CLINICAL REPORT

Acquisition and Clearance of Perianal Human Papillomavirus Infection in Relation to HIV-positivity in Men Who Have Sex with Men in the Netherlands

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This study was performed to establish the prevalence of perianal human papillomavirus (HPV) infection in relation to HIV-positivity in a group of men who have sex with men (MSM), and to correlate follow-up data with regard to acquisition and clearance of HPV infection. Data with regard to HPV prevalence and HIV serostatus during two visits were compared. At both visits participants underwent a routine venereological examination and swabs were taken from the perianal region for HPV DNA testing. During both visits HPV types 16, 18, 31, 33 and 52 were significantly more often detected in HIV-positive individuals. Persistence of HPV type 31 at the perianal region was significantly more often seen in HIV-positive MSM ($p=0.036$) while the incidence of type 16 may be associated with HIV positivity ($p=0.059$). In HIV-positive MSM significantly more high-risk HPV types were detected at the perianal region. *Key words: homosexual; persistence; perianal infection.*

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Human papillomavirus (HPV) infection is the most common sexually transmitted viral infection and has a steadily increasing prevalence (1). The prevalence of genital subclinical and latent HPV infection in sexually active women and men ranges from 10 to 46%, depending on country and population tested (2).

Anal cancer is an uncommon cancer in men in western European countries and the USA. The incidence of anal cancer in the general population is between 7 and 9 per million and has increased in recent decades (3). Anal cancer shares many features with cervical cancer, including a similar histology and a tendency to arise in the transformation zone, where the columnar epithelium changes to squamous epithelium (4). HPV DNA has been identified in 46–100% of all *in situ* and invasive squamous cell carcinomas of the anus (3, 5). There is evidence that the HPV types that are causally linked to cervical cancer may also be linked to anal cancer. In a

study in Denmark and Sweden, among 388 patients with invasive or *in situ* anal carcinomas in women and men, HPV-16 was detected by PCR in 73% (3). A large number of partners, young age at the time of first (receptive anal) sexual contact, unmarried status, a variety of concomitant (rectal) sexually transmitted diseases (STDs) and a history of rectal genital warts are all linked to the risk of anal cancer (3).

Probably, only persistent infections may trigger carcinogenic development (6). Suppressed cellular immunity in HIV-positive persons and organ transplant recipients receiving immunosuppressive therapy is associated with persistence of HPV infection in cervical cancer (7).

The incidence of anal cancer in men who have sex with men (MSM) was estimated to be at least 44 times higher than in the general population, while the incidence of anal cancer among HIV-positive MSM may be about twice that of HIV-negative MSM (8). In a population-based study linking HIV and cancer registries, the risk of anal cancer among persons with HIV was 84 times greater than in the general population (9, 10). According to Del Mistro & Chieco Bianchi (11), HIV-infected MSM are at increased risk for persistent HPV infection.

Goldie et al. (4, 12) stated that screening HIV-negative MSM for preneoplastic anal lesions with anal cytology every 2 or 3 years would provide life expectancy benefits, and would be cost-effective. In HIV-positive MSM annual screening was found to be cost-effective.

Most HPV infections are transient; about 70% of infections with HPV are cleared within 1 year and as many as 91% are cleared within 2 years (13). The mean duration of high-risk HPV infection is longer than that of low-risk HPV infection (6). Other data showed that HPV DNA positivity may be more persistent for high-risk HPV types 16, 18, 31, 33 and 35 (14).

To develop strategies for prevention and early treatment of HPV-associated preneoplastic anal lesions and anal carcinoma, a better understanding of clearance of HPV infection is needed. Knowledge of the prevalence of HPV in anal specimens could be the first step towards the development of an effective vaccine to prevent HPV-related anal carcinoma.

In this study data with regard to HPV prevalence and HIV serostatus of MSM at two planned cohort visits, with a median interval of 21 months, were compared. Data on the first visit were published elsewhere (15). The major goal was to study clearance and acquisition of anal HPV infection, and to investigate possible differences between HIV-positive and HIV-negative cohort participants.

MATERIALS AND METHODS

Study population and study design

The study was performed at the STD clinic of the Department of Dermatology and Venereology, Erasmus MC, Rotterdam, the Netherlands. From February 1999 to February 2000, we recruited 286 MSM to participate in the Rotterdam cohort study. MSM were recruited by trained volunteers at gay meeting places like bars and saunas. Both HIV-positive and HIV-negative individuals were asked to join the study. The way participants were recruited has been described in more detail elsewhere (15). At enrolment, all participants provided written informed consent. The ethics committee of our medical centre approved the protocol.

Cohort participants were tested for STD and HIV every 6 months, over a period of 3 years. HPV specimens were only taken during the third and sixth visit.

Data collection and questionnaires

Demographic and sexual behavioural data were collected. These included ethnic background, age, educational qualification, sexual orientation and number of sexual partners during the last 6 months. Also, data were collected about age of first sexual experience, estimated number of lifetime sexual partners and the presence of genital warts in the past by using self-administered questionnaires.

Venereological examination

At enrolment and at each semi-annual visit, all participants underwent a standardized venereological examination as described previously (16). In brief, the examination included testing for urethral, rectal and oropharyngeal gonorrhoea and urethral and rectal *Chlamydia trachomatis* infection. Blood samples were taken to be tested for HIV antibodies, syphilis and hepatitis B. In HIV-positive men blood samples were taken for a CD4+ lymphocyte count every 3 months.

HPV DNA sample collection

Specimens for assessment of HPV DNA were collected using a dry swab (Medical Wire & Equipment Co. Ltd, Corsham, Wiltshire, UK), swabbing the perianal area. The swabs were immediately placed into standard collecting tubes without transport medium and sent to the Department of Virology for further processing.

All third visit specimens were taken between March 2000 and September 2001 and all sixth visit specimens were taken during the period January 2002 to May 2003.

Detection and typing of HPV DNA

During the third visit HPV DNA testing was carried out in 119 of the 258 men (46.1%) by using a specific HPV type detection PCR for HPV-6, -11, -16, -18, -31 and -33, as described before

(15, 17) (Fig. 1). Later on, the SPF LiPA (18) HPV PCR-reverse hybridization test, detecting 25 different HPV types, i.e. 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, 74 and 'unclassified' (XX), was used in 139 of 258 men (53.9%).

Van Doorn et al. (19) showed that LiPA results are highly concordant with those of genotype-specific tests. The total nucleic acid DNA was extracted by using the total nucleic acid isolation kit on a MagnaPure LC system (Roche Applied Science, Penzberg, Germany).

We used the epidemiologic classification according to Munoz et al. (20), which grouped HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 as high-risk and considered types 26, 53 and 66 as probably high-risk. When high-risk and low-risk HPV types were compared in our study, HPV types 53 and 66 were considered as high-risk types. HPV types 26, 73 and 83 were not tested in this study. During the sixth visit, 213 participants were tested for HPV DNA with the SPF LiPA HPV PCR-reverse hybridization test (Fig. 1).

As two different HPV detection methods were used, all individuals tested for HPV types 6–33 are, for analytical purposes, referred to as group A. Those tested for HPV types 34–74 at both visits are referred to as group B.

Statistics

Data were compared to assess statistically significant differences in the prevalence of HPV related to HIV status. Prevalence was calculated as the number of positive tests by type per 100 tested individuals. Persistent cases of HPV were defined as cases which were prevalent at both visits. Fisher's exact test was used to test differences in prevalence of various HPV types between HIV-negative and HIV-positive MSM. A test was considered significant if the p value was < 0.05.

Incidence rates of infection with individual HPV types, and their associated 95% confidence intervals (CI), were calculated on the basis of the numbers of cases in which a given type was detected among MSM at the sixth visit who were free of that type at the third visit. Calculation of the CI was based on the Poisson distribution (21).

RESULTS

Fig. 1 shows a flow chart of the study material, starting with a cohort of 286 men at visit 1 and finally with a cohort of 213 men at visit 6. During the third visit, 258 men still participating in the cohort study (subcohort, 90.2% of the original cohort) were tested for HPV DNA and during the sixth visit 213 men were tested (82.6% of the subcohort). The median duration between the third and sixth visit was 21 months (range 7–32 months).

Population characteristics

During the third visit, 258 men (subcohort) were tested, including 17 HIV-positive men and 241 HIV-negative men. During the sixth visit, 213 men were tested (82.6% of the subcohort), including 17 HIV-positive men and 196 HIV-negative men. None of the MSM refused perianal testing for HPV DNA.

Forty-five participants (17.4% of the subcohort) were only tested for HPV at the third visit, as they dropped out before the sixth visit. These participants were
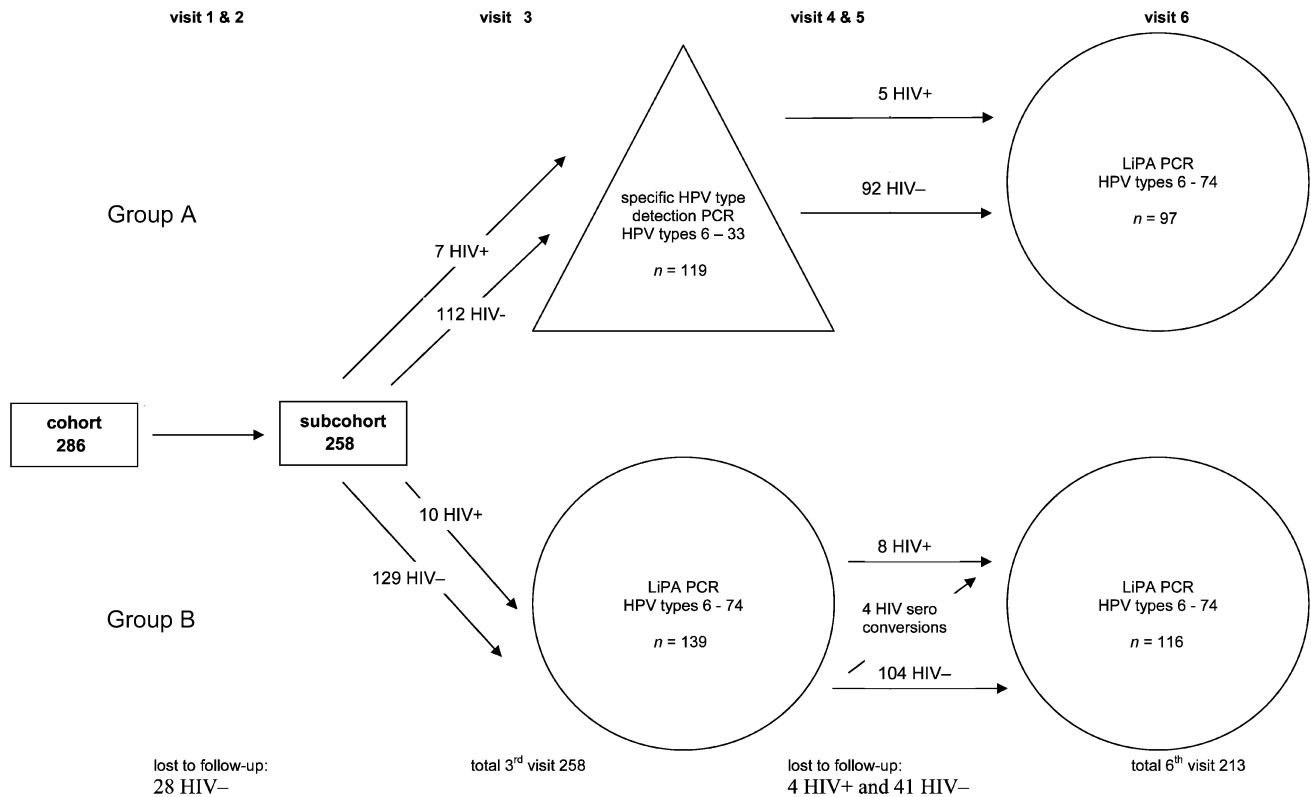
Fig. 1. Flow chart of the study material. The triangle symbols the number of men and HIV serostatus tested by using a specific HPV type detection PCR at the third visit. The circles indicates the number of men and HIV serostatus tested by using the SPF LiPA HPV PCR-Reverse Hybridization test at the third and/or sixth visit.

comparable to the rest of the subcohort with respect to sexual orientation (percentage bisexual: 20.0 versus 9.9%; $p=0.071$), median number of sexual partners during the last 6 months (5 versus 8; $p=0.090$) and median age at first sexual contact (both groups: 18 years). Those who were not followed up were significantly younger (median age 38 versus 43 years; $p=0.011$), less often had a college degree (28.9 versus 48.0%; $p=0.021$) and more often were of non-Dutch descent (11.1 versus 3.3%; $p=0.039$).

Of the subcohort tested during the third visit, four HIV-positive men did not show up and could therefore not be tested for the second time. Of the 17 HIV-positive men tested at the sixth visit, four were detected since their third semi-annual visit and had therefore seroconverted recently. The median CD4+ lymphocyte count of all tested HIV-positive participants at the third visit was $600/mm^3$ (range 340–1020) compared to $570/mm^3$ (range 230–1100) at the sixth visit. During the third visit, only one HIV-positive MSM had a CD4+ lymphocyte count of $\leqslant 350/mm^3$ compared with five HIV-positive MSM tested during the sixth visit. Viral loads were undetectable (RNA plasma viraemia below 50 copies/ml) during the third visit in five MSM. The mean viral load in the other men was 4.1×10^4 copies/ml. At both visits, 5 of

the 17 HIV-positive individuals were on antiretroviral therapy.

With regard to demographics, the 17 HIV-positive MSM at the third visit were comparable to the 241 HIV-negative MSM of the subcohort with respect to sexual orientation (percentage bisexual: 0 versus 12.4%; $p=0.23$), age (median age: 43 versus 42 years; $p=0.80$), ethnicity (MSM of non-Dutch descent: 5.9 versus 4.6%; $p=0.57$), educational qualification (college degree: 33.3 versus 45.2%; $p=0.43$), median age at first sexual contact (median age: 17 versus 18 years, $p=0.93$) and median number of sexual partners during the last 6 months (median number: 10 versus 6; $p=0.074$).

Of all HIV-positive MSM, 5 (29.4%) had had genital warts in the past versus 40 HIV-negative MSM (16.6%, $p=0.19$).

At the third visit perianal warts were found in six (2.3%) individuals compared to five (2.3%) MSM at the sixth visit. HPV-6 was detected in three individuals and HPV-11 in five. Surprisingly, in three of the MSM with perianal warts no HPV could be detected at the perianal region.

In this study no data were available about cigarette smoking. An estimated minority of <5% of the participants were circumcised.

HPV findings

Table I summarizes the detection of HPV DNA in perianal specimens in HIV-positive and HIV-negative men at the third and sixth visit. Data on HPV positivity at the third visit have been published elsewhere (15). At the third visit, 90 of the 258 (34.9%) perianal specimens in group A were positive for at least one HPV type compared to 78 of the 213 (36.6%) specimen at the sixth visit. Types most often detected were HPV-6 and -16 (13.2/12.2% and 10.5/11.7% at visit three and six, respectively). Of the 139 MSM in group B tested at the third visit 60 (43.2%) were positive for at least one HPV type compared to 57/116 MSM (49.1%) at the sixth visit. Most often detected at the last visit were HPV-51 (12.9%), HPV-53 (10.3%), HPV-44 (9.5%) and HPV-66 (8.6%).

Table I shows that HPV-16, -18, -31 and -33 were detected in perianal specimens significantly more often in HIV-positive participants during both visits in group A. In group B only HPV-52 was detected significantly more often in HIV-positive MSM during both visits. HPV types 39, 44, 51 and 66 were significantly more often detected in HIV-positive individuals but only during the sixth visit.

During the visit, two or more different types of HPV were more often found in perianal specimens of HIV-positive men than in HIV-negative men, namely 14 times (82.4%) versus 66 times (33.7%; $p < 0.0005$). High-risk HPV types at the perianal region were found in 15 HIV-positive men (88.2%) and in 91 (46.4%) of the HIV-negative men ($p = 0.001$).

Acquisition and clearance

The detection of the 12 most frequently detected HPV types (those with $\geq 7\%$ cumulative positivity) in perianal specimens at the third visit and the clearance and acquisition rates per 100 men-months for each HPV type are summarized in Table II. HPV-51 was the most frequent incident type (0.52% per month), followed by HPV-53 and HPV-16 (0.48 versus 0.37% per month). Clearance was seen most often for HPV-52 (5.15% per month), followed by HPV-68 (5.00% per month), HPV-39 and HPV-11 (both 4.79% per month).

Table II also classifies HPV types on the basis of the tendency for a given type to persist during both visits. The right-hand column in Table II shows the ratio between the frequency of positivity in both visits and

Table I. *Detection of human papillomavirus (HPV) types in perianal specimens in HIV-positive and HIV-negative men (n; %) at third and sixth visit*

Detected HPV type	Third visit HIV-negative	Third visit HIV-positive	*p* values	Sixth visit HIV-negative	Sixth visit HIV-positive	*p* values
Group A[a]	241	17		196	17	
HPV-6	30 (12.4)	4 (23.5)	n.s.	22 (11.2)	4 (23.5)	n.s.
HPV-11	19 (7.9)	1 (5.9)	n.s.	9 (4.6)	2 (11.8)	n.s.
HPV-16	22 (9.1)	5 (29.4)	0.022	19 (9.7)	6 (35.3)	0.007
HPV-18	9 (3.7)	4 (23.5)	0.006	13 (6.6)	7 (41.2)	<0.001
HPV-31	18 (7.5)	4 (23.5)	0.045	4 (2.0)	5 (29.4)	<0.001
HPV-33	8 (3.3)	4 (23.5)	0.005	7 (3.6)	3 (17.6)	0.036
Group B	129	10		104	12	
HPV-34	0	0	–	2 (1.9)	0	n.s.
HPV-35	5 (3.9)	0	n.s.	3 (2.9)	1 (8.3)	n.s.
HPV-39	7 (5.4)	2 (20)	n.s.	2 (1.9)	3 (25)	0.008
HPV-40	4 (3.1)	0	n.s.	0	0	–
HPV-42	0	0	–	1 (1.0)	0	n.s.
HPV-43	2 (1.6)	0	n.s.	4 (3.8)	2 (16.7)	n.s.
HPV-44	7 (5.4)	1 (10)	n.s.	7 (6.7)	4 (33.3)	0.015
HPV-45	5 (3.9)	1 (10)	n.s.	3 (2.9)	1 (8.3)	n.s.
HPV-51	6 (4.7)	1 (10)	n.s.	11 (10.6)	4 (33.3)	0.049
HPV-52	10 (7.8)	6 (60)	<0.0005	5 (4.8)	4 (33.3)	0.006
HPV-53	7 (5.4)	1 (10)	n.s.	9 (8.7)	3 (25)	n.s.
HPV-54	2 (1.6)	0	n.s.	1 (1.0)	0	n.s.
HPV-56	2 (1.6)	0	n.s.	3 (2.9)	1 (8.3)	n.s.
HPV-58	0	0	–	0	1 (8.3)	n.s.
HPV-59	3 (2.3)	0	n.s.	5 (4.8)	1 (8.3)	n.s.
HPV-66	2 (1.6)	0	n.s.	6 (5.8)	4 (33.3)	0.010
HPV-68	10 (7.8)	3 (30)	0.053	5 (4.8)	1 (8.3)	n.s.
HPV-70	2 (1.6)	0	n.s.	5 (4.8)	2 (16.7)	n.s.
HPV-74	2 (1.6)	0	n.s.	7 (6.7)	0	n.s.
HPV-XX	2 (1.6)	0	n.s.	3 (2.9)	0	n.s.

[a]Groups A and B are defined in Fig. 1.

Table II. *Frequency of human papillomavirus (HPV) types in perianal specimens in all men at third visit and cumulative positivity at sixth visit including ratio between frequency of positivity in both visits ("twice") and that in one visit ("once")*

Detected HPV type	Prevalence at 3rd visit (%)	Clearance at 6th visit (%)	Clearance rate/month*	Acquisition at 6th visit	Acquisition rate/month†	Positive twice (%)	Ratio twice/once
Group A (n=213)							
HPV-6	29 (13.6)	15 (51.7)	4.46	12 (5.6)	0.30	14 (6.6)	0.5
HPV-11	14 (6.6)	10 (71.4)	4.79	7 (3.3)	0.16	4 (1.9)	0.2
HPV-16	25 (11.7)	15 (60.0)	4.30	15 (7.0)	0.37	10 (4.7)	0.3
HPV-18	10 (4.7)	5 (50.0)	4.55	15 (7.0)	0.34	5 (2.3)	0.3
HPV-31	19 (8.9)	17 (89.5)	4.70	7 (3.3)	0.17	2 (0.9)	0.1
HPV-33	9 (4.2)	5 (55.6)	4.33	6 (2.8)	0.14	4 (1.9)	0.4
Group B (n=116)							
HPV-39	8 (6.9)	6 (75.0)	4.79	3 (2.6)	0.13	2 (1.7)	0.2
HPV-44	7 (6.0)	3 (42.9)	4.70	7 (6.6)	0.31	4 (3.4)	0.4
HPV-51	5 (4.3)	2 (40.0)	4.55	12 (10.3)	0.52	3 (2.6)	0.2
HPV-52	12 (10.3)	8 (66.7)	5.15	5 (4.3)	0.23	4 (3.4)	0.3
HPV-53	7 (6.0)	6 (85.7)	4.29	11 (9.5)	0.48	1 (0.9)	0.1
HPV-68	11 (9.5)	10 (90.9)	5.00	5 (4.3)	0.23	11 (9.5)	0.1

*Number of cleared cases prevalent cases at sixth visit per 100 men-months.
†Number of incident cases at sixth visit per 100 men-months.

that in one visit, as an indication of the tendency of a given type to persist. The ratio for all HPV types is <1. The ratio for HPV-6 was 0.5, indicating that an HPV-6 infection was twice as often detected at only one of the two test visits.

Relation with HIV serostatus

The tendency to persist of the 12 most frequently detected perianal HPV types in HIV-positive and HIV-negative men shows that the tendency of HPV-31 for being persistent was significantly stronger in HIV-positive MSM ($p=0.036$).

The incidence of perianal HPV-16 infection could possibly be associated with HIV positivity ($p=0.059$) (Table III). Other types did not show a (close to) significant relation in this table.

DISCUSSION

In this study we examined the persistence and incidence of perianal HPV infection in a Dutch cohort of MSM, and showed that HPV infection was relatively frequent: up to 50% of all MSM tested for HPV infection in group B at the perianal region were positive for one or more of the investigated HPV types. Nearly 40% of the HIV-positive participants tested were positive for HPV-16 or HPV-18 at the perianal site at both visits. During this visit two or more different types of HPV were more often found in perianal specimens of HIV-positive men than in HIV-negative men. The most common HPV infections at the perianal site were HPV-6, -16, -18 and -31.

The high-risk HPV-51 was the most frequent incident type in this study (0.52% per month). The incidence of perianal HPV-16 infection showed a strong tendency towards association with HIV positivity. In HIV-positive

Table III. *Incidence of perianal human papillomavirus (HPV) infection in both HIV-positive and HIV-negative men who have sex with men (MSM)*

Detected HPV type	MSM rate/1000 person months (95% CI)	
	HIV-positive	HIV-negative
Group A		
HPV-6	4.9 (0.1–27.4)	3.0 (1.5–5.3)
HPV-11	0.0 (0.0–11.3)	1.2 (0.4–2.9)
HPV-16	15.2 (3.1–44.5)	2.9 (1.5–5.2)
HPV-18	10.3 (1.2–37.0)	2.7 (1.3–4.8)
HPV-31	9.4 (1.1–33.9)	1.0 (0.3–2.6)
HPV-33	0.0 (0.0–15.7)	1.2 (0.4–2.8)
Group B		
HPV-39	8.1 (0.2–45.3)	0.5 (0.1–2.7)
HPV-44	6.5 (0.2–36.4)	1.9 (0.5–4.9)
HPV-51	6.6 (0.2–36.9)	4.7 (2.3–8.7)
HPV-52	11.1 (0.3–61.9)	1.5 (0.3–4.3)
HPV-53	14.2 (1.7–51.2)	3.9 (1.7–7.7)
HPV-68	0.0 (0.0–27.3)	1.9 (0.5–4.9)

MSM the tendency to persist was significantly higher for HPV type 31 at the perianal region, as compared with HIV-negative MSM.

We propose that a high incidence and a higher tendency to persist of high-risk perianal HPV infection (HPV-31 in this study) may be a reason for more frequent HPV-related disease in HIV-positive MSM. Our results are largely in accordance with the study on the prevalence of cervical HPV infection by Franco et al. (6), in which they showed that in a group of 1425 low-income women in a high-risk area for cervical cancer the mean infection duration was longer for high-risk HPV types.

In the HIV-positive participants a significantly higher persistence of high risk HPV type 31 was seen but not of other high-risk types or low-risk HPV types. Richardson

et al. (22) found that HPV type 16 was the most persistent, followed by HPV-31 and HPV-53 in cervical specimens of >600 female university students in Montreal, Canada.

High prevalences of high-risk HPV types, as found in HIV-positive individuals in this study, may go together with a high detection rate of abnormal perianal cytology (23). Anal cancer screening should be promoted, especially in HIV-positive individuals, to prevent HPV-related malignancies (4, 12, 24).

It is unlikely that variability in sample collection can explain the differences in HPV detection. During both visits, >90% of all perianal HPV specimens were collected by the same physician. All other men were examined by the same, thoroughly instructed, research nurse at both visits.

Given the fact that MSM from the Rotterdam region were invited to join the cohort, it is most likely that the cohort participants were not representative of the MSM population at large in the Netherlands. One of the major limitations of our study is the relatively small number of HIV-positive individuals; however, this is in concordance with the modest rate of HIV seropositivity amongst MSM in the Netherlands (25).

Another important issue in this study is that we cannot measure true persistence among those positive for a specific HPV type at enrolment. As yet it is still impossible to distinguish a new infection with a certain HPV type from a persistent infection, within the limitations of the HPV detection method. For this reason we can only measure the 'presumed' persistent rate of those positive at enrolment. Maybe type-specific serology will enable discrimination between a new infection and persistent infection in the future.

Understanding the epidemiology of perianal HPV infection is important for the prevention of HPV-related disease in MSM. Longitudinal studies in different geographic areas may provide information which can also be used for prevention and future immunization programmes (26). Further studies in MSM are needed to establish additional risk factors related to HPV persistence.

ACKNOWLEDGEMENT

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