

HPV Seroconversion Following Anal and Penile HPV Infection in HIV-Negative and HIV-Infected MSM

Sofie H. Mooij^{1,2}, Olivia Landén¹, Fiona R.M. van der Klis³, Marianne A.B. van der Sande^{3,4}, Hester E. de Melker³, Maria Xiridou³, Arne van Eeden⁵, Titia Heijman¹, Arjen G.C.L. Speksnijder¹, Peter J.F. Snijders⁶, and Maarten F. Schim van der Loeff^{1,2}

Abstract

Background: We assessed human papillomavirus (HPV) seroconversion following anal and penile HPV infection in HIV-negative and HIV-infected men who have sex with men (MSM).

Methods: MSM aged ≥ 18 years were recruited in Amsterdam, the Netherlands (2010–2011), and followed up semiannually. Antibodies against 7 high-risk HPV types in baseline and 12-month serum samples were tested using a multiplex immunoassay. Baseline, 6-, and 12-month anal and penile samples were tested for HPV DNA using the SPF₁₀-PCR DEIA/LiPA₂₅ system. Statistical analyses were performed using logistic regression with generalized estimating equations.

Results: Of 644 MSM included in the analysis, 245 (38%) were HIV-infected. Median age was 38 years for HIV-negative and 47 years for HIV-infected MSM ($P < 0.001$). Seroconversion against ≥ 1 of the 7 HPV types was observed in 74 of 396 (19%) HIV-negative and 52 of 223 (23%) HIV-infected MSM at risk ($P = 0.2$). Incident [adjusted OR (aOR) 2.0; 95% confidence interval (CI), 1.1–3.4] and persistent (aOR 3.7; 95% CI, 1.5–9.5) anal HPV infections were independently associated with type-specific seroconversion in HIV-negative MSM. In HIV-infected MSM, there was a nonsignificant positive association between penile HPV infection at any time point and seroconversion (aOR 1.7; 95% CI, 0.9–3.2).

Conclusions: Incident or persistent anal HPV infection was an independent determinant of seroconversion in HIV-negative MSM.

Impact: Our data support that seroresponse may vary per anatomic site and that persistent HPV infections are more likely to elicit a detectable humoral immune response. *Cancer Epidemiol Biomarkers Prev*; 23(11); 2455–61. ©2014 AACR.

Introduction

Infection with a human papillomavirus (HPV) is one of the most common sexually transmitted infections, and persistent infection with certain high-risk types is the

cause of many cancers worldwide (1). Most HPV infections are transient and are cleared by the human immune system within 1 or 2 years (1–3). Cellular immunity plays a major role in the clearance of HPV infection (4).

Humoral immune responses following natural HPV infection of the anogenital tract are not always observed: approximately 40% to 60% of women and probably a lower proportion of men develop type-specific antibodies (5–9). If seroconversion does occur, antibodies against HPV capsids may persist for years, and are therefore reflective not only of recent, but lifetime HPV exposure (5, 8, 10). HPV seropositivity has been associated with both virus-related (e.g., regarding persistence of infection, viral load, and viral type) and host-related factors (e.g., increasing age, HIV-induced immunosuppression, anatomic site of infection, and sexual risk behavior; refs. 7, 9, 11, 12). The very high antibody concentrations observed after HPV vaccination confer protection against incident HPV infection (13, 14), but to what extent naturally induced antibodies also confer protection is still a matter of debate (9).

Longitudinal studies analyzing HPV seroconversion following HPV infection are scarce, in particular in men and in HIV-infected individuals. More insight into the

¹Cluster of Infectious Diseases, Public Health Service of Amsterdam (GGD), Amsterdam, the Netherlands. ²Center for Infection and Immunity Amsterdam (CINIMA), Department of Internal Medicine, Academic Medical Center, Amsterdam, the Netherlands. ³Centre for Infectious Disease Control, National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM), Bilthoven, the Netherlands. ⁴Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands. ⁵Department of Internal Medicine, Jan van Goyen Medical Center, Amsterdam, the Netherlands. ⁶Department of Pathology, Vrije Universiteit-University Medical Center (VUmc), Amsterdam, the Netherlands.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

Current address for A. van Eeden: Department of Internal Medicine, DC Klinieken Oud Zuid, Amsterdam, the Netherlands.

Corresponding Author: Sofie Mooij, Public Health Service of Amsterdam, Nieuwe Achtergracht 100, 1018 WT, Amsterdam, the Netherlands. Phone: 31-20-555-5705; Fax: 31-20-555-5533; E-mail: somooij@ggd.amsterdam.nl

doi: 10.1158/1055-9965.EPI-14-0199

©2014 American Association for Cancer Research.

associations between HPV infection at various anatomic sites, HIV infection, and seroresponse is crucial to interpret results from serosurveillance, which can, for example, be used to monitor HPV prevention strategies. As men who have sex with men (MSM), and especially HIV-infected MSM, are at increased risk for HPV infection (15) and HPV-related diseases (16, 17), understanding these issues among MSM is important. In this study, we aimed to assess type-specific HPV seroconversion following anal and penile HPV infection in HIV-negative and HIV-infected MSM.

Materials and Methods

Study population

HIV-negative and HIV-infected MSM were recruited into the HIV & HPV in MSM (H2M) study from July 2010 to July 2011 at three study sites in Amsterdam, the Netherlands: the Amsterdam Cohort Study (ACS) among MSM (18), an outpatient infectious disease clinic (Jan van Goyen Medical Center), and the Amsterdam Sexually Transmitted Infection (STI) clinic (19). Inclusion criteria were having had sex with men, being 18 years or older, and speaking English or Dutch. All participants provided written informed consent before participation. The study was approved by the Medical Ethics Committee of the Academic Medical Center (AMC) Amsterdam (Amsterdam, the Netherlands).

Data collection

At enrollment, participants completed an extensive, self-administered questionnaire regarding sociodemographic characteristics, health-related issues, and sexual behavior. Venous blood was drawn for analyses of HPV antibodies. Participants were instructed to self-swab their anus and penile shaft using 2 separate swabs (regular flocked swab with 1 mL UTM medium, Copan) for HPV DNA analyses, as previously described (20). HIV-related data (e.g., CD4 cell count and HIV viral load) were obtained from the national HIV patient database of the Dutch Monitoring Foundation.

Participants were invited for follow-up every 3 to 6 months. During follow-up visits, swabs and blood samples were taken using the same method as during the baseline visit. Data described in this article are limited to 1-year follow-up: HPV DNA was analyzed in anal and penile self-swabs collected at baseline, 6 months, and 12 months, whereas HPV antibodies were analyzed in serum samples collected at baseline and at 12 months.

HPV serology

Serum samples were stored at -20°C and transported to the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands), where samples were stored at -80°C until analysis. Serology was performed as described by Scherpenisse and colleagues (21). HPV-specific serum antibodies against L1 virus-like particles (VLP) for 7 high-risk serotypes were measured using a VLP-based multiplex immunoassay (MIA). VLPs

(kindly donated by GlaxoSmithKline Biologicals) from a single batch were coupled to fluorescent microspheres (Luminex corporation). Antibodies were analyzed using Bioplex software (Bio-Rad Laboratories) and median fluorescent intensity was converted to luminex units (LU) for each analyte. Cutoff values were determined based on sera from children (1–10 years) highly likely to be seronegative, using a one-sided 99% prediction interval method (22). Cutoff values were determined at ≥ 9 , ≥ 13 , ≥ 27 , ≥ 11 , ≥ 19 , ≥ 14 , and ≥ 31 LU/mL for HPV types 16, 18, 31, 33, 45, 52, and 58, respectively.

HPV DNA detection

Swabs were stored at -20°C at the Public Health Service of Amsterdam. DNA from anal and penile samples was extracted using MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche) and a 65 base pair region within L1 was amplified by the highly sensitive SPF₁₀-PCR DEIA/LiPA₂₅ system, version 1 (Labo Bio-medical Products; ref. 23). The amplified PCR products were subjected to a DNA Enzyme Immunoassay (HPV DEIA, Labo Bio-medical Products) for detection of at least 68 different HPV genotypes as a pool. Positive samples were subsequently genotyped by a reverse hybridization line probe assay (LiPA₂₅; HPV LiPA₂₅, Labo Bio-medical Products). LiPA₂₅ allows simultaneous identification of 25 specific mucosal HPV genotypes. Two readers independently interpreted the results, visible as bands on a blot. In case of discordance, a third reader was decisive. Baseline anal and penile HPV prevalence data have recently been reported (20).

Statistical analyses

All analyses were performed on an HPV type-specific level, meaning that we assessed HPV-16 antibodies in response to HPV-16 infection, HPV-18 antibodies in response to HPV-18 infection, and so on. Analyses were limited to the 7 high-risk HPV types that could be detected by both the serology and DNA genotyping methods used, i.e., types 16, 18, 31, 33, 45, 52, and 58. Samples positive for one or more of these 7 HPV types were designated hrHPV-positive in this article; samples negative for all of these 7 high-risk types were categorized as hrHPV-negative.

To compare baseline characteristics between HIV-negative and HIV-infected MSM, χ^2 tests were used for categorical data and rank sum tests for continuous data. Type-specific seroconversion was analyzed using logistic regression, as we had only one serology measurement after the baseline measurement, and there was little variation in follow-up time. Generalized estimating equations (24) with an exchangeable correlation structure were applied to account for multiple hrHPV infections within one person. Univariable and multivariable estimates were obtained with 95% confidence intervals (CI). Each analysis was stratified by HIV status.

Univariable analyses were adjusted for HPV type. For multivariable analyses, the following variables were selected *a priori*, based on literature and biologic plausibility: age, smoking, HPV type, and anal and penile hrHPV infection.

Parameters of sexual behavior were not included, as the exposure of interest was hrHPV infection and sexual behavior is a predecessor of HPV acquisition.

We observed that some cases of seroconversion involved only a slight increase in antibody concentration leading to passing the cutoff limit. As this could reflect normal fluctuation in antibody concentration, leading to misclassification, we analyzed the data in two ways based on two different definitions of seroconversion: (i) using the cutoff limit as defined by RIVM, and (ii) using the same cutoff limit plus the criterion that the 12-month antibody concentration must be at least twice the baseline concentration. The second definition is hereafter referred to as "stricter definition of seroconversion."

All statistical analyses were performed using Stata software package version 11.2 (Stata Intercooled).

Definitions of anal and penile HPV infection patterns

Anal hrHPV infection patterns were categorized as "no infection," "incidence," "persistence," "clearance," or "other." Incident infection was defined as a hrHPV-positive visit following a hrHPV-negative visit. Persistence was defined as positivity for the same hrHPV type at all three visits. Clearance was defined as a hrHPV-positive baseline visit, followed by two negative visits, in accordance with Giuliano and colleagues (3). In all other cases, the anal infection was classified as "other." Penile hrHPV infection was, due to lower numbers, simplified as "no infection" or "infection at any time point" (see footnotes Table 1).

Results

Study population

A total of 795 MSM were enrolled in the H2M study and complete baseline data were available for 756 MSM. For the current analyses, 112 MSM were excluded because of missing 12-month serology results, leaving 644 MSM who could be analyzed in this study. The 151 excluded MSM were significantly more often HIV-infected compared with the 644 included MSM (52% vs. 38%; $P = 0.002$).

Of the 644 MSM, 245 (38%) were HIV infected. HIV-infected MSM were significantly older, with a median age of 47 years [interquartile range (IQR) 40–53] compared with 38 years (IQR 33–42) in HIV-negative MSM ($P < 0.001$). HIV-negative MSM were more likely to have been born in the Netherlands. HIV-infected MSM were more likely to be tobacco smokers and to have used cannabis or poppers in the preceding 6 months. They also had more lifetime and recent anal sex partners, and were more likely to have been fisted in the last 6 months. Their median CD4 cell count at enrollment was 540 cells/mm³ (IQR 410–697); 87% used combination antiretroviral therapy. A detailed description of the study population has been published previously (20, 25).

Anal and penile HPV infection patterns

In the 644 MSM, a total of 357 incident anal hrHPV infections were observed. A total of 143 of 417 (34%) infections present at baseline persisted, 129 of 417 (31%)

of these baseline infections were cleared, and 145 of 417 (35%) of these infections were classified as "other" due to ambiguous patterns or missing 6-month data. In total, 327 anal infection patterns were classified as "other." At the penile shaft, we observed a total of 275 hrHPV infections at any time point (Table 1).

HPV seroprevalence at baseline

HPV seroprevalence at baseline was high, as reported previously (25): 60% of HIV-negative and 87% of HIV-infected MSM had antibodies against one or more of the hrHPV types tested. The HPV seroprevalence at baseline was significantly higher in HIV-infected compared with HIV-negative MSM for each HPV type; type-specific seroprevalence varied between 8% (HPV-31 in HIV-negative MSM) and 60% (HPV-16 in HIV-infected MSM).

HPV seroconversion at 12 months

Seroconversion against one or more of the hrHPV types was observed in 74 of 396 (19%) HIV-negative and 52 of 223 (23%) HIV-infected MSM at risk ($P = 0.2$). In total, 119 and 95 seroconversions occurred in HIV-negative and HIV-infected MSM, respectively, as multiple seroconversions could occur per person. Seroconversion was more common in HIV-infected MSM, although this difference was only significant for HPV types 31, 52, and 58 (Table 2). The increase in antibody concentration in case of seroconversion was higher in HIV-infected MSM, but this was only significant for HPV-33 (Table 2).

Using the stricter definition of seroconversion, we observed 92 seroconversions in 52 of 396 (13%) HIV-negative and 80 seroconversions in 40 of 223 (18%) HIV-infected MSM at risk ($P = 0.1$). We observed a total of 391 seroreversions (i.e., seropositivity at baseline followed by seronegativity at 12 months; 30% of all type-specific seropositivities at baseline) in 240 MSM.

Univariable associations between HPV infection and seroconversion

In univariable analyses, an incident (OR 2.0; 95% CI, 1.1–3.4) or persistent (OR 3.7; 95% CI, 1.5–9.2) anal hrHPV infection was significantly associated with type-specific HPV seroconversion in HIV-negative MSM (Table 3). In HIV-infected MSM, anal infection was not significantly associated, but penile infection showed a positive, borderline significant association with type-specific seroconversion (OR 1.8; 95% CI, 1.0–3.2).

Multivariable associations between HPV infection and seroconversion

In multivariable analyses, both incident [adjusted OR (aOR) 2.0; 95% CI, 1.1–3.4] and persistent (aOR 3.7; 95% CI, 1.5–9.5) anal hrHPV infection was significantly associated with type-specific HPV seroconversion in HIV-negative MSM (Table 3). In HIV-infected MSM, anal infection was not significantly associated with seroconversion. Penile hrHPV infection was not associated with seroconversion in HIV-negative MSM, but showed a

Table 2. Type-specific 12-month HPV seroconversion and increase in antibody concentration in case of seroconversion in MSM, stratified by HIV status (H2M study, Amsterdam, 2010–2012)

HPV Type	HIV-negative MSM			HIV-infected MSM			<i>P</i> ^c	<i>P</i> ^d
	No. of seroconversion ^a	% (95% CI)	Median increase in Ab concentration (IQR) ^b	No. of seroconversion ^a	% (95% CI)	Median increase in Ab concentration (IQR) ^b		
16	21/255	8.2 (5.2–12.3)	11.7 (7.3–17.6)	12/97	12.4 (6.6–20.6)	16.2 (9.7–30.6)	0.2	0.3
18	24/293	8.2 (5.3–11.9)	18.4 (10.5–33.9)	15/140	10.7 (6.1–17.1)	22.1 (7.5–25.2)	0.4	0.8
31	7/366	1.9 (0.8–3.9)	29.5 (13.3–87.8)	15/181	8.3 (4.7–13.3)	43.0 (19.1–55.0)	<0.001	0.8
33	18/316	5.7 (3.4–8.9)	11.2 (6.2–17.5)	13/123	10.6 (5.7–17.4)	27.8 (18.6–49.5)	0.07	0.03
45	23/291	7.9 (5.1–11.6)	13.6 (6.1–22.7)	12/101	11.9 (6.3–19.8)	15.5 (5.2–52.6)	0.2	0.6
52	15/351	4.3 (2.4–7.0)	17.9 (9.6–24.8)	15/177	8.5 (4.8–13.6)	23.0 (12.9–40.2)	0.05	0.2
58	11/356	3.1 (1.6–5.5)	50.3 (27.9–57.5)	13/177	7.3 (4.0–12.2)	54.0 (33.7–81.0)	0.03	0.5

NOTE: Seroconversion was defined as passing the cutoff limit (i.e., ≥ 9 , ≥ 13 , ≥ 27 , ≥ 11 , ≥ 19 , ≥ 14 , and ≥ 31 LU/mL for HPV types 16, 18, 31, 33, 45, 52, and 58, respectively). Significant results ($P < 0.05$) are presented in bold type.

^aNumbers at risk for seroconversion vary per HPV type.

^bIncrease in antibody concentration (in IgG LU/mL) in case of seroconversion.

^c P values are based on χ^2 tests for difference in proportions of participants with seroconversions.

^d P values are based on rank sum tests for difference in increase in antibody concentration in case of seroconversion.

positive nonsignificant association in HIV-infected MSM (aOR 1.7; 95% CI, 0.9–3.2).

In sensitivity analyses using the stricter definition of seroconversion, similar associations were observed between anal and penile HPV infection and seroconversion, but now the association between penile hrHPV infection and seroconversion was significant in HIV-infected MSM (aOR 2.3; 95% CI, 1.2–4.3; Supplementary Table S1).

When adding HIV viral load and CD4 cell count to the multivariable models of the HIV-infected group, similar results were obtained (Supplementary Table S2). Furthermore, when adding variables for multiple anal and multiple penile hrHPV infections (i.e., ≥ 2 hrHPV types) at

baseline to the models, similar results were obtained (Supplementary Table S3).

Discussion

In this study, assessing the association between anal and penile HPV infection and humoral immune responses in MSM, we found that incident or persistent anal HPV infection was an independent determinant of seroconversion in HIV-negative MSM. In HIV-infected MSM, anal infection was not associated with seroconversion. In contrast, penile HPV infection was positively associated with seroconversion in HIV-infected MSM, although this

Table 3. Univariable and multivariable associations between anal and penile HPV infection and type-specific seroconversion in HIV-negative and HIV-infected MSM (H2M study, Amsterdam, 2010–2012)

HPV infection status ^a	HIV-negative MSM				HIV-infected MSM			
	OR (95% CI)	<i>P</i>	aOR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	aOR (95% CI)	<i>P</i>
Anal infection		0.007		0.009		0.5		0.5
No infection	1		1		1		1	
Incident infection	2.0 (1.1–3.4)		2.0 (1.1–3.4)		1.4 (0.7–2.6)		1.4 (0.7–2.8)	
Persistent infection	3.7 (1.5–9.2)		3.7 (1.5–9.5)		1.6 (0.7–3.6)		1.3 (0.5–3.3)	
Cleared infection	1.0 (0.3–3.2)		1.0 (0.3–3.2)		1.4 (0.5–4.0)		1.4 (0.4–4.6)	
Other	0.4 (0.1–2.0)		0.4 (0.1–2.1)		1.7 (0.8–3.7)		1.9 (0.9–4.1)	
Penile infection		0.7		0.8		0.07		0.1
No infection	1		1		1		1	
Infection at any time point	1.2 (0.5–2.4)		0.9 (0.4–2.0)		1.8 (1.0–3.2)		1.7 (0.9–3.2)	

NOTE: Seroconversion was defined as passing the cutoff limit (i.e., ≥ 9 , ≥ 13 , ≥ 27 , ≥ 11 , ≥ 19 , ≥ 14 , and ≥ 31 LU/mL for HPV types 16, 18, 31, 33, 45, 52, and 58, respectively). Univariable analyses were adjusted for HPV type. The multivariable model was adjusted for age, smoking, HPV type, and anal and penile HPV infection. Significant results ($P < 0.05$) are presented in bold type.

^aFor HPV types 16, 18, 31, 33, 45, 52, and 58.

association was only significant using a stricter definition of seroconversion.

Generally, the cross-sectional correlation between detection of HPV DNA and type-specific antibodies is poor (9, 26). However, recent data suggest that the anatomic site of HPV infection could influence seropositivity (12, 27, 28); mucosal (e.g., anal) infections might be more likely to elicit a humoral immune response than infections in keratinized epithelium (e.g., the penile shaft). In addition, it has been observed that persistent, rather than transient, HPV infection is associated with seroconversion (5, 9, 29). Our data are in line with these hypotheses, as persistent anal HPV infection was the strongest independent determinant for seroconversion in HIV-negative MSM. This strong association, combined with the fact that a persistent HPV infection is a prerequisite for development of HPV-related cancer, supports the use of HPV serology in monitoring HPV infection on a population level.

The finding that anal HPV infection was associated with seroconversion only in HIV-negative and not HIV-infected MSM was surprising but may partly be explained by differences in sample size or selection bias. HIV-infected MSM who are prone to seroconvert may have done so in the past, especially given their high level of HPV infection and sexual risk behavior. Indeed, relatively more HIV-infected MSM were already HPV-seropositive at baseline and not at risk for seroconversion, leaving HIV-infected MSM who might be less prone to seroconvert. However, this does not explain the positive, albeit non-significant, association between penile HPV infection and seroconversion in HIV-infected, but not HIV-negative MSM. Possibly, a higher HPV viral load or increased HPV persistence of penile infection due to HIV-related changes in immunity might lead to an increased risk of seroconversion in HIV-infected MSM. In addition, differences in sexual practices between HIV-negative and HIV-infected MSM might play a role.

One of the major strengths of our study was the longitudinal design, allowing us to assess associations between both anal and penile HPV infection and type-specific seroconversion within 12 months. The inclusion of HIV-negative and HIV-infected MSM enabled us to compare both groups. The large number of included MSM, and the fact that 7 HPV types were analyzed simultaneously, increased the power of this study.

This study also had some limitations. First, exposure and outcome were measured over the same time period (e.g., persistent infection was based on HPV DNA status measured at 0, 6, and 12 months follow-up, and seroconversion was based on serology measured at 0 and 12 months). Therefore, a causal relationship cannot be demonstrated. Second, the number of penile HPV infections was relatively low compared with the number of anal infections, so we were not able to use the same detailed characterization of infection patterns. Third, in case of new HPV detection, it was impossible to distinguish between HPV acquisition and reactivation of latent infection (30). Our study population was not HPV-naïve, and as a result a proportion of

"incident" infections might actually have been reactivations of long-standing infections, diluting our results. Finally, there is no standardized serologic assay for detection of HPV antibodies (9, 31). In a previous article, we compared 3 serologic assays, including the MIA used in the current study, and observed good correlations for both naturally induced and vaccine-derived HPV-specific antibody levels (32). However, there is overlap in antibody concentrations detected by MIA between seronegatives and sera from naturally infected individuals, and cross-reactivity between phylogenetically related HPV types is conceivable (21, 33).

In conclusion, we found that an incident or persistent anal high-risk HPV infection was an independent determinant of type-specific HPV seroconversion in HIV-negative MSM. Our data support that seroresponse may vary per anatomic site, and that persistent infections are more likely to elicit a detectable humoral immune response. More research is needed to improve understanding of natural immunity against HPV, more specifically to what extent naturally induced antibodies contribute to protection against incident HPV infection.

Disclosure of Potential Conflicts of Interest

M.F. Schim van der Loeff reports receiving commercial research grants from Sanofi-Pasteur MSD and Merck. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: S.H. Mooij, M.A.B. van der Sande, P.J.F. Snijders, M.F. Schim van der Loeff

Development of methodology: S.H. Mooij, M.A.B. van der Sande, M.F. Schim van der Loeff

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.H. Mooij, F.R.M. van der Klis, A. van Eeden, T. Heijman, A.G.C.L. Speksnijder

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.H. Mooij, O. Landén, M. Xiridou, M.F. Schim van der Loeff

Writing, review, and/or revision of the manuscript: S.H. Mooij, O. Landén, F.R.M. van der Klis, M.A.B. van der Sande, H.E. de Melker, M. Xiridou, T. Heijman, P.J.F. Snijders, M.F. Schim van der Loeff

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.H. Mooij, A.G.C.L. Speksnijder, M.F. Schim van der Loeff

Study supervision: A. van Eeden, M.F. Schim van der Loeff

Other (fundraising): A.G.C.L. Speksnijder

Acknowledgments

The authors thank Stichting HIV Monitoring (SHM), in particular Frank de Wolf and Colette Smit, for their contributions in data collection; the members of the H2M steering committee, in particular Roel Coutinho, for their advice; the personnel of the ACS, MC Jan van Goyen, and the Amsterdam STI clinic for their contributions to the implementation of the study and data collection; Martijn van Rooijen and Linda May for assisting in data management; Wilma Vermeulen (Public Health Service of Amsterdam) for HPV genotyping; Jan Sonsma and Marina Burger (RIVM) for DNA isolation; Rutger Schepp (RIVM) for HPV serology; Ronald Geskus for statistical advice and critically reading the manuscript; and Claire Buswell for editorial review. Above all, the authors thank all study participants for their cooperation.

Grant Support

This work was supported by Aids Fonds (grant number 2009029; to M.F. Schim van der Loeff) and additional funding from the Public Health Service of Amsterdam and RIVM. The Amsterdam Cohort Studies on HIV infection and AIDS, a collaboration between the Public Health Service of Amsterdam, the Academic Medical Center of the University of Amsterdam, Sanquin Blood Supply Foundation, and the University Medical

Center Utrecht, are part of the Netherlands HIV Monitoring Foundation and financially supported by the Netherlands National Institute for Public Health and the Environment.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked

advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 19, 2014; revised August 12, 2014; accepted August 22, 2014; published OnlineFirst August 28, 2014.

References

- Giuliano AR, Tortolero-Luna G, Ferrer E, Burchell AN, de Sanjosé S, Kjaer SK, et al. Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. *Vaccine* 2008;26 Suppl 10:K17–28.
- Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjosé S, Bruni L, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. *Vaccine* 2008;26 Suppl 10:K1–16.
- Giuliano AR, Lee JH, Fulp W, Villa LL, Lazcano E, Papenfuss MR, et al. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. *Lancet* 2011;377:932–40.
- Stanley M. HPV - immune response to infection and vaccination. *Infect Agent Cancer* 2010;5:19.
- Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis* 2000;181:1911–9.
- Edelstein ZR, Carter JJ, Garg R, Winer RL, Feng Q, Galloway DA, et al. Serum antibody response following genital {alpha}9 human papillomavirus infection in young men. *J Infect Dis* 2011;204:209–16.
- Markowitz LE, Sternberg M, Dunne EF, McQuillan G, Unger ER. Seroprevalence of human papillomavirus types 6, 11, 16, and 18 in the United States: National Health and Nutrition Examination Survey 2003–2004. *J Infect Dis* 2009;200:1059–67.
- Tiggelaar SM, Lin MJ, Viscidi RP, Ji J, Smith JS. Age-specific human papillomavirus antibody and deoxyribonucleic acid prevalence: a global review. *J Adolesc Health* 2012;50:110–31.
- Mollers M, Vossen JM, Scherpenisse M, van der Klis FR, Meijer CJ, de Melker HE. Review: current knowledge on the role of HPV antibodies after natural infection and vaccination: implications for monitoring an HPV vaccination programme. *J Med Virol* 2013;85:1379–85.
- Ho GY, Studentsov YY, Bierman R, Burk RD. Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. *Cancer Epidemiol Biomarkers Prev* 2004;13:110–6.
- Lu B, Viscidi RP, Lee JH, Wu Y, Villa LL, Lazcano-Ponce E, et al. Human papillomavirus (HPV) 6, 11, 16, and 18 seroprevalence is associated with sexual practice and age: results from the multinational HPV Infection in Men Study (HIM Study). *Cancer Epidemiol Biomarkers Prev* 2011;20:990–1002.
- Lu B, Viscidi RP, Wu Y, Nyitray AG, Villa LL, Lazcano-Ponce E, et al. Seroprevalence of human papillomavirus (HPV) type 6 and 16 vary by anatomic site of HPV infection in men. *Cancer Epidemiol Biomarkers Prev* 2012;21:1542–6.
- Giuliano AR, Palefsky JM, Goldstone S, Moreira ED Jr, Penny ME, Aranda C, et al. Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. *N Engl J Med* 2011;364:401–11.
- Schiller JT, Castellsague X, Garland SM. A review of clinical trials of human papillomavirus prophylactic vaccines. *Vaccine* 2012;30 Suppl 5:F123–38.
- Smith JS, Gilbert PA, Melendy A, Rana RK, Pimenta JM. Age-specific prevalence of human papillomavirus infection in males: a global review. *J Adolesc Health* 2011;48:540–52.
- Silverberg MJ, Chao C, Leyden WA, Xu L, Tang B, Horberg MA, et al. HIV infection and the risk of cancers with and without a known infectious cause. *AIDS* 2009;23:2337–45.
- Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. *Lancet Oncol* 2012;13:487–500.
- Jansen IA, Geskus RB, Davidovich U, Jurriaans S, Coutinho RA, Prins M, et al. Ongoing HIV-1 transmission among men who have sex with men in Amsterdam: a 25-year prospective cohort study. *AIDS* 2011;25:493–501.
- Heijman RL, Stolte IG, Thiesbrummel HF, van LE, Coutinho RA, Fennema JS, et al. Opting out increases HIV testing in a large sexually transmitted infections outpatient clinic. *Sex Transm Infect* 2009;85:249–55.
- Van Aar F, Mooij SH, van der Sande MA, Speksnijder AG, Stolte IG, Meijer CJ, et al. Anal and penile high-risk human papillomavirus prevalence in HIV-negative and HIV-infected MSM. *AIDS* 2013;27:2921–31.
- Scherpenisse M, Mollers M, Schepp RM, Boot HJ, de Melker HE, Meijer CJ, et al. Seroprevalence of seven high-risk HPV types in The Netherlands. *Vaccine* 2012;30:6686–93.
- Frey A, Di Canzio J, Zurakowski D. A statistically defined endpoint titer determination method for immunoassays. *J Immunol Methods* 1998;221:35–41.
- Van Doorn LJ, Molijn A, Kleter B, Quint W, Colau B. Highly effective detection of human papillomavirus 16 and 18 DNA by a testing algorithm combining broad-spectrum and type-specific PCR. *J Clin Microbiol* 2006;44:3292–8.
- Xue X, Gange SJ, Zhong Y, Burk RD, Minkoff H, Massad LS, et al. Marginal and mixed-effects models in the analysis of human papillomavirus natural history data. *Cancer Epidemiol Biomarkers Prev* 2010;19:159–69.
- Mooij SH, van der Klis FR, van der Sande MA, Schepp RM, Speksnijder AG, Bogaards JA, 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.
- Hagensee ME, Kiviat N, Critchlow CW, Hawes SE, Kuypers J, Holte S, et al. Seroprevalence of human papillomavirus types 6 and 16 capsid antibodies in homosexual men. *J Infect Dis* 1997;176:625–31.
- Vriend HJ, Bogaards JA, van der Klis FR, Scherpenisse M, Boot HJ, King AJ, et al. Patterns of human papillomavirus DNA and antibody positivity in young males and females, suggesting a site-specific natural course of infection. *PLoS ONE* 2013;8:e60696.
- Heiligberg M, Alberts CJ, Waterboer T, Speksnijder AG, de Vries HJ, Pawlita M, et al. Route of sexual exposure is independently associated with seropositivity to HPV-16 and HPV-18 among clients of an STI clinic in the Netherlands. *J Infect Dis* 2013;208:1081–5.
- de Grijl TD, Bontkamps HJ, Walboomers JM, Schiller JT, Stukart MJ, Groot BS, et al. Immunoglobulin G responses against human papillomavirus type 16 virus-like particles in a prospective nonintervention cohort study of women with cervical intraepithelial neoplasia. *J Natl Cancer Inst* 1997;89:630–8.
- Gravitt PE. The known unknowns of HPV natural history. *J Clin Invest* 2011;121:4593–9.
- Faust H, Jelen MM, Poljak M, Klavs I, Ucakar V, Dillner J. Serum antibodies to human papillomavirus (HPV) pseudovirions correlate with natural infection for 13 genital HPV types. *J Clin Virol* 2013;56:336–41.
- Scherpenisse M, Schepp RM, Mollers M, Mooij SH, Meijer CJ, Berbers GA, et al. Comparison of different assays to assess human papillomavirus (HPV) type 16- and 18-specific antibodies after HPV infection and vaccination. *Clin Vaccine Immunol* 2013;20:1329–32.
- 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.