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Human papillomavirus-associated squamous cell carcinoma of the larynx or hypopharynx: Clinical outcomes and implications for laryngeal preservation

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ABSTRACT

Objectives: Human papillomavirus (HPV) can be detected in approximately 25% of squamous cell carcinomas (SCC) of the larynx and hypopharynx. Though HPV is associated with improved survival and disease control in patients with oropharyngeal SCC, the role of HPV as a marker of favorable treatment outcomes in laryngeal and hypopharyngeal cancer is unclear.

Materials and Methods: Patients treated for laryngeal or hypopharyngeal SCC were reviewed. HPV status detected by p16 and/or HPV DNA PCR were abstracted from the medical record. A subset of samples (stage III-IV treated with primary radiotherapy) was retrospectively tested for p16 and HPV DNA. Overall survival (OS), disease-free survival (DFS), and locoregional control (LRC) were determined and compared between HPV-positive (p16+, PCR+ or both) and HPV-negative (p16- or PCR-) patients.

Results: In total, 279 patients were identified, 94 of which were tested for HPV. Eighty-two (87%) were negative and 12 (13%) were positive for HPV. At 3 years, there were no significant differences in OS (72% v. 83%), DFS (60% v. 71%) and LRC (80% v. 89%). Performance status, smoking history and stage predicted for OS, while performance status and stage predicted for DFS. Analysis of patients treated with primary radiotherapy revealed non-significantly higher rates of laryngeal preservation at 3 years (75% v. 100%).

Conclusion: HPV was detected in 13% of tested laryngeal/hypopharyngeal cancers. HPV does not appear to significantly impact survival or disease control in patients with SCC of the larynx or hypopharynx. Non-significant improvements in laryngeal preservation were observed in HPV-positive patients.

Introduction

An estimated 12,400 new cases of laryngeal cancer and 3000 new cases of hypopharyngeal cancer will be diagnosed in 2019 in the United States, the majority of which are squamous cell carcinomas (SCC) [1,2]. Early-stage disease is commonly managed with conservative surgical excision or limited radiotherapy. Locally advanced disease (stage III-IV) is often managed by aggressive surgical resection (laryngectomy/pharyngectomy with neck dissection and adjuvant therapy as indicated) or with definitive chemoradiotherapy (CRT), which offers the benefit of voice preservation in those with a functional larynx [3–5]. Laryngeal preservation in patients with advanced disease positively impacts quality of life [6]. Predictive biomarkers for favorable survival and improved disease control may provide a method to safely apply larynx preservation without compromising disease control.

Identification of human papillomavirus (HPV) infection as an oncogenic factor associated with significantly improved outcomes in oropharyngeal SCC has prompted a profound evolution in the management of that disease [7–12]. HPV is also detected in approximately 21–27% of laryngeal cancer cases [13–15] and its prevalence is increasing in non-oropharyngeal sites among certain populations [16]. The utility of HPV in laryngeal/hypopharyngeal cancer is unclear, as the current data are conflicting. Few reports demonstrate improved disease control in select cohorts [17,18]. The effect of HPV on survival of patients with laryngeal or hypopharyngeal SCC is controversial. Some studies found no differences in survival [13,19,20], yet others have noted improved overall survival for HPV-positive non-oropharyngeal and hypopharyngeal SCC [21,22]. These studies are

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heterogeneous in nature and employ a variety of HPV detection techniques. Identification of a low-risk subset of patients may facilitate further investigation into laryngeal preservation and the feasibility of treatment de-intensification [10,23].

In this study, we aimed to evaluate the incidence of HPV in a cohort of laryngeal and hypopharyngeal SCC patients. We analyzed the concordance of p16 with the presence of HPV DNA and compared survival and disease control outcomes in patients with HPV-associated disease compared to those with HPV-negative disease.

Material and methods

Patients with primary SCC of the larynx or hypopharynx treated consecutively with curative intent between May 2010 and October 2018 were queried from an Institutional Review Board (IRB)-approved head and neck cancer database (n = 279). Study data were collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at Wake Forest School of Medicine [24]. Exclusion criteria were: prior head and neck cancer, metastatic disease or synchronous non-head and neck primary malignancy. Patient demographic, disease, and treatment factors were abstracted from the medical record. Tobacco use (ever versus never smoker), pack-year smoking history, alcohol use (yes or no at the time of diagnosis), Eastern Cooperative Oncology Group (ECOG) performance status and Charlson comorbidity index were derived from medical record data at the time of diagnosis.

Patients were initially evaluated by a multidisciplinary head and neck oncology group comprised of otolaryngologists, medical oncologists, radiation oncologists, radiologists, pathologists, dentists, dieticians and speech and swallow therapists as part of routine standard of care at our institution. In general, conservative surgical or limited-field radiotherapeutic management was employed in early-stage (I-II) disease while combined modality therapy (including total laryngectomy followed by adjuvant radiotherapy as indicated or definitive chemoradiotherapy) was utilized for advanced stage III-IVB disease. Postoperative radiotherapy was generally recommended in the presence of pathologic features including lymphovascular invasion, perineural invasion, T3-4 primary disease, involvement of multiple lymph nodes or close margins (within 5 mm) [25]. Postoperative chemoradiotherapy was routinely recommended in the case of positive margin or extracapsular invasion, according to the accepted standard of care [26–28]. Overall survival was defined as the duration from diagnosis to death from any cause and disease-free survival was defined as the interval to death or any disease recurrence. Locoregional recurrence was defined as any clinically suspected, radiographically- and/or pathologically-confirmed evidence of disease within the primary site or neck. Second primary malignancies (arising from anatomically distinct sites within the head and neck) were defined separately. Laryngectomy-free survival was defined as the duration of time from diagnosis to salvage laryngectomy (for any reason) or death.

Pathologic assessment of HPV

HPV status was collected retrospectively from the medical record when available (n = 72). According to standard practice, there was no routine reflex testing performed at the time of diagnosis. As such, all testing obtained from the medical record had been performed at the request of the treating clinicians. Due to a lack of institutional standard for routine testing of non-oropharyngeal SCC, the utilization of p16 IHC or HPV DNA PCR varied.

We hypothesized that the subgroup of patients with stage III-IV disease treated with larynx-preserving definitive CRT would be most likely to show an improvement in outcomes associated with HPV positivity. Therefore, with IRB approval and for the purposes of a planned subgroup analysis, an additional subset of 46 laryngeal cancer specimens meeting these criteria within the original 279 patient cohort were tested retrospectively for p16 surrogate marker and HPV DNA using banked formalin-fixed paraffin-embedded tissue samples. This resulted in known HPV status for 51 patients eligible for a planned subset analysis. Immunohistochemistry (IHC) for p16 using clone E6H4 (Ventana Medical Systems, Tucson, Arizona) was interpreted as positive with > 70% nuclear and cytoplasmic tumor cell staining with at least moderate to strong intensity. HPV DNA PCR was performed using the Roche Cobas® 4800 system (Roche Molecular Diagnostics, Pleasanton, CA) which detects the presence of HPV types 16, 18 and a pooled group of other high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). Patients were categorized as HPV-positive if the biopsy or surgical specimen was positive by either p16 IHC or PCR for HPV DNA. If both IHC and PCR were performed, patients were only considered positive if both IHC and PCR were concordantly positive. Discordant results were considered negative, as this may represent a distinct HPV-independent subtype of disease [29].

Statistical analysis

Descriptive analyses were performed and compared between groups using the *t*-test for normally-distributed continuous variables and Fisher's exact and the chi-square tests for categorical variables. Sensitivity, specificity, positive predictive value and negative predictive value of the p16 surrogate marker were calculated. Bivariate logistic regression analyses were performed to identify factors associated with HPV positivity. Time-to-event outcomes were estimated using the Kaplan-Meier method and compared using the log-rank test. Follow-up was determined using the reverse Kaplan-Meier method. Univariate Cox proportional hazards models were performed to identify potential predictors of OS and DFS. Covariates identified on univariate analysis ($p \leq 0.05$) were entered into multivariate models to assess their impact on OS and DFS while adjusting for all potential predictors. The Cox proportional hazards assumption was tested and met for all models. All analyses were performed using R version 3.5 (R Foundation for Statistical Computing, Vienna, Austria).

Results

In total, 279 patients with laryngeal/hypopharyngeal SCC were identified, the majority of whom were male (79%), Caucasian (81%) and had a performance status of 0–1 (78%, Table 1). Most patients (93%) had a smoking history and 87% had smoked ≥10 pack-years. Laryngeal primary site predominated with 98% of these arising from the supraglottic and glottic regions. The AJCC 7th Edition clinical stage was III-IV in 62%. The primary treatment modality was surgery with adjuvant therapy as indicated in 58% and radiotherapy in 42%. The treatment paradigms utilized were as follows (number, %): surgery/local excision alone (111, 40%), surgery followed by adjuvant radiotherapy (25, 9%), surgery followed by chemoradiotherapy (30, 11%), definitive chemoradiotherapy (72, 26%) and radiotherapy alone (41, 15%). Median RT dose was 70 Gy (IQR 65.25–70) for definitive therapy and 60 Gy (IQR 60–66) for postoperative. Concurrent chemotherapy was delivered in 102 patients and included the following regimens: cisplatin (71%), carboplatin/paclitaxel (21%), cetuximab (6%), platinum-cetuximab combination (1%), unknown (4%). Median follow-up was 31.1 months (95% CI 26.8–36.7).

After supplementation of HPV status abstracted from the medical record with that determined by retrospective testing, the characteristics of 94 patients with known HPV status were compared to 185 patients with unknown HPV status. Unexpected differences between groups included age, laryngeal primary site, and year of diagnosis. Compared to those with unknown HPV, those with known HPV were younger (mean age 60 versus 64 years, $p = 0.01$), were more likely to have a supraglottic primary site (66% versus 41%, $p < 0.01$) and were more likely to be diagnosed between 2015 and 2018 (70% versus 49%, $p < 0.01$).

Table 1
Patient and Treatment Characteristics by HPV Status.

	Total (n = 279)	HPV-negative (n = 82)	HPV-positive (n = 12)	p-value
Age, mean (range)	62 (25–91)	61 (25–83)	58 (43–78)	0.40
Gender				
Male	220 (79)	66 (81)	9 (75)	0.70
Female	59 (21)	16 (20)	3 (25)	
Race				
Caucasian	227 (81)	69 (84)	11 (92)	0.77
African American	47 (17)	12 (15)	1 (8)	
Other	5 (2)	1 (1)	0 (0)	
ECOG Performance Status				
0	35 (17)	10 (13)	4 (44)	0.03
1	129 (61)	52 (67)	5 (56)	
2	47 (22)	16 (21)	0 (0)	
Unknown	68	4	3	
Charlson Comorbidity Index > 3	73	24 (32)	3 (25)	0.63
Unknown	46	7	–	
Rural Location	64 (27)	25 (31)	3 (33)	0.84
Unknown	41	–	–	
Smoking History				
No	20 (7)	2 (2)	2 (17)	0.08
Yes	254 (93)	80 (98)	10 (83)	
Unknown	5	–	–	
Pack-years > 10	225 (87)	75 (95)	7 (58)	< 0.01
Current Alcohol Use				
Yes	114 (42)	29 (35)	6 (50)	0.35
Unknown	7	–	–	
Primary Site				
Larynx	251 (90)	70 (85)	12 (1 0 0)	0.35
Hypopharynx	28 (10)	12 (15)	0 (0)	
Location within Larynx				
Supraglottic	124 (49)	49 (70)	5 (42)	0.10
Glottis	123 (49)	21 (30)	7 (58)	
Subglottic	3 (2)	–	–	
Tumor Stage				
T1	70 (25)	13 (16)	1 (8)	0.03
T2	62 (22)	17 (21)	7 (58)	
T3	85 (30)	32 (39)	1 (8)	
T4	62 (22)	20 (24)	3 (25)	
Nodal Stage				
N0	177 (63)	38 (46)	8 (67)	0.57
N1	21 (8)	10 (12)	1 (8)	
N2	74 (27)	30 (37)	3 (25)	
N3	7 (3)	4 (5)	0 (0)	
Clinical Stage (AJCC 7th Edition)				
I	63 (23)	7 (9)	1 (8)	0.03
II	43 (15)	9 (11)	5 (42)	
III	64 (23)	25 (31)	1 (8)	
IV	109 (39)	41 (50)	5 (42)	
Primary Treatment Modality				
Radiotherapy	113 (41)	53 (65)	9 (75)	0.74
Surgery	166 (59)	29 (35)	3 (25)	
Treatment Paradigm				
CRT	72 (26)	47 (57)	5 (42)	0.05
RT	41 (15)	6 (7)	4 (33)	
Surgery	111 (40)	15 (18)	3 (25)	
Surgery + RT	25 (9)	4 (5)	0 (0)	
Surgery + CRT	30 (11)	10 (12)	0 (0)	
Diagnosis Era				
2010–2014	123 (44)	25 (30)	3 (25)	0.70
2015–2018	156 (56)	57 (70)	9 (75)	

AJCC, American Joint Commission on Cancer; ECOG, Eastern Cooperative Oncology Group; HPV, human papillomavirus; PCR, polymerase chain reaction.

Table 2
HPV categorization by detection method for all patients and in patients with both p16 and PCR results.

All patients

	HPV Positive (n = 12)	HPV Negative (n = 82)	Total (n = 94)
p16	2 (7)	27 (93)	29
HPV DNA PCR	3 (10)	27 (90)	30
Both	7 (20)	28 (80)	35

Patients with both p16 and PCR results

		HPV DNA PCR		
		Positive	Negative	Total
p16 IHC	Positive	7	5	12
	Negative	3	20	23
	Total	10	25	35

IHC, immunohistochemistry; PCR, polymerase chain reaction.

HPV detection

Out of 94 patients with known HPV status, 82 were categorized as negative and 12 were categorized as positive. Twenty-nine were determined by p16 alone, 30 by HPV DNA PCR alone, and 35 by both p16 IHC and PCR (Table 2). Among the 29 tumors identified using p16 alone, 27 (93%) were negative and 2 (7%) were positive. Of the 30 tumors identified using HPV DNA PCR, 27 (90%) were negative and 3 (10%) were positive. Seventy-two (77%) patients had at least one HPV assay (either HPV DNA PCR or p16 IHC) performed as part of initial workup, 61 (74%) in the HPV negative group and 11 (92%) in the HPV positive group (p = 0.19). In the HPV-negative group, 41 had HPV DNA PCR and 29 had p16 testing at diagnosis. In the HPV-positive group, 9 patients had HPV DNA PCR and 6 patients had p16 testing at diagnosis.

There were similar baseline characteristics between patients with HPV-positive and HPV-negative cancers in terms of age, race, gender, primary site, smoking history (yes versus no), alcohol use, primary treatment modality (surgery or radiotherapy), treatment paradigm, and radiotherapy dose (p ≥ 0.05). HPV positive disease was associated with better ECOG performance status, earlier stage, and smoking history of fewer than 10 pack-years (Table 1). HPV type 16 was present in 7 of 13 (54%) patients testing positive by HPV DNA PCR, HPV type 18 was present in 1 (8%) and other (non-16/18) high-risk HPV type was present in the remaining 5 (38%).

Of 35 patients with both p16 and HPV DNA PCR results available, 20 (57%) were concordantly negative for HPV, 8 (23%) were discordant (either PCR+/p16− or PCR-/p16+) and 7 (20%) were concordantly positive for HPV (Table 2). Using high-risk HPV DNA detection by PCR as a reference, p16 immunohistochemistry detected HPV with 80% sensitivity, 70% specificity, 32% positive predictive value and a 95% negative predictive value.

Survival and locoregional control

Three-year OS in HPV-positive patients was 83% versus 72% for HPV-negative patients (log-rank p = 0.61, Fig. 1A) and 3-year DFS was 71% v. 60%, respectively (p = 0.37, Fig. 1B). Three-year overall survival was 85% for concordantly negative, 70% for discordant, and 100% for concordantly positive patients (log-rank p = 0.31). Analysis of the 65 patients with known HPV PCR status found no significant differences in OS (p = 0.29) or DFS (p = 0.36). Likewise, no significant differences in OS (p = 0.42) or DFS (p = 0.54) were found in the 64 patients with known p16 status. Locoregional control at 3 years was 89% in the HPV-positive group and 80% in the HPV-negative group (p = 0.35, Fig. 2).

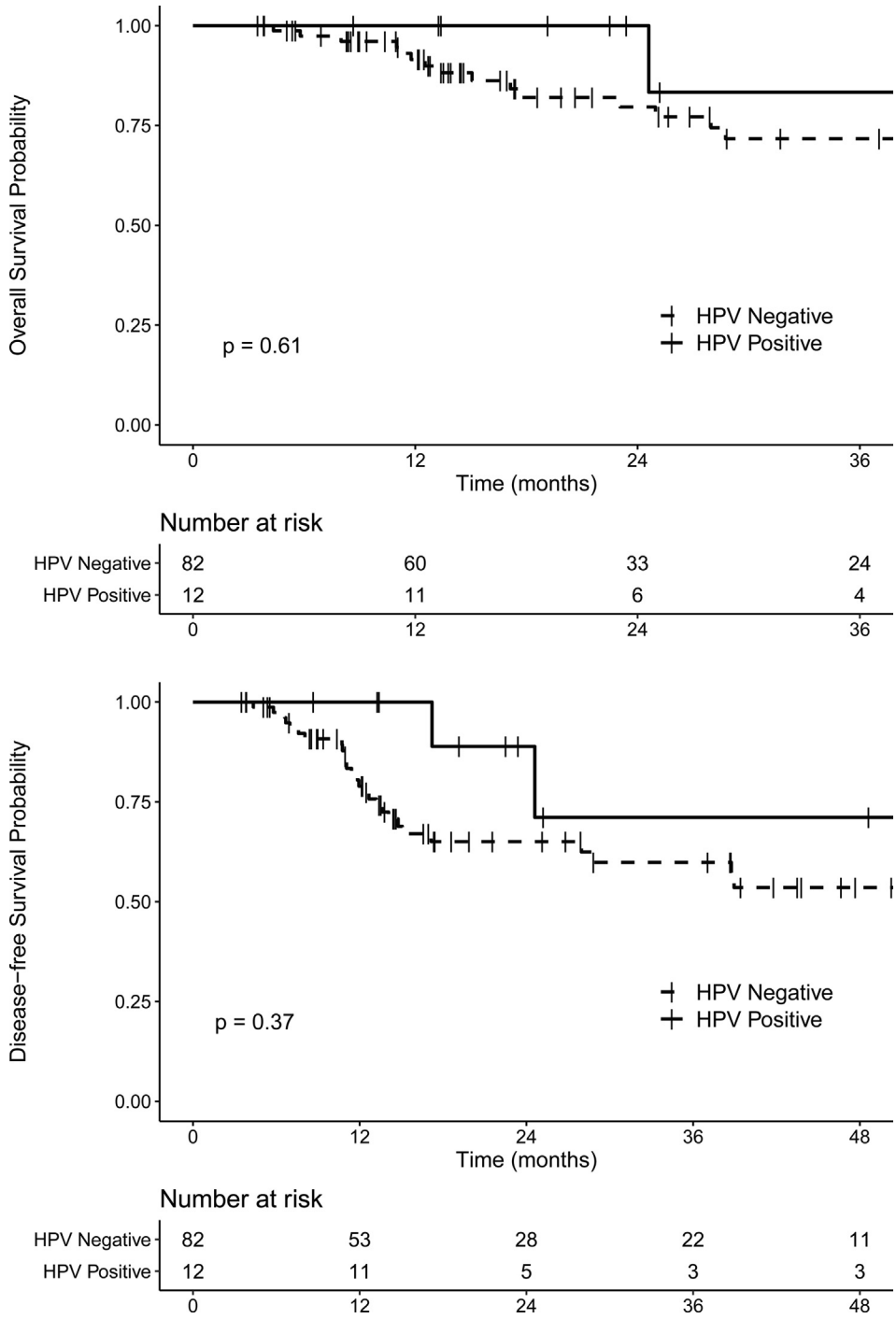

Fig. 1. Kaplan-Meier plots of overall survival (top) and disease-free survival (bottom) by HPV status.

Univariate Cox proportional hazards analyses of overall survival found ECOG 2 versus 0 (HR 6.84, 95% CI 2.05–22.82, $p < 0.01$), hypopharynx versus larynx primary site (HR 3.46, 95% CI 1.90–6.31, $p < 0.01$), ≥10 pack-years versus < 10 pack-years (HR 4.11, 95% CI 1.00–16.86, $p = 0.05$), stage III (versus I, HR 3.64, 95% CI 1.19–11.17, $p = 0.02$) and stage IVA/B (versus I, HR 6.51, 95% CI 2.33–18.23, $p < 0.01$) as predictive of OS. Similarly, univariate analyses of disease-free survival found ECOG 2 versus 0 (HR 4.22, 95% CI 1.61–11.03, $p < 0.01$), hypopharynx versus larynx primary site (HR 2.43, 95% CI 1.39–4.26, $p < 0.01$), stage III (versus I, HR 2.47, 95% CI 1.07–5.68, $p = 0.03$) and stage IVA/B (versus I, HR 4.06, 95% CI 1.92–8.58, $p < 0.01$) were predictive of DFS. Age, race, rural location, alcohol exposure, year of diagnosis and HPV status were not associated with OS or DFS on univariate analysis. Upon multivariate analysis, ECOG 2 versus 0 (adjusted HR 4.27) was associated with OS (Table 3). ECOG 2 versus 0 (adjusted HR 3.03) and stage IV (versus I, HR 5.10) were associated with DFS (Table 3).

Survival, control and larynx preservation in patients treated with chemoradiotherapy

Out of a subgroup of 51 patients with stage III-IV disease treated with larynx-preserving chemoradiotherapy and known HPV status, 5 (10%) were positive for HPV. No significant differences between HPV-positive and HPV-negative patients were noted in 3-year OS (100% v. 78%, $p = 0.29$), DFS (75% v. 63%, $p = 0.49$), or LRC (75% v. 78%,

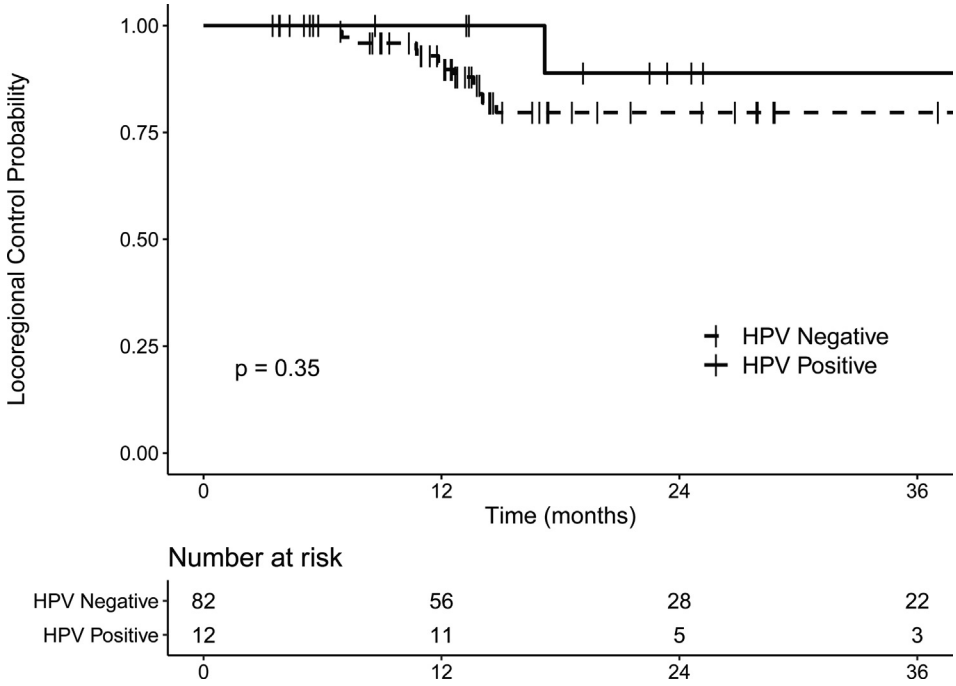

Fig. 2. Kaplan-Meier plot of locoregional control by HPV status.

Table 3
Multivariate Cox proportional hazards models of overall and disease-free survival.

	Overall Survival		Disease-free Survival	
	HR (95% CI)	p-value	HR (95% CI)	p-value
ECOG Performance Status				
0	Ref.	–	Ref.	–
1	1.48 (0.44–4.98)	0.53	1.61 (0.63–4.12)	0.32
2	4.27 (1.24–14.69)	0.02	3.03 (1.13–8.09)	0.03
Primary Site				
Larynx	Ref.	–	Ref.	–
Hypopharynx	1.25 (0.63–2.50)	0.52	1.19 (0.65–2.20)	0.57
Pack-years				
< 10	Ref.	–	–	–
≥10	2.92 (0.69–12.34)	0.14		
Clinical Stage				
I	Ref.	–	Ref.	–
II	1.21 (0.17–8.63)	0.85	2.28 (0.54–9.55)	0.26
III	2.09 (0.44–9.89)	0.35	3.03 (0.86–10.71)	0.09
IV	3.99 (0.93–17.16)	0.06	5.10 (1.55–16.76)	0.01

CI, confidence interval; HP, hypopharynx; HR, hazard ratio; L, larynx; Ref., reference.

p = 0.94). Laryngectomy-free survival at 3 years was 100% for HPV-positive patients and 75% for HPV-negative patients (p = 0.26, Fig. 3). Univariate Cox proportional hazards model did not identify HPV nor any other factors evaluated to be associated with OS.

Discussion

The role of HPV in defining a favorable group of patients with oropharyngeal squamous cell carcinoma (OPSCC) has been profound. Owing to significant improvements in disease control and survival [8], investigations into treatment de-escalation and organ preservation represent an exciting new era in the field of head and neck oncology. Chera et al. have reported their prospective experience with dose de-escalated chemoradiotherapy for low-risk HPV-associated oropharyngeal SCC, delivering 60 Gy in 30 fractions with concurrent weekly cisplatin [10]. Patients treated with this paradigm demonstrated excellent disease control and survival; pathologic complete response was noted in 86%, 3-year local and regional control, cause-specific survival, and distant metastasis-free survival were all 100%, and 3-year OS was 95%. Despite the inability to prove non-inferiority of RT with concurrent cetuximab, the standard arms of the RTOG 1016 and De-ESCALaTE HPV trials demonstrate excellent survival (5-year OS 85% and 2-year OS 97.5% in the two trials, respectively) compared to the HPV-negative cohort of RTOG 0129 (3-year OS 57%) [8,30,31]. Though the impact of HPV in OPSCC is well-established, its effect on clinical outcomes such as survival and disease control in the larynx and hypopharynx remain controversial.

While the prevalence of HPV detection in laryngeal SCC has been described with a wide range, it can be found in approximately 20–25% of patients [13,15,32]. In our patient population, the rate of HPV positivity was 13%. This may be related to the regional distribution of this patient population within the southern United States, where tobacco use is prevalent. In total, 93% of patients had a smoking history and 87% had smoked ≥10 pack-years. An inverse relationship between history of heavy smoking and HPV detection may account for the low proportion of HPV-associated disease described herein [33]. Rates of HPV-associated oropharyngeal SCC in this particular patient population are in line with those of the entire United States and demonstrate a similar association with smoking [34,35].

The presence of HPV was not associated with a statistically significant difference in survival or locoregional disease control in this cohort. This finding is in agreement with other institutional studies. Vlachtsis et al reported no association between HPV status and overall survival or time to progression [36]. Meshman and colleagues described a small (n = 31) cohort of locally advanced laryngeal or hypopharyngeal SCC patients, in whom HPV status by p16 was positive in 45% [37]. This is a fairly high proportion not often seen within the literature which may be related to patient demographic/geographic differences, or method of testing (HPV DNA in situ hybridization). Regardless, HPV was not significantly associated with 2-year overall survival (despite numeric differences, 91% versus 64%) or locoregional control. Similar to the current study, these findings were likely limited by sample size, though our cohort represents a much larger group with proportionally less HPV-positive tumors. Alternatively, other retrospective studies

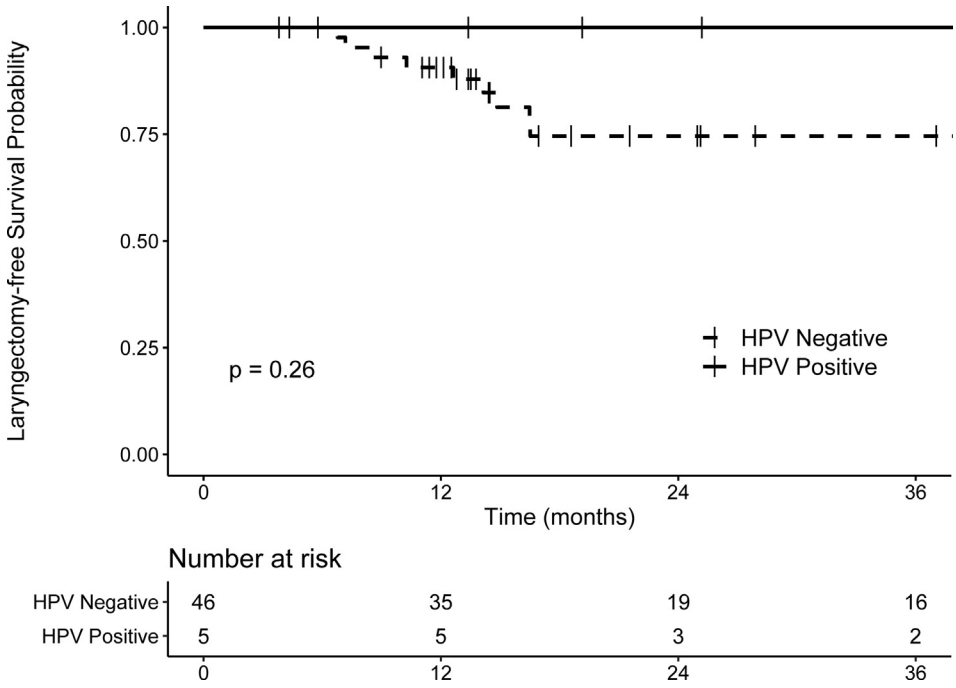

Fig. 3. Laryngectomy-free survival after treatment with CRT for Stage III-IV disease.

including patients managed both surgically and with radiotherapy have demonstrated differences in disease recurrence but not survival that our study did not detect [17,18]. This discrepancy may be related to the high prevalence of tobacco abuse in our cohort as noted above. It may also be related to relatively high rates of detection of HPV types other than 16. Whereas non-16 HPV types comprise only ~9% of HPV infection in OPSCC [38], they comprise 38% of HPV-positive laryngeal/hypopharynx tumors in the current study and up to 68% of laryngeal tumors in another [13]. Other high-risk HPV-associated OPSCC may represent a separate biological entity that does not carry the favorable prognostic implications typically associated with HPV-mediated disease [39]. Small sample sizes limit our power to detect differences by HPV type in this study.

Despite the fact that no significant differences in survival or disease control were noted between HPV positive and negative patients, multiple interesting trends were evident. First, OS and DFS were numerically greater in patients with HPV-positive tumors. This may be related to the population in which HPV association was more frequent, with less significant smoking history and lower stage of disease. In addition to performance status, these factors were found to be associated with survival. Among patients treated with laryngeal preservation for locally advanced disease, no HPV-positive patients required laryngectomy while only 75% of HPV-negative patients remained laryngectomy-free at 3 years. Indeed, the possibility remains that given this small sample size, this may be due to random chance or related to lack of statistical power to detect a true difference between these populations.

The use of the surrogate marker p16 has excellent accuracy in the detection of HPV-associated OPSCC [40]. In non-OPSCC head and neck malignancies, the sensitivity, specificity and negative predictive value of p16 are reasonably high, though it has a poor positive predictive value (83%, 95%, 99%, and 40%, respectively) [16]. In our cohort, p16 predicted the presence of HPV DNA by PCR with a concordance consistent with previous reports [37]. Our observation that 42% of p16 positive cases were negative for HPV by PCR suggests that p16 may not be a good surrogate marker for HPV status in the larynx and hypopharynx. However, it should be noted that these studies are not directly comparable due to the use of HPV DNA in situ hybridization rather than PCR, which was used in our study. There is moderate agreement between the two tests but a consensus has not been reached with regard to the optimal confirmatory assay [40,41]. A consensus guideline for a standard assay confirmatory high-risk HPV assay is needed. It should also be noted that the presence of HPA DNA is highly specific but not sensitive for HPV viral activity, potentially limiting the utility of HPV DNA PCR as the standard assay.

This study is limited by its retrospective nature, rendering it subject to implicit selection biases related to treatment and identification of HPV and unavoidably inherent testing bias. In an attempt to narrow the scope of the study and define a subgroup that would be most likely to benefit in the case of HPV positivity, we selected patients with stage III-IV disease treated with definitive chemoradiotherapy, adding further bias to the sample. The findings of this study are limited to hypothesis generation and should be confirmed or refuted by prospective study using a consensus guideline-adherent standard assay.

In summary, HPV-associated squamous cell carcinoma of the larynx or hypopharynx was identified in 13% of patients treated at an academic institution in the southern United States. Improvements in OS, DFS and LRC were noted, though none of these reached statistical significance. Laryngectomy-free survival in a cohort of patients treated with organ-preserving CRT was higher in HPV-positive compared to HPV-negative disease, though this difference was also not statistically significant. Poor concordance was found between the surrogate marker p16 and HPV DNA PCR, and a high proportion of other high-risk HPV types was detected. Prospective evaluation of the prevalence and clinical impact of HPV status on laryngeal and hypopharyngeal squamous cell carcinoma is warranted.

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Declaration of Competing Interest

RH: None Declared.
WB: None Declared.
SO: None Declared.
MP: None Declared.
TL: None Declared.
JW: None Declared.
BF: None Declared.
KG: None Declared.

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