Prevalence and Risk Factors for Anal Squamous Intraepithelial Lesions in Women

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Background: **Anal cancers are thought to arise from squamous intraepithelial lesions in the anal canal, and women infected with human immunodeficiency virus-1 (HIV) may be at higher risk of anal cancer. Our aim was to determine the prevalence of human papillomavirus (HPV)-related abnormalities of the anal canal in women and to characterize risk factors for these lesions.** ***Methods:*** **We evaluated HPV-related abnormalities in 251 HIV-positive and in 68 HIV-negative women. We completed physical examinations and obtained questionnaire data on medical history and relevant sexual practices. Univariate and adjusted relative risks (RRs) and 95% confidence intervals (CIs) were computed using the Mantel–Haenszel procedure and regression techniques. All statistical tests were two-sided.** ***Results:*** **Abnormal anal cytology, including atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesions, or high-grade squamous intraepithelial lesions (HSILs), was diagnosed in 26% of HIV-positive and in 8% of HIV-negative women. HSILs were detected by histology or cytology in 6% of HIV-positive and in 2% of HIV-negative women. HIV-positive women showed increased risk of anal disease as the CD4 count decreased ($P<.0001$) and as the plasma HIV RNA viral load increased ($P = .02$). HIV-positive women with abnormal cervical cytology had an increased risk of abnormal anal cytology at the same visit (RR = 2.2; 95% CI = 1.4 to 3.3). Abnormal anal cytology in HIV-positive women was associated with anal HPV RNA detected by the polymerase chain reaction and by a nonamplification-based test (RR = 4.3; 95% CI = 1.6 to 11). In a multivariate analysis, the history of anal intercourse and concurrent abnormal cervical cytology also were statistically significantly ($P = .05$) associated with abnormal anal cytology.** ***Conclusions:*** **HIV-positive women had a higher risk of abnormal anal cytology than did HIV-negative women with high-risk lifestyle factors. These data provide strong support for anoscopic and histologic assessment and careful follow-up of women with abnormal anal lesions. [J Natl Cancer Inst 2001;93:843–9]**

The incidence of anal cancer is higher among women than among men in the general population *(1–3);* women at high risk include those with high-grade cervical lesions *(4)* and cervical and vulvar cancers *(5,6).* Men who have sex with men and those who have human immunodeficiency virus-1 (HIV) infections also are at especially high risk for the development of anal cancer *(2,3,7),* and an increased incidence of anal cancer also has been reported in HIV-positive women *(8,9).* Several biologic similarities are shared between cervical and anal cancers, including an association with human papillomavirus (HPV) infection *(10–12).*

The current incidence of cervical cancer in the United States is approximately eight per 100 000 and varies by race and ethnicity from seven to 17 per 100 000 *(13).* Invasive cervical cancers, which arise from squamous intraepithelial lesions (SILs) *(14),* are a prominent cause of morbidity and mortality in the United States *(15).* SILs also may be detected in the anal canal and most likely represent the precursor to anal cancer. Cervical SILs (CSILs) or anal SILs (ASILs) range from low to high grade. High-grade SILs (HSILs) most likely represent the true invasive cancer-precursor lesion in the cervix and most likely in the anus. Low-grade SILs (LSILs) are not thought to progress directly to invasive cancer *(7).* However, anal LSILs are clinically important, since the disease in 62% of HIV-positive and 36% of HIV-negative homosexual men who had LSILs progressed to detectable HSILs within 2 years *(16).* Atypical squamous cells of undetermined significance (ASCUS) also may be found on cytologic examination in both the cervix and the anus *(16,17),* and these lesions often are accompanied by biopsy-proven SILs *(17).*

HIV-positive women have been shown to be at increased risk of CSILs when compared with their HIV-negative counterparts *(18–24),* and several reports *(9,20,21)* have confirmed that HPVs are the sexually transmitted agents that are etiologically related to cervical cancer. In contrast, relatively little is known about ASILs in HIV-positive women. However, recent work *(9)* has shown a 7.8-fold increased risk of *in situ* anal cancer in HIV-positive women. Several earlier studies *(21,25)* have shown that anal cytologic abnormalities were more common among HIV-positive women than among HIV-negative women, with most of the cytologic changes diagnosed as ASCUS and not ASIL. However, anoscopic and histologic assessments of anal lesions were not completed in these studies, and there were too few women to characterize risk factors by lesion type. Anoscopic and histologic assessment of anal lesions is critical to classify the lesions accurately, since the grade of anal cytology often does not correspond to that of histology, which remains the gold standard.

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See "Notes" following "References."

Routine cervical cytology screening of women followed by treatment of high-grade CSIL has substantially reduced the incidence of invasive cervical cancer. No comparable screening program for ASILs exists. However, an ASIL screening program similar to that for CSIL could help to prevent anal cancer in high-risk individuals. Establishment of such a program would require a thorough understanding of the prevalence and natural history of ASIL in populations at risk for ASIL. Invasive anal cancer has been reported recently to be 6.8 times greater among HIV-positive women *(9)*. The aim of this study was to determine the prevalence of HPV-related abnormalities of the anal canal in HIV-positive and HIV-negative women by use of cytology, high-resolution anoscopy, and histology and to characterize risk factors for these lesions.

Subjects and Methods

Study Design

This study was conducted with the approval of the University of California, San Francisco Committee on Human Research. Written informed consent was obtained from each participant. Participants in this anal lesion study were recruited from November 1995 to January 1997 from women who were participating at the San Francisco Bay Area site of the multicenter Women's Interagency HIV Study (WIHS), a natural-history cohort study of HIV disease in women *(26)*. The baseline visits (study entry) for the WIHS study occurred from October 1993 through November 1994, with follow-up evaluations at 6-month intervals. A total of 251 HIV-positive and 68 HIV-negative women were enrolled in this anal lesion study. In the WIHS study, the HIV-negative women were frequency matched to the HIV-positive women by age and HIV risk factors. The HIV-positive and HIV-negative cohorts were recruited from similar sources and were frequency matched on demographic and other key risk factors, including age, race/ethnicity, educational level, injection drug use since 1978, and the total number of sexual partners since 1980. Therefore, they constitute a group that is at high risk of acquiring HIV and other sexually transmitted diseases. As a part of the routine WIHS visit, each study participant was interviewed in person regarding her behavioral and medical history. A pelvic examination, including cervical and vaginal cytology, cervical HPV testing, and blood tests that included detection of HIV, measurement of HIV RNA viral load, and CD4 counts, was completed on all participants by trained research clinicians. In conjunction with this regular WIHS visit, women who signed consent forms to participate in the anal neoplasia study had samples taken for anal cytology and HPV tests, and a brief additional questionnaire was administered that covered medical history and sexual practices specific to the anal neoplasia study. To obtain samples for anal cytology and HPV, two consecutive Dacron swabs moistened in tap water were inserted into the anal canal. The first was used for anal cytology as described previously *(17)*. The second swab was used for HPV testing and was inserted into a 1-mL vial of sample transport medium (Digene Corp., Gaithersburg, MD) and frozen at –70 °C until analysis.

Clinical Examination

Anal cytology was evaluated by a pathologist (T. M. Darragh) who was experienced in interpretation of these specimens. The pathologist had no knowledge of the clinical status of the participants, their questionnaire responses, or other test results. Anal cytologic results were classified as normal, ASCUS, LSIL, or HSIL by use of current criteria to evaluate cervical cytology *(27)*. Women were asked to return for a more thorough anal examination if they had abnormal anal cytologic results. This examination included high-resolution anoscopy by use of 3% acetic acid and a colposcope as described previously *(10,28,29)*. Women who consented underwent an anal biopsy if a lesion was present. If more than one lesion was seen, biopsy specimens were obtained from areas with different colposcopic appearances. Biopsy specimens were fixed in 10% formalin for routine histopathologic examination. During some examinations, lesions were seen but no biopsy specimen was obtained because of participant refusal or medical contraindications, such as recent acetylsalicylic acid intake, neutropenia (<1000/mm^3), thrombocytopenia (platelet count <65 000/mm^3), or concurrent bacterial or viral infection in or around the anus. Anal histologic results were classified as normal, atypical, LSIL, or HSIL.

Detection of HPV

Because of its high sensitivity, polymerase chain reaction (PCR) was used in this study to detect low-level HPV infection *(30)*. Since a positive PCR result does not discriminate between low- and high-level infections, Hybrid Capture II™ (Digene Corp.), a nonamplification-based test that measures low and high levels of HPV infection, was used to provide information on the quantity of the virus that was present.

HPV Typing

Two hundred microliters of specimen in sample transport medium (Digene Corp.) was used for PCR, and the remainder was used for Hybrid Capture II. PCR was performed by use of MY09/MY11 consensus HPV L1 primers as well as primers for amplification of the human β-globin gene as described previously *(31)*. After 30 amplification cycles, specimens were probed with a biotin-labeled HPV L1 consensus probe mixture *(31)*. A separate membrane was probed with a biotin-labeled probe to the human β-globin gene. Specimens were studied to determine the specific HPV type by use of probes for 29 different HPV types (6, 11, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, AE2, Pap 155, and Pap 291) as well as the following 10 types together in a probe mixture (HPV 2, 13, 34, 42, 57, 62, 64, 67, 72, and W13B). Specimens negative for β-globin gene amplification were excluded from analysis. Hybrid Capture II was conducted according to the manufacturer's recommendations.

The results of Hybrid Capture II were expressed as a relative light unit (RLU) ratio that was determined by dividing the chemiluminescent signal of the test sample by that obtained with a control sample containing 10 pg/mL of HPV16 DNA. The magnitude of the RLU ratio increases with increasing quantity of HPV DNA in the specimen. The RLU ratio was computed, and HPV infection was classified as negative (<1.0) or positive (≥1.0); for some analyses, positive specimens were further categorized as 1.0 to less than 10, 10–100, and greater than 100.

Assays for Detection of HIV, Measurement of HIV RNA Viral Load, and CD4 Cell Count in Blood

Blood was obtained to determine HIV status, HIV RNA viral load, and CD4 level at the same time that the anal specimens were collected. HIV tests were completed by use of enzyme-linked immunosorbent assay, and all positive results were confirmed by western blot analysis, as described previously *(26)*. CD4 levels were measured by use of standardized two- or three-color fluorescence methods, and HIV RNA viral load in plasma (expressed as number of copies/mL) was measured by use of a nucleic acid-sequence-based amplification assay (Organon Teknika, Durham, NC) *(26)*.

Data Analysis

To evaluate potential risk factors for association with anal neoplasia, anal disease was considered to be present if the anal cytology diagnosis was ASCUS, LSIL, or HSIL and was considered to be absent if the diagnosis was normal. ASCUS was included in the abnormal category because it often is associated with HPV infection, is found in the presence of colposcopically or anoscopically detectable SIL, including HSIL, and frequently predates detection of HSIL *(10,16,17)*. A purpose of our analyses was to evaluate risk factors for anal disease to identify women who would benefit from cytologic screening; therefore, we included the ASCUS diagnosis in the disease category for these analyses. Study participants for whom the sample was missing or insufficient for evaluation were excluded from analysis. HIV-positive women were stratified by CD4 count or plasma HIV RNA viral load and were compared with HIV-negative women. Other risk factors were evaluated for HIV-positive women alone because the data for HIV-negative women were too sparse for analyses.

The lifetime presence or absence of a disease, treatment, or behavior was used to evaluate associations between anal disease and potential risk factors unless otherwise stated. To determine lifetime exposure, most risk factor data from the WIHS baseline interview were combined with WIHS follow-up interview data up to the time of the enrollment visit for the anal study. The total number of lifetime sexual partners was obtained from the WIHS baseline interview. A history of anal intercourse, hemorrhoids, fissures or fistulas, and blood in the stool was determined by use of data from the interview given at enrollment in the anal study. The study participants were asked about history of genital warts in both the WIHS interview and supplemental interview for the anal study. The

presence of warts, if any, was noted at the time of the physical examinations. Women were considered to have had a history of genital warts if reported during interviews or detected at examinations for either the WIHS or the anal lesion study. If the CD4 counts were not available for a particular visit, the average number of CD4 counts was used from visits 6 months earlier and from 6 months later.

The SAS statistical package was used for data analysis (SAS Institute, Cary, NC) to compute relative risks (RRs) and 95% confidence intervals (CIs). The Mantel–Haenszel procedure was used to adjust for one or two risk factors, and logistic regression was used to evaluate models with three or more risk factors. The Zhang and Yu correction *(32)* was applied to the odds ratios from logistic regression to approximate adjusted RRs. All statistical tests were two-sided.

RESULTS

A total of 319 of the 422 women at the San Francisco Bay Area site of the WIHS cohort agreed to participate in this anal lesion study: One hundred five women were recruited at the 6-month WIHS visit, 152 at the 1-year visit, 58 at the 18-month visit, and four at the 2-year visit. The distribution of socioeconomic and demographic characteristics was similar to that reported for the entire WIHS sample *(26),* except for age and Hispanic origin (Table 1). The demographic characteristics of the subjects in this study reflected closely those of the San Francisco Bay Area site of the WIHS cohort. The median age was 36 years at the baseline visit for the HIV-positive WIHS participants and 34 years for HIV-negative WIHS participants for all of the WIHS geographic sites *(26).* In contrast, the median age was 38 years at the baseline visit for all of the women in the San Francisco Bay Area WIHS cohort (6–18 months before the baseline visit for the anal lesion study) and 39 years for the participants in the anal lesion study.

Anal cytology was missing or insufficient for 16 (6%) HIV-positive and for seven (10%) HIV-negative women. The distribution of anal cytologic diagnoses for the 296 women with interpretable results showed that 26% (61 of 235) of the HIV-positive and 8% (five of 61) of the HIV-negative women had an abnormal anal cytologic result at their baseline examination (Table 2). Of the 66 women (61 HIV positive and five HIV negative) with an anal cytology diagnosis of ASCUS, LSIL, or HSIL, 46 (70%) completed their anal examination that included high-resolution anoscopy and biopsy of visible anal lesions within 6 months. Biopsy-confirmed HSILs were found in 14 (30%) of these women (Table 3), half of whom had only ASCUS when cytology was used for their initial diagnosis. HSILs were found in 23% (seven of 30) of the women with ASCUS by cytology. In total, at baseline, HSILs were detected by histology or cytology in 6% (14 of 235) of the HIV-positive and in 2% (one of 61) of the HIV-negative women who had interpretable cytology.

HIV-positive women were more likely to have been diagnosed with abnormal anal cytology than were HIV-negative women (RR = 3.2; 95% CI = 1.3 to 7.5; Table 4). When HIV-positive women were stratified by CD4 count, they showed an increased risk of anal disease as CD4 count decreased (test for trend $P<.0001$). HIV-positive women also showed an increased risk of anal disease as plasma HIV viral load increased (test for trend $P = .02$). Even women with CD4 counts greater than 500 cells/mm^3 or with an HIV viral load of less than or equal to 20 000 copies/mL showed elevated risks compared with HIV-negative women. Although the 95% CIs included unity, for these risk factor groups, the tests for trend were statistically significant.

Results of cervical cytologic diagnoses were compared with results from the anal samples for HIV-positive women (Table 5). HIV-positive women who had abnormal cervical cytologic results had an increased risk of abnormal anal cytology at the same visit (RR = 2.2; 95% CI = 1.4 to 3.3; data not shown in Table 5). The risk of abnormal anal cytology increased with increasing severity of abnormalities in the cervical specimen to a 2.9-fold increased risk (test for trend, $P = .0001$). Too few HIV-negative

Table 1. Baseline characteristics of HIV-positive and HIV-negative women*

	HIV positive (n = 251)		HIV negative (n = 68)	
Characteristic	No.	%	No.	%
Education				
< High school	73	29	23	34
High school graduate or GED	99	39	29	43
Some college	69	27	13	19
College graduate or graduate school	10	4	3	4
Household annual income, $				
≤6000	46	18	23	34
6001–12 000	135	54	30	44
12 001–24 000	39	16	8	12
>24 001	25	10	3	4
Unknown, decline to state	6	2	4	6
Currently employed	38	15	9	13
Currently receives public assistance	80	32	32	47
Marital status				
Married/living with partner	81	32	30	44
Widowed	23	9	3	4
Separated/divorced	76	30	13	19
Never married	71	28	22	32
Sexual orientation				
Heterosexual	189	75	55	81
Bisexual	42	17	8	12
Lesbian	15	6	3	4
Other	3	1	2	3
Race/ethnicity				
Black/African-American	148	59	41	60
White non-Hispanic	63	25	17	25
White Hispanic	20	8	6	9
Multiethnic	15	6	3	4
Other	4	2	1	1
Any Hispanic origin	31	12	6	9
Median age at anal study baseline, y (range)	40 (20–61)		40 (22–54)	

*Percentages in subsets described in different demographic factors may not add up to 100 because of rounding. Number of subjects may vary among factors because of missing values. HIV = human immunodeficiency virus-1; GED = general equivalency diploma.

Table 2. Anal cytologic diagnoses at baseline for HIV-positive and HIV-negative women*

	Normal		ASCUS		LSILs		HSILs		
HIV status	No.	%	No.	%	No.	%	No.	%	Total No.†
Positive	174	74	35	15	24	10	2	1	235
Negative	56	92	4	7	1	2	0	0	61

*ASCUS = atypical squamous cells of undetermined significance; LSILs = low-grade squamous intraepithelial lesions; HSILs = high-grade squamous intraepithelial lesions; HIV = human immunodeficiency virus-1.

†The anal cytology data were insufficient or missing in 16 of 251 HIV-positive participants and in seven of 68 HIV-negative participants.

Table 3. Anal histologic diagnosis at supplemental visit within 6 months of baseline for women with anal cytologic diagnosis of ASCUS, LSILs, or HSILs at baseline visit in HIV-positive women*

Cytology diagnosis at baseline	Total No.	No. of women with histology diagnoses within 6 mo					
		No. lesions	Lesion but no biopsy	Normal	Atypia	LSILs	HSILs
ASCUS	30	9	1	2	0	11	7
LSIL	14	1	0	0	1	6	6
HSIL	2	0	0	0	0	1	1

*ASCUS = atypical squamous cells of undetermined significance; LSILs = low-grade squamous intraepithelial lesions; HSILs = high-grade squamous intraepithelial lesions; HIV = human immunodeficiency virus-1.

Table 4. Relative risks (RRs) and 95% confidence intervals (CIs) for abnormal anal cytology by HIV status, CD4 counts, and HIV RNA viral load*

Risk factor	Anal cytology		
	Abnormal	Normal	RR (95% CI)†
HIV status			
Negative	5	56	1.0 (referent)
Positive	61	174	3.2 (1.3 to 7.5)
HIV-positive individuals			
CD4 >500/mm^3	9	62	1.6 (0.54 to 4.5)
CD4, 200–500/mm^3	25	78	3.0 (1.2 to 7.5)
CD4 <200/mm^3	27	33	5.5 (2.2 to 16)
Test for trend			P<.0001
HIV-positive individuals			
HIV viral load <4000	16	68	2.3 (0.88 to 6.1)
HIV viral load, 4000–20 000	8	31	2.5 (0.86 to 7.3)
HIV viral load >20 000–100 000	14	31	3.8 (1.6 to 10)
HIV viral load >100 000	11	17	5.5 (2.1 to 14)
Test for trend			P = .02

*HIV = human immunodeficiency virus-1.

†The referent group is HIV-negative women. Number of subjects may vary among factors because of missing values.

Table 5. Cervical cytology diagnosis versus risk for abnormal anal cytology in HIV-positive women*

Cervical cytology	Anal cytology, No.				Relative risk (95% confidence interval)†
	Normal	ASCUS	LSILs	HSILs	
Normal	122	17	8	2	1.0 (referent)
ASCUS	36	8	9	0	1.8 (1.04 to 3.0)
LSILs	14	9	6	0	2.9 (1.7 to 4.7)
HSILs	1	0	1	0	

*ASCUS = atypical squamous cells of undetermined significance; LSILs = low-grade squamous intraepithelial lesions; HSILs = high-grade squamous intraepithelial lesions; HIV = human immunodeficiency virus-1.

†Test for trend, P = .0001. Number of subjects may vary among factors because of missing values.

women had abnormal anal cytology to evaluate the association with cervical cytology or other risk factors.

Associations between anal HPV infection and anal cytology among HIV-positive women demonstrated that abnormal anal cytology was associated with the detection of any anal HPV by PCR (RR = 4.0; 95% CI = 1.5 to 11) as well as by Hybrid Capture II (RR = 4.3; 95% CI = 1.6 to 11; Table 6). Moreover, HPV DNA quantity, as measured by the Hybrid Capture II RLU ratio, also was associated with risk of abnormal anal cytology,

Table 6. Relative risks (RRs) and 95% confidence intervals (CIs) of abnormal anal cytology for HPV infection detected by PCR or by Hybrid Capture II™ in HIV-positive women*

Risk factor	Anal cytology, No.		
	Abnormal	Normal	RR (95% CI)
HPV detected by use of PCR consensus primer			
No	4	46	1.0 (referent)
Yes	51	109	4.0 (1.5 to 11)
PCR amount for consensus primer			
0	4	46	1.0 (referent)
1–2	5	35	1.6 (0.44 to 5.6)
3	7	30	2.4 (0.73 to 7.7)
4–5	39	44	5.9 (2.2 to 16)
Test for trend			P<.0001
Any HPV detected by Hybrid Capture II			
No	4	50	1.0 (referent)
Yes	56	119	4.3 (1.6 to 11)
RLU for HPV by Hybrid Capture II			
<1	4	50	1.0 (referent)
1 to <10	3	50	0.76 (0.17 to 3.4)
10 to <100	15	42	3.6 (1.2 to 10)
⩾100	38	27	7.9 (3.0 to 21)
Test for trend			P<.0001

*HPV = human papillomavirus; PCR = polymerase chain reaction; HIV = human immunodeficiency virus-1; RLU = relative light unit. Number of subjects may vary among factors because of missing values.

with an RR of 7.9 for an RLU ratio of 100 or more (test for trend, P<.0001). HPV infection remained highly associated with abnormal anal cytology after adjustment for CD4 counts (adjusted RR = 2.9 [95% CI = 1.1 to 7.7] for an RLU of 10–100 versus an RLU of <1; RR = 7.0 [95% CI = 3.4 to 15] for an RLU of >100 versus an RLU <1; data not shown in Table 6). HPV was detected by Hybrid Capture II in 80% (four of five) of the HIV-negative women with abnormal anal cytology compared with only 25% (14 of 55) of those with normal anal cytology (P = .03).

In univariate analysis (Table 7), increased risk of abnormal anal cytology was related to sexual activity or gynecologic disease (anal or genital warts, pelvic inflammatory disease, anal intercourse, and abnormal cervical cytology), lifetime or current use of several medications used to treat HIV and HIV-related diseases (zidovudine, didanosine, trimethoprim and sulfamethoxazole, luconazole, lamivudine, stavudine, dapsone, and ketaconazole), and history of several HIV-related diseases (oral candidiasis, diarrhea for >1 month, self-reported diagnosis of acquired immunodeficiency syndrome, and *Pneumocystis carinii* pneumonia [PCP]). Women who were less than 40 years of age were at increased risk, whereas those who had completed menopause were at decreased risk of abnormal anal cytology.

All RRs for the variables listed in Table 7 diminished in multivariate analysis after adjustment for CD4 counts or for anal HPV infection (as measured by Hybrid Capture II). However, history of genital warts, history of anal intercourse, abnormal cervical cytology, diarrhea for more than 1 month, and use of dapsone remained elevated after adjustment for HPV infection by use of Hybrid Capture II. Several additional factors were significant after adjustment for CD4. After adjusting for both HPV infection and CD4 counts, history of anal intercourse, diarrhea for more than 1 month, and ever use of dapsone were

Table 7. Univariate and adjusted relative risks (RRs) and 95% confidence intervals (CIs) for variables associated with abnormal anal cytology among HIV-positive women*

	Univariate RR (95% CI)	RR (95% CI) adjusted for Hybrid Capture II™†	RR (95% CI) adjusted for CD4‡	RR (95% CI) adjusted for CD4 and Hybrid Capture II§
		Risk factor with >30 exposed		
Genital warts	2.1 (1.4 to 3.2)	1.5 (1.0 to 2.2)	2.2 (1.4 to 3.3)	1.4 (0.98 to 2.1)
PID	1.7 (1.1 to 2.6)	1.2 (0.78 to 1.7)	1.7 (1.1 to 2.7)	
Anal intercourse	2.5 (1.5 to 4.2)	1.9 (1.2 to 3.0)	2.4 (1.5 to 3.8)	2.0 (1.3 to 3.1)
Abnormal cervical cytology	2.2 (1.4 to 3.3)	1.6 (1.1 to 2.3)	1.8 (1.1 to 2.8)	1.5 (0.97 to 2.2)
Menopause	0.45 (0.21 to 0.98)	0.54 (0.28 to 1.0)	0.42 (0.21 to 0.84)	
Age <40 y	1.9 (1.2 to 3.0)	1.4 (0.86 to 2.2)	2.0 (1.3 to 3.1)	
Oral candidiasis	2.0 (1.3 to 3.2)	1.3 (0.89 to 2.0)	1.7 (1.0 to 2.6)	
Diarrhea >1 mo	1.9 (1.2 to 2.9)	1.8 (1.3 to 2.7)	1.7 (1.1 to 2.7)	1.8 (1.2 to 2.7)
AIDS diagnosis	1.8 (1.2 to 2.8)	1.0 (0.70 to 1.5)	1.2 (0.70 to 1.9)	
Drug use				
AZT ever	2.1 (1.3 to 3.5)	1.4 (0.94 to 2.2)	1.6 (0.96 to 2.8)	
AZT current	1.8 (1.2 to 2.8)	1.2 (0.84 to 1.8)	1.4 (0.89 to 2.2)	
DDI ever	1.6 (1.0 to 2.5)	1.3 (0.90 to 2.0)	1.0 (0.64 to 1.7)	
Bactrim ever	1.8 (1.2 to 2.8)	1.2 (0.82 to 1.8)	1.2 (0.74 to 2.0)	
Bactrim current	1.7 (1.1 to 2.6)	1.2 (0.83 to 1.8)	1.2 (0.72 to 1.9)	
Fluconazole ever	2.1 (1.4 to 3.2)	1.2 (0.85 to 1.8)	1.6 (1.0 to 2.5)	
Fluconazole current	2.4 (1.6 to 3.6)	1.4 (0.97 to 2.1)	1.8 (1.1 to 2.8)	
3TC ever	2.2 (1.5 to 3.4)	1.3 (0.90 to 1.9)	1.8 (1.1 to 2.8)	
3TC current	2.4 (1.6 to 3.6)	1.4 (0.96 to 2.0)	1.8 (1.1 to 2.9)	
		Risk factors with <30 but ⩾20 subjects exposed		
Drug use				
D4T ever	1.7 (1.1 to 2.9)	1.2 (0.79 to 1.9)	1.3 (0.80 to 2.2)	
D4T current	1.8 (1.0 to 3.1)	1.4 (0.83 to 2.3)	1.4 (0.77 to 2.4)	
Dapsone ever	2.5 (1.6 to 3.8)	1.8 (1.2 to 2.7)	1.8 (1.1 to 2.8)	1.6 (1.1 to 2.5)
Ketaconazole ever	2.1 (1.4 to 3.2)	1.4 (0.87 to 2.2)	1.6 (1.0 to 2.5)	
		Risk factors with <20 subjects exposed		
Dapsone current	2.7 (1.7 to 4.1)	2.3 (1.4 to 3.6)	2.0 (1.2 to 3.3)	
PCP	1.9 (1.1 to 3.4)	1.5 (0.86 to 2.5)	1.3 (0.71 to 2.4)	

*PID = pelvic inflammatory disease; PCP = *pneumocystis carinii* pneumonia; AIDS = acquired immunodeficiency syndrome; HIV = human immunodeficiency virus-1; AZT = zidovudine; DDI = didanosine; 3TC = lamivudine; D4T = stavudine.

†Adjusted for Hybrid Capture II relative light unit categories of <1, 1–10, 10 to <100, and ⩾100.

‡CD4 categories are <200, 200–500, and >500 counts/mm^3.

§RRs and 95% CIs were computed only for variables that were related to abnormal anal cytology after adjustment for CD4 or Hybrid Capture II.

associated with an elevated risk of abnormal anal cytology (Table 7).

Other factors of interest were evaluated in univariate analyses and were found not to have substantially elevated RRs or statistically significant associations with abnormal anal cytology. These factors included history of genital herpes, gonorrhea, syphilis, chlamydial infections, vaginal trichomonas, bacterial vaginitis, or vaginal yeast infection; insertion of objects into the anus or oral sexual activities; cigarette smoking or use of alcohol, marijuana, amphetamines, and cocaine; intravenous drug use; history of hemorrhoids, anal fissures or fistulas, or blood in the stool; cancer of any type; conditions, such as shingles, skin rashes, pneumonia other than PCP, wasting syndrome, recent memory problems, peripheral numbness, weight loss, night sweats, asthma, tuberculosis, diabetes, lupus, or rheumatoid arthritis; pregnancy or use of oral contraceptives; race; marital status; sexual orientation; and use of zalcitabine. However, there were few exposed participants in many of these groups.

Other risk factors of interest for abnormal anal cytology were considered in a multivariate model that included HIV viral load, CD4 levels, HPV detected by Hybrid Capture II, history of anal intercourse, and the results of concurrent cervical cytology diagnosis (Table 8). Each of these risk factors was associated independently with abnormal anal cytology except for the CD4 cell count, which was associated with elevated risk estimates but had wide CIs that overlapped unity. Plasma HIV RNA levels above 100 000 copies/mL and Hybrid Capture II RLU ratios above 100 remained associated with the presence of anal cytologic abnormalities, with RLU levels of 10 to less or equal 100 of borderline significance.

Discussion

A screening program to detect ASILs in high-risk individuals may be of value to prevent anal cancer. A recent study *(33)* has concluded that anal cytologic screening among HIV-positive *(33)* and HIV-negative homosexual men should be cost-effective to prevent anal cancer when based on identification of a discrete high-risk population. Little is known of the magnitude of risk for anal squamous abnormalities among HIV-positive and high-risk HIV-negative women. However, recent evidence has shown that women with ASCUS had a 5.1% underlying prevalence of histologically confirmed cervical intraepithelial neoplasia grade 3 *(34)*. Knowledge of the prevalence and risk factors for anal lesions is important before initiating a screening program.

Before this study, few large studies of risk factors have been conducted for anal lesions in HIV-positive and HIV-negative women. In addition, we used PCR to detect low levels of HPV

Table 8. Relative risks (RRs) and 95% confidence intervals (CIs) for an abnormal anal cytology, multivariate model in human immunodeficiency virus-1 (HIV)-positive women

Risk factor	RR (95% CI) adjusted for all other risk factors in model*
HIV viral load, copies/mL	
<4000	1.0 (referent)
4000–20 000	0.98 (0.35 to 2.2)
20 000–100 000	1.5 (0.67 to 2.8)
>100 000	2.4 (1.1 to 3.9)
CD4 cell count/mm^3	
>500	1.0 (referent)
200–500	1.8 (0.65 to 4.0)
<200	2.2 (0.80 to 4.7)
RLU for HPV by HC†	
<1	1.0 (referent)
1 to <10	0.68 (0.14 to 2.8)
10 to <100	2.9 (0.98 to 6.3)
⩾100	4.6 (1.9 to 7.9)
Anal intercourse	
Never	1.0 (referent)
Ever	2.3 (1.2 to 3.6)
Concurrent cervical cytology	
Normal	1.0 (referent)
Abnormal	2.1 (1.0 to 3.6)

*Adjusted RRs and 95% CIs in a multivariate model, including HIV RNA viral load, CD4 counts, human papillomavirus (HPV) detected by HC with three levels of intensity measured in RLUs, history of anal intercourse, and concurrent cervical cytology diagnosis. Adjusted odds ratios were computed by logistic regression with the Zhang and Yu *(32)* correction to approximate the adjusted RR.

†RLU = relative light unit; HC = Hybrid Capture II™.

types and Hybrid Capture II to quantify HPV infection in the anal specimen. We also performed high-resolution anoscopy and biopsy of the majority of our patients with abnormal anal cytology. Similar to results found in cervical cytology *(17),* results in anal cytology do not always correlate with histology from biopsy specimens, and the combination of cytology and histology provides the most accurate assessment of anal lesions. In our study, 13 of 14 patients with histologically confirmed HSILs had ASCUS or LSILs on cytology. These data provide strong support for the importance of anoscopic and histologic assessment of women with ASCUS and LSILs on cytology as well as careful follow-up of these women.

HIV infection in the women who participated in this study was associated with a greater than threefold increased risk for abnormal anal cytology. HIV-positive women were analyzed separately because risk factors for anal lesions may differ between HIV-positive and HIV-negative women. While our study was of limited power to detect risk factors for anal lesions among HIV-negative women, the results show that women with anal HPV infection measured by Hybrid Capture II were much more likely to have abnormal anal cytology than were women with no HPV infection.

Our larger sample of HIV-positive women permitted detailed analyses of factors associated with anal lesions. In a multivariate analysis of HIV-positive women, risk factors for abnormal anal cytology included history of anal intercourse, anal HPV infection as detected by Hybrid Capture II, high levels of plasma HIV RNA, and concurrent abnormal cervical cytology. These results were similar to those found in our previous study of homosexual men *(35).* The findings of two earlier studies of men *(35,36)* showed that HIV-related CD4 cell depletion may have contributed to the development of ASIL, independent of its effects on the level of HPV replication. The mechanisms underlying the relationship between abnormal cervical cytology and abnormal anal cytology are not yet clear but may reflect concurrent HPV infection in the cervix and anus. Since the pathogenesis of SILs at these two sites may be similar, concurrent detection of lesions may reflect the presence of other common risk factors, such as vigor of immune response to HPVs or other unknown factors. We also observed that anal HPV infection and anal intercourse were independent risk factors for abnormal anal cytology. These data suggest that factors associated with anal intercourse other than HPV infection also may play a role in the pathogenesis of anal lesions. We speculate that an example of one such factor could be chronic anal irritation, which previously was shown to be a risk factor for invasive anal cancer *(2).*

A high proportion (26%) of HIV-positive women had abnormal anal cytology similar to that observed in a previous study *(37).* Most of these abnormalities were ASCUS or LSILs in 25% and HSILs in 1%. However, cytology may underestimate the severity of the anal lesions as demonstrated with a follow-up biopsy. Since we were unable to perform high-resolution anoscopy on all of the study participants who had abnormal anal cytology, it is likely that the overall severity of anal lesions in this population was underestimated.

This study was conducted before highly active antiretroviral therapy (HAART) became widely available. Our data showed that more than a quarter of HIV-positive women had abnormal anal cytology. Of interest, when we compared the prevalence of anal lesions in the pre-HAART era in HIV-positive women whose CD4 cell counts were under 500/mm^3 with comparable HIV-positive men who have sex with men, we found that the prevalence of anal disease in women was approximately 70% of that of the men (Palefsky JM, Holly EA, Ralston ML: unpublished data). This result was despite the men having had a larger number of insertive anal intercourse partners and more frequent receptive anal intercourse than the women. Earlier studies of HIV-positive men who have sex with men showed a high incidence of anal HSILs, suggesting that the incidence of HSIL may prove to be high among HIV-positive women with longer follow-up *(30).* A large population of HIV-positive women should be followed prospectively to determine the natural history of ASILs in this population, particularly in the HAART era. If the findings in women continue to resemble those in men who have sex with men, then it is likely that HIV-positive women may benefit from an anal cytology screening program similar to that described for HIV-positive and HIV-negative homosexual men *(33).*

In summary, our study indicates that HIV-positive women were at higher risk of abnormal anal cytology than high-risk HIV-negative women. Risk factors for abnormal anal cytology in HIV-positive women included detection of anal HPV DNA, a lower number of CD4 cells, a higher plasma level of HIV RNA, a history of receptive anal intercourse, and concurrent abnormal cervical cytology. Our study also showed that the HIV-negative women most likely to have abnormal anal cytology were those who were positive for anal HPV DNA. Further work is needed to determine the role of HPV testing to identify HIV-negative women at high risk of ASIL and to describe the natural history of ASILs in HIV-positive and high-risk HIV-negative women.

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NOTES

Supported in part by Public Health Service grants CA63933 (National Cancer Institute), 5M01RR00079 and 5M01RR00083–37 (Division of Research Resources), and U01AI34989 (National Institute of Allergy and Infectious Diseases), National Institutes of Health, Department of Health and Human Services.

Manuscript received September 6, 2000; revised March 16, 2001; accepted March 30, 2001.