Anal Intraepithelial Neoplasia in Heterosexual and Homosexual HIV-Positive Men with Access to Antiretroviral Therapy

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Background. Studies of human immunodeficiency virus (HIV)–positive men have demonstrated high rates of anal intraepithelial neoplasia (AIN), a precursor to anal carcinoma, mostly in white homosexual men and men not receiving effective antiretroviral therapy (ART).

Methods. Ninety-two participants—53% Latino, 36% African American, and 40% without a history of receptive anal intercourse (RAI)—were evaluated with a behavioral questionnaire, liquid-based anal cytological testing, Hybrid Capture 2 human papillomavirus (HPV) DNA assay and polymerase chain reaction, and anal colposcopy with biopsy of lesions.

Results. High-risk HPV DNA was identified in 61%, and this was associated with a history of RAI (78% vs. 33%; \( P < .001 \)); 47% had abnormal cytological results, and 40% had AIN on biopsy. In multivariate analysis, both were associated with a history of RAI (odds ratio [OR], 10 [\( P < .001 \]) and OR, 3.6 [\( P = .02 \)], respectively) and lower nadir CD4+ cell counts (\( P = .06 \) and \( P = .01 \)). Current ART use was protective (OR, 0.09; \( P < .01 \) and OR, 0.18; \( P = .02 \)).

Conclusions. Although anal infections with high-risk HPV and AIN in HIV-positive men are associated with a history of RAI, both conditions are commonly identified in HIV-positive men without this history. Both lower nadir CD4+ cell counts and lack of current ART were associated with AIN but not with the detection of anal HPV.

Anal carcinoma is strongly related to infection with high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) of human papillomavirus (HPV), as has been seen for cervical, vaginal, vulvar, and penile cancer [1–5]. The incidence of anal cancer in men who have sex with men (MSM) is estimated to be 35 cases/100,000 person-years [6]. This incidence is comparable to that observed for cervical cancer before the introduction of routine Papanicalaou screening. The rate of anal cancer is twice as high in HIV-positive than in HIV-negative MSM [7].

Investigators have used anal cytological testing and colposcopy to test for anal intraepithelial neoplasia (AIN), which are precancerous lesions that can progress to invasive cancer. A high incidence and prevalence of AIN have been reported in HIV-positive and -negative MSM [8–12]. These studies have included predominantly white men and have not included HIV-positive men without a history of sex with men, despite some evidence that these men are also at increased risk for anal cancer [7].

Our study was performed to determine the prevalence of anal HPV infection and AIN in a diverse population of HIV-positive men, including men of color, men with and without a history of sex with other men, and men receiving or not receiving effective ART.
SUBJECTS, MATERIALS, AND METHODS

Study population. Participants were enrolled from June 2001 through March 2002. Clinicians referred men from the Columbia-Presbyterian Medical Center Infectious Diseases Clinic, an urban clinic that serves ~1000 HIV-positive patients. The racial/ethnic breakdown of the referring clinic is 60% Hispanic or Latino, 35% African American, and 5% white. Sixty percent of the patients are male, ~30% of whom self-identify as gay or homosexual. Exclusion criteria included a platelet count <75,000 cells/mm$^3$ and an absolute neutrophil count <1000 cells/mm$^3$. Primary care providers from the referring clinic were asked to refer men without regard to the patient’s previous history of genital or perianal condyloma, other sexually transmitted diseases, or sexual behavior. Informed consent was obtained from all patients. Human-experimentation guidelines of the US Department of Health and Human Services (DHHS) and those of Columbia University were followed during our study.

Behavioral questionnaire. An investigator who was not the participant’s primary care provider administered a questionnaire that elicited a detailed sexual history, with questions on the history of receptive anal intercourse (RAI) and participant-defined sexual behavior (heterosexual, homosexual, or bisexual), lifetime number of female partners, lifetime number of male partners, lifetime number of RAI partners, age at onset of sexual activity, receptive oral-anal contact, and insertion of fingers or sex toys during sexual intercourse, as well as questions on tobacco use and history of sexually transmitted diseases.

Cytological testing. Anal cytological results were evaluated by use of 2 different methods: (1) a Dacron swab premoistened in saline was used to obtain cells from the anal canal that were then spread onto a glass slide and fixed with spray cytology fixative; and (2) a conical cytobrush with a blunt tip (cervical specimen collection kit; Digene) that was premoistened in saline was used to obtain cells from the anal canal that were subsequently transferred to a liquid-based cytology vial (Thin Prep; Cytyc) and processed according to the manufacturer’s instructions for cervical cytological testing. The liquid-based cytological testing led to an interpretable result more often than the conventional method and was chosen as the outcome of interest for the analysis.

HPV testing. After the cytological specimens were obtained, an additional cytobrush was used to collect a sample from the anal canal for the Hybrid Capture 2 (hc2) HPV DNA assay for high-risk types of HPV (Digene). The HPV types present in samples found to contain high-risk HPV DNA when hc2 was used were determined by use of polymerase chain reaction (PCR) with consensus Ll PGM09/11 primers and reverse-line blot hybridization (provided by Janet Kornegay, Roche Molecular Diagnostics, Pleasanton, CA). For PCR, DNA was isolated by use of spin columns (Qiagen) from an aliquot of the liquid cytological medium.

Anal colposcopy. Anal colposcopy was performed as described elsewhere on all participants [16]. In brief, a disposable anoscope coated with a mixture of surgical lubricant and 2% tetracaine gel was used to introduce an applicator stick wrapped in gauze that had been soaked in 3% acetic acid, which was then left in place for 2 min. A colposcope was then used to view the walls of the anal canal under magnification. Any areas of acetowhitraining or vascular changes suspicious for AIN were sampled by use of cervical biopsy forceps. If no visible lesions were seen, then no biopsy was done. Subsequently, if either of the 2 anal cytological specimens was diagnosed as atypical squamous cells (ASC) or greater and no biopsy had been performed at the time of anal colposcopy, the participant was asked to return for repeat anal colposcopy and biopsy.

Pathologic classification. The liquid-based and conventional cytological specimens were interpreted by use of the Bethesda criteria [17]. Anal biopsies were interpreted by use of a 2-tiered terminology (low-grade and high-grade AIN) with criteria established elsewhere for cervical biopsy samples [18]. Cytological specimens were initially screened by a cytotecnologist, with the final interpretation being made by a single pathologist who was blinded to all clinical information and other pathological results.

Medical record review. An investigator performed a standardized review of the participants’ medical records to ascertain the nadir CD4$^+$ cell count (i.e., the lowest CD4$^+$ cell count recorded), current CD4$^+$ cell count and log$_{10}$ HIV RNA load, current receipt of ART, and length of time of having an HIV RNA load <400 copies/mL.

Statistical analyses. The outcomes of interest were the presence of high-risk HPV DNA according to hc2 (relative light units $\geq$ 1 were considered to indicate positivity), abnormal results of liquid-based cytological testing (atypical squamous cells or low- or high-grade squamous intraepithelial lesions), and AIN according to the results of histological testing. A participant was classified as having normal results in the histology-based analysis if no biopsy was performed because of a lack of visible lesions on anal colposcopy with normal cytological results or if there was no evidence of condyloma accuminata, AIN, or cancer on any of the biopsy specimens. If the participant had an abnormal cytological result but did not return for the biopsy, he was excluded from the histology-based analysis. The association of univariate predictors was calculated by use of the $\chi^2$ test. Multivariate models were constructed by use of logistic regression, and the same model was selected for all 3 outcomes (the presence of high-risk HPV, abnormal cytological result, and AIN according to the results of histological testing). Current and nadir CD4$^+$ cell counts were analyzed as continuous variables after log transformation to normalize the dis-
RESULTS

Study population. Table 1 describes the study population of 92 men. Eighty-nine percent were either Latino or African American. Seventy-five (82%) were receiving ART at the time of the study, and most had a good virologic response (61% had an HIV RNA load <400 copies/mL). Of the 17 men not receiving ART, 7 had a CD4+ count <350 cells/mm³ and therefore met DHHS criteria for consideration for starting ART [19]. Sixty percent of participants reported a history of RAI, including all 46 homosexual men, 8 of 10 bisexual men, and 1 of 35 heterosexual men.

High-risk HPV analyses. Overall, 56 (61%) of 92 participants were found to have high-risk HPV DNA according to the results of hc2. The strongest predictor of positivity for high-risk HPV DNA was a history of RAI (table 2). Age ≤40 years was also associated with the detection of high-risk HPV DNA. In the multivariate analysis (table 2), only a history of RAI remained significant. There was a trend toward nadir CD4+ cell count being associated in the multivariate analysis. Other factors—such as race/ethnicity (Hispanic/Latino, African American, or white/other); tobacco use (current or lifetime); total number of sex partners; number of female, male, or RAI partners; history of receptive oral-anal intercourse or insertion of fingers or sex toys during intercourse; and age at onset of sexual activity—were not associated with high-risk HPV DNA positivity. Neither current plasma HIV RNA load, current CD4+ cell count, current receipt of ART, nor time receiving ART was associated with high-risk HPV. HPV DNA PCR was attempted on the 56 participants who tested positive for HPV DNA by hc2. Forty-six had high-risk HPV DNA typeable by PCR. The most common types of high-risk HPV detected were 16 (17% of participants), 52 (10%), 18 (8%), and 45 (8%).

Cytological analyses. Seven participants were excluded from the cytological analysis (5 had specimens unavailable for analysis, and 2 had liquid-based cytological specimens classified as being unsatisfactory for evaluation). Of the remaining participants, 47% (40/85) had abnormal results by liquid-based cytological testing: 18% ASC of undetermined significance (ASC-US), 24% low-grade squamous intraepithelial lesions (SILs), and 6% high-grade SILs. A history of RAI was the strongest predictor of abnormal cytological results in the univariate analysis. Other predictors of abnormal results in the univariate analysis were age ≤40 years and no current ART use (table 2). The current CD4+ cell count was not related to abnormal cytological results. In the multivariate model, RAI and no current ART use were significantly associated with abnormal cytological results, and nadir CD4+ cell count exhibited a trend toward being associated (table 2). High-risk HPV was strongly associated with abnormal cytological results—72% (38/53) of those with high-risk HPV had abnormal results, compared with only 6% (2/32) without high-risk HPV. When we restricted the analysis to those with high-risk HPV DNA according to hc2, RAI and no current ART use remained significantly related to abnormal results of hc2. The strongest predictor of positivity for high-risk HPV DNA according to hc2, RAI and no current ART use remained significantly related to abnormal results of hc2. The strongest predictor of positivity for high-risk HPV DNA according to hc2, RAI and no current ART use.
Table 2. Univariate and multivariate predictors of anal human papillomavirus (HPV) infection, abnormal cytological results, and anal intraepithelial neoplasia (AIN) on histological results.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Anal HPV infection</th>
<th>Abnormal cytological results&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AIN on histological results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. with anal HPV infection/total no. (%)</td>
<td>Univariate analysis, OR (95% CI); P</td>
<td>Multivariate analysis, Adjusted OR (95% CI); P</td>
</tr>
<tr>
<td>History of RAI&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>43/65 (67)</td>
<td>7.2 (2.8–18); &lt;.001</td>
<td>7.1 (2.6–20); &lt;.001</td>
</tr>
<tr>
<td>No</td>
<td>12/36 (33)</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>Current use of ART&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>45/75 (60)</td>
<td>0.82 (0.27–2.5); .7</td>
<td>0.48 (0.12–2); .3</td>
</tr>
<tr>
<td>No</td>
<td>11/17 (65)</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>Age &lt;40 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26/33 (78)</td>
<td>3.6 (1.4–9.6); .01</td>
<td>2.4 (0.8–7); .12</td>
</tr>
<tr>
<td>No</td>
<td>30/69 (45)</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>Nadir CD4&lt;sup&gt;+&lt;/sup&gt; cell count&lt;sup&gt;d&lt;/sup&gt;</td>
<td>NA</td>
<td>0.86 (0.63–1.2); .3</td>
<td>0.68 (0.44–1.1); .08</td>
</tr>
</tbody>
</table>

**NOTE.** The multivariate analyses are adjusted for the 4 predictors presented in the table. ART, antiretroviral therapy; CI, confidence interval; NA, not applicable; OR, odds ratio; RAI, receptive anal intercourse.

<sup>a</sup> Includes atypical squamous cells and low- or high-grade squamous intraepithelial lesions.

<sup>b</sup> Data on history of RAI were missing for 1 subject.

<sup>c</sup> An OR <1 indicates that the factor is associated with a lower odds of having the outcome of interest.

<sup>d</sup> Modeled as a continuous variable after log transformation to normalize the distribution.
Histological analyses. Five participants were excluded from the histological analysis (2 did not have a biopsy despite abnormal cytological results, and 3 had unavailable or unsatisfactory cytological results with no biopsy). Some 40% (35/87) of participants had AIN on histology (31% low-grade AIN and 9% high-grade AIN). A history of RAI and age ≥40 years were related to AIN on histological testing in the univariate analysis (table 2). Current CD4+ cell count was not related to abnormal histological results. In the multivariate analysis, lower nadir CD4+ cell count and no current ART use, in addition to RAI and younger age, were associated with AIN being found by histology (table 3). The association of ART with AIN was confounded by nadir CD4+ cell count. The mean nadir CD4+ cell count of participants who were receiving ART (63 cells/mm³) was lower than that of participants not receiving ART (290 cells/mm³). The association of ART use and AIN was apparent only when controlling for nadir CD4+ cell count. High-risk HPV was strongly associated with AIN on histology—64% (34/53) of those with high-risk HPV had AIN, compared with only 3% (1/33) without high-risk HPV. When we restricted the analysis to those with high-risk HPV DNA according to hc2, nadir CD4+ cell count and no current ART use remained significantly related to AIN on histology, but RAI did not (table 3).

DISCUSSION

We have demonstrated a high prevalence of AIN and anal HPV in a predominantly Latino and African American group of HIV-positive men. Although a history of RAI was strongly related to AIN, there was a significant prevalence (23%) in men without this history, 94% of whom self-identified as heterosexual. This suggests that RAI is not a necessary factor for anal HPV infection and AIN and is supported by the results of a recent study that found a high prevalence of high-grade AIN (18%) in heterosexual HIV-positive men with a history of intravenous drug use [20] and a study that found that husbands of women with cervical cancer are at an increased risk for anal cancer [21]. In a study of HIV-positive women, 23% of participants without a history of RAI were found to have abnormal anal cytological results [22]. The questionnaire used in the present study, which provides a much more detailed sexual history than would be obtained in clinical practice, did not identify factors that reliably discriminated those at low risk for AIN. These data strongly suggest that, if instituting an anal cancer screening program, all HIV-positive men, regardless of sexual orientation, should be offered participation. Other researchers have made a similar claim to include all HIV-positive women in anal cancer screening programs, regardless of history of RAI [23].

Our study also found that the nadir CD4+ cell count was significantly associated with AIN on histology in the multivariate analysis. Lower CD4+ cell counts have been demonstrated elsewhere to be associated with AIN [10, 24], and our results suggest that a lower nadir CD4+ cell count remains an independent risk factor for AIN, despite the use of effective ART. Other investigators have shown that, among patients with similar CD4+ cell counts, those with a lower nadir CD4+ cell count have decreased proliferative T cell responses, which are indicative of immune suppression, compared with those who have a higher nadir CD4+ cell count [25]. Although immune recovery sufficient for protection against opportunistic infections and other AIDS-defining illnesses generally occurs with ART, regardless of the nadir CD4+ cell count at the time of initiation, this suggests that a less-pronounced immunodeficiency related to lower nadir CD4+ cell counts remains. These immune deficits may allow the development of AIN in persons with persistent infection with high-risk types of HPV and may confer an elevated risk of invasive cancer.

Current use of ART was significantly associated with a lower prevalence of abnormal cytological results and AIN in the multivariate model. Substituting current plasma HIV RNA load for ART use yielded similar results (data not shown). This associ-

Table 3. Multivariate predictors of abnormal cytology and anal intraepithelial neoplasia (AIN) according to histological results in those with high-risk human papillomavirus (HPV) infection according to the results of Hybrid Capture 2 assay.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Abnormal cytological resultsa</th>
<th>AIN on histological results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted OR (95% CI) P</td>
<td>Adjusted OR (95% CI) P</td>
</tr>
<tr>
<td>History of RAI, yes vs. no</td>
<td>5.7 (1.1–29) .03</td>
<td>1.9 (0.4–8.9) .4</td>
</tr>
<tr>
<td>Current use of ART, yes vs. no</td>
<td>0.06 (0.01–0.87) &lt;.01</td>
<td>0.09 (0.01–0.75) .03</td>
</tr>
<tr>
<td>Age, &lt;40 vs. ≥40 years</td>
<td>1.8 (0.5–7.1) .4</td>
<td>3.6 (0.9–13) .06</td>
</tr>
<tr>
<td>Nadir CD4+ cell countb,c</td>
<td>0.6 (0.3–1.1) .06</td>
<td>0.5 (0.3–0.9) .03</td>
</tr>
</tbody>
</table>

NOTE. ART, antiretroviral therapy; CI, confidence interval; NA, not applicable; OR, odds ratio; RAI, receptive anal intercourse.

a Includes atypical squamous cells and low- or high-grade squamous intraepithelial lesions.

b An OR <1 indicates that the factor is associated with lower odds of having the outcome of interest.

c Modeled as a continuous variable after log transformation to normalize the distribution.
decreased to 500 cells/mm³ [29]. As a result of a growing realization of the acute and chronic toxicities of these regimens, recent major US national consensus guidelines for the treatment of HIV infection have favored delaying ART until later during the course of HIV infection, as the CD4⁺ count approaches 200 cells/mm³ [19, 30]. However, further research is needed to determine whether the delayed initiation of ART and lower nadir CD4⁺ cell counts will have a deleterious effect on HPV-associated malignancy.

Although ART was associated with a decreased prevalence of AIN, it was not associated with a decreased prevalence of high-risk HPV. This is interesting and may tell us something about the relationship between persistent HPV infection and neoplasia in HIV-positive individuals. We have previously reported that, in HIV-positive women, the persistence of HPV shedding into genital secretions was increased, compared with HIV-negative control subjects, even at relatively high CD4⁺ cell counts [31]. The risk of cervical intraepithelial neoplasia in those women with persistent HPV infection increased with lower CD4⁺ cell counts. Consistent with this, the present results suggest that ART may reduce the prevalence of AIN in those men with persistent HPV infection but does not appreciably reduce HPV persistence itself. Prospective cohort studies are needed to definitively answer this question.

Infection with high-risk HPV appears to be a necessary factor for having abnormal cytological results or AIN according to histological testing. When the analyses were restricted to participants with high-risk HPV, the odds ratios for a history of RAI to these outcomes were reduced by ~50%, which suggests that these relationships are partially mediated by increased exposure to HPV. In contrast, the odds ratios for nadir CD4⁺ cell count and ART did not change appreciably.

There are several limitations to our study. Primary care providers were more likely to refer subjects who reported sex with other men, which led to an overestimate of the overall prevalence of high-risk HPV, abnormal cytological results, and AIN. Providers may have been more likely to refer subjects with a prior history of genital condyloma, which may limit the generalizability of our study. Participants may have inaccurately reported their history of RAI, even though participants could report such a history even when they self-identified as heterosexual. The cross-sectional design does not establish temporality, so it is impossible to know whether ART is associated with decreased progression of AIN or increased regression.

Although a definitive diagnosis of AIN is based on histological results, we presented cytological results to illustrate the percentage of patients screened in a clinical setting that would require anal colposcopy. The histological outcome may underestimate the prevalence of AIN because of the limited number of anal biopsies performed in any given patient. Because of the limited number of cases of high-grade AIN that were identified, any grade of biopsy-confirmed AIN was used as our histological end point. High-grade AIN is the condition that is thought to precede invasive cancer and is the lesion that requires intervention. Although >50% of low-grade AINs will progress within 2 years [11], high-grade AIN would be the ideal outcome for these analyses.

Our study does not provide direct evidence as to whether screening for AIN is clinically beneficial. An anal cancer screening program should identify patients with high-grade AIN for which there is a surgical intervention, which will reduce the risk of invasive carcinoma. Studies by Goldie et al. [32, 33] have suggested that cytological screening for anal cancer is cost-effective for HIV-positive and -negative men. However, those studies assumed reasonable levels of effectiveness for the treatment of high-grade AIN. There are few data on the efficacy of various treatment modalities for high-grade AIN, including the topical application of 80% trichloroacetic acid or liquid nitrogen, electrocautery, infrared coagulation, or laser ablation. One study of HIV-positive and -negative men evaluated the surgical excision of extensive high-grade AIN that was too large for topical therapy with 80% trichloroacetic acid [34]. The surgery was effective in removing AIN and preventing recurrent lesions in all 8 HIV-negative men studied (median follow-up, 32 months) but was ineffective for HIV-positive men, with 23 of 29 having persistent or recurrent high-grade AIN (median follow-up, 29 months). The effect of ART use on the response to treatment of high-grade AIN is unknown. More information is needed on the recurrence rates of high-grade AIN and the relative morbidities for the various techniques before widespread screening programs can be implemented.

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References


