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Prognostic Indicators in Head and Neck Oncology Including the New 7th Edition of the AJCC Staging System

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Introduction

Head and neck squamous cell carcinoma (HNSCC) represents a number of different diseases each with unique clinical "niche" issues. Clinicopathologic or molecular studies based upon small numbers of patients which group together different anatomic sites and stages are not optimized to address these niche issues and are usually destined to have limited clinical impact. An underlying consideration for any investigation is, "Will this biomarker/indicator add prognostic value beyond known clinical confounders?" Will it retain prognostic significance after model building, which should include confounders such as site, stage, gender, age, and treatment, as well as other factors such as the continuation of smoking, race, Human Papillomavirus (HPV) status, and resection margin status? Only biomarkers/indicators that remain significantly associated with outcome after regression analysis have the potential for clinical usefulness. The number of confounders requiring consideration will impact the required cohort size to study any biomarker. It is startling how rarely this issue of sample size is mentioned in publications, and often omitted is the acknowledgment that the lack of statistical significance in small studies does not negate the possibility of a significant association.

The goal of this manuscript is to review the recent changes in the 7th edition of the AJCC staging criteria for HNSCC and to focus on some of the validated prognostic indicators for HNSCC. The following focused questions currently facing clinicians will be discussed:

(1) Which patients with stage I oral SCC will require elective neck dissection?
(2) Which patients with stage I/II oral/oropharyngeal SCC will fail primary surgical therapy?
(3) What impact does resection margin status have on outcome?
(4) Which oropharyngeal carcinomas are HPV+ driven cancers, and thus may be overtreated by current regimens?

The 7th AJCC Staging Criteria: General Issues

The recently published 7th AJCC staging criteria [1] contain the following major modifications for head and neck cancers: (1) Staging components for nasopharyngeal carcinoma have shifted (2) Thyroid T1 neoplasia are now subdivided into T1a and T1b, and (3) New staging criteria have been added for mucosal melanoma. A common minor change is the addition of the descriptors "moderately advanced" and "very advanced" to T4a and T4b tumors, respectively, for lip, oral cavity, oropharynx, nasal cavity, laryngeal, major salivary carcinomas and thyroid carcinomas. However, the actual anatomic landmarks defining T4a and T4b for each of these anatomic sites remain the same. Additionally, reporting prognostic factors ("site-specific factors") is recommended for oral and pharyngeal cancers, although not required for staging. These site-specific factors are related to the primary site, i.e. tumor thickness and HPV status, as well as nodal disease, i.e. node size, location/level, and extracapsular spread. Tumor thickness is germaine to only small T1 SCC, and expressed in

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millimeters. It is measured perpendicularly from the surface of the invasive SCC to the deepest area of involvement. This indicator measures both the exophytic and invasive tumor components. In distinction, the indicator of tumor depth of invasion is measured from the level of the mucosal surface to the deepest part of the tumor. Depth of invasion is also germaine to only small T1 SCC. With respect to HPV status as a site-specific factor, *there are no recommendations as to the specific method for HPV detection.*

Extracapsular spread has long been recognized as being associated with poorer prognosis, and can be recognized clinically by the presence of a "matted" lymph node mass, fixation to overlying skin, carotid sheath structures, soft tissues, or cranial nerve involvement. Pathologically, extracapsular spread should be classified as either gross (Eg), characterized by tumor apparent to the naked eye beyond the confines of the lymph node capsule, or microsopic (Em), defined as metastatic tumor beyond the lymph node capsule, associated with desmoplasia. No extracapsular spread should be designated as En. The reporting of the following features is also recommended: (1) Histological grade, (although subjectivity is acknowledged), (2) Vascular invasion and (3) Perineural invasion.

Changes in Staging for Nasopharyngeal Carcinoma

The staging criteria for nasopharyngeal carcinoma (NPC) has become more consolidated. Tumors previously classified as T2a (extension to oropharynx and nasal cavity without parapharyngeal extension) are now classified as T1; consequently, Stage IIA NPC is now Stage I. Tumors previously classified as T2b (parapharyngeal extension—beyond pharyngobailar fascia) are now classified as T2 and Stage IIB NPC is now Stage II. Lastly, bilateral retropharyngeal lymph node metastases, previously classified as N2, are now classified as N1 (Table 1). Histologically, NPC is classified according to the latest WHO criteria (keratinizing, nonkeratinizing, and basaloid). This replaces the previous terminology of WHO I (keratinizing carcinoma), WHO II (transitional cell carcinoma) and WHO III (undifferentiated or lymphoepithelial carcinoma).

Changes in Staging for Thyroid Carcinoma

For thyroid neoplasia, T1 tumors are now staged as either T1a (≤1 cm) or T1b (>1–2 cm), but the TNM staging groups are not affected. The most significant staging modifier for well-differentiated thyroid cancer (papillary and follicular carcinomas) remains patient age. Patients under 45 with well-differentiated thyroid cancer, any T, any N, and M0 have Stage I disease; while those patients with distant metastases (M1) are classified as Stage II. Patients 45 years of age or older are classified either as Stage I, II, III, or IV by other grouping definitions. Finally, descriptors of solitary (s) versus multifocal (m) tumor have been added to the pathologic staging.

Table 1 AJCC staging criteria for nasopharyngeal carcinoma

T1	Confined to nasopharynx, or extends to oropharynx and/or nasal cavity, no parapharyngeal extension		
T2	With parapharyngeal extension		
T3	Skull base and/or paranasal sinuses		
T4	Intracranial extension and/or cranial nerves, hypopharynx, orbit, infratemporal fossa/masticator space		
Nx	Cannot be assessed		
N0	Negative LN		
N1	LN mets, ≤6 cm either unilateral cervical above supraclavicular fossa, or unilat/bilat retropharynx		
N2	Bilateral cervical LN ≤6 cm above supraclavicular fossa		
N3	+LN >6 cm and/or to supraclavicular fossa		
N3a	>6 cm		
N3b	+Supraclavicular fossa		
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T1	N1	M0
	T2	N0	M0
	T2	N1	M0
Stage III	T1	N2	M0
	T2	N2	M0
	T3	N0	M0
	T3	N1	M0
	T3	N2	M0
Stage IVA	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage IVB	Any T	N3	M0
Stage IVC	Any T	Any N	M1

New Staging Criteria for Head and Neck Mucosal Melanoma

Primary head and neck MM are rare and aggressive tumors which have not yet had a practical or useful staging system. The new staging criteria reflect the aggressive nature of head neck mucosal melanoma, as all of these tumors are classified as high-stage (Table 2). Mucosal melanomas are commonly polypoid; T stage criteria are based on the degree of tumor extension, rather than size. Primary tumors are staged as either T3 (mucosal disease), T4a (moderately advanced disease involving deep soft tissue, cartilage, bone, or skin) or T4b (very advanced disease involving brain, dura, skull base, cranial nerves, masticator space, carotid artery, prevertebral space, or mediastinum). Although not specified, it is reasonable to assume that the

Table 2 AJCC staging criteria for head and neck mucosal melanoma

T3	Mucosal disease		
T4a	Moderately advanced—soft tissue, cartilage, bone, skin		
T4b	Very advanced—brain dura, skull base, lower cranial nerves, masticator space, carotid artery, prevertebral space, mediastinum		
Nx	Cannot be assessed		
N0	Negative		
N1	Regional LN+		
Stage III	T3	N0	M0
Stage IVa	T4a	N0	M0
	T3–T4a	N1	M0
Stage IVb	T4b	Any N	M0
Stage IVc	Any T	Any N	M1

classification as T4a may be based solely on microscopy. The sinonasal tract is the most common site for head neck mucosal melanoma. A polypoid sinonasal melanoma with only microscopic evidence of nasal septal invasion would then be classified as T4a.

Regional lymph nodes are classified as either NX (cannot be assessed), N0 (no node metastases) or N1 (positive regional nodes). Table 2 demonstrates the combined staging groupings. Additional recommended reported site specific factors are tumor thickness and size, level, and extracapsular spread, with respect to positive nodes.

Prognostic Indicators

Elective Neck Dissection for T1cN0 Oral Squamous Carcinoma

Oral squamous cell carcinoma (OSCC) is approached as a primary surgical disease. Cervical lymph node dissection is usually included in the primary surgery for T2/T3/T4 OSCC. What is the optimum treatment for T1 OSCC with clinically N0 (node negative) necks? It is generally accepted that a risk of occult cervical metastases $\geq$20% is an indication for elective neck dissection, and efforts have been centered upon primary tumor characteristics to assess risk on an individual basis. These factors have included lymphovascular invasion, perineural invasion, tumor depth of invasion, and tumor thickness. If the risk of occult metastases is >20%, what are the risks and benefits of elective neck dissection? There is little local morbidity from this surgical procedure although it does increase operative time. However, it is debatable whether elective neck dissection affects long-term survival; comprehensive review of the published clinical data examining this issue is beyond the scope of this manuscript. Patients who are followed closely and develop locoregional recurrence can be adequately treated by salvage surgery if diagnosed early and treated aggressively. Alternatively, elective neck dissection can be viewed as a staging procedure providing prognostic information for locoregional disease-status, adjuvant therapy, and potentially accomplish the same outcomes as salvage neck surgery with far less morbidity.

Yuen et al. [2] recently published a randomized clinical trial addressing elective neck dissection for patients with Stage I/II tongue carcinoma and clinical N0 necks. The necks were treated either by elective dissection (36 patients) or observation (35 patients). Occult metastases were found in 22% of the elective neck dissection group, and the majority (88%) of these patients went onto receive adjuvant radiotherapy. The locoregional recurrence rate was higher in the observational group, 11/35 (31%), as compared to the elective neck dissection group, 2/36 (6%). Most patients in the observation arm who developed locoregional recurrence were successfully salvaged and there were no differences in the 5-year survival rates for either group. They concluded that an advantage of elective neck dissection is that it allows for less extensive surgery (selective neck dissection) as compared to salvage radical neck dissection. There is additional controversy regarding whether patients with pN1 lacking extracapsular spread require adjuvant radiotherapy. As this study has a small number of patients in each group, caution must be maintained in overgeneralizing the results. Additionally, the authors advocated future studies addressing the extent of extracapsular spread and locoregional recurrence.

Predictors of Occult Metastases: Tumor Depth of Invasion

Which indicators predict occult metastases to the cervical lymph nodes? Ideally, these indicators should be derived from studies limited to patients with T1 and T2 tumors and cN0 necks, and therefore the inclusion of data from patients with clinically positive lymph nodes possibly skews the results. Optimally, subsites should also be considered independently, as anatomic differences might influence the rate of occult metastases. Occult metastases can be predicted by tumor depth of invasion, tumor thickness, and also tumor pattern of invasion. Tumor depth of invasion is considered a better predictor than tumor thickness.

Tumor Depth of Invasion and Tongue SCC

Tumor depth of invasion is an accepted indicator which is significantly associated with occult cervical metastases. Fakih reported on 70 patients with T1/T2 cN0 tongue SCC and found that a cut-off for depth of invasion of 4 mm was predictive of occult cervical metastases [3]. This was the

very first study to randomize patients into elective neck dissection and observation arms; however, unlike the study by Yuen [2], no significant differences in disease-free survival were observed between the two treatment groups. This may be due to the inclusion of more T2 patients (66%) as compared to Yuen's randomized trial (39%). Fukano [4] studied 34 patients with tongue SCC, all stages, but separated out the data for cN0 patients . No occult metastases were associated with depth of invasion <3 mm. Occult metastases were found in 6% of tumors with <5 mm depth of invasion and 65% of tumors with depth of invasion ≥5 mm. Sparano studied 45 patients with T1/T2 cN0 tongue SCC and found a significant cut off at 4 mm for both depth of invasion and tumor thickness [5]. No occult metastases were found for tumors with <4 mm depth of invasion and/or tumor thickness, whereas occult metastases were present in 42 and 41% of tumors with depth of invasion and tumor thickness ≥4 mm, respectively. The above studies support a cut-off of 4–5 mm for depth of invasion in tongue SCC. More recently, An et al. [6] studied 63 patients with T1/T2 cN_o tongue SCC. While this study did not investigate predictors of occult metastases, it did find that a depth of invasion cut-off of 3 mm and/or invasion into intrinsic muscle was associated with regional recurrence. Given these results, and others, the generally accepted tumor depth of ≥4 mm is commonly used as the major criterion for elective neck dissection in T1N0M0 tongue SCC.

Tumor Thickness and Floor of Mouth SCC

Only one study focused on patients with low-stage floor of mouth cancers with cN0 necks. Mohit-Tabatabai reported on 84 patients with T1/T2 cN0 floor of mouth SCC, examining the indicator of tumor thickness [7]. Occult metastases were found in one of 57 (2%) tumors ≤1.5 mm thick, four of 12 (33%) tumors between 1.6 and 3.5 mm thick, and nine of 15 (60%) tumors thicker than >3.5 mm; this supports a cut-off >1.5 mm. This one study suggests than thinner cancers in the floor of mouth SCC may metastasize, as compared to the tongue. However, appropriate validation is lacking, and the same indicators (depth of invasion) should be compared. Suzuki, and Wallwork, studied patients with T1/T2 floor of mouth SCC, however these studies are suboptimal as they included patients with clinically positive lymph nodes which possibly could skew significant cut-off values [8, 9].

Predicting Treatment Failure in Low-Stage Oral/Oropharyngeal SCC: Our Risk Model

Patients with Stage I/II oral/oropharyngeal SCC are usually treated with single modality protocols. As many as 25 and 37% of Stage I and Stage II oral cavity cancer patients, respectively, develop locoregional recurrence, suggesting that more aggressive protocols are warranted for a subset of low-stage patients [10]. Offering adjuvant radiotherapy to all low-stage patients would expose a larger subpopulation to unnecessary, harmful toxicities. Therefore the identification of low-stage patients who are at a high risk of disease progression or recurrence would represent a tremendous advantage in the management of this disease, as they could be candidates for targeted administration of multimodality treatment protocols.

Multiparameter histological predictive models have been advocated in Europe since the 1970s. Could these models be used to predict which low-stage patients are at risk for disease-progression? Tumor pattern of invasion at the tumor host interface is a consistent component of these models [11–20]. Other components have included lymphocytic host response, exophytic versus endophytic growth pattern, mitotic rate, degree of keratinization, and nuclear pleomorphism. Interestingly, perineural invasion, a component of our Risk Model (see below) was never part of these models. Most data supporting associations of perineural invasion with outcome are derived from univariate analyses [21]. Few studies demonstrate that perineural invasion maintains prognostic significance after multivariable modeling [22–24].

Jakobsson's original model evaluated 8 variables in a 4-tiered scoring system. This cumbersome system was modified into a four-variable four-tiered system by Bryne [12–14, 16] Attempts at validating the Bryne model, and pattern of invasion as a single indicator, however, were not always successful. Problems with the validation studies included small sample size, heterogeneous tumor sites and primary treatment modalities, plus evaluation of different specimen types, as some studies evaluated only biopsies, others only resection specimens, and still others evaluated both biopsies and resection specimens.

We published a novel Risk Model in 2005 that extended previous models and contained unique features [25]. The Risk Model classified patients into low-, intermediate-, and high-risk groups, and correlated significantly with locoregional recurrence ($p = 0.0004$) and overall survival ($p = 0.0001$). The greater levels of statistical significance indicated that the performance of our Risk Model was better than the Bryne Model. While the Bryne and Jacobsson models are linear, assigning equal weight to each variable, the Risk Model is non-linear. It groups together non-aggressive patterns of invasion, while assigning greater point value for carcinomas that spread beyond the main mass (Worst pattern of invasion type 5—3 points) as compared to carcinomas forming small islands which remain close to the main tumor (Worst pattern of invasion type 4—1 point). Worst pattern of invasion type 5

is unique to our model, describing a tumor phenotype with a dispersed pattern of invasion. We found worst pattern of invasion type 5 in 25% of tumors and it is significantly associated with poorer outcome. Perineural invasion of large nerves (>1 mm diameter) is another unique feature of our model. It is also significantly associated with poorer outcome as compared to small nerve involvement or no perineural invasion. We have recently completed the first validation study on a cohort of 305 patients with primary HNSCC of the oral cavity, oropharynx, and larynx, from three different institutions [26]. The median follow-up period for all patients was 27 months. The outcome data confirm that the risk categories are predictive of time to disease progression ($p = 0.0005$), locoregional recurrence ($p = 0.013$), and overall survival ($p = 0.0000$) by Kaplan–Meier analysis. Cox regression analysis confirms that the high-risk status is significantly associated with decreased time to disease progression ($p = 0.015$, HR 2.32 95% CI 1.18, 4.58) compared to collapsed intermediate- and low-risk groups (Table 3).

In response to the original question, it is reasonable to expect that high-risk status confers the same prognostic implications when application is limited to patients with low-stage disease. This has been demonstrated in the above study since the confounder of stage was considered in the regression analysis. However, this first validation study was not powered to demonstrate added predictive value *within* a low-stage cohort. Over 500 Stage I/II patients will need to be assessed to provide adequate power and multi-institutional accrual is underway. However, an interim subgroup analysis of low-stage patients ($n = 107$) reveals that high-risk status predicts decreased time to overall survival ($p = 0.0239$) and disease progression ($p = 0.0070$) on univariate analysis. Once the high-risk category has demonstrated predictive value for low-stage patients, widespread application can be advocated as a tool to be used in the development of new treatment protocol.

The Prognostic Significance of Resection Margins

The disease-free survival benefit of adequate resection margins been demonstrated in a number of large studies. Using the definition of "tumor cut-through" for positive margins, Byers [27] reported on 216 patients, and demonstrated local recurrence rates of 80% in the positive margin group, compared to 12-18% in the negative margin group. The definition of ≥5 mm for margin adequacy is a common standard, and was used in the following statistically significant studies [28–31]. Chen reported on 270 patients and demonstrated locoregional recurrence of 55 versus 17%, and 5-year disease-free survival of 7 versus 39%, for inadequate, versus negative margin groups, respectively [28]. Loree reported on 303 patients and demonstrated locoregional recurrence of 30 versus 18%, and 5-year overall survival of 52 versus 60%, for inadequate, versus negative margin groups, respectively [29]. Garzino [30] reported on 245 patients and demonstrated 5-year overall survival of 48 versus 65%, for inadequate, versus negative margin groups, respectively. El-Husseiny reported on 66 patients and demonstrated 5-year disease-free survival of 0 versus 63%, and 5-year overall survival of 21% versus 72%, for adequate versus inadequate margin groups, respectively.

No study has ever directly compared different margin distances. For instance, is there any benefit in achieving 10 mm clearance? McMahon [24] studied 237 patients using a 10 mm standard for margin adequacy. This study confirmed that resection margin status was associated with

Table 3 Cox proportional hazard model: risk model and outcome for 305 patients with primary squamous carcinoma of the oral cavity, oropharynx, and larynx

	Disease progression			Overall survival		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
High-risk	2.32	(1.18, 4.58)	0.015	1.69	(0.94, 3.05)	0.079
Female	0.87	(0.49, 1.54)	0.638	0.83	(0.51, 1.36)	0.466
Age ≥60	1.22	(0.70, 2.11)	0.480	1.59	(0.98, 2.57)	0.060
Margins <5 mm	1.28	(0.70, 2.37)	0.422	2.09	(1.22, 3.59)	0.008
Positive margins	0.92	(0.40, 2.13)	0.844	2.14	(1.08, 4.25)	0.030
Oropharynx*	0.64	(0.27, 1.55)	0.325	0.49	(0.25, 0.97)	0.039
Larynx*	0.74	(0.33, 1.65)	0.467	0.45	(0.24, 0.85)	0.014
T stage 3/4	1.75	(0.94, 3.29)	0.079	1.53	(0.92, 2.53)	0.100
N stage ≥1	1.00	(0.53, 1.89)	0.993	1.42	(0.84, 2.41)	0.196
Surgery alone, no adjuvant therapy	1.38	(0.73, 2.61)	0.315	0.74	(0.45, 1.24)	0.256

HR hazard ratio; *CI* confidence interval

* Anatomic sites are compared to oral cavity as baseline

local recurrence and disease-specific survival on univariate analysis. Unfortunately, the data presented does not allow for comparison of corresponding outcomes with other centers using a 5 mm clearance standard.

Conversely, some studies fail to demonstrate significant outcome differences relating to resection margin status. Amaral and colleagues studied 188 patients with clinical Stage I/II oral SCC; they found that the disease-free and overall survival rates were unaffected by margin status (5 mm standard) [32]. Weijers reported on 68 patients, all stages, with oral SCC, and found no significant differences in locoregional recurrence between patient groups for negative (2/30 or 6.6%) and close margins (<5 mm, 3/38 or 7.9%), after the exclusion of patients with frankly positive ("cut through") margins [33]. Similarly, we found no significant differences in locoregional recurrence between patient groups for negative (28/119, or 23.5%) and close margins (<5 mm, 4/30 or 13%), after the exclusion of 19 patients with frankly positive ("cut through") margins [25]. The local recurrence rates for patients with inadequate margins in these two studies [25, 33] are better than for the corresponding inadequate margin groups in the previous studies [28, 29], although the same 5 mm margin standard was used. Why would some "inadequate" resection margins be associated with better outcome than others?

Comparing outcome data by resection margin status across different institutions is extremely complex. In addition to considering the usual confounders (age, gender, site, T and N stage, indications for adjuvant therapy, type of adjuvant therapy, smoking, HPV status), other layers of confounders require consideration such as the method of margin assessment and surgical techniques. It would be reasonable to expect at least some differences in techniques between surgeons. This would require adjusting analyses for each surgeon, which would greatly increase the required sample size. We hypothesize that the more optimal the surgical resection, and the more optimal the intraoperative margin assessment, the better the outcome for patients with "inadequate" margins. This would be seen as a lack of significant difference in outcome between patients with adequate and inadequate margins. We speculate that the degree of margin "inadequacy" was underestimated in the studies by Chen, Loree, and Garzino, which is reflected as significantly poorer outcomes for these patients [28–30]. Testing this hypothesis requires a well-designed multicenter study, ideally limited to one anatomic site, which would be adequately powered to adjust for the many anticipated confounders.

Importantly, there is no accepted standard method for margin assessment. Our practice is to perform intraoperative margin surveillance and procure supplemental tissues to address close margins. We advocate that the resection margin surveillance should be "specimen driven". The surgeon personally hands-off the specimen to the pathologist, and participates in specimen mapping. The pathologist cuts into the specimen at 5–10 mm intervals perpendicular to the resection margin plane. This gross assessment yields important preliminary information. The surgeon may elect to return to the defect at this time to harvest more tissue based on the gross examination, while the slides are being processed. This is followed by microscopic examination which further refines the information.

Human Papillomavirus

Increasing incidence of HPV-associated HNSCC has lead to shifts in overall patient demographics. The annual percentage increase for the incidence of tonsil/tongue base SCC is +3.0% ($p < 0.05$) [34]. A four-fold increased incidence of tonsillar SCC is observed for woman (1945–1994), as compared to the 1.3 fold increase for men [35]. These studies are indirect evidence of the impact of HPV on HNSCC carcinogenesis. The mean age of diagnosis for patients with known HPV + HNSCC is younger than for patients with HPV negative tumors [36]. Numerous studies have demonstrated a strong association with HPV16 and oropharyngeal SCC, especially tonsillar SCC. Table 4 summarizes 23 studies of oropharyngeal SCC (OPSCC) and HPV, published from 2000 to 2007; the HPV detection rates range from 13 to 82% [37–59]. Histologically, HPV + SCC tend to be basaloid, and nonkeratinizing, however the keratinizing phenotype may also be associated with HPV.

The clinical and therapeutic importance of HPV-driven carcinogenesis relates to the observation that patients with HPV + HNSCC have better outcome than patients with tobacco-mediated HNSCC carcinogenesis. Furniss [56] demonstrated improved survival for patients with HPV + OPSCC (Hazard ratios of 0.4 and 0.5 for serum and tumor data, respectively). This improvement is specifically associated with *productive* HPV infection. Weinberger demonstrated dramatically improved outcome when patients were stratified according to HPV viral load plus p16 status, as compared to classification by HPV status alone [54]. High HPV copy number plus p16 overexpression is associated with 5-year overall survival of 75%, as compared to 15% for HPV negative cases, or 13% for cases with low HPV copy number and limited p16 expression (Hazard ratio 5.26). One could argue that direct measurement of HPV becomes irrelevant, and p16 alone may be a sufficient prognosticator. Fischer recently demonstrated 5-year overall survival rates for patients with p16 + OPSCC of 57.1% (95% CI: 37%, 73%) as compared to 26.8% (95% CI: 15%, 41%) for p16 negative OPSCC (based on 5% nuclear expression cut-off) [60].

Overexpression of p16, a cell-cycle checkpoint regulator which inhibits cyclin-dependent kinase, has emerged as an important surrogate marker of productive HPV infection in

Table 4 HPV detection rates in patients with oropharyngeal squamous cell carcinoma

Author	Year	Country	Serum Ab	Pos	Total	%	Comments
Gillison [37]	2000	US		34	60	57	Most HPV 16. HPV more likely in nonsmokers (7/8) than smokers (27/51)
Mellin [38]	2000	Sweden		26	60	43	More women in HPV+ group
Schwartz [39]	2001	US		18	44	41	HPV more common in never smokers 14/42 than ever smokers
Mork [40]	2001	Scandinavia	Yes	9	18	50[t]	
Ringstrom [41]	2002	US		15	29	52	All HPV16
Dahlstrom [42]	2003	US	Yes	41	70	59	HPV16 more common in never smokers (24/35) than ever smokers (17/35)
							Serum HPV16 L1 Ab predictive for oropharyngeal SCC, OR 59.53 (95% CI: 5.71-620-2)
El-Mofty [43]	2003	US		10	11	91	All tonsil HPV16
Herrero [44]	2003	Multiple	Yes	26	142	18[t]	Serum HPV16 E6 or E7Ab predictive for oropharyngeal SCC, OR = 9.2 (95% CI: 4.8–17.7)
Begum [45]	2005	US		37	45	82	
Hansson [46]	2005	Sweden		21	46	46[o]	HR HPV DNA in oral mouthwash samples predictive for oropharyngeal SCC, OR = 230 (44–1,200) on multivariate regression analysis
Ibieta [47]	2005	Mexico		4	9	44	Most HPV16, no tonsillar SCC included, only retromolar trigone
Koppikar [48]	2005	India		2	6	33	Most were HPV16, but 14% were multiple HPV infections, (including HPV18, 8, 38)
Kreimer [49]	2005	Multiple	Yes	25	141	18[s]	Serum HPV VLP Ab plus high tumor viral load predictive for oropharyngeal SCC, OR 12.0 (95% CI 5.2–27.5)
Tachezy [50]	2005	Czech		32	56	57	HPV more common in never smokers (7/7) than ever smokers 28/61
El-Mofty [51]	2006	US		12	20	60	HPV 16
Licitra [52]	2006	Italy		17	90	19	All HPV16, 2 SCC integrated only, remaining both episomal and integrated
Nemes [53]	2006	Hungary		1	8	13	Most HPV16, most integrated
							HPV more common in never smokers (7/18) than ever smokers 26/61
Weinberger [54]	2006	US		48	79	61	
DeSouza [55]	2007	US		72	100	72	
Furniss [56]	2007	US	Yes	116	288	40.3[s]	High HPV16 L1 serum Ab titer predictive for oropharyngeal SCC, OR = 30.3 (95% CI 12.4–73.6)
Pintos [57]	2007	Canada	Yes	9	17	53[s]	Serum HPV16 VLP predictive for oropharyngeal SCC, OR = 27.68 (95% CI 7.5–102.2) by crude analysis
Smith [58]	2007	US	Yes	35	62	56[s]	Serum HPV16 E6/E7Ab predictive for oropharyngeal SCC, OR = 72.8 (95% CI 16.0–330)
Schlecht [59]	2007	US		7	17	41	Most HPV16

OR overall risk; *Ab* serum antibodies; *HR* high-risk; *s* seropositivity results; *t* tumor results; *o* oral mouthwash results; *VLP* viral like particles; *CI* confidence intervals

OPSCC. The mechanism of p16 overexpression relates to HPV E7 protein, which is formed during productive infection. E7 binds and inactivates Rb protein, which leads to escape from cell cycle G1 arrest and S-phase entry. The overexpression of p16 is the result of loss of feedback inhibition following Rb inactivation.

In response to the original question, how can the surgical pathologist utilize immunohistochemical (IHC) p16 expression to guide future therapeutic planning? What is the sensitivity and specificity of p16 expression by IHC as compared to the gold standard of reverse-transcription polymerase chain reaction (RT-PCR)? It is important to emphasize that the presence of HPV DNA alone, as determined by PCR or in situ hybridization (ISH) is insufficient to confirm productive infection. What cut-offs should be applied for p16 to deem a carcinoma HPV positive? Most researchers agree that *nuclear* p16 expression is required, however, the cut-off for nuclear expression varies between studies and has been as low as 5%. The sensitivity and specificity for nuclear tumor p16 expression as a surrogate biomarker for HPV is 69% (95% CI 61%, 75%) and 90% (95% CI 81%, 95%) [51, 61–64].

As patients with HPV-mediated cancers have a better prognosis, are they being over-treated by current protocols? Can they be offered less aggressive therapy? The treatment goal would be to decrease the risk of treatment-related toxicities while not compromising outcome. To the best of our knowledge, clinical trials designed to offer less intense treatment ("deintensification") to patients with HPV-induced HNSCC, are still in the planning phases. The AJCC staging recommendation to include the reporting of HPV status speaks to the inevitability that this biomarker will become a very important stratifier for treatment planning.

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