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Genital Tract HIV RNA Levels and Their Associations with Human Papillomavirus Infection and Risk of Cervical Pre-Cancer

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Abstract

Objective—Plasma HIV RNA levels have been associated with risk of human papillomavirus (HPV) and cervical neoplasia in HIV-seropositive women. However, little is known regarding local genital tract HIV RNA levels and their relation with cervical HPV and neoplasia.

Design/Methods—In an HIV-seropositive women's cohort with semi-annual follow-up, we conducted a nested case-control study of genital tract HIV RNA levels and their relation with incident high-grade squamous intraepithelial lesions sub-classified as severe (severe HSIL), as

Conflicts of Interest
None of the authors have any conflicts of interest to disclose.

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The list of authors reflects the six sites that collected the data and the data analysis center in this multicenter cohort study, as well as the HPV Working Group members who headed this specific project. J.G., X.X., B.C.H., J.M.P., and H.D.S. were the primary leaders of this study, involved in generation of the hypotheses and every aspect of data analysis and writing and review of the manuscript. A.K., R.B., L.S.M, H.M., G.D., X.X., D.H.W., A.M.L., M.H.E., C.C., K.A., and I.E. were involved in writing and review of the manuscript. A.K., R.B., L.S.M, H.M., D.H.W., A.M.L., C.C., K.A., and I.E were additionally involved in collection of the data.

provided for under the Bethesda 2001 classification system. Specifically, 66 incident severe HSIL were matched to 130 controls by age, CD4+ count, HAART use, and other factors. We also studied HPV prevalence, incident detection, and persistence in a random sample of 250 subjects.

Results—Risk of severe HSIL was associated with genital tract HIV RNA levels (odds ratio comparing HIV RNA ≥ the median among women with detectable levels versus undetectable [OR_{VL}] 2.96; 95% CI: 0.99–8.84; P_{trend}=0.03). However, this association became non-significant (P_{trend}=0.51) following adjustment for plasma HIV RNA levels. There was also no association between genital tract HIV RNA levels and the prevalence of any HPV or oncogenic HPV. However, the incident detection of any HPV (P_{trend}=0.02) and persistence of oncogenic HPV (P_{trend}=0.04) were associated with genital tract HIV RNA levels, after controlling plasma HIV RNA levels.

Conclusion—These prospective data suggest that genital tract HIV RNA levels are not a significant independent risk factor for cervical pre-cancer in HIV-seropositive women, but leave open the possibility that they may modestly influence HPV infection, an early stage of cervical tumoriogenesis.

Key words/phrases

Genital tract HIV viral load; cervical neoplasia; HPV natural history

INTRODUCTION

Women with HIV have high rates of infection by human papillomavirus (HPV), the viral cause of cervical cancer,[1,2] and several-fold increased risks of cervical pre-cancer and cancer compared with women in the general population.[3–6] Further, the prevalence, incident detection, and persistence of HPV and cervical neoplasia among HIV-seropositive women increases with lower CD4+ T-cell count and higher plasma HIV RNA levels.[1,7] A significant relation of incident HPV detection and neoplasia with plasma HIV levels can be detected even when the CD4+ T-cell count is very low.[1] These associations with plasma HIV levels are thought to be indirect; i.e., reflecting the detrimental effects of HIV on host immunity beyond total CD4+ T-cells.[8]

In contrast, little is known regarding local HIV RNA levels in the genital tract and its associations with cervical HPV infection and neoplasia.[9,10] One prior study reported that high cell free genital tract HIV RNA levels were significantly related to HPV detection.[9] However, the analysis did not carefully control for plasma HIV viral load. Another small study of cervical intraepithelial neoplasia (CIN) and cervical HIV RNA detection (present versus not present) was reported and had null results, but the study was cross-sectional and could not address temporality.[11] Thus, it remains unclear whether cervical HIV RNA levels are independently associated with HPV infection or the incident development of cervical disease. This is important, especially since there is evidence of compartmentalization of HIV in the genital tract.[10–15] Several studies, for example, have reported differences in both the level of HIV RNA and the distribution of HIV quasispecies (i.e., distinct but inter-related variants of HIV within a single individual) between the genital tract and the systemic circulation. In our own prior study, we found that HIV RNA levels in

both cervicovaginal lavage (CVL) and endocervical swab specimens had only a correlation of 52% with those in plasma,[10] and other research groups have reported similar results.[9, 16]

These prior data are consistent with evidence of local production of HIV in the microenvironment of the genital tract,[13,17] which is reported to take place in T-cells that are present in cervicovaginal fluid.[18,19] It has also been proposed that differences in local genital tract HIV RNA levels may induce or reflect changes in the cervicovaginal milieu, including cytokines, alpha- and beta-defensins, and local adaptive and innate cellular immune function, which may impact control of HPV.[20,21] Therefore, we studied the associations of local genital tract HIV RNA levels with the risk of incident high-grade squamous intraepithelial lesions sub-classified as severe dysplasia (severe HSIL), controlling for CD4+ T-cell count and plasma HIV RNA levels, as well as other covariates. Our investigation focused on data and specimens within 2 years of diagnosis, consistent with recent studies by our group and others showing that the effects of host immune status on cervical cancer risk in HIV-seropositive women were greatest in the period shortly preceding disease detection.[22,23] We also examined the relation of genital tract HIV RNA levels with the natural history of HPV, including the prevalence, incident detection, and persistence of HPV.

METHODS

Women's Interagency HIV Study (WIHS)

Specimens and clinical data were obtained from the WIHS, an ongoing prospective cohort of HIV-seropositive and HIV-seronegative women enrolled through 6 clinical consortia (Bronx, NY; Brooklyn, NY; Chicago, IL; Los Angeles, CA; San Francisco, CA; and Washington, DC). The initial enrollment was conducted between October 1, 1994 and November 15, 1995 (N=2054 HIV-seropositive women and N=569 HIV-seronegative women), and a second enrollment was separately conducted between October 1, 2001 and September 30, 2002 (N=737 HIV-seropositive women and N=406 HIV-seronegative women). Details of the WIHS data collection and recruitment methods are described in detail elsewhere.[24] Women in the WIHS undergo semiannual visits during which physical and gynecologic examinations are conducted that include the collection of cervical and blood specimens, as well as the completion of an interviewer-administered questionnaire. CVL specimens were collected by spraying 10 mL of sterile, non-bacteriostatic saline against the cervical os and endocervix and aspirating it from the posterior vaginal fornix.[10] Only HIV-seropositive women were included in the current investigation.

Nested Case-Control Study of Incident Severe HSIL

A nested case-control design was used to efficiently study the relation of genital tract HIV RNA levels with incident severe HSIL. Although for clinical purposes all women with HSIL are referred to colposcopy, the Bethesda cytology classification system allows for sub-classification of lesions into moderate HSIL (akin to a histologic diagnosis of CIN-2) and severe HSIL (akin to CIN-3), with important research implications.[25–27] A diagnosis of

severe HSIL has been shown to have high specificity for pre-cancer.[27] We focused on cytology as an endpoint, since all Pap tests were evaluated centrally.

Control subjects (n=130) were individually matched 2:1 to incident severe HSIL cases (n=66) based on the following criteria: age (± 5 years), CD4+ T-cell count by strata (<200 cells/μL, 200–500 cells/μL, ≥500 cells/μL), current HAART use, as well as time in the WIHS cohort (year of enrollment). The presence of low grade cervical lesions (i.e., atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesion (LSIL) at baseline and other risk factors were addressed as covariates in the statistical analysis (described below). For the current study, we tested the CVL samples collected 6 months, 12 months, 18 months, and 24 months prior to diagnosis of incident severe HSIL to determine genital tract HIV RNA levels, with similar testing in the matched controls. HPV DNA was assessed at every visit in all subjects in the WIHS. Thus, multiple time points and lag times prior to diagnosis were evaluated.

HPV Natural History Study

To study the relation of genital tract HIV RNA levels with HPV natural history (i.e. prevalence, incident detection, and persistence of HPV infection as previously described [1]), we selected a simple random sample (N=250) of all HIV-seropositive WIHS women present at visit 12, the first visit at which cervical swab specimens were collected for measurement of HIV RNA levels. Swabs collected during visits 12 through 15 were then tested for HIV RNA levels. As reported below, the correlations of plasma HIV RNA levels with those in CVL and cervical swabs were both within the expected range based on prior studies (range: 40%–64%). [10,16] Cervical swabs had the possible advantage of being obtained directly from the cervix, but they were not available for cases of severe HSIL diagnosed before visit 12 and, therefore, were only used in the HPV natural history study.

Detection of HIV RNA

HIV quantitation for plasma and genital tract specimens was conducted in the central laboratory of the University of Southern California School of Medicine, Los Angeles, CA, which participates in the Division of AIDS (DAIDS) Quality Assurance Program. As previously described in detail, [10,24,28] CVL and fluid from swab specimens were defrosted and spun for 10 minutes at 400×g at room temperature to remove cellular elements. Then, 500 μl of CVL or swab fluid was used to determine HIV-1 RNA viral load using the isothermal nucleic acid sequence-based amplification method, NucliSens HIV-1 QT™ (Biomerieux, Durham, NC). [28] Lower limit of detection is 25 copies/mL and reliable quantification is achieved between 176 to 3.5×106 copies/ml. The accuracy and reproducibility of the plasma HIV RNA assay in the laboratory is regularly assessed on an ongoing basis through the DAIDS Quality Assurance Program.

Genital tract HIV RNA levels were categorized as (i) undetectable, (ii) detectable but below the median, and (iii) detectable above the median. This approach was chosen, in part, due to the large number of women with undetectable genital tract HIV RNA levels in both the nested case-control study and the natural history study (see Results), which made it difficult to meaningfully categorize these levels as a continuous variable. Furthermore, women with

undetectable cervical HIV are a discrete group, and the absence of detectable HIV is likely to be biologically relevant. Likewise, the median is a convenient, commonly employed cutoff for examining a gradient, and having groups of relatively balanced size is statistically efficient.

Detection of HPV DNA

The HPV DNA assay used has been described in detail elsewhere.[21] Briefly, HPV DNA was detected in CVL specimens using L1 consensus primer MY09/MY11/HMB01 PCR assays.[29] To serve as an internal control for amplification, a control primer set PC04/GH20, amplifying a 268 base pair cellular beta globin DNA fragment, was included in each assay. Following proteinase K digestion, 2–10 μL of each cell digest was used in reactions containing 10 mM Tris-HCL, 50 mM KCL, 4 mM MgCl2, 200 μM of each deoxyribonucleotide triphosphate, 2.5U Taq DNA polymerase, 0.5μM of each primer. There were 40 amplification cycles (95°C for 20 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with a 5-minute extension period at 72°C on the last cycle). Amplified material was then detected using filters individually hybridized with biotinylated type-specific oligonucleotide probes for more than 40 individual HPV types. Oncogenic HPV types were defined as HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, and all other HPV types were considered non-oncogenic HPV, consistent with recent recommendations.[30,31]

Clinical Laboratory Data

Pap specimens were obtained using a spatula and endocervical brush. All available Pap smears were centrally examined at Dianon Systems (New York, NY) and categorized according to the 1991 Bethesda system for cervicovaginal diagnosis, including reporting whether moderate or severe dysplasia was favored.[25] An expert cytopathologist reviewed all Pap smears that were considered abnormal and 10% of Pap smears considered normal during screening by a cytotechnologist.

Plasma HIV RNA levels and CD4+ T-cell counts were measured at baseline and at all subsequent visits at laboratories participating in the DAIDS Quality Assurance Program.

Statistical Methods

Nested Case-Control Study of Severe HSIL—Preliminary data analysis examined the distribution of the baseline (i.e., enrollment) characteristics of cases and matched controls. Differences within strata of matching groups were assessed using the Cochran–Mantel–Haenszel tests. The correlation between plasma and genital tract HIV RNA levels was assessed using a statistical model that addressed left truncation (i.e., the threshold for detectability), as previously described in detail.[32] The relation of genital tract HIV RNA levels and risk of incident severe HSIL was studied using multivariate conditional logistic regression models. In addition to the matching variables, the covariates included race/ethnicity, smoking, lifetime number of male sexual partners at baseline, number of male sexual partners in the past 6 months, condom use in the past 6 months, the presence of ASC-US or LSIL at baseline, plasma HIV RNA level, and phase of menstrual cycle.[33,34] Specifically, we characterized phase of menstrual cycle as either (i) follicular (within 14 days of the last menstrual period (LMP), or (ii) luteal (14–28 days since LMP) among

women who reported regular menstrual cycles, whereas other women were characterized as having (iii) irregular menses, or (iv) no menses. All models were run separately using cervical HIV RNA levels at 6 months, 12 months, 18 months, and 24 months prior to diagnosis as the main exposure variable, in order to assess multiple time points and possible lag times before disease development.

HPV Natural History Study (Prevalence, incidence, and persistence)—The prevalence of HPV DNA was expressed as the percentage of women with adequate HPV test results (i.e., those in whom amplification of β-globin was detected). Incident detection of HPV was defined as a positive test result for any HPV type that was not present at baseline or at any other earlier visits in a given woman. HPV persistence was studied based on the time to clearance (at least 2 sequential negative results) of a newly detected HPV type.[1] Generalized estimating equation (GEE) logistic regression models were used to assess the associations of genital tract HIV RNA levels with prevalent HPV infection, while adjusting for within-individual correlation related to repeated observations (multiple visits and multiple HPV types at each visit), as well as covariates. The incident detection of HPV was assessed using Cox proportional hazard models that incorporated the Wei-Lin-Weissfeld (WLW) method to adjust for within-individual correlations related to multiple HPV types. The mid-point between two consecutive visits was used as the time of incident infection. Subjects who had missing data for two visits in a row were censored at the last visit with adequate data. Persistence of HPV infection was studied with GEE models that adjusted for whether the HPV infection was prevalent or incident. All statistical tests were two sided, and $p<0.05$ was considered statistically significant.

RESULTS

Nested Case-control Study of Incident Severe HSIL

Table 1 shows the characteristics of the 66 incident severe HSIL cases and 130 matched controls at baseline. Both groups were similar in terms of the matching variables (i.e., age, CD4+ T-cell count, use of HAART, and year of enrollment), lifetime number of male sex partners, number of male sex partners in the past 6 months, alcohol and injection drug use, cigarette smoking, and condom use in the past 6 months. As expected, however, the cases were more likely to test positive for oncogenic HPV, to have high plasma HIV RNA levels (>100,000 copies/mL), and have ASC-US or LSIL at the baseline visit. Cases were also more likely to be black and less likely to be Hispanic.

Cervical HIV RNA levels were measured using stored CVLs collected at visits 6, 12, 18, and 24 months prior to diagnosis of incident severe HSIL. To assess the relationship between plasma and genital tract HIV RNA levels, we analyzed data in the control women; those most representative of the general population of HIV-seropositive women. HIV RNA was undetectable in 38% of plasma and 78% of CVL specimens in control women (a 40% difference). However, the discordance was largely limited to samples with low detectable levels in plasma. For example, the median detectable plasma level was 13,000 copies/mL when HIV RNA was detectable in the genital tract versus 570 copies/mL when HIV RNA was not detectable in the genital tract ($p <0.001$). Overall, the correlation of plasma and

genital tract HIV RNA levels was 58% ($p<0.001$) using a statistical model that accounted for left truncation (i.e., the lower limit of detection) and for repeated observations of the same subjects over time.[32] No relation of HIV RNA level in CVL with phase of menstrual cycle was observed, whether characterized as follicular versus luteal phase, or incorporating those with either irregular menstrual cycles or no menstrual cycles as two additional strata (all $p>0.4$).

We then assessed the relation of incident severe HSIL with cervical HIV RNA level at (i) the most recent visit (a median of 6 months prior to diagnosis) and (ii) the visit with the longest lag time (a median of 18 months prior to diagnosis; note: the maximum possible was 24 months based on the study design). The initial multivariate conditional GEE logistic regression model adjusted for the matching variables (age, CD4+ T-cell count, year of enrollment, and HAART use), as well as race/ethnicity, smoking, lifetime number of male sexual partners at baseline, number of male sexual partners in the past 6 months, and condom use in the past 6 months (Table 2). Based on this model, genital tract HIV RNA levels at the most recent prior visit were significantly associated with incident severe HSIL (odds ratio comparing HIV RNA ≥ the median among women with detectable levels versus undetectable [OR_{VL}] 2.96; 95% CI: 0.99–8.84; $P_{trend}=0.03$). However, this relationship was non-significant ($OR_{VL}=1.54$; 95% CI: 0.43–5.46; $P_{trend}=0.51$) after additional adjustment for plasma HIV RNA levels. Analysis of genital tract HIV RNA levels from the visit with the greatest possible lag time provided similar results as the analysis at the most recent visit prior to the diagnosis of severe HSIL. Adjustment for the presence of ASCUS or LSIL at enrollment had no affect on the findings (data not shown).

HPV Natural History Study

Table 3 shows selected baseline characteristics of the 250 randomly selected women to study the relationship between genital tract HIV viral load and HPV natural history. Most women (73%) had normal cytology, whereas 16% had ASC-US, 10% LSIL, and 1% HSIL. Approximately 21% of these women had CD4+ T-cell count <200 cells/mm^3, and 24% had undetectable plasma HIV RNA levels. Genital tract HIV RNA levels were undetectable in 62% of the samples; 38% more than in plasma. This is similar to the 40% difference in undetectable rates between plasma and cervical specimens reported above among the controls in the nested case-control study, and as above the discordant results between plasma and cervical specimens were largely limited to women with low detectable plasma level. The overall correlation between genital tract and plasma HIV RNA levels in the natural history study was 66% ($p<0.001$). No relationship of HIV RNA levels in cervical swabs and phase of menstrual cycle was observed, whether characterized as follicular versus luteal phase, or also incorporating those with either irregular menstrual cycles or no menstrual cycles as two additional strata (all $p>0.5$).

As shown in Table 4, no association between genital tract HIV RNA levels and prevalence of either any HPV or any oncogenic HPV types was observed, after adjustment for plasma HIV viral load, CD4+ T-cell count, and other factors. However, cervical HIV RNA levels were positively associated with incident detection of any HPV (OR 1.82, 95% CI 1.12–2.98, $P_{trend}=0.02$) as well as any oncogenic HPV (OR=1.99; 95% CI: 1.15–3.43; $P_{trend}=0.02$).

Note that while the relation with incident oncogenic HPV became non-significant with adjustment for covariates, the effect estimate itself changed approximately 10% or less, indicating that the initial (statistically significant) model was the most parsimonious. While no relationship between genital tract HIV RNA levels and persistence of any HPV was observed, there was a significant association between oncogenic HPV persistence and genital tract HIV RNA levels (OR=2.84; 95% CI: 1.03–57.83, p=0.04), after adjusting for CD4+ T-cell count and other variables (based on the most parsimonious model).

DISCUSSION

This prospective investigation found no significant independent associations between local genital tract HIV RNA levels and the risk of incident severe HSIL after statistical adjustment for circulating HIV RNA levels measured in plasma. There were also no significant associations between genital tract HIV RNA levels and the prevalence of any HPV or any oncogenic HPV. Nonetheless, we observed a positive relation of genital tract HIV RNA levels with incident detection of HPV, and with the persistence of oncogenic HPV, each of moderate strength.

The discrepancy between the HPV prevalence results and those for incident detection and persistence is surprising. In prior studies in the WIHS cohort we have found that host immune status (i.e., CD4+ T-cell count and plasma HIV RNA levels) was more strongly associated with HPV prevalence than either incident detection or persistence, which is expected given that the impact of immune status on prevalence reflects its combined effects on both incidence and persistence.[1] For example, we compared HPV prevalence rates in HIV-seropositive women with CD4+ T-cell count <200/HIV RNA level >100,000 to those who were HIV-seronegative, and found that the relative difference in prevalence was OR = 7.88 (5.98 to 10.38), incidence HR=4.27 (3.24 to 5.62), whereas for persistence the HR was never more than 2.5.[1] While it is possible that the relation of genital tract HIV RNA levels with HPV reflects other factors, such as cytokines, chemokines, or other inflammatory mediators,[20] these too would for the same reasons be expected to have a greater impact on HPV prevalence than incidence or persistence alone. Overall, while the current data do not entirely resolve whether cervical HIV RNA levels might be independently associated with HPV infection, the inconsistent, moderate associations observed argue against the existence of any strong effects, and may be a spurious finding.[1]

Moreover, our results are clear as they pertain to the incident development of severe HSIL and are consistent with a recent study that found no association with HIV genital shedding and CIN 2/3 among 44 cases and 44 controls matched for age.[11] That is, they suggest that local genital tract HIV RNA levels have little if any impact on the risk of severe HSIL. It is important to point out, therefore, that the development of pre-cancer and cancer involves not only infection by HPV, but also the aggregation of genetic and epigenetic changes necessary for tumoriogenesis [35], which may be less associated than HPV with either systemic or local host immune factors.

Certain limitations to this study should be considered in the interpretation of the results. In particular, due to their well-controlled HIV, many women had undetectable genital tract

HIV RNA levels and low or undetectable plasma HIV RNA levels, which may have limited our ability to detect their effects on cervical HPV and neoplasia. We also cannot exclude the possibility that the use of CVL versus swabs in the nested case-control study provided less accurate estimates of HIV RNA levels. That is, while the correlations between plasma and cervical HIV RNA were highly significant ($p<0.001$) and consistent with prior reports [10,16] using both types of cervical specimens, these correlations were nonetheless somewhat higher when using swabs (66%) than CVL (58%). However, despite these differences, the null results for severe HSIL were consistent with the null results for HPV prevalence (based on cervical swabs). It is unlikely that any strong relationships would have remained undetected due to minor differences in CVL versus swab results. Further, the study also had several strengths, including the large, prospective cohort of HIV-seropositive women with long follow-up, centralized expert pathology review, repeated serial testing for type-specific HPV DNA and HIV RNA levels in expert laboratories.

Taken together, the results suggest that genital tract HIV RNA levels are not an important independent risk factor for the incident development of cervical pre-cancer/cancer, though the current data do not entirely resolve whether cervical HIV RNA levels might modestly influence risk of HPV infection - an early stage of cervical tumoriogenesis. Therefore, from a clinical perspective while cervicovaginal HIV levels may be important for other reasons (e.g., risk of HIV transmission), efforts to control genital tract HIV levels are unlikely to impact cervical cancer risk.

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Table 1

Selected baseline characteristics of incident severe HSIL cases and matched controls.[a]

Characteristic	Cases (N=66)	Controls (N=130)	p-value
Age, mean (SD)[b]	34 (7)	35 (7)	0.43
Race, n (%)			0.03
White	5 (8)	9 (7)	
Hispanic	11 (17)	47 (36)	
Black	47 (71)	73 (56)	
Other	3 (5)	1 (1)	
Current alcohol use, n (%)			0.30
None	39 (59)	63 (48)	
Light	16 (24)	47 (36)	
Moderate	6 (9)	14 (11)	
Heavy	5 (8)	6 (5)	
Smoking status, n (%)			0.44
Never smoked	17 (26)	41 (32)	
Former smoker	10 (15)	24 (18)	
Current smoker	39 (59)	65 (50)	
IDU[c] in past 6 month, n (%)			0.14
No	60 (91)	125 (96)	
Yes	6 (9)	5 (4)	
IDU[c] ever, n (%)			0.32
No	38 (83)	78 (74)	
Yes	8 (17)	27 (26)	
Lifetime No. male sex partners, n (%)			0.41
<5	12 (18)	28 (22)	
5–9	9 (14)	28 (22)	
10–49	23 (35)	35 (27)	
≥50	21 (32)	37 (29)	
No. of male sex partners in past 6 month, n (%)			0.69
None	23 (35)	36 (28)	
1(married)	9 (14)	18 (14)	
1(single)	25 (38)	55 (43)	
2+	9 (14)	20 (16)	
Condom use in past 6 month, n (%)			0.77
No	22 (34)	43 (33)	
Yes	43 (66)	87 (67)	
Positive for Any HPV, n (%)			<.0001
No	14 (25)	64 (56)	

Characteristic	Cases (N=66)	Controls (N=130)	p-value
Yes	43 (75)	50 (44)	
Positive for Oncogenic HPV, n (%)			<.0001
No	27 (47)	87 (76)	
Yes	30 (53)	27 (24)	
Presence of ASC-US or LSIL, n (%)[d]			<.0001
No	20 (32)	98 (80)	
Yes	42 (68)	25 (20)	
Current antiretroviral therapy, n (%)[b]			0.80
None	26 (39)	49 (38)	
Monotherapy	17 (26)	42 (32)	
Combination, not HAART	19 (29)	31 (24)	
HAART	4 (6)	8 (6)	
CD4+ T-cell count, n (%)[b]			0.17
>500	20 (31)	36 (30)	
200–500	26 (40)	62 (51)	
<200	19 (29)	24 (20)	
Plasma HIV RNA level, n (%)			0.01
≤4000	15 (23)	48 (38)	
4001–20000	11 (17)	33 (26)	
20001–100000	26 (39)	33 (26)	
>100000	14 (21)	12 (10)	

[a] Controls matched to cases 2:1 on the following criteria: age, CD4+ T cell count, year of enrollment, and HAART use; Controls defined as having either a negative Pap, ASCUS, or LSIL, but not incident severe HSIL.

[b] Matching variables

[c] Injection drug use

[d] At the time of matching to cases a total of 16% of the controls had ASC-US/LSIL and 84% had normal cytology.

Table 2

The incident development of severe high grade squamous intra-epithelial lesions (HSIL) and its associations with genital tract HIV RNA levels measured at (i) the most recent semi-annual visit prior to the visit at diagnosis (i.e., a minimum of 6 months prior), or (ii) the visit with the longest lag time prior to diagnosis (a maximum of 24 months).[a]

	Cervical HIV RNA Level: *Measured at Most Recent Prior Visit (Least Lag Time)*[b]			
	Undetectable	**Detectable Below the Median[d]**	**Detectable Above the Median**	
Model 1[c]	ref	2.39 (0.80–7.19)	2.96 (0.99–8.84)	0.03
Model 1 + plasma HIV RNA level	ref	0.69 (0.11–4.53)	1.32 (0.20–8.82)	0.84
Model 1 + ASCUS/LSIL + plasma HIV RNA level	ref	0.70 (0.11–4.62)	1.37 (0.20–9.17)	0.82
	Cervical HIV RNA Level: *Measured at the Visit with Longest Lag Time*[e]			
	Undetectable	Detectable Below the Median[f]	Detectable Above the Median	p-trend
Model 1[c]	ref	2.83 (0.98–8.17)	1.83 (0.60–5.59)	0.11
Model 1 + plasma HIV RNA level	ref	2.59 (0.24–27.89)	1.63 (0.26–10.33)	0.61
Model 1 + ASCUS/LSIL + plasma HIV RNA level	ref	2.76 (0.26–29.80)	1.67 (0.26–10.60)	0.59

[a] Genital tract HIV RNA levels were measured 6, 12, 18, and 24 months prior to the diagnosis of incident severe HSIL.

[b] Median “least lag time” was 6 months prior to diagnosis of incident severe HSIL.

[c] Model 1: a multivariable conditioned logistic regression model that adjusted for race/ethnicicty, cigarette smoking, lifetime number of male sex partners, number of male sex partners in the past six months, condom use in the past six months,

[d] Median cervical HIV viral load = 485 copies/mL.

[e] Median “longest lag time” was 18 months prior to diagnosis of incident severe HSIL.

[f] Median cervical HIV viral load = 915 copies/mL.

Table 3

Selected baseline characteristics of the random sample of WIHS women studied to determine the relation of genital tract HIV RNA levels and the natural history of HPV.

Characteristic	HIV+ Women (N=250)
Age, mean (SD)	41 (8)
Race, n (%)	
White	43 (17)
Hispanic	70 (28)
Black	134 (54)
Other	3 (1)
Current alcohol use, n (%)	
None	143 (57)
Light	66 (27)
Moderate	29 (12)
Heavy	11 (4)
Smoking status, n (%)	
Never smoked	51 (23)
Former smoker	63 (28)
Current smoker	108 (49)
IDU[a] in past 6 month, n (%)	
No	244 (98)
Yes	6 (2)
IDU[a] ever, n (%)	
No	144 (65)
Yes	78 (35)
Lifetime No. male sex partners, n (%)	
<5	46 (19)
5–9	50 (20)
10–49	90 (36)
≥50	61 (25)
No. of male sex partners in past 6 month, n (%)	
None	85 (34)
1(married)	43 (17)
1(single)	95 (38)
2+	25 (10)
Cytologic diagnosis, n (%)	
Normal	182 (73)
ASC-US	40 (16)
LSIL	25 (10)
HSIL	3 (1)

Characteristic	HIV+ Women (N=250)
Positive for Oncogenic HPV, n (%)	
No	201 (80)
Yes	49 (20)
Condom use in past 6 month, n (%)	
No	96 (39)
Yes	153 (61)
Current antiretroviral therapy, n (%)	
None	71 (28)
Monotherapy	2 (1)
Combination, not HAART	23 (9)
HAART	154 (62)
CD4+ T-cell count, n (%)	
>500	77 (31)
200–500	117 (48)
<200	51 (21)
Plasma HIV RNA level, n (%)	
≤4000	135 (55)
4001–20000	49 (20)
20001–100000	40 (16)
>100000	20 (8)
Cervical HIV RNA level, n (%)	
Undetectable	152 (62)
Below median	39 (16)
Above median	56 (23)

Injection drug use

Table 4

The associations of genital tract HIV RNA levels with the natural history (i.e., prevalent and incident detection and persistence) of “any HPV” and “any oncogenic HPV”.

	Prevalence			
	***Any HPV*[a]**	**p-trend**	***Oncogenic HPV*[a]**	**p-trend**
Model 1[b]	1.57 (1.17–2.11)	0.005[c]	2.03 (1.23–3.36)	0.01
Model 1 + CD4 count	1.17 (0.85–1.60)	0.41	1.36 (0.83–2.23)	0.28
Model 1 + CD4 count + plasma HIV viral load	1.20 (0.88–1.62)	0.36	1.36 (0.82–2.25)	0.29
	Incident Detection			
	Any HPV[a]	p-trend	*Oncogenic HPV*[a]	p-trend
Model 1[b]	2.52 (1.70–3.74)	<0.001[c]	1.99 (1.15–3.43)	0.02
Model 1 + CD4 count	1.74 (1.15–2.65)	0.01[c]	1.51 (0.80–2.88)	0.23
Model 1 + CD4 count + plasma HIV viral load	1.82 (1.12–2.98)	0.02[c]	1.79 (0.73–4.39)	0.22
	Persistence			
	Any HPV[a]	p-trend	*Oncogenic HPV*[a]	p-trend
Model 1[b]	1.19 (0.71–2.00)	0.41	2.49 (0.93–6.68)	0.07
Model 1 + CD4 count	1.04 (0.60–1.82)	0.78	2.84 (1.03–7.83)	0.04
Model 1 + CD4 count + plasma HIV viral load	1.14 (0.63–2.08)	0.58	2.66 (0.88–8.07)	0.11

[a]Odds ratio contrasting those women with genital tract HIV RNA levels above the median (545 copies/mL) found in subjects who had detectable values versus those women with undetectable levels

[b]Model 1: a multivariable model adjusted for age, race, smoking, lifetime number of male sex partners, number of male sex partners in the past six months, condom use in the past six months, and cervical treatment in the past six months, and use of highly active anti-retroviral therapy (HAART).