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Squamous cell carcinoma of the oral cavity often overexpresses p16 but is rarely driven by human papillomavirus

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Abstract

Objective—Human papillomavirus (HPV) is a causal and prognostic factor for oropharyngeal cancer, but its role in squamous cell carcinoma of the oral cavity (SCCOC) is unclear. We sought to clarify HPV's role in SCCOC.

Materials and Methods—Patients with newly diagnosed SCCOC (N=460) were prospectively recruited, treated, and followed at one institution. p16/HPV status was determined by p16 immunohistochemistry (IHC) (N=210), PCR-based HPV 16/18 E6/7 DNA testing (N=403), and/or HPV in situ hybridization (ISH) (N=178). Kaplan-Meier curves and log-rank tests were used to compare survival by p16/HPV status.

Results—p16 expression was detected in 30% of tumors (62/210) and HPV 16/18 E6/7 DNA in 28% (114/403), although correlation between these two assays was poor ($r=-0.01$). Patients with p16-positive tumors were more likely to be younger and have primary tumors of the oral tongue. Only 4% of tumors (7/171) were positive for HPV by ISH. Comparisons of patients with p16-positive and p16-negative tumors, patients with HPV-positive and HPV-negative tumors by PCR, and patients with HPV-positive and HPV-negative tumors by ISH showed no significant differences in disease-specific or disease-free survival by p16/HPV status. When we applied a

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more stringent definition of HPV positivity based on a combination of assay results, only 10 of 166 tumors were HPV positive, and there were no significant differences in demographic, exposure, clinical, or survival characteristics between these patients and the 156 HPV-negative patients.

Conclusions—Very few patients with SCCOC have HPV-driven tumors. SCCOC that overexpresses p16 may be a unique subset deserving of further study.

Keywords

p16 protein; human papillomavirus; oral cavity cancer; oral tongue cancer

Introduction

Human papillomavirus (HPV) type 16 (HPV 16) plays an etiologic role in the majority of cases of squamous cell carcinoma of the oropharynx (SCCOP), particularly those occurring in middle-aged patients and patients with no smoking history [1-6]. Chaturvedi et al, in an analysis of US incidence trends for oral cavity and oropharynx cancers stratified by age, demonstrated that the incidence of squamous cell carcinoma of the oral cavity (SCCOC) decreased overall but increased significantly among adults younger than 40 years, consistent with an increasing incidence of SCCOP across age groups [6]. Shiboski et al analyzed data from the Surveillance, Epidemiology, and End Results database and found that the incidence of oral tongue cancer among whites under 45 years of age increased between 1973 and 2001 [7]. In an earlier study, Schantz and Yu noted a 12% increase in oral tongue cancer in young adult non-Hispanic whites [8]. Additionally, Most broad epidemiologic studies have shown that the oral cavity is the second most common head and neck mucosal subsite for SCC among younger patients without a history of tobacco use [9-13]. A recent analysis of SCCOC specimens from 3 Radiation Therapy Oncology Group cooperative group trials found that 21 (26%) of 80 oral cavity cancers were p16 positive and 13 (15%) of 89 oral cavity cancers were HPV positive by in situ hybridization [14]. These observations lead to the hypothesis that HPV may also play a role in the etiology of SCCOC, but an etiologic link between HPV 16 and SCCOC has not been established.

In a previous meta-analysis, HPV 16 infection was associated with increased risk for “oral” cancer in the pooled population (odds ratio [OR] = 2.0, 95% confidence interval [CI]: 1.2-3.4), but there was significant between-study heterogeneity with respect to the magnitude of the association and considerable between-study variation in HPV 16 prevalence (0%-48%) [15]. A later meta-analysis by Syrjanen et al found a significant association between HPV 16 and “oral” cancer, to a stronger extent for the presence of oral HPV 16 DNA (OR = 3.9, 95% CI: 2.2-6.9), with between-study heterogeneity [16]. A potential explanation for the between-study heterogeneity is misclassification of oropharyngeal cancers as “oral” resulting from the common practice of describing cancers of the base of tongue or soft palate (oropharyngeal sites) more generally as cancers of the “tongue” or “palate” respectively [17]. Use of different HPV detection methods presents another potential classification bias. In the meta-analysis by Syrjanen et al [16], the pooled HPV prevalence among patients with oral cancer, in most cases determined by polymerase chain reaction (PCR) amplification of HPV 16 DNA, was 34%, which is much higher than

the 6% rate of HPV positivity (6%) in among patients with SCCOC determined on the basis of HPV E6/7 expression level [18]. PCR-based detection of HPV DNA, when used alone, has poor specificity for causal integrated HPV. Measurement of HPV E6/7 expression, on the other hand, is generally regarded as the gold standard for detection of causal HPV, but clinical application of HPV E6/7 testing is challenging because of technical limitations [19]. In the clinic, p16 immunohistochemistry (IHC) has emerged as a practical surrogate marker of HPV status because of its high sensitivity and specificity in the detection of HPV in SCCOP [19]. However, in non-oropharyngeal head and neck SCCs, in which the HPV positivity rate is much lower, p16 IHC alone as a surrogate marker for HPV status suffers from low specificity [14, 18].

In this study, through analysis of a large cohort of SCCOCs from a single tertiary cancer center, we sought to determine whether HPV plays an important role in SCCOC. We applied p16 IHC, PCR-based detection of HPV 16/18 genotypes (“HPV PCR”), and HPV ISH, and we used these methods in combination in an effort to optimize the overall reliability of HPV detection. HPV-positive and HPV-negative SCCOCs were compared to evaluate whether they differed in clinical presentation and outcomes.

Materials and Methods

Patients with histopathologically confirmed SCCOC were recruited at our institution as part of a prospective molecular epidemiologic study of incident squamous cell carcinoma of the head and neck. All cases of SCCOC were confirmed as cancers of the oral cavity and not oropharynx by a single head and neck surgeon on staff as well as during a multidisciplinary treatment planning conference. Subjects were U.S. residents and signed institutional review board–approved informed consent forms; subjects were recruited without regard to age, sex, ethnicity, or cancer stage, except that patients with known distant metastases were excluded. Patients with any prior cancer except nonmelanoma skin cancer were excluded.

Demographic and exposure data were obtained using a prospective self-administered epidemiologic questionnaire at enrollment. Clinical data were obtained from review of the medical record. “Ever smokers” were defined as those who had smoked at least 100 cigarettes in their lifetime. “Ever drinkers” were defined as those who had drunk at least one alcoholic beverage per week for at least one year during their lifetime.

After histopathologic verification of SCCOC, paraffin-embedded tumor specimens were assessed for the presence of HPV 16 and HPV 18 DNA. In brief, DNA was extracted using a tissue DNA extraction kit (Qiagen Inc., Valencia, CA) and tested for the presence of HPV 16 and HPV 18 DNA using two separate PCR assays with primers specific for the E6 and E7 regions and positive and negative controls, as described previously [20]. Samples were run in triplicate along with positive and negative controls and β -actin as a quality control. Paraffin-embedded tumor specimens were also assessed for p16 by IHC and for HPV by ISH, using methods described previously [21, 22]. p16 IHC was performed with a Bond Max automated immunostainer (Vision BioSystems, San Francisco, CA), and HPV ISH (genotypes 16, 18, 31, 33, 45, 51, 52, 56, 58, and 66) was performed with Ventana Inform HPV III probes (Ventana Medical Systems, Tucson, AZ).

We tested for group differences in demographic, exposure, and clinical characteristics using Mantel-Haenszel chi-square or Fisher exact test as appropriate for categorical variables and Student t-test for continuous variables. A phi coefficient was calculated to determine the correlation between different HPV detection methods. For analysis, age at presentation was categorized as young (< 45 years), typical age (45-69 years), or elderly (≥ 70 years). For the survival analysis, disease-specific survival (DSS) was defined as time from first appointment at the cancer center to death from the disease or last follow-up, and disease-free survival (DFS) was defined as time from first appointment at the cancer center to detection of recurrence or death from any cause. Kaplan-Meier curves and log-rank tests were used to detect statistically significant differences in survival between specified groups. Statistical significance was preset at $p < 0.05$, and all tests were two-sided. All statistical analyses were carried out using Stata 12.0 (StataCorp, College Station, TX).

Results

Nine hundred eighteen patients with incident (newly diagnosed, previously untreated) SCCOC were prospectively enrolled in the parent molecular epidemiologic study. Of these, 460 patients had at least one HPV test. Supplemental Table 1 summarizes demographic, exposure, and clinical characteristics of patients with SCCOC with and without HPV testing. Patients with HPV testing were more likely to be younger and have primary tumors of the oral tongue. Otherwise, there were no significant differences in the distribution of characteristics between patients with and without HPV testing.

Results of p16/HPV testing and demographic, exposure, and clinical characteristics by p16/HPV status are summarized in Table 1. Of the 210 tumors analyzed with p16 IHC, 62 (30%) overexpressed p16. Of the 403 tumors analyzed with PCR for the E6 and E7 regions, 114 (28%) were positive for E6 and/or E7 (only six tumors were positive for HPV 18, and two of these were also positive for HPV 16). Of the 178 tumors analyzed with HPV ISH, only 7 tumors (4%) were positive for HPV.

There were no significant differences in the distribution of demographic, exposure, and clinical variables between patients with p16/HPV-positive and p16/HPV-negative tumors, with the exception that patients with p16-positive tumors were more likely than patients with p16-negative tumors to be younger and to have primary tumors of the oral tongue. Furthermore, there were no significant differences in DSS or DFS between patients with p16-positive and p16-negative tumors (DSS, $p = 0.512$, Figure 1A; DFS, $p = 0.054$, Figure 1C), between patients with HPV PCR-positive and HPV PCR-negative tumors (DSS, $p = 0.634$, Figure 1B; DFS, $p = 0.646$, Figure 1D), or between patients with HPV ISH-positive and HPV ISH-negative tumors (DSS, $p = 0.241$; DFS, $p = 0.767$; survival curves not shown as there were only seven HPV ISH-positive tumors). Additionally, there were no significant differences in DSS or DFS between patients with and without a p16/HPV-positive tumor (by p16 IHC, HPV PCR, or HPV ISH) among patients who had surgery alone (log-rank for DSS, $p = 0.393$, $p = 0.581$, and $p = 0.663$, respectively; log-rank for DFS, $p = 0.426$, $p = 0.776$, and $p = 0.054$, respectively) or among patients who had surgery with adjuvant therapy (log-rank for DSS, $p = 0.668$, $p = 0.721$, and $p = 0.224$, respectively; log-rank for DFS, $p = 0.090$, $p = 0.704$, and $p = 0.162$, respectively).

The overwhelming majority of tumors considered HPV positive by either p16 IHC or HPV PCR were HPV negative by each of the other two assays (Table 2). The correlation between these two assays was poor ($r = -0.01$, Table 2). All HPV ISH-positive tumors were also positive by p16 IHC, though this was a group of very limited sample size ($n = 7$). Because these data suggested that most likely a significant number of oral cavity cancers are falsely positive for HPV by HPV PCR and/or p16 IHC, we chose to classify tumors according to a more robust algorithm for determining HPV status previously described for SCCOP [23]. According to this algorithm, tumors are considered HPV negative if p16 is not overexpressed. Tumors are considered HPV positive if they are p16 IHC positive and HPV ISH positive or if they are p16 IHC positive, HPV ISH negative, and HPV PCR positive. Only 10 (6%) of 166 patients could be considered to have an HPV positive tumor by this algorithm (Figure 2). However, there were no significant differences in demographic, exposure, or clinical characteristics between these 10 patients and the remaining 156 patients with HPV-negative tumors (Table 3). Finally, there were no differences in survival between patients with HPV-positive and HPV-negative tumors according to this algorithm (DSS, $p = 0.166$; DFS, $p = 0.398$). Demographic, exposure, and clinical characteristics of the 10 patients with HPV-positive tumors according to this algorithm are provided in Supplementary Table 2.

Discussion

Our results indicate that HPV-driven SCCOC was rare in a cohort of 460 patients who underwent p16/HPV testing at a single institution. Only 6% of patients in this cohort could be considered to have an HPV-positive tumor according to an algorithm previously described for SCCOP [23]. Furthermore, the clinical profiles and survival outcomes of patients considered to have HPV-positive tumors according to this algorithm were similar to those of patients with HPV-negative tumors, suggesting that 6% may represent an overestimation of the true proportion of SCCOC cases driven by HPV.

It is well established that patients with HPV-positive SCCOP, as compared to patients with HPV-negative SCCOP, tended to be of younger age, male sex, non-Hispanic white ethnicity, no history of smoking, less advanced T category, more advanced N category, and poor pathologic tumor differentiation, and to be associated with better survival [24-26]. However, in the current study, patients who were considered to harbor HPV-positive SCCOC according to the robust algorithm for determining HPV status did not differ significantly from patients with HPV-negative SCCOC with respect to age, sex, ethnicity, smoking history, T category, N category, or pathologic tumor grade. Only 20% of patients with HPV-positive SCCOC according to the robust algorithm had poorly differentiated tumors, whereas it was previously reported that approximately 59% of patients with HPV-driven SCCOP had poorly differentiated tumors [4]. Furthermore, of the 10 patients (Supplemental Table 2) with HPV-positive tumors according to the robust algorithm, none fit the abovementioned classic profile that has emerged for HPV-driven SCCOP. Only two patients among this group had no history of cigarette smoking or smokeless tobacco use, and of these, only one had an HPV-positive tumor by HPV ISH, a 36-year-old woman with an SCC of the oral tongue. In addition, a review of the sexual behavior history of these patients (data not shown) showed that among the six patients with available data, the median number of lifetime sexual

partners was 3.5, and the median number of lifetime oral sexual partners was 2. In contrast, patients with HPV-associated SCCOP have been reported to have more than 8 to 10 lifetime sexual partners and more than 4 oral sexual partners [27, 28]. Finally, patients with HPV-positive tumors according to the robust algorithm did not have better survival than patients with HPV-negative tumors.

Our findings differ from those of Chung et al, who reported that in patients with head and neck SCC at non-oropharyngeal sites, p16 expression was significantly associated with better overall survival and progression-free survival [14]. Approximately 26% of SCCOC cases in their study were p16 positive [14], similar to the 30% rate of p16 positivity among SCCOC patients in our current study. However, in subgroup analysis by primary tumor sites, Chung et al found that the significantly better survival in patients with p16-positive tumors was limited to patients with cancer of the hypopharynx, a site with potential overlap with oropharyngeal sites. Chung et al found that only 8 (38%) of 21 p16-positive SCCOCs were also HPV ISH positive, leaving open the possibility that p16 overexpression is driven by mechanisms other than HPV infection. In fact, in their study, the correlation between p16 status and HPV status was quite poor (correlation coefficient = 0.35, 95% CI: 0.13-0.57). Furthermore, there was no correlation between p16 status and either overall survival ($p = 0.34$) or progression-free survival ($p = 0.52$) among patients with SCCOC. However, Chung et al observed a surprisingly high 15% rate of HPV positivity according to HPV ISH among SCCOC patients [14]. In our study, the rate of HPV positivity by HPV ISH was higher in patients with primary tumors at gingivobuccal sites than in patients with primary tumors at other sites. Between-study difference in distribution of tumor subtypes might partly explain the difference in HPV ISH results.

Initial studies of HPV-associated “oral” SCCs were confounded by failure to differentiate well between SCCOC and SCCOP [29]. More recent studies have generally demonstrated a relatively lower prevalence of HPV-positive SCCOC [30, 31]. In a meta-analysis on HPV and head and neck cancer, Mehanna et al found that HPV prevalence increased over time for SCCOP but decreased over time for non-oropharyngeal head and neck cancers, from 22% prior to 2000 to 6% in 2005 and later [32]. The authors suggested that site misclassification of SCCOC and SCCOP might have contributed to this trend.

The data herein support the findings of previous authors who have demonstrated that p16 status is a poor surrogate for HPV status of tumors in the oral cavity [30, 33]. We advise against the use of p16 as a surrogate marker for HPV for SCCOC. In this study, among patients who had both p16 IHC and HPV ISH testing, all seven patients who were positive by ISH were also positive by p16, but 76% (22 of 29) of the patients who were positive by p16 IHC were negative by HPV ISH. This suggests that p16 IHC has a very high false-positive rate in SCCOC. Chung et al recently found that among all non-oropharyngeal sites of head and neck SCC, the site with the lowest correlation between HPV ISH and p16 IHC findings was the oral cavity (correlation coefficient, 0.35, compared to 0.54 for the hypopharynx and 0.52 for the larynx) [14]. Lingen et al reported that the positive predictive value of p16 as a potential indicator of HPV status in the oral cavity was approximately 40% [18]. Sgaramella et al found no evidence of HPV 16 DNA in 71 SCCs of the tongue, whereas 36 of these 71 SCCs (51%) showed p16 staining [34]. Various mechanisms likely

contribute to the poor concordance between p16 IHC and HPV ISH findings, including overlapping oral cavity/oropharyngeal disease whose epicenter in the oropharynx cannot be determined due to tumor size at presentation, true HPV-positive tumors that originate in reticular epithelium in oral cavity sites, and low sensitivity of the methods (particularly p16) in detection of the truly HPV-driven cases [18, 35]. We could not exclude the possibility that the poor concordance between p16 IHC and HPV ISH findings in our study was due to other mechanisms of p16 activation independent of HPV in oral cavity carcinogenesis. More studies are expected to explore the potential underlying mechanisms.

The study had notable strengths and several limitations. The strengths include the large group of patients all treated at a single tertiary care institution, the prospective design with long follow-up, and the well-defined tumor site classification and pathology classification. One limitation of this study is that not all patients had HPV testing by all three assays. However, this limitation appears not to be major given that the comparison between patients with different HPV testing results showing no significant differences. One could argue that the lack of significant difference in survival between HPV-positive and HPV-negative patients was likely due to the small number of HPV-positive patients, but since the HPV-positive rate among patients with SCCOC is very low, it is in practice very hard to achieve adequate power for survival analysis. Future work is needed to corroborate our findings with findings from other cohorts. In addition, a slight underestimation of HPV-related SCCOC could not be excluded because our PCR testing covered only high-risk HPV types 16 and 18, though HPV ISH covered multiple oncogenic types. A second possible cause of underestimation is that our PCR testing was on paraffin-embedded tumor specimens and the sensitivity of PCR-based assays is generally lower in paraffin-embedded specimens than fresh specimens as a result of DNA fragmentation and DNA-protein cross-linking caused by formaldehyde and paraffin. Finally, our study did not further confirm HPV status using a gold-standard HPV E6/7 mRNA expression assay.

In summary, our findings from this study support a very low incidence of true HPV-driven SCCOC. The 6% HPV-positive prevalence may be an overestimation, as overall clinical and survival comparisons between patients with HPV-positive and HPV-negative tumors, as well as a study of the individual patients who tested HPV positive by a well-defined algorithm for SCCOP, raises some doubt as to whether even this small minority of patients had truly HPV-driven tumors. This study also casts doubt on whether HPV assays that have high specificity and sensitivity in the oropharynx can be reliably applied to non-oropharyngeal head and neck sites. p16 expression in particular does not have a clinically useful correlation with HPV positivity in SCCOC. Next-generation sequencing emerges as a promising method to assess HPV status given its ability to determine both viral load and co-detection of multiple viral types using a limited amount of DNA [36]. Future studies are expected to comprehensively evaluate HPV involvement in SCCOC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DFS	disease-free survival
DSS	disease-specific survival
HPV	human papillomavirus
IHC	immunohistochemistry
ISH	in situ hybridization
OR	odds ratio
PCR	polymerase chain reaction
SCCOC	squamous cell carcinoma of the oral cavity
SCCOP	squamous cell carcinoma of the oropharynx

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Highlights

- Very few patients with oral cavity cancer have HPV-driven tumors.
- HPV status is not correlated with survival outcomes of oral cavity cancer.
- p16 does not clinically correlate with HPV positivity in oral cavity cancer.

Figure 1.

Survival of patients with squamous cell carcinoma of the oral cavity by tumor p16/HPV DNA status. A and B, Disease-specific survival of (A) patients with tumors positive and negative for p16 by immunohistochemistry ($p = 0.512$) and (B) patients with tumors positive for HPV 16 and/or HPV 18 or negative for both by PCR ($p = 0.634$). C and D, Disease-free survival of (A) patients with tumors positive and negative for p16 by immunohistochemistry ($p = 0.054$) and (D) patients with tumors positive for HPV 16 and/or HPV 18 or negative for both by PCR ($p = 0.646$).

Figure 2.

Tumor HPV status determined according to a robust algorithm in 210 patients with squamous cell carcinoma of the oral cavity whose tumors were tested for p16 by immunohistochemistry.

Table 1

p16 status by immunohistochemistry (IHC), HPV status by polymerase chain reaction (PCR) for HPV 16/18 E6/7, and HPV status by in situ hybridization (ISH) in patients with squamous cell carcinoma of the oral cavity, together with demographic, exposure, and clinical characteristics by p16/HPV status.

Characteristic	p16 IHC (N = 210)			p	HPV PCR (N = 403)			p	HPV ISH (N = 178)			p ^a
	-	+	(%+)		-	+	(%+)		-	+	(%+)	
Total	148	62	(29.5)		289	114	(28.3)		171	7	(3.9)	
Age, years				<0.001 ^a				0.157				0.567
<45	39	35	(47.3)		61	28	(31.5)		37	2	(5.1)	
45-69	74	23	(23.7)		157	68	(30.2)		99	5	(4.8)	
70	35	4	(10.3)		71	18	(20.2)		35	0	(0.0)	
Sex				0.622				0.945				0.424
Female	53	20	(27.4)		105	41	(28.1)		63	1	(1.6)	
Male	95	42	(30.7)		184	73	(28.4)		108	6	(5.3)	
Ethnicity				0.757				0.907				1.000
Non-Hispanic white	124	53	(29.9)		242	96	(28.4)		141	6	(4.1)	
Other	24	9	(27.3)		47	18	(27.7)		30	1	(3.2)	
Smoking history ^b				0.567				0.849				1.000
Never smoker	56	26	(31.7)		96	39	(28.9)		63	2	(3.1)	
Ever smoker	90	35	(28.2)		193	75	(28.0)		106	4	(3.6)	
Alcohol use history ^c				0.772				0.556				1.000
Never user	53	21	(28.4)		100	43	(30.1)		52	2	(3.7)	
Ever user	92	40	(30.3)		189	71	(27.3)		116	4	(3.3)	
Primary tumor site				0.021				0.107				0.119
Oral tongue	82	47	(36.3)		142	65	(31.4)		108	2	(1.8)	
Floor of mouth	21	5	(19.2)		39	19	(32.8)		19	1	(5.0)	
Gingivobuccal	45	10	(18.2)		108	30	(21.7)		44	4	(8.3)	
Grade ^d				0.650				0.341				0.634
Poorly differentiated	32	15	(31.9)		71	24	(25.3)		34	2	(5.6)	
Moderately or well differentiated	113	45	(28.5)		204	89	(30.4)		134	5	(3.6)	

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Characteristic	p16 IHC (N = 210)			HPV PCR (N = 403)			HPV ISH (N = 178)			^a p
	-	+	p	-	+	p	-	+	p	
T category			0.256			0.921				0.117
1-2	82	39 (32.2)		179	70 (28.1)		105	2 (1.9)		
3-4	66	22 (25.0)		110	44 (28.6)		66	5 (7.0)		
N category			0.319			0.416				0.701
0	78	28 (26.4)		180	66 (26.8)		96	3 (3.0)		
1-3	70	34 (32.7)		109	48 (30.6)		75	4 (5.1)		

^aFisher exact test.

^bSmoking data missing for 6 patients.

^cAlcohol use data missing for 8 patients.

^dGrade not reported for 23 patients.

Table 2

Relationships between p16 status by immunohistochemistry (IHC), HPV status by polymerase chain reaction (PCR) for HPV 16/18 E6/7, and HPV status by in situ hybridization (ISH) in patients with squamous cell carcinoma of the oral cavity.

	p16 IHC status			HPV status by ISH		
HPV status	Positive (%)	Negative (%)	Total	Positive (%)	Negative (%)	Total
By PCR						
Positive	13 (29.5)	31 (70.5)	44	2 (4.8)	40 (95.2)	42
Negative	34 (43.0)	79 (57.0)	113	1 (1.2)	81 (98.8)	82
Phi coefficient (95% CI)	−0.01 (−0.16 - 0.17)			0.11 (−0.07 - 0.21)		
By ISH						
Positive	7 (100)	0 (0)	7			
Negative	22 (17.1)	107 (82.9)	129			
Phi coefficient (95% CI)	0.45 (0.21 - 0.45)					

Table 3

Demographic, exposure, and clinical characteristics of patients with squamous cell carcinoma of the oral cavity by HPV status determined according to a robust algorithm.

Characteristic	HPV positive (N = 10)		HPV negative (N = 156)		p
	No.	(%)	No.	(%)	
Age, years					0.760
<45	3	(30.0)	42	(26.9)	
45-69	6	(60.0)	78	(50.0)	
70	1	(10.0)	36	(23.1)	
Sex					0.498
Female	2	(20.0)	54	(34.6)	
Male	8	(80.0)	102	(65.4)	
Ethnicity					1.000
Non-Hispanic white	9	(90.0)	131	(84.0)	
Other	1	(10.0)	25	(16.0)	
Smoking history ^a					1.000
Never smoker	3	(33.3)	60	(39.0)	
Ever smoker	6	(66.7)	94	(61.0)	
Alcohol use history ^b					0.495
Never user	2	(22.2)	55	(36.0)	
Ever user	7	(77.8)	98	(64.1)	
Primary tumor site					0.895
Oral tongue	5	(50.0)	88	(56.4)	
Floor of mouth	1	(10.0)	21	(13.5)	
Gingivobuccal	4	(40.0)	47	(30.1)	
T category					0.343
1-2	4	(40.0)	88	(56.4)	
3-4	6	(60.0)	68	(43.6)	
N category					0.521
0	4	(40.0)	83	(53.2)	
1-3	6	(60.0)	73	(46.8)	
Grade ^c					1.000
Poorly differentiated	2	(20.0)	35	(23.0)	
Moderately or well differentiated	8	(80.0)	117	(77.0)	

^aSmoking history not reported for 1 patient with an HPV-positive tumor and 2 patients with HPV-negative tumors.

^bAlcohol use history not reported for 1 patient with an HPV-positive tumor and 3 patients with HPV-negative tumors.

^cGrade not reported for 4 patients with HPV-negative tumors.