Oncogenic Oral Human Papillomavirus Clearance Patterns over 10 Years

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ABSTRACT

Background: Effective screening for oropharyngeal cancer is lacking. Four oncogenic HPV clearance definitions were explored to understand long-term natural history for persistent oncogenic oral HPV (oncHPV), the precursor of oropharyngeal cancer.

Methods: Prospective multicenter cohort of participants living with/at-risk for HIV, with oral rinse and gargle samples collected every 6 to 12 months for up to 10 years and tested for oncHPV. HPV clearance definitions included 1 (clear1), 2 (clear2), 3 (clear3) consecutive negatives, or being negative at last two visits (clearlast).

Results: Median time to clearance of oncHPV exceeded 2 years for conservative definitions (clear3: 2.38, clearlast: 2.43), but not lenient (clear1: 0.68, clear2: 1.15). By clear3, most incident infections cleared at 2, 5, 8 years (55.1%, 75.6%, 79.1%), contrary to prevalent infections (37.1%, 52.5%, 59.5%, respectively). In adjusted analysis, prevalent oncHPV, older age, male sex, and living with HIV were associated with reduced clearance. Of 1,833 subjects screened, 13.8% had prevalent oncHPV and 47.5% of those infections persisted ≥5 years, representing 6.5% of persons screened. Two men with prevalent oral HPV16 developed incident oropharyngeal cancer [IR = 1.62 per 100 person-years; 95% confidence interval (CI), 0.41–6.4]. Many with oral HPV16 persisted ≥5 years (and/or developed HPV-oropharyngeal cancer) among those with 2 (72.2%), ≥2 of first 3 (65.7%), or 3 (80.0%) consecutive positive oHPV16 tests, but not after 1 (39.4%).

Conclusions: In our 10-year study, most incident infections cleared quickly. However, half of prevalent oncHPV persisted ≥5 years, suggesting increased risk with persistent oncHPV at >2 visits.

Impact: We identified groups with persistent oncHPV at increased risk of oropharyngeal cancer and contextualized risk levels for those with oral HPV16 infection.

Introduction

Oncogenic oral HPV (oncHPV) infection precedes diagnoses of HPV-associated oropharyngeal cancer. A large nested case–control study detected oncHPV a median of 42 months prior to HPV-associated oropharyngeal cancer diagnosis (1). In addition, oncHPV has been detected among healthy adults who have steadily increasing HPV viral loads prior to HPV-associated oropharyngeal cancer development (2). Natural history studies suggest that most infections clear spontaneously within two years and few persist beyond 4 years (3). Long-term oncHPV natural history and progression to cancer remain unexplored. While there is no recognized HPV-associated oropharyngeal cancer precancer (4), persistent oncHPV is a potential surrogate. Therefore, screening for oncHPV may be a viable strategy for earlier detection of HPV-associated oropharyngeal cancer. Indeed, type-specific oncHPV persistence is a surrogate endpoint for oropharyngeal cancer risk in preventive vaccine trials (5), and in recurrent HPV-associated oropharyngeal cancer, persistent oncHPV is considered equivalent to subclinical microscopic (i.e., low-volume) disease (6, 7).

To understand risk factors for progression to HPV-associated oropharyngeal cancer, defining HPV clearance is key. Previously, six-month sampling compared with two-week intervals estimated short-term persistence well, although cumulative prevalence of oral HPV DNA is higher with more frequent interval sampling (8). Because one negative test can overestimate clearance, the anogenital HPV literature commonly defines HPV persistence as two or more positive tests at six-month intervals (9). Using 10 years of oncHPV natural history data, we examine the impact of differing definitions on inferences of oncHPV clearance.

Materials and Methods

Study design and population

This longitudinal observational cohort study collected oral rinse and gargle samples tested for oncHPV DNA for up to 10 years. Persistent Oral Papillomavirus Study (POPS; ref. 10) consisted of 1833 men and women living with or without HIV from the multi-institutional

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MACS WIHS Combined Cohort Study (MWCCS). Participants underwent semiannual visits for four years. Inclusion criteria allowed all interested participation at participating MWCCS study sites (MACS: Baltimore, Chicago, Pittsburgh, Los Angeles; WIHS: Brooklyn, Bronx, San Francisco, Chicago, Chapel Hill, Atlanta) to enroll if interested.

131 participants with persistent HPV DNA at the end of POPS were enrolled in the Men and Women Understanding Throat HPV (MOUTH; ref. 11) study, after a median of 2.5 years (IQR = 1.9–3.0) between studies, and had three additional years of annual visits Supplementary Fig. S1). The study population included participants with ≥1 oncogenic oral HPV type detected and ≥1 follow-up visit(s) thereafter (n = 692 infections among 435 of 1,833 participants in POPS). This study was approved by the institutional review boards of all sites, and informed written consent was obtained from all participants. The study was conducted in accordance with recognized ethical guidelines of the Declaration of Helsinki.

HPV testing

HPV detection was performed using PCR amplification in POPS (PGMY09/11 primer system; ref. 10) and MOUTH (SPF_{10}, Labo Biomedical Products) and genotyped using the Roche linear array (POPS) and SPF10 DEIA/LIPA system (MOUTH) for oncogenic HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, as previously described (3, 12). HPV16 copy number (HPV16CN) was measured by qPCR and standardized to CN/cell using RNase P for MOUTH and using ERV3 for POPS.

Blood samples were obtained from a subset of participants at POPS baseline (n = 133, 30.5% of 435 subjects in this analysis), and additionally from MOUTH baseline among MOUTH reenrolled persons (n = 120, 92% of the 131 in MOUTH extension), and tested for HPV antibodies. HPV antibody testing was performed centrally by the German Cancer Research Center (DKFZ) using multiplex serology method (13). Serum was tested for E1, E2 antibodies of HPV types 16 and 18, and E6 and E7 oncoprotein antibodies for oncogenic HPV types 16, 18, 31, 33, 35, 45, 52, 58, as previously described (12).

Statistical analysis

The primary analysis evaluated time to clearance of oncHPV DNA. All analyses were type-specific HPV infection, clustered by person to account for multiple infections within the same person. Type-specific oncHPV was classified as prevalent if detected at first study visit and incident if it was absent at first visit but detected later. oncHPV clearance was explored using four different definitions: "clear1", "clear2", and "clear3", meaning the occurrence of 1, 2, and 3 consecutive negative results after a positive result. The fourth definition, "clearlast" only considered any infection cleared if it was negative at the last two visits (i.e., not cleared if detected at either of last two visits despite intervening negatives). For all four definitions, time of clearance was considered at the first negative of consecutive negatives meeting that clearance definition.

Data were organized on the basis of the assumption of multiple unordered events of similar types per participant. Hence, a participant could have multiple oncHPV types and could clear them in any order. Upon clearing a type-specific HPV, participants remain in the risk set for clearance of other types, if any. Differences in time to clearance were explored using Kaplan–Meier curves and log-rank tests. Clear3 was used as the primary clearance definition for exploring risk factors for oncHPV clearance, using Cox proportional hazard models with robust SEs, clustered by participant. Risk factors explored included HIV characteristics (HIV serostatus, current CD4 count, HIV RNA viral load), demographics (age, sex), HPV characteristics (incident/-prevalent, HPV16/non-16 HPV type, HPV16 E6 seropositivity), and behaviors (current smoking, alcohol and marijuana use). Among HPV16 infections, HPV16CN was explored using qPCR results. Given that HPV16CN was always 0 at visit of clearance, its effect was considered at first detection carried forward to explore utility as a predictor of clearance in an HPV-associated oropharyngeal cancer screening context.

A subanalysis of subjects who developed an incident oropharyngeal cancer during follow-up was performed to examine timing of cancer diagnosis related to oncHPV. Oropharyngeal cancer incidence rates with 95% confidence intervals (95% CI) were estimated. For groups with 0 events, Hanley's rule of 3 was used to estimate the upper confidence interval limit (14). Among participants persistent by clear3, we examined oncHPV detection patterns to visualize differences in those considered persistent by clear3 but not by other definitions. All analyses were conducted using STATA16.1 and R4.2.2.

Data availability

The data generated in this study are available upon request from the corresponding author.

Results

This analysis included 435 people with 692 type-specific oncHPVs. At the visit of first detection, participants had a median age of 49.6 years (IQR = 43.3–55.5), and the majority were male (58.4%), living with HIV (73.8%), current/ever smokers (45.6%/81.3%) and current drinkers (69.5%; **Table 1**). Among those living with HIV, median current CD4 cell count was 528 cells/μL (IQR = 326–723), 61.6% had undetectable current HIV viral load (<50 copies per mL), and 81.6% were on ART. 58.2% of subjects with oncHPV had ≥1 prevalent HPV type, representing 13.8% of subjects tested (**Table 1**). About half of the infections detected were incident (52.5%). At time of this analysis, two participants had been diagnosed with an HPV-associated oropharyngeal cancer (**Fig. 1**).

Median follow-up for participants was 4.4 years (IQR, 2.7–7.1; range, 0.4–11.5) from study entry to censor, and 8.8 years (IQR, 7.7–9.7; range, 3.9–11.5) among the subset reenrolled in MOUTH. Reasons for censoring among the 435 enrolled included: study completion (n = 362, 83%), loss to follow-up (n = 68, 16%; COVID-related missed visits and closures of study sites. Characteristics of those lost to follow-up were similar to those completing the study. There were five deaths, all were HIV-related.

Median time to clearance varied dramatically by clearance definition. When using more stringent definitions requiring three repeated negative oral rinses (clear3) or absence of oncHPV at last samplings (clearlast), median clearance was over two years: (clear3: 2.38 years, IQR = 0.52, undetermined; clearlast: 2.43 years, IQR = 0.52–7.78). In contrast, requiring two consecutive negatives (clear2), had median clearance of 1.15 years (IQR = 0.50–4.04). Requiring only one negative oral rinse (clear1) reduced median time to clearance to less than one year (0.68 years; IQR, 0.49–1.98). Median times to clearance were similar by clear3 and clearlast definitions, but both were significantly longer than those by clear2 or clear1 (**Fig. 2**). Among all visits from first detection of type-specific oncHPV to clearance (by clear3) or censoring, 38.4% of tests were intermittently negative for that type, explaining the higher clearance by clear1 and clear2 than clear3, (**Fig. 3**). Among persistent infections (i.e., never observed to clear during study), 28.6%

Table 1. Characteristics of 692 oncogenic oral HPV at visit of first detection (among 435 participants included in this analysis).

Characteristics	Number of people		Number of type-specific infections			
	N	%	*N*	% (of 692 observed)	% (of 5,655 tested)	% among 1,833 screened
Sex						
Female	181	41.6	289	41.8	5.1	—
Male	254	58.4	403	58.2	7.1	—
Age in years						
22–45	138	31.7	215	31.1	3.8	—
46–55	179	41.2	281	40.6	5.0	—
>55	118	27.1	196	28.3	3.5	—
HIV						
HIV negative	114	26.2	163	23.5	2.8	—
Person Living with HIV (PLWH)	321	73.8	529	76.5	9.4	—
HIV and current CD4 cell count						
HIV negative	114	26.3	163	23.6	2.8	—
PLWH, CD4>500	171	39.4	266	38.5	4.7	—
PLWH, CD4 250–500	90	20.7	144	20.8	2.5	—
PLWH, CD4<250	59	13.6	118	17.1	2.1	—
Current antiretroviral therapy (among PLWH)						
No	59	18.4	92	17.4	1.6	—
Yes	261	81.6	436	82.6	7.7	—
HIV viral load (among PLWH)						
Undetectable	197	61.6	305	57.9	5.4	—
50–9,999 copies/mL	70	21.8	122	23.1	2.2	—
10,000+ copies/mL	53	16.6	100	19.0	1.8	—
Current smoker						
No	236	54.4	356	51.5	6.3	—
Yes	198	45.6	335	48.5	5.9	—
Current pack/day (among ever smokers)						
None	170	48.3	254	45.1	4.5	—
Packsday	98	27.8	180	32.0	3.2	—
0.5–<1 pack/day	54	15.3	80	14.2	1.4	—
1+ packs/day	30	8.5	49	8.7	0.9	—
Current drinker						
No	131	30.5	214	31.2	3.8	—
Yes	298	69.5	471	68.8	8.3	—
Oncogenic oral HPV						
Incident	182	41.8	363	52.5	6.4	9.9
Prevalent	253	58.2	329	47.5	5.8	13.8
Oncogenic oral HPV type						
Ever HPV16	107	24.6	107	15.5	1.9	5.8
Ever non-HPV16 oncogenic types	328	75.4	585	84.5	10.3	17.9
HPV vaccination status (self-reported)						
Never vaccinated	360	85.7	—	—		—
Ever vaccinated	17	4.1	—	—		—
Unsure	43	10.2	—	—		—
Diagnosed with oropharyngeal cancer anytime after initial oncogenic oral HPV infection detection	2	0.4	—	—		0.1

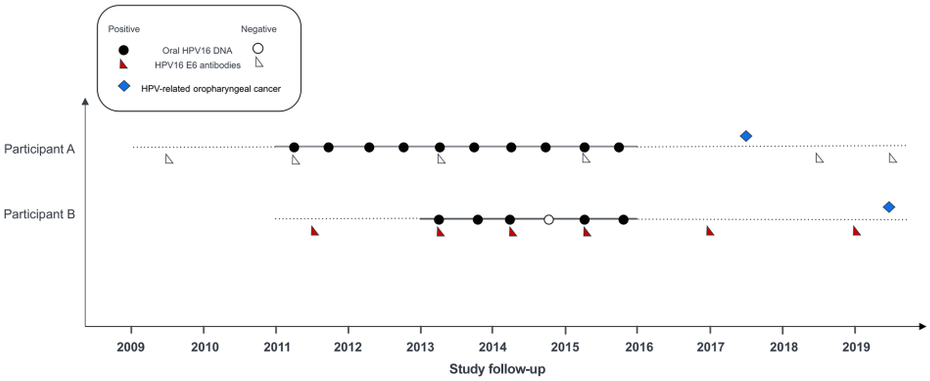
of tests from first visit detected to censoring were HPV DNA negative.

Intermittent HPV16 detection ("flickering") was significantly more common among those with low oral HPV16CN (55% of those with ≤0.0001 copies/cell, 26% of those with 0.0001–<0.001, 16% of those with 0.001–<0.1) than those with high oral HPV16CN (3.2% of those with ≥0.1 copies/cell). Higher HPV16CN was associated with significantly lower odds of "flickering" (i.e., $P_{trend} < 0.0001$). Odds of flickering were significantly reduced in those with with ≥0.1 copies/cell (OR, 0.05; 95% CI, 0.01–0.27) and 0.0001–<0.1 copies/cell (OR, 0.25; 95% CI, 0.11–0.57) compared with those with <0.0001 copies/cell.

Most oncHPVs cleared during follow-up, even when using clear3 as the definition. Clearance of incident oncHPV was: 55.1%, 75.6%, and 79.1% at 2, 5, and 8 years, respectively. Clearance of prevalent oncHPV was lower with 37.1%, 52.5%, and 59.5%, respectively at 2, 5, and 8 years (**Fig. 2**). Thus, by 8 years, most (79.1%) incident infections had cleared, while only 59.5% of prevalent infections had cleared ($P < 0.001$). The group without clearance of oncHPV had variable patterns of HPV DNA detected, some consistently detected and others fluctuating (intermittently detected; **Fig. 3**).

Among the 1,833 participants, 13.8% had prevalent oncHPV (Supplementary Table S1) and 47.5% of those did not clear by clear3 at

Figure 1.
Natural history of oral HPV16 (shaded circle indicates positive and unshaded circle indicates negative) in semi-annual testing and HPV16 E6 serology results (red triangle indicates positive and unshaded triangle indicates negative) among participants later diagnosed with HPV-related oropharyngeal cancer (blue diamond).



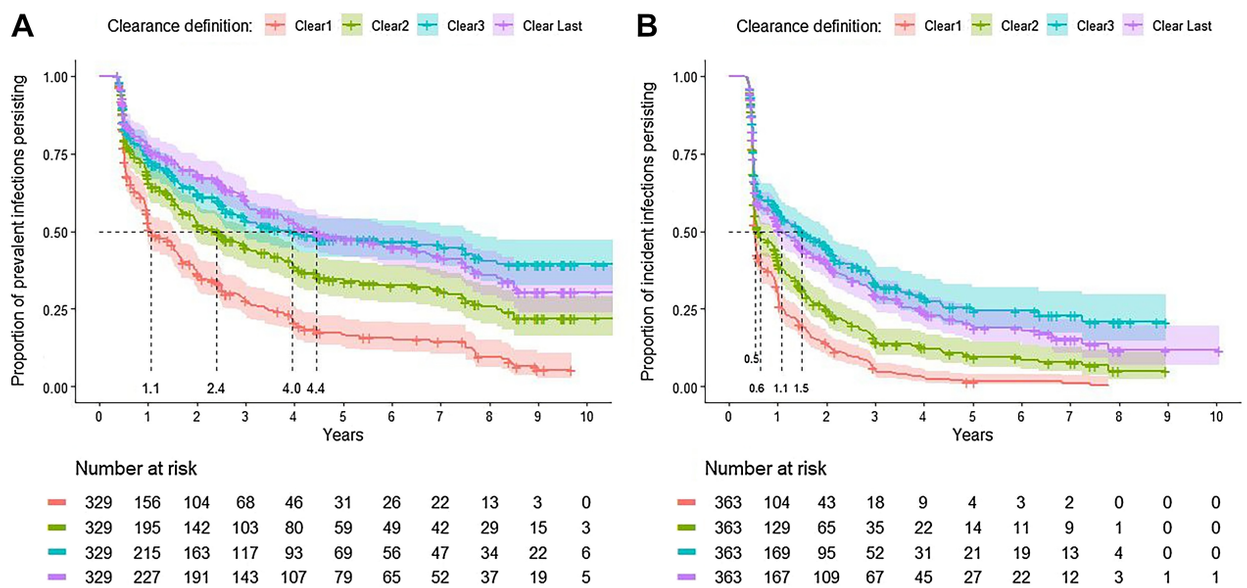


Figure 2.
Clearance of prevalent (**A**) and incident (**B**) oncogenic oral HPV infections over 10 years.

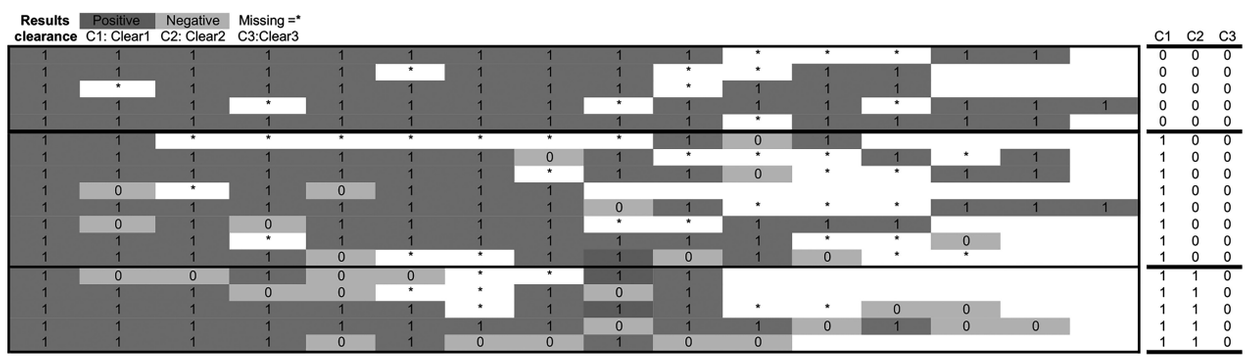


Figure 3.
Examples of the pattern of oncogenic oral HPV DNA among subjects who did not clear their infections (by clear3). Included here are participants with oral HPV16 detected, who had at least 5 years of follow-up, stratified by whether clear1 and clear2 also considered these infections not to have cleared, or whether these alternative definitions considered the infections to have cleared. Top, infections considered persistent (i.e., not to have cleared) by all three definitions (clear1, clear2 and clear3). Middle, infections considered persistent by clear2 and clear3 but cleared by clear 1. Bottom, infections considered persistent by clear3 but cleared by clear2 or clear 1. Legend: 1, HPV16 positive (dark gray); 0, HPV16 negative (light gray); *, missed visit; no test result (white).

5 years, representing 6.5% of those screened. Oral HPV16 (oHPV16) prevalence was lower with 56 prevalent oHPV16 detected (3.1%), 53.6% of which persisted 5 years (Supplementary Fig. S2), representing 1.6% of subjects screened. Among 35 male subjects with prevalent oHPV16 (123.4 years of follow-up), two developed HPV-associated oropharyngeal cancer. Risk of oHPV16 persisting ≥5 years and/or developed HPV-associated oropharyngeal cancer (5-year-risk) was high among those with 2 (72.2%), ≥2 of first 3 (65.7%), 3 (80.0%), or ≥3 of first 4 (75.8%) consecutive positive oHPV16 tests, compared with only 39.4% after 1 oHPV16-positive test. Among subjects with oHPV16persisting 1 year, 5-year risk was >50% by clear2 (57.2%) and clear3 (68.2%), but not clear1 (25.4%). Among subjects with oHPV16 persisting 2 years, 5-year risk was >70% (clear2 = 72.1%, clear3 = 75.4%). Allowing intervening negatives expanded the proportion defined as oHPV16 persisting ≥5 years from 11.1% (clear1) to 31.3% (clear2) and 39.4% (clear3). Among men with prevalent oHPV16, the incidence rate (IR) of HPV-associated oropharyngeal cancer was 1.62 per 100 person-years (95%CI, 0.41–6.47) overall, and was higher in 26 men living with HIV than 9 men without HIV (2.28 per 100 person-years; 95% CI, 0.57–9.11 vs. 0). IR was also 0 among 21 women with prevalent oHPV16 and among 19 men and 32 women with incident oHPV16.

Both subjects who developed HPV-associated oropharyngeal cancer had oHPV16 detected at first visit and persisted at follow-up visits (**Fig. 1**). At study entry, both were men living with HIV, former smokers, and had a history of performing oral sex on ≥20 lifetime partners. Subject A was HPV16 E6- and E7-seronegative (L1 and E4 positive). At first HPV16 detection, oHPV16CN was detectable by RT-PCR at a low level (0.00038 copies per cell). Six years after first oHPV16 detection, while in his 60s, on ART, with CD4 count >500 cells/μL, he was diagnosed with HPV-associated oropharyngeal cancer. At first visit, subject B was HPV16 E1, E2, E6, E7, and L1 seropositive, oHPV16 was detected with low HPV16CN/cell (<RT-PCR limit). Six years later when diagnosed with HPV-associated oropharyngeal cancer, he was in his 40s, on ART, with a CD4 count of 189 cells/μL.

Older age, male sex, prevalent oncHPV, and higher oHPV16CN were each associated with reduced oncHPV clearance in unadjusted analysis (**Table 2**; Supplementary Figs. S3–S7). Risk factors were similar when explored by all four clearance definitions, except that HPV16 was less likely to clear than other oncogenic types by clear1 and clear2, but not clear3 (Supplementary Table S2). In adjusted analysis, prevalent oncHPV [adjusted HR (aHR) = 0.58; 95% CI, 0.46–0.73], older age (aHR = 0.82; 95% CI, 0.74–0.91), living with HIV (aHR, 0.77; 95% CI, 0.60–0.99), male sex (aHR, 0.83; 95% CI, 0.66–1.03), and increasing oHPV16CN (per category of CN: aHR = 0.63; 95% CI, 0.46–0.86; $P_{trend} = 0.004$) remained significant predictors of reduced oncHPV clearance. Smoking, drinking, current, and nadir CD4 cell count were not associated with oncHPV clearance (**Table 2**; Supplementary Figs. S6 and S7).

Discussion

With 692 oncHPV infections among 435 individuals followed for up to ten years, this analysis is the longest and largest existing natural history study of oncHPV, to our knowledge. The majority of oncHPV cleared within two years when using less stringent definition of clearance. Notably, we identified a subset of persons with long-term persistent oncHPV DNA detected for ≥8 years, who may be at increased risk of HPV-associated oropharyngeal cancer. This included almost half of those with prevalent oncHPV and oHPV16, representing 6.5% and 1.6% of participants eligible for this study, respectively. When oHPV16 is detected at two consecutive visits, approximately 72% persist, therefore representing an at-risk group for HPV-associated oropharyngeal cancer. These findings inform future screening strategies of visit intervals, dynamics of infections over time, and factors affecting clearance.

Our findings imply that in a large-scale HPV-associated oropharyngeal cancer screening scenario, discernment between one-time versus continued detection of oncHPV infection is crucial. The definitions explored demonstrate that more stringent definitions are less likely to be impacted by intermittently negative tests and may more accurately capture true risk. When considering populations to screen, age, sex, and HIV serostatus may enrich for individuals at increased risk for development of HPV-associated oropharyngeal cancer (15, 16).

Our analysis underscores the importance of detection of prevalent infection (at first visit). While most incident oncHPVs cleared quickly, clearance was significantly reduced for prevalent oncHPV, especially by conservative definitions. This suggests adults with prevalent oncHPV represent a population at increased risk for long-term persistence and HPV-associated oropharyngeal cancer particularly for those with persistent oHPV16, which is responsible for most HPV-associated oropharyngeal cancer in the United States (17). Overall, 63.1% of oncHPV infections cleared within 5 years, suggesting low specificity for HPV-associated oropharyngeal cancer (many false positives) and a low positive predictive rate for oncHPV persistence among persons with a single detection of oncHPV DNA. It may be feasible to identify individuals at greatest risk for HPV-associated oropharyngeal cancer by screening for oHPV16 and following only those individuals with prevalent oHPV16. Not all cases would be identified as there is a broad range of oral rinse sensitivity (18, 19) using PCR-based method and, among persistent infections, 28.6% of visits involved a negative HPV test, suggesting some false-negative testing. However, screening for prevalent infections among older adult men (given the role of sex and age) would capture most individuals at risk and potentially have a low false-negative rate. In a screening program, oncHPV testing could be done for oHPV16 DNA alone due to its higher specificity and positive predictive value compared with non-16 oncHPV (17). Recent research suggests the 10-year risk of oropharyngeal cancer following HPV16 E6 seropositivity is high in some groups (e.g., 17% among 50-year-old men; ref. 20); therefore, combination of oHPV16 and E6 seropositivity may be especially predictive of oropharyngeal cancer. Oral rinse and gargle assess the mucosal compartment (i.e., represents the oral and oropharyngeal mucosa), while plasma HPV DNA detection may reflect locoregional or systemic sites. Next-generation sequencing, droplet digital PCR (ddPCR), and/or methylation markers (21) may offer improved performance characteristics. Acknowledging limitations of current testing and sampling paradigms, it is clear that persistent oHPV16 is a precursor to HPV-associated oropharyngeal cancer.

The large sample size of this study permitted the calculation of an incidence rate among individuals with HPV-associated oropharyngeal cancer precursor (persistent infection). While previous work used different population-based studies to estimate risk (22), this is the first study to provide an estimate of HPV-associated oropharyngeal cancer incidence among persons with oHPV16 DNA. Because this estimate is based upon two cases of HPV-OPC and 107 subjects with oHPV16 detected, it warrants caution and further validation. Nonetheless, this represents an important

Table 2. Unadjusted and adjusted predictors of oncogenic oral HPV clearance (as defined by clear3).

Characteristics	# visits	HR (95% CI)	*P*	aHR (95% CI)	*P*
Gender					
Female	898	1		1	
Male	1,441	**0.77 (0.62–0.96)**	**0.02**	0.83 (0.66–1.03)	0.097
Age (per 10-year increase)	2,339	**0.81 (0.73–0.90)**	**<0.0001**	**0.82 (0.74–0.91)**	**<0.0001**
Age categories, in years					
22–39	213	1			
40–49	527	1.13 (0.81–1.57)	0.46		
50–59	911	0.79 (0.57–1.09)	0.15		
≥60	688	**0.53 (0.36–0.79)**	**0.002**		
HIV					
HIV negative	516	1		1	
PLWH	1823	0.83 (0.64–1.07)	0.15	**0.77 (0.60–0.99)**	**0.046**
HIV and current CD4 cell count (cells/μL)					
HIV negative	516	1			
PLWH, CD4>500	995	0.84 (0.63–1.10)	0.21		
PLWH, CD4 250–500	482	0.85 (0.60–1.18)	0.34		
PLWH, CD4<250	315	0.83 (0.59–1.16)	0.28		
HIV and Nadir CD4 cell count (cells/μL)					
HIV negative	516	1			
PLWH, nadir CD4>500	221	0.74 (0.46–1.17)	0.21		
PLWH, nadir CD4250–500	829	0.79 (0.59–1.05)	0.11		
PLWH, nadir CD4<250	743	0.92 (0.69–1.23)	0.59		
HIV Viral load (copies/mL)					
Undetectable	1216	1			
50–10,000 copies	360	0.90 (0.66–1.22)	0.50		
>10,000 copies	213	1.01 (0.71–1.43)	0.96		
Current smoker					
No	1226	1			
Yes	1072	1.11 (0.89–1.36)	0.34		
Current drinker					
No	822	1			
Yes	1453	1.16 (0.93–1.46)	0.17		
HPV16 E6 serum antibodies[a]					
Seronegative	1389	1			
Seropositive	49	1.66 (0.83–3.32)	0.15		
Oncogenic oral HPV					
Incident	819	1		1	
Prevalent	1520	**0.55 (0.44–0.69)**	**<0.0001**	**0.58 (0.46–0.73)**	**<0.0001**
Oncogenic oral HPV type					
Non-16 oncogenic HPV type	1913	1		1	
Type 16	426	1.01 (0.76–1.33)	0.94	1.00 (0.75–1.34)	0.97
Among those with oral HPV 16 DNA detected only[a]					
HPV16 copy number/cell (qPCR) at first detection (carry forward)					
<0.0001	96	1			
0.0001–<0.001	113	0.57 (0.31–1.04)	0.07		
0.001–<0.1	137	**0.25 (0.12–0.52)**	**<0.0001**		
≥0.1 copy/cell	62	**0.17 (0.06–0.48)**	**0.001**		
Change per qPCR category increase: B and 95% CI	408	**0.53 (0.40–0.69)**	**<0.0001**	**0.63 (0.46–0.86)**	**0.004**

[a]Characteristics in this were run among those with HPV16 DNA only. For the multivariate result, HPV16CN was adjusted for gender, age, HIV status, and infection type. Other multivariate results in the top of table were not adjusted for HPV16CN because the primary model in this table includes all oncogenic oral HPV infections, not just HPV16. Bold text indicates statistical significance.

step toward understanding level of risk and natural history among those with oHPV16.

Previous studies of oncHPV natural history involved short follow-up (≤3 years), and fewer infections (<200 oncHPV infections studied for clearance; refs. 23–29). For example, a study of healthy young adults showed 24% of infections persisted one year (27). Longer-term persistence in our study may be explained by the increased biologic risk due to older age and HIV-related immunosuppression. A shorter study of 114 prevalent oncHPV infections among a similar population (adult men who have sex with men, 40% of whom were living with HIV) used a clear2 definition and reported a similar estimate of 1-year clearance (53%; ref. 29).

oncHPV clearance estimates were similar to previous anogenital HPV studies in comparable populations which reported that most infections clear within 4 years (30–33), with faster median clearance <2 years in some (31, 34). Slower clearance of oral compared to genital HPV might explain later onset (after HPV infection) of HPV-associated oropharyngeal cancer compared with anogenital cancers.

Similar to our findings, anogenital HPV clearance is lower in people living with HIV and older individuals (33–36). While smoking is associated with HPV persistence in several studies, no association was observed here. Higher HPV viral load was less likely to clear, similar to several anogenital HPV studies (37–39), implying that higher or increasing viral load over time may be an important marker for HPV-associated oropharyngeal cancer risk.

Our analysis has several limitations. oncHPV detection was PCR-based which has moderate sensitivity (18). Newer more costly technologies including genetic sequencing or ddPCR (21), may ultimately improve performance characteristics. Our study population included many people living with HIV and was not representative of the general population but was chosen to enrich for HPV infections and to allow sufficient power for studying long-term persistence of oncHPV and oropharyngeal cancer risk, rare events in the general population despite increasing overall oropharyngeal cancer incidence.

The opportunity to study oncHPV natural history as represented in this report is unique because HPV vaccination efforts should prevent oncHPV infection in younger age cohorts and thereby preclude such study in the future. However, our findings support the assertion that persistent oncHPV may represent a condition justifying surveillance, where no oropharyngeal precancer has yet been identified. These data underscore that prevalent infections have lower clearance and are of potential concern, especially infections that persist longer than two years that may represent a group who may benefit from screening if such a surveillance strategy is developed.

This analysis used a stringent definition of clearance (clear3) to better reflect the dynamic nature of oncHPV DNA detection. Many type-specific infections are intermittently undetectable despite an overall pattern of positivity. Whether an intermittent detection of HPV ("flickering") represents variation in immunologic control, sampling limitations or intermittent fluctuations at the limits of detection is uncertain. In addition, a false-negative HPV at first visit which was flickering could have misclassified an infection as incident, so some of the persistent incident infections may have been these intermittently detected infections, suggesting clearance estimates for truly incident infections may be underestimated. Using an overly lenient definition of clearance could therefore underestimate risk. It is possible that intermittent HPV presence within an individual may represent clearance and new acquisition of the same HPV type; however, data from this and previous studies suggest new infection of the same type is less likely given the "flickering" patterns were apparent even among subjects who reported no new sexual partners. Older clearance definitions used less sensitive PCR methods, but with assay sensitivity advancement, flickering results may be more common. Hence, clearance definitions may need revisions to account for that. In sum, this study, the longest and largest existing natural history study of oncHPV, revealed important risk factors for oropharyngeal cancer development and estimated oropharyngeal cancer incidence rate among those at risk. This highlights the importance of persistent oHPV16 infection and the possible role for oHPV16 for future targeted screening programs.

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Disclaimer

The contents of this publication are solely the responsibility of the authors and do not represent the official views of the NIH. The authors assume full responsibility for analyses and interpretations of these data.

Authors' Contributions

G. D'Souza: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, writing–original draft, project administration, writing–review and editing. **S.R. Tewari:** Data curation, formal analysis, investigation, visualization, methodology, writing–original draft, writing–review and editing. **T. Troy:** Resources, data curation, methodology, project administration, writing–review and editing. **J. Webster-Cyriaque:** Writing–review and editing. **D.J. Wiley:** Writing–review and editing. **C.D. Lahiri:** Writing–review and editing. **F.J. Palella:** Writing–review and editing. **M.L. Gillison:** Writing–review and editing. **H.D. Strickler:** Writing–review and editing. **L. Struijk:** Writing–review and editing. **T. Waterboer:** Writing–review and editing. **K. Ho:** Writing–review and editing. **J. Kwait:** Writing–review and editing. **J. Lazar:** Writing–review and editing. **K.M. Weber:** Writing-review and editing. **C. Fakhry:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, writing–original draft, project administration, writing–review and editing.

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Note

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