Title

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Journal

Clinical Infectious Diseases, 78(3)

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Publication Date

2024-03-20

DOI

10.1093/cid/ciad614

Peer reviewed

Clinical Infectious Diseases

MAJOR ARTICLE

Two-Year Incidence and Cumulative Risk and Predictors of Anal High-Grade Squamous Intraepithelial Lesions (Anal Precancer) Among Women With Human Immunodeficiency Virus

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Background. Detection and treatment of anal histologic high-grade squamous intraepithelial lesions (hHSIL) prevents anal cancer. However, anal hHSIL incidence among women with human immunodeficiency virus (HIV, WHIV) remains unknown. Performance of anal high-risk human papillomavirus ([hr]HPV), anal cytology (anal-cyt), and both for hHSIL detection longitudinally over 2 years also remains undetermined.

Methods. We determined 2-year incidence and cumulative risk estimates (2-y-CR) of anal hHSIL among WHIV using prevalence and incidence (per 100 person-years [py]) observations stratified by baseline hrHPV and/or anal-cyt results.

Results. In total, 229 WHIV with complete baseline data were included in the analysis; 114 women without prevalent anal hHSIL were followed with 2 annual evaluations. Median age was 51, 63% were Black, and 23% were Hispanic. Anal hrHPV or abnormal anal-cyt was associated with an increased risk of incident anal hHSIL at 2 years (18.9/100py [95% confidence interval {CI} 11.4–31.3] and 13.4/100py [95% CI 8.0–22.7], respectively) compared with no detection of anal HPV or negative cytology (2.8/100py [95% CI 1.1–7.4] and 4.2 [95% CI, 1.8–10.2]) The presence of anal hrHPV with abnormal cytology was associated with 2-y-CR of anal hHSIL of 65.6% (95% CI 55.4%–75%); negative hrHPV with negative cytology was associated with 2-y-CR of anal hHSIL of 9.2% (95% CI 7.0–16.0).

Conclusions. Detection of anal hrHPV or abnormal anal cytology are comparable predictors for 2-y-CR of anal hHSIL. The absence of anal hrHPV combined with negative cytology was predictive of a lower (but measurable) risk of developing anal hHSIL. These findings provide important data to inform anal cancer screening guidelines for WHIV.

Keywords. women with HIV; anal HSIL; anal high-risk HPV test; anal cytology; anal cancer screening.

Women with human immunodeficiency virus (HIV, WHIV) are at a disproportionately elevated risk of developing squamous cell carcinoma of the anus (SCCA). Foundational data from the ANal Cancer/HSIL Outcomes Research (ANCHOR) trial confirm that treatment of anal high-grade squamous intraepithelial lesion (HSIL) among persons with HIV (including women) reduces SCCA risk [1]. Algorithms and guidelines similar to those for cervical cancer screening and prevention are needed for detection of anal histologic (h)HSIL, in which at-risk individuals are screened using an anal swab for cytology and/or high-risk (hr) human papillomavirus (HPV), and patients with positive screening tests are referred for diagnostic high-resolution anoscopy (HRA) with directed biopsies. If anal hHSIL is detected, then the patients are subsequently treated.

Screening tests are important for detecting not only prevalent anal hHSIL but also predicting incident (ie, future) anal hHSIL risk, particularly as triage procedures are resource intensive and

Received 11 June 2023; editorial decision 29 August 2023; published online 8 October 2023

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Clinical Infectious Diseases® 2024;78(3):681–9

https://doi.org/10.1093/cid/ciad614

screening tests that accurately predict the absence of hHSIL detection for several years may decrease the need for yearly screening and triage. Understanding the natural history of hHSIL is essential to inform SCCA prevention and guide optimal screening algorithms. Currently, anal hHSIL incidence and predictors of risk among WHIV remain undetermined. Robust estimation of anal hHSIL incidence and associated predictors from longitudinal cohorts are crucial to understand drivers of anal hHSIL that may contribute to SCCA among WHIV but remain undetermined.

The AIDS Malignancy Consortium (AMC) study, "AMC-084: Screening HIV-positive women for anal cancer precursors (AMC084)," was a multi-center national trial that was designed to provide robust estimation of performance of various screening tests for prevalent hHSIL and SCCA [2], including hHSIL prevalence [3] and incidence and associated predictors of hHSIL risk. All enrolled women underwent annual screening evaluations for 2 years including anal cytology, anal HPV tests, and concurrent HRA with biopsy (in contrast to prior studies where HRAs were only conducted in women with abnormal cytology), thus providing unbiased estimates of anal hHSIL prevalence and incidence. In this article, we describe the incidence (one of the three primary endpoints for the AMC-084 protocol) and the 2-year risk of anal hHSIL among the cohort of women enrolled in national study, AMC 084.

METHODS

Study Design and Participants

AMC-084 recruitment occurred between 2014 and 2016. Detailed methods, HSIL prevalence, and performance of screening tests are previously reported in papers presenting the two of the other primary endpoints [2, 3]. Briefly, WHIV were recruited at twelve US sites. The clinicians responsible for performing HRA at each site were certified using a standardized approach focused on quality assessments developed and implemented by the AMC HPV Working Group [3]. The study protocol was approved by the US National Cancer Institute, Cancer Therapy Evaluation Program and by institutional review boards for each participating institution. Potential participants were screened using a standardized questionnaire and medical records review. At baseline, all women were evaluated using a standard protocol. Women without hHSIL were then followed semiannually for 2 years. Follow-up was discontinued if incident anal hHSIL, the primary endpoint, was diagnosed.

Sample and Subjects

Eligible women were 18 years old or older, diagnosed with HIV, had no history of anal HSIL determined based on cytology or histology [2, 3]. Study participants without prior anal hHSIL detection and with at least 1 follow-up visit with HRA and biopsies were eligible for analysis of incident anal hHSIL.

Procedures

At the baseline visit, data was collected for HIV, HPV-related, smoking and sexual history as previously reported [2, 3]. Recent HIV viral load and CD4 count data were collected. Participants underwent a targeted physical exam, including exams of the vulva, vagina/cervix, anus and perianus for signs of HPV-related lesions. Anal specimens were collected for cytology and hrHPV analysis, digital anorectal exam (DARE) and HRA of the anal canal and perianus with biopsies as previously described [3]. Participants were seen every 6 months for 2 years. At each 6-month visit, participants underwent anal evaluations including collecting specimens for anal cytology and hrHPV as well as DARE. At the 12- and 24-month visits, participants underwent collection of specimens for cervical cytology and hrHPV testing, and HRA with at least 2 directed or random biopsies as previously described [2, 3].

Laboratory Testing

Local pathology departments processed the cervical and anal cytology specimens and evaluated the cytology using the Bethesda Classification System [4]. Abnormal cytology was defined as atypical squamous cells of undetermined significance (ASC-US) or higher grade (ASC-US+). Cervical and anal hrHPV analyses were performed at the manufacturers" laboratories (Hybrid Capture 2 (HC2), Qiagen Corporation, Gaithersburg, MD and HPV-Aptima, Hologic Inc., Marlborough, MA) and results were reported to the investigators. The methods of hrHPV testing and histology assessments have been previously reported [2]. Briefly, HPV-HC2 is a signal amplification assay that detects ≥1 picogram of HPV-DNA for a pool of 13 different high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) [5]. HPV-HC2 is hereafter referred to as HPV DNA. HPV-Aptima assay is a nucleic acid amplification test that detects the HPV E6/E7 messenger RNA (mRNA) for a pool of 14 high-risk types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) [6]. HPV detection specimens underwent further analysis with the HPV-Aptima 16 18/45 Genotype Assay [7] to identify specimens with HPV genotypes 16 and 18/45 (designated 16/18/45+). HPV-Aptima is hereafter referred to as HPV mRNA. The study outcome of interest was anal hHSIL (vs no evidence of intraepithelial lesions or low-grade squamous intraepithelial lesions) as determined by the central pathology consensus review. If central review was not available, then local review was used for analysis. Among the 64 with prevalent anal HSIL, 59 were diagnosed on central pathology review and 5 women through local pathology review only.

Statistical Analysis

The primary endpoint for this study is to estimate the incidence *and* the 2-year cumulative risk calculated from prevalent and incident anal HSIL cases observed.

We used the approach by Clarke et al to calculate the 2-year cumulative risk [8]:

$$\text{Cumulative Risk (2years; } x) = p(x) + \{1 - p(x)\}\{1 - S(2\text{years}; x)\}$$

where $p(x)$ is the probability of prevalent anal hHSIL for those with test values x, and $S(2\text{years};x)$ is the hHSIL probability at 2 years for those without prevalent disease and with test values x, estimated from Cox proportional hazards model. Test value x is defined as the characteristic or test result as denoted in column 1 of Tables 1–3. For each cumulative risk estimate, we generated 100 bootstrap samples [9] to calculate 95% confidence intervals (Cis).

The analyses of incident anal hHSIL were restricted to the women whose initial histology results were classified as negative for anal hHSIL at baseline. Incidence rates were computed by dividing the total number of events observed by the total number of person-years of observed follow-up, and comparisons of the incidence rates were performed using Poisson regression [10]. Results from these incidence analyses are reported as incidence rate ratios (IRRs) and 95% CIs.

The prevalence by test value × for this cohort was defined as the ratio of the number of women diagnosed with anal hHSIL at baseline to the number of enrolled women with test values x. Log-binomial regression was used to estimate the prevalence and 95% CI [11].

All statistical analyses except bootstrapped 95% CI around cumulative risk were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). Bootstrapped 95% CI around 2-year cumulative risk was generated using R (version 4.2.1). *P* values less than .05 were interpreted as indicating statistical significance.

RESULTS

Of the 229 WHIV enrolled in the study with complete baseline data, 64 (28%) women had prevalent anal hHSIL (by local or central pathology read), 51 (22%) were excluded because of lack of follow-up, 114 (50%) women without prevalent anal hHSIL and having at least 1 follow-up HRA were included in the analysis of incident anal HSIL (see Figure 1), and the full cohort of 229 was used for the 2-year cumulative risk calculations. The median age of WHIV in the follow-up cohort of the 114 women was 51 years (interquartile range [IQR] 44–55). Women were predominantly non-Hispanic African Americans (72 or 63%) and current or former smokers (67 or 60%). Nearly half (54 or 48%) reported a history an abnormal cervical cytology, 58% (65) reported at least 1 lifetime male anal sex partner, and nearly half (50 or 45%) reported a prior sexual assault (see Table 1). The majority had well controlled HIV infection (viral load <200 copies/mL); the median CD4 count was 691 (IQR 533–920) cells/μL, and 90% (102) of the women had CD4 counts >350 cells/μL. In total, 27% (30) had abnormal cervical/vaginal cytology, and 23% (26) tested positive for cervical/vaginal hrHPV-DNA. The 51 participants excluded from incident hHSIL analyses due to lack of follow-up had similar demographic and clinical characteristics compared with the studied cohort (data not shown).

The overall incidence (per 100 py) of anal hHSIL among WHIV was 8.5 per 100 person-years (py) [95% CI, 5.45–13.40]. Anal hHSIL incidence (per 100 py) did not differ by age categories (<50-year-old vs ≥50-year-old: 9.1 [95% CI, 4.74–17.51] vs 8.1 [95% CI, 4.35–15.04]) or race/ethnicity (non-Hispanic Black [11.9 {95% CI, 7.26–19.34}]) when compared to Hispanic women (5.7 [95% CI, 1.82–17.55]). Incidence estimates did not significantly differ by smoking status (former/current and never); or nadir CD4 count (≤200 and >200 cells/μL) (Table 1). Similarly, the 2-year cumulative risk did not differ by age, race, smoking status. or nadir CD4 (Supplementary Table).

Table 2 shows the relationship between cervical cytology and hrHPV test at baseline and incident anal hHSIL. Although abnormal cytology (defined as ASC-US+) and any hrHPV were not significantly associated with hHSIL, atypical squamous cells cannot rule out HSIL (ASC-H), and HSIL cytology and HPV-16 were associated with anal hHSIL (incidence rate ratio [IRR] 10.5, 95% CI, 2.39–46.27) and (IRR 8.7, 95% CI, 1.68–44.82, respectively) (Table 2). Detection of any cervical hrHPV (compared with no detection of cervical hrHPV) was associated with a higher 2-year cumulative risk of anal hHSIL (49.2%, 95% CI, 41.4%–59.2% and 33.5% CI 27.9–39.6, respectively) (Supplementary Table).

The hHSIL incidence (per 100 py) among participants with abnormal anal cytology (13.4 [95% CI 8.0–22.7]) differed significantly as compared to participants having negative anal cytology (4.2 [95% CI 1.8–10.2]) (Table 3). Similarly, a statistically significant difference in hHSIL incidence was observed by anal hrHPV positivity status at baseline. HPV detection by DNA was associated with an increased risk of incident anal hHSIL (incidence of 18.9 [95% CI 11.4–31.3]) compared with no detection of anal HPV (2.8 [95% CI 1.1–7.4]); the mRNA results were similarly associated (see Table 3). Co-testing with cytology and hrHPV testing demonstrated that the incidence was 11-fold greater (IRR = 10.8; 95% CI, 2.4–48.5) higher with detection of hrHPV (DNA testing) and abnormal cytology (incidence 25.0 [95% CI 14.2–44.1]) compared to negative cytology in combination with no HPV detection (incidence 2.3 [95% CI, .7–9.2]).

The 2-year cumulative risk of anal hHSIL was similarly high for detection of anal hrHPV (58.7% [95% CI 51.2%–70.3%]) or abnormal anal cytology (52.1% [95% CI, 44.0%–59.5%]) compared with absence of anal hrHPV (18.7% [95% CI 15.0%–25.4%]) or negative cytology (18.3% [95% CI, 13.5%–5.3%]). Assessing the different combinations of co-testing (concurrent hrHPV and cytology results) demonstrated that the combination of detection of anal hrHPV and abnormal cytology was most likely to be associated with anal hHSIL at 2 years

Table 1. Anal hHSIL Incidence and Incidence Rate Ratio by Demographic and Clinical History

Characteristics	Non-Prevalent Anal hHSIL Cohort N = 114	Incident hHSIL n (%)	Person Years	Incidence per 100 py	95% CI (Incidence per 100 py)	Incidence Rate Ratio	95% CI (Incidence Rate Ratio)	*P* Value
Overall	114 (100)	19 (16.7)	222.3	8.5	5.45–13.40			
Demographic characteristics								
Age								
<40 y	14 (12)	3 (21.4)	26.5	11.3	3.64–35.04	1.4	.38–5.07	.61
40–49 y	36 (32)	6 (16.7)	72.2	8.3	3.73–18.5	1.0	.37–2.82	.96
≥50 y	64 (56)	10 (15.6)	123.5	8.1	4.35–15.04	ref		
Race/Ethnicity								
NH Black	72 (63)	16 (22.2)	135.0	11.9	7.26–19.34	2.1	.61–7.19	.24
NH White or Other	16 (14)	0 (0)	34.3	0.0	0.0	NA	NA	NA
Hispanic	26 (23)	3 (11.5)	53.0	5.7	1.82–17.55	ref		
Smoking status								
Former/Current	67 (60)	12 (17.9)	130.1	9.2	5.23–16.23	1.2	.45–2.94	.76
Never	45 (40)	7 (15.5)	87.9	8.0	3.80–16.71	ref		
Education								
High school diploma or less	59 (53)	7 (11.9)	118.8	5.9	2.81–12.36	0.5	.20–1.36	.19
Some college or higher	52 (47)	11 (21.1)	98.5	11.2	6.18–20.15	ref		
Annual income								
<$20K	90 (84)	16 (17.78)	171.7	9.3	5.71–15.21	1.1	.33–3.84	.86
≥$20K	17 (16)	3 (17.65)	36.1	8.3	2.68–25.78	ref		
Marital status								
Married/Not married, living with someone	26 (23)	6 (23.1)	49.0	12.2	5.50–27.27	1.6	.62–4.30	.32
Divorced/Widowed/ Single	88 (77)	13 (14.8)	173.3	7.5	4.34–12.92	ref		
HIV characteristics								
Current CD4 T-cell count								
≤200 cells/mm^3	4 (3)	1 (25.0)	8.9	11.2	1.57–79.36	1.2	.16–9.12	.85
201–350 cells/mm^3	8 (7)	0 (0)	17.3	NA	NA	NA	NA	NA
>350 cells/mm^3	102 (90)	18 (17.6)	196.1	9.2	5.78–14.57	ref		
Viral load								
Suppressed (≤200 copies/mm^3)	99 (88)	17 (17.2)	192.5	8.8	5.49–14.21	1.2	.28–5.32	.78
Unsuppressed (>200 copies/mm^3)	14 (12)	2 (14.3)	27.8	7.2	1.79–28.73	ref		
Nadir CD4 T-cell count								
≤200 cells/mm^3	46 (42)	7 (15.2)	91.5	7.6	3.64–16.04	0.8	.33–2.18	.73
>200 cells/mm^3	63 (58)	11(17.4)	121.6	9.0	5.01–16.33			
Current cART user								
Yes	107 (95)	18 (94.7)	207.7	8.7	5.46–13.75	1.1	.15–8.21	.93
No/unsure	6 (5)	1 (16.7)	12.7	7.9	1.11–56.11	ref		
Reported clinical history								
Lifetime male anal sex partners								
0	48 (42)	11 (22.92)	90.2	12.2	6.75–22.02	ref		
1+	65 (58)	8 (12.31)	129.9	6.1	3.1–12.3	0.5	.20–1.25	.14
History of anogenital warts								
Yes	22 (20)	6 (27.3)	41.1	14.6	6.56–32.51	2.1	.80–5.71	.13
No	89 (80)	12 (13.5)	176.1	6.8	3.87–12.0	ref		
History of abnormal cervical cytology								
Yes	54 (48)	11 (20.4)	10.5.4	10.4	5.78–18.83	1.5	.60–3.71	.39

Table 1. Continued

Characteristics	Non-Prevalent Anal hHSIL Cohort N = 114	Incident hHSIL n (%)	Person Years	Incidence per 100 py	95% CI (Incidence per 100 py)	Incidence Rate Ratio	95% CI (Incidence Rate Ratio)	*P* Value
No/unsure/declined	59 (52)	8 (13.5)	114.5	7.0	3.49–13.97	ref		
History of sexual assault								
Yes	50 (45)	10 (20.0)	96.0	10.4	5.60–19.35	1.4	.56–3.42	.47
No/declined	61 (55)	9 (14.7)	120.0	7.5	3.90–14.40	ref		

Abbreviations: cART, combination antiretroviral therapy; CI, confidence interval; hHSIL, high-grade squamous intraepithelial lesions; HIV, human immunodeficiency virus; NH, non-hispanic; ref, reference.

Table 2. Anal hHSIL Incidence and Incidence Rate Ratio by Baseline Cervical/Vaginal Screening Results

Baseline Cervical/vaginal Screening Results	Non-Prevalent Anal hHSIL Cohort N = 114	Incident hHSIL n (%)	Person Y	Incidence per 100 py	95% CI (Incidence per 100 py)	Incidence Rate Ratio	95% CI (Incidence Rate Ratio)	*P* Value
Cervical/vaginal cytology								
ASC-H/HSIL	2 (2)	2 (100)	2.2	92.3	23.08–369.0	10.5	2.39–46.27	<.001
ASC-US/LSIL	28 (25)	3 (10.7)	56.2	5.3	1.72–16.54	0.6	.17–2.11	.43
NILM[a]	82 (73)	14 (17.1)	159.5	8.8	5.20–14.82	ref		
Cervical /vaginal cytology								
Not NILM	30 (27)	5 (16.7)	58.4	8.6	3.56–20.57	0.9	.35–2.71	.96
NILM	82 (73)	14 (17.1)	159.5	8.8	5.20–14.82	ref		
Cervical/vaginal HPV DNA								
HPV+	26 (23)	6 (23.1)	47.3	12.6	5.69–28.20	1.7	.65–4.49	.28
HPV–	88 (77)	13 (14.7)	175	7.4	4.31–12.80	ref		
Cervical /vaginal HPV (mRNA)								
HPV+	26 (23)	7 (26.9)	49.3	14.2	6.77–29.79	2.1	.81–5.20	.13
HPV–	88 (77)	12 (13.6)	173	6.9	3.94–12.21	ref		
Cervical /vaginal HPV positive (mRNA)								
HPV16+	2 (8)	2 (100)	2.2	92.2	23.08–369.01	8.7	1.68–44.82	.0097
HPV+ (not HPV16)	24 (92)	5 (20.8)	47.1	10.6	4.42–25.49	ref		

Abbreviations: ASC-US, atypical cells of undetermined significance; CI, confidence interval; DNA, Deoxyribonucleic acid; hHSIL, high-grade squamous intraepithelial lesions; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy, ref, reference.

[a]NILM: Negative for intraepithelial lesion or malignancy.

(65.6% [95% CI, 55.4%–75%]) and conversely negative hrHPV with negative cytology was least likely to be associated with anal hHSIL (9.2% [95% CI 7.0%–16.0%]) (see Table 3).

DISCUSSION

To our knowledge, this study is the first to report the 2-year incidence, 2-year cumulative risk, and predictors for anal hHSIL in a cohort of women with HIV. Anal hHSIL incidence was 8.5 per 100 py. Baseline detection of anal hrHPV (with HPV-Aptima or HC2) or abnormal anal cytology were associated with development of anal hHSIL (incidence of 18.4/100py and 13.4/100py, respectively), whereas the absence of anal hrHPV or negative cytology were predictive of a significantly lower incidence of anal hHSIL (2.8/100py and 4.2/100py, respectively). The 2-year cumulative incidence was significantly higher for detection of anal HR HPV DNA (52%) or abnormal anal cytology (59%) but remained over 18% for women with baseline absence of HR HPV DNA or negative baseline anal cytology.

Previously published prospective longitudinal studies of people with HIV included only men who have sex with men (MSM). The Study of the Prevention of Anal Cancer (SPANC) study

Table 3. Prevalence, Incidence, Incidence Rate Ratio and Cumulative Risk of Anal hHSIL by Baseline Anal HPV Test and/or Anal Cytology

	Study Cohort (N = 229)	Prevalent Anal hHSIL (%) (N = 64)	95% CI Prevalence	Study Cohort Incidence N = 114	Incidence per 100 py (95% CI)	Incidence Rate Ratio (95% CI)	*P* Value*	2-y Cumulative Risk (%) (95% CI)	*P* Value*
Overall	229	64 (27.9)	22.1–33.8	114	8.5 (5.4–13.4)	—	—	38.3 (33.7–43.8)	—
Baseline anal screening results									
Anal cytology									
Abnormal cyrology	135 (59)	53 (39.3)	31.8–48.4	57 (50)	13.4 (8.0–22.7)	3.17 (1.1–8.8)	.0268	52.1 (44.0–59.5)	<.001
NILM[a]	94 (41)	11 (11.7)	6.7–20.4	57 (50)	4.2 (1.8–10.2)	Ref		18.3 (13.5–25.3)	
Anal HPV (DNA)									
HPV+	111 (48)	46 (41.4)	33.2–51.7	45 (39)	18.9 (11.4–31.3)	6.8 (2.2–20.4)	<.001	58.7 (51.2–70.3)	<.001
HPV–	118 (52)	18 (15.2)	10.0–23.3	69 (61)	2.8 (1.1–7.4)	Ref		18.7 (15.0–25.4)	
Anal HPV (mRNA)									
HPV+	102 (45)	48 (47.1)	38.3–57.8	35 (31)	18.4 (10.2–33.3)	3.75 (1.5–9.3)	.0044	64.7 (56.5–73.2)	<.001
HPV–	127 (55)	16 (12.6)	8.0–19.9	79 (69)	4.9 (2.4–9.8)	Ref		18.5 (14.1–21.9)	
Anal HPV 16+[b]	30 (13.1)	22 (73.3)	59.1–90.9	5 (4.4)	34.9 (11.2–108.3)	4.7 (1.3–16.0)	.0143	87.6 (NE)	NE
Anal HPV 16–	199 (86.9)	42 (21.1)	16.1–27.6	109 (95.6)	7.4 (4.6–12.2)	Ref		30.5 (25.3–36.9)	Ref
Anal HPV (DNA) and/or cytology									
HPV+ and abnormal cytology	84 (36.7)	40 (47.6)	38.1–59.6	29 (25.4)	25.0 (14.2–44.1)	10.8 (2.4–48.5)	.0018	65.6 (55.4–75.3)	<.001
HPV+ and NILM	27 (11.8)	6 (22.2)	11.0–45.0	16 (14.0)	9.6 (3.1–29.7)	4.1 (.7–24.8)	.1191	36.8 (22.0–54.0)	.001
HPV– and abnormal cytology	51 (22.3)	13 (25.5)	15.9–40.8	28 (24.6)	3.5 (.9–14.2)	1.5 (.2–10.9)	.6665	30.5 (25.0–38.7)	.03
HPV– and NILM	67 (29.3)	5 (7.5)	3.2–17.3	41 (36.0)	2.3 (.7–9.2)	Ref	Ref	9.2 (7.0–16.0)	Ref
Anal HPV (mRNA) and/or cytology									
HPV+ and abnormal cytology	83 (35.8)	44 (53.7)	43.9–65.6	26 (22.8)	21.2 (11.0–40.7)	7.1 (1.9–26.3)	.0032	69.6 (58.2–79.1)	<.001
HPV+ and NILM	20 (8.7)	4 (20.0)	8.3–48.0	9 (7.9)	11.7 (2.9–46.7)	3.9 (.7–23.5)	.1388	40.2 (20.1–64.0)	.005
HPV– and abnormal cytology	53 (23.1)	9 (17.0)	9.4–30.8	31 (27.2)	8.1 (3.3–19.4)	2.7 (.7–11.4)	.1702	25.1 (17.0–34.3)	.07
HPV– and NILM	74 (32.3)	7 (9.5)	4.7–19.1	48 (42.1)	3.0 (.9–9.2)	Ref		12.7 (9.0–20.0)	Ref
Anal HPV 16 (mRNA) and/or cytology									
HPV16+ and abnormal cytology	25 (10.9)	19 (76.0)	60.9–94.7	4 (3.5)	30.3 (7.6–121.3)	8.8 (1.6–48.0)	.0120	86.5 (NE)	NE
HPV16+ and NILM	5 (2.2)	3 (60.0)	29.3–122.7	1 (0.9)	50.1 (7.1–355.9)	14.5 (1.5–130.1)	.0166	Model did not converge	NE
HPV16– and abnormal cytology	110 (48.0)	34 (30.9)	23.4–40.9	53 (46.5)	12.3 (7.0–21.6)	3.6 (1.1–11.0)	.0277	44.4 (36.2–54.1)	<.001
HPV16– and NILM	89 (38.9)	8 (9.0)	4.6–17.4	56 (49.1)	3.4 (1.3–9.2)	Ref	Ref	13.9 (9.0–18.7)	Ref

Abbreviations: CI, confidence interval; DNA, deoxyribonucleic acid; hHSIL, high-grade squamous intraepithelial lesions; HPV, human papillomavirus; NE, could not be estimated; NILM, negative for intraepithelial lesion or malignancy; Ref, reference.

[a]NILM: Negative for intraepithelial lesion or malignancy.

[b]HPV16 was only tested on those specimens where HR HPV was detected.

[9] conducted in Australia included 397 HIV negative and 220 HIV positive gay and bisexual (GBM) men followed with annual HRA evaluations over 3 years to determine the natural history of anal HPV and anal HSIL (defined as composite (c) HSIL, ie, either with HSIL cytology and/or HSIL histology). The SPANC study found that for those without prevalent anal cHSIL, the incidence of anal cHSIL was 10.1/100py in HIV-negative GBM compared with 14.0/100py for GBM with HIV [12]. Risk factors for anal cHSIL incidence included age younger than 45 years, as well as diagnosed with HIV. Anal hrHPV status at the current visit was a strong predictor of concurrent anal cHSIL; cHSIL incidence was 26.2, 11.0, and 2.8 per 100py in men with HPV16, other hrHPV or no hrHPV detected, respectively. Jongen et al [13] reported a study from the Netherlands of 107 MSM with HIV without prevalent anal hHSIL who had a follow-up HRA 1–4.5 years from the first HRA and found that the incidence of anal hHSIL was 15.9 per 100py (95% CI, 10.7–23.5) and was not associated with any clinical or demographic factors. Only 107 of the 807 study participants without prevalent hHSIL had a follow-up HRA.

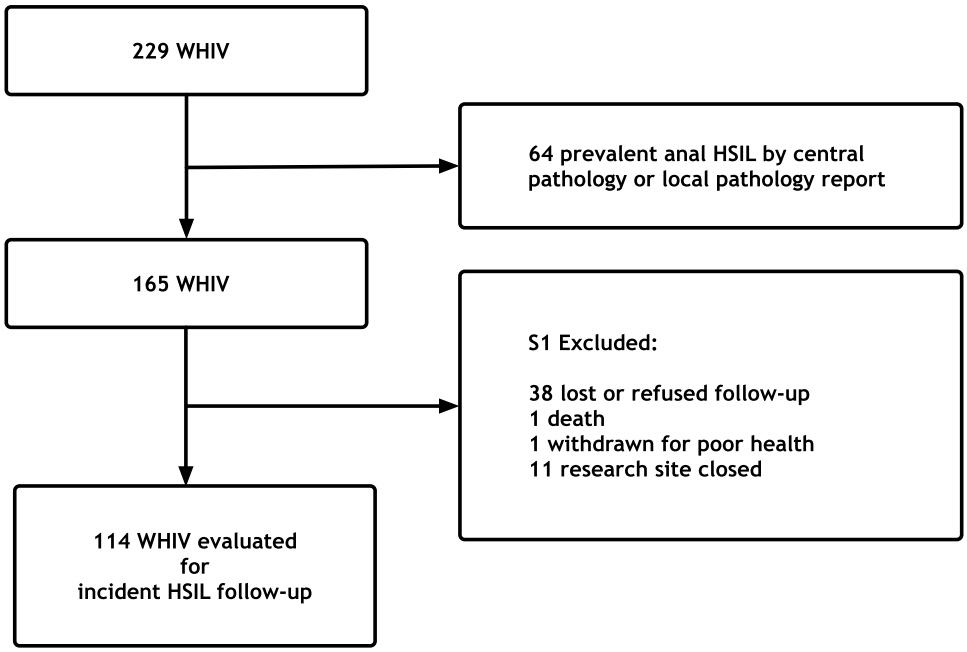

Figure 1. Consort diagram. Abbreviations: HSIL, high-grade squamous intraepithelial lesions; WHIV, women with human immunodeficiency virus.

Clark et al reported on a cohort of 135 (of the 259) MSM LWH without prevalent hHSIL who had at least 1 follow-up HRA and found that 25 had incident anal hHSIL over a median of 2.1 years [8]. In that study, predictors of incident anal hHSIL included detection of HPV (incident HSIL at 2 years HPV positive—10% and HPV negative at 3.3%) and abnormal anal cytology (ASC-US+ 10.6% and normal 3.9%). We found that the incidence of anal hHSIL in WHIV is similar to that of GBM without HIV (from the SPANC study).

Our findings also suggest that ASC-H or HSIL cervical cytology, or cervical HPV-16 detection were associated with a higher risk of anal hHSIL. Thus, because other demographic variables (including age and prior abnormal cytology) do not appear to be associated with anal hHSIL, recent cervical ASC-H/HSIL or HPV-16 results may increase the clinical suspicion for anal HSIL.

Multiple studies have reported on the prevalence and risk factors for anal hHSIL in WHIV however we are unaware of any studies reporting anal hHSIL incidence among WHIV. Liu et al [14] report prevalence data from a cohort of 381 WHIV, where women with ASC-US+ (68%) were referred for HRA; 42% of whom were found to have anal hHSIL (comparable to 37% of women with abnormal anal cytology having anal hHSIL in our cohort). In a collaborative pooled analysis by Lin et al [15] described an hHSIL prevalence of 7.7% among 1003 WHIV included in the analysis, and that detection of cervical HPV was associated with anal hHSIL. Interestingly, although we did find that cervical HPV was significant in univariate analysis for prevalent anal hHSIL, the results were not significant in multivariate analysis [3].

Unlike in cervical cancer screening, where HPV testing alone is significantly predictive of future cervical hHSIL (compared with cervical cytology), we found that anal hrHPV testing, cytology, or cotesting with cytology and HPV are all comparable predictors of future hHSIL. The lack of detection of anal hrHPV in combination with normal cytology had the lowest 2-year risk of anal hHSIL, yet co-testing would still miss 9.2% and 12.7% of hHSIL cases, respectively, using the Qiagen and Aptima tests. The number of HPV-negative anal hHSIL, especially for prevalent cases was higher than expected (when compared to negative co-testing in cervical samples). Because the hrHPV detection thresholds utilized for the Qiagen and Aptima tests are optimized for cervical specimens, the anal hrHPV testing threshold may need to be decreased in order to adjust for the relatively acellular anal specimens. A more precise (sensitive) biomarker for anal hHSIL would greatly reduce the number of patients referred for repeated HRAs in the future. Promising candidates include optimized HPV assays (using restrictive genotyping and/or alternate cycle thresholds) [16], methylation markers [17, 18] and automated dual staining [19].

The strengths of this study include the racial and ethnic diversity of the participants and that all women enrolled in the study underwent high-resolution anoscopy (HRA) and biopsy (not only women with abnormal cytology). Furthermore, all the clinicians who performed HRAs underwent a rigorous

certification process before study sites were activated. In addition, our study is the first to measure the 2-year anal hHSIL cumulative risk, a methodology that has been utilized in large cervical screening cohorts, which provides a basis for comparing screening tests at the 2 anatomic sites. The limitations of the study include: no central review of the local cytology interpretations, the use of commercial non-polymerase chain reaction (PCR)-based HRHPV assays, 21.8% loss to follow-up, small number of HPV 16 cases, small number of different populations, and the small number of incident anal hHSILs, limiting the study's power, particularly to determine the additional predictive value of HPV and cytology cotesting.

CONCLUSION

WHIV have a high incidence of anal hHSIL, and detection of anal HPV and abnormal anal cytology predict prevalent and incident anal hHSIL. However, the converse, negative hrHPV and negative cytology, do not assure the absence of prevalent or incident anal hHSIL. These crucial data suggest that further improvement in anal HR HPV testing sensitivity is needed. In addition, the current study will inform anal cancer screening guidelines and suggest that annual screening with hrHPV testing or cytology may be needed, and women with a recent cervical ASC-H/HSIL cytology or cervical HPV-16 detection should be prioritized for anal cancer screening . Longer follow-up and further evaluation of additional biomarkers are needed to improve algorithms for anal cancer prevention.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. E. Y. C., J. L., E. A. S., and A. A. D. planned the study; E. C., H. J., M. J., and E. A. S. wrote the manuscript; E. A. S., M. H. E., N. J., J. M. B.-L., JMP, T.W., G. E., A. L. F., L. F. B., R. L., H. M. G., and E. Y. C. did acquisition of data; H. J. and M. J. designed the analysis; H. J. and M. J. analyzed the data; E. Y. C. and E. A. S. interpreted the data. All authors read and approved the final manuscript and critically revised the paper.

Financial support. This work was supported by the National Cancer Institute at the National Institutes of Health (NIH), U54 grant number U54CA242646 to J. M. P.; grant number CA163103 to E. Y. C. and E. A. S. (principal investigator [PI]: Dr Elizabeth Chiao); and AIDS Malignancy Consortium grant number UM1CA121947 to E. Y. C., E. A. S. (also reported by A. L. F., J. L., R. A. L., J. M. B.-L., and H. M. G.). (PI: Dr Joseph Sparano). H. J. was supported by the AIDS Malignancy Consortium as an employee of Mount Sinai Hospital. Hologic, Qiagen, and HPV-Aptima provided in-kind contributions for HPV testing.

Potential conflicts of interest. J. M. P. reports consulting fees from Vir Biotechnologies, Roche Diagnostics, Abbott, Antiva Biosciences, and Merck and Co.; consulting fees and stock options from Virion Therapeutics; travel support from Merck and Co.; an unpaid role with the International Papillomavirus Society; research support to University of California, San Francisco (UCSF) and gifts from Atila Biosystems. M. H. E. reports research support to institution from J&J, Pfizer, Inovio, PDS Biotechnologies, AstraZeneca, Imvax, Iovance, B-D, and Zentalis; consulting fees (paid to institution) from Papivax, Merck, B-D, PDS Biotechnologies, and World Health Organization; travel support for meetings from Merck and Zentalis; and participation on Merck's Advisory Board for HPV research. T. W. reports institutional grants or contracts from Merck; payment or honoraria for speaking engagements from ViiV Healthcare and Merck; travel support and donation of vaccine for trials from Merck. E. A. S. reports travel support from National Cancer Institute and ASCCP; and board membership with ASCCP. R. A. L. reports a role on their institutions data safety monitoring board. H. M. G. reports a contract with the University of Puerto Rico; honoraria for lectures from Salud Integral de la Montana, American College of Physicians, and Infectious Diseases Society of Puerto Rico; payment for expert testimony (21-CV-001169-FAB; CG2020CV01235 (Caguas, 101); C DP2015-0062, Arecibo); travel support from University of Puerto Rico School of Medicine; and unpaid roles as President of Infectious Diseases Society of Puerto Rico and membership on the Puerto Rico Medical Task Force for COVID-19. A. L. F. reports institutional grants from NIH. G. E. reports honoraria from Northeast/Caribbean AIDS Education and Training Center (NECA AETC) and International AIDS Society – USA (IAS-USA). A. A. D. reports consulting fees from Value Analytics Lab unrelated to the current work. LFB reports an unpaid role as President of International Anal Neoplasia Society. N. J. reports Board membership for International Anal Neoplasia Society. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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