Human Papillomavirus, Anal Squamous Intraepithelial Lesions, and Human Immunodeficiency Virus in a Cohort of Gay Men

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Cross-sectional associations between human papillomavirus (HPV), anal squamous intraepithelial lesions (SIL), and human immunodeficiency virus (HIV) were studied in a cohort of gay men. HPV DNA was detected by generic and type-specific polymerase chain reaction (PCR) probes and hybrid capture assay (HC). HPV virus load was estimated by HC relative light unit (RLU) ratio. HPV prevalence, number of HPV types detected, and HC RLU ratios were each greater in HIV-positive than HIV-negative participants. Further, among HIV-positive men, HC RLU ratio was inversely associated with CD4 cell count. SIL was more frequent in HIV-positive participants, particularly those with a CD4 cell count $<200/\mu$L and was positively associated with HPV. Men with a high HC RLU ratio were nearly 3 times more likely to have SIL than were those both PCR- and HC-negative. These data support that HIV augments HPV-associated anal disease in this population.

Human papillomaviruses (HPV), particularly HPV-16 and -18, are causally associated with invasive cervical cancer and its precursors [1–4]. Since the squamocolumnar junction between the rectum and anus are histologically similar to the transformation zone of the uterine cervix, attention has been focused on the role of HPV in anal carcinoma [4]. HPV DNA sequences have been detected in anal canal tumors and in their putative precursors, squamous intraepithelial lesions (SIL) [4]. In a case-control study comparing anal cancer specimens with those from rectal adenocarcinomas, HPV DNA was detected in 84% of the former and none of the latter [5].

Although anal cancer is rare among men in general, gay men who practice receptive anal intercourse appear to be at increased risk for this neoplasm [6–8]. In addition, concomitant infection with human immunodeficiency virus (HIV), which is prevalent in gay men, may facilitate and/or accelerate the pathologic consequences of HPV infection in the anus as has been shown for the cervix [9]. In a population-based study linking AIDS and cancer registries, the risk of anal cancer among persons with AIDS was 84 times greater than that in the general population [10]. While that study could not assess the risk associated with HIV independently from that associated with homosexuality, cross-sectional studies have demonstrated both an increased prevalence of anal HPV infection and of SIL among HIV-seropositive men, particularly those who are symptomatic or have decreased CD4 cell counts [11, 12].

HPV DNA has been detected in 78% of HIV-seronegative (HIV-negative) and 92% of HIV-seropositive (HIV-positive) men using the sensitive polymerase chain reaction (PCR) assay [11], while less-sensitive methods have detected HPV infection in 6%–38% of the former and 26%–64% of the latter [11–15]. In a prospective study of 305 gay or bisexual men, 5% of HIV-negative and 15% of HIV-positive participants developed high-grade SIL. The risk of SIL among HIV-positive men was associated with high levels of HPV-16 or -18 and with depressed CD4 cell counts [16]. In addition, the incidence of anal SIL among HIV-negative men with high levels of these HPV types was similar to that previously reported for cervical neoplasia in HIV-negative women.

We undertook the current study to estimate the prevalence of HPV and SIL in a well-established and characterized cohort of gay men, to examine whether associations between HPV and SIL varied by HPV viral type determined by a PCR-based assay, and to use the hybrid capture system (HC), a semiquantitative HPV DNA detection method, to assess the association of SIL with HPV virus load in this population.

Materials and Methods

Population

Men who attended follow-up visits between November 1992 and October 1994 for the Study to Help the AIDS Research Effort

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The written informed consent procedure at entry into SHARE included explanation of studies of HIV-related or potentially HIV-related conditions, and human experimentation guidelines of the US Department of Health and Human Services and Johns Hopkins University were followed.

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(SHARE) were eligible for the present study. SHARE, one of four sites participating in the Multicenter AIDS Cohort Study, enrolled men from October 1984 to April 1985, and reopened enrollment in 1988 to increase participation from minority populations. As described in detail elsewhere, SHARE followed gay and bisexual men at semiannual visits to investigate the natural history of HIV infection and AIDS [17, 18]. At baseline and at each semiannual visit, participants completed a detailed standardized interviewer-administered questionnaire on demographics, behavioral, and medical events, underwent a focused physical examination, and provided biologic specimens. All eligible HIV-positive men and a subset of eligible HIV-negative men were provided with information about the present study and invited to participate.

Specimen Collection

At each SHARE visit, participants had blood drawn for T cell phenotyping and detection of HIV antibody, as indicated. In addition, at each visit during the study period, 2 anal specimens were collected by inserting a saline-moistened Dacron swab into the anal canal beyond the pectinate line and rotating it several times against the anorectal wall. The first swab, an anal epithelial cell specimen, was used for the detection of cytologic abnormalities. Cytology specimens obtained from November 1992 to July 1993 were prepared by conventional smear methodology. However, because a large proportion were unsatisfactory for diagnostic interpretation, these are not included in the present study [19]. Swabs collected after July 1993 were placed in fluid (Cytolite; CYTYC, Boxborough, MA) and subsequently processed as single thin-layer slides (ThinPrep; CYTYC) as described in the manufacturer's instructions. The second anal swab was placed into a tube containing normal saline and frozen at −70°C until processed for detection of HPV DNA.

Laboratory Methods

HIV testing and lymphocyte phenotyping. Serum collected at each study visit was tested by ELISA in blinded fashion for antibody to HIV, and standard techniques were used to determine CD4 cell counts. Details of laboratory procedures are described elsewhere [17, 18].

Cytologic diagnosis. The thin-layer slides were stained with a modified Papanicolaou procedure and diagnosed according to the Bethesda System [20] by two independent reviewers. Discrepancies in the initial readings were adjudicated in joint review by the 2 pathologists at a multihead microscope. Abnormalities of squamous intraepithelial cells were diagnosed as atypical squamous cells of undetermined significance or SIL. The latter were further classified as low-grade SIL (mild dysplasia) or high-grade SIL (moderate or severe dysplasia or carcinoma in situ). Slides were considered inadequate if <10% of the specimen area was covered with nucleated cells.

Detection and typing of HPV DNA. Specimens were screened for HPV DNA by two methods: the FDA-approved, tube-based HC (Digene Diagnostics, Silver Spring, MD) and PCR amplification. HC methods are described in detail elsewhere [21]. Briefly, specimens were hybridized to two HPV RNA probe cocktails; probe A hybridizes to the low-risk HPV-6, -11, -42, -43, and -44, while probe B hybridizes to the intermediate- and high-risk HPV-16, -18, -31, -33, -35, -45, -51, -52, and -56. The number of relative light units (RLUs) for each specimen was divided by the number from an appropriate positive control, and RLU ratios <1.0 were considered negative for the targeted HPV DNA. RLUs are proportional to the amount of targeted DNA in the specimen so that greater ratios indicate greater numbers of DNA copies.

PCR amplification of HPV DNA sequences was performed using MY09/MY11/HMB01 L1 consensus primers [22, 23]. Products were hybridized individually to type-specific probes to HPV-6, -11, -16, -18, -31, -33, -35, -39, -40, -45, -51, -52, -53, -54, -55, -56, -58, -59, -66, -68, and -73; unnumbered types PAP155, PAP291, and w13b; and to a generic probe capable of detecting almost all genital HPVs. Hybridization signal strength was coded 0–4 for the generic and each type-specific probe; a signal strength of 0 indicates an absence of the targeted HPV DNA, while codes 1–4 indicate increasing signal intensity. In addition, for each specimen, human β-globin DNA was amplified and hybridized as a test of the specimen's suitability for amplification of HPV DNA sequences. Specimens from which viral DNA was detected were considered adequate for amplification, regardless of whether β-globin DNA was detected, while specimens from which no HPV DNA and no β-globin DNA could be detected were considered nonamplifying and thus inadequate for detection of HPV DNA. Participants with nonamplifying specimens were excluded from analyses of HPV infection.

HPV DNA Analyses

HPV detection by PCR was considered for individual DNA types, any DNA type, total number of DNA types, and HPV types grouped in risk categories based on their association with cervical cancer [1, 24, 25]. For these analyses the low-risk category included HPV-6, -11, -40, -53, -54, -55, and -66, and PAP155 and PAP291; intermediate risk included HPV-33, -35, -39, -51, -52, -56, -58, -59, -68, and -73 and w13b; and high risk included HPV-16, -18, -31, and -45. In addition, a crude measure of virus load was made separately for each risk category by summing the individual PCR signal strengths for each of the HPV types present in each specimen included in that risk category. For HC, each specimen was scored positive or negative for probes A and B, and the RLU ratio of the specimen was used to estimate virus load. The association of SIL with virus load was also examined using a combination PCR/HC variable. The analytic sensitivity of HC is on the order of ~100,000 genome copies compared with 10–100 copies for PCR. Thus, with specimens negative for both assays serving as a referent group, HC-positive specimens (regardless of PCR results) were considered to have high virus loads, and PCR-positive HC-negative specimens were considered to have intermediate virus load. HC-positive specimens were further distinguished as those with a probe RLU ratio >5.0 or those with both probe RLU ratios between 1.1 and 5.0.

Statistical Methods

Fisher's exact test was applied to comparisons of two proportions, and linear trends in proportions were assessed by a χ^2 statis-

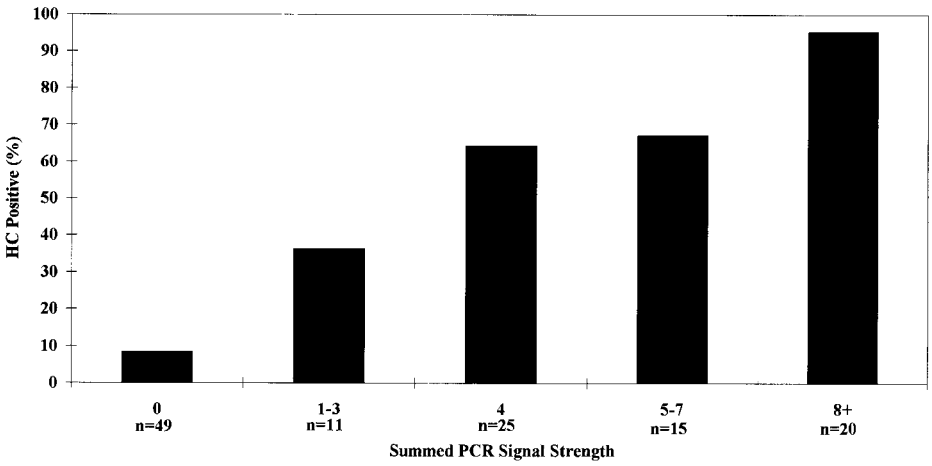

Figure 1. Hybrid capture (HC) probe B, % positive by summed polymerase chain reaction (PCR) signal strength to probe B HPV DNA types.

tic [26]. Distributions of continuous variables were compared using the Wilcoxon rank sum test or for >2 groups, the Kruskal-Wallis rank test [27]. Confidence intervals (CIs) for prevalence rate ratios (RRs) were based on a logarithmic transformation [28]. The simultaneous influence of HIV and HPV infections on the presence of SIL was assessed by logistic regression [29].

Results

Population Characteristics

Men who attended at least one study visit between November 1992 and January 1994 were asked to participate in the present study by providing anal specimens for the detection of HPV DNA and cytologic abnormalities. By design, HIV-positive men were overrepresented in the final study population; 184 (72%) of 256 HIV-positive and 79 (46%) of 171 HIV-negative attendees participated. Participants were similar to nonparticipants with respect to age (37% <40 years), race (13% black), and number of male partners in the previous 6 months (27% none, 10% >6).

HPV Findings

Prevalence of HPV DNA and the specific viral types were determined by dot blot hybridization following PCR amplification. Human β-globin DNA sequences were successfully amplified from specimens collected for HPV DNA detection from 116 (63%) of 184 HIV-positive and 45 (57%) of 79 HIV-negative participants (Fisher's exact test, $P = .4$). Further observations about HPV as detected by PCR are confined to these 161 specimens plus an additional 20 (20%) of 102 β-globin–negative specimens that were HPV-positive. HC was performed on specimens from 112 HIV-positive and 62 HIV-negative participants.

As expected, PCR was more sensitive than HC in detecting HPV infection. This can be directly assessed for the HPV types included in HC probe B. PCR and HC results were available for 120 specimens; 45 were negative for HPV types included in probe B by both HC and PCR, while infection was detected by both methods in 49 specimens. HPV DNA was detected by PCR only in 22 specimens, and by HC only in 4 (3 of which were PCR-positive for one or more HPV types not contained in HC probe B). In addition, HC positivity was significantly associated with virus load as crudely measured by summed PCR signal strengths to the HPV types included in probe B (figure 1). HC probe B positivity ranged from 8% among 49 specimens negative by PCR for each of the HPV types to 95% among 20 specimens with summed PCR signal strengths of $\geqslant 8$ (χ^2 for trend, $P < .001$).

HPV associated with HIV and CD4 cell count. HPV was highly prevalent among study participants, with 90% of HIV-positive and 70% of HIV-negative men positive to the generic PCR probe (Fisher's exact test, $P < .002$) (table 1). With the type-specific probes, a broad spectrum of HPV types was detected, with low-, intermediate-, and high-risk HPV types present in roughly equal proportions. All types were more often detected in HIV-positive men. For instance, HPV-16, -18, -31, and -53 were each detected in >15% of HIV-positive participants, while none of the HPV types sought were found this often in HIV-negative participants. HIV-positive participants also had multiple HPV types detected in their specimens; roughly one-third (37%) of HIV-positive but only 9% of HIV-negative participants were positive for three or more types (Wilcoxon rank sum test, $P = .001$).

The contrast in HPV prevalence between HIV-positive and HIV-negative participants was more pronounced when less-sensitive methods were used to detect HPV infection (figure 2). One or more type-specific HPV probes were positive in 83% of 135 HIV-positive and 50% of 46 HIV-negative partici-

Table 1. Prevalence of HPV DNA as detected by polymerase chain reaction among HIV-positive and HIV-negative participants.

HPV DNA category*	HPV type	HIV		$P^{\dagger}$
		Positive (n = 135), no. (%)	Negative (n = 46), no. (%)	
Any HPV DNA		122 (90)	32 (70)	.001
High risk	16	41 (30)	7 (15)	
	18	23 (17)	2 (4)	
	31	27 (20)	5 (11)	
	45	14 (10)	0	
	Any	76 (56)	12 (26)	<.001
Intermediate risk	33	12 (9)	0	
	35	2 (2)	0	
	39	5 (4)	0	
	51	19 (14)	4 (9)	
	52	9 (7)	2 (4)	
	56	5 (4)	0	
	58	16 (12)	2 (4)	
	59	3 (2)	1 (2)	
	68	12 (9)	1 (2)	
	73	5 (4)	0	
	w13b	6 (4)	0	
	Any	64 (47)	8 (17)	<.001
Low risk	6	17 (13)	5 (11)	
	11	12 (9)	2 (4)	
	40	1 (1)	0	
	53	26 (19)	4 (9)	
	54	8 (6)	1 (2)	
	55	2 (1)	0	
	66	12 (9)	0	
	PAP155	12 (9)	1 (2)	
	PAP291	6 (4)	0	
	Any	67 (50)	13 (28)	.012
Total no. of types detected	0	3 (17)	23 (50)	.001[‡]
	1	8 (21)	14 (30)	
	2	34 (25)	5 (11)	
	3	27 (20)	3 (7)	
	⩾4	23 (17)	1 (2)	

* HPV DNA category: Any HPV DNA, generic probe; low risk, 6, 11, 26, 40, 53, 54, 55, 66, PAP155, and PAP291; intermediate risk, 33, 35, 39, 51, 52, 56, 58, 59, 73, and w13b; high risk, 16, 18, 31, and 45.

† Fisher's exact test for prevalence of any HPV DNA, any high-risk, any intermediate-risk, and any low-risk HPV DNA in HIV-positive vs. HIV-negative participants. For each comparison, referent group is men who were negative for indicated risk group, regardless of presence of HPV types in other risk groups.

‡ Wilcoxon rank sum test for total number of types detected in HIV-positive vs. HIV-negative participants.

pants (RR = 1.7; 95% CI, 1.2–2.2). HPV was detected by HC probe A and HC probe B, respectively, in 30% and 50% of 112 HIV-positive men compared with 8% and 10% of 62 HIV-negative men. Thus, HIV-positive participants were nearly 4 times more likely to be positive by HC probe A (RR = 3.8; 95% CI, 1.6–9.1) and 5 times more likely to be positive by HC probe B (RR = 5.2; 95% CI, 2.4–11.4) than their HIV-negative counterparts.

Among HIV-positive participants, CD4 cell count was broadly distributed (10% >800 and 10% <50 cells/μL) and was inversely associated with detection of HPV DNA (figure 2). By PCR, one or more HPV types were detected in 90% of 39 men with CD4 cell counts <200 cells/μL compared with 81% of 91 with higher counts (Fisher's exact test, $P >$ .05). CD4 cell counts were lower among 109 men with HPV (mean ± SD: 347 ± 261 cells/μL) than among 21 who were PCR-negative (492 ± 311 cells/μL, Wilcoxon rank-sum test, $P <$ .05). Using the less-sensitive HC technique, intermediate- and high-risk HPV types included in HC probe B were detected in 83% of 24 participants with CD4 cell counts <200/μL compared with 41% of 87 men with higher counts (RR = 2.0; 95% CI, 1.5–2.7). Mean CD4 cell count was 353 ± 282 cells/μL among participants positive by HC probe B compared with 509 ± 290 cells/μL in those who were negative by probe B (Wilcoxon rank sum test, P = .003). However, the presence of low-risk HPV types at levels detectable by HC probe A was not associated with decreased CD4 cell counts.

HPV virus load associated with HIV and CD4 cell count. The hypothesis that HPV is not only more common but present in greater amounts in HIV-positive than HIV-negative men was tested by comparing HC probe B RLU ratios among participants known to be HPV-positive (table 2). Among participants with PCR and/or HC evidence of the HPV types included in probe B, HC RLU ratios were positively associated with HIV (Wilcoxon rank sum test, P = .009). For instance, 29% of HIV-positive compared with 7% of HIV-negative participants had HC probe B RLU ratios >5.0. There was also some evidence that HPV virus load was greater among HIV-positive men with CD4 cell counts <200/μL compared with those with greater CD4 cell counts (Wilcoxon rank sum test, $P <$ .07). HC probe B failed to detect infection in only 5% of 21 men with CD4 cell counts <200/μL compared with 25% of 48 participants with greater counts. It is possible that these observations reflect differences in the number of HPV types present. Therefore, we calculated mean HPV virus loads for each specimen by dividing the total RLU ratio by the number of HC probe B types detected by PCR. This measure produced patterns similar to those seen with total RLU ratio (data not shown).

Cytologic Findings

SIL associated with HIV. Liquid-based anal cytology specimens were collected from 69 (84%) of 79 HIV-negative and 122 (66%) of 184 HIV-positive participants. Of these, 54 (78%) from HIV-negative and 102 (84%) from HIV-positive participants were adequate for cytologic diagnosis. The remaining 33 slides were unsatisfactory: 21 had insufficient cellularity, 11

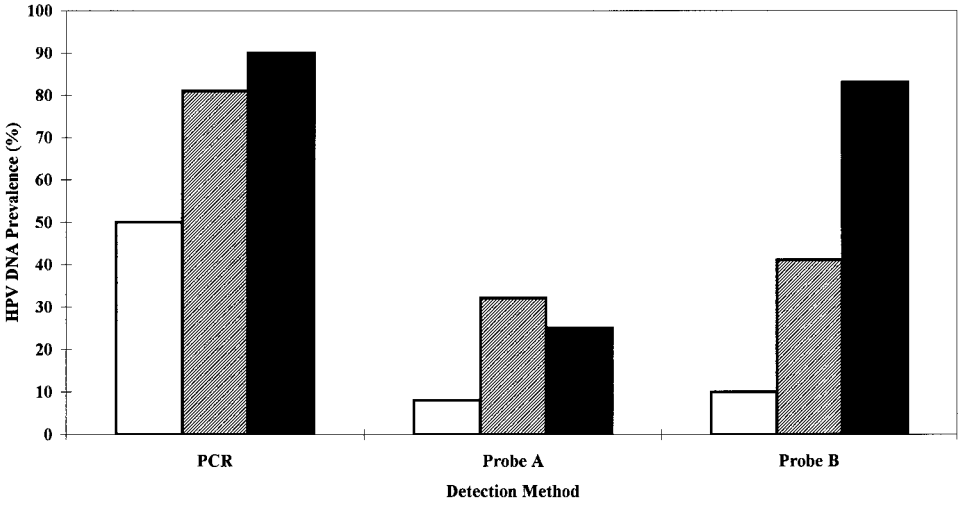

Figure 2. Prevalence of HPV DNA as detected by polymerase chain reaction (PCR), hybrid capture (HC) probe A, and HC probe B among HIV-negative and HIV-positive (CD4 cell count, ⩾200 or <200/μL) participants.

consisted mainly of anucleated squamous cells, and 1 was poorly preserved.

Abnormal anal cytology was common among study participants and was positively associated with HIV infection (table 3). Overall, 41% of HIV-positive compared with 17% of HIV-negative participants had SIL (RR = 2.5; 95% CI, 1.3–4.7), and the only two high-grade (AIN II) lesions detected occurred in HIV-positive men. The prevalence of SIL increased with decreasing CD4 cell count (χ^2 for trend, $P = .002$); however, when confined to HIV-positive participants, this association did not reach statistical significance (Fisher's exact test, $P > .2$). Mean CD4 cell count among the 40 participants with normal cytology was 469 ± 319 cells/μL compared with 402 ± 292 cells/μL among 20 with atypical squamous cells of undetermined significance and 363 ± 286 cells/μL among 42 with SIL (Kruskal-Wallis test, $P > .2$). There was also a weak association between SIL and time since HIV seroconversion; among 21 participants who acquired HIV during follow-up, 9 seroconverters with SIL had been HIV-positive an average of 6.8 years, while 12 without SIL had been infected an average of 5.2 years (Wilcoxon rank sum test, $P = .13$).

Table 2. Hybrid capture (HC) probe B relative light unit (RLU) ratio among participants positive for probe B HPV types by either polymerase chain reaction or HC.

HC probe B RLU ratio	HIV		HIV-positive CD4 count/μL	
	Positive, no. (%)	Negative, no. (%)	<200 no. (%)	⩾200 no. (%)
⩽1.0	13 (19)	9 (60)	1 (5)	12 (25)
1.1–2.0	16 (23)	2 (13)	3 (14)	13 (27)
2.1–3.0	6 (9)	0	2 (10)	4 (8)
3.1–4.0	8 (12)	2 (13)	3 (14)	5 (10)
4.0–5.0	6 (9)	1 (7)	5 (24)	1 (2)
>5.0	20 (29)	1 (7)	7 (33)	13 (27)
P	.009[†]		.061[‡]	

NOTE. Probe B HPV (human papillomavirus) types 16, 18, 31, 33, 35, 45, 51, 52, and 56.

[†] Wilcoxon rank sum test for HC probe B RLU ratio in HIV-positive vs. HIV-negative participants.

[‡] Wilcoxon rank sum test for HC probe B RLU ratio in HIV-positive participants with CD4 cell count <200 vs. ⩾200/μL.

SIL associated with HPV. SIL was positively associated with HPV infection as detected by PCR, HC, and a combined PCR/HC variable (table 3). The prevalence of SIL increased with increasing number of HPV types detected by PCR (χ^2 for trend $P = .025$). However, there was no evidence that this association was type-specific, as SIL was no more common in participants with high-risk types only (35% of 17) than among those with low-risk types only (31% of 13). Similarly, virus load as measured by both HC probe A (χ^2 for trend, $P = .001$) and HC probe B (χ^2 for trend, $P = .038$) was positively associated with SIL. Finally, considering a combined PCR/HC variable, men with HC probe A and/or B RLU ratios >5.0 were nearly three times more likely to have SIL than were those both PCR- and HC-negative.

High-grade SIL was detected in 2 participants; one was PCR-positive for HPV-16 alone and negative to both HC probes, and the other was PCR-positive for HPV-35, -51, -58, and -59; negative for HC probe A; and weakly positive for HC probe B (RLU ratio, 1.23).

We constructed logistic regression models with SIL as the dependent variable to assess whether its relationship with HPV virus load could be explained by HIV or other potential confounders. The association of SIL with the combined PCR/HC variable was similar when adjusted for HIV serostatus, age at

Table 3. Prevalence of cytologic diagnoses by HIV and HPV DNA detection.

Characteristic	*N*	Cytologic diagnosis: Negative, no. (%)	ASCUS no. (%)	SIL no. (%)	RR (95% CI) SIL*
HIV[†]					
Negative	54	38 (70)	7 (13)	9 (17)	Reference
Positive, CD4 cells ⩾200/μL	75	31 (41)	16 (21)	28 (37)	2.2 (1.2–4.4)
Positive, CD4 cells <200	25	9 (36)	4 (16)	12 (48)	2.9 (1.4–5.9)
PCR[‡]					
Negative	26	18 (69)	1 (4)	7 (27)	Reference
1 or 2 types detected	53	22 (42)	15 (28)	16 (30)	1.1 (0.5–2.4)
⩾3 types detected	38	10 (26)	8 (21)	20 (53)	2.0 (1.0–3.9)
HC probe A[§]					
⩽1.0	110	66 (60)	19 (17)	25 (23)	Reference
1.1–5.0	18	6 (33)	3 (17)	9 (50)	2.2 (1.2–3.9)
>5.0	16	3 (19)	4 (25)	9 (56)	2.5 (1.4–4.3)
HC probe B[‖]					
⩽1.0	88	54 (61)	12 (14)	22 (25)	Reference
1.1–5.0	36	15 (42)	10 (28)	11 (31)	1.2 (0.7–2.3)
>5.0	20	6 (30)	4 (20)	10 (50)	2.0 (1.1–3.5)
PCR/HC[¶]					
PCR^-/HC^-	22	17 (77)	1 (5)	4 (18)	Reference
PCR^+/HC^-	28	15 (54)	6 (21)	7 (25)	1.4 (0.5–4.1)
HC					
1.1–5.0	38	15 (39)	10 (26)	13 (34)	1.8 (0.7–5.1)
>5.0	31	8 (26)	7 (23)	16 (52)	2.8 (1.1–7.3)

NOTE. ASCUS, atypical squamous cells of undetermined significance; SIL, squamous intraepithelial lesions; RR, relative risk; CI, confidence interval; PCR, polymerase chain reaction; HC, hybrid capture.

* RR of SIL vs. negative or ASCUS for each characteristic.

χ^2 for trend, prevalence of SIL: [†] $P = .002$, [‡] $P = .025$, [§] $P = .001$, [‖] $P = 0.38$, [¶] $P = .007$.

examination, and number of male partners in the previous 6 months. Similarly, when limited to HIV-positive participants, the PCR/HC variable remained associated with SIL when adjusted for CD4 cell count, age, and number of partners. Further, none of these covariates was independently associated with SIL.

Discussion

In this cross-sectional examination of a cohort of gay men, we found HPV and SIL to be common and to be associated with HIV. The greater prevalence of HPV among HIV-positive men was more pronounced by HC probe A (RR = 3.8; 95% CI, 1.6–9.1) and probe B (RR = 5.2; 95% CI, 2.4–11.4) than by PCR (RR = 1.7; 95% CI, 1.2–2.2). Since the analytic sensitivity of HC is low relative to PCR, this suggests that HPV is present in greater amounts in individuals coinfected with HIV. Southern or dot blot positivity in PCR-positive specimens has been used as an indication of high HPV virus load in previous studies [11, 13]. By using hybrid capture, which utilizes chemiluminescent detection, we were able to estimate HPV virus loads more directly and on a continuous scale. Among men PCR-positive for the viral types included in HC probe B, RLU ratios were greater among those with HIV than in those who were HIV-negative. Further, among HIV-positive men, HC RLU ratios were greater in those with CD4 cell counts <200/μL. Given the high rate of detection by PCR of HPV in HIV-negative men, these findings suggest that preexistent HPV infection is reactivated or up-regulated by the immunosuppression that is a hallmark of HIV infection. Analogously, a low CD4 cell count is a risk factor for repeated detection of cervical HPV in HIV-infected women [30, 31]. HIV may also affect HPV expression by molecular interaction between the two viruses [32].

It has been hypothesized that the effect of HIV on HPV infection will lead to an increased incidence of anal cancer in men infected with both viruses. The results of this and other cross-sectional studies support this hypothesis [11–15, 33]. In our study population, anal cytologic abnormalities were more frequent in HIV-positive men, particularly those with CD4 cell counts <200/μL. As in other cross-sectional studies, high-grade SIL, the putative precursor to anal carcinoma, was rare, and the low-grade lesions we observed were not selectively associated with high-risk HPV types [11–15, 33]. Rather, SIL was most common among men infected with multiple HPV

types or who were infected at levels detectable by HC, regardless of viral type. To date, a single prospective study has compared the incidence of SIL in HIV-positive to that in HIV-negative men [16]. In that study, high-grade SIL was nearly 3 times more likely to develop in HIV-positive participants. Higher levels (i.e., detection by Southern transfer hybridization) of HPV-16 or -18 and repeated detection of high levels of HPV-16 or -18 were associated with SIL in both HIV-negative and HIV-positive men. Further, SIL remained associated with HIV or HIV-related immunosuppression (low CD4 cell count) once adjusted for these measures of high HPV virus load.

The recognition that gay men are at increased risk of anal cancer coincided with the onset of the HIV epidemic [6, 7, 34]. Since that time, investigators have used a variety of methods to assess whether HIV-positive gay men might represent a particularly high-risk population. Although both cross-sectional and prospective studies in gay men support this hypothesis, it is not clear whether this effect has or will result in an actual increased incidence of anal cancer in these men. Population-based studies have demonstrated an excess risk in gay men, including those diagnosed with AIDS, but these studies could not distinguish between the effects of HIV and other correlates of homosexuality [10, 35]. There is one published study that could examine the independent effects of these variables; in that cohort of young homosexual men in San Francisco, the annual incidence of anal cancer was 13.4 and 16.6 per 100,000 HIV-positive and HIV-negative men, respectively [36]. Thus, while the association of anal cancer with homosexuality was confirmed, there was no increased incidence of overt cancer among HIV-positive men.

In summary, in this study all HPV types were more frequent in HIV-positive than HIV-negative participants, and the types most strongly associated with anogenital cancer were 2 times more likely to be detected in these men. In addition, HIV-positive men had greater HC RLU ratios than did men without HIV, and among HIV-positive participants, this measure of HPV virus load was associated with a low CD4 cell count. In turn, high HPV virus load was associated with anal SIL.

Anal cancer likely progresses from SIL over the course of many years. This long latency may explain why, despite an increased risk of SIL among HIV-positive individuals, a corresponding increase in anal cancer has not been observed. Historically, men with HIV and AIDS may not have survived long enough to develop clinically detectable anal neoplasia. However, improved antiretroviral therapy may increase survival and thus increase the incidence of anal cancer in this group. Alternatively, since this therapy slows the immunosuppressive effects of HIV [37], infected men may continue to demonstrate the same incidence of anal cancer as those without HIV. Thus, gay men in general represent a group that may benefit from screening and early intervention for anal cancer. However, any proposal for screening will require a better understanding of the clinical significance of the spectrum of anal lesions and the utility of HPV DNA and cytologic testing to detect lesions that require intervention.

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References

1. Bosch FX, Manos MM, Muñoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J Natl Cancer Inst **1995**; 87:796–802.
2. Koutsky LA, Holmes KK, Critchlow CW, et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. N Engl J Med **1992**;327:1272–8.
3. Schiffman MH, Bauer HM, Hoover RN, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst **1993**;85:958–64.
4. International Agency for Research on Cancer. Human papillomaviruses. In: IARC monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 64. Lyon, France: World Health Organization, **1995**.
5. Frisch M. Sexually transmitted infection as a cause of anal cancer. N Engl J Med **1997**;337:1350–8.
6. Austin DF. Etiological clues from descriptive epidemiology: squamous carcinoma of the rectum or anus. Natl Cancer Inst Monograph **1982**; 62:89–90.
7. Peters RK, Mack TM. Patterns of anal carcinoma by gender and marital status in Los Angeles County. Br J Cancer **1983**;48:629–36.
8. Daling JR, Weiss NS, Hislop TG, et al. Sexual practices, sexually transmitted diseases, and the incidence of anal cancer. N Engl J Med **1987**;317: 973–7.
9. Petry KU, Scheffel D, Bode U, et al. Cellular immunodeficiency enhances the progression of human papillomavirus–associated cervical lesions. Int J Cancer **1994**;57:836–40.
10. Melbye M, Coté TR, Kessler L, Gail M, Biggar RJ, AIDS/Cancer Working Group. High incidence of anal cancer among AIDS patients. Lancet **1994**;343:636–9.
11. Kiviat NB, Critchlow CW, Holmes KK, et al. Association of anal dysplasia and human papillomavirus with immunosuppression and HIV infection among homosexual men. AIDS **1993**;7:43–9.
12. Palefsky JM, Shiboski S, Moss A. Risk factors for anal human papillomavirus infection and anal cytologic abnormalities in HIV-positive and HIV-negative homosexual men. J Acquir Immune Defic Syndr **1994**;7:599–606.
13. Caussy D, Goedert JJ, Palefsky J, et al. Interaction of human immunodeficiency and papilloma viruses: association with anal epithelial abnormality in homosexual men. Int J Cancer **1990**;46:214–9.
14. Melbye M, Palefsky J, Gonzales J, et al. Immune status as a determinant of human papillomavirus detection and its association with anal epithelial abnormalities. Int J Cancer **1990**;46:203–6.

15. Palefsky JM, Gonzales J, Greenblatt RM, Ahn DK, Hollander H. Anal intraepithelial neoplasia and anal papillomavirus infection among homosexual males with group IV HIV disease. JAMA **1990**;263:2911–6.
16. Critchlow CW, Surawicz CM, Holmes KK, et al. Prospective study of high grade anal squamous intraepithelial neoplasia in a cohort of homosexual men: influence of HIV infection, immunosuppression and human papillomavirus infection. AIDS **1995**;9:1255–62.
17. Chmiel JS, Detels R, Kaslow RA, et al. Factors associated with prevalent human immunodeficiency virus (HIV) infection in the Multicenter AIDS Cohort Study. Am J Epidemiol **1987**;126:568–77.
18. Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. Am J Epidemiol **1987**;126:310–8.
19. Sherman ME, Friedman HB, Busseniers AE, Kelly WF, Carner TC, Saah AJ. Cytologic diagnosis of anal intraepithelial neoplasia using smears and Cytyc thin-preps. Mod Pathol **1995**;8:270–4.
20. National Cancer Institute Workshop. The 1988 Bethesda System for reporting cervical/vaginal cytological diagnoses. JAMA **1989**;262:931–4.
21. Cox JT, Lorincz AT, Schiffman MH, Sherman ME, Cullen A, Kurman RJ. Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance. Am J Obstet Gynecol **1995**;172:946–54.
22. Muñoz N, Bosch FX, de Sanjosé S, et al. The causal link between human papillomavirus and invasive cervical cancer: a population-based case-control study in Columbia and Spain. Int J Cancer **1992**;52:743–9.
23. Hildesheim A, Schiffman MH, Gravitt PE, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis **1994**;169:235–40.
24. Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol **1992**;79:328–37.
25. Wright TC, Kurman RJ. A critical review of the morphologic classification systems of preinvasive lesions of the cervix: the scientific basis for shifting the paradigm. Papillomavirus Report **1994**;5:175–82.
26. Mantel, M. Chi-square tests with one degree of freedom; Extensions of the Mantel-Haenszel Procedure. J Am Stat Assoc **1963**;58:690–700.
27. Lehmann EL. Nonparametrics: statistical methods based on ranks. San Francisco: Holden-Day, **1975**.
28. Kleinbaum DG, Kupper LL, Morgenstern H. Epidemiologic research: principles and quantitative methods. Belmont: Wadsworth, **1982**.
29. Hosmer DW, Lemeshow S. Applied logistic regression. New York: John Wiley & Sons, **1989**; Wiley Series in Probability and Mathematical Statistics.
30. Vernon SD, Reeves WC, Clancy KA, et al. A longitudinal study of human papillomavirus DNA detection in human immunodeficiency virus type 1–seropositive and –seronegative women. J Infect Dis **1994**;169:1108–12.
31. Sun XW, Kuhn L, Ellerbrock TV, Chiasson MA, Bush TJ, Wright TC. Human papillomavirus infection in women infected with the human immunodeficiency virus. N Engl J Med **1997**;337:1343–9.
32. Vernon SD, Hart CE, Reeves WC, Icenogle JP. The HIV-1 *tat* protein enhances E2-dependent human papillomavirus 16 transcription. Virus Res **1993**;27:133–45.
33. Palefsky JM, Holly EA, Ralston ML, Arthur SP, Hogeboom CJ, Darragh TM. Anal cytological abnormalities and anal HPV infection in men with Centers for Disease Control group IV HIV disease. Genitourin Med **1997**;73:174–80.
34. Daling JR, Weiss NS, Klopfenstein LL, Cochran LE, Chow WH, Daifuku R. Correlates of homosexual behavior and the incidence of anal cancer. JAMA **1982**;247:1988–90.
35. Biggar RJ, Curtis RE, Cote TR, Rabkin CS, Melbye M. Risk of other cancers following Kaposi's sarcoma: relation to acquired immunodeficiency syndrome. Am J Epidemiol **1994**;139:362–8.
36. Koblin BA, Hessol NA, Zauber AG, et al. Increased incidence of cancer among homosexual men, New York City and San Francisco, 1978–1990. Am J Epidemiol **1996**;144:916–23.
37. Collier AC, Coombs RW, Schoenfeld DA, et al. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. N Engl J Med **1996**;334:1011–7.