC, invasive cervical cancer; LSILs, low-grade squamous intraepithelial lesions.

^a Results were classified as described elsewhere [34].

^b ICC was diagnosed by either histologic or cytologic testing.

^c Women with dysplasia (cytologic diagnosis of LSILs, HSILs, or ICC) vs. women without dysplasia (cytologic diagnosis of negative or ASCUS) (*t* test for continuous variables or the Mantel-Haenszel χ^2 test for trend for categorical variables).

^d Where no SD is given, data are mean only, because they are based on a single observation. Missing data are as follows: CD4 cell count, 26; plasma HIV-1 RNA load, 18; and plasma HIV-2 RNA load, 12.

DISCUSSION

To our knowledge, this is the first study of African women to examine associations among the presence of HSILs or ICC and HIV-1 or HIV-2 infection, plasma HIV RNA levels, and HIV-induced immunosuppression (CD4 cell count). Among women who had not been screened previously by cytologic testing and who had never received antiretroviral therapy, we found that those with HIV-1, HIV-2, or HIV dual infection all were at increased risk for prevalent HSILs or ICC, compared with HIV-negative women. This increased risk was confined to those in whom high-risk HPV types were detected. In addition, we found that, for a given CD4 cell count, HIV-2-positive women were at significantly higher risk for prevalent HSILs or ICC than were HIV-1-positive women.

Although several previous studies have investigated the relationship between CIN and HIV infection [8–20, 37], most have included limited numbers of women with severe cervical disease, making it difficult to separately examine risk of HSILs and LSILs, the latter, in most women, being transient with little risk of progression [38, 39]. Studies that have examined associations between HIV and HSILs or ICC in the developing world include that of Temmerman et al. [40], who reported a ~5-fold-increased risk of prevalent HSILs associated with HIV-1 among women attending a family planning clinic in Kenya, and that of Womack et al. [41], who reported a 3-fold-increased

risk of HSIL among HIV-1-positive women in Zimbabwe. In addition, Sitas et al. [27] recently reported that HIV-1 infection was associated with a 1.6-fold-increased risk of prevalent ICC among Ugandan women. In contrast, in a case-control study from Mexico, no association was observed between HIV infection and risk of prevalent HSILs [42]. La Ruche et al. [16] reported an increased risk of prevalent HSILs associated with HIV-1 but not HIV-2 infection, after adjustment for age. In the present study, HIV-2-positive women were at greater risk of prevalent HSILs or ICC than were HIV-1-positive women, which possibly reflects the fact that the HIV-positive women studied by La Ruche were less severely immunosuppressed than were those in the present study.

In the present study, among women with HIV-1 and (in a separate analysis) HIV-2 infection, increasing degree of cervical abnormality was associated with low CD4 cell count and high plasma HIV load. These factors have been shown to be associated with cervical abnormality in previous studies in the developed world [17, 19, 37, 43–47] but only sporadically in African populations [16, 18, 48]. Of importance, among HIV-positive women, we found that HSILs were detected only in women with CD4 cell counts <500 cells/ μ L and that ICC was detected only in women with CD4 cell counts <300 cells/ μ L. In multivariable analyses, low CD4 cell count was a better predictor of prevalent HSILs than was high plasma HIV RNA level,

as has been reported elsewhere [47]. These findings have important implications for developing screening strategies for identifying those women at highest risk of developing ICC.

Although some researchers have suggested that HIV increases the risk of developing CIN, independent of HPV infection [18, 37, 49], most available data suggest that the molecular pathogenesis of ICC [50] and the role of oncogenic HPV types in the pathogenesis of disease [33] are similar among HIV-positive and HIV-negative women. We and other researchers hypothesize that the increased risk of HPV-related neoplasia due to HIV infection results from HIV-induced immunosuppression and the loss of the ability to control HPV expression [12, 51–54]. In most women, HPV infections, even with high-risk types of HPV, are self-limited, with few long-term consequences. However, in individuals unable to control HPV infection (i.e., women with HIV-induced immunosuppression), persistent HPV expression allows prolonged exposure to HPV oncoproteins E6 and E7, which interfere with the host's normal cell-cycle controls and lead to the accumulation of populations of genetically abnormal cells [55–58]. In the present study, we found cervical infection with high-risk (“oncogenic”) HPV types to be common in African women, with HPV detected in 52% and 15% of women with and without HIV, respectively. Detection of high-risk HPV types was highly associated with the presence of HSILs or ICC, both among HIV-negative (OR, 5.0) and HIV-positive (OR, 10.1) women. However, the association between HIV and HSILs was confined to women with high-risk HPV types detected. Infection with high-risk HPV was strongly associated with both HIV-1 and HIV-2 infection, and, among women with HIV, the presence of high-risk HPV types was associated with low CD4 cell count and high plasma HIV load, which strongly suggests that the effect of HIV on ICC risk is related to control of HPV.

Of interest, we found that HIV-2–positive women were at greater risk of prevalent HSILs or ICC than were HIV-1–positive women, which may be due, in part, to the fact that HIV-2 infection is associated with slower CD4 cell count decline [32]. For a given CD4 cell count, an HIV-2–positive woman is likely to have been infected, and thus immunosuppressed (albeit, mildly) for a much longer period of time than an HIV-1–positive woman. Because even mild immunosuppression is associated with a decreased ability to control HPV [53], among women with similar CD4 cell counts, it is likely that a woman with HIV-2 would have experienced longer periods of exposure to HPV oncoproteins than would have women with HIV-1 infection, and thus, would be at higher risk of developing HSILs or ICC. These findings could have implications for HIV-1–positive women receiving highly active antiretroviral therapy (HAART). In other words, similar to women infected with HIV-2, HIV-1–positive women receiving HAART will likely expe-

rience many years of mild immunosuppression with long-term alteration of HPV control. Such women may thus be at substantially increased risk of HPV-associated neoplasia and ICC, compared with HIV-negative women.

A potential limitation of our study is the small number of women who underwent biopsy; thus, we were unable to use histologically confirmed HSILs as our outcome of interest. Although it is well established that a cytologic diagnosis of ICC is highly specific, most studies show that cytologic testing has low sensitivity for identifying women with high-grade lesions, especially if lesions are small [59–61], which suggests that we may have underestimated the number of women with HSILs. If misclassification of cervical lesions was nondifferential with respect to HIV status, our observed OR would most likely be an underestimate of the true relative risk. However, it may be that the observed rates of cytologic abnormalities reported among HIV-positive women underestimate the true prevalence of cervical disease, because the performance of Pap smears may be unusually poor in HIV-positive women [62, 63]. However, if high-grade lesions were, indeed, more likely to be missed in our HIV-positive women, then our observed association likely underestimates the true risk of having developed HSILs or ICC associated with HIV-1 or HIV-2 infection.

Even though our study is one of the largest to be conducted to date, we were still limited by the relatively small number of women with HSILs and ICC, especially women with these lesions who also had HIV-2 or dual HIV infection. Hence, we had little power to adequately evaluate potential HIV type-specific differences in the effect of factors, such as CD4 cell count or plasma HIV load on the risk of the presence of HSILs or ICC, or the degree to which these factors are independently associated with the risk of having developed neoplasia among women with HIV-1 versus those with HIV-2 infection. Finally, measurements of plasma HIV RNA levels were not conducted on all study participants, although women with and without these laboratory measurements were similar in regard to other potential risk factors associated with the presence of HSILs or ICC.

In summary, we found that women with HIV-1, HIV-2, and HIV-1 and HIV-2 dual infection were all at increased risk of prevalent HSILs or ICC, compared with HIV-negative women. Of note, this increased risk of having developed HSILs or ICC was only seen among women with high-risk HPV types detected and was seen most often in women with CD4 cell counts of <200 cells/ μ L. Finally, the fact that HIV-2–positive women were at greater risk of diagnosis with HSILs or ICC than were HIV-1–positive women, perhaps as a result of many additional years of mild immunosuppression, may have important implications for HIV-1–positive women receiving HAART therapy. Results from ongoing cohort studies of HIV-negative women and women with HIV-1 and/or HIV-2 infection will be useful in

clarifying temporal relationships between progression of HIV-induced immunosuppression and detection and load of high-risk HPV types in the development of high grade cervical lesions, including ICC.

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