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Prevalence and Risk Factors for Neutralizing Antibodies to Human Papillomavirus Types 16 and 18 in HIV-positive Men who have Sex with Men

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Abstract

Objective—HPV vaccination is routinely recommended in HIV-positive MSM ≤ 26 years old. Levels of prior HPV exposure in older HIV-positive MSM are assumed to be too high to warrant routine HPV vaccination. However, little is known about the prevalence of and risk factors for neutralizing antibody seropositivity to HPV-16 or HPV-18, a key measure of prior exposure to these types.

Methods—Cross-sectional analysis of baseline visit for 296 HIV-positive MSM participating in a prospective cohort study of anal squamous intraepithelial lesions (ASIL) at a university-based research clinic. Participants completed a questionnaire detailing behaviors and medical history. Phlebotomy, anal cytology, HPV DNA testing with quantitation, and high resolution anoscopy with biopsy were performed. A pseudovirion-based neutralizing antibody (PBNA) assay was used to measure HPV-16 and HPV-18 neutralizing antibodies.

Results—132/296 (45%) men were HPV-16-seropositive and 141/296 (48%) were HPV-18-seropositive. 175/296 (59%) of the men were positive for HPV-16 antibodies or DNA, and 167/296 (56%) were positive for HPV-18 antibodies or DNA. In multivariable analysis, HPV-16 seropositivity did not correlate with age, years of HIV positivity, CD4+ level or HIV viral load. Significant risk factors included HPV-16 DNA positivity with higher DNA levels (p_{trend}<.001) and higher number of receptive sexual partners in the last year (p_{trend}=.012).

Conclusions—A high proportion of HIV-positive MSM >26 years are DNA-negative and seronegative to HPV-16 and HPV-18 even when using a sensitive PBNA assay. Prospective

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studies are needed to determine the clinical- and cost-effectiveness of HPV vaccination in HIV-positive MSM > 26 years old.

Keywords

Human papillomavirus; pseudovirions; neutralizing antibodies; HIV; MSM

Introduction

Like cervical cancer, anal cancer is associated with human papillomavirus (HPV) infection, particularly HPV-16 and HPV-18 [1]. The incidence of anal cancer is increased in certain groups of the population, particularly, men who have sex with men (MSM) and those who are immunocompromised due to medication to prevent solid organ transplant rejection, or HIV infection. Prior to the advent of highly active antiretroviral therapy (HAART), the incidence of anal cancer was increased in HIV-positive MSM relative to MSM but the difference in incidence was modest [2]. It is now clear that the HAART has not led to a reduction in the incidence of anal cancer, with several studies reporting an increase in the incidence of anal cancer in the post-HAART era. The incidence of anal cancer was recently reported to be 131/100,000 among HIV-positive MSM [3], and is 80-fold higher in this population than the general population of men.

The quadrivalent HPV (qHPV) vaccine was recently shown to be effective to prevent persistent anal HPV-16 and HPV-18 infection and anal squamous intraepithelial lesions caused by these HPV types in healthy mostly HIV-negative MSM aged 16 to 26 years [4]. HPV vaccination therefore has the potential to prevent a large percentage of anal cancers.

All of the randomized placebo-controlled trials of the bivalent or quadrivalent HPV vaccines demonstrated their highest efficacy among those considered naïve to a given vaccine type, i.e., those who were both DNA-negative and seronegative to that type [5-8]. To most accurately assess prior HPV exposure, it is necessary to assess seropositivity to these types, since individuals may become DNA test-negative over time. An understanding of current or prior exposure to HPV-16 and HPV-18 among HIV-positive MSM older than 26 years is therefore critical to guide vaccination policy in this group given its high incidence of anal cancer.

Several methods have been used to measure seropositivity to HPV. Antibodies to a variety of linear HPV proteins may be measured [9-12]. More recently there has been increasing interest in measuring neutralizing antibodies since their presence or absence would presumably best reflect potential for benefit from vaccination. A commonly used method is a competitive Luminex immunoassay (cLIA) that employs a monoclonal antibody to a single L1 neutralizing epitope [13]. While convenient for high-throughput assays and highly specific, this assay has limited sensitivity given its ability to detect antibodies to only one epitope. More recently ELISAs designed to detect a wider array of neutralizing antibodies have been developed to address this limitation [14, 15].

Here we report the prevalence of, and risk factors for seropositivity to HPV-16 and HPV-18 in a group of HIV-positive MSM using a pseudovirion-based neutralizing assay (PBNA).

Since the PBNA likely measures a wider range of antibodies with neutralizing capability than cLIA or ELISAs it may have higher sensitivity and thus may better reflect prior HPV exposure to HPV-16 and HPV-18 [16]. World Health Organization guidelines for HPV vaccines have indicated that neutralization assays are the 'gold standard' for unbiased assessment of the protective potential of vaccine-induced antibodies [17, 18].

Methods

Study participants

All procedures were performed after obtaining informed consent and with the approval of the Committee on Human Research of the University of California, San Francisco (UCSF). MSM were recruited into this cohort study between February 1998 and January 2000. Interviews were conducted in person at baseline and included medical history, sexual practices and substance use [19].

Laboratory testing

Each participant had a swab specimen collected for anal HPV testing and anal cytology. High resolution anoscopy with biopsy of visible lesions were performed at each visit. The severity of disease reflected the most severe grade of either the cytology or the biopsy [19-21]. Blood was obtained for serology, HIV status, HIV viral load and CD4+ lymphocyte level. HIV viral load was measured using the branched-chain Chiron assay. When the study was initiated, the lower limit of sensitivity was 500 copies/ml. CD4+ levels were measured by standardized two- or three-color fluorescence methods as described previously [19]. HPV DNA testing of anal swab specimens was performed as described previously using the polymerase chain reaction with L1 consensus primers and probes specific for 29 individual HPV types and a mixture of 10 other types [21,22]. The intensity of the HPV DNA signal on dot-blot was recorded on a standardized scale of 1-4 with 4 representing the most intense signal. This has been validated as an indicator of HPV DNA quantity [22,23].

HPV-16 and HPV-18 serum neutralizing antibody titers were determined using a pseudovirion-based neutralizing assay (PBNA). The 293TT cell line, p16sheLL, p18sheLL and pYSEAP plasmids were kind gifts from John Schiller, NIH. HPV-16 and HPV-18 pseudovirions (PsVs) were prepared as described [24-26]. The p16sheLL or p18sheLL plasmids were co-transfected with the pYSEAP plasmid in 293TT cells. The resulting HPV-16 and HPV-18 PsVs carried the secreted alkaline phosphatase (pYSEAP) plasmid. PsVs were purified by loading the crude extract on a 2% agarose gel column as described previously [26, 27]. The integrity of the PsVs was confirmed using electron microscopy. Neutralization assays were performed as previously described [24, 25]. PsVs carrying the SEAP-encoding plasmid were incubated with test sera for 1 hour on ice and the mix was added to the 293TT cells. After 72 hours SEAP protein secreted into the cell culture medium was quantified as a measure of PsV cell entry using the Great Escape SEAP 2.0 detection kit (Clontech, Mountain View, CA) per the manufacturer's instructions. Chemiluminescence was read on a microplate reader (Dynatech Laboratories, Chantilly, VA). Negative controls consisted of sera known to lack neutralizing HPV antibodies. Positive controls consisted of assays performed with addition of heparin (1mg/ml) and sera known to contain neutralizing

antibodies from HPV-16- and HPV-18-vaccinated individuals (a kind gift from Dr. John Schiller, NIH). The neutralization titer was defined as the reciprocal of the highest dilution of test serum that reduced the SEAP activity by at least 50% in comparison with reactivity in the wells that received only PsVs but no antibody [24, 25]. All assays were done in duplicate at four different dilutions − 1:100, 1:200, 1:400 and 1:800. For samples where the titers were greater than 1:800 additional assays were performed at higher serum dilutions. Data represented an average of the duplicate wells. Samples that did not reduce SEAP activity by 50% or more at a 1:100 dilution were considered to be seronegative.

Statistical analysis

Relative risks (RRs) were used as the measure of association between selected characteristics and serostatus. Confidence intervals for RRs were estimated by Poisson regression using robust error variances [28]. Neutralizing titers were expressed as mean ± standard deviation (SD) among those who were seropositive. Mean differences between/among groups were tested by T-tests and analysis of variance (ANOVA) methods. Where appropriate, p-for-trend was computed by a likelihood ratio procedure (RRs) or by a generalized linear model trend test (means) [29]. Fisher's test was used to compute exact p-values for 2×2 tables. A cubic spline model was used to graphically assess the association between HPV 16 and HPV-18 titer levels. Statistical significance was defined as $p < 0.05$. SAS version 9.3 (Cary, NC) was used for all analyses.

Results

Relationship between study population demographics, HIV disease status and HPV serostatus

296 HIV-positive MSM were included in the analysis. 86% were White, non-Hispanic, 6% were Hispanic and 8% were “other” (Table 1). The mean age was 42 years and 85% reported having had at “some college” or “college” education. The mean number of years of HIV positivity was 9.5 at the time of the study. Overall 132/296 (45%) of the men were seropositive to HPV-16 and 141/296 (48%) were seropositive to HPV-18. 78/296 (26%) were positive for both types and 101/296 (34%) were seronegative for both. Overall, 175 of 296 (59%) of the men were positive for HPV-16 antibodies or HPV-16 DNA, and 167 of 296 (56%) of the men were positive for HPV-18 antibodies or HPV-18 DNA. Conversely, 41% of the men were DNA-and seronegative to HPV-16, 44% of the men were DNA-and seronegative to HPV-18. Twenty-two % were DNA-and seronegative to both HPV-16 and HPV-18.

Titers to HPV-16 in this unvaccinated population were generally less than 1:2000, and titers to HPV-18 were generally less than 1:1000.

There was no relationship between age or ethnic/racial background and rates of seropositivity to HPV-16 or HPV-18, or titers to these HPV types. Although there was no relationship between level of education and rates of seropositivity to HPV-16 or HPV-18, HPV-16 neutralization titers were significantly lower (p_{trend}=.032) among those who reported a college education than those who reported high school or some college education.

There was no relationship between years of HIV positivity, current CD4+ level and current HIV viral load, and HPV-16 or HPV-18 seropositivity or titers (Table 2).

Relationship between anal HPV infection, anal squamous intraepithelial lesions and HPV serostatus

There was no relationship between HPV-16 or 18 seropositivity or titers, prevalent anal squamous intraepithelial lesions or lesion severity (Table 3). History of anogenital warts was associated with higher HPV-18 titers but not overall HPV-16 or HPV-18 seropositivity or HPV-16 titers.

HPV-16 seropositivity was associated with HPV-16 DNA positivity. 76 of 119 (64%) men who were HPV-16-DNA positive were HPV-16-seropositive, compared with 56 of 177 (32%) of men who were HPV-16 DNA-negative ($p_{\text{Fisher exact}} < 0.001$). Likewise, HPV-18 seropositivity was associated with HPV-18 DNA positivity. 53 of 79 (67%) men who were HPV-18 DNA-positive were HPV-18-seropositive, compared with 88 of 217 (41%) of men who were HPV-18 DNA-negative ($p_{\text{Fisher exact}} < 0.001$). Compared with those who were HPV-16 DNA-negative, there was a significant increase in HPV-16 seropositivity among those who were HPV-16 DNA-positive whether or not they were also positive for HPV-18 DNA. HPV-16 seropositivity was not associated with HPV-18 DNA positivity alone. HPV-16 titers were significantly increased among those who were HPV-16 DNA-positive and HPV-18 DNA-negative compared with those who were HPV-16 and HPV-18 DNA-negative.

As with the significant and specific relationship between HPV-16 DNA positivity and HPV-16 seropositivity, there was a significant and specific relationship between HPV-18 DNA positivity and HPV-18 seropositivity. Compared with those who were HPV-16 DNA-negative and HPV-18 DNA-negative, there was a significant increase in HPV-18 seropositivity among those who were HPV-18 DNA-positive and those who were positive for both HPV-16 and HPV-18 DNA. In contrast, HPV-18 seropositivity was not associated with HPV-16 DNA positivity alone in the absence of concurrent HPV-18 positivity. HPV-18 titers were significantly increased among those who were HPV-18 DNA-positive but not those who were HPV-16 DNA-positive and HPV-18 DNA-negative, or positive for both HPV-16 and HPV-18 DNA.

HPV-16 DNA signal intensity correlated with the proportion of study participants who were HPV-16-seropositive ($p_{\text{trend}}<0.001$) and to a lesser extent, the proportion who were HPV-18 DNA-positive ($p_{\text{trend}}=0.013$). Higher HPV-16 DNA signal intensity also correlated with higher HPV-16 titers ($p_{\text{trend}}=0.008$) but not HPV-18 titers. HPV-18 DNA signal intensity correlated with the proportion of men seropositive for HPV-18 ($p_{\text{trend}}<0.001$) and HPV-18 titers ($p_{\text{trend}}=0.003$) but not with the proportion who were seropositive for HPV-16 or HPV-16 titers.

Relationship between sexual behavior and HPV serotatus

A higher number of receptive sexual partners in the last year ($p_{\text{trend}} < 0.001$), but not lifetime receptive sexual partners, was associated with a higher proportion of HPV-16 seropositivity (Table 4). Both lifetime receptive partners ($p_{\text{trend}} = 0.010$) and receptive partners within the

last year (p_{trend} =0.003) were associated with a higher proportion of HPV-18 positivity. The number of receptive partners did not correlate with HPV-16 or HPV-18 titers.

A higher number of lifetime insertive sexual partners (p_{trend} =0.004) and insertive sexual partners in the last year (p_{trend} =0.006), and were associated with a higher proportion of HPV-16 seropositivity, whereas neither insertive partners within the last year nor lifetime insertive partners were associated with a higher proportion of HPV-18 positivity. The number of insertive partners did not correlate with either HPV-16 or HPV-18 titers.

Multivariable model of risk factors for HPV-16 or HPV-18 seropositivity

Using selected risk factors shown to be significant in univariate analysis, as well as measures of HIV status, we assembled a multivariable model of risk factors HPV-16 and HPV-18 seropositivity (Table 5). When including years of HIV positivity, current CD4 level and HIV viral load, risk factors that remained significant for increased risk of HPV-16 seropositivity included positivity for HPV DNA with increasing signal intensity (p_{trend}<0.001) and number of receptive partners in the last year (p_{trend}=0.012). Risk factors for HPV-18 seropositivity included HPV-18 positivity with increasing HPV-18 DNA signal intensity (p_{trend}<.001), and number of receptive partners within the last year (p_{trend}<0.001), but also included being older (p_{trend}=0.019).

Association between HPV-16 and HPV-18 neutralization results

The neutralizing titer levels were significantly higher for HPV-16 (mean=1148, interquartile range=1400) than HPV-18 (mean=758, interquartile range=650) genotypes (p<0.0001). However, a statistically significant cubic correlation between HPV-16 and HPV-18 titer levels was not observed (adjusted R^2=0.08, p=0.7629) (Figure 1, supplemental digital content).

Discussion

HPV vaccination with the quadrivalent HPV vaccine is not recommended routinely for HIV-positive MSM older than 26 years. This age cutoff was made based on the assumption that HIV-positive men older than 26 years would derive little benefit from vaccination given the likelihood of prior exposure to multiple sexual partners. A higher number of sexual partners is a risk factor for HPV acquisition and reduced likelihood of benefiting from vaccination [30-32]. Consistent with this assumption, nearly all HIV-positive men have prevalent anal HPV infection with at least one HPV type, and often have multiple HPV types. However, most of these studies show that HPV-16 or HPV-18 DNA is detected in fewer than half of men [33], and it is possible some might benefit from HPV vaccination if they are both DNA-negative and seronegative to HPV-16 or HPV-18. Our study shows that 41% of HIV-positive MSM are both DNA-negative and seronegative for HPV-16 and 44% were DNA-negative and seronegative for HPV-18. It is therefore important that both the DNA and serostatus of these men to these types be assessed.

This is the first study using the PBNA assay to measure neutralizing antibodies in HIV-positive MSM, an assay that has been shown to be more sensitive than cLIA or ELISA [13]. In this study, as expected, the proportion of HIV-positive MSM with a mean age of 42 years

who were seropositive to HPV-16 or HPV-18 was substantially higher than a healthy population of unvaccinated women aged 9 to 26 years in British Columbia, among whom 1.9% were seropositive to HPV-16 and 1.1% to HPV-18 [13]. However, a surprisingly high proportion would also be considered naïve to HPV-16 (41%) and HPV-18 (44%) given their extensive history of prior sexual exposure. A recently published AIDS Malignancy Consortium (AMC) multisite study of similarly-aged HIV-positive MSM showed an even higher rate of being "naïve" to HPV-16 (62%) or HPV-18 (78%) [34]. The lower proportion of men putatively naïve to HPV-16 and HPV-18 in our study may reflect higher sensitivity for measurement of seropositivity using the PBNA assay compared with the ELISA assay. It may also reflect a higher prevalence of HPV-16 and HPV-18 DNA positivity in our study, since participants with anal high-grade squamous intraepithelial lesions were excluded from the AMC study. In an earlier study of HIV-positive MSM, 41% were HPV-16-seropositive using a capsid antibody ELISA assay but this assay did not measure neutralizing antibodies [35].

In theory individuals who are "naive" to HPV-16 or HPV-18 may benefit from HPV vaccination if they remain at risk of *de novo* exposure to these types. The incidence of HPV-16 (10.8 cases/1000 person-months) and HPV-18 DNA (4.4 cases/1000 person-months) measured at a single time point was high in an earlier study of HIV-positive MSM of similar age to those in our study [36] suggesting that the risk of exposure remains high in this population. However, we speculate that many if not most men who would currently be classified as "naïve" to HPV-16 or HPV-18 were previously seropositive to these types, and sero-reverted to negative, a process that has been clearly demonstrated to occur over time in healthy women [37,38]. Consistent with this possibility is our observation that HPV-16 seropositivity was associated in multivariate analysis with the number of receptive sexual partners in the last 12 months but not lifetime partners. If these men are not truly naïve, the value of vaccination is not clear and studies are needed to determine if vaccination affords these men the same protection that they would have received if they were truly naïve.

In our study there was a clear relationship between being positive for HPV-16 or 18 DNA and seropositivity to that particular type. HPV-16 seropositivity was not associated with HPV-18 DNA positivity alone.. HPV-16 titers were also significantly increased in association with higher HPV-16 DNA levels. Similar results were found for HPV-18. There was no relationship between presence of and severity of lesions and seropositivity, and it is possible that the relationship with HPV DNA quantity may reflect lesion size, which was not measured in this study, and potentially quantity of viral capsid antigens to which a serologic response is being mounted. Consistent with this, serologic titers also correlated with increasing DNA quantity.

Seropositivity did not vary with age, years of HIV positivity, CD4 level, and HAART level. The proportion that was seropositive to HPV-16 or HPV-18 did not change from 31 to 60 years of age. A smaller proportion was positive after age 60 years but we had too few participants in this age group to show that this was statistically significant. It is clear that HIV-positive MSM can mount a serologic response to HPV-16 or HPV-18 through a wide range of CD4 levels and after many years of HIV positivity. The titers that were found were considerably lower than those found after vaccination of similarly-aged HIV-positive MSM

[34] and reflect the response to natural HPV infection, and possibly, an anamnestic response after re-exposure to HPV-16 or HPV-18.

In summary, while a high proportion of HIV-positive MSM are seropositive to HPV-16 and HPV-18, a high proportion would also be considered naïve to one or both types despite a high likelihood of prior exposure to these types. Serologic responses were maintained over a wide CD4 levels and HIV viral loads. Incident detection of HPV-16 and 18 remains high among men in this demographic. Studies have shown that HPV vaccination is projected to be cost-effective in HIV-negative and HIV-positive MSM up to age 26 years [39]. Prospective, randomized studies in HIV-positive MSM in different age strata over the age of 26 years are needed to determine if HPV vaccination can reduce the risk of incident high-grade squamous intraepithelial lesions and ultimately have an impact on the high incidence of anal cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Rachna Sharma performed the neutralization assays, compiled the data and co-wrote the manuscript. Jimmy Efird performed statistical analysis of the data. Aung Chein assisted in performing neutralization assays. Elizabeth Holly helped to design the study and assisted in data analysis. Mel Krajden assisted in development of the PBNA. Michael Berry, Teresa Darragh and Naomi Jay assisted in the collection of anal cytology and biopsy samples. Teresa Darragh performed cytologic and histologic interpretation of the cytology and biopsy samples. Dr. Palefsky designed the study, assisted in data analysis and co-wrote the manuscript. The experimental work was performed in Joel Palefsky's laboratory and he is the corresponding author.

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References

1. Hoots BE, Palefsky JM, Pimenta JM, et al. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. Int J Cancer. 2009; 124:2375–83. [PubMed: 19189402]
2. Ioannidis JP, O'Brien TR, Goedert JJ. Evaluation of guidelines for initiation of highly active antiretroviral therapy in a longitudinal cohort of HIV-infected individuals. AIDS. 1998; 12:2417–23. [PubMed: 9875579]
3. Silverberg MJ, Lau B, Justice AC, et al. Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America. Clin Infect Dis. 2012; 54:1026–34. [PubMed: 22291097]
4. Palefsky JM, Giuliano AR, Goldstone S, et al. HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. N Engl J Med. 2011; 365:1576–85. [PubMed: 22029979]
5. Wheeler CM, Castellsagué X, Garland SM, et al. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol. 2012; 13:100–10. [PubMed: 22075170]
6. Paavonen J, Naud P, Salmerón J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet. 2009; 374:301–14. [PubMed: 19586656]

7. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med. 2007; 356:1928–43. [PubMed: 17494926]
8. The FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med. 2007; 356:1915–27. [PubMed: 17494925]
9. Dillner J, Wiklund F, Lenner P, et al. Antibodies against linear and conformational epitopes of human papillomavirus type 16 that independently associate with incident cervical cancer. Int J Cancer. 1995; 60:377–82. [PubMed: 7530234]
10. Dillner J. Mapping of linear epitopes of human papillomavirus type 16: the E1, E2, E4, E5, E6 and E7 open reading frames. Int J Cancer. 1990; 46:703–11. [PubMed: 1698732]
11. Kulski JK, Sadleir JW, Kelsall SR, et al. Type specific and genotype cross reactive B epitopes of the L1 protein of HPV16 defined by a panel of monoclonal antibodies. Virology. 1998; 243:275–82. [PubMed: 9568027]
12. Christensen ND, Dillner J, Eklund C, et al. Surface conformational and linear epitopes on HPV-16 and HPV-18 L1 virus-like particles as defined by monoclonal antibodies. Virology. 1996; 223:174–84. [PubMed: 8806551]
13. Krajden M, Cook D, Yu A, et al. Human papillomavirus 16 (HPV-16) and HPV-18 antibody responses measured by pseudovirus neutralization and competitive Luminex assays in a two- versus three-dose HPV vaccine trial. Clin Vaccine Immunol. 2011; 18:418–23. [PubMed: 21248158]
14. Di Bonito P, Grasso F, Mochi S, et al. Serum antibody response to Human papillomavirus (HPV) infections detected by a novel ELISA technique based on denatured recombinant HPV16 L1, L2, E4, E6 and E7 proteins. Infect Agent Cancer. 2006; 1:6. [PubMed: 17150135]
15. Monroy-García A, Gómez-Lim MA, Weiss-Steider B, et al. A novel HPV-16 L1-based chimeric virus-like particle containing E6 and E7 seroreactive epitopes permits highly specific detection of antibodies in patients with CIN 1 and HPV-16 infection. Virol J. 2011; 8:59. [PubMed: 21306638]
16. Schiller JT, Lowy DR. Immunogenicity Testing in Human Papillomavirus Virus-Like-Particle Vaccine Trials. J Infect Dis. 2009; 200:166–171. [PubMed: 19519255]
17. Dessy FJ, Giannini SL, Bougelet CA, et al. Correlation between direct ELISA, single epitope-based inhibition ELISA and pseudovirion assay for measuring anti-HPV-16 and anti-HPV-18 antibody response after vaccination with the AS04-adjuvanted HPV-16/18 cervical cancer vaccine. Hum Vaccin. 2008; 4:425–434. [PubMed: 18948732]
18. World Health Organization. WHO, Geneva: 2008. Who meeting on the standardization of HPV assays and the role of WHO HPV LabNet in supporting vaccine introduction. URL: http://www.who.int/biologicals/areas/vaccines/hpv_labnet/FINAL%20repor_%20HPV_%2023+24%20+25%20Jan%202008%20_CLEAN_.pdf [last accessed July 2013]
19. Palefsky JM, Holly EA, Efird JT, et al. Anal intraepithelial neoplasia in the highly active antiretroviral therapy era among HIV-positive men who have sex with men. AIDS. 2005; 19:1407–14. [PubMed: 16103772]
20. Palefsky JM, Holly EA, Hogeboom CJ, et al. Virologic, immunologic, and clinical parameters in the incidence and progression of anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual men. J Acquir Immune Defic Syndr Hum Retrovirol. 1998; 17:314–9. [PubMed: 9525431]
21. Palefsky JM, Holly EA, Ralston ML, et al. Prevalence and risk factors for human papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. J Infect Dis. 1998; 177:361–7. [PubMed: 9466522]
22. Morrison EA, Goldberg GL, Kadish AS, et al. Polymerase chain reaction detection of human papillomavirus: quantitation may improve clinical utility. Journal of Clinical Microbiology. 1992; 30:2539–43. [PubMed: 1328278]
23. Palefsky JM, Holly EA, Ralston ML, et al. Prevalence and risk factors for anal HPV infection in HIV-positive and high-risk HIV-negative women. J Infec Dis. 2001; 183:383–391. [PubMed: 11133369]

24. Pastrana DV, Buck CB, Pang YY, et al. Reactivity of human sera in a sensitive, high-throughput pseudovirus-based papillomavirus neutralization assay for HPV16 and HPV18. Virology. 2004; 2:205–16. [PubMed: 15051381]
25. Buck CB, Pastrana DV, Lowy DR, et al. Generation of HPV pseudovirions using transfection and their use in neutralization assays. Methods Mol Med. 2005; 119:445–62. [PubMed: 16350417]
26. Buck CB, Thompson CD. Production of papillomavirus-based gene transfer vectors. Curr Protoc Cell Biol. 2007; Chapter 26:Unit 26.1. [PubMed: 18228512]
27. Buck CB, Cheng N, Thompson CD, et al. Arrangement of L2 within the papillomavirus capsid. J Virol. 2008; 82:5190–7. [PubMed: 18367526]
28. Zou G. A modified Poisson regression approach to prospective studies with binary data. Am J Epidemiol. 2004; 159:702–706. [PubMed: 15033648]
29. Graybill, F. Theory and Application of the Linear Model. North Scituate, MA: Duxbury Press; 1976.
30. Porras C, Bennett C, Safaeian M, et al. Determinants of seropositivity a.ng HPV-16/18 DNA positive young women. BMI Infect. 2010; 10:238.
31. Lu B, Viscidi RP, Lee JH, 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. [PubMed: 21378268]
32. Goldstone S, Palefsky JM, Giuliano AR, et al. Prevalence of and Risk Factors for Human Papillomavirus (HPV) Infection Among HIV-Seronegative Men Who Have Sex With Men. J Infect Dis. 2011; 203:66–74. [PubMed: 21148498]
33. Machalek DA, Poynten M, Jin F, 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. [PubMed: 22445259]
34. Wilkin T, Lee JY, Lensing SY, et al. Safety and immunogenicity of the quadrivalent human papillomavirus vaccine in HIV-1-infected men. J Infect Dis. 2010; 202:1246–53. [PubMed: 20812850]
35. Hagensee ME, Kiviat N, Critchlow CW, et al. Seroprevalence of human papillomavirus types 6 and 16 capsid antibodies in homosexual men. J Infect Dis. 1997; 176:625–31. [PubMed: 9291307]
36. de Pokomandy A, Rouleau D, Ghattas G, et al. Prevalence, clearance, and incidence of anal human papillomavirus infection in HIV-infected men: the HIPVIRG cohort study. J Infect Dis. 2009; 199:965–73. [PubMed: 19239366]
37. Stone KM, Karem KL, Sternberg MR, et al. Seroprevalence of human papillomavirus type 16 infection in the United States. J Infect J. 2002; 186:1396–402.
38. Wang SS, Schiffman M, Herrero R, et al. Determinants of human papillomavirus 16 serological conversion and persistence in a population-based cohort of 10 000 women in Costa Rica. Br J Cancer. 2004; 91:1269–74. [PubMed: 15292929]
39. Kim JJ. Targeted human papillomavirus vaccination of men who have sex with men in the USA: a cost-effectiveness modelling analysis. Lancet Infect Dis. 2010; 10:845. [PubMed: 21051295]

Table 1

Univariable relationship between selected study demographics and serostatus for HPV 16 and 18 among HIV-positive men who have sex with men

Characteristic (n)	HPV 16+n (%)	HPV 16-n (%)	RR (95% CI)‡	HPV 16+ Neut. Titer Mean ± SD	HPV 18+n (%)	HPV 18-n (%)	RR (95% CI)‡	HPV 18+ Neut. Titer Mean ± SD
Age at enrollment (yrs)								
≤30 (9)	5 (56)	4 (44)	1.0 Referent	840 ±750	4 (44)	5 (56)	1.0 Referent	500 ±258
31-40 (117)	50 (43)	67 (57)	0.77 (0.41-1.4)	1514 ±1675	53 (45)	64 (55)	1.0 (0.48-2.2)	621 ±821
41-50 (137)	63 (46)	74 (54)	0.83 (0.45-1.5)	839 ±2211	66 (48)	71 (52)	1.1 (0.51-2.3)	608 ±801
51-60 (24)	11 (46)	13 (54)	0.83 (0.40-1.7)	800 ±835	13 (54)	11 (46)	1.2 (0.54-2.8)	385 ±435
>60 (9)	3 (33)	6 (67)	0.60 (0.20-1.8)	3333 ±4086	9 (56)	4 (44)	1.3 (0.49-3.2)	240 ±114
Mean±SD	42±7.5	43±8.0 $p_{t\text{-}test}$=0.77†	p_{trend}=0.82§	p_{trend}=0.15^	43±8.3	42±7.3 $p_{t\text{-}test}$=0.56†	p_{trend}=0.35§	p_{trend}=0.47^
Education								
≤High school (43)	21 (49)	22 (51)	1.0 Referent	2010 ±3632	22 (51)	21 (49)	1.0 Referent	525 ±512
Some college (87)	36 (41)	51 (59)	0.85 (0.57-1.3)	1035 ±1822	33 (38)	54 (62)	0.74 (0.50-1.1)	508 ±666
College (166)	75 (45)	91 (55)	0.93 (0.65-1.3) p_{trend}=0.89§	961 ±1238 p_{trend}=0.032^	86 (52)	80 (48)	1.0 (0.73-1.4) p_{trend}=0.37§	616 ±844 p_{trend}=0.62^
Race/ethnicity								
White n/Hisp. (254)	115 (45)	139 (55)	1.0 Referent	1200 ±2100	122 (48)	132 (52)	1.0 Referent	610 ±796
White Hisp. (19)	8 (42)	11 (58)	0.93 (0.54-1.6)	938 ±597	10 (53)	9 (47)	1.1 (0.70-1.7)	405 ±473
Other (23)	9 (39)	14 (61)	0.84 (0.51-1.5)	667 ±589 p_{ANOVA}=0.71*	9 (39)	14 (61)	0.81 (0.48-1.4)	306 ±300 p_{ANOVA}=0.39*

‡Confidence intervals for relative risks (RR) were computed using robust error variances.

†T-test comparing mean values for seropositive and seronegative individuals for indicated characteristic.

§Likelihood ratio trend test for relative risk.

^Generalized linear model trend test.

*Analysis of variance F-test.

Table 2

Univariable relationship between HIV disease status and serostatus for HPV 16 and 18 among HIV-positive men who have sex with men

Characteristic (n)	HPV 16+n (%)	HPV 16-n (%)	RR (95% CI)‡	HPV 16+ Neut. Titer Mean ± SD	HPV 18+n (%)	HPV 18-n (%)	RR (95% CI)‡	HPV 18+ Neut. Titer Mean ±SD
Years of HIV positivity¶								
≤5 (42)	22 (52)	20 (48)	1.0 Referent	1723 ±2217	25 (60)	17 (40)	1.0 Referent	496 ±683
>5-10 (79)	34 (43)	45 (57)	0.82 (0.56-1.2)	1087 ±1564	36 (46)	43 (54)	0.77 (0.54-1.1)	440 ±609
>10-15 (162)	68 (42)	94 (58)	0.80 (0.57-1.1)	1059 ±2176	74 (46)	88 (54)	0.77 (0.57-1.04)	688 ±860
>15 (6)	3 (50)	3 (50)	0.95 (0.41-2.2)	467 ±306	4 (67)	2 (33)	1.1 (0.60-2.1)	400 ±283
Mean±SD	9.5±4.2	10±3.7 $p_{t\text{-}test}$=0.30†	p_{trend}=0.34§	p_{trend}=0.31^	9.5±4.2	10±3.6 $p_{t\text{-}test}$=0.27†	p_{trend}=0.29§	p_{trend}=0.97^
CD4 Level$^{\beta}$								
>500 (6)	44 (41)	63 (59)	1.0 Referent	1415 ±2088	48 (45)	59 (55)	1.0 Referent	575 ±810
200-500 (107)	70 (51)	67 (49)	1.2 (0.94-1.6)	1099 ±2128	69 (50)	68 (50)	1.1 (0.86-1.5)	564 ±735
<200 (137)	14 (30)	32 (70)	0.74 (0.45-1.2)	668 ±541	21 (46)	25 (54)	1.0 (0.70-1.5)	681 ±781
Mean±SD	447±236	444±277 $p_{t\text{-}test}$=0.94†	p_{trend}=0.62§	p_{trend}=0.23^	438±252	452±266 $p_{t\text{-}test}$=0.66†	p_{trend}=0.74§	p_{trend}=0.60^
HIV viral load$^{\rho}$								
<500 (154)	64 (42)	90 (58)	1.0 Referent	1259 ±2295	74 (48)	80 (52)	1.0 Referent	405 ±426
500-4k (52)	23 (44)	29 (56)	1.1 (0.74-1.5)	900 ±1637	24 (46)	28 (54)	96 (0.69-1.3)	1196 ±1273
>4k-20k (47)	25 (53)	22 (47)	1.3 (0.92-1.8)	1014 ±1573	24 (51)	23 (49)	1.1 (0.77-1.5)	548 ±747
>20k (35)	14 (40)	21 (60)	0.96 (0.62-1.5)	1621 ±2004	15 (43)	20 (57)	0.89 (0.59-1.4)	577 ±604
Mean±SD	14180±52149	18764±61689 $p_{t\text{-}test}$=0.50†	p_{trend}=0.57§	p_{trend}=0.53	17200±60310	16358±55341 $p_{t\text{-}test}$=0.90†	p_{trend}=0.79§	p_{trend}=0.84

‡Confidence intervals for relative risks (RR) were computed using robust error variances.

†T-test comparing mean values for sero-positive and sero-negative individuals for indicated characteristic.

§Li kelihood ratio trend test for relative risk.

^Generalized linear model trend test.

*Analysis of variance F-test.

¶n=7 observations had unknown values and were excluded from analyses.

$\beta_{n=46}$ observations had unknown values and were excluded from analyses.

$\rho_{n=8}$ observations had unknown values and were excluded from analyses.

Table 3

Univariable relationship between anal squamous intraepithelial lesions, anal HPV 16 and 18 DNA positivity and serostatus for HPV 16 and 18 among HIV-positive men who have sex with men

Characteristic (n)	HPV 16+N (%)	HPV 16-n (%)	RR (95% CI)‡	HPV 16+ Neut. Titer Mean ± SD	HPV 18+n (%)	HPV 18-n (%)	RR (95% CI)‡	HPV 18+ Neut. Titer Mean ±SD
Anal disease								
None (28)	11 (39)	17 (61)	1.0 Referent	832 ±898	13 (46)	15 (54)	1.0 Referent	338 ±417
Normal/Inflam (8)	6 (75)	2 (25)	1.9 (1.03-3.5)	517 ±560	1 (13)	7 (88)	0.27 (0.04-1.8)	200 (NA)
Atypia/ASCUS(21)	5 (24)	16 (76)	0.61 (0.25-1.5)	400 ±235	9 (43)	12 (57)	0.93 (0.49-1.7)	611 ±994
Condyl/AIN 1 (86)	32 (37)	54 (63)	0.95 (0.55-1.6)	855 ±460	42 (49)	44 (51)	1.1 (0.67-1.7)	619 ±850
AIN 2 or 3 (153)	78 (51)	75 (49)	1.3 (0.80-2.1) p_{trend}=0.19§	1410 ±2340 p_{trend}=0.34^	76 (50)	77 (50)	1.1 (0.70-1.6) p_{trend}=0.31§	594 ±730 p_{trend}=0.30^
History of anogenital warts								
No (79)	32 (41)	47 (59)	1.0 Referent	944 ±1447	37 (47)	42 (53)	1.0 Referent	359 ±383
Yes (217)	100 (46)	117 (54)	1.1 (0.84-1.5)	1214 ±2121 P_{ANOVA}=0.50*	104 (48)	113 (52)	1.0 (0.78-1.3)	653 ±841 P_{ANOVA}=0.043*
HPV 16/18 DNA								
−/− (141)	44 (31)	97 (69)	1.0 Referent	534 ±490	52 (37)	89 (63)	1.0 Referent	413 ±537
−/+ (36)	12 (33)	24 (67)	1.1 (0.63-1.8)	1342 ±2176	22 (61)	14 (39)	1.7 (1.2-2.3)	1098 ±1044
+/− (76)	48 (63)	28 (37)	2.0 (1.5-2.7)	1309 ±717	36 (47)	40 (53)	1.3 (0.93-1.8)	393 ±601
+/+ (43)	28 (65)	15 (35)	2.1 (1.5-2.9) p_{trend}<0.001§	1754 ±3236 p_{trend}=0.020^	31 (72)	12 (28)	2.0 (1.5-2.6) p_{trend}=0.001§	692 ± 843 p_{trend}=0.81^
HPV 16 DNA intensity								
Negative (177)		121 (68)						
+1 (24)	56 (32)	16 (67)	1.0 Referent	707 ±1117	74 (42)	103 (58)	1.0 Referent	617 ±784
+2-3 (26)	8 (33)	9 (35)	1.1 (0.57-1.9)	463 ±272	12 (50)	12 (50)	1.2 (0.77-1.8)	329 ±333
+4-5 (69)	17 (65)	18 (26)	2.1 (1.5-2.9)	1912 ±2478	15 (58)	11 (42)	1.4 (0.95-2.0)	977 ±1101
	51 (74)		2.3 (1.8-3.0) p_{trend}<0.001§	1485 ± 2508 p_{trend}=0.008^	40 (58)	29 (42)	1.4 (1.06-1.8) p_{trend}=0.013§	425 ± 584 p_{trend}=0.89^
HPV 18 DNA intensity								
Negative (217)	92 (42)	125 (58)	1.0 Referent	939 ±1337	88 (41)	129 (59)	1.0 Referent	405 ±561
+1 (17)	7 (41)	10 (59)	0.97 (0.54-1.8)	2014 ±2711	8 (47)	9 (53)	1.2 (0.68-2.0)	675 ±492
+2-3 (14)	8 (57)	6 (43)	1.3 (0.83-2.2)	813 ±1025	9 (64)	5 (36)	1.6 (1.03-2.4)	906 ±1009
+4-5 (48)	25 (52)	23 (48)	1.2 (0.90-1.7) p_{trend}=0.16§	1784 ±3402 p_{trend}=0.43	36 (75)	12 (25)	1.8 (1.5-2.3) p_{trend}<0.001§	890 ±1016 p_{trend}=0.003^

‡Confidence intervals for relative risks (RR) were computed using robust error variances.

†T-test comparing mean values for sero-positive and sero-negative individuals for indicated characteristic.

§Li kelihood ratio trend test for relative risk.

^ Generalized linear model trend test.

* Analysis of variance F-test.

Table 4

Univariable relationship between sexual behaviors and serostatus for HPV 16 and 18 among HIV-positive men who have sex with men

Characteristic (n)	HPV 16+ n (%)	HPV 16-n (%)	RR (95% CI)‡	HPV 16+ Neutralization Titer Mean ± SD	HPV 18+ n (%)	HPV 18-n (%)	RR (95% CI)‡	HPV 18+ Neutralization Titer Mean ± SD
No. of lifetime anal receptive partners								
≤10 (56)	18 (32)	38 (68)	1.0 Referent	1453 ± 1862	20 (36)	36 (64)	1.0 Referent	493 ±735
11-50 (84)	38 (45)	46 (55)	1.4 (0.90-2.2)	1287 ±1823	39 (46)	45 (54)	1.3 (0.85-2.0)	690 ±957
51-200 (69)	33 (48)	36 (52)	1.5 (0.95-2.3)	1000 ±1530	31 (45)	38 (55)	1.3 (0.81-1.9)	492 ±681
>200 (87)	43 (49)	44 (51)	1.5 (0.99-2.4) p_{trend}=0.065§	1012 ±2447 p_{trend}=0.36^	51 (59)	36 (41)	1.6 (1.1-2.4) p_{trend}=0.010§	574 ±643
No. of anal receptive partners in last year								
None (96)	32 (33)	64 (67)	1.0 Referent	919 ±1499	36 (38)	60 (63)	1.0 Referent	489 ±646
1 (55)	22 (40)	33 (60)	1.2 (0.78-1.8)	898 ±781	25 (45)	30 (55)	1.2 (0.82-1.8)	646 ±913
2-3 (45)	21 (47)	24 (53)	1.4 (0.92-2.1)	1223 ±2307	21 (47)	24 (53)	1.2 (0.83-1.9)	793 ±1043
≥4 (100)	57 (57)	43 (43)	1.7 (1.2-2.4) p_{trend}<0.001§	1346 ±2382 p_{trend}=0.27^	59 (59)	41 (41)	1.6 (1.2-2.1) p_{trend}=0.003§	523 ±624 p_{trend}=0.64^
No. Of lifetime anal insertive partners								
≤10 (51)	21 (41)	30 (59)	1.0 Referent	1338 ±1034	22 (43)	29 (57)	1.0 Referent	668 ±925
11-50 (79)	24 (30)	55 (70)	0.74 (0.46-1.2)	1908 ±2540	30 (38)	49 (62)	0.88 (0.58-1.3)	852 ±976
51-200 (90)	43 (48)	47 (52)	1.2 (0.78-1.7)	586 ±611	48 (53)	42 (47)	1.2 (0.85-1.8)	466 ±578
>200 (76)	44 (58)	32 (42)	1.4 (0.96-2.1) p_{trend}=0.004§	1192 ±2634 p_{trend}=0.28^	41 (54)	35 (23)	1.3 (0.86-1.8) p_{trend}=0.056§	455 ±624 p_{trend}=0.10^
No. of anal insertive partners in last year								
(None(93)	33 (35)	60 (65)	1.0 Referent	930 ±966	42 (45)	51 (55)	1.0 Referent	617 ±854
1 (48)	20 (42)	28 (58)	1.2 (0.76-1.8)	1145 ±1708	19 (40)	29 (60)	0.88 (0.58-1.3)	429 ±494
2-3 (51)	22 (43)	29 (57)	1.2 (0.80-1.8)	1273 ±2240	27 (53)	24 (47)	1.2 (0.83-1.7)	974 ±1110
≥4 (104)	57 (55)	47 (45)	1.5 (1.1-2.1) p_{trend}=0.006§	1227 ±2387 p_{trend}=0.48^	53 (51)	51 (49)	1.1 (0.84-1.5) p_{trend}=0.27§	394 ±385

‡Confidence intervals for relative risks (RR) were computed using robust error variances.

§Li kelihood ratio trend test for relative risk002E

^Generalized linear model trend test

Table 5

Multivariable relationship between study characteristics and serostatus for HPV 16 and 18 among HIV-positive men who have sex with men

Characteristic[¶]	HPV 16-seropositive RR (95% CI)[‡]	HPV 18-seropositive RR (95% CI)[‡]
Age at enrollment (yrs)		
≤30	1.0 Referent	1.0 Referent
31-40	1.2 (0.59-2.4)	1.0 (0.47-2.2)
41-50	1.4 (0.68-2.8)	1.3 (0.60-2.8)
51-60	1.6 (0.72-3.5)	1.4 (0.62-3.4)
>60	1.2 (0.44-3.4)	2.0 (0.79-5.1)
	$p_{trend}=0.21^{§}$	$p_{trend}=0.019^{§}$
Years of HIV positivity		
≤5	1.0 Referent	1.0 Referent
>5-10	0.75 (0.48-1.1)	0.77 (0.53-1.1)
>10-15	0.74 (0.51-1.1)	0.69 (0.50-0.95)
>15	0.75 (0.36-1.5)	0.89 (0.46-1.7)
	$p_{trend}=0.17^{§}$	$p_{trend}=0.087^{§}$
CD4 Level		
>500	1.0 Referent	1.0 Referent
200-500	1.2 (0.91-1.6)	1.2 (0.87-1.5)
<200	0.69 (0.42-1.2)	1.0 (0.69-1.6)
	$p_{trend}=0.53^{§}$	$p_{trend}=0.46^{§}$
HIV viral load		
<500	1.0 Referent	1.0 Referent
500-4K	1.1 (0.78-1.6)	0.96 (0.69-1.3)
>4K-20K	1.3(0.97-1.9)	1.1 (0.75-1.5)
>20K	1.1 (0.68-1.9)	1.1 (0.72-1.7)
	$p_{trend}=0.14^{§}$	$p_{trend}=0.73^{§}$
HPV 16 DNA intensity		
Negative	1.0 Referent	----
+1	1.3 (0.68-2.4)	----
+2-3	2.2 (1.5-3.2)	----
+4-5	2.4 (1.8-3.1)	----
	$p_{trend}<0.001^{§}$	----
HPV 18 DNA intensity		
Negative	----	1.0 Referent
+1	----	1.2 (0.74-1.8)
+2-3	----	1.9 (1.2-3.1)
+4-5	----	2.1 (1.6-2.7)
	----	$p<0.001^{§}$

Characteristic[¶]	HPV 16-seropositive RR (95% CI)[‡]	HPV 18-seropositive RR (95% CI)[‡]
No. of anal receptive partners in last year		
None	1.0 Referent	1.0 Referent
1	1.3 (0.88-2.1)	1.3 (0.87-1.9)
2-3	1.5 (0.97-2.2)	1.3 (0.88-2.0)
≥4	1.5 (1.1-2.1)	1.8 (1.3-2.5)
	p_{trend}=0.012[§]	p_{trend}<0.001[§]

[‡]Confidence intervals for relative risks (RR) were computed using robust error variances.

[§]Li kelihood ratio trend test for relative risk.

[¶]Observations with unknown values excluded from analyses.