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Impact of p16 expression in oropharyngeal cancer in the postoperative setting: the necessity of re-evaluating traditional risk stratification

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Abstract

Objective: To evaluate the impact of p16 expression as a surrogate marker of human papillomavirus status in oropharyngeal squamous cell carcinoma patients underwent surgery followed by postoperative radiotherapy.

Methods: We identified 126 consecutive patients with histologically confirmed, newly diagnosed oropharyngeal squamous cell carcinoma who received surgery followed by radiotherapy and had p16 expression data available. All patients were treated between 2001 and 2011. Patients with high-risk factors (positive surgical margin and/or extracapsular extension) or other risk factors (multiple positive lymph nodes, perineural/lymphovascular invasion) were offered postoperative radiotherapy with or without concurrent chemotherapy.

Results: One hundred and four (82.5%) patients were p16-positive (p16 (+)) and 22 (17.5%) were p16-negative (p16 (−)). With a median follow-up of 56 months, patients with p16 (+) oropharyngeal squamous cell carcinoma exhibited a significantly better 5-year disease-free survival (80.7% vs. 57.6%, $P < 0.001$) and overall survival (84.9% vs. 59.1%, $P < 0.001$) than those with p16 (−) tumors. The p16 (+) oropharyngeal squamous cell carcinoma with high-risk factors ($n = 64$) showed no difference in disease-free survival (79.7% vs. 68.3%; $P = 0.531$) and overall survival (82.1% vs. 76.2%; $P = 0.964$) between postoperative radiotherapy and postoperative radiotherapy with concurrent chemotherapy.

Conclusions: Expression of p16 is a strong independent prognostic factor of survival in the postoperative setting of oropharyngeal squamous cell carcinoma. The favorable prognosis of p16 (+) oropharyngeal squamous cell carcinoma suggests a need to re-examine traditional risk stratification for determining optimal adjuvant treatment.

Key words: oropharyngeal squamous cell carcinoma, HPV, p16, postoperative setting, risk stratification

Introduction

Over several decades, there has been a change in the etiology of oropharyngeal squamous cell carcinoma (OPSCC). Overall, this change may be attributed to decreased smoking and alcohol consumption and increased infection of human papillomavirus (HPV) (1–3). An estimated 70% of all OPSCCs are due to HPV. In particular, cancers of the palatine tonsils and the base of tongue may be up to 90% HPV-positive (2,4).

The presence of HPV infection in OPSCC is a major determinant in prognosis. Generally, patients with HPV-positive OPSCC have a superior outcome compared with patients with HPV-negative disease (3,5–7). However, this favorable prognosis of OPSCC patients with HPV-positive have been demonstrated in clinical studies that were focused on patients treated by primary radiotherapy (RT) or chemoradiotherapy (CRT). Therefore, the report of patients with HPV-associated OPSCC treated with surgical resection followed by postoperative RT (PORT) is relatively decimal.

In our institution, the application of surgery followed by PORT for OPSCC is the preferred course of treatment. It has been used increasingly under the influence of recent advances in robotic and transoral laser microsurgery. We evaluated the clinical outcome of patients with HPV-associated OPSCC based on p16 expression as surrogate marker of HPV status in the oropharynx who underwent surgical resection followed by PORT.

Methods

Study population

We identified 150 consecutive patients with histologically confirmed, newly diagnosed, and previously untreated OPSCC who received surgery followed by PORT with curative intent at our institution between January 2001 and December 2011. All clinical and pathological data regarding disease and treatment characteristics were reviewed. This retrospective review was approved by the Institutional Review Board. Five patients who received induction chemotherapy before surgical resection were excluded. And we excluded 19 patients with unavailable HPV tumor status via p16 immunohistochemical analysis. The 126 remaining patients were included for analysis.

All 126 patients underwent surgical resection for OPSCC. In our institution, transoral robotic surgery (TORS) has not been used commonly until 2011, although TORS was first applied in 2008. Based on final pathologic reports after surgery, patients with risk factors such as incomplete resection, extracapsular extension (ECE), close/positive surgical margin, multiple positive lymph node, perineural invasion (PNI) and lymphovascular invasion (LVI) were offered PORT. Since 2007, concurrent chemotherapy has been generally administered to patients with high-risk factors (positive surgical margin and/or ECE) and other combined risk factors, according to the clinical discretion of physician. RT doses for high-risk areas (residual tumor, positive surgical margin area, positive lymph node bed, especially with ECE and unresectable positive retropharyngeal lymph node) were 60–70 Gray (Gy). The doses for intermediate risk areas (positive tumor bed area, positive lymph node area and minimum of first nodal echelons beyond positive lymph node area) were 54–60 Gy. The doses for low-risk areas (contralateral neck node, lower neck) were 50–54 Gy.

Immunohistochemistry for p16

Pathological review was carried out by two pathologists who specialize in head and neck cancer. Eligible samples included histopathologically confirmed invasive OPSCC, and 126 tumor tissues could be retrieved from the pathology archives. To assess HPV status of each tumor, we examined p16 expression, which is recognized as a surrogate marker for HPV in the oropharynx, using formalin-fixed, paraffin-embedded (FFPE) surgical tissue. The p16-immunostaining was carried out with a CINtec TM Histology Kit (Roche MTM laboratories AG, Heidelberg, Germany), which contains the mouse monoclonal antibody INK4A that recognizes p16. Representative 4-μm tumor sections cut from FFPE tissue blocks were de-paraffinized. After heat-induced epitope retrieval, immunohistochemistry for $p16^{INK4a}$ was performed with a primary antibody dilution of 1:7 per manufacturer's protocol. Samples were considered p16-positive (p16 (+)) if strong and diffuse nuclear and cytoplasmic immunostaining was observed in at least 70% of the carcinoma tissue (8,9). Tissues with only faintly diffuse or no reactivity were considered to be p16-negative (p16 (−)).

Statistical analysis

Disease-free survival (DFS) was defined as the amount of time from the start of treatment to the date of any disease recurrence or death from any cause. Overall survival (OS) was calculated as the amount of time from the start of treatment to the date of death from any cause or last day when the patient was known to be alive. OS and DFS were estimated using the Kaplan–Meier method, and survival curves were compared using the log-rank test. To determine the effects of distinct prognostic factors on survival, multivariate analysis was performed according to the Cox's regression model in a stepwise backward elimination method. Differences in patient characteristics between p16 (+) and p16 (−) tumors were assessed using the Pearson χ^2-test. In all statistical analyses, $P < 0.05$ was considered to be significant. Statistical analysis was carried out using IBM SPSS Statistics version 20 (SPSS Inc., Chicago, IL, USA).

Results

Patient, tumor and treatment characteristics

Of the 126 patients with established p16 status, 104 (82.5%) were p16 (+) and 22 (17.5%) were p16 (−). Patients who were p16 (+) more commonly complained of a neck mass as initial symptom, whereas those who were p16 (−) had symptoms related to the primary tumor site ($P = 0.017$). Patients who were p16 (+) had tumors confined to the tonsil or base of tongue, while p16 (−) patients had tumors in all oropharyngeal sites ($P < 0.001$). The distribution of age at diagnosis ($P = 0.621$) and smoking history ($P = 0.536$) were not significantly different between the two cohorts. With respect to tumor characteristics, there were no significant differences in the distribution of risk factors between both groups. The patient and tumor characteristics were summarized in Table 1.

An open surgical approach was undertaken in 85.7%, whereas 14.3% underwent transoral robotic surgery. All patients, except for one, underwent neck dissection. PORT after surgical approach was offered to 116 patients with risk features of a close/positive margin, ECE, PNI, LVI and multiple positive lymph nodes, while the remaining 10 patients received PORT at the discretion of the physician. Of these 10 patients, 9 had a primary tumor larger than 3 cm and 1 had a pathological T4a tumor. Among all patients, 89 patients (70.6%) received PORT alone and 37 patients (29.4%) received PORT with concurrent chemotherapy or targeted agent. PORT was delivered by 3-dimensional conformal RT in 27 patients (21.4 %) and by intensity-modulated RT in 99 patients (78.6%). Of the

Table 1. Patient and tumor characteristics

Variables		All, $N = 126$	p16 expression		P
			p16 (+), $N = 104$	p16 (−), $N = 22$	
Age, years	Median (range)	58 (32–78)	58 (32–78)	62 (41–74)	
Age group, *n* (%)	<60 years	69 (54.8)	58 (55.8)	11 (50.0)	0.621
	≥60 years	57 (45.2)	46 (44.2)	11 (50.0)	
Sex, *n* (%)	Male	110 (87.3)	89 (85.6)	21 (95.5)	0.206
	Female	16 (12.7)	15 (14.4)	1 (4.5)	
Subsite, *n* (%)	Tonsil	10 (80.2)	89 (85.6)	12 (54.5)	<0.001
	Base of tongue	19 (15.1)	15 (14.4)	4 (18.2)	
	Soft palate	4 (3.2)		4 (18.2)	
	Posterior wall	2 (1.6)		2 (9.1)	
Initial symptoms, *n* (%)	Neck mass	63 (50.0)	57 (54.8)	6 (27.3)	0.017
	Sore throat/dysphagia	42 (33.3)	29 (27.9)	13 (59.1)	
	Tonsil lesion	21 (16.7)	18 (17.3)	3 (13.6)	
Smoking group, *n* (%)	Never	75 (59.5)	60 (57.7)	15 (68.2)	0.536
	<10 PY	3 (2.4)	3 (2.9)		
	≥10 PY	48 (38.1)	41 (39.4)	7 (31.8)	
ECOG, *n* (%)	0–1	122 (96.8)	100 (96.2)	22 (100)	0.350
	2–4	4 (3.2)	4 (3.8)		
Histologic differentiation, *n* (%)	WD	16 (12.7)	10 (9.6)	6 (27.3)	0.059
	MD	75 (59.5)	65 (62.5)	10 (45.5)	
	PD	30 (23.8)	26 (25.0)	4 (18.2)	
	UE	5 (4.0)	3 (2.9)	2 (9.1)	
Tumor size, cm	Mean (±SD)	3.0 (±1.1)	3.0 (±1.0)	3.4 (±1.7)	0.076
Metastatic LN size, cm	Mean (±SD)	2.2 (±1.3)	2.3 (±1.3)	1.8 (±1.3)	0.440
cT stage, *n* (%)	II/III	115 (91.2)	95 (91.3)	20 (91.0)	0.947
	IV	11 (8.8)	9 (8.7)	2 (9.0)	
cStage, *n* (%)	II/III	33 (26.2)	26 (25.0)	7 (31.8)	0.509
	IV	93 (73.8)	78 (75.0)	15 (68.2)	
pT stage, *n* (%)	II/III	110 (87.3)	90 (86.5)	20 (91.0)	0.576
	IV	16 (12.7)	14 (13.5)	2 (9.0)	
pStage, *n* (%)	II/III	31 (24.6)	25 (24.0)	6 (27.3)	0.749
	IV	95 (75.4)	79 (76.0)	16 (72.7)	
Surgical margin, *n* (%)	Negative	78 (61.9)	62 (59.6)	16 (72.7)	0.250
	Positive	48 (38.1)	42 (40.4)	6 (27.3)	
ECE, *n* (%)	Absent	77 (61.6)	64 (61.5)	14 (63.6)	0.854
	Present	48 (38.4)	40 (38.5)	8 (36.4)	
PNI, *n* (%)	Absent	112 (88.9)	93 (89.4)	19 (86.4)	0.678
	Present	14 (11.1)	11 (10.6)	3 (13.6)	
LVI, *n* (%)	Absent	93 (73.8)	74 (71.2)	19 (86.4)	0.140
	Present	33 (26.2)	30 (28.8)	3 (13.6)	

PY, pack-years; ECOG, Eastern Cooperative Oncology Group; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; UE, unevaluable; LN, lymph node; cT stage, clinical tumor stage; cStage, clinical stage; pT stage, pathological tumor stage; pStage, pathological stage; ECE, extracapsular extension; PNI, perineural invasion; LVI, lymphovascular invasion.

37 patients (40%) with PORT and concurrent chemotherapy, 34 received cisplatin-based chemotherapy, whereas 2 received cetuximab and 1 received TS-1. The detailed treatment characteristics are shown in Table 2. In addition, we summarized the characteristics of the patients according to adjuvant therapy (PORT vs. PORT with chemotherapy) in Table 3. Distributions for each risk factor and combined risk factors were significantly different between patient groups receiving both adjuvant treatments

Outcomes and prognostic factors in entire patients

With a median follow-up of 56 months (range, 4–157 months), 20 patients (15.9%) developed recurrences, including 9 loco-regional recurrences, 9 distant metastases and 2 both loco-regional and distant metastases. We identified 13 recurrences (12.5%; 5 loco-regional, 6 distant metastases, and 2 both loco-regional and distant metastases) in the p16 (+) patients and 7 recurrences (31.8%; 4 loco-regional and 3 distant metastases) in the p16 (−) patients. By analyzing these recurrences, we found significantly fewer recurrences in p16 (+) patients than in p16 (−) patients (12.5% vs. 31.8%, $P = 0.024$). However, there was no significant difference in patterns of failure ($P = 0.062$) between the two groups. Furthermore, we observed that both loco-regional recurrence rate (6.7% vs. 18.2%, $P = 0.084$) and distant metastasis rate (7.7% vs. 13.6%, $P = 0.370$) between both groups were comparable. The 5-year DFS was 76.7% for the entire patient cohort. Patients with p16 (+) OPSCC exhibited a significantly better 5-year DFS than those with p16 (−) OPSCC (80.7% vs. 57.6%, $P < 0.005$, Fig. 1A). Univariate analysis revealed other independent predictors of DFS, including pathologic T stage ($P = 0.024$), PNI ($P = 0.007$) and LVI ($P = 0.028$). On multivariate Cox regression model in a stepwise method, p16 (+) status (hazard ratio [HR], 0.19; 95% confidence interval [CI], 0.08–0.44, $P < 0.001$), pathologic T4 stage (HR, 2.67; 95% CI, 1.09–7.34,

Table 2. Treatment characteristics

Variables		All, $N = 126$	p16 expression		P
			p16 (+), $N = 104$	p16 (−), $N = 22$	
Surgical approach, *n* (%)	Open procedure	108 (85.7)	87 (83.7)	21 (95.5)	0.151
	Transoral robotic surgery	18 (14.3)	17 (16.3)	1 (4.5)	
Type of neck dissection, *n* (%)	Selective neck dissection	19 (15.1)	15 (14.4)	4 (18.2)	0.827
	Modified neck dissection	61 (48.4)	52 (50.0)	9 (40.9)	
	Radical neck dissection	45 (35.7)	36 (34.6)	9 (40.9)	
	NA[a]	1 (0.8)	1 (1.0)		
Extent of neck dissection, *n* (%)	Unilateral	74 (58.7)	64 (61.5)	10 (45.5)	0.314
	Bilateral	51 (40.5)	39 (37.5)	12 (54.5)	
	NA[a]	1 (0.8)	1 (1.0)		
Number of dissected neck node[b]	Median (range)	52 (10–149)	51 (12–118)	59 (10–149)	0.447
Number of metastatic neck node[b]	Median (range)	2 (0–20)	2 (0–20)	3 (0–7)	0.836
Modality of RT, *n* (%)	Conventional	27 (21.4)	19 (18.3)	8 (36.4)	0.060
	IMRT	99 (78.6)	85 (81.7)	14 (63.6)	
Total dose of RT, Gy	Median (range)	63 (75.9–50.4)	63 (75.9–50.4)	63 (68.4–54.0)	0.852
Fractionated dose of RT, Gy	Median (range)	1.8 (1.5–2.3)	1.8 (1.5–2.2)	1.9 (1.5–2.3)	0.120
Adjuvant therapy, *n* (%)	RT alone	89 (70.6)	71 (68.3)	18 (81.8)	0.205
	CRT	37 (29.4)	33 (31.7)	4 (18.2)	
Regimen of concurrent chemotherapy[c], *n* (%)	Cisplatin	34 (91.9)	31 (93.9)	3 (75.0)	0.179
	Cetuximab	2 (5.4)	1 (3.0)	1 (25.0)	
	TS-1	1 (2.7)	1 (3.0)		

NA, not applicable; RT, radiotherapy; IMRT, intensity-modulated radiotherapy; Gy, Gray; CRT, chemoradiotherapy; TS-1, oral fluoropyrimidine anticancer drug.
[a]One patient refused the neck node dissection.
[b]Calculation only includes patients underwent neck dissection ($n = 125$).
[c]Calculation only includes patients treated with postoperative CRT ($n = 40$).

$P = 0.033$) and positive LVI (HR, 2.74; 95% CI, 1.25–6.00, $P = 0.01$) were significant prognosticators for DFS (Table 4).

Twenty-five patients died of the disease during the follow-up period. The 5-year OS in all patients was 80.4%. Patients with p16 (+) OPSCC had a significantly better 5-year OS than patients with p16 (−) OPSCC (84.9% vs. 59.1%, $P < 0.001$, Fig. 1B). In addition, univariate analysis demonstrated that both p16 expression status ($P < 0.001$) and PNI ($P = 0.048$) correlated significantly with OS rates, while surgical margin ($P = 0.053$) exhibited a correlative trend with OS. The stepwise multivariate analysis identified both p16 (+) expression (HR, 0.09; 95% CI, 0.03–0.23, $P < 0.001$) and positive surgical margin (HR, 2.50; 95% CI, 1.03–6.07, $P = 0.043$) as independent prognostic factors of OS (Table 4).

Outcomes in p16 (+) OPSCC patients with high-risk factors

To gain insights into optimized adjuvant therapy, we analyzed 64 patients with p16 (+) OPSCC with high-risk factors. The 5-year DFS and OS were 75.1% and 80.7%, respectively. In terms of adjuvant therapy (PORT alone vs. PORT with adjuvant chemotherapy), the full details of patients and their tumor characteristics treated with both treatments are listed in the Supplementary Table 1. The 5-year DFS did not differ significantly for patients who received PORT alone ($n = 35$) compared with those who received PORT with adjuvant chemotherapy ($n = 29$) (79.7% vs. 68.3%; $P = 0.531$) (Fig. 2A). Similarly, there were no significant differences in 5-year OS for patients who received PORT alone versus PORT with adjuvant chemotherapy (82.1% vs. 76.2%; $P = 0.964$) (Fig. 2B). The modality of adjuvant treatment (PORT vs. PORT with chemotherapy) did not significantly affect either DFS or OS in a multivariate Cox regression model in a stepwise method. Only LVI was identified as an independent risk factor for DFS (HR, 6.41; 95% CI, 1.27–32.41, $P = 0.025$) and OS (HR, 3.26; 95% CI, 1.14–9.30, $P = 0.027$). Data on multivariate analyses to assess potential prognostic factors for survival in p16 (+) OPSCC patients with high-risk factors are shown in Supplementary Table 2.

Discussion

This study showed the clinical significance of p16 expression in a retrospective, unselected cohort of 126 patients with OPSCC. In the period 2000–11, p16 positivity in our cohorts was 82.5%. This prevalence rate is comparable with that in the USA and Europe (1,2,10). Additionally, a meta-analysis suggests that the proportion of OPSCC associated with HPV has increased from 40.5% before the year 2000 to 72.2% after 2005 (2). We reported that patients with p16 (+) OPSCC, managed with surgery followed by PORT with or without chemotherapy, showed significantly better 5-year DFS (80.7% vs. 57.6%, $P < 0.001$) and OS survival (84.9% vs. 59.1%, $P < 0.001$) than those with p16 (−) tumors. This could be understood in the same context that, in the literature, HPV infection and/or p16 positivity are associated with improved survival in OPSCC patients treated with primary RT and CRT (5,6,11). Indeed, p16 (+) OPSCC are a distinct type of cancer with a generally better outcome than p16 (−) disease, which may be independent of the treatment modality chosen.

The standard of care of OPSCC is multimodality therapy based on several factors, including clinical stage, individual patient factors such as comorbidities and preferences, and particularly, the institutional preference based on clinical discretion of the physician. Recently data suggest that most institutions prefer primary RT/CRT to surgery followed by PORT, as the former helps to preserve the

Table 3. Comparison of characteristics according to adjuvant therapy ($N = 126$)

Variables		Adjuvant therapy		*P*
		RT alone, $N = 89$	CRT, $N = 37$	
Age, *n* (%)	<60 years	49 (55.1)	20 (54.1)	0.918
	≥60 years	40 (44.9)	17 (45.9)	
Smoking, *n* (%)	<10 PY[a]	59 (66.3)	19 (51.4)	0.116
	≥10 PY	30 (33.7)	15 (48.6)	
Histologic differentiation, *n* (%)	WD	11 (12.4)	5 (13.5)	0.736
	MD	55 (61.8)	20 (54.1)	
	PD	19 (21.3)	11 (29.7)	
	UE	4 (4.5)	1 (2.7)	
p16 expression	Negative	18 (20.2)	4 (10.8)	0.205
	Positive	71 (79.8)	33 (89.2)	
Tumor size, *n* (%)[b]	< 3 cm	38 (42.7)	18 (48.6)	0.475
	≥ 3 cm	48 (53.9)	19 (51.4)	
Metastatic LN size, *n* (%)[b]	< 2 cm	35 (42.7)	7 (20.0)	0.012
	≥ 2 cm	47 (57.3)	28 (80.0)	
pT stage, *n* (%)	II/III	76 (85.4)	34 (91.9)	0.318
	IV	13 (14.6)	3 (8.1)	
Surgical margin, *n* (%)	Negative	56 (62.9)	22 (59.5)	0.716
	Positive	33 (37.1)	15 (40.5)	
ECE, *n* (%)	Absent	67 (75.3)	11 (29.7)	<0.001
	Present	22 (24.7)	26 (70.3)	
Multiple metastatic LN	Absent	33 (37.1)	3 (8.1)	<0.001
	Present	56 (62.9)	34 (91.9)	
PNI, *n* (%)	Absent	86 (96.6)	26 (70.3)	<0.001
	Present	3 (3.4)	11 (29.7)	
LVI, *n* (%)	Absent	77 (86.5)	16 (43.2)	<0.001
	Present	12 (13.5)	21 (56.8)	
High-risk factors[c]	Absent	45 (50.6)	5 (13.5)	<0.001
	Present	44 (49.4)	32 (86.5)	
Risk factors[d]	Absent	34 (38.2)	5 (13.5)	
	Present	55 (61.8)	32 (86.5)	0.006

[a]Calculation includes patients had smoking history that both non-smoker and below 10 PY.

[b]Calculation includes only patients had pathological report.

[c]Includes features such as positive surgical margin and ECE.

[d]Includes features such as positive surgical margin, ECE, multiple metastatic LN, PNI and LVI.

organ in advanced OPSCC patients. Consequently, the majority of publications have focused on patients with OPSCC who received definitive RT. Generally, these conclusions regarding prognosis in HPV-positive OPSCC were based on results from tumors treated with definitive CRT (5–7,11). The prognosis of HPV-positive OPSCC after surgical resection and adjuvant PORT is relatively unclear because of a small number of clinical studies although this might be presumed good prognosis. Previous literature implied that HPV positivity is a predictor of prognosis for OPSCC in the postoperative setting. Haughey et al. reported that 171 p16 (+) OPSCC patients treated with transoral laser microsurgery had excellent survival outcomes including 5-year OS, disease-specific survival (DSS) and DFS of 91%, 94%, 88%, respectively (12). Rahmati et al. reported that patients who were p16 (+) had superior OS and DSS compared with patients who were p16 (−) (5-year OS, 74% vs. 47%; $P = 0.04$ and 5-year DSS, 89% vs. 66%; $P = 0.08$) (13). In our institution, surgery followed by RT was the mainstay for treating patients with OPSCC, based on clinical factors and physician discretion, including concern for the short-/long-term toxicity of RT. Recently, surgical management of OPSCC has seen increasing application with advances in minimally invasive surgery, such as robotic and transoral laser microsurgery. So, we assessed the prognosis of OPSCC managed with surgery followed by PORT stratified by p16 expression status. We confirmed that p16 (+) expression in OPSCC is an independent and favorable prognostic factor related to DFS and OS in these patients regardless of whether they received chemotherapy.

Currently, patients with HPV-positive OPSCC are treated similarly to age- and stage-matched HPV-negative counterparts although HPV testing of OPSCC is recommended for prognostic purposes. However, treatment goals and selection of therapy are debatable in these patients because HPV-positive OPSCC has a superior prognosis and a distinct patient profile, including younger age and good performance status (14). In other words, because patients with HPV-positive OPSCC are expected to live longer after treatment, avoiding late toxicity and maintaining quality of life (QOL) are particularly important. Accordingly, de-intensification of therapy may be appropriate for these HPV-positive OPSCC with good prognosis to improve associated morbidity and QOL (7,15).

Treatment strategies, including PORT and adjuvant chemotherapy, for HPV-positive OPSCC patients who have undergone surgical

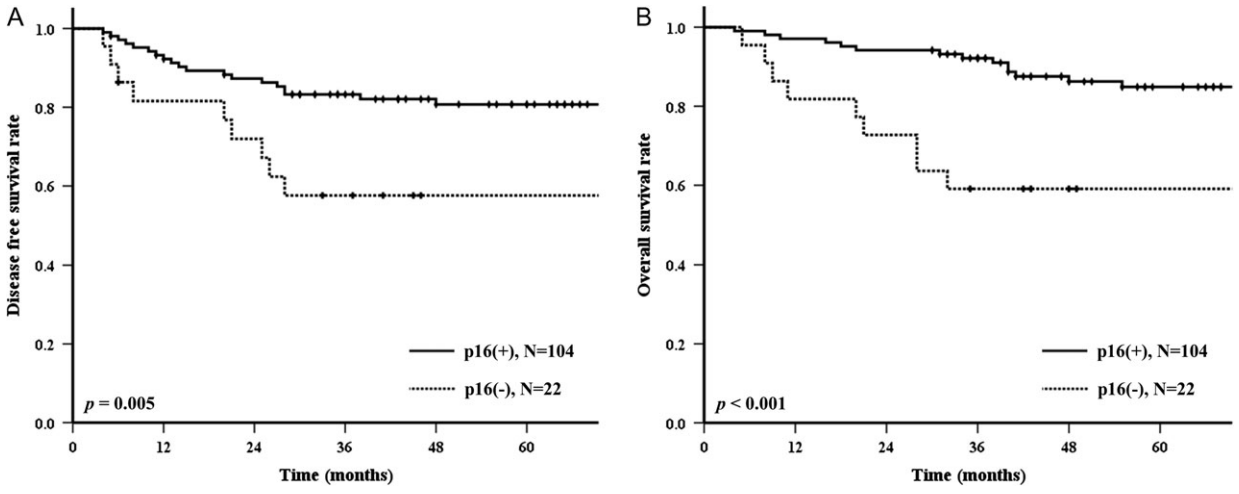

Figure 1. Disease-free survival (DFS) (A) and overall survival (OS) (B) according to p16 expression status.

Table 4. Univariate and multivariate analyses of potential prognostic factors for DFS and OS

Variable		DFS				OS			
		UVA		MVA*		UVA		MVA*	
		5-year DFS	*P*	HR (95% CI)	*P*	5-year OS	*P*	HR (95% CI)	*P*
Age	<60 years	81.0	0.112	2.070 (0.996–4.304)	0.051	82.7	0.209	NI	
	≥60 years	70.8				77.5			
Sex	Male	74.1	0.463	NI		77.7	0.439	NI	
	Female	93.8				100.0			
Smoking	<10 PY[a]	78.7	0.560	NI		81.2	0.979	NI	
	≥10 PY	72.8				78.4			
Histologic differentiation	WD	93.8	0.333	NI		93.8	0.509	NI	
	MD	75.9				79.3			
	PD	69.0				75.4			
p16 expression	Negative	57.6	0.005	Ref	<0.001	59.1	< 0.001	Ref	<0.001
	Positive	80.7		0.186 (0.078–0.443)		84.9		0.087 (0.033–0.229)	
cT Stage	II–III	78.8	0.047	NI		81.0	0.465	NI	
	IV	54.5				72.7			
cStage	II–III	87.5	0.361	NI		89.0	0.359	NI	
	IV	72.9				77.4			
pT Stage	I–III	80.0	0.024	Ref	0.033	83.3	0.063	Ref	0.047
	IV	56.3		2.823 (1.085–7.342)		62.5		2.847 (1.012–8.007)	
pStage	I–III	92.4	0.070	Ref	0.081	92.8	0.195	NI	
	IV	71.7		2.669 (0.887–8.032)		76.5			
Surgical margin	Negative	82.5	0.161	Ref	0.091	87.9	0.053	Ref	0.043
	Positive	68.1		1.955 (0.898–4.259)		69.1		2.500 (1.029–6.069)	
ECE	Absent	82.9	0.074	NI		84.7	0.140	NI	
	Present	66.1				73.3			
PNI	Absent	80.5	0.007	NI		83.0	0.048	NI	
	Present	42.9				56.8			
LVI	Absent	82.3	0.028	Ref	0.012	84.4	0.147	NI	
	Present	60.1		2.740 (1.252–5.998)		66.9			
Adjuvant therapy	RT	80.5	0.221	NI		81.4	0.852	NI	
	CRT	65.4				74.6			

DFS, disease-free survival; OS, overall survival; UVA, univariate analysis; MVA, multivariate analysis; HR, hazard ratio; CI, confidence interval; NI, not included; Ref, reference.

[a]Calculation includes patients had smoking history that both non-smoker and below 10 PY.

*Variables were entered into the multivariate Cox regression model in a stepwise method if $P < 0.10$ and were removed at any point if $P \geq 0.10$.

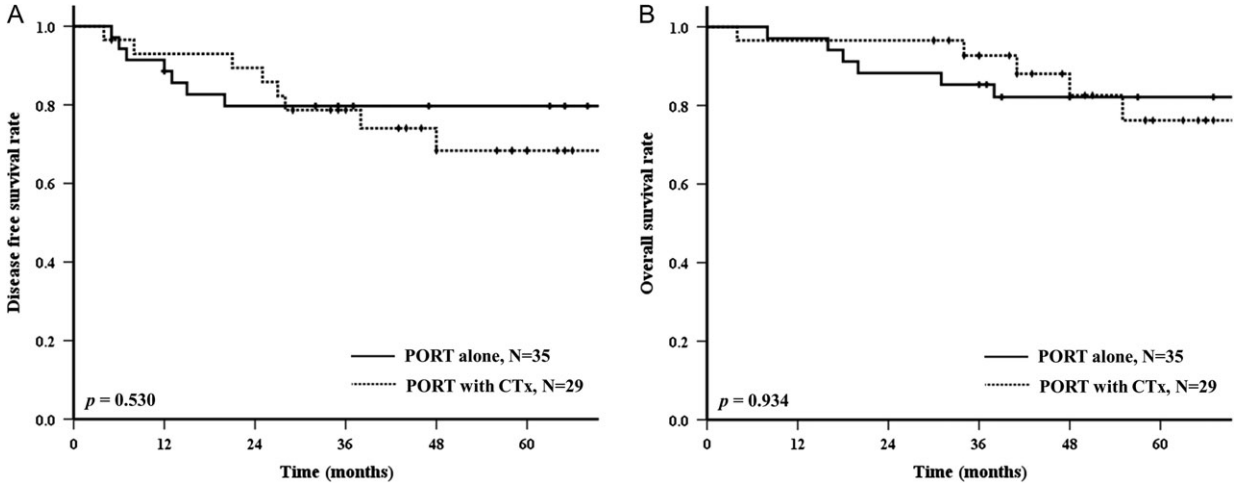

Figure 2. DFS (A) and OS (B) in p16 (+) oropharyngeal squamous cell carcinoma patients with high-risk factors that were treated with postoperative radiotherapy (PORT) alone versus PORT with chemotherapy (CTx).

resection have not yet emerged. Traditionally, PORT has been the standard adjuvant approach postoperatively for OPSCC patients with risk factors such as positive surgical margin, ECE, multiple positive LN, PNI and LVI. Thereafter, two major Phase III randomized trials including RTOG 9501 (16) and European Organization for Research and Treatment of Cancer (EORTC) 22931 (17)

identified that high-risk patients with positive surgical margins and/or ECE in lymph node metastasis had benefit from the addition of cisplatin. However, these two randomized trials did not consider the significance of HPV status. Our cohort showed a 20–30% increase in the 5-year DFS and OS over those of RTOG 9501 and EORTC 22931 trials. These outcomes came from a high percentage of p16 (+) disease in this cohort. The sharp increase in the proportion of HPV-positive OPSCC occurred after the year 2000, and the percentage of carcinogenic HPV in the etiology of OPSCC has doubled over the last decade (2). The cohorts of RTOG 9501 and EORTC 22931 were treated with PORT or PORT with chemotherapy before 2000, while the cohort in this study was treated after the year 2000.

We observed that patients with p16 (+) OPSCC and high-risk factors (n = 63) had excellent DFS and OS of 75.1% and 80.7%, respectively, although only 45% of these patients received PORT with chemotherapy. It is reasonable to propose that de-intensification adjuvant treatment may be considered in the management of select p16 (+) OPSCC with high-risk features showed favorable outcome regardless of concurrently adjuvant chemotherapy. Based on our findings, a re-evaluation of the routine application of concurrent chemotherapy during PORT for p16 (+) OPSCC with high-risk factors may be warranted. Also, in this study, it is noteworthy that LVI, which is traditionally considered a minor or moderate risk factor, was a more important risk factor for survival than ECE in p16 (+) OPSCC. Maxwell reported that ECE, a long-established major risk factor, was not significantly associated with worse DSS in p16-positive OPSCC patients (18). Ultimately, the superior prognosis associated with p16 (+) disease may indicate a need to re-examine traditional risk factors and stratification in the postoperative setting.

Despite the overall good prognosis for HPV-positive OPSCC, some aggressive subtypes have been described, characterized by distant spread (19) and advanced nodal stage (20). Likewise, some patients with HPV-positive OPSCC remain at risk of poor outcome, complicating de-intensification efforts. Therefore, we should classify risk group for studies testing de-intensification approaches. Currently, de-intensification trials are being conducted for HPV-positive OPSCC based on risk factors in the postoperative setting. The Phase III ADEPT (NCT01687413) trial (21) is investigating a treatment de-intensification strategy by comparing RT alone to CRT in HPV-positive OPSCC patients with ECE in lymph node metastasis and a negative surgical margin who also underwent surgery. Also, the Eastern Cooperative Oncology Group (ECOG) 3311 (NCT01898494) (22) is conducting a Phase II trial in which patients with resectable p16 (+) OPSCC are stratified into four-arm treatments according to their surgical pathology after transoral surgery. In that study, patients are randomized into either low-dose or standard-dose PORT, with or without chemotherapy.

Our study has some limitations. Owing to data with retrospective in nature, we could not assess functional outcomes of swallowing, salivation, speech and diet. In addition, it comprised relatively small patient cohorts. Next, the use of p16 immunochemistry as a sole marker for HPV positivity is unsatisfactory. Although p16 overexpression is a sensitive technique to detect the presence of HPV in OPSCC, polymerase chain reaction testing and *in situ* hybridization would further improve the validation (23,24). Finally, our data did not examine an association between p16 expression and molecular biomarkers such as epidermal growth factor receptor and p53 in OPSCC, which could provide important prognostic information (25–27).

In conclusion, HPV positivity based on expression of p16 is a strong and independent prognosticator of survival in OPSCC treated with surgical resection followed by PORT. Future research will confirm whether the traditional risk factors and risk stratification applies equally to the HPV-positive cohort. Additional studies will be able to validate optimal de-intensification approaches according to the risk group for p16 (+) OPSCC in the postoperative setting.

Supplementary data

Supplementary data are available at http://www.jjco.oxfordjournals.org.

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Conflict of interest statement

None declared.

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