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Detailed Analysis of Clinicopathologic Factors Demonstrate Distinct Difference in Outcome and Prognostic Factors Between Surgically Treated HPV-Positive and Negative Oropharyngeal Cancer

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Abstract

Background—Oropharyngeal cancers (OPC) secondary to human papillomavirus (HPV) infections likely represent a completely different disease compared with conventional head and neck cancers. Our objective was to analyze a surgically treated cohort to determine predictors of outcome in HPV-positive versus HPV-negative patients.

Methods—HPV positivity was inferred based on p16-immunohistochemistry. Data was available for 201 patients with OPC treated with surgical resection with/without adjuvant radiotherapy between 1985 and 2005. Subsite distribution was: 66 (33 %) tonsil, 46 (23 %) soft palate, and 89 (44 %) tongue base. Patients were classified into low-, intermediate-, and high-risk groups based on p16 status and smoking history. Outcomes stratified by p16 status and risk groups were determined by the Kaplan–Meier method. Factors predictive of outcome were determined by univariate and multivariate analyses.

Results—In this cohort, 30 % had locally advanced disease (pT3/T4) and 71 % had nodal metastasis. The 5-year overall (OS), disease-specific, and recurrence-free survival rates were 60, 76, and 66 %, respectively. There were 22 % low-, 34 % intermediate-, and 44 % high-risk patients. Patients who were p16-positive had better survival compared with p16-negative (OS, 74 vs. 44 %; $p < .001$). Similarly, low-risk group patients had a better survival compared with intermediate- and high-risk groups (OS, 76, 68, 45 %, respectively, $p < .001$). Independent

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predictors of survival in p16-negative patients included margin status, lymphovascular invasion, pN status, and extracapsular spread. In contrast, none of these were predictive in p16-positive patients.

Conclusions—Surgically treated patients with p16-positive OPC have superior survival compared with p16-negative patients. Outcomes in p16-positive and p16-negative OPC are determined by different prognostic factors supporting the notion that these are very different diseases. These should be incorporated into future clinical trials design.

The changing trend in the epidemiology of oropharyngeal cancer (OPC) and association with the human papillomavirus is a major paradigm shift in head and neck cancer.^{1,2} Unlike other head and neck squamous cell cancers (HNSCC), the incidence of OPC has been steadily increasing over the last 2 decades, with an annual incidence of 8500 cases/year in the United States alone, and it is estimated that 40 %–70 % of these are due to HPV infection.^{1,3,4} Outcome data have shown that HPV positivity confers a better prognosis than HPV-negative tumors, an effect that is usually demonstrated in patients treated with primary radiotherapy.^{5,6} These data have called for oncologists to re-evaluate management protocols and consider de-escalation of current treatment regimens in HPV-associated OPCs. Currently, most centers use regimens of either cisplatin-based concurrent chemoradiotherapy (CRT) or taxol/cisplatin/5-fluorouracil (TPF)-based induction CRT. These are fraught with complications not commensurate with a new disease where patients are younger, live longer, rarely develop second primary cancers and will experience delayed effects of radiation.^{7–9} Apart from de-escalation using lower radiation doses or less toxic agents, an alternative involves the use of primary surgery as the primary modality of treatment.¹⁰ With the advent of new techniques and introduction of transoral laser (TLS) and robotic surgery (TORS), it is now possible to resect tumors without the need for mandibulotomy. Excellent preliminary results have been reported for tonsil and base of tongue cancers.^{11–15} Several trials are currently underway or completed accrual in the United States and Europe, and results from these are currently awaited.

Primary surgery also offers additional staging information from the histopathological specimen, from the primary tumor and/or nodal stations.^{16,17} Surgical staging can be used to determine subsequent adjuvant therapy or avoid it altogether.¹⁸ However, in order for this concept to work, 2 further steps have to take place. First, there is a need for robust analysis of surgical data, in multivariable models to determine the major prognostic factors in the current setting of HPV-associated OPCs. Second is to conduct prospective studies based on these prognostic factors. Previous studies aggregate OPCs into a single entity, and instead these need to be re-examined by separating into HPV-positive and HPV-negative cases, analyzing them as separate and distinct diseases.

Here, we re-examined surgically managed OPCs treated at Memorial Sloan-Kettering Cancer Center (MSKCC) from 1985 to 2005. The objectives were to determine p16 status in this surgically treated cohort, determine its effect on prognosis, and determine predictors of outcome in HPV-positive versus HPV-negative patients.

PATIENTS AND METHODS

After IRB approval, 300 patients with OPC treated with open surgical resection with or without adjuvant radiotherapy (PORT) at MSKCC between 1985 and 2005 were identified. Only patients who underwent primary surgery with curative intent were included in this study. Patients who had previous treatment elsewhere for their cancer, did not complete all components of their treatment at MSKCC, or underwent surgery for salvage treatment were excluded. Patients with recurrent tumors at presentation, with tumors arising from other sites with extension to the oropharynx or who had a previous attempt at definitive surgery were also excluded. Tissue was available for p16 analysis in 201 patients. All pathology specimens were re-examined by a pathologist specializing in head and neck pathology (SD) for p16 status and high-risk histopathologic features: T- and N-stage, extracapsular spread (ECS), perineural (PNI), lymphovascular invasion (LVI), margin status, and tumor grade. p16 immunohistochemistry was performed: The 4-micrometer tumor sections were deparaffinized, and after heat-induced epitope retrieval, immunohistochemistry for p16INK4a was performed with the primary antibody dilution of 1:7 as per manufacturer's protocol (CINtec Histology Kit, catalog No. 9517, Roche mtm Laboratories AG, Heidelberg, Germany). Cases with nuclear and cytoplasmic immunolabeling in at least 70 % of the tumor cells were considered positive for p16. In this cohort, there were 66 (33 %) tonsil, 46 (23 %) soft palate, and 89 (44 %) base of tongue cancers. All patients had surgery with 181 (90 %) having neck dissections and 138 (69 %) having adjuvant RT. None had postoperative chemotherapy.

Median age was 58 years (range 27–84 years) with 138 (69 %) males, 164 (82 %) ever-alcohol drinkers, and 167 (83 %) ever-smokers. Also, 80 (40 %) had advanced stage primary tumors (T3/T4) and 136 (68 %) had clinically positive neck disease (Supplementary Table 1); 60 (30 %) patients had pT3/4 tumors with 94 (47 %) having close [38 (19 %)] or positive [56 (28 %)] margins (Supplementary Table 2). Close margins were defined as any margin <5 mm. PNI and LVI were present in 42 (21 %) and 49 (24 %) patients, respectively. Nodes were pathologically positive in 142 (71 %) patients, with ECS in 69 (34 %). A total of 106 tumors (53 %) were p16 positive. From 1985 to 2005 the prevalence of p16 positivity increased from 37 % (1985–1989) to 69 % (2000–2005) (Supplementary Fig. 1). Patients were reclassified into low-, intermediate-, and high-risk groups based on p16 status and smoking history (low risk: p16 positive, <10 pack/years tobacco consumption [pyr]; high risk: p16 negative, >10 pyr; intermediate risk: p16 positive, >10 pyr or p16 negative, < 10 pyr). There were 196 patients for whom smoking and p16 status was available: 43 (22 %) low-, 66 (34 %) intermediate-, and 87 (44 %) high-risk patients.

Factors predictive of outcome in p16-positive and p16-negative cohorts were determined separately. Statistical analysis was carried out using SPSS (ver21, IBM Corp., Armonk, NY). Pearson's χ^2 test was used to compare variables within groups. Overall survival (OS), disease-specific survival (DSS), and recurrence-free survival (RFS) were determined by the Kaplan–Meier method. Univariate and multivariate analyses were carried out by log-rank test and Cox proportional hazard model, respectively; *p* values less than .05 were considered significant.

RESULTS

There were 123 deaths (47 cancer-specific). A total of 67 (33 %) patients had recurrences: 37 (15 %) local, 20 (10 %) regional, and 28 (14 %) distant. The 5-year OS, DSS, and RFS for the entire cohort were 60, 76, and 66 %, respectively (Supplementary Fig. 2). The 5-year local, regional, and distant RFS were 89, 90, and 89 %, respectively.

p16-positive tumors tend to be seen in younger patients ($p < .001$), males ($p = .012$), nonsmokers ($p < .001$), and nondrinkers ($p = .012$), are more likely to occur in the tonsils, and are uncommon in the soft palate ($p < .001$) (Supplementary Table 1). These are often poorly differentiated ($p < .001$) and lower T stage (cT, $p = .031$; pT, $p = .028$) but show more lymph node metastasis (cN and pN, $p = .001$) (Supplementary Table 2). The incidence of positive/close margins, LVI, and ECS were similar ($p = .646$, $p = .674$, and $p = .365$, respectively).

Patients who were p16 positive had better survival compared with p16-negative patients: OS, 74 vs. 44 % ($p < .001$) and DSS, 84 vs. 66 % ($p < .008$), respectively (Fig. 1A). Stratifying by risk groups, low-risk patients had a better 5-year OS and DSS compared with intermediate-risk and high-risk groups: OS, 76 vs. 68 vs 45 % ($p < .001$) and DSS, 89 vs. 78 vs. 75 % ($p = .04$), respectively (Fig. 1B).

Predictors of Outcome in p16-Negative Patients

For OS, age, margin status, LVI, and ECS were prognostic on univariate analysis (Table 1). In multivariate analysis, LVI, margin status, and ECS remained significant (Table 3). Patients with LVI were 2.1 times more likely to die compared with those with no LVI ($p = .010$) or close/positive margins ($p = .020$). Patients with ECS were 1.7 times more likely to die compared with those without ($p = .05$).

For DSS, pathological N status, margin status, LVI and ECS were prognostic on univariate analysis (Table 1). In multivariate analysis, margin status and ECS remained significant (Table 3). Patients with close/positive margins were 3.2 times more likely to die compared with those with negative margins ($p = .015$). Patients with ECS were 4.7 times more likely to die compared with those without ($p = .019$).

For RFS, margin status and LVI were prognostic on univariate analysis (Table 1). On multivariate analysis, only LVI remained significant (Table 3). Patients with LVI were 2.7 times more likely to recur compared with patients without ($p = .01$).

Predictors of Outcome in p16-Positive Patients

For OS, pT status, PNI, and PORT were prognostic on univariate analysis (Table 2). In multivariate analysis (Table 3), pT status and PORT remained significant. Patients with pT3/4 tumors were 3.7 times more likely to die compared with pT1/2 ($p < .001$). Patients who did not receive PORT were 2.7 times more likely to die compared with those who did ($p = .015$).

For DSS, tumor subsite, cT status and pT status were prognostic on univariate analysis (Table 2). In multivariate analysis, both tumor subsite and pT status remained significant (Table 3). Patients with pT3/4 tumors were 3.5 times more likely to die compared with pT1/2 ($p = .004$). Patients with soft palate cancers were 4.8 times more likely to die compared with those with tonsil and base of tongue cancers ($p = .016$).

For RFS, cT status, pT status, and PNI were prognostic on univariate analysis (Table 2). On multivariate analysis, both pathological T status and PNI remained significant (Table 3). Patients with pT3/4 tumors were 5.2 times more likely to recur compared with pT1/2 ($p < .001$). Patients with PNI were 3 times more likely to recur compared with those without ($p = .016$).

Differences in Prognostic Factors Between p16+ and p16– Oropharyngeal Cancers

Conventional prognostic factors of poor outcome in HNSCC were compared between p16-positive and p16-negative patients for DSS (Fig. 2). Marked differences were seen between the 2 cohorts when stratifying by pT, pN, margin, and ECS status. Kaplan–Meier plots show pT status to be a significant predictor of survival in p16-positive patients, while having no impact in p16-negative patients. pN status showed a trend toward significance in p16-negative patients while having no impact on p16-positive patients. Margin status was significant in p16-negative patients, while having minimal effect on prognosis in p16-positive patients. ECS was also significant in p16-negative patients with a lesser effect (if any) in p16-positive patients.

DISCUSSION

The main hypothesis in this study is that HPV-associated OPC is a distinct disease compared with HPV-negative cancers. We therefore examined a series of patients who underwent conventional surgery and had full clinicopathologic data available. The objectives were to establish the incidence of HPV, determine if HPV/p16-positivity and risk classification defined by p16 and smoking status were prognostic in a surgical cohort, and identify prognostic factors in p16-positive compared with p16-negative cohorts through rigorous multivariate analysis.

Not surprisingly, data from this cohort show that the rate of p16-associated oropharyngeal cancers has been increasing during the last 2 decades, in accordance with national and international data.^{2,5,19–21} p16 positivity is largely confined to SCC arising from the tonsils and tongue base, while the proportion of p16-positive soft palate cancer is only 8 %. These data support the notion that HPV-associated disease predominates in subsites with mucosa-associated lymphoid tissue and should be considered differently, rather than defining oropharynx as a purely anatomic site, which includes the soft palate and lateral and posterior pharyngeal walls.^{16,17} In agreement with previous reports, patients with p16-positive tumors were younger, nonsmokers, had lower T-stage, and had higher likelihood for nodal metastases.^{2,22} Interestingly, these tumors tend to be more common in males ($p = .012$), providing a strong basis for advocating HPV vaccination in boys, as well as girls.²³

Notwithstanding higher nodal metastasis rates, p16 positivity confers better prognosis compared with p16-negative tumors in this surgically treated cohort. HPV-associated oropharyngeal cancers have good outcomes, and there are a number of studies that have shown that the same excellent prognosis is also seen in patients managed with primary surgery, either open or transoral laser/robotic surgery.^{6,24} Indeed, in patients who are exclusively p16-positive, Haughey and Sinha demonstrated 5-year OS, DSS, and DFS of 91, 94, and 88 %, respectively.²⁵ These figures also compare favorably with large series from MSKCC and MD Anderson Cancer Center, in which patients were treated with primary radiotherapy.^{26,27} These data provide compelling evidence for the use of primary surgery for oropharyngeal cancer in selected cases. Importantly, we also went on to validate the risk-stratification system applied to patients in a prospective trial (RTOG0129) in a surgically treated cohort, albeit using a simplified risk-stratification based on HPV and smoking status.⁵ Outcome data showed a statistically significant difference between the 3 groups for overall and disease-specific survival. A striking 31 % difference in 5-year overall survival was seen between low-risk versus high-risk groups, not taking into account any other clinicopathological features. This concept is important in the current environment where a significant amount of effort and cost is spent on imaging to establish clinical staging, while a simple history on tobacco consumption and single immunohistochemistry test may provide equally robust if not better prognostication of disease.¹⁸

The final objective of this study was to identify clinicopathologic factors that determine outcome using the data available from the surgical specimen. Association data demonstrates that p16-positive and p16-negative cancers have vastly different epidemiological and pathologic profiles, suggesting that these are different diseases. We therefore hypothesized that prognostic factors would also differ. This is an issue of current interest because of the increasing practice of using TLS/TORS in managing OPC. The first advantage is the potential to avoid or reduce doses of adjuvant treatment in well-selected groups, which at the moment extends only to patients with completely resected T1/T2 tumors with no nodal metastasis or N1 disease with no ECS.^{12,28} Previous studies have shown this approach can spare unnecessary radiation in 20–40 % of well-selected cohorts.¹⁶ Secondly, the availability of a surgical specimen allows meticulous identification of other high-risk features that may guide adjuvant treatment. However, there is a long list of putative high-risk features, and the relative contribution of each of these has not been previously established through multivariable analysis. The study presented here sets out to identify these and would therefore provide benchmark outcome figures for future trials using TLS or TORS.

The analyses of this surgical cohort show striking differences between the p16-positive and p16-negative cohorts. In the p16-negative cohort, traditional risk factors such as margin status, LVI, and ECS were prognostic.²⁹ Surprisingly, none of these were prognostic in the p16-positive cohort. Instead, pathological T status and tumor subsite were the only prognostic factors. The recognition that margin status and pathological neck disease were not prognostic is a surprising observation and illustrates that traditional risk factors associated with head and neck squamous cell cancer do not apply in the HPV-positive population. Although pN status was not prognostic in the p16-positive group, it is important to point out that the presence of ECS had a negative impact on DSS in p16-negative patients and to a lesser extent on p16-positive patients (statistically not significant). These data

suggest that the size and number of pathological nodes (which determine N status currently) is of less importance in p16-positive disease compared with the presence of ECS, and perhaps it is timely that staging for p16-positive disease be reconsidered in light of such data. This has important implications when planning adjuvant treatment and for the design of future trials. The recommendation of postoperative concurrent chemoradiation therapy is based on 2 studies published in 2004 (RTOG-9501 and EORTC-22931).^{28,30,31} In both studies, margin status (and ECS) was shown to be one of the consistent pathologic risk factors where there appears to be advantage in the addition of chemotherapy to adjuvant radiotherapy.²⁸ This is corroborated in our p16-negative cohort, but is not the case in p16-positive disease. Although ECS is important, the impact that margin status has on DSS is of less importance, and this raises questions about the use of margin status as an indication for adjuvant chemoradiation in these patients.

The data presented here also shows LVI to be an important risk factor for outcome, in p16-negative disease. Based on Bernier's and Cooper's trials, LVI alone would not have been one of the pathologic criteria for adjuvant chemoRT and hence ignored in future trial design. Our data strongly suggests that this needs to be examined in larger surgical cohorts and revisited in light of the data presented here.

Although our study has produced important comparisons of prognostic factors in the p16-positive and p16-negative oropharynx cancer patients, there are a number of limitations. Firstly, the data is retrospective and susceptible to inherent biases. However, given the large sample size, long follow-up, and consistent surgical management philosophy over 20 years, there is significant merit to this data. The choice of surgical treatment and postoperative management was carried out during a time when the impact of HPV on outcome was unknown, and therefore the influence of HPV on treatment decisions will be absent. This will limit any selection bias associated with this cohort. Secondly, with retrospective data the level of detail in pathology reporting is often heterogeneous. To overcome this, one pathologist (S.D.) reviewed all cases and reported margin status, PNI, pathological N status, and ECS in available slides, hence limiting any bias associated with pathological reporting.

We therefore conclude that the HPV/p16 status and risk factor stratification based on smoking and HOV/p16 status holds true for surgically managed oropharyngeal cancers. Importantly, patients with p16-positive and p16-negative tumors have different predictors of outcome. While traditional prognostic factors of outcome hold true in the p16-negative patients, this is not the case for patients with p16-positive oropharynx cancer. These differences must be taken into account when adjuvant treatments are being considered. Factors, including LVI, should be re-examined and incorporated in future trial design for adjuvant therapy in surgically managed oropharyngeal cancers. The current staging system used for p16-positive/HPV-associated oropharyngeal cancers also needs to be revised to reflect our better understanding of a very different disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1. Kaplan-Meier plots showing overall (OS) and disease-specific (DSS) survival stratified by p16 status (**a**) and stratified by risk group category (**b**). *p* values based on log-rank test

FIG. 2. Kaplan-Meier plots showing impact of prognostic factors on disease-specific survival (DSS) in p16-positive and p16-negative patients: **a** pT classification. **b** pN classification. **c** Margin status. **d** Extracapsular spread (ECS). 5-year DSS and p values based on log-rank test

Factors predictive of overall survival (OS), disease-specific survival (DSS), recurrence-free survival (RFS) in p16-negative patients based on univariate analysis

TABLE 1

	<i>n</i>	5-year OS (%)	<i>p</i> value	5-year DSS (%)	<i>p</i> value	5-year RFS (%)	<i>p</i> value
Subsite							
Tonsil	18	47.2	.250	66.0	.385	73.3	.362
Soft palate	38	44.3		70.7		42.5	
Base of tongue	39	42.2		60.6		53.0	
Gender							
Female	38	45.5	.749	63.3	.893	48.8	.863
Male	57	42.9		68.1		54.6	
Age							
60	42	60.9	.000 ^a	72.2	.162	57.6	.471
>60	53	30.2		58.2		46.9	
Smoking							
Low risk (<10 py)	7	42.9	.824	66.7	.830	25.7	.082
High risk (>10 py)	87	44.7		66.8		54.8	
Alcohol							
Never	10	40.0	.369	56.0	.450	42.0	.670
Ever	85	44.4		67.2		53.8	
cT							
T1	22	46.7	.863	82.6	.310	62.9	.728
T2	27	50.1		63.3		47.4	
T3	31	36.9		58.4		50.1	
T4	15	42.9		61.1		49.0	
cN							
N0	40	47.0	.314	80.1	.067	58.0	.817
N1	25	46.2		59.2		50.1	
N2	28	37.1		55.4		45.3	
N3	2	50.0		50.0		50.0	
N–	40	47.0	.238	80.1	.013 ^a	58.0	.335

	<i>n</i>	5-year OS (%)	<i>p</i> value	5-year DSS (%)	<i>p</i> value	5-year RFS (%)	<i>p</i> value
N+	55	41.6		56.5		48.4	
pT							
T1	23	45.2	.701	70.7	.691	46.6	.889
T2	40	46.3		65.4		53.3	
T3	15	43.1		76.2		56.3	
T4	17	37.5		54.2		56.3	
pN							
N0	25	51.4	.248	76.7	.051 ^a	58.8	.436
N1	17	47.1		72.2		61.1	
N2/N3	39	32.7		50.0		49.5	
N-	25	51.4	.097	76.7	.134	58.8	.428
N+	56	37.2		56.7		52.9	
pTNM							
Stage 1	17	49.4		81.5		45.7	
Stage 2	14	54.2		79.5		53.9	
Stage 3	18	55.6		78.0		65.2	
Stage 4	46	37.1	.358	52.8	.053 ^a	50.0	.565
Grade							
Well	14	42.9	.549	64.3	.917	57.1	.897
Moderately	47	47.1		65.9		54.7	
Poorly	50	38.9		63.7		49.0	
Margins							
Negative	48	59.5	.000 ^a	79.0	.019 ^a	63.1	.102
Close	18	43.9		61.1		38.3	
Positive	28	18.6		39.0		45.5	
Negative	48	59.5	.000 ^a	79.0	.009 ^a	63.1	.035 ^a
Close/positive	46	28.1		50.0		40.6	
PNI							
No	52	48.2	.466	61.1	.754	49.6	.470
Yes	21	37.5		59.5		61.4	
LVI							

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	<i>n</i>	5-year OS (%)	<i>p</i> value	5-year DSS (%)	<i>p</i> value	5-year RFS (%)	<i>p</i> value
No	51	57.0	.001 ^a	73.9	.002 ^a	63.0	.000 ^a
Yes	24	18.5		33.3		28.6	
ECS							
No	61	53.3		76.1		57.3	
Yes	29	28.6	.047 ^a	41.2	.002 ^a	41.3	.137
PORT							
No	38	48.4	.456	73.7	.113	49.7	.691
Yes	57	41.2		61.2		54.0	

^aStatistically significant

Factors predictive of overall survival (OS), disease-specific survival (DSS), recurrence-free survival (RFS) in p16-positive patients based on univariate analysis

TABLE 2

	<i>n</i>	5-year OS (%)	<i>p</i> value	5-year DSS (%)	<i>p</i> value	5-year RFS (%)	<i>p</i> value
Subsite							
Tonsil	48	74.1	.238	89.4	.037 ^a	87.1	.181
Soft palate	8	42.9		51.4		53.6	
Base of tongue	50	77.8		83.8		73.2	
Gender							
Female	25	67.8	.393	81.6	.845	72.7	.995
Male	81	75.8		84.7		79.1	
Age							
60	67	73.7	.457	83.4	.658	76.7	.710
>60	39	73.8		85.0		79.3	
Smoking							
Low risk (<10 py)	43	75.9	.221	89.0	.266	81.1	.537
High risk (>10 py)	59	70.5		79.2		73.2	
Alcohol							
Never	23	77.6	.965	89.5	.926	75.9	.553
Ever	79	72.6		82.7		78.2	
cT							
T1	21	79.3	.116	94.4	.014 ^a	95.0	.051 ^a
T2	51	77.8		81.2		76.5	
T3	21	76.2		94.1		76.9	
T4	13	46.2		60.0		50.0	
cN							
N0	25	68.0	.525	82.0	.467	64.3	.177
N1	18	70.6		79.0		79.4	
N2	60	75.8		85.4		81.9	
N3	3	100.0		100.0		100.0	
N–	25	68.0	.396	82.0	.145	64.3	.032

	<i>n</i>	5-year OS (%)	<i>p</i> value	5-year DSS (%)	<i>p</i> value	5-year RFS (%)	<i>p</i> value
N+	81	75.6		84.6		81.9	
pT							
T1	41	87.5	.010 ^a	92.2	.026 ^a	89.9	.003 ^a
T2	37	80.4		90.5		80.5	
T3	6	50.0		62.5		44.4	
T4	22	45.5		60.6		55.6	
pN							
N0	14	78.6	.762	92.3	.909	76.9	.876
N1	15	78.6		84.6		84.4	
N2/N3	71	73.9		83.9		78.8	
N-	14	78.6	.473	92.3	.681	76.9	.611
N+	86	74.6		84.0		80.0	
pTNM							
Stage 1	7	71.4		83.3		85.7	
Stage 2	7	85.7		85.7		57.1	
Stage 3	12	72.7		80.0		80.8	
Stage 4	80	73.1	.830	84.2	.830	78.4	.740
Grade							
Well	4	50.0	.516	100.0	.137	66.7	.568
Moderately	47	65.7		76.3		69.9	
Poorly	50	83.2		90.9		86.0	
Margins							
Negative	54	79.6	.256	84.1	.787	78.1	.648
Close	20	68.0		82.6		66.7	
Positive	28	62.7		82.1		82.1	
Negative	54	79.6	.396	84.1	.592	78.1	.438
Close/positive	48	64.9		82.3		74.9	
PN1							
No	67	75.2	.009 ^a	86.6	.113	83.7	.009 ^a
Yes	21	61.5		76.5		63.3	
LVI							

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	<i>n</i>	5-year OS (%)	<i>p</i> value	5-year DSS (%)	<i>p</i> value	5-year RFS (%)	<i>p</i> value
No	63	77.0	.322	84.1	.791	80.5	.771
Yes	25	59.3		84.8		76.2	
ECS							
No	64	80.9		91.2		80.8	
Yes	40	63.2	.300	74.0	.116	73.7	.753
PORT							
No	25	62.5	.017 ^a	72.7	.245	66.0	.176
Yes	81	77.3		86.7		80.3	

^aStatistically significant

TABLE 3

Factors predictive of overall survival (OS), disease-specific survival (DSS), recurrence-free survival (RFS) in p16-negative and p16-positive patients based on multivariate analysis

Variable	OS ^b			DSS ^c			RFS ^d		
	RR	95 % CI	p value	RR	95 % CI	p value	RR	95 % CI	p value
<i>HPV or p16 negative</i>									
Age									
60	Reference								
>60	1.7	1.0–3.1	.071 ^a						–
LVI									
No	Reference			Reference			Reference		
Yes	2.1	1.2–3.8	.010 ^a	2.1	0.9–5.0	.082	2.7	1.3–5.8	.01
Margins									
Negative	Reference			Reference			Reference		
Close/positive	2.1	1.1–3.9	.020 ^a	3.2	1.3–7.9	.015 ^a	1.6	0.7–3.4	.234
pN group									
N0	–			Reference					–
N+				0.5	0.1–1.9	.300			
ECS									
No	Reference			Reference			Reference		
Yes	1.7	1.0–2.9	.053 ^a	4.7	1.3–17.1	.019 ^a	1.5	0.8–3.1	.244
Variable	OS ^e			DSS ^f			RFS ^g		
	RR	95 % CI	p value	RR	95 % CI	p value	RR	95 % CI	p value
<i>HPV or p16 positive</i>									
Subsite									
Tonsil/BOT	–			Reference					–
Soft palate				4.8	1.3–17.2	.016			
PNI									

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No	Reference		Reference	–	Reference	–	
Yes	1.7	0.8–3.5	.185	–	3.0	1.2–7.5	.016
pT group							
T1/T2	Reference		Reference		Reference	–	
T3/T4	3.7	1.8–7.6	<.001 ^a	3.9	5.2	2.1–12.7	<.001
PORT				1.5–10.0	.004		
Yes	Reference					–	
No	2.7	1.2–5.9	.015	–			
ECS							
No							
Yes	–			–			

^aStatistically significant

^bModel for OS: age, LVI, margin, ECS

^cModel for DSS: LVI, margin, pN, ECS

^dModel for RFS: LVI, margin, ECS

^eModel for OS: pT, PNI, PORT

^fModel for DSS: pT, subsite

^gModel for RFS: pT, PNI