Prevalence, Clearance, and Incidence of Anal Human Papillomavirus Infection in HIV-Infected Men: The HIPVIRG Cohort Study

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Background. Human immunodeficiency virus (HIV)–seropositive men who have sex with men (MSM) are at higher risk of human papillomavirus (HPV) infection. This study was conducted to better understand the natural history of type-specific HPV infection in the anus.

Methods. A cohort study was conducted among HIV-seropositive MSM in Montreal to investigate acquisition and loss of anal HPV infection. Participants were followed up every 6 months for 3 years for risk behaviors, HIV-related parameters, and HPV testing.

Results. HPV DNA was detected in 97.9% of the 247 participants at baseline (median, 5 HPV types). The most common types were HPV-16 (38.2%) and HPV-6 (35.3%). Prevalent HPV-16 infections had the lowest clearance rate (12.2 cleared episodes per 1000 person-months [95% confidence interval {CI}, 8.5–17.7]) and a mean retention time of 36 months (95% CI, 32.7–38.8). The highest incidence rates were found for HPV-16 (10.8 new cases per 1000 person-months [95% CI, 8.0–14.7]), HPV-52 (10.8 new cases per 1000 person-months [95% CI, 8.2–14.1]), and HPV-53 (9.8 new cases per 1000 person-months [95% CI, 7.4–13.0]), with cumulative incidences at 36 months of ~30%.

Conclusions. Multiple HPV types were common in the anal canals of HIV-seropositive MSM. Incidence and clearance rates were not similar among HPV types. Ongoing surveillance of this cohort will help our understanding of the determinants of HPV persistence and progression to lesions.

The incidence of anal cancer has increased in recent decades [1, 2]. Men who have sex with men (MSM) are particularly at risk for this cancer [3]. The risk further doubles in HIV-infected MSM [4, 5]. Unfortunately, control of HIV infection via highly active antiretroviral therapy (HAART) does not seem to protect against anal cancer, as it does for many other AIDS-defining malignancies [4–6].

Anal infection with oncogenic genotypes of human papillomavirus (HPV) is a key causal precursor of anal cancer via the same mechanism as for cervical cancer [7, 8]. HPV infection and associated cellular changes can be identified by analyzing exfoliated cells from the anal canal and biopsy samples obtained during high-resolution anoscopy [9–11]. Similar to cervical cancer, anal cancer is suspected of progressing from anal intraepithelial neoplasia (AIN). The spectrum of AIN includes low-grade lesions (AIN1) and high-grade lesions (AIN2 and AIN3). Previous studies have demonstrated a high prevalence of AIN2 and AIN3 in HIV-seropositive MSM [6, 12–14], and some findings have suggested that low blood CD4 cell counts is the main risk factor for AIN [15, 16].

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The prevalence of anal HPV infection in HIV-infected men is very high, with estimates >86%, as is the prevalence of anal infections with multiple HPV types [6, 12, 17, 18]. Findings for clearance of cervical HPV in women [19] cannot be extrapolated to MSM, because the prevalence of anal HPV infection in the latter is higher than the prevalence of cervical HPV infection in the former. Furthermore, the high anal HPV prevalence in MSM does not decline with age, as happens for cervical HPV infection in nonimmunocompromised women [20]. MSM also have risk behaviors that place them at higher risk of infection. Moreover, we have recently shown that detection of high-risk HPV types in anal biopsy samples but not anal swab samples at a single time point was associated with high-grade anal disease [21]. A better understanding of the natural history of each HPV type infecting the anal canal is thus needed to clarify the role played by HPV in anal precancerous and cancerous lesions.

Publications on anal HPV clearance rates in MSM are scant. One of the few studies that specifically recorded HPV infections in the anal canal prospectively [18] did not estimate duration of infection with actuarial methods and assessed only 13 HPV types. Only 13% of HIV-seropositive men who were initially HPV positive became HPV negative over a period of 1860 days. Repeated-measurement cohort studies of the natural history of anal HPV infection and lesions in MSM can help us understand the dynamics of anal infections and their progression to lesions and can provide the basis for management strategies.

In 2002, we began a longitudinal cohort study in Montreal of anal HPV infection and AIN in HIV-seropositive MSM receiving HAART. The HIPVIRG (Human Immunodeficiency and Papilloma Virus Research Group) cohort study has the following objectives: (1) to measure the prevalence of anal HPV infection and AIN in a Montreal population of HIV-infected MSM; (2) to assess risks and determinants for intermediate end points in the natural history of HPV infection and AIN; (3) to examine the influence that lifestyle factors (smoking and sexual practices) and HIV infection (including immune function and HAART) has on the progression of HPV infection and AIN; (4) to study the impact that HPV infection (specific types and viral burden) has on development of AIN; and (5) to compare different techniques for studying HPV and AIN and identify the best methods to screen and manage these patients clinically. We have already described the concordance of HPV DNA detection between anal biopsy samples and anal swab samples at a single time point in a subset of men from this cohort [21].

We describe here the design and methods of the HIPVIRG study and present results concerning the prevalence, clearance, and incidence of type-specific HPV infection among all participants. The determinants of anal lesions during the course of HPV infection will be the subject of future articles.

METHODS

Study design and population. The HIPVIRG cohort study is a longitudinal study involving repeated measurements via questionnaires, blood tests, and anal examinations every 6 months for 3 years, for a total of 7 visits. Recruitment was initiated in January 2002, and accrual was completed in January 2005. Subjects were recruited through 4 main outpatient clinics that specialized in HIV/AIDS care in Montreal: Clinique Médicale du Quartier-Latin; Clinique Médicale l'Actuel; Unité d'Hospitalisation de Recherche et d'Enseignement sur les Soins du SIDA (UHRESS), Centre Hospitalier de l'Université de Montréal; and UHRESS, Royal Victoria Hospital, McGill University Health Center. Men were invited to participate if they were aged 18–65 years, had a history of sexual intercourse with other men, had a CD4 cell count <500 cells/μL of blood if not receiving HAART, and were either currently receiving HAART or scheduled to begin HAART within the next 6 months. Men were excluded if they had undergone radiotherapy, surgery, or laser surgery for anal cancer, AIN, or high-grade squamous intraepithelial lesions; if they were receiving systemic immunomodulatory agents (interleukin, interferon, or >10 mg/day prednisone or equivalent); or if they were being treated with cidofovir. Written informed consent was obtained from all participants. The project and consent forms were approved by the review boards and ethics committees of participating institutions. Participants received Can$10 at each visit, to reimburse them for transportation costs and increase compliance.

The lowest blood CD4 cell count and HIV load before the beginning of current HAART were obtained from each patient's medical file. Current CD4 cell counts and HIV loads were obtained during participants' usual clinical follow-up visits at their respective clinics within 1 month of the study visit. All CD4 cell counts were assessed by standard flow cytometry, and plasma HIV RNA loads were determined by the branched-chain DNA signal amplification assay (Quantiplex HIV RNA Assay; Chiron).

Questionnaires. Participants completed a detailed self-administered baseline questionnaire at the enrollment visit and follow-up questionnaires at each subsequent visit. The follow-up questionnaires contained a reduced set of questions and also elicited responses aimed at verifying the consistency of the information given at baseline. The information collected referred to the 4 main categories of risk factors for HIV infection, HPV infection, and AIN: sociodemographic characteristics, smoking and injection drug use, sexual history, and HIV history (including HIV treatment).

Anal samples for HPV testing. The technique used to obtain cell samples for HPV testing was the same as used for anal cytology. At each visit, a saline-moistened anal Dacron swab was inserted 3 cm into the anal canal and then removed with a twirling motion that applied gentle pressure on the walls of the canal

to maximize the yield of anal epithelial cells [9]. The swab sample was then agitated in 1.5 mL of PreservCyt solution (Cytyc). This sample was kept at 4°C for at most 5 days. After centrifugation at 13,000 *g* for 15 min at 22°C, the supernatant was discarded, and the cell pellet was left to dry and resuspended in 300 μL of 20 mmol/L Tris buffer (pH 8.3). DNA was purified using a MasterPure Kit (Epicentre) [22] and then tested in each polymerase chain reaction (PCR) assay.

Anal cytology and high-resolution anoscopy. Anal cytology was also performed at each visit, as described for the HPV testing, but the swab was then rolled on a glass slide and fixed with a cytological fixative spray. High-resolution anoscopy was performed at baseline [9]. It was repeated yearly thereafter, unless AIN2 or AIN3 was revealed, in which case high-resolution anoscopy was repeated every 6 months. The findings of cytology and high-resolution anoscopy will be the focus of future reports.

HPV testing. HPV DNA testing and genotyping was done by PCR. DNA extracted from samples was first tested with primers PC04 and GH20, which target a 268-bp fragment of β-globin. Samples negative for β-globin were considered inadequate. Samples positive for β-globin were amplified with the L1 consensus HPV PGMY09/PGMY11 primer set [23] in a TC9600 thermal cycler (PerkinElmer) at 95°C for 9 min, denaturated for 1 min at 95°C, annealed for 1 min at 55°C, and extended at 72°C for 1 min for a total of 40 cycles. Amplification was followed by a 5-min terminal extension step at 72°C. HPV genotyping was performed with the reverse line-blot detection system, as described elsewhere [21], for 36 genital HPV types: 6, 11, 16, 18, 26, 31, 33–35, 39, 40, 42, 44, 45, 51–54, 56, 58, 59, 61, 62, 66–73, 81, 82 (2 subtypes, including IS39 [24]), 83, 84, and 89. Samples that were not positive for any of these types were considered HPV negative.

Statistical analysis. The analysis of the prevalence, clearance, and incidence of HPV infections was based on HPV testing results for the swab samples. High-risk HPV types included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82 [25]. Clearance of a prevalent type-specific HPV infection at baseline was considered to have occurred at the first follow-up visit at which that infection was no longer detected. The incidence of infection with a given HPV type was calculated exclusively among patients whose first available HPV test result was negative for that type. Clearance and incidence rates by type were determined using appropriate incidence density calculation based on person-time denominators (person-months). Actuarial analysis of mean and median retention times by type took into account censoring for incomplete observations (i.e., those in whom clearance had not occurred at the last recorded follow-up visit). Kaplan-Meier curves depicting cumulative incidence and clearance were constructed for each type to derive the above-indicated statistics and rates of cumulative incidence at 36 months. Statistical analyses were performed using Stata software (version 9.0; StataCorp).

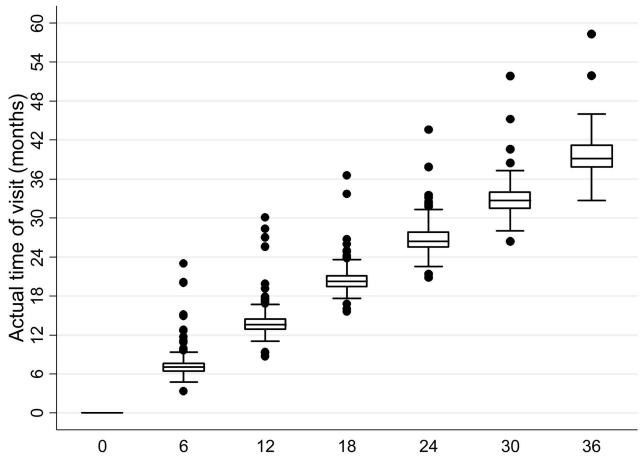

Figure 1. Box-and-whisker-plot representation of the distribution of actual follow-up times for the study-planned follow-up visits. The boxes extend from the 25th to the 75th percentile (i.e., the interquartile range [IQR]), and the lines inside the boxes represent median values. The vertical lines emerging from the boxes extend to 1.5 times the IQR above the third quartile and below the first quartile. Values outside these limits were considered outliers *(black circles)*. The no. of men attending each visit were 247 at 0 months (baseline), 217 (87.9%) at 6 months, 213 (86.2%) at 12 months, 199 (80.6%) at 18 months, 192 (77.7%) at 24 months, 152 (61.5%) at 30 months, and 137 (55.5%) at 36 months.

RESULTS

Subject recruitment and follow-up compliance. Of the 259 HIV-seropositive MSM approached for participation, 249 (96.1%) signed the informed consent form, and 2 did not complete the baseline visit, leaving 247 evaluable men in the cohort. At the end of January 2007, 1357 visits had been completed, for a total of 7612 person-months of follow-up, a mean follow-up time of 30.8 months (SD, 13.0) per subject, and a median follow-up time of 37.0 months. With respect to compliance to follow-up, 78.1% of patients completed ⩾5 visits (2 years), and 55.5% completed all 7 study visits. Figure 1 shows the distribution of actual follow-up times for the study-programmed follow-up visits.

Characteristics of participants. Table 1 presents the baseline characteristics of the study population. The mean and median age of participants was 43 years (range, 21–66). Most men were white, with at least a junior college or university education. Very few were injection drug users, but 38.0% were current smokers. The median age at first intercourse with a man was 17 years (range, 5–38), and the majority of men reported having an average of ⩾10 sex partners per year (not necessarily involving anal penetration).

The median time since HIV diagnosis was 10.7 years (range, 4 months to 25 years), and AIDS had been diagnosed in 36.1% of subjects. The median baseline CD4 cell count was 380 cells/mL of blood (range, 0–1440), and 56.2% of subjects had an undetectable HIV load; 93.1% were receiving HAART, and the me-

Table 1. Characteristics of subjects at enrollment in the HIPVIRG cohort study and of those completing ⩾5 follow-up visits.

Characteristic	Enrollment (n = 247)	⩾5 follow-up visits completed (n = 193)
Age		
20–29 years	9 (3.6)	6 (3.1)
30–39 years	72 (29.1)	48 (24.9)
40–49 years	113 (45.7)	95 (49.2)
50–59 years	48 (19.4)	41 (21.2)
60–69 years	5 (2.0)	3 (1.6)
Ethnicity		
White	221 (93.2)	173 (92.5)
Other	16 (6.8)	14 (7.5)
Education		
High school or less	84 (34.4)	63 (32.8)
Junior college or university	160 (65.6)	129 (67.2)
Smoking history		
Never	73 (30.2)	61 (32.1)
Current	92 (38.0)	65 (34.2)
Past	77 (31.8)	64 (33.7)
Injection drug use		
Never	220 (90.9)	173 (90.6)
Current	6 (2.5)	6 (3.1)
Past	16 (6.6)	12 (6.3)
Average no. of male sex partners per year		
1	7 (2.9)	6 (3.1)
2–9	77 (31.6)	57 (29.5)
10–25	84 (34.4)	67 (34.7)
>25	76 (31.1)	63 (32.6)
History of receptive anal intercourse		
Yes	224 (92.2)	180 (93.8)
No	19 (7.8)	12 (6.2)
History of condylomas		
Yes	163 (66.0)	124 (64.3)
No	53 (21.5)	43 (22.3)
Unknown	31 (12.6)	26 (13.5)
Past STI other than HIV infection[a]		
Yes	157 (63.6)	127 (65.8)
No	68 (27.5)	51 (26.4)
Unknown	22 (8.9)	15 (7.8)
Time since HIV diagnosis		
<5 years	48 (19.9)	36 (19.0)
5 to <10 years	63 (26.1)	48 (25.4)
10 to <15 years	59 (24.5)	50 (26.5)
⩾15 years	71 (29.5)	55 (29.1)
CD4 cell count at baseline		
<200 cells/μL of blood	38 (15.7)	29 (15.3)
200–500 cells/μL of blood	113 (46.7)	87 (46.0)
>500 cells/μL of blood	91 (37.6)	73 (38.6)
HIV load at baseline		
Undetectable (<50 HIV RNA copies/mL of plasma)	136 (56.2)	113 (59.5)
Detectable (⩾50 HIV RNA copies/mL of plasma)	106 (43.8)	77 (40.5)
AIDS diagnosis		
Yes	87 (36.1)	67 (35.4)
No	154 (63.9)	122 (64.6)

NOTE. Data are no. (%) of subjects. Nos. do not necessarily add up to the cohort total of 247 because of missing values.

[a] Sexually transmitted infections (STIs) include chlamydia, gonorrhea, herpes, and syphilis.

Table 2. Prevalence at enrollment and clearance of type-specific human papillomavirus (HPV) infection.

HPV type	Prevalence at enrollment, no. (%) of subjects	Clearance (for subjects positive at enrollment)			HPV infection retention time, months	
		Cleared episodes, no.	Person-months of follow-up, no.	Clearance rate, cleared episodes/1000 person-months (95% CI)	Median (95% CI)	Mean (95% CI)
High risk						
16	92 (38.2)	28	2286.3	12.2 (8.5–17.7)	ND (37.4–ND)	35.8 (32.7–38.8)
18	59 (24.5)	28	1375.2	20.4 (14.1–29.5)	34.2 (20.6–ND)	29.8 (25.9–33.7)
31	42 (17.4)	25	801.4	31.2 (21.1–46.2)	22.8 (12.8–36.5)	23.7 (19.2–28.2)
33	40 (16.6)	20	690.1	29.0 (18.7–44.9)	23.4 (7.7–ND)	24.6 (18.9–30.4)
35	33 (13.7)	23	593.9	38.7 (25.7–58.3)	19.4 (12.5–25.1)	21.7 (17.0–26.4)
39	49 (20.3)	26	968.1	26.9 (18.3–39.4)	28.0 (14.8–36.9)	25.8 (21.1–30.4)
45	51 (21.2)	36	891.4	40.4 (29.1–56.0)	19.6 (12.9–25.6)	21.3 (17.2–25.3)
51	35 (14.5)	20	596.1	33.6 (21.6–52.0)	18.2 (9.4–31.8)	22.1 (16.7–27.6)
52	52 (21.6)	29	1096.6	26.4 (18.4–38.1)	25.1 (16.7–37.3)	26.4 (22.2–30.5)
56	42 (17.4)	26	728.0	35.7 (24.3–52.5)	14.4 (13.3–32.2)	21.4 (17.1–25.8)
58	48 (19.9)	25	892.8	28.0 (18.9–41.4)	21.9 (15.2–ND)	24.6 (20.3–28.9)
59	47 (19.5)	21	1075.1	19.5 (12.7–30.0)	32.8 (20.6–ND)	29.4 (25.0–33.9)
66	23 (9.5)	18	314.5	57.2 (36.1–90.8)	13.3 (6.9–22.3)	15.5 (11.1–19.9)
68	36 (14.9)	16	606.7	26.4 (16.2–43.0)	20.5 (12.5–ND)	24.7 (19.3–30.1)
82	14 (5.8)	10	254.8	39.3 (21.1–73.0)	13.5 (6.6–ND)	20.2 (12.5–27.9)
Low risk						
6	85 (35.3)	26	1931.4	13.5 (9.2–19.8)	ND (35.2–ND)	33.6 (30.3–36.8)
11	56 (23.2)	18	1276.0	14.1 (8.9–22.4)	44.2 (31.1–ND)	33.4 (29.1–37.7)
40	19 (7.9)	11	199.1	55.2 (30.6–99.7)	11.1 (6.8–13.5)	15.1 (8.4–21.8)
42	69 (28.6)	30	1499.1	20.0 (14.0–28.6)	36.3 (20.7–ND)	30.4 (26.4–34.4)
44	47 (19.5)	22	1031.2	21.3 (14.0–32.4)	33.1 (24.8–41.5)	29.2 (25.0–33.4)
54	26 (10.8)	21	447.6	46.9 (30.6–72.0)	14.6 (8.5–21.1)	18.9 (14.1–23.8)
61	48 (19.9)	20	795.2	25.1 (16.2–39.0)	25.3 (13.6–ND)	26.8 (21.5–32.0)
70	28 (11.6)	19	524.9	36.2 (23.1–56.7)	17.1 (7.8–27.9)	20.8 (15.9–25.8)
72	21 (8.7)	15	384.6	39.0 (23.5–64.7)	20.3 (9.1–28.0)	20.8 (15.5–26.2)
81	26 (10.8)	14	411.0	34.1 (20.2–57.5)	18.2 (7.6–ND)	21.6 (15.9–27.4)
89	40 (16.6)	25	660.0	37.9 (25.6–56.0)	15.5 (9.6–20.8)	22.5 (16.6–28.4)
Undetermined risk						
26	13 (5.4)	10	178.2	56.1 (30.2–104.3)	12.5 (6.9–25.6)	15.0 (9.5–20.6)
34	3 (1.2)	3	26.0	115.3 (37.2–357.5)	7.4 (5.1–ND)	8.7 (4.6–12.7)
53	44 (18.3)	26	858.5	30.3 (20.6–44.5)	25.6 (8.1–34.7)	23.3 (19.0–27.6)
62	21 (8.7)	12	440.2	27.3 (15.5–48.0)	20.2 (14.2–ND)	26.1 (19.3–32.9)
67	16 (6.6)	12	308.7	38.9 (22.1–68.4)	19.2 (13.0–27.5)	21.4 (15.5–27.3)
69	22 (9.1)	12	395.2	30.4 (17.2–53.5)	26.0 (6.4–39.6)	24.6 (17.7–31.5)
71	6 (2.5)	0	103.9	0 (ND)	ND	42.7 (42.7–42.7)
73	40 (16.6)	22	801.3	27.5 (18.1–41.7)	28.9 (12.8–40.5)	25.3 (20.7–30.0)
83	16 (6.6)	9	220.6	40.8 (21.2–78.4)	16.9 (7.4–ND)	18.2 (12.5–23.9)
84	51 (21.2)	33	769.3	42.9 (30.5–60.3)	14.1 (11.7–20.6)	20.6 (16.2–25.0)

NOTE. HPV types are classified by risk of anogenital cancer according to Munoz et al. [25]. CI, confidence interval; ND, not determined.

dian duration of HAART was 1.8 years (range, 0–10 years). The median CD4 cell count before beginning HAART was 280 cells/mL (range, 10–1470).

The distributions of characteristics were similar for all participants and for those who completed ⩾5 visits, indicating that compliance with follow-up was not a function of most key risk factors or disease correlates. However, treatment of HIV infection resulted in improved immunological parameters over time in our participants; CD4 cell counts at the 24-month visit were higher than at baseline (median, 490 cells/mL; range, 35–1800), and 70.9% of participants had an undetectable HIV load at this visit.

HPV prevalence at baseline. HPV testing was completed at the baseline visit for 241 participants. The first column of table 2 shows the frequency distribution for all HPV types. HPV infec-

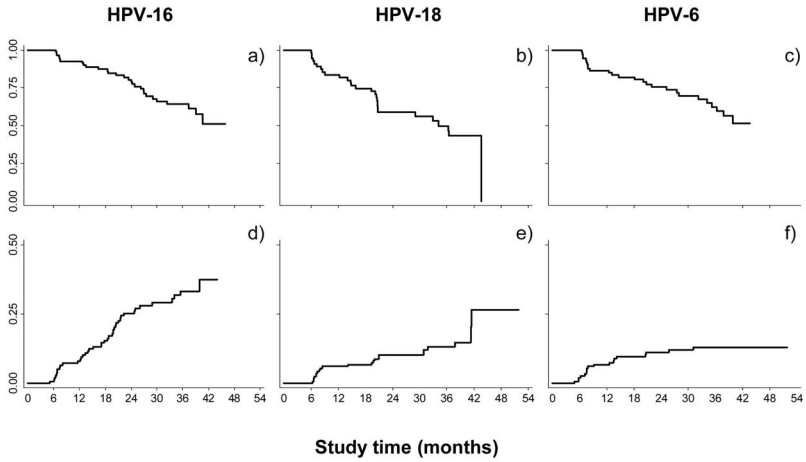

Figure 2. Kaplan-Meier estimates of the clearance times for human papillomavirus (HPV)–16 *(a),* HPV-18 *(b),* and HPV-6 *(c)* and of the cumulative incidence of infection with HPV-16 *(d),* HPV-18 *(e),* and HPV-6 *(f).*

tion was present in 97.9% of participants. Of 5 men who were not infected with HPV at baseline, only 2 never acquired HPV during this study (including 1 who completed only 2 visits). Most patients (90.9%) were infected with multiple HPV types, with a median number of 5 types per sample (range, 0–18). The median number of high-risk HPV types was 3 per sample (range, 0–12). The following were the most prevalent genotypes, being detected in at least 50 patients: HPV-16 (38.2%), HPV-6 (35.3%), HPV-42 (28.6%), HPV-18 (24.5%), HPV-11 (23.2%), HPV-52 (21.6%), HPV-45 (21.2%), and HPV-84 (21.2%). Age was not associated with type-specific HPV prevalence in stratified analysis (data not shown).

HPV clearance. As shown in table 2, HPV-16 had the lowest clearance rate, with 12.2 cleared episodes per 1000 person-months and a mean retention time of 36 months. HPV-6 and HPV-11 closely followed, with rates of 13.5 and 14.1 cleared episodes per 1000 person-months, respectively, and a mean duration of infection of 33.5 months. In comparison, the clearance rate for HPV-18 was 20.4 cleared episodes per 1000 person-months, with a mean duration of 30 months. However, these differences were not significant, as indicated by the overlapping 95% confidence intervals (CIs). HPV-26, HPV-34, HPV-40, and HPV-66 had the highest clearance rates, all >50 cleared episodes per 1000 person-months, with mean retention times between 9 and 16 months. The top part of figure 2 presents Kaplan-Meier curves depicting infection clearance patterns for 3 of the most common HPV types.

HPV incidence. Table 3 shows the incidence rates for type-specific HPV infections. These can represent new infections, reinfections, or reactivation of latent HPV infections. Again, HPV-16 had one of the highest incidence rates along with HPV-52 and HPV-53, with rates of 10.8, 10.8, and 9.8 newly detected episodes per 1000 person-months, respectively. HPV-18, HPV-6, and HPV-11 had significantly lower incidence rates, of 4.4, 4.0, and 2.4 new cases per 1000 person-months. The lowest incidence rates were for HPV-26, HPV-34, and HPV-71, with rates of <1.0 new case per 1000 person-months. With regard to the cumulative incidence of HPV infection, 33.2% of patients negative for HPV-16 at baseline became positive within 36 months, compared with only 13.1% for HPV-18. The lower part of figure 2 shows the cumulative incidence for 3 of the most common HPV types in the cohort.

DISCUSSION

This article is the first report on the epidemiology of anal HPV infections in the HIPVIRG cohort, one of the few ongoing cohorts worldwide in which the natural history and determinants of anal HPV infection and AIN in HIV-infected men receiving HAART are being studied prospectively. A high rate of participation was obtained, and compliance with follow-up was relatively high. The population characteristics and the predominance of multiple HPV types in coinfections in our participants are in agreement with findings of other studies [6, 12, 14].

The prevalence estimates for anal HPV infection at study entry may have reflected the more persistent HPV types. This is the first report of calculated incidence and clearance rates of type-specific anal HPV in HIV-seropositive MSM. We expected that HPV types with relatively low clearance rates would be most prevalent at baseline, although variations in incidence also affect prevalence. Prevalence and mean and median retention times in our cohort were higher than those for cervical HPV infection in HIV-infected women [19]. The low clearance rate and a mean retention time of close to 3 years for HPV-16 are consistent with the high prevalence of this type in the anal canal. HPV-16, being one of the most oncogenic types, has also been demonstrated to be a key precursor for the development of AIN in this population, as it is for cervical intraepithelial neoplasia in women [26]. Only 4 types of HPV had mean retention times <16 months, although the wide CIs precluded firm conclusions concerning

Table 3. Incidence of human papillomavirus (HPV) infection among subjects negative for the indicated HPV type at enrollment.

HPV type	New cases, no.	Person-months of follow-up, no.	Incidence rate, new cases/1000 person-months (95% CI)	Cumulative incidence at 36 months, % (95% CI)
High risk				
16	41	3794.2	10.8 (8.0–14.7)	33.2 (25.3–42.8)
18	22	4989.6	4.4 (2.9–6.7)	13.1 (8.5–20.0)
31	30	5325.8	5.6 (3.9–8.1)	18.1 (12.9–25.1)
33	11	5845.8	1.9 (1.0–3.4)	7.0 (3.9–12.3)
35	24	5881.8	4.1 (2.7–6.1)	12.5 (8.3–18.7)
39	20	5369.0	3.7 (2.4–5.8)	11.3 (7.4–17.2)
45	23	5236.5	4.4 (2.9–6.6)	13.8 (9.3–20.3)
51	33	5608.1	5.9 (4.2–8.3)	17.9 (12.7–24.9)
52	53	4909.6	10.8 (8.2–14.1)	30.4 (23.3–39.1)
56	26	5386.9	4.8 (3.3–7.1)	14.8 (10.1–21.4)
58	19	5319.2	3.6 (2.3–5.6)	10.8 (6.9–16.6)
59	22	5326.0	4.1 (2.7–6.3)	12.4 (8.3–18.4)
66	34	5966.8	5.7 (4.1–8.0)	18.6 (13.5–25.2)
68	22	5789.3	3.8 (2.5–5.8)	12.5 (8.4–18.4)
82	14	6544.9	2.1 (1.3–3.6)	6.5 (3.6–11.6)
Low risk				
6	18	4471.2	4.0 (2.5–6.4)	13.1 (8.4–20.0)
11	13	5372.3	2.4 (1.4–4.2)	8.1 (4.8–13.5)
40	13	6515.1	2.0 (1.2–3.4)	6.6 (3.7–11.4)
42	37	4498.4	8.2 (6.0–11.4)	24.0 (17.7–32.2)
44	28	5346.6	5.2 (3.6–7.6)	17.9 (12.4–25.5)
54	39	5518.7	7.1 (5.2–9.7)	21.5 (15.9–28.5)
61	28	5574.1	5.0 (3.5–7.3)	18.8 (13.2–26.3)
70	29	5587.5	5.2 (3.6–7.5)	16.4 (11.6–22.8)
72	19	6248.7	3.0 (1.9–4.8)	9.1 (5.7–14.2)
81	11	6309.6	1.7 (1.0–3.1)	5.9 (3.2–10.9)
89	41	5381.3	7.6 (5.6–10.3)	23.5 (17.7–30.9)
Undetermined risk				
26	5	6660.2	0.8 (0.3–1.8)	2.5 (1.0–5.8)
34	6	6991.3	0.9 (0.4–1.9)	2.6 (1.1–6.2)
53	49	4976.9	9.8 (7.4–13.0)	28.8 (22.2–36.8)
62	23	6144.4	3.7 (2.5–5.6)	12.3 (8.2–18.2)
67	18	6418.2	2.8 (1.8–4.5)	9.6 (6.0–15.1)
69	10	6302.3	1.6 (0.9–2.9)	5.3 (2.9–9.7)
71	3	6979.4	0.4 (0.1–1.3)	1.8 (0.6–5.7)
73	26	5518.3	4.7 (3.2–6.9)	15.8 (11.0–22.5)
83	15	6539.0	2.3 (1.4–3.8)	9.1 (5.5–14.8)
84	39	5084.9	7.7 (5.6–10.5)	23.3 (17.2–31.0)

NOTE. HPV types are classified by risk of anogenital cancer according to Munoz et al. [25]. CI, confidence interval.

differences in persistence across types. The low-risk types HPV-6 and HPV-11 also had mean retention times approaching 3 years. Although HPV-16 and HPV-18 may cause a significant proportion of AIN lesions, the high prevalence of concurrent HPV types, the high number of multiple-type infections, and the prolonged retention time for most HPV types suggest that many genotypes other than HPV-16 and HPV-18 may also be involved in anal carcinogenesis. Previous studies have found that multiple-type infections were associated with high-grade AIN [6].

The incidence rates for HPV-16, HPV-31, HPV-39, and HPV-53 obtained in this study are >3 times higher than those previously reported for perianal HPV infection in mostly HIV-seronegative men [27]. As was also suggested by the results of another longitudinal study comparing overall anal HPV incidence in HIV-positive and HIV-negative men [18], it is possible

that HIV infection not only increases susceptibility to HPV persistence but also increases the risk of acquisition of new HPV infections and reactivation of latent infections.

It is important to note that incidence rates cannot be directly compared with clearance rates because the denominators are not the same (all subjects at risk for incidence rates vs. only those with infections for clearance rates). HPV-16 was the type with the highest incidence rate estimate, although the CIs for type-specific incidence rates did overlap. Other highly prevalent types or types with relatively low clearance rates were not necessarily of high incidence in the cohort. Infections with HPV-6 and HPV-11, for example, were very prevalent at baseline and had low clearance rates but were relatively uncommon as incident events. Of concern are the high incidence rates for HPV-52 and HPV-53, which are comparable to that of HPV-16.

There are several limitations to our study. Although the number of participants was small, the HIPVIRG study is among the large cohorts of HIV-seropositive MSM, and compliance with follow-up visits has been excellent. It is possible that our defining clearance on the basis of only 1 negative sample underestimated the true duration of type-specific infections. Use of a more conservative definition, such as 3 consecutive negative samples for a target HPV type [28], would have reduced our precision by limiting the number of available visits and increased right censoring. As our study progresses and additional follow-up visits are accrued, our estimates will become more stable. Calculation of the mean retention time of HPV infection with the enrollment visit included also does not provide a true estimate of the average duration of HPV infections, because duration of infection before accrual is unknown. Another caveat is that most participants were >30 years old and had already acquired HIV infection, thus being past the onset of sexual activity. It is thus likely that apparent incident infections were not true new HPV infections but, in fact, reactivations of low-level infections that were below our assay's threshold of detection. This limitation, however, is nearly universal to all studies of HIV-seropositive men, regardless of age.

HPV testing using anal swab samples tends to yield a more diverse sampling of HPV types, in addition to the ones directly involved in anal carcinogenesis. This is because cells from a very wide area are collected, in contrast to AIN lesions, which occur preferentially at the junction of the anal and rectal mucosa. In a recent publication, our group demonstrated that the distributions of HPV types among anal swab samples and biopsy samples collected concurrently were similar [21]. In contrast to anal biopsy samples, most swab samples contained >1 type, and nearly all swab samples were positive for HPV. However, anal swab samples are easily obtained and identify HPV genotypes infecting the anal canal. They are better suited for studying the natural history of anal HPV infection irrespective of the presence and grade of anal lesion. The interval between visits could also change the assessment of clearance and persistence, because it is a determinant of HPV persistence in women [29]. Shorter intervals could result in earlier loss of infection or acquisition of a new type. A 6-month interval between visits is consistent with current clinical guidelines for the follow-up of AIN [30]. Shorter intervals would have resulted in more frequent anoscopies and possibly a greater loss to follow-up. Another limitation of the study is the use of the line blot assay instead of the linear array, which allows a more sensitive detection of HPV types, especially in mixed infections [24]. However, the linear array was not available at the beginning of our cohort study, and concordance between results obtained with both assays was excellent.

Cohort studies such as the HIPVIRG study will provide useful insights into preventive strategies to counter the increasing incidence of anal cancer in this vulnerable population. Further analysis regarding determinants of HPV infection and AIN in this cohort will follow, because we have demonstrated in a subset of our participants that HPV detected in anal swab samples at a single visit is not predictive of the presence of high-grade AIN, in contrast to detection of HPV DNA in biopsy samples [21]. Determinants that may prove relevant include HPV persistence, HPV load, and HPV variants.

HIPVIRG STUDY GROUP

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