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ORIGINAL PAPER

Oropharyngeal Squamous Cell Carcinoma in 390 Patients: Analysis of Clinical and Histological Criteria Which Significantly Impact Outcome

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Abstract

This study evaluates the prognostic impact of several factors in oropharyngeal squamous cell carcinoma (OPSCC), controlling for human papillomavirus (HPV)-associated tumors and stage (American Joint Committee on Cancer 8th edition). All patients in Southern California Permanente Medical Group diagnosed with OPSCC between 2006 and 2012 tested for p16 immunohistochemistry were included. Review of all pathology materials was combined with central p16 testing. Multivariable analyses were performed. The cohort of 390 patients included 342 p16-positive and 48 p16-negative tumors. For all-comers, on univariate analysis, the following factors, when present, were associated with improved patient survival: p16-positive tumor (n = 324, p < 0.001); crypt versus surface tumor location (n = 312, p = 0.004); nonkeratinizing type (n = 309, p < 0.0001); nonkeratinizing *with* maturation type (n = 37, p < 0.0001); basaloid pattern (n = 284, p = 0.005); and a broad, pushing border of infiltration (n = 282, p = 0.004). Inferior survival outcomes were observed with: age ≥ 55 years (p < 0.0001); ≥ 10 pack-year smoking history (n = 183, p = 0.003); increasing tumor stage (p < 0.0001); overt radiographic extranodal extension (ORENE) (n = 58, p < 0.0001); low level IV/Vb lymph node involvement (n = 45, p = 0.0002); a jagged pattern of infiltration (n = 76, p = 0.0004); tumor ulceration (n = 76, p = 0.0004); absent lymphocytic infiltrate (p < 0.0001); and concurrent dysplasia (n = 125, p = 0.009). On multivariable analysis, accounting for patient age, smoking history ≥ 10 pack-years, and TNM stage, for patients with p16-positive disease, advanced TNM stage (p = 0.007), the presence of ORENE (p = 0.0002), and low-neck lymphadenopathy (p = 0.0001) were independent negative prognostic factors for disease free survival (DFS). Older age (p < 0.0001), smoking history ≥ 10 pack-years (p = 0.02), advanced TNM stage (p = 0.0002), ORENE (p = 0.004), and low-neck lymphadenopathy (p = 0.002) were independent negative prognostic factors for OS. Among patients with p16-positive OPSCC, older age, smoking history, advanced stage, ORENE, and low-neck lymphadenopathy were significant negative prognostic factors for DFS and/or OS. Further refinement of staging to incorporate additional lymph node findings may be warranted.

Keywords Squamous cell carcinoma · Oropharyngeal neoplasms · Mouth neoplasms · Prognosis · Disease-free survival · Lymphadenopathy · Smoking · Cohort studies · Neoplasm staging · Human papillomavirus (HPV)

Introduction

It is well recognized that transcriptionally active human papillomavirus (HPV)-associated oropharyngeal squamous cell carcinoma (OPSCC) is a distinct cohort of head and neck squamous cell carcinomas that, as a group, portend a better prognosis than HPV-independent tumors [1–4]. HPV-associated OPSCC tends to affect younger, healthy men who often present with cervical lymph node metastases despite small primary tumors. The histologic features of these tumors have not been well characterized and correlated to patient disease free survival (DFS) and overall survival (OS) in a large

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group of patients managed in a standardized approach by a single health care delivery system [5–12]. It is the goal of this study to evaluate the clinical and histologic features of 390 patients with OPSCC and identify potential statistically significant factors which may predict patient outcome.

Materials and Methods

All patients from the 11 Southern California Permanente Medical Group hospitals with locally advanced OPSCC diagnosed between 2006 and 2012 were evaluated. Patients were excluded if: (1) they had received any chemoradiation prior to biopsy (i.e., no recurrent tumor or different tumor type) or did not receive initial treatment within our organization; (2) they had a history of head and neck cancer or prior head and neck radiotherapy; or (3) the patient had left the care of Southern California Permanente Medical Group before completion of therapy. An oropharyngeal centered tumor must have involved the base of tongue, palatine tonsillar fossa, pharyngeal wall and/or soft palate [13].

This clinical investigation was conducted in accordance with all guidelines of an Internal Review Board authorization (#5968 and 11178). It was in part supported by a grant from the Regional Research Committee of Kaiser Permanente Southern California (KP-RRC-20161103). Electronic medical records were reviewed with additional information obtained as needed.

The resulting 390 patients' records were analyzed. All patients were treated with curative intent after initial surgery with definitive concurrent chemoradiation (n = 345), induction chemotherapy followed by chemoradiation (n = 44), or radiotherapy alone (n = 1). Specific chemotherapy protocols were employed and are separately reported [14–17]. Importantly, all patients were retrospectively clinically restaged using American Joint Committee on Cancer (AJCC) 8th edition staging, including any subsequent corrections, for both HPV-associated and HPV-independent oropharyngeal squamous cell carcinomas [18].

Review was performed of all histology materials available with preference given to excisional samples of the primary tumor (i.e., if primary and metastatic tumor were both available, core biopsy versus resection, the evaluation focused on the primary resection tumor). The histologic features were evaluated based on hematoxylin and eosin stained slides from surgical pathology material. This was done with the understanding of the limitations of identifying pertinent features in a biopsy versus resection sample. The data extracted included patient demographics along with tobacco use at the time of diagnosis (ever or never; if ever, current or former; pack-year history was aggregated into $<$ or $\geq$ 10 pack-years); alcohol use (ever or never; if ever, light, moderate or heavy use), anatomic centering of the primary tumor and laterality, and metastatic disease with location, number, and size of involved lymph nodes. Imaging findings, types of treatment, current patient status (alive or dead, with or without disease and whether local, regional or disseminated) were also recorded. While alcohol use was recorded, non-standardized reporting of light, moderate, or heavy use made this error prone, thus these data were not incorporated into the reported results.

For imaging findings, the largest lymph node was documented in addition to the presence of low-neck lymphadenopathy, retropharyngeal lymphadenopathy, overt radiographic extranodal extension (ORENE), and matted lymphadenopathy. Low-neck lymphadenopathy was defined as radiographic involvement of level IV and/or Vb in the neck. ORENE was defined radiographically as a clear loss of the integrity of the nodal capsule with infiltration of tumor into the adjacent fat planes or musculature. Matted lymph nodes were defined radiographically as multiple abutting lymph nodes with the loss of intervening fat planes.

Pathology features included histologic type (as previously described [11, 19]), tumor pattern or subtype [8], crypt versus surface centered, nuclear anaplasia and tumor cell multinucleation (as previously defined [19]), comedonecrosis, type of infiltration (jagged/single cell, broad front, blended), desmoplastic stromal reaction (present or absent), extent of lymphocytic infiltrate (absent, mild, moderate, brisk), mitotic figures (number of mitotic figures per 2 mm^2 [magnification at $\times$40 with a $\times$10 objective lens using Olympus BX41 microscope and a field of view of 22]), and other features. p16-negative tumors were graded as well (grade 1), moderately (grade 2), or poorly (grade 3) differentiated; p16-positive tumors were not graded by definition (although they would be considered grade 1 when nonkeratinizing, since they are similar to normal lymphoepithelium).

p16 immunohistochemistry was performed on a 4-µm-thick section of a formalin-fixed, paraffin-embedded (FFPE) tumor block with sufficient material for successful evaluation using the E6H4 Ventana (Tucson, AZ) monoclonal antibody to p16INK4a at a 1:1 dilution on a Ventana Benchmark LT automated immunostainer according to the manufacturer supplied standard protocol. This included antigen retrieval using a Ventana epitope retrieval solution. Positive and negative controls (normal tonsil) were used throughout. The tumors were interpreted to be either p16-positive or negative using established cutoffs: positive defined as $>$ 70% strong, diffuse nuclear and cytoplasmic reactivity in the neoplastic cells and negative defined as anything less than this cutoff. Of the 48 cases interpreted to be negative, all showed staining in $<$ 20% of the neoplastic cells, including no staining at all. Evaluated prospectively, the criteria match those published in the College of American Pathologists Guidelines [20].

Statistical Evaluation

Fischer's Exact test and unpaired *t* test were used to compare frequencies of histopathologic subtypes by p16 status. The OS and DFS were defined as the time interval between the date of initial pathologic diagnosis of the patient's tumor and the date of death due to any cause (primary end point) or the date of first tumor recurrence (secondary end point). Recurrences included locoregional failure or distant metastasis in a patient who had successfully completed primary treatment and was identified as being disease free. If a patient failed therapy and thus was never disease free, they were classified as having persistent disease and locoregional failure for statistical purposes. These parameters were identified in patients with a minimum follow-up of 60 months or to death. OS and DFS were determined by the Kaplan–Meier method with log-rank tests used to examine survival differences between cohorts. Time intervals were computed from the date of treatment completion to event occurrence. Multivariable analyses were performed using a Cox proportional hazard model to estimate the risk of disease recurrence or death. A regression model was constructed to identify histopathologic prognostic factors using a stepwise selection algorithm after accounting for confounding factors of patient age, tumor stage, and smoking history (≥ 10 pack-years vs. < 10 pack-years). All histopathologic predictors eligible for inclusion in the regression model demonstrated a p-value < 0.10 on univariable analysis. Factors in the final regression model demonstrated a p-value of < 0.05 on multivariable analysis.

Results

Clinical Findings

The clinical findings are summarized in Table 1. The vast majority of patients were male (87.4%) with a median age of 59 years at presentation (Fig. 1). Most were white (88.7%) with blacks (9.2%), Asians (1.8%), and Native Americans (0.3%) comprising the remaining patients. There were more black patients proportionately who had p16-negative tumors (33.3%) than white patients (10.4%) (p = 0.0005). Patients presented with a variety of symptoms, referable to the primary tumor or metastatic disease in the cervical lymph nodes. A neck mass was the presenting clinical finding in 49.5% of patients. The lesions were evaluated by fine needle aspiration (n = 169, 43%) or a core needle biopsy (n = 29, 7%), the result of which informed additional management. The majority of patients were ever smokers (n = 259, 66.4%), with 89 current and 170 former smokers. 131 patients were never smokers. As previously described [21–24], all patients with < 10 pack-years of smoking history, including the never smokers, were combined to a cohort of 207 patients for statistical purposes. The remaining 183 patients reported a smoking history of ≥ 10 pack-years. Based on criteria described in the materials and methods, 98 patients were never users and 290 patients were ever users of alcohol. Overall, p16-positive OPSCC patients who smoked ≥ 10 pack-years had inferior OS (HR 1.76 [1.10–2.82], p = 0.02) compared to < 10 pack-years smokers (see Table 2).

The majority of OPSCCs arose in the tonsil (n = 201, 51.5%) or the base of tongue (n = 169, 43.3%). Only a few tumors affected the pharynx or soft palate (Table 1). Most tumors were centered in a single site (n = 364, 93.3%) but may have expanded to involve more than one site (likely the case with the soft palate tumors). The remaining tumors presented with multiple separate sites including 2.6% (n = 10) that presented with bilateral disease. The particular subsite affected, even when controlled for p16 reactivity and stage, did not yield a statistically significant difference in DFS or OS (Supplemental Table A).

Overall, using the AJCC 8th edition, the majority of tumors were clinically low stage (Supplemental Table A). Specifically, for cT-categories, 211 patients (54%) were T1 or T2, comprising 57% of p16-positive tumors and 31.3% of p16-negative tumors. Of p16-positive OPSCC patients, only 18 (5.3%) were cN0. Thus 94.7% of all p16-positive OPSCC patients had lymph node metastases at the time of diagnosis even though only 49.5% presented with a neck mass clinically. Similarly, 8 patients (16.7%) with p16-negative OPSCC tumors were cN0. The remaining 83.3% had lymph node metastases although only 54% presented with a neck mass clinically.

Imaging Findings

At least an MRI or CT scan was available for evaluation with concurrent PET/CT in many patients. The definition of ORENE was used to evaluate the 342 patients with p16-positive tumors. There were 22 patients with clinical stage I extranodal extension (ENE), 7 patients with clinical stage II-ENE, and 23 patients with clinical stage III-ENE (see Supplemental Table B; Fig. 2). Analyzing only the patients with p16-positive tumors, ORENE was a poor prognostic factor for DFS and OS (HR = 2.88 [1.83–4.54]; p < 0.001). Clinical stage-matched analysis of p16-positive cases demonstrated worse survival among patients with ORENE compared to patients in the same clinical stage group *without* ORENE (mean years): Stage I: 132 (7.6) vs. I-ENE: 22 (6.2); II: 76 (7.5) vs. II-ENE: 7 (5.9); III: 52 (6.1) vs. III-ENE: 23 (4.2) (HR = 2.35 [1.43-3.87]; p = 0.0007) (Supplemental Table B).

Evaluating the entire cohort, clinical low-neck disease (lymph node levels IV/Vb; Fig. 3) was a poor prognostic factor for survival after adjusting for p16 status (HR 2.60 [1.64–4.11], p < 0.0001, n = 45). Analyzing

Table 1 Clinical findings of 390 oropharyngeal squamous cell carcinoma patients

Clinical finding	Number of cases (percent)	p16-positive (n = 342)	p16-negative (n = 48)
Sex (p = 0.19)			
Male	341 (87.4%)	302	39
Female	49 (12.6%)	40	9
Race (p = 0.42)			
White	346 (88.7%)	310	36
Black	36 (9.2%)	24	12
Asian	7 (1.8%)	7	0
Native American	1 (0.3%)	1	0
Age (years) (p < 0.0001)			
Range	35–83	35–81	41–83
Mean	58.9	58.8	60.2
Median	59	58	61
Smoking history (p = 0.09)			
Never	131 (33.6%)	124	7
Ever	259 (66.4%)	218	41
≥ 10 pack-years	183 (46.9%)	146 (p = 0.02)	37
< 10 pack-years	207 (53.1%)	196	11
Anatomic site (p = 0.10)			
Tonsil	201 (51.5%)	181	20
Base of tongue	169 (43.3%)	153	16
Pharynx	12 (3.1%)	3	9
Soft palate	8 (2.1%)	5	3
Focality			
Single site only	364 (93.3%)	321	43
Mixed/multiple sites	26 (6.7%)	21	5
Laterality			
Left	190 (48.7%)	165	25
Right	182 (46.7%)	164	18
Midline	8 (2.1%)	5	3
Bilateral	10 (2.6%)	8	2
Size (cm) (T)			
Range	0.2–9.8	0.2–6.0	0.4–9.8
Mean	2.6	2.6	3.0
Median	2.5	2.5	2.7
T-stage Clinical			
T1	70 (17.9%)	64	6
T2	141 (36.3%)	132	9
T3	84 (21.5%)	69	15
T4	77 (19.7%)	77	N/A
T4a	11 (2.8%)	N/A	11
T4b	7 (1.8%)	N/A	7
N-stage Clinical			
cN0	26 (6.7%)	18	8
cN1	249 (63.8%)	239	10
cN2	71 (18.2%)	71	N/A
cN2a	0	N/A	0
cN2b	11 (2.8%)	N/A	11
cN2c	12 (3.1%)	N/A	12
cN3	14 (3.6%)	14	N/A

Table 1 (continued)

Clinical finding	Number of cases (percent)	p16-positive (n = 342)	p16-negative (n = 48)
cN3a	0	N/A	0
cN3b	7 (1.8%)	N/A	7
Overall staging group (p = 0.0002)			
I	164 (42.1%)	164	0
II	94 (24.1%)	94	0
III	95 (24.4%)	84	11
IV	37 (9.5%)	0	0
IVA	24 (6.2%)	N/A	24
IVB	13 (3.3%)	N/A	13
IVC	0	N/A	0

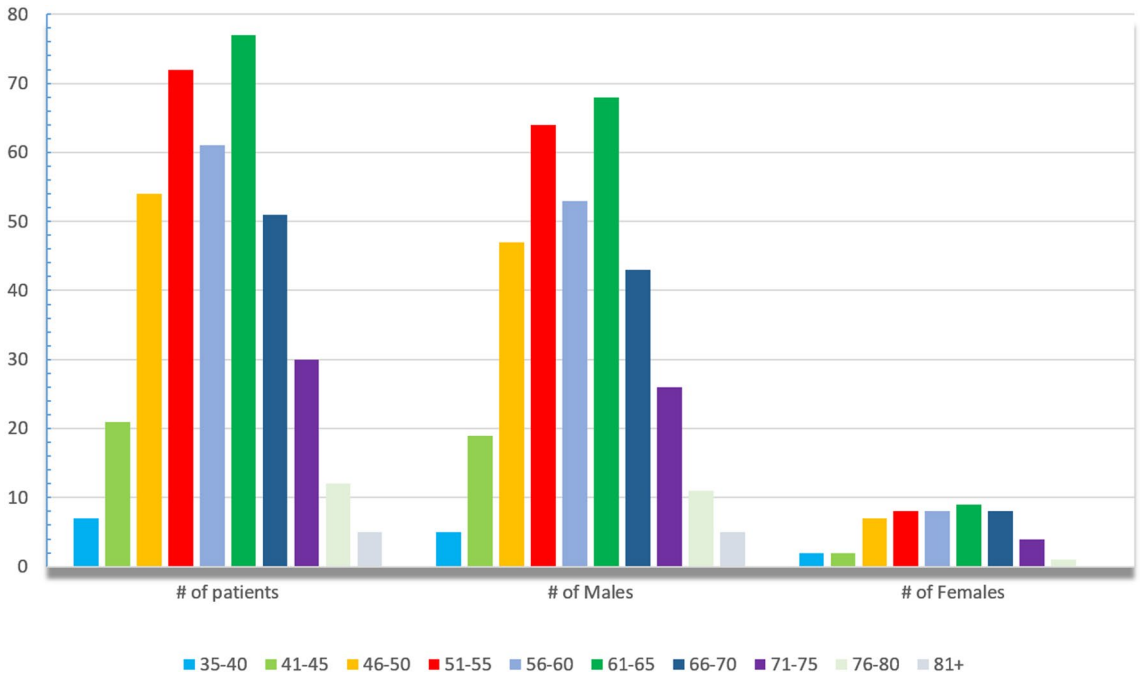

Fig. 1 Patient quintile age distribution between men and women. The majority are between 51 and 65 years

Table 2 Clinical and histopathologic prognostic factors on multivariable analysis of patients with p16-positive oropharyngeal squamous cell carcinomas

Parameter evaluated	Disease-free survival		Overall survival	
	Hazard ratio	p-value	Hazard ratio	p-value
Age	1.03 (0.99–1.06)	0.058	1.07 (1.04–1.10)	< 0.0001
≥ 10 pack-year smoker	1.37 (0.80–2.34)	0.25	1.76 (1.10–2.82)	0.02
Stage	1.58 (1.13–2.21)	0.007	1.77 (1.32–2.38	0.0002
Overt radiographic extranodal extension	2.90 (1.66–5.07)	0.0002	2.16 (1.29–3.63)	0.004
Low neck (IV/Vb) lymphadenopathy	2.61 (1.42–4.80)	0.0001	2.37 (1.36–4.15)	0.01
Lymphoid infiltrate				
Absent	1.00		1.00	
Mild	0.01 (0.002–0.05)	< 0.0001	0.01 (0.002–0.05)	< 0.0001
Moderate	0.02 (0.004–0.10)	< 0.0001	0.01 (0.002–0.06)	< 0.0001
Brisk	0.01 (0.002–0.06)	< 0.0001	0.01 (0.002–0.05)	< 0.0001

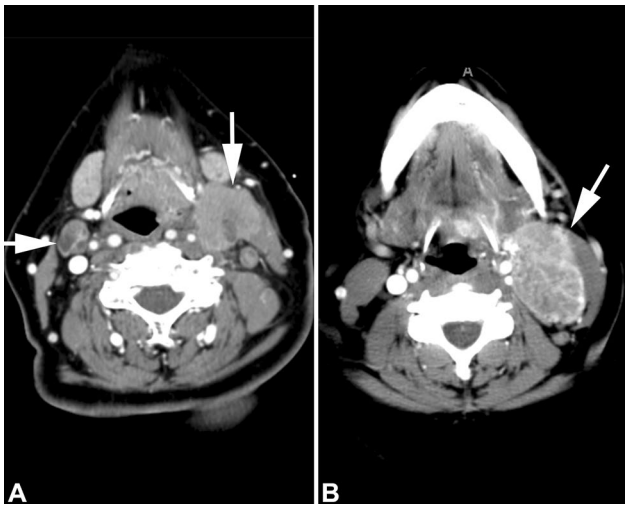

Fig. 2 **a** A 72 year old white female never smoker with light alcohol use history had a left tonsil nonkeratinizing p16-positive pT4 OPSCC and demonstrates a 3 cm left neck lymph node with extranodal extension (white arrow) in a setting of bilateral neck disease (arrowhead). **b** A 39 year old black woman with a negative alcohol and tobacco use history had a left base of tongue nonkeratinizing, spindled, p16-positive pT4 OPSCC and shows a 7 cm, matted lymph node group involving level II–Vb with extranodal extension (white arrow)

only those patients with p16-positive disease, low-neck disease remained prognostic for worse survival (HR 2.95 [1.81–4.80], $p < 0.0001$). Furthermore, clinical stage-matched analysis of p16-positive cases demonstrated worse survival among patients with low-neck disease compared to patients in the same stage group without this feature (see Supplemental Table C).

Pathologic Features

OPSCCs were separated into nonkeratinizing (n = 309), nonkeratinzing with maturation (n = 37), and keratinizing (n = 44) (Table 3 and Supplemental Table D). Importantly, separation was based on the largest volume specimen submitted, but the majority of patients did not have surgical resections. The vast majority of tumors were nonkeratinizing, with 290 p16-positive and 301 tumors of the tonsil and base of tongue (see Supplemental Table D) showing a "blue-cell" appearance. This was characterized by large, well defined lobules, nests and trabeculae of tumor that often showed a sharp edge or pushing border with the surrounding stroma or lymphoid tissue (Fig. 4). An "inside-out" or "reverse maturation" appearance, with central basaloid cells surrounded at the periphery by cells with squamous maturation (Fig. 5), was quite common. The cells were arranged in a syncytial pattern of intermediate sized polygonal cells with a relatively high nuclear to cytoplasmic ratio. The cytoplasm was eosinophilic to amphophilic and surrounded round to oval nuclei with heavy chromatin and inconspicuous nucleoli. Mitoses were abundant, including atypical forms. Central comedonecrosis was common. Nonkeratinizing tumors

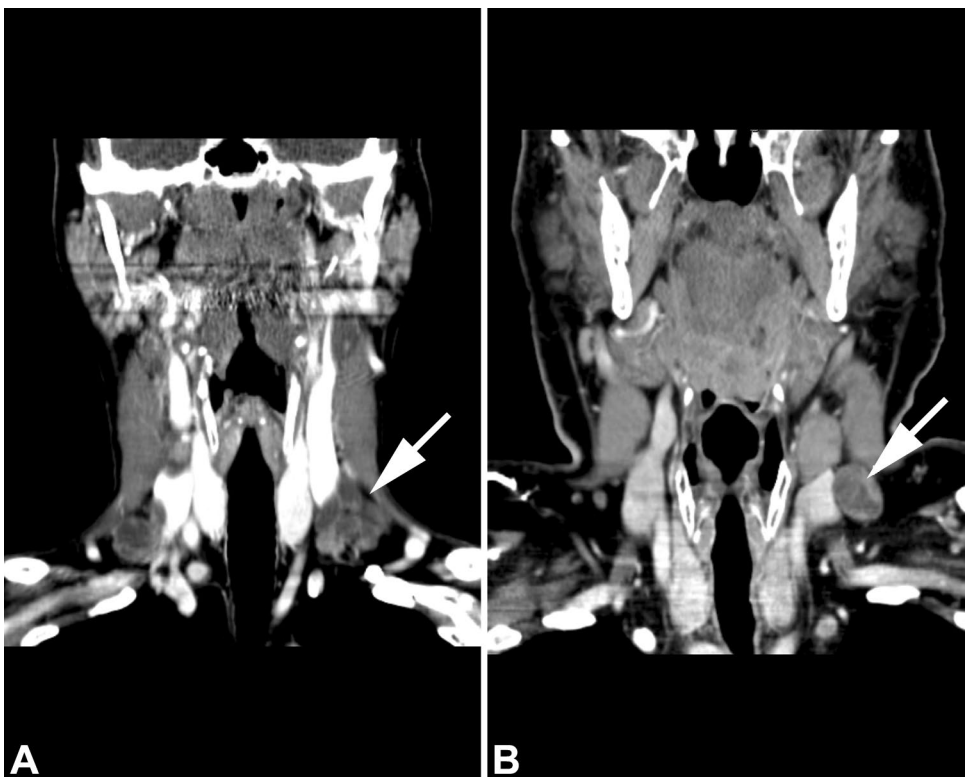

Fig. 3 **a** A 56 year old Asian man with a 40 pack-year smoking history had a right tonsil nonkeratinizing p16-positive pT3 OPSCC and shows a 1.7 cm low level neck lymph node (white arrow), along with bilateral disease. **b** A 62 year old white man with a 10 pack-year smoking history and light alcohol use had a left base of tongue nonkeratinizing (lymphoepithelial pattern), p16-positive pT3 OPSCC and shows a 2.6 cm level Vb enlarged lymph node (white arrow), along with a 3 cm level II lymph node

Table 3 Histopathologic features in p16-positive and p16-negative oropharyngeal squamous cell carcinomas

Histological feature	p16-positive (n = 342)	p16-negative (n = 48)	p-value
Histologic type			< 0.0001
Nonkeratinizing	290	19	
With maturation	35	2	
Keratinizing	25	19	
Basaloid appearance			< 0.0001
Yes	264	20	
No	78	28	
Papillary type			0.09
Yes	22	0	
No	320	48	
Lymphoepithelial type			0.01
Yes	52	1	
No	290	47	
Spindle cell type			0.55
Yes	5	1	
No	337	47	
Basaloid squamous cell carcinoma			> 0.99
Yes	6	0	
No	336	48	
Adenosquamous type			0.12
Yes	0	1	
No	342	47	
Location			< 0.0001
Crypt-centered	293	19	
Surface-centered	49	29	
Ulceration			< 0.0001
Yes	48	28	
No	294	20	
Concurrent dysplasia/surface			< 0.0001
Yes	95	30	
No	247	18	
Squamous pearls/eddies			0.02
Yes	219	39	
No	123	9	
Dyskeratotic cells present			0.20
Yes	287	44	
No	55	4	
Nuclear anaplasia			0.87
Yes	109	16	
No	233	32	
Tumor cell multinucleation			0.85
Yes	73	9	
No	269	39	
Comedonecrosis			0.11
Yes	255	41	
No	87	7	
Jagged/single cell			< 0.0001
Yes	45	31	
No	297	17	
Broad front			< 0.0001
Yes	282	23	
No	60	25	
Blended			0.40
Yes	31	2	
No	311	46	
Desmoplastic stromal reaction			0.004
Yes	229	42	
No	113	6	
Lymphocytic infiltrate			< 0.0001
Brisk	224	13	
Moderate	51	7	
Mild	65	28	
Absent	2	0	
Mitotic figures			0.01
Range	3–387	3–260	
Mean (per 2 mm^2)	69.9	49.4	

still displayed areas with dyskeratosis, squamous eddies, or pearls. By contrast, keratinizing SCC showed a jagged, angulated infiltration by variably sized nests of neoplastic cells that elicited a strong desmoplastic stromal reaction. These neoplastic cells were large and polygonal with abundant, opacified, eosinophilic cytoplasm surrounding remarkably irregular nuclei with coarse, inky-black, hyperchromatic chromatin (Fig. 6). Distinct cell borders with easily identified spinous processes (intercellular bridges) and dyskeratosis, keratin pearl formation, and keratin debris were usually present. Most of these keratinizing tumors were p16-negative (59.5%) but still affected the tonsil and base of tongue (83.3%). The "nonkeratinizing with maturation" term was employed to cover hybrid tumors that showed a "basaloid" appearance but with easily identified areas of maturing squamous differentiation (Fig. 7). The majority of these tumors (92.1%) were p16-positive, and, again, involved the tonsil and base of tongue most commonly (89.5%).

Several different histological subtypes were identified including basaloid, papillary, lymphoepithelial [undifferentiated], spindle cell, and adenosquamous (Table 3 and Supplemental Table D). Basaloid SCC is a distinct entity, different from the basaloid features in OPSCC, and six tumors showed this pattern. Specifically, the basaloid SCC has more rounded tumor cells arranged within islands that show central comedonecrosis. The cells form a jigsaw-puzzle pattern of nests that mold to one another around hyalinized

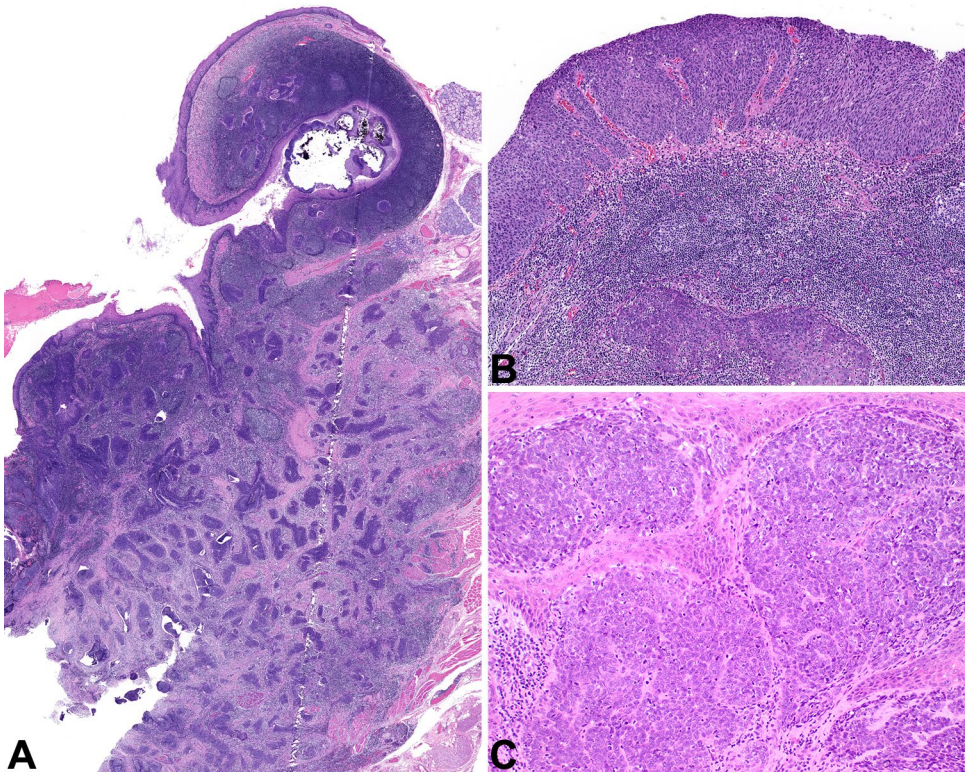

Fig. 4 Nonkeratinizing OPSCC p16-positive. **a** The majority of the tumor shows a deep crypt origin/centering and a basaloid morphology with multiple well defined nests of tumor with a sharp border. **b** This nonkeratinizing OPSCC shows well developed surface derivation with extension into the underlying stroma. **c** The surface epithelium is immediately overlying the basaloid proliferation and intimately associated with it

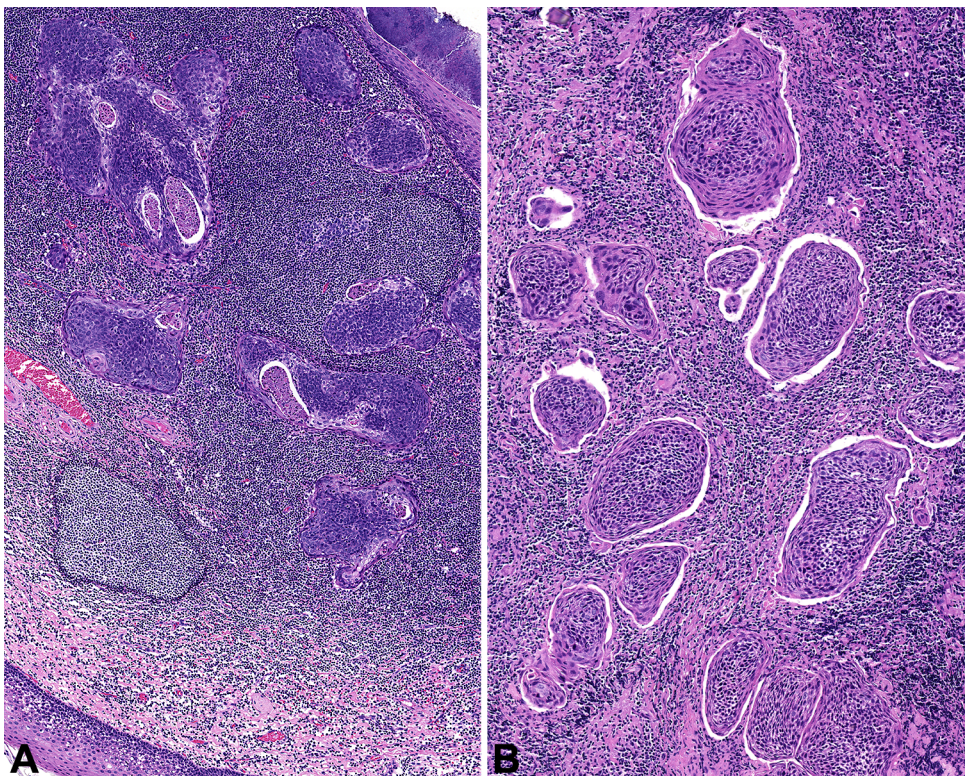

Fig. 5 Nonkeratinizing OPSCC p16-positive. **a** Uninvolved surface epithelium is seen (top and bottom) with islands of neoplastic cells showing an "inside-out" pattern of central basaloid cells surrounded by maturing squamous cells. **b** The inside out pattern shows a syncytial architecture of cells with a high nuclear to cytoplasmic ratio, frequently showing a cleft from the surrounding tissue

basement membrane type material. The stroma is paucicellular (Fig. 8) [7, 19, 25, 26]. Papillary SCCs (n = 22) were histologically identical to previous descriptions [27] and showed delicate fibrovascular cores lined by cytologically pleomorphic squamous cells, all of which were p16-positive (Fig. 9). The lymphoepithelial pattern was detected in 53

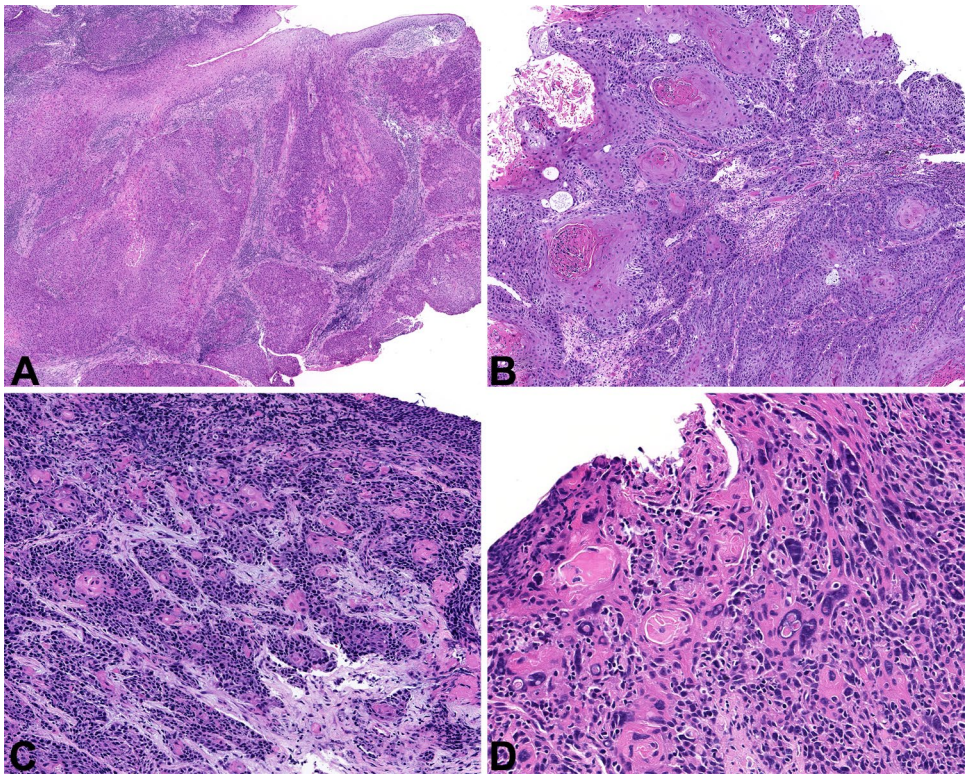

Fig. 6 Keratinizing OPSCC p16-positive. **a** Surface centered tumor with areas of central keratinization. **b** Irregular infiltration into stroma with desmoplasia and limited inflammation. **c** Jagged infiltration into the stroma of a keratinizing epithelium. **d** Nuclear pleomorphism, including multinucleation and giant cells, in this keratinizing OPSCC

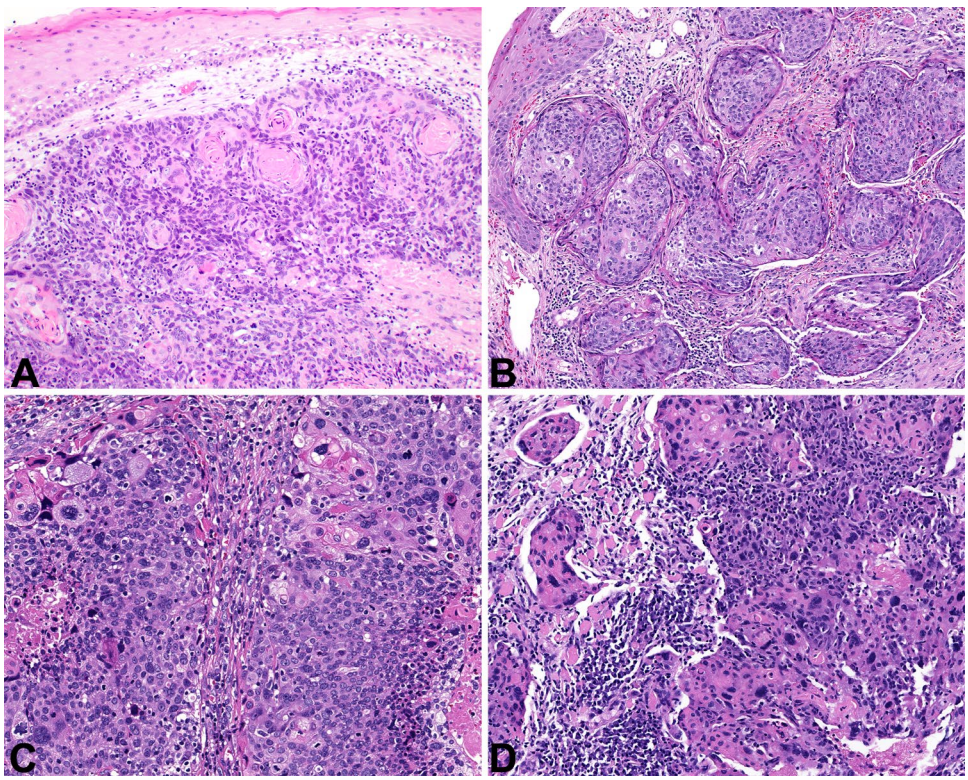

Fig. 7 Nonkeratinizing with maturation OPSCC p16-positive. **a** The surface is uninvolved, while limited keratinization can be seen. **b** A basaloid appearance predominates, but keratinization is noted. **c** Comedonecrosis with areas of keratinization and profound pleomorphism. **d** Keratinization is easily identified in a basaloid neoplasm, with areas of lymphovascular invasion identified

tumors (n = 52 p16-positive; n = 1 p16-negative). This histologic presentation was identical to nasopharyngeal undifferentiated carcinoma, with solid sheets and irregular islands of discohesive cells intimately blended with lymphocytes and plasma cells. There was usually a syncytial appearance with indistinct cell borders and large, round to oval nuclei

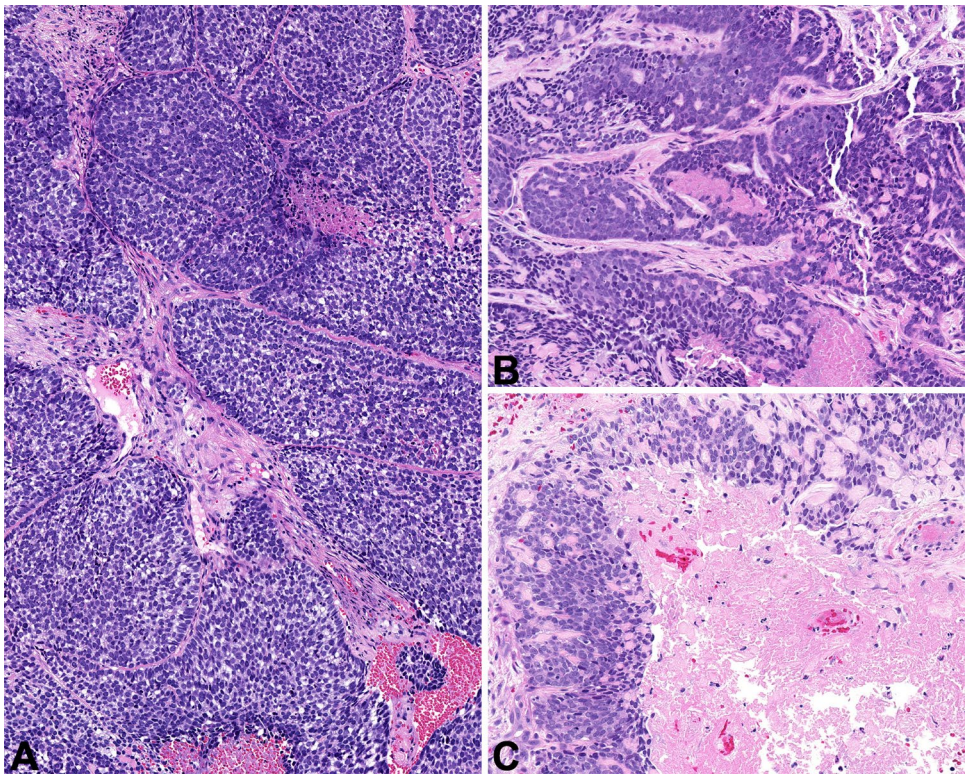

Fig. 8 Basaloid SCC p16-positive. **a** A jigsaw-puzzle configuration with central comedonecrosis and basement membrane-type material. **b** Reduplicated basement membrane separates the tumor islands and is seen as small droplets within the tumor nests. **c** Central comedonecrosis surrounded by basaloid cells with basement membrane material easily identified

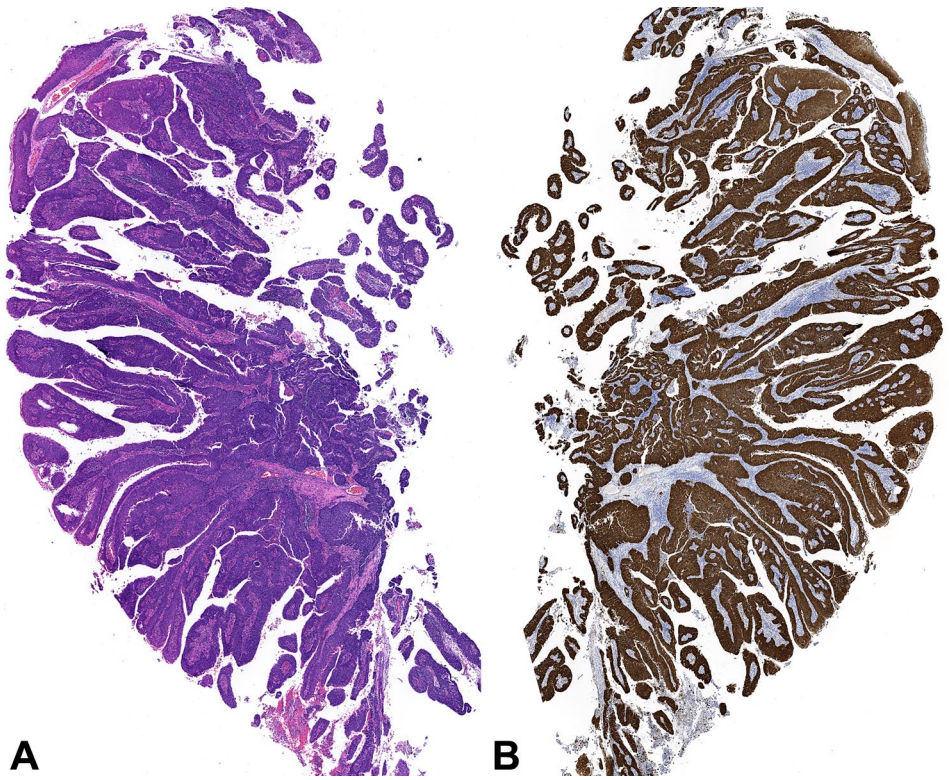

Fig. 9 Papillary OPSCC p16-positive. **a** There are numerous, delicate, filiform papillary projections of atypical squamous epithelium, showing **b** strong and diffuse nuclear and cytoplasmic p16 immunoreactivity

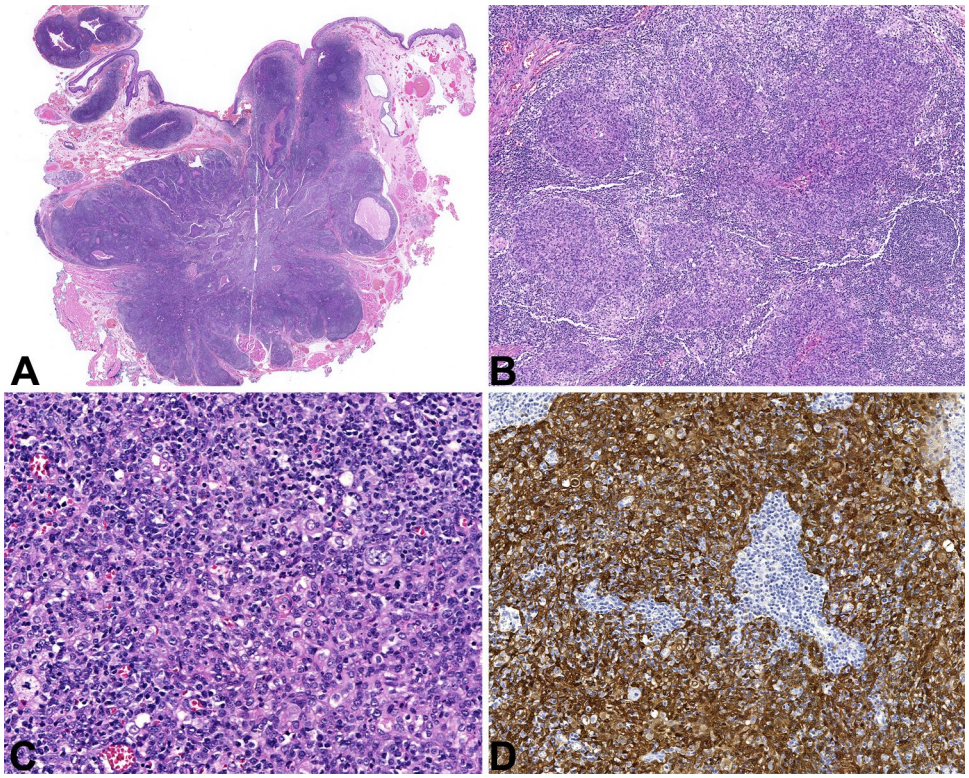

Fig. 10 Lymphoepithelial OPSCC p16-positive. **a** A predominantly deep, crypt-centered tumor. **b** The neoplastic cells are intimately associated with the lymphoid elements. **c** The intimate relationship of the neoplastic cells with the lymphoid cells makes definitive separation between the elements difficult. **d** There is a strong and diffuse, nuclear and cytoplasmic reaction of the neoplastic cells with p16, with negative areas representing lymphoid cells

with vesicular-open chromatin and large central nucleoli (Fig. 10). Adenosquamous carcinoma, a combination of both adenocarcinoma and squamous cell carcinoma in the same tumor, was present only in a single p16-negative tumor.

Of all tumors, 312 were crypt-centered (Fig. 4) while 78 were surfaced-centered (Table 3; Fig. 6). Of the p16-positive tumors, 293 were crypt-centered and 49 were surface-centered and of the p16-negative tumors, 19 were crypt-centered and 29 were surface-centered. This difference was statistically significant ($p < 0.0001$). Further, the vast majority of p16-positive tumors were crypt-centered irrespective of subsite (see Supplemental Table D). There was no statistically significant difference in patient outcome within subsite when stratified by crypt-versus surface-centered tumors, controlling for stage and p16 status.

Tumor surface ulceration was identified more commonly in p16-negative tumors (28/48) than in p16-positive tumors (48/342). This undoubtedly correlated to the surface versus crypt-centering of the tumor, and thus ulceration was associated with p16-negative tumors ($p < 0.0001$, Table 3 and Supplemental Table D). Similarly, concurrent dysplasia of the adjacent epithelium was more common in p16-negative than in p16-positive tumors (30/48 vs. 95/342, $p < 0.0001$) and, again, related to tumor centering and keratinizing versus nonkeratinizing histology. Squamous pearls/eddies were more likely to be present in the p16-negative than p16-positive tumors (39/48 vs. 219/342, $p = 0.02$). Tumor cell profound anaplasia and tumor cell multinucleation (Fig. 11), as previously defined [19], were easily identified in many tumors but were not specifically associated with p16-positive versus p16-negative tumors ($p = 0.87$ and $p = 0.85$, respectively). Furthermore, their presence did not yield any statistically significant difference in OS. Central comedonecrosis within the neoplastic islands (Figs. 7 and 8) was a common occurrence and unrelated to p16 status ($p = 0.11$). The different patterns of stromal infiltration correlated to p16 status: jagged and/or single cell infiltration significantly correlated to p16-negative tumors ($p < 0.0001$) and a broad-pushing front to p16-positive tumors ($p < 0.0001$, Table 3 and Supplemental Table D; Figs. 4, 6 and 7). A strong desmoplastic stromal reaction was seen in both tumor types when extending beyond the lymphoid stroma but was much more common in p16-negative tumors than in p16-positive tumors ($p = 0.004$). In crypt-centered tumors, there was always a brisk lymphoid infiltrate, usually in p16-positive tumors, while a scant to limited response was more common in the p16-negative and surface origin tumors ($p < 0.0001$; Fig. 6).

Using the criteria established for mitoses per 2 mm^2, there was a range of 3–387 mitoses/2 mm^2 with an average of 69.9 in p16-positive tumors and 49.4 in p16-negative tumors. Thus, there were more mitoses in the p16-positive tumors ($p = 0.01$) (Table 3). When using incremental increasing groups of 20 mitoses/2 mm^2, however, there was no impact on patient DFS or OS.

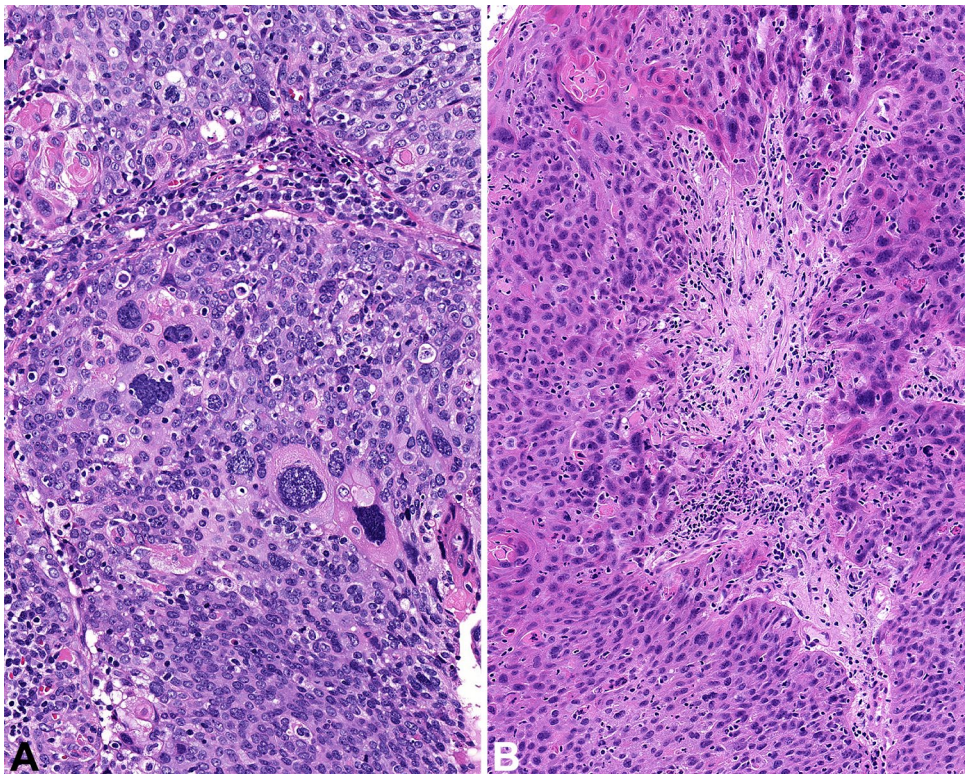

Fig. 11 Multinucleation and profound anaplasia in OPSCC. **a** There is profound nuclear pleomorphism and multinucleated in this field. **b** In this keratinizing OPSCC, there are numerous multinucleated and anaplastic cells

Clinical Treatment

See Supplemental Table E for management protocols. Patients underwent treatment after initial biopsy for curative intent with definitive concurrent chemoradiation (n = 345), induction chemotherapy followed by chemoradiation (n = 44), or radiotherapy alone (n = 1). Patients who underwent upfront chemoradiation received high-dose cisplatin (n = 143), triweekly carboplatin (n = 140), cetuximab (n = 32), weekly cisplatin (n = 25), weekly carboplatin (n = 1), and carboplatin/docetaxel (n = 1). Patients who received induction chemotherapy received either docetaxel and platinum-based induction chemotherapy with (n = 39) or without (n = 5) 5-fluorouracil. Following induction chemotherapy, concurrent regimens consisted of weekly carboplatin (n = 27), triweekly carboplatin (n = 9), weekly cisplatin (n = 4), high-dose cisplatin (n = 3), and cetuximab (n = 1). Patient follow-up for all patients was 7.2 years (average 6.7, range 0.02 up to 13.0 years) and 7.8 years for surviving patients (range 1.2–13.0 years). When separated by p16 status, the average OS was 7.0 and 4.9 years for p16-positive and p-16 negative patients, respectively (Fig. 12a). Within the p16-positive patient group, 284 were without disease at last contact (whether alive or dead), with an average follow-up of 7.8 years, and 58 patients were with disease at last contact (whether alive or dead), with an average of 2.9 years (see Supplemental Table B). The vital status at the time of last follow-up and the presence of recurrent/metastatic disease is included in Table 4. By Kaplan–Meier analysis, the 5-year DFS was 76% for p16-positive disease versus 46% for p16-negative disease ($p < 0.0001$; Fig. 12b). Five-year OS was 81% for p16-positive disease vs. 48% for p16-negative disease ($p < 0.0001$). Recurrences occurred in 84 patients, 65 of 342 (19.0%) p16-positive patients and 21 of 48 (43.8%) p16-negative patients. This shows that recurrences are more common in p16-negative patients ($p = 0.0003$). Locoregional recurrence developed in 47 patients (12.1%), 31 (9%) and 16 (33%) each in p16-positive and p16-negative, respectively ($p < 0.0001$). Distant metastases developed in 58 patients (14.9%), 49 (14%) and 9 (19%) in p16-positive and p16-negative, respectively ($p = 0.39$). Thus, the frequency of distant metastases was no different between p16-positive or negative tumors. There was a median time to recurrence of 0.74 years, and a median time to distant metastasis of 1.03 years; 96% of recurrences developed within 4 years, and 97% of distant metastases developed within 4 years.

On univariate analysis, baseline clinical features predictive of OS and DFS with statistical significance are included in Table 4. For p16-positive tumors, the DFS was worse and the OS shorter in older patients (using ≥ 55 years as a cutoff) compared to younger patients: hazard ratio 2.65 (1.62–4.33), $p < 0.0001$ and 3.21 (1.84–5.61), $p < 0.0001$, respectively. There was no difference in DFS or OS between males and females (n = 341 vs. 49; HR 0.67 [CI 0.42–1.08],

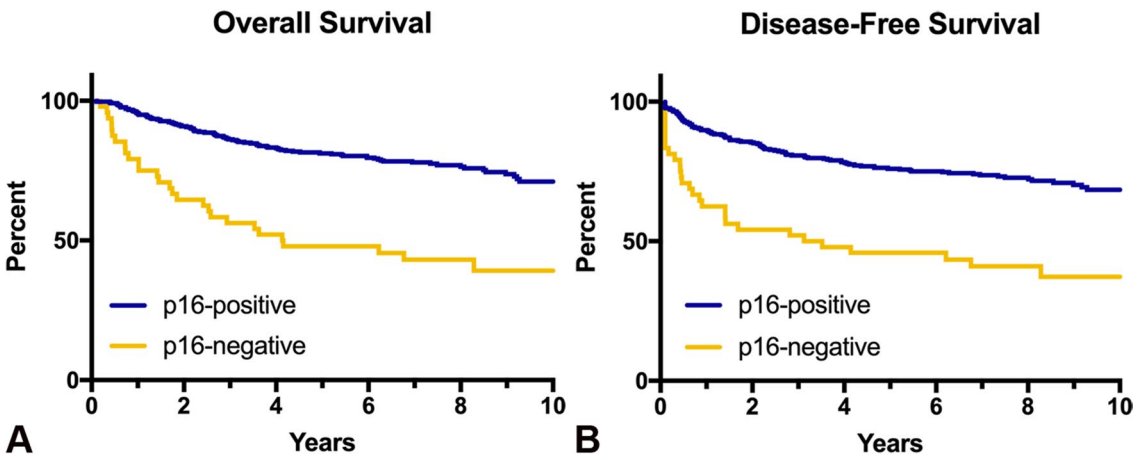

Fig. 12 Kaplan Meier analysis showing the overall survival (**a**) and disease free survival (**b**) for patients with p16-positive and p16-negative tumors

p = 0.095 and HR 0.71 [0.43–1.18], p = 0.19, respectively), nor between races (white, black, Asian, Native Indian; p = 0.57 and p = 0.42, respectively).

Among all-comers, the 5-year DFS and OS was 77% vs. 67% (p = 0.003) and 83% vs. 71% (p = 0.003) for < 10 pack-year smokers vs. ≥ 10-pack-year smokers, respectively. There was no patient outcome difference between *ever* (n = 290) versus *never* (n = 98) drinkers for DFS (p = 0.23) or OS (p = 0.18).

For patients with p16-positive disease, 5-year OS was 88% for clinical stage I disease, 86% for stage II disease, and 63% for stage III disease (p < 0.0001; Fig. 13a); and 5-year DFS was 84% for clinical stage I disease, 81% for stage II disease, and 55% for stage III disease (p < 0.0001; Fig. 13b). Among patients with p16-negative disease, 5-year OS was 55% for clinical stage III disease, 61% for stage IVA disease, and 21% for stage IVB disease (p = 0.04; Fig. 14a), and 5-year DFS was 55% for clinical stage III disease, 57% for stage IVA disease, and 21% for stage IVB disease (p = 0.02; Fig. 14b).

For patients with p16-positive tumors, ORENE was a poor prognostic factor for OS (HR 2.78 [1.68–4.46], p < 0.0001) seen within stage-matched patients without ORENE (HR 2.35 [1.43–3.87], p = 0.0007). ORENE remained a prognostically significant factor on multivariate analysis (HR 2.16 [1.29–3.63], p = 0.004; Fig. 15).

There was a worse survival outcome for patients with low level IV/Vb lymph node involvement when matched to same stage patients and controlled for p16 status; univariate analysis: HR 2.31 [1.47–3.63], p = .0003; controlling for p16 status HR 2.60 [1.65–4.11], p < 0.0001; controlling for stage: HR 2.01 [1.28–3.17], p < 0.001; and multivariate analysis: HR 2.37 [1.36–4.15], p = 0.002 (Fig. 16).

There was no statistically significant difference in patient DFS (p = 0.54) or OS (p = 0.10) when stratified by tumor site on univariable analysis. In p16-positive tumors, 293 were crypt centered and 49 were surface centered, but there was no clinically significant difference in DFS or OS (average 7.0 years overall survival). Histopathologic subtypes are listed in Table 3 and Supplemental Table D by p16 status. Overall, tumors that were p16-positive were more likely to be nonkeratinizing and crypt-centered with a broad front of invasion, moderate-to-brisk lymphocytic infiltrate, and higher mitotic index. Tumors that were p16-negative were more likely to be surface-centered, ulcerated, and keratinzing. They more commonly showed jagged/single cell infiltration, squamous pearl formation, a desmoplastic stromal reaction, concurrent dysplasia, mild lymphocytic infiltration, and a lower mitotic index.

On multivariable analysis of patients with p16-positive tumors, age (HR 1.07 [1.04–1.10], p < 0.0001), smoking history ≥ 10 pack-years (HR 1.76 [1.10–2.82], p = 0.02), stage (HR 1.77 [1.32–2.38], p = 0.0002), ORENE (HR 2.16 [1.29–3.63], p = 0.004), low-neck lymphadenopathy (HR = 2.37 [1.36-4.15], p = 0.002), and lymphocytic responses (mild vs. absent: HR 0.01 [0.002–0.05], p < 0.0001; moderate vs. absent: HR 0.01 [0.002–0.06], p < 0.0001; brisk vs. absent: HR 0.01 [0.002–0.05], p < 0.0001; Fig. 17a) were prognostic for OS. TNM stage (HR 1.58 [1.13–2.21], p = 0.007), the presence of ORENE (HR 2.90 [1.66–5.07], p = 0.0002), low-neck lymphadenopathy (HR 2.61 [1.42–4.80], p = 0.0001), and lymphocytic response (mild vs. absent: HR 0.01 [0.002–0.05], p < 0.0001; moderate vs. absent: HR 0.02 [0.004–0.10], p < 0.0001; brisk vs. absent: HR 0.01 [0.002–0.06], p < 0.0001; Fig. 17b) were prognostic for DFS. Five-year DFS/OS was 0%/0% for absent lymphocytic response, 66%/71% for mild lymphoid infiltrate, 72%/78% for moderate lymphoid infiltrate, and 80%/86% for brisk lymphoid response (Fig. 17).

Table 4 Overall patient outcomes for patients with oropharyngeal squamous cell carcinomas by clinical and histopathologic parameters

Factor (average follow-up in years)	Category	p16	All patients	Dead or alive *with* disease	Dead or alive *without* disease
Sex (p = 0.19)	Male	P	302 (7.0)	51 (2.8)	251 (7.9)
		N	39 (5.1)	14 (1.3)	25 (7.3)
	Female	P	40 (6.5)	7 (3.3)	33 (7.2)
		N	9 (4.0)	5 (1.5)	4 (7.1)
Race (p = 0.42)	White	P	310 (6.9)	54 (2.9)	256 (7.8)
		N	36 (4.4)	16 (1.5)	20 (6.7)
	Black	P	24 (7.4)	3 (3.4)	21 (7.9)
		N	12 (6.5)	3 (0.7)	9 (8.4)
	Asian	P	7 (7.1)	1 (1.3)	6 (8.1)
		N	0	0	0
	Native American	P	1 (4.3)	0	1 (4.3)
		N	0	0	0
Smoking (p = 0.09)	Ever	P	218 (6.8)	37 (2.8)	181 (7.6)
		N	41 (5.0)	16 (1.3)	25 (7.4)
	Never	P	124 (7.3)	21 (3.1)	103 (8.2)
		N	7 (4.5)	3 (1.7)	4 (6.5)
Tumor site (p = 0.10)	BOT	P	153 (6.8)	29 (3.0)	124 (7.7)
		N	16 (4.1)	6 (1.1)	10 (5.9)
	Pharynx	P	3 (6.5)	0	3 (6.5)
		N	9 (4.7)	4 (1.5)	5 (7.2)
	Soft palate	P	5 (5.7)	1 (2.0)	4 (6.6)
		N	3 (9.2)	0	3 (9.2)
	Tonsil	P	181 (7.1)	28 (2.7)	153 (8.0)
		N	20 (5.0)	9 (1.5)	11 (8.0)
Histologic classification (p < 0.0001)	Non-keratinizing	P	290 (7.0)	48 (2.9)	242 (7.8)
		N	20 (6.4)	3 (1.9)	17 (7.2)
	With maturation	P	35 (7.2)	6 (2.6)	29 (8.2)
		N	3 (4.9)	1 (0.9)	2 (6.9)
	Keratinizing	P	17 (6.6)	4 (2.6)	13 (7.8)
		N	25 (3.7)	15 (1.3)	10 (7.4)
Basaloid appearance (p = 0.005)	Yes	P	264 (6.9)	43 (2.8)	221 (7.6)
		N	20 (5.7)	4 (1.6)	16 (6.7)
	No	P	78 (7.3)	15 (3.0)	63 (8.4)
		N	28 (4.4)	15 (1.3)	13 (7.9)
Basaloid SCC (p = 0.53)	Yes	P	6 (7.2)	0	6 (7.2)
		N	0	0	0
	No	P	336 (7.0)	58 (2.9)	278 (7.8)
		N	48 (4.9)	19 (1.3)	29 (7.3)
Papillary type (p = 0.25)	Yes	P	22 (7.3)	2 (1.0)	20 (7.9)
		N	0	0	0
	No	P	320 (7.0)	56 (2.9)	264 (7.8)
		N	48 (4.9)	19 (1.3)	29 (7.3)
Lymphoepithelial type (p = 0.25)	Yes	P	52 (7.8)	9 (3.3)	43 (8.7)
		N	1 (10.0)	0	1 (10.0)
	No	P	290 (6.8)	49 (2.8)	241 (7.6)
		N	47 (4.8)	19 (1.3)	28 (7.2)

Table 4 (continued)

Factor (average follow-up in years)	Category	p16	All patients	Dead or alive *with* disease	Dead or alive *without* disease
Spindle cell type (p = 0.89)	Yes	P	5 (5.7)	2 (4.2)	3 (6.7)
		N	1 (10.8)	0	1 (10.8)
	No	P	337 (7.0)	56 (2.8)	281 (7.8)
		N	47 (4.8)	19 (1.3)	28 (7.1)
Adenosquamous (p = 0.57)	Yes	P	0	0	0
		N	1 (7.7)	0	1 (7.7)
	No	P	342 (7.0)	58 (2.9)	284 (7.8)
		N	47 (4.9)	19 (1.3)	28 (7.2)
Location (p = 0.004)	Crypt-centered	P	293 (6.9)	49 (3.0)	244 (7.7)
		N	19 (5.4)	6 (1.6)	13 (7.1)
	Surface-centered	P	49 (7.1)	9 (2.3)	40 (8.2)
		N	29 (4.6)	13 (1.2)	16 (7.4)
Ulceration (p = 0.0004)	Yes	P	48 (6.8)	6 (3.2)	42 (7.4)
		N	28 (4.7)	13 (1.1)	15 (7.8)
	No	P	294 (7.0)	52 (2.8)	242 (7.9)
		N	20 (5.2)	6 (1.9)	14 (6.7)
Concurrent dysplasia (p = 0.009)	Yes	P	95 (7.1)	19 (3.5)	76 (8.1)
		N	30 (4.7)	13 (1.2)	17 (7.4)
	No	P	247 (6.9)	39 (2.6)	208 (7.7)
		N	18 (5.3)	6 (1.6)	12 (7.1)
Squamous pearls/eddies (p = 0.47)	Yes	P	219 (7.0)	29 (2.5)	190 (7.7)
		N	39 (4.3)	18 (1.3)	21 (7.0)
	No	P	123 (6.9)	29 (3.2)	94 (8.0)
		N	9 (7.4)	1 (2.4)	8 (8.1)
Dyskeratotic cells (p = 0.16)	Yes	P	287 (7.1)	41 (3.0)	246 (7.8)
		N	44 (4.7)	18 (1.3)	26 (7.1)
	No	P	55 (6.1)	17 (2.5)	38 (7.7)
		N	4 (7.1)	1 (2.4)	3 (8.6)
Nuclear anaplasia (p = 0.79)	Yes	P	109 (7.0)	18 (3.1)	91 (7.7)
		N	16 (4.8)	6 (1.2)	10 (6.9)
	No	P	233 (7.0)	40 (2.8)	193 (7.8)
		N	32 (5.0)	13 (1.4)	19 (7.4)
Multinucleation (p = 0.99)	Yes	P	73 (6.8)	13 (3.5)	60 (7.5)
		N	9 (4.6)	4 (1.2)	5 (7.3)
	No	P	269 (7.0)	45 (2.7)	224 (7.9)
		N	39 (5.0)	15 (1.4)	24 (7.2)
Comedonecrosis (p = 0.48)	Yes	P	255 (7.0)	40 (3.0)	215 (7.8)
		N	41 (4.9)	16 (1.5)	25 (7.0)
	No	P	87 (6.8)	18 (2.5)	69 (7.9)
		N	7 (5.3)	3 (0.5)	4 (8.8)
Infiltrative pattern: Jagged/single cell (p = 0.0004)	Yes	P	45 (7.0)	6 (2.4)	39 (7.7)
		N	31 (4.4)	14 (1.4)	17 (6.9)
	No	P	297 (7.0)	52 (2.9)	245 (7.8)
		N	17 (5.8)	5 (1.3)	12 (7.7)

Table 4 (continued)

Factor (average follow-up in years)	Category	p16	All patients	Dead or alive *with* disease	Dead or alive *without* disease
Infiltrative pattern: broad front (p = 0.004)	Yes	P	266 (6.9)	46 (3.0)	220 (7.8)
		N	15 (6.1)	4 (1.6)	11 (7.7)
	No	P	76 (7.1)	12 (2.4)	64 (8.0)
		N	33 (4.4)	15 (1.3)	18 (7.0)
Infiltrative pattern: blended (p = 0.82)	Yes	P	31 (7.3)	6 (2.5)	25 (8.5)
		N	2 (4.1)	1 (0.2)	1 (8.0)
	No	P	311 (6.9)	52 (2.9)	259 (7.7)
		N	46 (5.0)	18 (1.4)	28 (7.2)
Desmoplastic stromal reaction (p = 0.09)	Yes	P	229 (7.0)	42 (2.8)	187 (7.9)
		N	42 (4.8)	17 (1.3)	25 (7.1)
	No	P	113 (7.0)	16 (3.2)	97 (7.6)
		N	6 (6.0)	2 (1.6)	4 (8.2)
Lymphocytic infiltrate (p < 0.0001)	Brisk	P	224 (7.2)	30 (2.9)	194 (7.9)
		N	13 (6.8)	3 (1.9)	10 (8.2)
	Moderate	P	51 (6.9)	12 (3.1)	39 (8.1)
		N	7 (5.7)	2 (2.1)	5 (7.2)
	Mild	P	65 (6.3)	14 (2.9)	51 (7.2)
		N	28 (3.9)	14 (1.1)	14 (6.6)
	Absent	P	2 (0.5)	0	2 (0.5)
		N	0	0	0
Mitotic figures (p = 0.31)	0–20	P	38 (7.8)	4 (3.6)	34 (8.3)
		N	13 (3.8)	8 (1.1)	5 (8.2)
	21–40	P	79 (6.8)	18 (2.5)	61 (8.1)
		N	12 (6.0)	4 (2.0)	8 (8.0)
	41–60	P	71 (6.9)	12 (3.3)	59 (7.6)
		N	11 (5.0)	3 (1.6)	8 (6.2)
	61–80	P	38 (6.7)	7 (2.5)	31 (7.6)
		N	5 (8.6)	0	5 (8.6)
	81–100	P	42 (6.7)	6 (1.7)	36 (7.6)
		N	1 (0.2)	1 (0.2)	0
	101–120	P	26 (6.7)	6 (3.6)	20 (7.6)
		N	2 (4.7)	1 (2.8)	1 (6.7)
	> 120	P	48 (7.3)	5 (3.5)	43 (7.7)
		N	4 (1.8)	2 (0.5)	2 (3.1)

P positive, *N* negative

On multivariable analysis of p16-negative disease, low-neck lymphadenopathy (HR 5.59 [1.01–31.05], p = 0.049) was prognostic for DFS, with 5-year DFS of 33% vs. 44% with and without low-neck lymphadenopathy, respectively. Other histopathologic factors did not demonstrate prognostic significance.

Discussion

Tumors that were p16-positive were more likely to be crypt-centered and nonkeratinizing with a broad, pushing front of invasion, moderate-to-brisk lymphocytic infiltrate, and high mitotic index [11]. In fact, nonkeratinizing tumors were nearly all (94%) p16-positive, a finding that may have been even higher if further high-risk HPV mRNA testing had been performed [28]. Tumors that were p16-negative were more likely to be ulcerated and surface-centered. Their

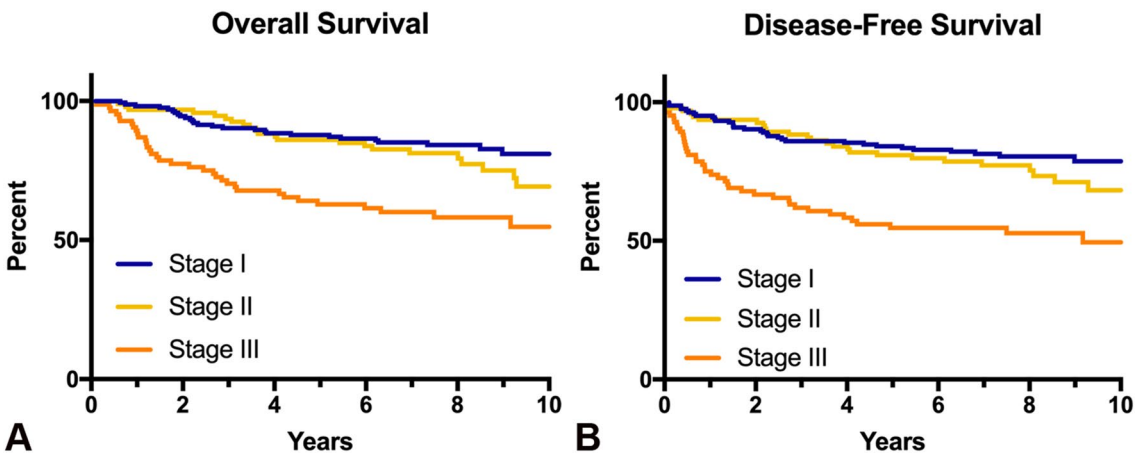

Fig. 13 Kaplan Meier analysis showing the overall survival (**a**) and disease free survival (**b**) for patients with p16-positive tumors separated by stage

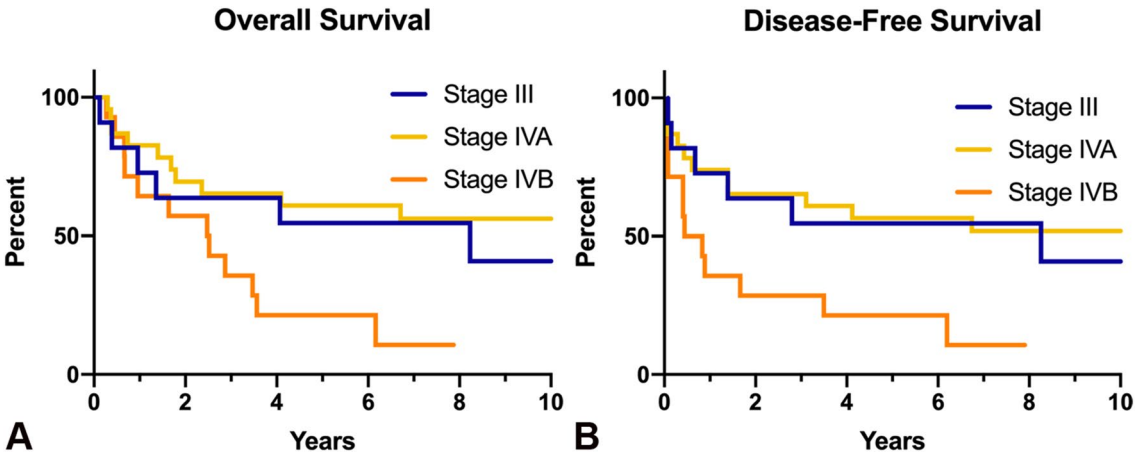

Fig. 14 Kaplan Meier analysis showing the overall survival (**a**) and disease free survival (**b**) for patients with 16-negative tumors separated by stage

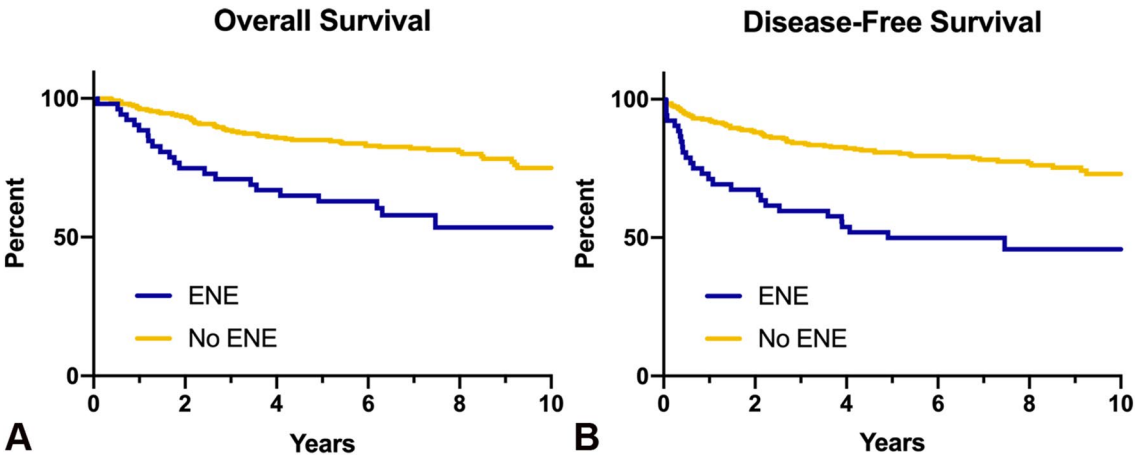

Fig. 15 Kaplan Meier analysis showing the overall survival (**a**) and disease free survival (**b**) for patients with p16-positive tumors with and without overt radiographic extranodal extension

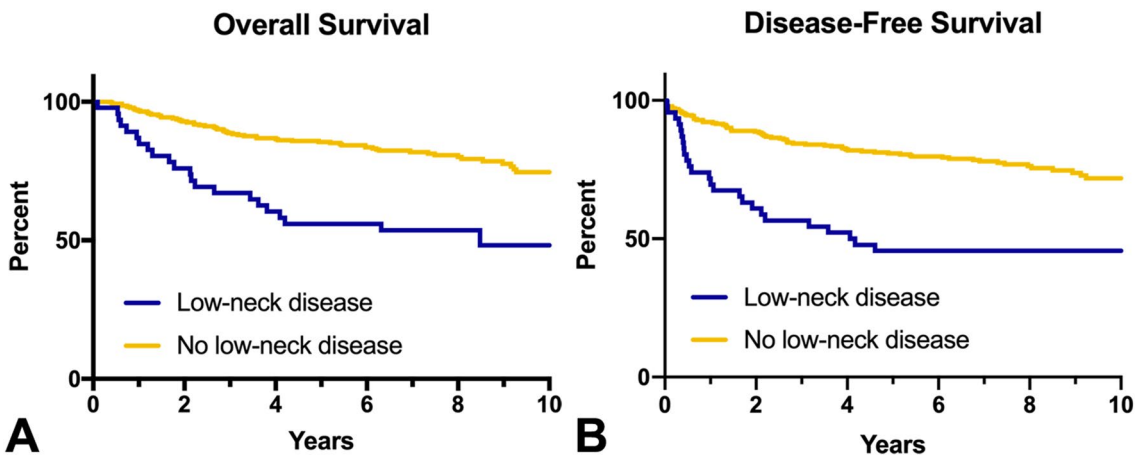

Fig. 16 Kaplan Meier analysis showing the overall survival (**a**) and disease free survival (**b**) for patients with p16-positive tumors with and without low level IV/Vb lymph node involvement

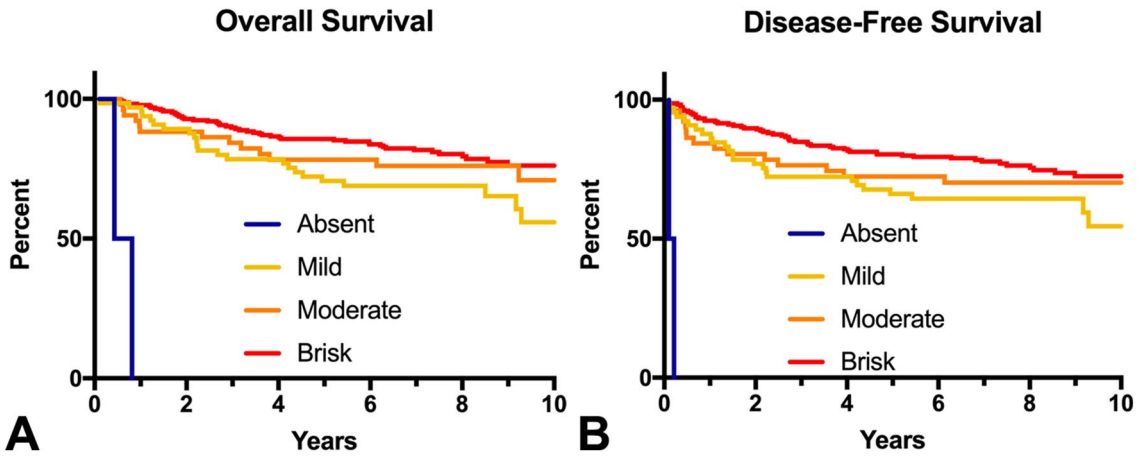

Fig. 17 Kaplan Meier analysis showing the overall survival (**a**) and disease free survival (**b**) for patients with p16-positive tumors with variably lymphocytic infiltrate responses

histologic features included jagged/single cell infiltration, squamous pearls/eddies, a desmoplastic stromal reaction, concurrent dysplasia, mild lymphocytic infiltration, and a lower mitotic index. As already described, the lack of a well-developed basement membrane in the lymphoepithelium of the oropharynx [13] results in a high incidence of lymphovascular invasion and early lymph node metastasis. This finding was seen in 364/390 (93.3%) of the patients in this cohort. Even though the lack of an inflammatory infiltrate was statistically correlated with a poor prognosis (DFS and OS), it was identified in only 2 patients, and thus may not be an event with sufficient power to warrant such a statement.

Reported 5-year overall survival for p16-positive OPSCC ranges from 60 to 94% for all comers [4, 5, 29–32]. Disease free or progression free survival ranges from 57 to 85% [5, 30–32]. In this study, recurrence occurred in 86 (22%) patients with 47 (12%) patients experiencing locoregional disease and 58 (15%) with distant metastases. Nineteen patients (5%) experienced both. The average time to recurrence for all comers was 15.0 months; 7.2 months for p16-negative tumors and 17.6 months for p16-positive tumors, with an average time to locoregional recurrence and distant metastasis of 11.2 months and 18.2 months, respectively. Specifically, 96% of recurrences developed within 48 months and 97% of distant metastases developed within 48 months. These findings suggest that post-treatment oncologic surveillance for at least 48 months is prudent [30, 33].

As previously reported, patient age (defined by < 55 and ≥ 55 years) is a significant factor in DFS and OS, but only for p16-positive patients in this cohort of patients ($p < 0.0001$). These findings are similar to those reported by others, although with different cutoffs amongst studies [6, 29–32, 34–46].

The majority of patients were white (88.7%), although black patients disproportionately had p16-negative tumors (50%) when compared to white patients (9%). Patients in this series were stratified based on smoking status [4]. Recognizing that pack year history, smoking duration, and interval from quitting impact patient outcomes [4, 5, 22, 24, 36, 38, 47, 48], the patients were further categorized into never smokers or < 10 pack-year smokers as a single group while all other patients were placed in the ≥ 10 pack-year smoking history. This stratification identified 46.9% of all patients had a ≥ 10 pack-year smoking history in this cohort, a significant disease cofactor. Specifically in the p16-positive tumors, a smoking history ≥ 10 pack-years was an independent predictor of inferior survival (HR 1.76 [1.10–2.82], p = 0.02), confirming smoking as an adverse prognostic factor in HPV-associated OPSCC.

The AJCC 8th edition separated the HPV-associated OPSCC from HPV-independent tumors in recognition of the different variables that need to be considered in management as treatment options are modified based on HPV-status. For HPV-associated cancers, this is predicated on whether the disease is clinically unilateral (N1), bilateral (N2), or the size of any node being over 6.0 cm (N3). Based on findings in this patient cohort, specifically a worse outcome associated with the presence of ORENE and low level IV/Vb neck lymphadenopathy, the lymph node categories may need to be further stratified.

In p16-positive OPSCC primary tumors, additional studies have identified clinical extranodal extension/matted nodes to be a strong predictor of worse outcome [49–55] although size within 6 cm and number of affected lymph nodes does not seem to adversely affect outcome [56, 57]. One suggested reason is that large cystic metastases, a common finding in these patients, do not carry the same disease burden as solid metastases of a similar size [58]. In this cohort, this clinical finding was encompassed by ORENE. Patients who had no ORENE had the best overall survival. Analyzing only those patients with p16-positive tumors, ORENE was a poor prognostic factor for overall survival (HR 2.74 [1.68–4.46], p < 0.0001). The prognostic impact of ORENE in the cohort of p16-positive tumors remained significant on multivariate analysis (HR 2.16 [1.29–3.63], p = 0.004), even after adjusting for potential confounding factors (age, stage, tobacco status) [58]. This suggests the cN categories may need to be modified or upstaged to account for ORENE or that de-intensified therapy be selectively applied to patients without ORENE if other findings are similar.

In p16-positive OPSCC primary tumors, involvement of low lying level cervical lymph nodes, specifically IV/Vb, is a predictor of worse outcome and development of distant metastasis [14, 59]. In this cohort of patients, especially in patients with p16-positive tumors, level IV/Vb disease was prognostic for inferior survival (HR 2.95 [1.81–4.80], p < 0.001). The prognostic impact of level IV/Vb lymph node involvement in p16-positive disease remained significant on multivariate analysis (HR 2.37 [1.36–4.15], p = 0.002). Again, this finding suggests that the cN categories may need to be modified or upstaged to account for level IV/Vb lymph node disease.

In a large national registry report of OPSCC, 1.1% of tumors presented as bilateral primaries [60]. In this cohort of patients, 2.6% (10 of 390) presented with bilateral tumors, 8 of which were p16-positive and 2 were p16-negative. Therefore, it would seem that bilateral presentation is uncommon, but not rare.

Nearly all of the tumors arose from the tonsil (n = 201) or base of tongue (n = 169). The remaining 20 cases arose from the pharynx (n = 12) or soft palate (n = 8). When broken down further, a significant proportion of tumors were p16-negative in the pharynx (75%; 9/12) and soft palate (37.5%; 3/8) when compared to the base of tongue and tonsil (9.5%; 16/169 and 10%; 20/201). However, large primary tumors may have been centered with the bulk of tumor in an anatomic site, even though adjacent sites may also be involved. As recently suggested [61], regions of the oropharynx that do not harbor tonsillar tissues, such as the soft palate, may be separated from this anatomic designation [13] and included in the oral cavity instead.

All our patients were treated in a unified and consistent approach and all were centrally reviewed prior to evaluation. Thus, even though retrospective cohort analysis from large groups of patients suggest there is a difference in OS by subsite, it is probably due to other confounding factors that are not consistently evaluated, treatment changes over the past two decades, or perhaps aggregated groupings that are not easily evaluated [62–64]. Further, several authors have suggested that within p16-positive tonsillar tumors, the specific location of crypt (referred to as specified tonsillary SCC [STSCC] based on tonsillar crypts and lymphoid tissue with germinal centers) versus surface (referred to as non-specified tonsillary SCC [NSTSCC] when tonsillary crypts and lymphoid tissue are absent) may be associated with a better outcome when crypt-centered versus surface located [35, 64–66].

In this cohort of patients, the nonkeratinizing histologic appearance was strongly correlated with p16-positive tumors (p < 0.0001) and was shown to be associated with patient outcome [9, 11, 28, 67]. There was no difference between p16-positive tumors classified as nonkeratinizing and nonkeratinizing with maturation when evaluating patient outcome, thus, while a recognized group, they can be collapsed into a single category of nonkeratinizing for management purposes [11, 19, 28, 68].

Several different histological subtypes were identified including basaloid, papillary, lymphoepithelial

(undifferentiated) [8, 69], spindled cell, and adenosquamous [70, 71]. The true basaloid squamous cell carcinoma is a distinct pathological entity [7]. In this cohort of patients, this subtype did not experience a worse outcome than the remaining patients, a similar finding for any of the other variants.

Conceptually, many of the tumors were originally diagnosed as "poorly differentiated," but these nonkeratinizing SCC are actually the best differentiated, most closely resembling the normal lymphoepithelium of the tonsil/base of tongue region. As such, tumor grade is conceptually no longer an issue for HPV-associated (p16-positive) OPSCC. The lymphoepithelium, and especially the deep crypts of the tonsil, have a morphologic appearance that is indistinguishable from the vast majority of tumors that arise from this epithelium. Thus, by definition, they are "well differentiated" tumors as they so closely approximate and resemble the epithelium native to the region [11, 19, 20, 28, 67, 68, 72–75]. However, by classical histologic standards for other anatomic sites, these squamous cell carcinomas have historically been interpreted as poorly differentiated [76] due to a basaloid appearance and cells with a high nuclear to cytoplasmic ratio, prominent nucleoli, vesicular to coarse nuclear chromatin, and increased mitoses. It has been shown in this cohort that the traditional tumor grading for p16-positive tumors does not inform prognosis, and thus no differentiation or tumor grade is used for these tumors. Instead, grade is only assigned to p16-negative tumors.

By univariate analysis, old age (≥ 55 years), smoking status (≥ 10 pack-year history), and advanced stage were all statistically significant clinical features associated with a shorter OS. These findings are also documented in other smaller studies [22, 30, 31, 50, 77]. Other potential variables, such as sex [31, 37, 41], race [37], lymphovascular invasion [50], and more than 4 lymph nodes with metastatic disease [30, 50] have been suggested as factors associated with treatment failure and a worse outcome. In this cohort of 390 patients managed centrally, these other factors were not significant.

By multivariable analysis, p16-positive tumors, older age (≥ 55 years), ≥ 10 pack-year history smoking status, overt radiographic extranodal extension, low neck (level IV/Vb) lymph node involvement, and high tumor stage remained prognostically predictive. These findings are similar to those previously reported in smaller cohorts of patients, irrespective of geographic distribution [22, 37, 38, 50, 77–79].

The specific pattern of the tumor is not associated with patient outcome. Specifically, when using the term "lymphoepithelial-like" or "undifferentiated" carcinoma in the oropharynx, a concept similar to the pattern of growth seen in nasopharyngeal tumors (nasopharyngeal carcinoma, undifferentiated type) [69], there is no difference in patient outcome, as seen in the 53 patients in this series who showed this pattern. All except one were p16-positive, showing an overall 7.8 years of follow-up without a statistically significant difference in outcome in comparison to the remaining OPSCC, findings that are similar to other authors [8].

In cases demonstrating a high tumor inflammatory response with low stromal desmoplasia, patients seemed to have a better disease specific 5-year survival, a finding that we did not encounter in this cohort of patients [35, 80]. This may be due to the limitation of examining biopsy samples rather than resection samples. Because the desmoplastic stromal reaction is usually only detected in HPV-associated tumors that have invaded all the way through the lymphoid stroma before expanding into the soft tissue, the biopsy sample may not have included the area where the desmoplastic stromal reaction is identified [35, 80].

The specific treatment modalities and comparisons between therapies in this cohort of patients and smaller subsets of the cohort have already been published, specifically addressing cisplatin-based chemoradiotherapy and cetuximab-based bioradiotherapy [15], high-dose cisplatin versus triweekly carboplatin-based chemotherapy [17], induction chemotherapy followed by concurrent chemoradiation versus concurrent chemoradiation alone in the definitive management of p16-positive oropharyngeal squamous cell carcinoma with low-neck or N3 disease [14], and radiographic nodal prognostic factors in stage I HPV-associated oropharyngeal squamous cell carcinoma [58].

Conclusion

OPSCC is strongly associated with p16 positivity, and, by extension, HPV-associated. The majority of the patients are younger, white, and likely to present with lymph node metastatic disease. There is a worse OS in patients with ≥ 10 pack-year smoking history, overt radiographic extranodal extension, and low-neck (IV/Vb) lymph node involvement. Histologically, among patients with p16-positive disease, OS is worse in patients with an absent lymphocytic infiltrate and improved in p16-negative patients with non-keratinizing histology. Thus, it seems that the AJCC 8th edition criteria pose potential problems in classification, treatment, and patient outcome as it relates to clinical lymph node status. Specifically, radiographically detectable overt extranodal extension and low-neck lymph node disease are parameters which are associated with a worse overall survival and disease-free survival.

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Compliance with Ethical Standards

Conflict of interest All authors declare that he/she has no conflict of interest as it relates to this research project. The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of Southern California Permanente Medical Group.

Ethical Approval All procedures performed in this retrospective data analysis involving human participants were in accordance with the ethical standards of the institutional review board (IRB #5968 and #11178), which did not require informed consent.

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