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Prevalent Serum Antibody Is Not a Marker of Immune Protection against Acquisition of Oncogenic HPV16 in Men

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Abstract

In women, naturally induced anti–human papilloma virus (HPV) serum antibodies are a likely marker of host immune protection against subsequent HPV acquisition and progression to precancerous lesions and cancers. However, it is unclear whether the same is the case in men. In this study, we assessed the risk of incident genital infection and 6-month persistent genital infection with HPV16 in relation to baseline serostatus in a cohort of 2,187 men over a 48-month period. Genital swabs were collected every 6 months and tested for HPV presence. Incidence proportions by serostatus were calculated at each study visit to examine whether potential immune protection attenuated over time. Overall, incidence proportions did not differ statistically between baseline seropositive and seronegative men at any study visit or over the follow-up period. The risk of incident and 6-month persistent infection was not associated with baseline serostatus or baseline serum antibody levels in the cohort. Our findings suggest that baseline HPV seropositivity in men is not associated with reduced risk of subsequent HPV16 acquisition. Thus, prevalent serum antibodies induced by prior infection may not be a suitable marker for subsequent immune protection against genital HPV16 acquisition in men.

Introduction

Genital human papilloma virus (HPV) infection is one of the most common sexually transmitted infections (STI) worldwide (1). Prevalence of up to 73% has been documented in men globally (2), with HPV16 being the most frequently detected oncogenic HPV type (3, 4). Evidence from a growing number of studies has supported the etiologic role of genital

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HPV in penile cancer and its precursor lesions. HPV DNA is detected in 29% to 82% of penile carcinoma (5–12), in 70% to 100% of penile intraepithelial neoplasia (PIN; refs. 13–15), and in 80% to 100% of genital warts (condyloma acuminata) in men (16–19). Immunization with HPV L1 virus-like particles (VLP) elicits strong serum antibody response and provides high degree of protection against subsequent genital HPV infection, precancerous lesions, and cancers associated with vaccine-targeted genital HPV types (20–22). Among vaccine recipients, anti-HPV serum antibodies measured by VLP-based immunoassay are highly correlated with neutralizing antibodies that are critical for viral neutralization and a key factor in protection mechanism (23). Thus, naturally induced serum antibodies measured by VLP-based ELISA, though at much lower levels than those generated by immunization, are a likely marker of host immune protection against subsequent genital HPV infection and progression.

In women, findings on the protective role of anti-HPV16 serum antibodies have been inconsistent, with moderate protection observed in a limited number of studies (24–27).Whether a man's risk of acquiring genital HPV16 infection is altered by the presence of anti-HPV16 serum antibodies remains largely unknown. We have previously reported that the detection of HPV16 seropositivity was not associated with risk of subsequent genital HPV16 infection among a small cohort of U.S. men (28). However, limitations of that study with respect to the duration of follow-up, sample size, and unavailability of quantitative measurement of serum antibody levels impeded our ability to fully assess associations between circulating anti-HPV serum antibodies and subsequent risk of infection.

There is also growing evidence that HPV infection acquired at various anatomic sites may differentially contribute to circulating antibody levels observed in men (29–33). Findings from previous HPV serology studies also suggest that men who had same-sex intercourse were more likely to have detectable antibodies to HPV types 6, 11, 16, or 18 than heterosexual men (29–33). As a result, any potential protection conferred by detectable serum antibodies may differ between men with different sexual practices.

To determine whether serum antibodies detectable by VLP-based immunoassay are a marker of immune protection and whether the protection varies by sexual practice, we evaluated the risk of incident genital HPV16 infection among a large cohort of men according to their enrollment serum antibody status and sexual practices using data from the HIM Study.

Methods

Study population

We analyzed data from the HIM Study, an ongoing multinational natural history study of HPV infection in men conducted in Tampa, FL, São Paulo, Brazil, and Cuernavaca, Mexico. Details of the study cohort have been reported previously (34). In brief, healthy men were recruited from several population sources in each study site and followed every 6 months for a maximum of 4 years. Men were eligible to participate if the following criteria were met: (i) 18–70 years of age; (ii) residents of 1 of the 3 study sites; (iii) no prior diagnosis of penile or anal cancers; (iv) no prior diagnosis of genital or anal warts; (v) no symptoms of or current treatment for an STI; (vi) no concurrent participation in an HPV vaccine study; (vii) no history of HIV or AIDS; (viii) no history of imprisonment, homelessness, or drug treatment during the past 6 months; and (ix) willingness to comply with 10 scheduled visits every 6 months for 4 years with no plans to relocate within 4 years. All eligible men signed an informed consent prior to enrollment. At the enrollment visit and each follow-up visit, an extensive sexual history and health questionnaire was administered using a Computer-Assisted Self-Interviewing (CASI) system. Ten milliliters venous blood was collected for serum antibody testing, and the external genitalia were sampled for HPV testing by study

clinicians. The informed consent and the study protocol were reviewed and approved by appropriate Internal Review Boards and human subject committees at each study site.

A total of 4,074 men residing in Tampa, São Paulo, and Cuernavaca were enrolled in the HIM Study between June 2005 and September 2009. As of August 31, 2010, 2,546 of the 4,074 men had completed one or more follow-up visits and were available for the present analysis; the remaining 1,528 men had yet to return for their first follow-up visit. Of 2,546 men, we excluded 204 men who were HPV16 DNA positive at enrollment and 155 men who self-identified as virgins and provided survey responses consistent with this classification, leaving 2,187 baseline HPV16 DNA–negative men with HPV16 serology and survey information from the baseline and HPV DNA results from one or more follow-up visits for inclusion in the present study. Comparison of participant characteristics indicated that this subset is comparable with the full HIM Study cohort with respect to sociodemographic characteristics, sexual behaviors, and lifestyle factors, except that a slightly higher percentage of Brazilian and a lower percentage of U.S. participants were represented in the current cohort (Supplementary Table S1).

HPV serum antibody testing

Testing of serum antibodies to HPV type 16 was conducted using VLP-based ELISA (35) in the laboratory of Dr. Viscidi at Johns Hopkins University, Baltimore, MD. HPV16 VLPs were produced in insect cells from recombinant baculoviruses expressing HPV16 L1 capsid proteins as described previously (36). Specimens were tested in duplicate on separate plates, with retesting of specimens showing results exceeding a preset, acceptable coefficient of variation (CV) of 25%. Seroreactivity was measured by absorbance values expressed in optical density (OD). The mean and SD of absorbance values were estimated on the basis of seroreactivity of serum samples from children 1 to 10 years of age. Five SDs above the mean absorbance value were used as the cutoff point for seropositivity. Quality control of the serology assays was assured by inclusion of laboratory-prepared seropositive and seronegative controls in each run of the assay. No blind replicates of serum samples were generated to estimate within- and between-batch reliability. However, the laboratory staff was blind to HPV DNA status of individuals whose serum samples were to be tested for serum antibodies.

HPV DNA sampling and testing

Three prewetted Dacron swabs were used to collect exfoliated skin cells from the penis and scrotum and later combined to form a single specimen. All specimens were stored at −70°C until PCR analyses and genotyping were conducted. DNA was extracted from exfoliated skin cell samples using the QIAamp DNA Mini Kit (QIAGEN) and tested for HPV DNA using PCR for amplification of a fragment of the HPV L1 gene (37). HPV genotyping was conducted using the Linear Array HPV Genotyping Protocol (Roche Diagnostics) to detect 37 genital HPV types (38). Human β-globin was tested to assure the integrity of DNA and was detected in 94.9% (2,076 of 2,187) of baseline HPV samples and 92.0% to 100% of the samples collected through the 48-month visit.

Statistical analysis

Participants were classified as men who had sex with women (MSW, 89.3%), men who had sex with men (MSM, 5.0%), and men who had sex with men and women (MSMW, 5.7%) on the basis of their responses to multiple survey questions about their recent and lifetime sexual behaviors at enrollment. Because of the small number of MSM and MSMW and their shared practice of same-sex sexual behaviors, MSM and MSMW were combined into the group "MSM" in the current analysis. Characteristics of seronegative and seropositive men

were compared using χ^2 and Fisher exact tests among the overall cohort, MSW, and MSM, respectively.

Two virological endpoints were evaluated: incidence of HPV16 infection and incidence of 6-month persistent HPV16 infection. An incident HPV16 infection was defined as the first detection of HPV16 DNA by the Linear Array assay at a follow-up visit, assuming the date of detection as the date of infection. An incident 6-month persistent HPV16 infection was defined as the detection of HPV16 DNA at 2 or more consecutive follow-up visits, using the date of first positive DNA detection as the date of infection. Incidence proportion for both endpoints was summarized for each study interval up to the month 24 using the number of incident cases detected at the end of each study interval as the numerator and the number of participants who tested HPV16 DNA negative at the beginning of the study interval as the denominator. The number of incident cases was assumed to follow the Poisson distribution. Cox proportional hazard regression was applied to estimate risk of HPV16 infection associated with participant baseline serostatus for the entire cohort, MSW, and MSM, respectively, controlling for potential confounders. Serostatus was included in the Cox models as a binary variable (seropositive vs. seronegative) as well as a continuous variable measuring antibody levels, expressed as mean optical density (OD) and log-transformed. Men who tested negative for HPV16 DNA throughout all follow-up visits were censored at the last visit. Potential confounders considered included (i) sociodemographic characteristics such as age, country of residence, race, ethnicity, marital status, and educational attainment and (ii) lifestyle and behavioral factors including alcohol consumption, smoking, circumcision, age at first sexual intercourse, sexual practice, the number of recent and lifetime sex partners, frequency of sexual intercourse, condom use, and history of other STIs. The values of confounding variables were determined on the basis of participant information obtained from the baseline survey. Individual factors that showed statistical significance at the level of 0.1 along with serostatus in Cox models were considered for inclusion in the multivariable models. Partial likelihood ratio and Wald tests were used for covariate selection using a backward elimination procedure. Hazard ratio (HR) and its 95% confidence intervals (CI) were estimated from Cox regression models. A total of 2,187 men were included in the analysis of incident HPV16 infection. Men (1,834 of 2,187) with HPV DNA results for at least 2 consecutive follow-up visits remained in the analysis of incident 6-month persistent HPV16 infection.

Results

The cohort of 2,187 men contributed a total of 10,086 visits, equivalent to 4,424 person-years. Seven hundred and thirtynine (33.8%) men were followed for approximately 12 months (2 follow-up visits), 728 (33.3%) for 24 months (4 visits), 559 (25.6%) for 36 months (6 visits), and 161 (7.4%) for 48 months (8 visits). The mean and median duration of follow-up was 2.0 years (range, 0.4–4.1; interquartile range, 1.2–3.0). The median interval between visits was 6.2 months.

Characteristics of seronegative and seropositive men are summarized in Table 1. Baseline seroprevalence of HPV16 was 12.3% overall, 10.0% among MSW, and 31.2% among MSM. Overall, seronegative and seropositive men differed significantly by age at enrollment, country of residence, the number of lifetime sex partners (either sex), and new sex partners (either sex) in the past 6 months. Seropositive men were more likely to be 25 years or older, Brazilian, and have a large number of lifetime sex partners and 2 or more new sex partners in past 6 months. Similarly, significant differences were observed in age at enrollment, country of residence, and the number of lifetime sex partners (either sex) and lifetime female sex partners between seronegative and seropositive MSW. Among MSM, seropositive men were significantly older than seronegative men at enrollment.

Overall, a total of 221 (10.1%) men developed incident HPV16 infections (9.8% of seronegative men vs. 12.6% of seropositive men) and 72 (3.9%) men developed 6-month persistent HPV16 infections (3.9% of seronegative men vs. 3.8% of seropositive men) during the study period. Timing of incident and 6-month persistent HPV16 detection over the study period is summarized in Table 2 to determine if protection of serum antibodies is dependent upon the time of infection. A smaller proportion of seropositive men than seronegative men had incident HPV16 infections during the first year of follow-up (6-month visit: overall, 3.0% vs. 3.3%; MSW, 3.1% vs. 3.2%; MSM, 2.7% vs. 5.0%; 12-month visit: overall, 2.2% vs. 3.1%; MSW, 1.8% vs. 2.9%; MSM, 3.1% vs. 5.8%). The differences, though more apparent among MSM, were not statistically significant. However, the reduction in HPV16 incidence was not sustained throughout the study period. Likewise, seropositive MSM had a lower incidence of 6-month persistent HPV16 infection than seronegative MSM (6-month visit: 0% vs. 2.1%; 12-month visit: 1.5% vs. 2.8%) in the first year which was not retained for the remaining study period.

The risk of incident HPV16 infection and 6-month persistent infection according to baseline serostatus is presented in Table 3. Overall, baseline serostatus was not associated with risk of incident HPV16 infection among the 2,187 men in either univariate or multivariable analyses (HR, 1.23; 95% CI, 0.85–1.77; adjusted HR, 1.22; 95% CI, 0.83–1.79). Likewise, incidence of 6-month persistent HPV16 infection was not associated with baseline seropositivity in either univariate (HR, 0.96; 95% CI, 0.48–1.93) or multivariable analyses (adjusted HR, 1.05; 95% CI, 0.51–2.16).

When we examined the associations according to sexual practices, the risk of incident HPV16 infection was not associated with baseline serostatus among MSW (adjusted HR, 1.60; 95% CI, 0.98–2.60) or MSM (adjusted HR, 1.23; 95% CI, 0.60–2.50; Table 3). Likewise, no associations were detected in the analysis of 6-month persistent HPV16 infection among MSM or MSW. In univariate analyses, a negative association with the risk of 6-month persistent infection was observed among MSM (HR, 0.53; 95% CI, 0.11–2.51). However, the association did not reach statistical significance in multivariable analyses (adjusted HR, 1.03; 95% CI, 0.21–4.98). No protection against 6-month persistent HPV16 infection was observed among MSW.

We further determined whether the associations between baseline serostatus and HPV16 incidence were impacted by the inclusion of potentially reactivated latent infections from the baseline. We reassessed the risk of incident and 6-month persistent HPV16 infection in association with baseline serostatus after restricting incident HPV infections to those that had 2 or more prior consecutive negative HPV16 DNA results (Table 4). The associations in the restricted analysis were consistent with the previous analysis and showed no statistical significance.

In addition, we evaluated whether higher serum antibody levels were associated with a lower likelihood of subsequent HPV16 infection. We did not observe significant associations between serum antibody levels and risk of incident HPV16 infection or 6-month persistent HPV16 infection for the overall cohort, MSW, or MSM (Supplementary Table S2). Nor did we observe significant associations after we restricted the analysis to seropositive men only (data not shown).

Associations of enrollment HPV16 serostatus with the risk of group-specific HPV infection (alpha-9 HPV, alpha-9 HPV excluding HPV16, and other HPV) were also examined. No cross-protection of HPV16 enrollment serostatus against group-specific HPV infection was observed (data not shown).

Discussion

In this multinational prospective study of healthy men, we assessed the risk of genital HPV16 infection over a 48-month study period by baseline HPV16 serostatus. We sought to determine whether anti-HPV16 serum antibodies induced by prior infection conferred protection against subsequent incident HPV16 infection and 6-month persistent HPV16 infection. Our study is one of the few to investigate the potential protective role of serum antibodies in the natural history of genital HPV in men. Overall, incidence of HPV16 infection and 6-month persistent HPV16 infection did not differ statistically between baseline seropositive and seronegative men at any study visit or over the follow-up period. We did not detect statistically significant associations between baseline HPV16 seropositivity and risk of incident HPV16 infection or 6-month persistent HPV16 infection. Nor did we find that higher HPV16 serum antibody levels were significantly associated with lower risk of infection.

While we were not able to show statistically significant differences in the risk of HPV16 infection by baseline serostatus, our data showed that HPV16 incidence was lower among seropositive men than seronegative men during the first year of follow-up and then became higher among seropositive men in the months that follow. Previous studies in women have shown that in the absence of viral antigen, serum IgG antibody levels attenuate over time. For example, the study of Shoultz (39) assessed serostatus in female STI clinic attendees prospectively with a 4-month interval. Among 197 women exhibiting 2 or more consecutive HPV16 seropositivities, the median antibody level declined by nearly 30% to 35% within 12 to 18 months following initial detection and remained relatively stable afterward. In contrast, among 223 women who exhibited single seropositivity, the median antibody level declined by approximately 70% and remained below the positive cutoff point in the first year and then rebounded and fluctuated around the cutoff point throughout the study period (39). Similarly, Ho and colleagues reported that for female university students who tested seropositive at least once during the study period, the cumulative probabilities of losing anti-HPV16 IgG seropositivity by 12, 24, and 36 months were 38.5%, 40.0%, and 48.2% (40). These findings suggest that while the observed difference in incidence over time between seropositive and seronegative men in our study could be due to chance alone, it could also be explained by the loss of serum antibodies over time. The latter possibility, however, must be tested in future studies where repeated measurements of serum antibody and DNA status are available.

Consistent with our previous findings in men, we found that the risk of both incident HPV16 infection and 6-month persistent HPV16 infection was not associated with baseline HPV16 serostatus or serum antibody levels in our cohort (incidence rate ratio, 1.1; 95% CI, 0.3–4.0; ref. 28). Findings from serology studies in women have been mixed (24–27, 41, 42). Using L1/L2 VLP-based direct ELISA, Ho and colleagues showed that high anti-HPV16 antibody level at 2 or more consecutive visits was associated with lower risk of subsequent HPV16 infection [adjusted relative risk (ARR), 0.49; $P = 0.037$] among female university students (25). Wentzensen and colleagues showed a significant reduction in the risk of subsequent HPV16 infection or HPV16-positive $CIN2^{+}$ for seropositive women compared with seronegative women using a VLP-based ELISA (adjusted OR, 0.60; 95% CI, 0.40–0.90) and a competitive Luminex-based immunoassay (cLIA; adjusted OR, 0.44; 95% CI, 0.21–0.93) in the Guanacaste Natural History Study (24). Evidence from HPV vaccine trials also supports the protective role of anti-HPV16 serum antibodies in women. Safaeian and colleagues reported a 50% reduction of HPV16 infection risk among seropositive women with high antibody levels (≥60 EU/mL; ARR, 0.50; 95% CI, 0.26–0.86) in the control group of the HPV16/18 Costa Rica Vaccine Trial (26). Velicer and colleagues detected a lower incidence rate of HPV types 6/11/16/18 infection and 6-month persistent infection in

baseline seropositive women than in seronegative women enrolled in the placebo arm of an HPV vaccine trial using cLIA [incident infection, 1.0 (95% CI, 0.31–2.23) vs. 5.7 (95% CI, 4.68–6.84) per 100 woman-years; 6-month persistent infection, 0.4 (95% CI, 0.05–1.37) vs. 2.5 (95% CI, 1.89–3.35) per 100 woman-years; ref. 27]. In contrast, no significant protective effect was reported by Viscidi and colleagues among 7,046 Guanacaste cohort participants (risk ratio, 0.74; 95% CI, 0.45–1.20; ref. 41) or by Trottier and colleagues among 1,902 Ludwig–McGill cohort participants both using an L1/L2 VLP-based direct ELISA assay [incidence rate: lowest tertile of antibody levels 1.6 (95% CI, 1.3–2.1) vs. highest tertile 2.1 (95% CI, 1.7–2.6) per 1,000 woman-months; ref. 42].

The lack of protection in men observed in the present study, in contrast to the moderate protection found in a number of studies in women, could be due to several factors including differential gender-related immune response, assay differences, and varying duration of serum antibody presence. Gender differences in seroprevalence have been reported in multiple studies where seroprevalence of oncogenic and nononcogenic HPV was consistently higher among women than men from the same source population (31–33, 43–45). The observed gender-specific immune response may be an indication that HPV infection at keratinized epithelium is less likely to induce immune responses than infection of mucosal epithelium or that heterosexual women may be at higher risk than heterosexual men to have HPV exposures at multiple anatomic sites, mostly mucous membranes such as cervix, oral cavity, and anus, eliciting stronger antibody responses. Furthermore, differences in serologic assays used across studies may have contributed to inconsistent results observed across publications. The VLP-based direct-binding ELISA assay used in the current study measures total type-specific binding IgG antibodies, including neutralizing and nonneutralizing antibodies, whereas the competitive neutralization assay used by Velicer and colleagues (27) measures IgG antibodies that bind to known neutralizing epitopes in HPV viral capsid. The inclusion of nonneutralizing antibodies in the present study could have led to overestimation of functional neutralizing antibody levels and biased the association of protection toward the null. Another explanation for the inconsistent results observed could be that prolonged presence of serum antibodies had played a role in conferring protection. The protective effect of serum antibodies reported by Ho and colleagues was only restricted to women who showed high antibody level at 2 or more consecutive visits using a direct ELISA assay (25). Conversely, no protection was detected in other studies of women that determined serostatus only once at the baseline using a similar assay (41, 42).

The assumption of incidence in the present study was largely based on a single negative HPV16 DNA result obtained at the baseline prior to the first detection of HPV16 DNA by PGMY09/11 and Linear Array assays. Among incident HPV16 infections detected over the study period, about 35% of cases in the seronegative group and 24% of cases in the seropositive group occurred within the first 6 months. It was plausible that some of these clinically apparent incident infections may have been latent infections that were undetected at the baseline but later became detectable. In an attempt to distinguish reactivated latent infection from genuine incident infection, we evaluated the risk of incident and 6-month persistent HPV16 infection by baseline serostatus after restricting incident cases to those who showed 2 or more prior consecutive negative HPV16 DNA results. The use of more stringent incidence definition did not alter the null associations observed previously. Despite evidence that HPV establishes a cycle of latency and reactivation in animal models (46) and recurrent human respiratory papillomatosis (47), little is known about latent genital HPV infection and its reactivation. Accurate identification and classification of reactivation events would require knowledge of prior infection, reliable information on sexual behaviors, an intervening period with no evidence of viral shedding, and subsequent confirmation of active infection (46). Furthermore, reactivated latent infection may present low level of viral

load or a short time frame for detection. These technical and logistic challenges need to be addressed in future studies.

The present study is unique in its longitudinal design, the size of cohort, the long duration of follow-up completed for a large percentage of participants, and the availability of repeated measurements of HPV16 DNA status. Yet, a few limitations must be addressed. We used a less conservative definition of antibody presence than that used by Ho and colleagues, a single detection of seropositivity at enrollment. Because of waning antibody response, time lags in antibody development following infection, and generally less than 100% seroconversion rate, some men who had been previously exposed to HPV16 and as a result had acquired immunity were likely to have been protected but misclassified as seronegative men. Furthermore, there could be possible misclassification of HPV infection status in the presence of low viral load, both among seropositive and seronegative men. The misclassification of both baseline serostatus and HPV16 infection status may have contributed to the null association observed. Finally, despite the relatively large sample size, our stratified analyses were statistically underpowered to obtain stable estimates of time-specific incidence and to detect potential associations of HPV16 acquisition with baseline serostatus among MSM due to low incidence of HPV16 and the small number of MSM in the current study.

In conclusion, our data showed that in the cohort of 2,187 healthy men, the presence of seropositivity to HPV16 or higher anti-HPV16 serum antibody levels at baseline were not associated with reduced risk of genital HPV16 acquisition over a 48-month period. However, our findings should be interpreted with caution. Potential protection conferred by serum antibodies may depend on the proximity to the last HPV exposure, the duration of serum antibody presence, and the timing of subsequent infection from seroconversion. These results likely suggest that prevalent serum antibody status is not a suitable marker for subsequent immune protection against oncogenic HPV16 acquisition at the external genitalia in men. Additional studies that prospectively assess serum antibody status are necessary to confirm the role of serum antibody response in the natural history of genital HPV infection in men.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Select participant characteristics comparing seronegative and seropositive men in Tampa, Cuernavaca, and São Paulo

Characteristics	Overall (*N* = 2,187), *n* (%)		MSW (*N* = 1,953), *n* (%)		MSM (*N* = 234), *n* (%)	
	Seronegative	Seropositive	Seronegative	Seropositive	Seronegative	Seropositive
Overall	1,918 (87.7)	269 (12.3)	1,757 (90.0)	196 (10.0)	161 (68.8)	73 (31.2)
Age, y						
18–24	597 (31.1)	46 (17.1)	551 (31.4)	39 (19.9)	46 (28.6)	7 (9.6)
25–44	1,057 (55.1)	183 (68.0)	957 (54.5)	125 (63.8)	100 (62.1)	58 (79.5)
45–70	264 (13.8)	40 (14.9)	249 (14.2)	32 (16.3)	15 (9.3)	8 (11.0)
P[a]		**<0.001**		**0.004**		**0.006**
Country of residence						
Brazil	671 (35.0)	148 (55.0)	561 (31.9)	89 (45.4)	110 (68.3)	59 (80.8)
Mexico	682 (35.6)	53 (19.7)	655 (37.3)	44 (22.4)	27 (16.8)	9 (12.3)
United States	565 (29.5)	68 (25.3)	541 (30.8)	63 (32.1)	24 (14.9)	5 (6.8)
P[a]		**<0.001**		**<0.001**		0.113
Marital status						
Single	853 (44.6)	122 (45.4)	752 (42.9)	74 (37.8)	101 (62.7)	48 (65.8)
Cohabiting	243 (12.7)	37 (13.8)	228 (13.0)	29 (14.8)	15 (9.3)	8 (11.0)
Married	663 (34.7)	81 (30.1)	630 (36.0)	72 (36.7)	33 (20.5)	9 (12.3)
Divorced/separated/widowed	154 (8.1)	29 (10.8)	142 (8.1)	21 (10.7)	12 (7.5)	8 (11.0)
P[a]		0.289		0.392		0.424
Education						
Less than high school	410 (21.4)	54 (20.1)	380 (21.7)	39 (19.9)	30 (18.6)	15 (20.5)
High school graduate	520 (27.2)	73 (27.1)	477 (27.2)	52 (26.5)	43 (26.7)	21 (28.8)
College or higher	982 (51.4)	142 (52.8)	894 (51.1)	105 (53.6)	88 (54.7)	37 (50.7)
P[a]		0.860		0.772		0.851
Alcohol drinking[b]						
Light	612 (43.5)	80 (41.0)	566 (43.7)	51 (37.0)	46 (41.4)	29 (50.9)
Moderate	502 (35.7)	72 (36.9)	461 (35.6)	56 (40.6)	41 (36.9)	16 (28.1)
Heavy	293 (20.8)	43 (22.1)	269 (20.8)	31 (22.5)	24 (21.6)	12 (21.1)

Characteristics	Overall (*N* = 2,187), *n* (%)		MSW (*N* = 1,953), *n* (%)		MSM (*N* = 234), *n* (%)	
	Seronegative	**Seropositive**	**Seronegative**	**Seropositive**	**Seronegative**	**Seropositive**
P[a]		0.803		0.309		0.443
Cigarette smoking						
Never	1,115 (58.3)	156 (58.0)	1,022 (58.3)	113 (57.7)	93 (57.8)	43 (58.9)
Current	432 (22.6)	57 (21.2)	388 (22.2)	40 (20.4)	44 (27.3)	17 (23.3)
Former	366 (19.1)	56 (20.8)	342 (19.5)	43 (21.9)	24 (14.9)	13 (17.8)
P[a]		0.758		0.678		0.743
Circumcision[c]						
No	1,251 (65.2)	186 (69.1)	1,127 (64.1)	125 (63.8)	124 (77.0)	61 (83.6)
Yes	667 (34.8)	83 (30.9)	630 (35.9)	71 (36.2)	37 (23.0)	12 (16.4)
P[a]		0.217		0.938		0.300
Lifetime sex partners (either sex), *n*						
None	104 (5.5)	17 (6.4)	100 (5.7)	14 (7.2)	4 (2.7)	3 (4.3)
1–3	495 (26.0)	36 (13.6)	474 (27.0)	31 (15.9)	21 (14.0)	5 (7.1)
4–7	407 (21.4)	47 (17.7)	386 (22.0)	38 (19.5)	21 (14.0)	9 (12.9)
8–18	459 (24.1)	67 (25.3)	420 (23.9)	51 (26.2)	39 (26.0)	16 (22.9)
≥19	440 (23.1)	98 (37.0)	375 (21.4)	61 (31.3)	65 (43.3)	37 (52.9)
P[a]		**<0.001**		**0.001**		0.484
Lifetime female sex partners among MSW, *n*						
None	–	–	2 (0.1)	0 (0)		
1–3	–	–	478 (28.9)	32 (17.8)		
4–17	–	–	789 (47.7)	87 (48.3)		
≥18	–	–	385 (23.3)	61 (33.9)		
P[a]		–		**0.001**		
Lifetime male anal sex partners, *n*						
None	–	–	–	–	5 (3.4)	4 (6.1)
1–2	–	–	–	–	26 (17.7)	6 (9.1)
3–10	–	–	–	–	70 (47.6)	26 (39.4)
≥11	–	–	–	–	46 (31.3)	30 (45.5)
P[a]		–		–		0.100

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Characteristics	Overall (*N* = 2,187), *n* (%)		MSW (*N* = 1,953), *n* (%)		MSM (*N* = 234), *n* (%)	
	Seronegative	Seropositive	Seronegative	Seropositive	Seronegative	Seropositive
New sex partners (either sex) in past 6 mo, *n*						
None	1,183 (61.9)	152 (57.4)	1,122 (64.0)	126 (64.6)	61 (39.1)	26 (37.1)
1	492 (25.8)	63 (23.8)	454 (25.9)	43 (22.1)	38 (24.4)	20 (28.6)
≥2	235 (12.3)	50 (18.9)	178 (10.1)	26 (13.3)	57 (36.5)	24 (34.3)
P[a]		**0.012**		0.256		0.798

NOTE: Bolded *P* values denote statistical significance at $\alpha = 0.05$. Cells that do not add up to 100% are due to missing values.

[a] *P* values were derived from χ^2 or Fisher exact test statistics.

[b] Light drinking was defined as less than one half drink per day; moderate drinking as half to 2 drinks per day; and heavy drinking as more than 2 drinks per day on average.

[c] Circumcision status was assessed by study clinician.

Table 2

Incidence proportion of HPV16 infection and 6-month persistent infection at each study visit by baseline serostatus

Time of DNA detection	6 mo		12 mo		18 mo		24 mo	
	No. at risk[a]	Incidence % (95% CI)[b]	No. at risk[a]	Incidence % (95% CI)[b]	No. at risk[a]	Incidence % (95% CI)[b]	No. at risk[a]	Incidence % (95% CI)[b]
Incident infection								
Overall								
Seronegative	1,918	3.3 (2.6–4.3)	1,539	3.1 (2.3–4.1)	1,183	2.9 (2.0–4.0)	830	2.7 (1.7–4.0)
Seropositive	269	3.0 (1.3–5.9)	228	2.2 (0.7–5.1)	174	4.0 (1.6–8.3)	127	4.7 (1.7–10.3)
MSW								
Seronegative	1,757	3.2 (2.4–4.1)	1,401	2.9 (2.0–3.9)	1,080	2.9 (2.0–4.1)	754	2.5 (1.5–3.9)
Seropositive	196	3.1 (1.1–6.7)	164	1.8 (0.4–5.3)	127	4.7 (1.7–10.3)	93	2.2 (0.3–7.8)
MSM								
Seronegative	161	5.0 (2.1–9.8)	138	5.8 (2.5–11.4)	103	2.9 (0.6–8.5)	76	3.9 (0.8–11.5)
Seropositive	73	2.7 (0.3–9.9)	64	3.1 (0.4–11.3)	47	2.1 (0.1–11.9)	34	11.8 (3.2–30.1)
6-mo persistent infection								
Overall								
Seronegative	1,598	1.3 (0.8–1.9)	1,576	1.1 (0.7–1.8)	1,231	1.1 (0.6–1.9)	867	0.7 (0.3–1.5)
Seropositive	236	1.3 (0.3–3.7)	232	1.3 (0.3–3.8)	178	1.1 (0.1–4.1)	133	0 (0–2.8)
MSW								
Seronegative	1,453	1.2 (0.7–1.9)	1,434	1.0 (0.5–1.6)	1,125	1.2 (0.7–2.1)	786	0.6 (0.2–1.5)
Seropositive	170	1.8 (0.4–5.2)	166	1.2 (0.1–4.4)	128	0.8 (0.02–4.4)	97	0 (0–3.8)
MSM								
Seronegative	145	2.1 (0.4–6.0)	142	2.8 (0.8–7.2)	106	0 (0–3.5)	81	1.2 (0.03–6.9)
Seropositive	66	0 (0–5.6)	66	1.5 (0.03–8.4)	50	2.0 (0.1–11.1)	36	0 (0–10.2)

NOTE: No incident infections were detected at month 40 and later, and the number of participants at risk for months 30 and 36 were very small for MSM, and likely result in unstable estimates. Therefore, data were not presented for months 30 and 36.

[a] No. at risk was defined as the number of men who were followed until the stated visit or later and were HPV16 negative at the beginning of the study interval.

[b] Percentage represents the number of incident infections that occurred during the stated study interval over the number of participants at risk.

Table 3

Association of incident HPV16 infection and 6-month persistent HPV16 infection with baseline serostatus among 2,187 men in Tampa, Cuernavaca, and São Paulo

	Overall				MSW				MSM			
	No. of men	**No. of infections**	**HR (95% CI)**	**Adjusted HR (95% CI)**	**No. of men**	**No. of infections**	**HR (95% CI)**	**Adjusted HR (95% CI)**	**No. of men**	**No. of infections**	**HR (95% CI)**	**Adjusted HR (95% CI)**
Incident infection	2,187				1,953				234			
Seronegative	1,918	187	1.00	1.00	1,757	160	1.00	1.00	161	27	1.00	1.00
Seropositive	269	34	1.23 (0.85–1.77)	1.22[a] (0.83–1.79)	196	22	1.18 (0.76–1.84)	1.60[b] (0.98–2.60)	73	12	0.91 (0.46–1.80)	1.23[c] (0.60–2.50)
6-mo persistent infection	1,834				1,623				211			
Seronegative	1,598	63	1.00	1.00	1,453	55	1.00	1.00	145	8	1.00	1.00
Seropositive	236	9	0.96 (0.48–1.93)	1.05[d] (0.51–2.16)	170	7	1.09 (0.49–2.38)	1.05[e] (0.45–2.46)	66	2	0.53 (0.11–2.51)	1.03[f] (0.21–4.98)

[a] Adjusted for age at enrollment, sexual practice (MSW and MSM), and lifetime number of sex partners.

[b] Adjusted for age at enrollment and lifetime number of female sex partners.

[c] Adjusted for age at enrollment and circumcision.

[d] Adjusted for age at enrollment, sexual practice (MSW and MSM), and the number of new sex partners in past 6 months.

[e] Adjusted for age at enrollment and the number of new female sex partners in past 6 months.

[f] Adjusted for age at enrollment.

Table 4

Association of incident HPV16 infection and 6-month persistent HPV16 infection with baseline serostatus in the restricted analysis (only including HPV16 infections detected following 2 or more consecutive negative HPV16 DNA results)

	Overall		MSW		MSM	
	HR (95% CI)	**Adjusted HR (95% CI)**	**HR (95% CI)**	**Adjusted HR (95% CI)**	**HR (95% CI)**	**Adjusted HR (95% CI)**
Incident infection						
Seronegative	1.00	1.00	1.00	1.00	1.00	1.00
Seropositive	1.41 (0.92–2.15)	1.37[a] (0.88–2.13)	1.31 (0.77–2.22)	1.48[b] (0.87–2.53)	1.06 (0.49–2.29)	1.60[c] (0.71–3.61)
6-mo persistent infection						
Seronegative	1.00	1.00	1.00	1.00	1.00	1.00
Seropositive	0.93 (0.40–2.19)	1.01[d] (0.42–2.43)	0.90 (0.32–2.52)	0.76[e] (0.23–2.50)	0.85 (0.16–4.36)	1.66[f] (0.32–8.77)

[a] Adjusted for age at enrollment, sexual practice (MSW and MSM), and lifetime number of sex partners.

[b] Adjusted for age at enrollment and lifetime number of female sex partners.

[c] Adjusted for age at enrollment and circumcision status.

[d] Adjusted for age at enrollment, sexual practice (MSW and MSM), and the number of new sex partners in past 6 months.

[e] Adjusted for age at enrollment and the number of new female sex partners in past 6 months.

[f] Adjusted for age at enrollment.