The Journal of Infectious Diseases

MAJOR ARTICLE

Detection of Incident Anal High-Risk Human Papillomavirus DNA in Men Who Have Sex With Men: Incidence or Reactivation?

Denise E. Twisk,[1] Marianne A. B. van der Sande,[2,3,4] Arne van Eeden,[5] Daniëlle A. M. Heideman,[6] Fiona R. M. van der Klis,[2] Henry J. C. de Vries,[1,2,7] and Maarten F. Schim van der Loeff[1,8]

[1]Department of Infectious Diseases, Public Health Service Amsterdam, [2]Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, and [3]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands; [4]Department Public Health, Institute of Tropical Medicine, Antwerp, Belgium; [5]Department of Internal Medicine, DC Klinieken Oud Zuid, Amsterdam, [6]Department of Pathology, Cancer Center Amsterdam, VU University Medical Center, Amsterdam, and [7]Department of Dermatology, and [8]Amsterdam Infection and Immunity Institute (AIII), Academic Medical Center, The Netherlands.

(See the Editorial Commentary by Burchell, on pages 1015–7.)

Background. We aimed to assess whether sexual exposure may explain all incident anal human papillomavirus (HPV) detections among men who have sex with men (MSM).

Methods. A longitudinal study among MSM was conducted between 2010 and 2013 with visits every 6 months and up to 24 months of follow-up. Risk-factor questionnaires, blood samples, and anal and penile self-swabs were collected at each visit. Self-swabs were used for detection and genotyping of HPV by the broad spectrum L1 based SPF_{10} PCR DNA/enzyme immunoassay $LiPA_{25}$ system. Serum samples were tested for high-risk HPV (hrHPV) antibodies. Incident anal HPV detection rates among sexually non-, low, and highly exposed MSM were compared. Factors associated with incident anal hrHPV detection were assessed using multivariable Cox regression.

Results. Seven hundred fourteen men (median age, 40 years; 39% human immunodeficiency virus [HIV] infected) were included in the analysis. Incident anal detections of all hrHPV types were observed among both sexually nonexposed and exposed MSM. In multivariable analyses, being highly sexually exposed, being HIV infected, and having a penile HPV infection were positively associated with incident anal HPV detection; those reporting more sex partners had a nonsignificantly increased risk of HPV detection.

Conclusions. Incident anal hrHPV detection is common among recently nonexposed MSM, suggesting that a reactivated latent HPV infection instead of an incident infection may underlie incident HPV detection.

Keywords. HPV; HIV; incidence rate; men who have sex with men; latency.

Human papillomavirus (HPV) infections are very common among sexually active people [1]. Persistent high-risk HPV (hrHPV) infections of the cervix and anus are associated with high-grade dysplasia and cancer [2, 3]. Only a small proportion of all HPV infections persist. Up to 90% of all high- or low-risk HPV infections become undetectable after acquisition within about 2 years [4–6]. It is generally assumed that undetectable infections are cleared, but latency may provide another explanation [7–10].

Typically, individuals with a positive HPV DNA test are considered to be HPV infected, whereas a negative HPV DNA test represents HPV-uninfected individuals [7]. To avoid false-negative test results, some studies require at least 2 consecutive negative tests to declare clearance. However, ≥2 negative test results do not rule out an HPV infection as HPV might persist in tissues below the limit of detection, displaying viral latency [7].

The current analysis aims to increase understanding of the meaning of incident anal hrHPV detection among men who have sex with men (MSM) by using data of the longitudinal HPV in HIV-Negative and HIV-Positive Infected MSM (H2M) study. We assessed the incidence of anal hrHPV infections in MSM in relation to the level of sexual exposure.

METHODS

The current study is based on data from the H2M study, a longitudinal cohort study with a follow-up time of 24 months. The overall aim of the H2M study was to assess the epidemiology of HPV in human immunodeficiency virus (HIV)–negative and HIV-infected MSM. Details on enrollment, data collection, HPV DNA detection and genotyping, and HPV serology were previously described [11–13]. Upon cohort entry, all men reported having had sex with men.

Study Participants

Between July 2010 and July 2011, 317 HIV-infected and 461 HIV-negative men aged ≥18 years were enrolled in the H2M

Received 22 December 2017; editorial decision 2 April 2018; accepted 8 May 2018; published online May 16, 2018.

Correspondence: M. F. Schim van der Loeff, MD, PhD, Department of Infectious Diseases, Public Health Service Amsterdam, PO Box 2200, 1000 CE, Amsterdam, The Netherlands (mschim@ggd.amsterdam.nl).

The Journal of Infectious Diseases® 2018;218:1018–26

DOI: 10.1093/infdis/jiy276

study [11]. All participants provided written informed consent prior to participation. The Medical Ethics Committee of the Academic Medical Center Amsterdam approved the H2M study.

Data Collection

Data were collected at baseline and at 6-month intervals for up to 24 months. At each visit, participants completed a self-administered questionnaire regarding sociodemographic characteristics, health-related issues, and sexual behavior. Participants collected 1 anal and 1 penile shaft self-swab during each visit. In addition, a venous blood sample was collected for serum antibody testing. HIV-related data (CD4 cell count, HIV RNA load, and use of combination antiretroviral therapy [cART]) were obtained, at baseline, from the Dutch HIV Monitoring Foundation's national HIV patient database [14].

HPV DNA Detection and HPV Serology

Anal and penile swabs were tested and typed using the highly sensitive broad spectrum L1 based SPF_{10} PCR DNA/enzyme immunoassay $LiPA_{25}$ system (version 1, Labo Biomedical Products, Rijswijk, the Netherlands), which is able to recognize at least 68 HPV genotypes [15]. Serum samples of 7 hrHPV types (16, 18, 31, 33, 45, 52, and 58) were tested for HPV antibodies to the major HPV capsid protein L1 by a virus-like particle–based multiplex immunoassay [16–18].

Statistical Methods

For analyses we focused on 7 hrHPV types: 16, 18, 31, 33, 45, 52, and 58. Detection of type-specific incident anal HPV DNA (hereafter referred to as incident anal HPV detection) was defined as an HPV-positive visit preceded by at least 2 consecutive HPV-negative visits for this specific HPV type. We assumed that incidence occurred at the midpoint between the date of the last negative sample and the date of the positive sample. For type-specific HPV detection rates, only participants at risk for type-specific incident anal HPV detection were included. Men were considered at risk if they completed at least 3 visits and were negative for this specific HPV type on their first 2 study visits. Time at risk for an HPV type ended when that specific HPV type was detected using the midpoint date, or at the date of the last collected sample. Type-specific anal HPV incident detection rates were calculated by dividing the number of incident detections by the number of person-years (PY) at risk, and expressed as events per 100 PY with 95% confidence intervals (95% CI). The same method was used to calculate incident detection rates of type-specific penile HPV. To account for possible autoinoculation from penile mucosa, we calculated incident anal HPV detection rates in a risk set limited to men without penile HPV DNA detection at the 2 preceding visits or at the current visit.

We calculated anal HPV incident detection rates for MSM by recent sexual anal exposure (3 groups). The sexually non-exposed group included men who reported no receptive and no insertive anal intercourse in the 6 months prior to the visit, and were not rimmed and not fisted within this period. High sexual exposure was defined as reporting ≥2 anal sex partners (receptive and/or insertive anal intercourse) and having been rimmed and/or fisted in the 6 months prior to the visit. Low exposure was defined as having had anal sex or having been rimmed and/or fisted but not meeting the criteria for high sexual exposure. A sensitivity analysis was performed using a stricter definition of exposure status: no receptive and no insertive anal intercourse in the 12 months prior to the visit, and not rimmed and not fisted within this period. Although condoms protect against most sexually transmitted infections, condoms only partially protect against HPV infections [19, 20]. Therefore, reported condom use was ignored in the definitions of anal sexual exposure, meaning that men reporting anal intercourse were regarded as exposed, whether they had used condoms or not.

Univariable and multivariable Cox proportional hazard regression analyses using the Wei-Lin-Weissfeld method were used to assess risk factors for incident anal HPV detection [21]. This method is an elaboration of Cox proportional hazard regression with generalized estimating equations and an exchangeable correlation structure, which adjusts for correlations between multiple observations within a subject; a subject is simultaneously at risk for up to 7 different hrHPV events and at risk for each event until this event occurs [21]. All variables that were associated with incident anal HPV detection (P value < .2, Wald test) in univariable analyses were included in the multivariable model; some variables were forced into the multivariable model because they were a priori considered relevant based on literature or our specific research question: sexual exposure status (the main variable of interest), number of lifetime male sex partners [22–24], HPV serostatus [10, 25, 26], and HIV status [27, 28]. Other (candidate) variables included demographic and health-related characteristics (age, tobacco smoking, cannabis use in the previous 6 months, popper use in the previous 6 months, and alcohol use in the previous 6 months) and sexual behavior characteristics (age at first anal intercourse and years since anal sexual debut). In addition, penile HPV DNA detection during the 2 preceding visits or at the current visit was included as autoinoculation from penis to anus is possible. We were not able to take autoinoculation from oral sites to anus into account, as oral HPV status was lacking for the fourth and fifth visits. Candidate variables were retained in the final multivariable model if they had a P value <.05 (backward-selection method). Interaction was tested between exposure status and all variables that remained in the final model.

Multicollinearity in the multivariable analysis was evaluated by a variance inflation factor. A variance inflation factor of ≥10

Table 1. Baseline Characteristics of the Study Population[a] (N = 714)

Characteristic	No.	(%)
Sociodemographic characteristics		
Age, y		
≤34	182	(25.5)
35–44	321	(45.0)
≥45	211	(29.5)
Median (IQR)	40.1	(34.8–47.4)
Country of birth		
The Netherlands	572	(81.0)
Any other country	134	(19.0)
Missing	8	
Health-related characteristics		
Tobacco smoking		
Never	249	(38.4)
In the past	172	(26.5)
Current	228	(35.1)
Missing	65	
Cannabis use previous 6 mo		
No	474	(70.3)
Yes	200	(29.7)
Missing	40	
Popper use previous 6 mo		
No	349	(57.1)
Yes	262	(42.9)
Missing	103	
Alcohol use previous 6 mo		
No	113	(17.0)
Yes	552	(83.0)
Missing	49	
Self-reported history of anal warts		
No	465	(66.2)
Yes	237	(33.8)
Missing	12	
HIV infected		
No	439	(61.5)
Yes	275	(38.5)
HPV seropositive		
HPV-16	253	(42.8)
HPV-18	201	(31.6)
HPV-31	82	(13.6)
HPV-33	195	(30.8)
HPV-45	247	(38.1)
HPV-52	98	(16.1)
HPV-58	107	(16.1)
Penile shaft HPV DNA positive		
HPV-16	42	(5.9)
HPV-18	20	(2.8)
HPV-31	23	(3.2)
HPV-33	17	(2.4)
HPV-45	18	(2.5)
HPV-52	31	(4.3)
HPV-58	2	(0.3)
Penile shaft HPV DNA negative for all above HPV types	601	(84.2)
Sexual behavior characteristics		
No. of male sex partners during life		
≤15	42	(6.3)
16–50	116	(17.5)
51–100	118	(17.8)
101–500	224	(33.8)
≥501	162	(24.5)
Median (IQR)	200	(60–500)
Missing	52	
Age at first anal intercourse, y		
≤18	198	(30.6)
19–21	170	(26.2)
22–25	177	(27.3)
≥26	103	(15.9)
Median (IQR)	21	(18–24)
Missing	66	
Years since anal sex debut		
≤10	83	(12.8)
11–20	293	(45.2)
≥21	272	(42.0)
Median (IQR)	18.4	(13.1–25.6)
Missing	66	
No. of male sex partners during previous 6 mo		
0	113	(16.1)
1	184	(26.1)
2–5	224	(31.8)
≥6	183	(26.0)
Median (IQR)	2	(1–6)
Missing	10	
Anal intercourse in previous 6 mo		
None	113	(16.0)
Only insertive	123	(17.4)
Only receptive	96	(13.6)
Both insertive and receptive	374	(53.0)
Missing	8	
Condom use previous 6 mo		
No anal sex	113	(16.1)
Anal sex, always protected	187	(26.6)
Anal sex, sometimes protected	285	(40.6)
Anal sex, never protected	117	(16.7)
Missing	12	
Being rimmed previous 6 mo		
No	230	(32.6)
Yes	475	(67.4)
Missing	9	
Being fisted previous 6 mo		
No	629	(90.1)
Yes	69	(9.9)
Missing	16	
HIV-related characteristics		
CD4 cell count at enrollment, cells/μL		
≤350	35	(12.7)
>350	240	(87.3)
Median (IQR)	535	(410–690)
Nadir CD4 cell count, cells/μL		
<200	85	(35.1)
200–349	108	(44.6)
≥350	49	(20.3)
Median (IQR)	230	(170–320)
Missing	33	
HIV RNA load		
Undetectable (<50 copies/mL)	182	(78.8)
Detectable (≥50 copies/mL)	49	(21.2)

Table 1. Continued

Characteristic	No.	(%)
Missing	44	
Use of cART		
No	31	(13.1)
Yes	205	(86.9)
Missing	39	

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: cART, combination antiretroviral therapy; HIV, human immunodeficiency virus; HPV, human papillomavirus; IQR, interquartile range.

[a]Study population was men at risk for an incident anal high-risk HPV detection. Men at risk are all men with ≥2 consecutive HPV type-specific negative tests followed by an HPV test, which can either be positive or negative for at least 1 of the high-risk HPV types.

was the criterion used for multicollinearity. No variables in the models presented here reached this criterion.

All statistical analyses were performed using Stata version 13.1 software (StataCorp, College Station, Texas).

RESULTS

Baseline Characteristics

Of the 778 men enrolled in the H2M study, we excluded 64 (8.2%) men because they had <3 study visits. Remaining in the current analysis were 714 (91.8%) men, all at risk for incident anal hrHPV detection, given they were negative for at least 1 hrHPV type on their first 2 study visits. Excluded men had more male sex partners in the previous 6 months ($P < .001$), were more often HIV infected ($P < .001$), had a lower nadir CD4 cell count ($P = .03$), and had more often a detectable plasma HIV RNA load ($P < .001$).

Table 1 presents the distribution of sociodemographic, health-related, sexual behavior, and HIV-related characteristics at baseline of men included in this study. At enrollment, the median number of lifetime male sex partners was 200 (interquartile range [IQR], 60–500), and the median number of male sex partners in the previous 6 months was 2 (IQR, 1–6). Two-thirds (n = 470 [66.6%]) reported receptive anal sex (with or without insertive anal sex) in the 6 months before enrollment. For most HIV-infected men, HIV RNA load was undetectable (182 of the 275 HIV-infected participants), and 86.9% were on cART.

Type-Specific Incident Anal HPV Detection by Anal Sexual Exposure

The 714 included men contributed 3444 visits with information on anal and penile swabs typed for HPV. For 257 visits (7.5%), there were incomplete data on sexual exposure status, leaving 3187 visits with information on sexual exposure. Of these, 371 visits by 157 men were regarded as sexually non-exposed in the preceding 6 months, 1339 visits by 494 men as low sexually exposed and 1477 visits by 481 men as highly sexually exposed. Some men contributed visits to >1 exposure group. In total, there were 354 incident anal hrHPV detections. The most frequently detected incident HPV types were HPV-31 and HPV-52 (ie, each nearly 20% of detections). Person-years at risk varied by HPV type. The mean time at risk was 1.3 years (range, 0.1–2.2 years). Table 2 shows the type-specific incident anal HPV detection rates stratified by sexual exposure. For the highly sexually exposed group, HPV-31 had the highest incidence detection rate with 13.6 cases per 100 PY, followed by HPV-52 (12.3/100 PY). For the low and non–sexually exposed groups, HPV-52 had the highest incidence detection rate (6.9/100 PY and 6.6/100 PY, respectively). The next highest incidence detection rate in the sexually nonexposed group

Table 2. Number of Incident Anal High-Risk Human Papillomavirus (HPV) Detections[a], Incidence Detection Rates, and Incidence Detection Rate Ratios of Type-Specific Anal High-Risk HPV Types

	Total	Sexually Nonexposed MSM[b]		Low Sexually Exposed MSM[c]		Highly Sexually Exposed MSM[d]		Highly Sexually Exposed MSM vs Nonexposed MSM	
HPV Type	Total No. of Incident Detections	No. of Incident Detections	Incidence Rate per 100 PY (95% CI)	No. of Incident Detections	Incidence Rate per 100 PY (95% CI)	No. of Incident Detections	Incidence Rate per 100 PY (95% CI)	IRR (95% CI)	*P* Value
HPV-16	56	5	5.7 (2.4–13.6)	19	5.6 (3.6–8.8)	32	9.9 (7.0–14.0)	1.8 (.6–4.5)	.24
HPV-18	47	2	2.1 (.5– 8.4)	18	4.9 (3.1–7.7)	27	7.4 (5.1–10.7)	3.5 (.8–14.8)	.07
HPV-31	68	5	5.6 (2.3–13.4)	20	5.9 (3.8–9.1)	43	13.6 (10.1–18.3)	2.4 (1.0–6.1)	.05
HPV-33	38	3	3.0 (1.0–9.3)	10	2.7 (1.4–4.9)	25	6.8 (4.6–10.1)	2.3 (.7–7.5)	.17
HPV-45	52	2	2.0 (.5–8.0)	17	4.6 (2.8–7.4)	33	3.6 (6.5–12.9)	4.6 (1.1–19.1)	**.02**
HPV-52	70	6	6.6 (3.0–14.8)	24	6.9 (4.6–10.3)	40	12.3 (9.1–16.8)	1.9 (.8–4.4)	.15
HPV-58	23	4	3.8 (1.4–10.2)	5	1.3 (.5–3.0)	14	3.5 (2.1–5.9)	0.9 (.3–2.8)	.87

Significant results ($P < .05$) are represented in bold font.

Abbreviations: CI, confidence interval; HPV, human papillomavirus; IRR, incidence rate ratio; MSM, men who have sex with men; PY, person-years.

[a]An incident anal high-risk HPV detection was defined as a positive HPV test result preceded by at least 2 consecutive HPV negative visits of that specific high-risk HPV type.

[b]Sexual nonexposure was defined as reporting no receptive and no insertive anal sexual exposure (ie, no anal intercourse, not being rimmed, not being fisting) in the 6 months prior to the visit. Data of 157 men were included.

[c]Low sexual exposure was defined as reporting sexual exposure in the 6 months prior to the visit (receptive and/or insertive anal intercourse or having been fisted and/or having been rimmed), but not meeting the criteria for high sexual exposure. Data of 494 men were included.

[d]High sexual exposure was defined as reporting ≥2 anal sex partners (receptive and/or insertive anal intercourse), and having been fisted and/or rimmed in the 6 months prior to the visit. Data of 481 men were included.

was found for HPV-16 with 5.7 cases per 100 PY. This was comparable to the HPV-16 detection rate for the sexually low exposed group (5.6/100 PY). Among highly sexually exposed men, the HPV-16 incidence detection rate was higher (9.9/100 PY; $P = .24$). For all assessed HPV types, except HPV-58, the incident anal HPV detection rates were higher in the highly sexually exposed group than in the sexually nonexposed group (Table 2), but this difference was only significant for 1 type. Similar patterns were observed with a stricter definition of exposure status (Supplementary Table 1).

Subgroup and Sensitivity Analyses

Among HPV-seronegative men no significant differences in incidence detections rates were observed between nonexposed MSM and highly exposed MSM; neither was this the case among HPV-seropositive men (data not shown). Using the stricter definition of exposure status (based on the preceding 12 months) did not change this (data not shown).

Incident penile HPV infections were frequently detected in our study population (Supplementary Table 2). An analysis on incident anal hrHPV detection was done excluding observations from men with current or recent penile HPV infection (Supplementary Table 3). Incident anal HPV infections were also detected among nonexposed men without penile infection.

Risk Factors for Incident Anal HPV Detection

Results from univariable and multivariable models are summarized in Table 3. The multivariable model showed that HIV-infected men and men with a penile HPV infection were more likely to have an incident anal HPV detection. Men who were highly sexually exposed were almost 2 times more likely to have an incident anal HPV detection than nonexposed men ($P = .01$). The incidence rate in men with low sexual exposure was similar compared to that of nonexposed men. HPV serostatus at baseline and lifetime number of sex partners were not significantly associated with incident HPV detection. Although none of the tested interaction terms were significant (all $P > .3$), we also assessed univariable and multivariable models stratified by exposure status (Supplementary Table 4). In the model for sexually nonexposed men, no risk factors were associated with incident anal HPV detection. For MSM with low sexual exposure, HIV infection was significantly associated with incident anal HPV detection in the multivariable model. In the highly sexually exposed group HIV infection, penile HPV infection, and younger age were associated with incident anal HPV detection.

DISCUSSION

This study revealed that incident anal HPV detection is common in both sexually nonexposed and sexually exposed MSM. The incident anal detection rates of individual hrHPV types tended to be higher in the highly sexually exposed group than in the sexually nonexposed group, but this difference was mostly nonsignificant. In multivariable analysis, including all 7 hrHPV types, recent high sexual exposure was significantly associated with incident anal hrHPV detection, as were being HIV infected and penile HPV detection. Number of sex partners showed a trend toward significance.

From a naive point of view, one might expect that men without recent receptive anal exposure would not have HPV detections, or have a very low incident detection rate. However, our study demonstrates that sexually nonexposed MSM have a considerable incident anal HPV detection rate (eg, HPV-16: 5.7/100 PY). In a sensitivity analysis using a stricter definition of sexual exposure, we found similar patterns. This suggests that incident anal HPV detection can occur without recent receptive anal exposure. This finding supports the hypothesis that incident detection of anal HPV may be due to reactivation of a previous anal HPV infection [7], and does not always represent an incident infection.

Other studies also observed incident HPV detections among recently sexually inactive individuals [26, 29], but none of these studies was conducted among men. One study among HIV-infected MSM observed a similar anal HPV detection prevalence in sexually inactive MSM compared with sexually active MSM [30]. This was a cross-sectional study, reporting prevalence rates, which is weaker evidence compared to our cohort study reporting incidence rates. Our finding suggests, as did other studies [26, 29, 30], that finding HPV detections among sexually nonexposed individuals could indicate reactivation of previously acquired HPV. That reactivation is a likely explanation for incident HPV detection among sexually nonexposed individuals is strengthened by our multivariable analysis. We found that having a higher number of lifetime sex partners showed a trend toward significance and being HIV infected was significantly associated with incident anal HPV detections. Both more lifetime sex partners [22–24] and HIV infection [27, 28] could be seen as indicators for the probability of prior infection. We also expected that having a positive HPV serostatus at baseline, a marker for (previous) infection, would be positively associated with incident anal HPV detection, but we did not find this. Immunosuppression is often invoked as explanation for reactivation of a latent HPV infection, as immunological control of an acquired type-specific HPV infection may be affected by HIV infection. Strickler et al and Theiler et al observed strong associations between HPV detection and markers of immunity [26, 29]. Memory CD4 cells for HPV may be important in the control of latent HPV infections, as it has been shown before that HPV detection increases rapidly after acute HIV infection [31, 32]. Although the CD4 cell count generally increases after starting cART in HIV-infected individuals, a complete reconstitution of the immune systems to preinfection levels usually does not happen [33]. If immune memory controls HPV infections below limits of detection

Table 3. Associations Between Incident Anal High-Risk Human Papillomavirus Detection[a] and Possible Risk Factors

	Univariable		Multivariable[b,c]	
Characteristic	HR (95% CI)	*P* Value[d]	aHR (95% CI)	*P* Value[d]
Sociodemographic characteristics				
Age group, y		.62		
≤34	Ref			
35–44	0.92 (.68–1.25)			
≥45	0.85 (.61–1.18)			
Health-related characteristics				
Tobacco smoking		.25		
Never	Ref			
In the past	0.78 (.56–1.09)			
Current	1.03 (.78–1.37)			
Cannabis use previous 6 mo	1.33 (1.02–1.76)	**.04**[e]		
Popper use previous 6 mo	1.72 (1.32–2.21)	**<.001**[e]		
Alcohol use previous 6 mo	1.32 (.96–1.85)	.09[e]		
HPV seropositive at baseline	1.09 (.85–1.41)	.49[e]	0.89 (.67–1.17)	.39
Sexual behavior characteristics				
Anal sexual exposure (6 mo)[f]		**<.001**[e]		
Sexually nonexposed	Ref		Ref	
Low sexually exposure	1.14 (.72–1.81)		1.09 (.67–1.79)	.73
Highly sexually exposed	2.22 (1.43–3.42)		1.90 (1.19–3.02)	**.01**
No. of male sex partners during life (per increase of natural log)	1.19 (1.11–1.28)	**<.001**[e]	1.07 (.98–1.67)	.16
No. of male sex partners during previous 6 mo		**<.001**		
0	Ref			
1	1.12 (.73–1.74)			
2–5	2.00 (1.34–2.98)			
≥6	2.26 (1.51–3.37)			
Age at first anal intercourse, y		.25		
≤18	Ref			
19–21	0.80 (.57–1.12)			
22–25	0.91 (.65–1.26)			
≥26	0.68 (.45–.02)			
Years since anal sex debut		.39		
≤10	Ref			
11–20	1.13 (.70–1.82)			
>20	1.31 (.81–2.10)			
Being rimmed previous 6 mo	1.86 (1.41–2.44)	**<.001**		
Being fisted previous 6 mo	1.38 (.92–2.08)	.12		
Condom use previous 6 mo		**<.001**		
No anal sex	Ref			
Anal sex, always protected	1.42 (.94–2.15)			
Anal sex, sometimes protected	2.23 (1.52–3.26)			
Anal sex, never protected	1.30 (.81–2.08)			
Penile HPV detection[g]	1.61 (1.27–2.05)	**<.001**[e]	1.41 (1.06–1.89)	**.02**
HIV-related characteristics				
HIV infected	1.71 (1.34–2.18)	**<.001**[e]	1.49 (1.13–1.98)	**.004**
HIV status and CD4 cell count		**<.001**		
HIV negative	Ref			
HIV infected, CD4 ≥350 cells/μL	2.22 (1.43–3.44)			
HIV infected, CD4 200–349 cells/μL	1.70 (1.26–2.30)			
HIV infected, CD4 <200 cells/μL	1.41 (.94–2.11)			

Results are from Cox regression analyses using generalized estimating equations. Significant results ($P < .05$) are represented in bold font.

Abbreviations: aHR, adjusted hazard ratio; CI, confidence interval; HIV, human immunodeficiency virus; HPV, human papillomavirus; HR, hazard ratio; Ref, reference.

[a]An incident anal high-risk HPV detection was defined as a positive HPV test result preceded by at least 2 consecutive HPV negative visits of that specific high-risk HPV type.

[b]Based on data of 630 men.

[c]Interaction was tested between anal exposure status and all variables in the final model; no such interactions were detected (all $P > .3$).

[d]Based on Wald test.

[e]Variables associated at $P < .20$ or variables forced into the model because they were a priori considered relevant based on previous literature to be included in the multivariable model.

[f]Anal sexual exposure. Sexually nonexposed was defined as men reporting no receptive and no insertive anal sexual exposure (ie, no anal intercourse, not being rimmed, not being fisting) in the 6 months prior to the visit. Low sexual exposure was defined as reporting sexual exposure in the 6 months prior to the visit (receptive and/or insertive anal intercourse or having been fisted and/or having been rimmed), but not meeting the criteria for high sexual exposure. High sexual exposure was defined as reporting ≥2 anal sex partners (receptive and/or insertive anal intercourse), and having been fisted and/or rimmed in the 6 months prior to the visit.

[g]Penile HPV detection at 12 and/or 6 months prior to current visit and/or at current visit.

under normal conditions, immune system weakening may allow an increase or restart of HPV viral replication resulting in (renewed) HPV detection. In line with the studies referred to above, we found that incident anal HPV detection was more likely in HIV-infected MSM. We also considered lifetime number of male sex partner as an important potential risk factor for anal HPV detection, since it can be seen as proxy for past HPV exposure [22–24]. In the current study, we observed a (nonsignificant) increased risk for incident anal HPV detection with increasing number of lifetime sex partners. A possible explanation for not finding a strong association could be saturation of risk for anal HPV, as our study population is very sexually active; only 3.6% had ≤10 lifetime sex partners.

Having a penile HPV infection was positively associated with incident anal HPV detection. Since autoinoculation from other epithelial sites could also contribute to a fraction of incident HPV detections [34], we assessed penile infection during the 2 preceding visits and/or the current visit. We found that MSM with a penile infection had an increased risk of incident anal HPV detection (hazard ratio, 1.41 [95% CI, 1.06–1.89]). This is in agreement with a recent study based on mathematical modeling of genital male infections by Ranjeva et al [34], which concluded that observed epidemiological patterns could only be explained by autoinoculation or latency. Unfortunately we were not able to incorporate autoinoculation from other sites such as the oral cavity, since oral HPV status was missing for the fourth and fifth visits.

The current study adds to the understanding of anal HPV viral latency. Our study is among the first with longitudinal data among men that combines HPV status with detailed information about sexual exposure. This enabled us to evaluate differences in incident anal HPV detections among subgroups of MSM with low and high sexual risk for recent HPV infection, by using a sexual exposure definition that carefully considered all relevant exposures. Another strength was the strict definition of incidence. We required that one should have at least 2 consecutive hrHPV type-negative tests to be at risk for incidence for that HPV type. A proper characterization of truly incident anal HPV infections would have required that we would have observed our study population from the onset of sexual exposure, which is (in most cases) not feasible. The various sensitivity analyses (stricter definition of sexual exposure, incidence stratified by baseline HPV serostatus, and analysis excluding men with possible autoinoculation) did not lead to different results, thus strengthening our findings.

Some limitations of this study must be recognized. We required at least 3 visits to be included in our analysis. This led to an important difference between participants included in our analyses and those excluded. Excluded men were significantly more often HIV infected compared to included men. This may have reduced power to find associations, but should not have affected the strength of observed observations. A second limitation is that we were unable to estimate the proportion of HPV detections caused by reactivation of previous (sexually) acquired infections in the sexually exposed groups. The most likely explanation for HPV detection in these groups is (new) recent sexual exposure. This combined sexual exposure (past and recent) as explanation for newly detected HPV infections is also suggested by other studies [24, 35]. González et al, in a study among women in Costa Rica, could only explain 21% of the incident infections by new sex partners; another 21% was explained by the lifetime number of sex partners [35]. Furthermore, false-negative HPV tests may have overestimated the incidence rate in the nonexposed group. However, we limited the risk of false-negatives by using an incidence definition requiring 2 consecutive preceding HPV negative visits; a single negative test can be due to chance. Another limitation is that our HPV detection was on type level, therefore we could not exclude that someone could become infected with another variant of the same HPV type. Analysis on HPV molecular variant incidence would be more accurate. In the current study, we did not report separate incident HPV detection rates for HIV-infected and HIV-uninfected MSM, as the number of type-specific HPV events was too low to compare reliable estimates. The last limitation is that our exposure group classification relied on self-reported sexual behavior. However, considering the high HPV incident detection rates among sexually nonexposed men, it would have required a gross misinterpretation among a large proportion of participants to account for these incidence rates. In addition, we were unable to account for exposure/transmission by sex toys in our exposure definition [36] as our questionnaire lacks questions about sex toys or other stimulation methods.

In conclusion, we observed high incident anal HPV detection rates among recently sexually nonexposed MSM. This strongly suggests that (re-)detection of anal HPV in MSM may be due to reactivation of an anal HPV infection and is not always a truly incident infection. This may have implications for the interpretation of longitudinal studies on incidence and clearance of anal HPV.

Supplementary Data

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

Notes

Author contributions. M. F. S. L. designed and led the H2M study as principal investigator. M. A. B. S., H. J. C. V., and D. A. M. H. contributed to the study design. H. J. C. V. and A. E. contributed to the implementation of the study and data collection. F. R. M. K. planned and supervised the serological analyses. D. E. T., together with M. F. S. L., performed the statistical

analyses and interpreted the data. D. E. T. wrote a draft manuscript, which was revised by M. F. S. L. All other authors contributed to revision of the manuscript (M. A. B. S., A. E., D. A. M. H., F. R. M. K., H. J. C. V.).

Acknowledgments. The authors thank all of the H2M study participants. Above all, we gratefully thank Sofie Mooij for setting up the H2M study.

Financial support. This work is supported by a grant from Aids Fonds (grant number 2009029) and additional funding by the Public Health Service of Amsterdam and the National Institute for Public Health and the Environment.

Potential conflicts of interest. D. A. M. H. serves occasionally on the scientific advisory board of Pfizer and Bristol-Meyers Squibb; has been on the speakers' bureau of Qiagen; and is minority stakeholder of Self-screen B.V., a spin-off company of VU University Medical Center. H. J. C. V. has received grants from Sanofi Pasteur MSD concerning HPV vaccine studies and from ZonMW. The institution of M. F. S. L. received study funding from Sanofi Pasteur MSD; he is a coinvestigator in a Merck-funded investigator-initiated study; he is an investigator on a Sanofi Pasteur MSD–sponsored trial; he served on a vaccine advisory board of GSK; his institution received in-kind contribution from Stichting Pathologie Onderzoek en Ontwikkeling for an HPV study; and his institution received research funding from Janssen Infectious Diseases and Vaccines. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Einstein MH, Schiller JT, Viscidi RP, et al. Clinician's guide to human papillomavirus immunology: knowns and unknowns. Lancet Infect Dis **2009**; 9:347–56.
2. Giuliano AR, Tortolero-Luna G, Ferrer E, et al. Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. Vaccine **2008**; 26(Suppl 10):K17–28.
3. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med **2003**; 348:518–27.
4. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet **2007**; 370:890–907.
5. Bosch FX, Burchell AN, Schiffman M, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine **2008**; 26(Suppl 10):K1–16.
6. Giuliano AR, Lee JH, Fulp W, et al. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet **2011**; 377:932–40.
7. Gravitt PE. The known unknowns of HPV natural history. J Clin Invest **2011**; 121:4593–9.
8. Maglennon GA, McIntosh P, Doorbar J. Persistence of viral DNA in the epithelial basal layer suggests a model for papillomavirus latency following immune regression. Virology **2011**; 414:153–63.
9. Maglennon GA, Doorbar J. The biology of papillomavirus latency. Open Virol J **2012**; 6:190–7.
10. Gravitt PE. Evidence and impact of human papillomavirus latency. Open Virol J **2012**; 6:198–203.
11. van Aar F, Mooij SH, van der Sande MA, et al. Anal and penile high-risk human papillomavirus prevalence in HIV-negative and HIV-infected MSM. AIDS **2013**; 27:2921–31.
12. Mooij SH, Boot HJ, Speksnijder AG, et al. Oral human papillomavirus infection in HIV-negative and HIV-infected MSM. AIDS **2013**; 27:2117–28.
13. Mooij SH, van Santen DK, Geskus RB, et al. The effect of HIV infection on anal and penile human papillomavirus incidence and clearance: a cohort study among MSM. AIDS **2016**; 30:121–32.
14. van Sighem AI, van de Wiel MA, Ghani AC, et al. Mortality and progression to AIDS after starting highly active antiretroviral therapy. AIDS **2003**; 17:2227–36.
15. Molijn A, Kleter B, Quint W, van Doorn LJ. Molecular diagnosis of human papillomavirus (HPV) infections. J Clin Virol **2005**; 32(Suppl 1):S43–51.
16. Scherpenisse M, Mollers M, Schepp RM, et al. Seroprevalence of seven high-risk HPV types in the Netherlands. Vaccine **2012**; 30:6686–93.
17. Scherpenisse M, Schepp RM, Mollers M, Meijer CJ, Berbers GA, van der Klis FR. Characteristics of HPV-specific antibody responses induced by infection and vaccination: cross-reactivity, neutralizing activity, avidity and IgG subclasses. PLoS One **2013**; 8:e74797.
18. Mooij SH, van der Klis FR, van der Sande MA, et al. Seroepidemiology of high-risk HPV in HIV-negative and HIV-infected MSM: the H2M study. Cancer Epidemiol Biomarkers Prev **2013**; 22:1698–708.
19. Burchell AN, Tellier PP, Hanley J, Coutlée F, Franco EL. Human papillomavirus infections among couples in new sexual relationships. Epidemiology **2010**; 21:31–7.
20. Pierce Campbell CM, Lin HY, Fulp W, et al. Consistent condom use reduces the genital human papillomavirus burden among high-risk men: the HPV infection in men study. J Infect Dis **2013**; 208:373–84.
21. Xue X, Gange SJ, Zhong Y, et al. Marginal and mixed-effects models in the analysis of human papillomavirus natural history data. Cancer Epidemiol Biomarkers Prev **2010**; 19:159–69.
22. Giuliano AR, Anic G, Nyitray AG. Epidemiology and pathology of HPV disease in males. Gynecol Oncol **2010**; 117:S15–9.

23. Heywood W, Smith AM. Anal sex practices in heterosexual and male homosexual populations: a review of population-based data. Sex Health **2012**; 9:517–26.
24. Rositch AF, Burke AE, Viscidi RP, Silver MI, Chang K, Gravitt PE. Contributions of recent and past sexual partnerships on incident human papillomavirus detection: acquisition and reactivation in older women. Cancer Res **2012**; 72:6183–90.
25. Safaeian M, Porras C, Schiffman M, et al. Epidemiological study of anti-HPV16/18 seropositivity and subsequent risk of HPV16 and -18 infections. J Natl Cancer Inst **2010**; 102:1653–62.
26. Strickler HD, Burk RD, Fazzari M, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. J Natl Cancer Inst **2005**; 97:577–86.
27. Brickman C, Palefsky JM. Human papillomavirus in the HIV-infected host: epidemiology and pathogenesis in the antiretroviral era. Curr HIV/AIDS Rep **2015**; 12:6–15.
28. Maglennon GA, McIntosh PB, Doorbar J. Immunosuppression facilitates the reactivation of latent papillomavirus infections. J Virol **2014**; 88:710–6.
29. Theiler RN, Farr SL, Karon JM, et al. High-risk human papillomavirus reactivation in human immunodeficiency virus-infected women: risk factors for cervical viral shedding. Obstet Gynecol **2010**; 115:1150–8.
30. Ong JJ, Chen M, Tabrizi SN, et al. Anal HPV detection in men who have sex with men living with HIV who report no recent anal sexual behaviours: baseline analysis of the Anal Cancer Examination (ACE) study. Sex Transm Infect **2016**; 92:368–70.
31. Wang C, Wright TC, Denny L, Kuhn L. Rapid rise in detection of human papillomavirus (HPV) infection soon after incident HIV infection among South African women. J Infect Dis **2011**; 203:479–86.
32. Nowak RG, Gravitt PE, Morrison CS, et al. Increases in human papillomavirus detection during early HIV infection among women in Zimbabwe. J Infect Dis **2011**; 203:1182–91.
33. Okoye AA, Picker LJ. CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. Immunol Rev **2013**; 254:54–64.
34. Ranjeva SL, Baskerville EB, Dukic V, et al. Recurring infection with ecologically distinct HPV types can explain high prevalence and diversity. Proc Natl Acad Sci U S A **2017**; 114:13573–8.
35. González P, Hildesheim A, Rodríguez AC, et al. Behavioral/lifestyle and immunologic factors associated with HPV infection among women older than 45 years. Cancer Epidemiol Biomarkers Prev **2010**; 19:3044–54.
36. Liu Z, Rashid T, Nyitray AG. Penises not required: a systematic review of the potential for human papillomavirus horizontal transmission that is non-sexual or does not include penile penetration. Sex Health **2016**; 13:10–21.