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Article

Long-term Persistence of Oral HPV Over 7 Years of Follow-up

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Abstract

Background: Human papillomavirus–related oropharyngeal cancer (HPV-OPC) incidence is increasing, but the natural history of the precursor—oral HPV—has not been well described. **Methods:** This observational cohort study of people living with HIV and at-risk HIV uninfected people evaluated participants semiannually using 30-second oral rinse and gargle specimens over 7 years. Initially, 447 participants were followed for 4 years as part of the Persistent Oral Papillomavirus Study, and a subset of 128 who showed persistent infections at the last Persistent Oral Papillomavirus Study visit had an additional visit, as part of the Men and Women Understanding Throat HPV Study, on average 2.5 years later. Extracted DNA from oral rinse and gargle specimens was amplified using polymerase chain reaction and type specification of 13 oncogenic HPV types. Risk factors for oncogenic oral HPV clearance were evaluated using Cox models. **Results:** The majority of oncogenic oral HPV infections cleared quickly, with a median time to clearance of 1.4 years (interquartile range = 0.5-3.9 years). After 7 years of follow-up, 97% of incident and 71% of prevalent infections had cleared. Lower HPV-16 viral load was statistically significantly associated with clearance (per 10-fold decrease in copy number: adjusted hazard ratio [aHR] = 2.51, 95% confidence interval [CI] = 1.20 to 5.26; $P = .01$). Adjusted analyses showed that oncogenic oral HPV clearance was lower among prevalent than incident-detected infections (aHR = 0.44, 95% CI = 0.35 to 0.55), among men than women (aHR = 0.74, 95% CI = 0.60 to 0.91), for older participants (aHR per 10 years increasing age = 0.81, 95% CI = 0.74 to 0.89), and among people living with HIV (aHR = 0.76, 95% CI = 0.60 to 0.95). One participant who had oral HPV-16 consistently detected at 10 study visits over 4.5 years was subsequently diagnosed with HPV-OPC. **Conclusions:** This prospective study of oncogenic oral HPV infection is the longest and largest quantification of oral HPV-16 infections to date.

Persistent oncogenic oral human papillomavirus (HPV) infection is a risk factor for oropharyngeal squamous cell carcinoma, more than 70% of which are HPV-related (HPV-OPC) (1), yet risk factors for oncogenic HPV persistence remain poorly understood. Persistence of cervical HPV serves as a strong biomarker for cervical cancer risk (2,3). As such, oncogenic cervical HPV detection is now incorporated as an option for screening in the United States (4). This model was implemented after understanding the long-term natural history of cervical HPV, cytologic change, and risk of malignancy. Whether such a paradigm applies to oral HPV and oropharyngeal cancer is unknown. Oral HPV natural history data are limited; three studies report variable follow-up (≤4 years) and limited number of oral HPV-16 infections (range = 10-48 infections) (5-8).

Initial natural history studies suggest that factors associated with acquisition and persistence of oral HPV infection include male sex, cigarette smoking, and immunosuppression (5,9). For example, people living with HIV (PLWH) have 2-3 times higher oral HPV prevalence than HIV-uninfected individuals and the risk of both incident oral HPV detection and its persistence in PLWH increases with diminishing CD4 cell count after controlling for sexual behavior and other relevant covariates (9). Short-term natural history studies have shown that most people clear oral HPV infections within 1-2 years (5-7,9,10); however, there is

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Table 1. Descriptive characteristics of 264 men from the Multicenter AIDS Cohort Study (MACS) and 183 women from the Women's Interagency HIV Study (WIHS) with oncogenic oral HPV detected at any point during the Persistent Oral Papillomavirus Study, compared with only those 128 MACS and WIHS participants enrolled in the Men and Women Understanding Throat HPV (MOUTH) study[a]

	No.	%			
Participant characteristics at time of first oral HPV detection	All N = 447	All N = 447	Men n = 264	Women n = 183	Participants in MOUTH follow-up only n = 128
Sex					
Men (MACS)	264	59.1	100	0	47.7
Women (WIHS)	183	40.9	0	100	52.3
Race					
White non-Hispanic	148	33.1	51.5	6.6	35.2
Black non-Hispanic	225	50.3	34.1	73.8	57.8
Other (Hispanic, Other)	74	16.6	14.4	19.7	7.0
Education level					
<High School	95	21.9	4.4	46.2	21.8
High School Graduate (or GED)	210	48.5	47.8	49.5	49.2
College	52	12.0	17.5	4.4	13.7
Advanced or professional degree	76	17.6	30.3	0.0	15.3
Smoking status					
Never	82	18.6	24.8	9.5	15.5
Former	160	36.3	42.8	26.8	30.9
Current	199	45.1	32.4	63.7	53.7
Current alcohol use					
No	131	30.1	16.7	49.2	30.6
Yes	305	70.0	83.3	50.8	69.4
Ever performed oral sex					
No	24	5.8	2.1	10.9	7.1
Yes	392	94.2	97.9	89.1	92.9
HIV status					
HIV-uninfected	119	26.6	28.4	24.0	25.0
PLWH	328	73.4	71.6	76.0	75.0
Currently on HAART (among PLWH)					
No	61	18.9	21.9	14.8	12.1
Yes	261	81.1	78.1	85.2	87.9
Infection characteristics					
Oral HPV-16 prevalence	447	19.9	17.1	24.0	31.3
Oral non-16 oncogenic HPV	447	83.9	86.7	79.8	72.7
Age (in years): median (IQR)	441	50 (43, 56)	51 (44, 58)	47 (42, 54)	50 (44, 57)
Current CD4 cell count, cells/ mm^3, among PLWH: median (IQR)	322	522 (326, 715)	555 (385, 746)	434 (228, 640)	439 (264, 657)
Current HIV RNA, among PLWH, median (IQR)	321	und (und, 1170)	und (und, 263)	48 (und, 2887)	48 (und, 600)

[a]HAART = highly active antiretroviral therapy; HPV = human papillomavirus; IQR = interquartile range; PLHW = person living with HIV; und = undetectable .

a paucity of long-term natural history studies. We therefore aimed to describe for the first time the natural history of persistent oral HPV over 7 years.

Methods

Study Population

The study population was a subcohort comprising 1833 participants enrolled from 2 prospective, multicenter cohort studies of PLWH and at-risk HIV-uninfected individuals in the United States: the Multicenter AIDS Cohort Study and the Women's Interagency HIV Study (11). Participants were followed prospectively every 6 months with oral rinse sample collection for 4 years between October 2009 and March 2016 as part of the Persistent Oral Papillomavirus Study (POPS; as previously described) (9, 12). After completion of POPS, the 128 participants who had persistent oncogenic oral HPV infection at the last POPS visit were enrolled in an additional follow-up visit as part of the Men and Women Understanding Throat HPV (MOUTH) study (ClinicalTrials.gov Identifier NCT03644563) (13). As in POPS, at the baseline MOUTH study visit, participants provided an oral rinse specimen for HPV testing, completed a questionnaire, and had CD4 cell count and HIV viral load tested. There was a median of 2.5 years (interquartile range [IQR] = 1.9-3.0 years) between the last POPS visit and the additional MOUTH visit, and the 128 MOUTH participants shared similar characteristics to the POPS participants as a whole (Table 1). All participants completed informed consent forms, and the study was approved by the institutional review board at each study site.

The current analysis includes 447 participants from POPS who had at least one oncogenic oral HPV infection detected, including 128 participants who subsequently had an additional visit as part of the MOUTH study (between October 2017 and September 2018; Figure 1) (9,12). At each visit, participants completed a computer-assisted self-interview questionnaire, which included questions on behaviors, medication use, and clinical outcomes; had a physical examination and CD4 cell count; and had the HIV viral load tested.

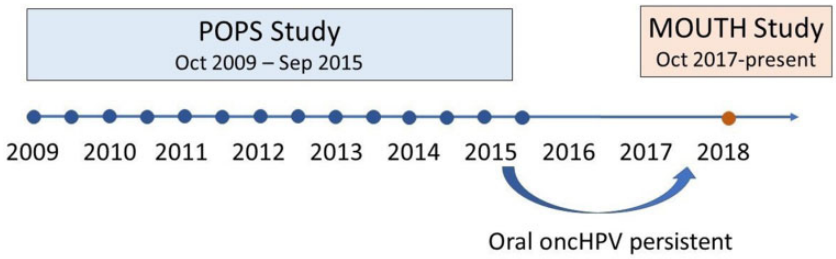

Figure 1. A total of 447 Multicenter AIDS Cohort Study and Women's Interagency HIV Study cohort study participants with 676 oncogenic oral human papillomavirus (HPV) infection. The Persistent Oral Papillomavirus Study (POPS) was from 2009 to 2015 and Men and Women Understanding Throat HPV (MOUTH) study visit included data collected from 2017 to 2018. The **blue dots** represent study visits that occurred at 6-month intervals during the POPS study. There was a lapse between POPS and MOUTH symbolized by the absence of dots. During the MOUTH study there was a one-time visit, which ranged between October 2017 and September 2018.

Oral Rinse Sample Collection, Processing, and Oral HPV Detection

Oral rinse samples were collected using 10 mL saline or Scope and a 30-second oral rinse and gargle as previously described (14). Samples were stored at 4°C until processed. Oral rinse samples were tested in the laboratory of Dr Maura Gillison at The Ohio State University (for the POPS study) and in the DDL Diagnostic Laboratory (https://www.ddl.nl for the MOUTH study). Oral HPV DNA detection involved polymerase chain reaction (PCR) with the PGMY09/11 primer system in the Gillison Lab (12) and with the SPF10 primer system in the DDL lab (version 1, Labo Bio-medical Products, Rijswijk, the Netherlands) (14). HPV type specification was conducted using Roche linear array in the Gillison lab and the SPF10 DEIA/LIPA system in the DDL lab. Oncogenic oral HPV types were defined identically for data from both labs, to include types 16/18/31/33/35/39/45/51/52/56/58/59/66 (15). Similar methods were used in both laboratories; 100 samples were tested in both and yielded 97% concordance. HPV-16 viral load was tested for all samples with any HPV detected using either linear array band intensity or TaqMan quantitative PCR (LightCycler® 480 Probes Master kit on Roche LC480 II instrument).

Statistical Analysis

Oral HPV prevalence and incidence in the POPS study have been reported elsewhere (9,12). The current analyses included 676 oncogenic oral HPV among 447 individuals, who had at least 1 follow-up visit after the infection was detected. All analyses were type specific, ie, following the same HPV type in the same person until it cleared. Infections detected at the first study visit were defined as prevalent, and infections detected for the first time during follow-up were defined as incident. Incident infections detected only at the participant's last visit were excluded from analysis of clearance and risk factors for clearance.

Time-to-clearance was calculated using Kaplan-Meier for each type-specific oncogenic infection as the time from first detection until cleared, where the primary definition for clearance was defined as "two consecutive negative tests." For infections considered to be cleared, the visit of clearance was the first of the 2 consecutive negative visits. As the MOUTH baseline visit was several years after the last sample collection, infections still detected at the MOUTH baseline visit were considered persistent whereas those with a single negative result at the MOUTH baseline visit were considered cleared for this analysis. Risk factors for clearance were explored using Cox proportional hazard models and clustered by ID to account for multiple infections within the same person. The multivariable model considered all variables with P values less than .05 in unadjusted Cox proportional hazard models and those known to be relevant based on previous literature (both current smoking and pack-years of tobacco were considered in multivariable models based on their importance in a priori literature but as both showed no association in adjusted models they were not retained in the final model). All significance tests were two-sided. Variables were removed one at a time to develop the final multivariable model.

HPV-16 viral load was tested at the visit of first HPV-16 detection. The number of HPV-16 copies in each oral rinse sample was standardized to the number of human cells in the sample to calculate the number of HPV-16 copies per cell as a measure of HPV-16 viral load standardized across samples. Effect of HPV-16 viral load on HPV-16 persistence was evaluated using Cox proportional hazard models, and adjusted for sex, age, and infection type. The number of HPV-16 copies was categorized by 10-fold changes per cell as at least 1 HPV-16 copy per every human cell, 0.1 to 0.99 copies per cell, 0.01 to 0.099 copies per cell, 0.001 to 0.0099 copies per cell, and less than 0.001 copies per cell.

Oral HPV detection and viral load were also considered in a graphic where intensity of the line blot results were plotted by color to visualize the pattern and strength (viral load) of infection. For viewability, this figure was restricted to 99 (of the 110) people with HPV-16 infection detected and at least 4 oral rinses collected in the study (results were similar when examined for all oncogenic oral HPV infections). For POPS samples, signal intensity of Roche HPV Linear Array (LA), an established surrogate for HPV DNA viral load (signal strength) (16–18) available at every study visit, was analyzed on a semiquantitative 4-point scale: strong [3,4], medium [1,2], weak [-1], and negative. For MOUTH samples, quantitative HPV-16 viral load was measured by quantitative PCR (among those with HPV-16 detected by Linear Array, otherwise assumed to be 0) and categorized on the same semiquantitative 4-point scale (defined by number of HPV-16 copies per cell: $\geq$1, <1 but >0, target detected but no quantification, 0).

Results

Participant Characteristics

Median follow-up for all participants in the current analysis was 4.2 years (IQR = 2.6-6.0 years) for POPS and MOUTH combined, including 7 years (IQR = 6.3-7.6 years) median follow-up for those in MOUTH. Participants were 59.1% men, 50.3% Black,

and 33.1% White, and median age was 50 years (Table 1). Current smoking (45.1%) and alcohol consumption (70.0%) were common, and 73.4% of participants were living with HIV (of whom 81.1% were currently using highly active antiretroviral therapy . The median CD4 cell count was 522 cells/mm^3, and HIV viral load was undetectable in 53.0% of participants.

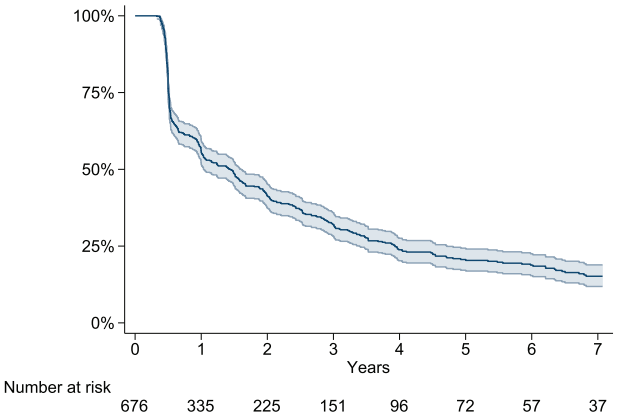

Figure 2. Time to clearance of any oncogenic human papillomavirus among 676 infections in the 447 Multicenter AIDS Cohort Study/Women's Interagency HIV Study participants enrolled in the Persistent Oral Papillomavirus Study and/or Men and Women Understanding Throat HPV study overall. Color band indicates the 95% confidence interval.

Time to Clearance of Oncogenic Oral HPV Infections

HPV-16 was the most common oncogenic infection detected and represented 15.5% of all oncogenic infections (105 of 676). Other common oncogenic types detected included HPV-33, -35, -45, -52, -59, and -66 (8%-9% of infections each). The majority of oncogenic oral HPV infections cleared within 1.4 years (IQR = 0.5-3.9 years). However, after 7 years of follow-up, 5.5% (n = 37) of oncogenic oral HPV infections were still persistently detected (Figure 2); these 37 infections were among 31 individuals, as some individuals had multiple persistent infections. Median time to clearance was more rapid for incident (0.7 years [IQR = 0.5-2.5]) than prevalent (2.4 years [IQR = 0.7-7.5]) infections (Figure 3, A). Most (70%) incident infections cleared by 2 years, with 93% and 97% clearance by 5 and 7 years, which suggests that long-term persistence of incident infections is rare. In contrast, only 47% of prevalent infections cleared by 2 years, and clearance was 65% and 71% at 5 and 7 years, respectively.

Among oral HPV-16 infections detected, clearance was 42%, 51%, 68%, and 76% at 1, 2, 5, and 7 years, respectively (Figure 3, B). This represents 1-year oral HPV-16 persistence of 58% (95% confidence interval [CI] = 48% to 67%). Among those with a persistent infection at 1 year, 55% (95% CI = 40% to 68%) remained persistent at 5 years. Five-year oral HPV-16 persistence was 32%

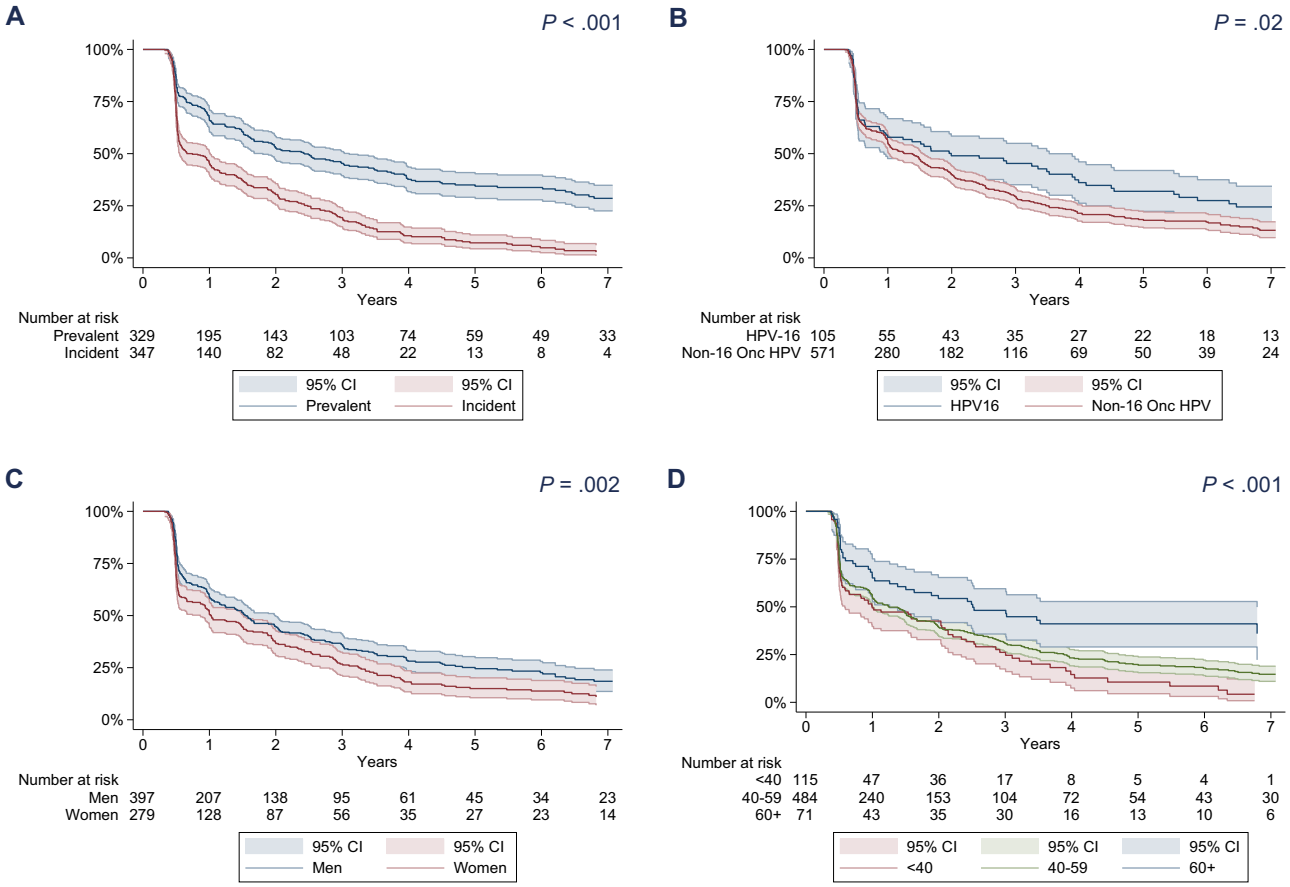

Figure 3. Time to clearance of any oncogenic human papillomavirus (HPV) among 676 infections in the 447 Multicenter AIDS Cohort Study/Women's Interagency HIV Study participants enrolled in the Persistent Oral Papillomavirus Study and/or Men and Women Understanding Throat HPV study by risk factor. By infection characteristics (prevalent vs incident: panel **A**); HPV-16 vs non-16 oncogenic HPV type (panel **B**); demographic characteristics (men vs women: panel **C**); and age (panel **D**). CI = confidence interval.

Table 2. Unadjusted and adjusted associations between selected characteristics and oncogenic HPV clearance, Multicenter AIDS Cohort Study and Women's Interagency HIV Study, 2009-2018

Characteristic	No. of visits	HR (95% CI)	aHR (95% CI)
Sex			
Female	942	1.00 (Referent)	1.00 (Referent)
Male	1558	0.63 (0.51 to 0.79)	0.74 (0.60 to 0.91)
PLWH			
HIV-negative	551	1.00 (Referent)	1.00 (Referent)
PLWH, current CD4 count ≥500	933	0.91 (0.70 to 1.12)	0.74 (0.58 to 0.95)
PLWH, current CD4 count <500	879	0.88 (0.62 to 1.25)	0.69 (0.53 to 0.92)
Oral HPV infection type			
Incident	928	1.00 (Referent)	1.00 (Referent)
Prevalent	1572	0.43 (0.35 to 0.53)	0.44 (0.35 to 0.55)
Age (by increasing decade—ref 20–29 year olds)	2363	0.81 (0.74 to 0.89)	
Age, y			
22-40	292	1.00 (Referent)	1.00 (Referent)
40-49	675	0.78 (0.58 to 1.05)	0.73 (0.54 to 0.99)
50-59	934	0.76 (0.57 to 1.02)	0.76 (0.57 to 1.01)
60-79	462	0.42 (0.30 to 0.61)	0.40 (0.27 to 0.59)
Oncogenic oral HPV type			
Non-16 HR HPV[a]	2051	1.00 (Referent)	1.00 (Referent)
HPV-16	449	1.48 (1.03 to 2.14)	1.19 (0.89 to 1.61)
History of tonsillectomy			
No	1717	1.00 (Referent)	—
Yes	603	0.78 (0.58 to 1.04)	—
Frequency of toothbrushing			
≥2 times per day	2083	1.00 (Referent)	—
<2 times per day	337	0.92 (0.65 to 1.29)	—
Current smoker			
No	1231	1.00 (Referent)	—
Yes	1130	1.05 (0.86 to 1.29)	—
Current alcohol use			
No	746	1.00 (Referent)	—
Yes	1589	1.08 (0.86 to 1.35)	—
Current marijuana use			
No	1620	1.00 (Referent)	—
Yes	702	1.00 (0.80 to 1.26)	—

[a]Non-16 oncogenic oral HPV types included all oncogenic types expect HPV-16: HPV-18/31/33/35/39/45/51/52/56/58/59/66. CI = confidence interval; HPV = human papillomavirus; HR = hazard ratio; aHR = adjusted hazard ratio; PLWH = person living with HIV.

(95% CI = 22% to 42%) overall, with 50% of prevalent infections and 16% of incident infections persisting at 5 years ($P < .001$).

Risk Factors for Oral HPV Clearance

Univariate factors associated with clearance of oncogenic oral HPV infection were explored (Figure 3 and Table 2). HPV-16 infections were statistically significantly less likely to clear than other (non-16) oncogenic HPV types (5-year persistence = 32% vs 18%, HR = 1.48, 95% CI = 1.03 to 2.14, $P = .02$, Figure 3B). Consistent with prior shorter natural history studies, infections were statistically significantly less likely to clear among men than women (5-year persistence = 25% vs 15%, HR = 0.63, 95% CI = 0.51 to 0.79, $P = .002$, Figure 3C), and among older than younger individuals (5-year persistence in ≥60 vs <40; 41% vs 11%, HR = 0.42, 95% CI = 0.30 to 0.61, $P < .001$, Figure 3D). Current tobacco, alcohol, marijuana use, HIV, history of tonsillectomy, and oral hygiene did not influence oral HPV clearance (P_{range} = .60-.72; Table 2).

Time-to-clearance curves were similar in magnitude when analyses were restricted to HPV-16 or prevalent infections; however, the effect of sex was not statistically different (Supplementary Figures 1–2, available online). When examining incident infections only, sex, age, and infection type (HPV-16 vs non-16) were not statistically significant predictors of clearance (Supplementary Figure 3, available online), perhaps because clearance was rapid for most incident infections, among both men and women and all age groups (each median < 12 months).

In multivariable analysis (Table 2), oncogenic oral HPV clearance was statistically significantly lower among prevalent than incident detected infections (adjusted hazard ratio [aHR] = 0.44, 95% CI = 0.35 to 0.55), among men than women (aHR = 0.74, 95% CI = 0.60 to 0.91), for older participants (aHR per 10 years increasing age = 0.81, 95% CI = 0.74 to 0.89), and among people living with HIV (aHR = 0.76, 95% CI = 0.60 to 0.95). However, type of oncogenic HPV infection (HPV-16 vs other oncogenic types) was no longer statistically significant.

Viral Load and Pattern of Oral HPV-16 Infection

Given the importance of HPV type 16 specifically, we next considered the signal strength (viral load) and pattern of HPV-16

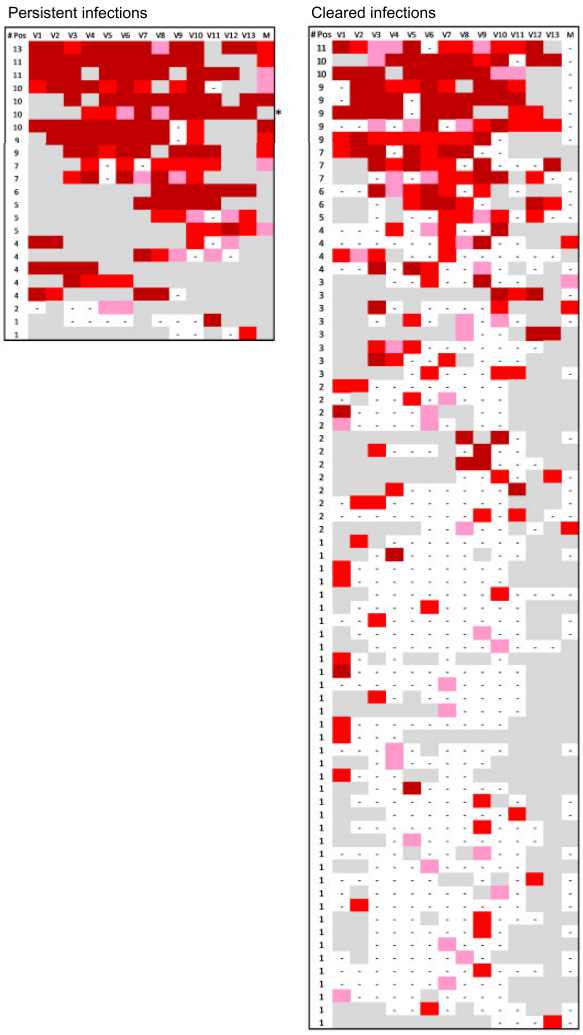

Figure 4. Oral HPV-16 viral load across study visits, among participants with persistent and cleared infections. Each row is a distinct subject in the study who had oral HPV-16 DNA detected. Semi-quantitative signal intensity depicted by color as follows: strong (**dark red**), medium (**red**), weak (**pink**), and negative (**white with -**). **Gray** represents visits with no oral rinse sample collection. The asterisk (*) denotes the subject diagnosed with HPV-OPC. The figure is restricted to participants with at least 4 oral rinse samples tested. Participants joined the study at variable times. Oral rinse results are shown by calendar visit, labeled "V1" for the first biannual visit. Gray represents visits where oral rinse sample was not collected or tested (this includes visits before substudy enrollment for those who entered later, as well as some missed visits, and visits after study completion). Men and Women Understanding Throat HPV baseline study visit labeled "M." HPV = human papillomavirus.

infections across visits (Figure 4). Among the 99 infections included in this analysis, nearly half of infections (n = 53, 54%) were detected at only 1 or 2 visits. There were 23 oral HPV-16 infections that did not clear during the study (ie, detected at the last study visit; Figure 4A), including 11 that were detected at ≥7 visits (representing at least 3.5 years of persistent infection). Oral HPV-16 clearance was 51%, 55%, 64%, 68%, 73%, 76% at 2, 3, 4, 5, 6, and 7 years, respectively. The longest oral HPV-16 infection observed was a prevalent infection that was persistently detected at each of 13 visits for more than 7.5 years (see row 1 in Figure 4).

Infections with a lower HPV-16 viral load were statistically significantly more likely to clear than those with higher viral load. Each 10-fold decrease in copy number was associated with a 3-fold increase in odds of clearance (HR = 3.3, 95% CI = 1.7 to 6.3). After adjusting for sex, age, and infection type, lower HPV-16 copy number remained a statistically significant predictor of clearance (aHR = 2.5, 95% CI = 1.2 to 5.2). Among 53 oral HPV-16 infections detected at high-intensity level at any point during the study, only 54.7% were observed to clear (Figure 4). Clearance was statistically significantly lower among the 53 participants with a high signal strength for HPV-16 than among the 52 participants with only medium to low strength results (5-year clearance = 39.4% vs 95.3%, $P < .001$).

Incident Case of HPV-OPC

Among those with oral HPV-16 persistence at 5 years, there was one histologically confirmed incident case of HPV-OPC (4.5%, 95% CI = 0.1% to 23%; 1 of 22 cases) detected as part of clinical care, not by this observational study (Figure 4). HPV tumor status was determined by p16 immunohistochemistry and oncogenic DNA in situ hybridization (PATHO-GENE HPV screening probe). This participant had oral HPV-16 consistently detected at each of the 10 study visits (Figure 4A—denoted by *) spanning over 4.5 years. The strength of oral HPV-16 detection during the first 2 years of detection was intermittently medium to low (4 times) but then had a persistently high intensity in the last 2 years (4 times) preceding the diagnosis of tonsillar cancer (American Joint Committee on Cancer 7th edition stage T2 N1 M0).

The participant was male, living with HIV, and a former smoker reporting 44 pack-years of tobacco use, daily alcohol use, at least 100 lifetime male oral sex partners, a nadir CD4+ T-lymphocyte count of 211 cells/mm^3, and CD4+ count at diagnosis of 1146 cells/mm^3.

Discussion

This is the longest natural history study of oncogenic oral HPV infection to date, with the largest number of oral HPV-16 infections, and the first temporal description of persistent oral HPV-16 infection followed until clinical presentation of HPV-OPC. It provides the first estimates of long-term oncogenic oral HPV persistence; 32% of oral HPV-16 infections were persistently detected for 5 or more years, and when persistent, were often found at high viral load. These data inform commonly asked questions about the implications of detecting oncogenic oral HPV infection and provide estimates necessary to design future potential screening trials.

Oncogenic oral HPV infection is the precursor to HPV-OPC, based on the cervical HPV-cancer paradigm, as well as two lines of evidence for oral HPV and OPC. First, in a nested case-control study with a single oral rinse sample tested, oral HPV-16 preceded the diagnosis of incident OPC by an average of 3.9 years (1). Second, multiple retrospective and prospective clinical studies of HPV-OPC patients suggest detection of oncogenic oral HPV infection is equivalent to microscopic evidence of malignancy (19–22). Indeed, 50% of HPV-OPC cases with oral oncogenic HPV infection detected after treatment go on to recur, suggesting oral HPV DNA heralds its recurrence. This is the first study to demonstrate longitudinal visits with consistent detection of oncogenic oral HPV infection, the surrogate of microscopic disease, over many years.

Not only was oncogenic oral HPV detected over multiple time points, but an increasing HPV-16 viral load was observed,

before progressing to HPV-OPC. Indeed, increasing viral load in this analysis was associated with reduced clearance of infection, consistent with the cervical cancer literature (23). Notably, one-third of those with oral HPV-16 infection remained persistent 5 years later. This suggests that there is a group of individuals with persistent infection who are at increased risk of HPV-OPC; if effective screening methods are developed, this could be a potential group on which to focus trials for prevention of HPV-OPC.

Cervical HPV persistence is known to be a necessary cause for malignancy and has emerged as an acceptable screening test for cervical cancer (24). In contrast, screening for HPV-OPC is not presently endorsed (25,26) because several important criteria have not yet been met (27), including 1) a screening test with sufficient sensitivity and specificity (28), 2) identified at-risk population (29), 3) screening leads to diagnosis at earlier stage, 4) screening reduces morbidity and mortality, 5) cost-effective, and 6) how to clinically evaluate biomarker-positive participants. This analysis identifies an at-risk population using one potential biomarker, but other critical barriers and limitations remain.

The biomarker used in this study—oncogenic oral HPV DNA—has moderate sensitivity but good specificity (14,28) for HPV-OPC, which would limit its utility (as a stand-alone test) for any screening program. Even among at-risk groups, oral HPV-16 prevalence is low, at no more than 4% (14,29). Although oral HPV persistence is clearly a prerequisite to development of HPV-OPC, our data suggest a large number is needed to screen to detect a single cancer (poor positive predictive value). Other biomarkers may be necessary to improve early identification of adults at increased risk of HPV-OPC (14). For example, the HPV-16 E6 antibody appears to be a promising biomarker and, although too rare in the general population to justify use, might be considered for more targeted screening (14,30,31)because of high sensitivity (14,29). Prior studies have shown that HPV-16 E6 antibodies are detectable up to 10 years before clinical diagnosis of OPC and that after seroconversion titers either remain stable or increase (32,33), thus potentially enabling a one-time assay to identify individuals at increased risk. Follow-up of these individuals might then rely on serial HPV testing in oral rinse and/or plasma.

Consistent with prior long-term cervical HPV and shorter oral HPV natural history studies, our study suggests that oral HPV persistence is increased among men, older individuals, PLWH, and prevalent compared with incident infections (23). Clearance for oral HPV in this study population was similar to that for cervical HPV among PLWH (2-year cumulative incidence of clearance of 59% for oral and approximately 60% for cervical; quick median time to clearance for oral [1.4 years] and cervical [0.8 years]) (34). This is the first study to show that higher viral load is a strong predictor of oral HPV persistence, which suggests it may serve as a marker of microscopic HPV-OPC (21,35–37). Differences in immune response by sex have been observed for other infections (38), supporting the possibility of immunologic differences to viral infection among men and women.

This study had several strengths and limitations. The study included a diverse population (sex and race or ethnicity), with a median age younger than that of HPV-OPC diagnosis. POPS (which collected most of the reported data) was not looking for cancers and did not perform clinical examinations. The study population was selected to be at increased risk of oral HPV and included PLWH in whom persistent oral HPV infection is expected to be higher than in the general population. Misclassification of our outcome (persistence) cannot be ruled out, as it could include clearance and reinfection with the same type-specific infection. Semiannual visits permit the evaluation of multiyear persistence but does not inform short-term dynamics within a 6-month window. The HPV testing in the POPS and MOUTH studies were performed in different labs, while using similar but not identical testing methods.

This study suggests that there is a subset of individuals with long-term persistent oncogenic oral HPV infection that can develop into HPV-OPC. For the first time we have estimates of oral HPV persistence over 7 years, based upon the largest number of oral oncogenic HPV infections to date. These data could be used to inform studies examining whether and how screening might be appropriate in select populations.

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