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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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Objective: Human papillomavirus (HPV) vaccination is routinely recommended in HIV-positive men who have sex with men (MSM) aged ≤ 26 years. Levels of previous 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 previous 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 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 assay was used to measure HPV-16 and HPV-18 neutralizing antibodies.

Results: One hundred thirty-two of 296 (45%) men were HPV-16 seropositive and 141 of 296 (48%) were HPV-18 seropositive. One hundred seventy-five of 296 (59%) of the men were positive for HPV-16 antibodies or DNA and 167 of 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 ($p_{\text{trend}} < 0.001$) and higher number of receptive sexual partners in the last year ($p_{\text{trend}} = 0.012$).

Conclusions: A high proportion of HIV-positive MSM aged >26 years are DNA negative and seronegative to HPV-16 and HPV-18 even when using a sensitive pseudovirion-based neutralizing antibody assay. Prospective studies are needed to determine the clinical- and cost-effectiveness of HPV vaccination in HIV-positive MSM aged >26 years.

Key Words: human papillomavirus, pseudovirions, neutralizing antibodies, HIV, MSM

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R.S. performed the neutralization assays, compiled the data, and cowrote the article. J.T.E. performed statistical analysis of the data. A.C. assisted in performing neutralization assays. E.A.H. helped to design the study and assisted in data analysis. M.K. assisted in development of the pseudovirion-based neutralizing antibody. J.M.B., T.M.D., and N.J. assisted in the collection of anal cytology and biopsy samples. T.M.D. performed cytological and histological interpretation of the cytology and biopsy samples. J.M.P. designed the study, assisted in data analysis, and cowrote the manuscript.

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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. Before 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 of 100,000 among HIV-positive MSM³ 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 16–26 years.⁴ HPV vaccination therefore has the potential to prevent a large percentage of anal cancers.

All the randomized placebo-controlled trials of the bivalent or qHPV vaccines demonstrated their highest efficacy among those considered naive to a given vaccine type, that is, those who were both DNA negative and seronegative to that type.^{5–8} To most accurately assess previous HPV exposure, it is necessary to assess seropositivity to these types because individuals may become DNA test negative over time. An understanding of current or previous 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 because 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 Although convenient for high-throughput assays and highly specific, this assay has limited sensitivity given its ability to detect antibodies to only 1 epitope. More recently, enzyme-linked immunosorbent assays (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 antibody (PBNA) assay. Because the PBNA assay likely measures a wider range of antibodies with neutralizing capability than cLIA or ELISAs, it may have higher sensitivity and thus may better reflect previous HPV exposure to HPV-16 and HPV-18.¹⁶ Guidelines of World Health Organization 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. 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 per milliliter. CD4⁺ levels were measured by standardized 2- or 3-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 PBNA assay. The 293TT cell line, p16sheLL, p18sheLL, and pYSEAP plasmids were kind gifts from John Schiller, National Institutes of Health. HPV-16 and HPV-18 pseudovirions (PsVs) were prepared as described.^{24–26} The p16sheLL or p18sheLL plasmids were cotransfected 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 mixture 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) as 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 (1 mg/mL) and sera known to contain neutralizing antibodies from HPV-16- and HPV-18-vaccinated individuals (a kind gift from Dr John Schiller, National Institutes of Health). 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 wells at 4 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 $\pm$ SD among those who were seropositive. Mean differences between/among groups were tested by *T* tests and analysis of variance 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 exact 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

Two hundred ninety-six HIV-positive MSM were included in the analysis. Eighty-six percent of MSM were white and non-Hispanic, 6% MSM were Hispanic, and 8% MSM 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 of 296 (45%) of the men

were seropositive to HPV-16 and 141 of 296 (48%) were seropositive to HPV-18. Seventy-eight of 296 (26%) of the men were positive for both types and 101 of 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 negative and seronegative to HPV-16 and 44% of the men were DNA negative and seronegative to HPV-18. Twenty-two percent of the men 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} = 0.032) among those who reported a college education than those who reported high school or some

TABLE 1. Univariable Relationship Between Selected Study Demographics and Serostatus for HPV-16 and HPV-18 Among HIV-Positive MSM

Characteristic (n)	HPV-16 Positive, n (%)	HPV-16 Negative, n (%)	RR (95% CI)*	HPV-16 Positive Neutralization Titer, Mean ± SD	HPV-18 Positive, n (%)	HPV-18 Negative, n (%)	RR (95% CI)*	HPV-18 Positive, Neutralization 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			43 ± 8.3	42 ± 7.3		
	<i>p</i> _{t test} = 0.77†		<i>p</i> _{trend} = 0.82‡	<i>p</i> _{trend} = 0.15§	<i>p</i> _{t test} = 0.56†		<i>p</i> _{trend} = 0.35‡	<i>p</i> _{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)	961 ± 1238	86 (52)	80 (48)	1.0 (0.73–1.4)	616 ± 844
			<i>p</i> _{trend} = 0.89§	<i>p</i> _{trend} = 0.032§			<i>p</i> _{trend} = 0.37§	<i>p</i> _{trend} = 0.62§
Race/ethnicity								
White non-Hispanic (254)	115 (45)	139 (55)	1.0 (referent)	1200 ± 2100	122 (48)	132 (52)	1.0 (referent)	610 ± 796
White Hispanic (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	9 (39)	14 (61)	0.81 (0.48–1.4)	306 ± 300
				<i>P</i> _{ANOVA} = 0.71				<i>P</i> _{ANOVA} = 0.39

* Confidence intervals (CIs) for RRs were computed using robust error variances.

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

‡ Likelihood ratio trend test for RR.

§ Generalized linear model trend test.

|| Analysis of variance *F* test.

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 HPV-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. Seventy-six 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. Fifty-three 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

TABLE 2. Univariable Relationship Between HIV Disease Status and Serostatus for HPV-16 and HPV-18 Among HIV-Positive MSM

Characteristic (n)	HPV-16 Positive, n (%)	HPV-16 Negative, n (%)	RR (95% CI)*	HPV-16 Positive Neutralization Titer, Mean $\pm$ SD	HPV-18 Positive, n (%)	HPV-18 Negative, n (%)	RR (95% CI)*	HPV-18 Positive Neutralization Titer, Mean $\pm$ SD
Years of HIV positivity†								
≤5 (42)	22 (52)	20 (48)	1.0 (referent)	1723 $\pm$ 2217	25 (60)	17 (40)	1.0 (referent)	496 $\pm$ 683
>5–10 (79)	34 (43)	45 (57)	0.82 (0.56–1.2)	1087 $\pm$ 1564	36 (46)	43 (54)	0.77 (0.54–1.1)	440 $\pm$ 609
>10–15 (162)	68 (42)	94 (58)	0.80 (0.57–1.1)	1059 $\pm$ 2176	74 (46)	88 (54)	0.77 (0.57–1.04)	688 $\pm$ 860
>15 (6)	3 (50)	3 (50)	0.95 (0.41–2.2)	467 $\pm$ 306	4 (67)	2 (33)	1.1 (0.60–2.1)	400 $\pm$ 283
Mean $\pm$ SD	9.5 $\pm$ 4.2	10 $\pm$ 3.7			9.5 $\pm$ 4.2	10 $\pm$ 3.6		
	$p_{\text{T test}} = 0.30^{\ddagger}$		$p_{\text{Trend}} = 0.34^{\S}$	$p_{\text{Trend}} = 0.31^{\parallel}$	$p_{\text{T test}} = 0.27^{\ddagger}$		$p_{\text{Trend}} = 0.29^{\S}$	$p_{\text{Trend}} = 0.97^{\parallel}$
CD4 level¶								
>500 (6)	44 (41)	63 (59)	1.0 (referent)	1415 $\pm$ 2088	48 (45)	59 (55)	1.0 (referent)	575 $\pm$ 810
200–500 (107)	70 (51)	67 (49)	1.2 (0.94–1.6)	1099 $\pm$ 2128	69 (50)	68 (50)	1.1 (0.86–1.5)	564 $\pm$ 735
<200 (137)	14 (30)	32 (70)	0.74 (0.45–1.2)	668 $\pm$ 541	21 (46)	25 (54)	1.0 (0.70–1.5)	681 $\pm$ 781
Mean $\pm$ SD	447 $\pm$ 236	444 $\pm$ 277			438 $\pm$ 252	452 $\pm$ 266		
	$p_{\text{T test}} = 0.94^{\ddagger}$		$p_{\text{Trend}} = 0.62^{\S}$	$p_{\text{Trend}} = 0.23^{\parallel}$	$p_{\text{T test}} = 0.66^{\ddagger}$		$p_{\text{Trend}} = 0.74^{\S}$	$p_{\text{Trend}} = 0.60^{\parallel}$
HIV viral load#								
<500 (154)	64 (42)	90 (58)	1.0 (referent)	1259 $\pm$ 2295	74 (48)	80 (52)	1.0 (referent)	405 $\pm$ 426
500–4,000 (52)	23 (44)	29 (56)	1.1 (0.74–1.5)	900 $\pm$ 1637	24 (46)	28 (54)	0.96 (0.69–1.3)	1196 $\pm$ 1273
>4,000–20,000 (47)	25 (53)	22 (47)	1.3 (0.92–1.8)	1014 $\pm$ 1573	24 (51)	23 (49)	1.1 (0.77–1.5)	548 $\pm$ 747
>20,000 (35)	14 (40)	21 (60)	0.96 (0.62–1.5)	1621 $\pm$ 2004	15 (43)	20 (57)	0.89 (0.59–1.4)	577 $\pm$ 604
Mean $\pm$ SD	14,180 $\pm$ 52,149	18,764 $\pm$ 61,689			17,200 $\pm$ 60,310	16,358 $\pm$ 55,341		
	$p_{\text{T test}} = 0.50^{\ddagger}$		$p_{\text{Trend}} = 0.57^{\S}$	$p_{\text{Trend}} = 0.53$	$p_{\text{T test}} = 0.90^{\ddagger}$		$p_{\text{Trend}} = 0.79^{\S}$	$p_{\text{Trend}} = 0.84$

* Confidence intervals (CIs) for RRs were computed using robust error variances.

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

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

§ Likelihood ratio trend test for RR.

|| Generalized linear model trend test.

¶ n = 46 observations had unknown values and were excluded from analyses.

n = 8 observations had unknown values and were excluded from analyses.

TABLE 3. Univariable Relationship Between Anal Squamous Intraepithelial Lesions, Anal HPV-16 and HPV-18 DNA Positivity, and Serostatus for HPV-16 and HPV-18 Among HIV-Positive MSM

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

* Confidence intervals (CIs) for RRs were computed using robust error variances.

† Likelihood ratio trend test for RR.

‡ Generalized linear model trend test.

§ Analysis of variance *F* test.

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 Serostatus

A higher number of receptive sexual partners in the last year ($p_{\text{trend}} < 0.001$), but not lifetime receptive sexual part-

ners, 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 ($p_{\text{trend}} = 0.003$) 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 ($p_{\text{trend}} = 0.004$) and insertive sexual partners in the last year ($p_{\text{trend}} = 0.006$) 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 and 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 ($p_{\text{trend}} < 0.001$) and number of receptive partners in the last year ($p_{\text{trend}} = 0.012$). Risk factors for HPV-18 seropositivity not only included HPV-

18 positivity with increasing HPV-18 DNA signal intensity ($p_{\text{trend}} < 0.001$), and number of receptive partners within the last year ($p_{\text{trend}} < 0.001$), but also included being older ($p_{\text{trend}} = 0.019$).

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 $R^2 = 0.08$, $P = 0.7629$) (see **Figure S1, Supplemental Digital Content**, <http://links.lww.com/QAI/A452>).

TABLE 4. Univariable Relationship Between Sexual Behaviors and Serostatus for HPV-16 and HPV-18 Among HIV-Positive MSM

Characteristic (n)	HPV-16 Positive, n (%)	HPV-16 Negative, n (%)	RR (95% CI)*	HPV-16 Positive Neutralization Titer, Mean $\pm$ SD	HPV-18 Positive, n (%)	HPV-18 Negative, n (%)	RR (95% CI)*	HPV-18 Positive Neutralization Titer, Mean $\pm$ SD
No. of lifetime anal receptive partners								
≤ 10 (56)	18 (32)	38 (68)	1.0 (referent)	1453 $\pm$ 1862	20 (36)	36 (64)	1.0 (referent)	493 $\pm$ 735
11–50 (84)	38 (45)	46 (55)	1.4 (0.90–2.2)	1287 $\pm$ 1823	39 (46)	45 (54)	1.3 (0.85–2.0)	690 $\pm$ 957
51–200 (69)	33 (48)	36 (52)	1.5 (0.95–2.3)	1000 $\pm$ 1530	31 (45)	38 (55)	1.3 (0.81–1.9)	492 $\pm$ 681
>200 (87)	43 (49)	44 (51)	1.5 (0.99–2.4)	1012 $\pm$ 2447	51 (59)	36 (41)	1.6 (1.1–2.4)	574 $\pm$ 643
			$P_{\text{trend}} = 0.065^{\dagger}$	$P_{\text{trend}} = 0.36^{\ddagger}$			$P_{\text{trend}} = 0.010^{\dagger}$	$P_{\text{trend}} = 0.94^{\ddagger}$
No. of anal receptive partners in last year								
None (96)	32 (33)	64 (67)	1.0 (referent)	919 $\pm$ 1499	36 (38)	60 (63)	1.0 (referent)	489 $\pm$ 646
1 (55)	22 (40)	33 (60)	1.2 (0.78–1.8)	898 $\pm$ 781	25 (45)	30 (55)	1.2 (0.82–1.8)	646 $\pm$ 913
2–3 (45)	21 (47)	24 (53)	1.4 (0.92–2.1)	1223 $\pm$ 2307	21 (47)	24 (53)	1.2 (0.83–1.9)	793 $\pm$ 1043
≥ 4 (100)	57 (57)	43 (43)	1.7 (1.2–2.4)	1346 $\pm$ 2382	59 (59)	41 (41)	1.6 (1.2–2.1)	523 $\pm$ 624
			$P_{\text{trend}} < 0.001^{\dagger}$	$P_{\text{trend}} = 0.27^{\ddagger}$			$P_{\text{trend}} = 0.003^{\dagger}$	$P_{\text{trend}} = 0.64^{\ddagger}$
No. of lifetime anal insertive partners								
≤ 10 (51)	21 (41)	30 (59)	1.0 (referent)	1338 $\pm$ 1034	22 (43)	29 (57)	1.0 (referent)	668 $\pm$ 925
11–50 (79)	24 (30)	55 (70)	0.74 (0.46–1.2)	1908 $\pm$ 2540	30 (38)	49 (62)	0.88 (0.58–1.3)	852 $\pm$ 976
51–200 (90)	43 (48)	47 (52)	1.2 (0.78–1.7)	586 $\pm$ 611	48 (53)	42 (47)	1.2 (0.85–1.8)	466 $\pm$ 578
>200 (76)	44 (58)	32 (42)	1.4 (0.96–2.1)	1192 $\pm$ 2634	41 (54)	35 (23)	1.3 (0.86–1.8)	455 $\pm$ 624
			$P_{\text{trend}} = 0.004^{\dagger}$	$P_{\text{trend}} = 0.28^{\ddagger}$			$P_{\text{trend}} = 0.056^{\dagger}$	$P_{\text{trend}} = 0.10^{\ddagger}$
No. of anal insertive partners in last year								
None (93)	33 (35)	60 (65)	1.0 (referent)	930 $\pm$ 966	42 (45)	51 (55)	1.0 (referent)	617 $\pm$ 854
1 (48)	20 (42)	28 (58)	1.2 (0.76–1.8)	1145 $\pm$ 1708	19 (40)	29 (60)	0.88 (0.58–1.3)	429 $\pm$ 494
2–3 (51)	22 (43)	29 (57)	1.2 (0.80–1.8)	1273 $\pm$ 2240	27 (53)	24 (47)	1.2 (0.83–1.7)	974 $\pm$ 1110
≥ 4 (104)	57 (55)	47 (45)	1.5 (1.1–2.1)	1227 $\pm$ 2387	53 (51)	51 (49)	1.1 (0.84–1.5)	394 $\pm$ 385
			$P_{\text{trend}} = 0.006^{\dagger}$	$P_{\text{trend}} = 0.48^{\ddagger}$			$P_{\text{trend}} = 0.27^{\dagger}$	$P_{\text{trend}} = 0.81^{\ddagger}$

* Confidence intervals (CIs) for RRs were computed using robust error variances.

† Likelihood ratio trend test for RR.

‡ Generalized linear model trend test.

DISCUSSION

HPV vaccination with the qHPV vaccine is not recommended routinely for HIV-positive MSM aged 26 years or older. This age cutoff was made based on the assumption that HIV-positive men aged 26 years or older would derive

little benefit from vaccination given the likelihood of previous 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 1 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 that 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 9–26 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 naive to HPV-16 (41%) and HPV-18 (44%) given their extensive history of previous sexual exposure. A recently published AIDS Malignancy Consortium multisite study of similarly aged HIV-positive MSM showed an even higher rate of being “naive” to HPV-16 (62%) or HPV-18 (78%).³⁴ The lower proportion of men putatively naive 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 because participants with anal high-grade squamous intraepithelial lesions were excluded from the AIDS Malignancy Consortium 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 “naive” 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 naive, the value of vaccination is not clear and studies are needed to determine if vaccination

TABLE 5. Multivariable Relationship Between Study Characteristics and Serostatus for HPV-16 and HPV-18 Among HIV-Positive MSM

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)
500–4,000	1.1 (0.78–1.6)	0.96 (0.69–1.3)
>4,000–20,000	1.3 (0.97–1.9)	1.1 (0.75–1.5)
>20,000	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 _{trend} < 0.001‡
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‡

* Observations with unknown values excluded from analyses.

† Confidence intervals (CIs) for RRs were computed using robust error variances.

‡ Likelihood ratio trend test for RR.

affords these men the same protection that they would have received if they were truly naive.

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 the 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. 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 MSM³⁴ and reflect the response to natural HPV infection, and possibly, an anamnestic response after re-exposure to HPV-16 or HPV-18.

In summary, whereas a high proportion of HIV-positive MSM are seropositive to HPV-16 and HPV-18, a high proportion would also be considered naive to 1 or both types despite a high likelihood of previous exposure to these types. Serologic responses were maintained over a wide CD4 levels and HIV viral loads. Incident detection of HPV-16 and HPV-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 26 years.³⁹ Prospective randomized studies in HIV-positive MSM in different age strata over the age of 26 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.

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