p16 Not a Prognostic Marker for Hypopharyngeal Squamous Cell Carcinoma

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Objective: To investigate the prognostic significance of p16 in patients with hypopharyngeal squamous cell carcinoma (HPSCC) and to evaluate the relationship between p16 and human papillomavirus (HPV). Unlike in oropharyngeal SCC (OPSCC), the prognostic significance of p16 in HPSCC and its association with HPV is unclear.

Design: Retrospective medical chart review.

Setting: University tertiary referral center.

Patients: A total of 27 patients with HPSCC treated with definitive radiation therapy between 2002 and 2011 whose tissue was available for immunohistochemical analysis.

Interventions: Twenty-two patients were treated with chemoradiation, and 5 with radiation alone. All tumor biopsy specimens were analyzed for p16 and, when sufficient tissue was available, for HPV DNA.

Main Outcome Measures: Overall survival (OS), locoregional control (LRC), disease-free survival (DFS), and laryngoesophageal dysfunction–free survival (LEDFS) were analyzed according to p16 status.

Results: Findings for p16 were positive in 9 tumors and negative in 18 tumors. Median follow-up was 29.3 months. There was no significant difference in OS, LRC, DFS, or LEDFS for patients with p16-positive vs p16-negative tumors. Only 1 of the 19 tumors tested for HPV was found to be HPV positive. When used as a test for HPV, p16 had a positive predictive value of 17%.

Conclusions: In contrast to OPSCC, p16 expression in patients with HPSCC has a low positive predictive value for HPV and did not predict improved OS, LRC, DFS, or LEDFS. Thus, for HPSCC, p16 is not a prognostic biomarker. Caution must be taken when extrapolating the prognostic significance of p16 in patients with OPSCC to patients with head and neck SCC of other subsites.


In contrast to OPSCC, little is known about the significance of p16 and HPV in other tumor subsites in the head and neck. Specifically, it is unclear whether the correlations found in OPSCC can be extrapolated to hypopharyngeal SCC (HPSCC). To our knowledge, no studies have published the prognostic significance of p16 in HPSCC. The only published study that we know of on the prognostic signifi-
cance of HPV in patients with HPSCC retrospectively analyzed 65 patients with hypopharyngeal or laryngeal SCC and found the presence of HPV to be associated with poorer outcomes, which contradicts what is known about HPV in OPSCC. The present study evaluates patients with HPSCC treated with intensity-modulated radiation therapy (IMRT) to determine the correlation between p16 and HPV status and the prognostic significance of p16 expression for this anatomic subsite.

METHODS

PATIENTS

Data were retrospectively collected under a protocol approved by an institutional human investigations committee. Prior to treatment, patient care was discussed by members of a multidisciplinary head and neck cancer tumor board. All patients in this analysis were treated with definitive IMRT for historically confirmed HPSCC between 2002 and 2011. Patients with a history of neck dissection or primary resection before irradiation, distant metastases at diagnosis, or absent biomarker data were excluded from the study. Twenty-nine patients were eligible by clinical criteria, but 2 had no biomarker data, leaving 27 patients available for analysis. Twenty-two patients were treated with chemotherapy and radiotherapy (81%), and 5 patients were treated with radiation alone (19%).

Of the 22 patients who received chemotherapy, 21 received induction chemotherapy and were treated with 1 to 4 cycles of induction chemotherapy as tolerated. Concurrent chemotherapy was delivered weekly.

The radiation dose to the primary tumor was 68 to 70 Gy in 34 to 35 fractions with the exception of 1 patient who failed to respond after a 50-Gy dose and underwent immediate surgical salvage. Doses of 68 to 70 Gy were delivered to the pathologic lymph nodes. Clinically uninvolved cervical lymph nodes were treated at doses up to 50 Gy. Regimens of IMRT were optimized so that at least 95.0% of the planned treatment volume received the prescribed dose.

Neck dissections were performed (1) on necks with stage N2b or higher nodal disease regardless of response to irradiation, and (2) on necks with apparent residual nodal disease on neck computed tomography imaging at 4 to 6 weeks after completion of radiation therapy. These were our institutional standards for neck dissection and were not part of a prospective protocol.

TISSUE MICROARRAY (TMA) AND IMMUNOHISTOCHEMICAL ANALYSIS

 Archived formalin-fixed and paraffin-embedded pretreatment primary tumor biopsy specimens were available for 27 patients. A TMA was constructed using three 0.6-mm cores of tumor per case. Immunohistochemical analysis was performed using a Dako Autostainer with primary antibodies for p16 (BD Biosciences, catalog No. 550834; titration: 1:100; pressure retrieval). Automated in situ hybridization (ISH) for high-risk HPV was performed using the Ventana HR HPV III probe set. Results of p16 testing were considered positive if strong nuclear and cytoplasmic staining was present in more than 60% of tumor cells, as previously described. The HPV ISH result was interpreted as positive in the presence of a punctate staining pattern of confluent groups of more than 20 cells.

STATISTICAL ANALYSIS

The following definitions were used for statistical analyses: overall survival (OS) was the time between the date of diagnosis and the date of death from any cause; locoregional control (LRC) was the time between the date of diagnosis and the date of first local or regional recurrence; disease-free survival (DFS) was the time between the date of diagnosis and the date of first disease recurrence or death from any cause; laryngoesophageal dysfunction–free survival (LEDFS) was the time between the date of diagnosis and the date of laryngectomy, death from any cause, or percutaneous endoscopic gastrostomy tube and/or tracheostomy dependence at 2 years of follow-up. Patients who were living and were without evidence of recurrence at the time of analysis were censored at the date of last follow-up. Two-sided t-tests and Fisher exact tests were used to assess differences in patient and tumor characteristics. The log-rank test was used to compare Kaplan-Meier survival curves. P values of .05 or smaller were considered significant. SPSS software (version 19.0.0; SPSS Inc) and SAS software (version 9.2; SAS Institute Inc) were used for statistical calculations.

Characteristics of patients and their tumors are summarized in Table 1. Eighteen of 27 tumors were p16-negative (67%), while 9 of 27 were p16-positive (33%). There were significantly more women in the p16-positive group than in the p16-negative group (33% vs 0%) (P = .03). Otherwise, there were no significant differences in cancer stage, tumor site or histologic findings, or patient age, smoking status, or alcohol use between patients with p16-positive and p16-negative tumors. Median follow-up for the entire cohort was 29.3 months (range, 2.6-95.6 months).

There was no statistically significant difference in mean OS (56.7 vs 58.7 months) (P = .88), mean LRC (65.3 vs 76.8 months) (P = .52), mean DFS (45.8 vs 54.2 months) (P = .60), or mean LEDFS (53.1 vs 54.5 months) (P = .97) for patients with p16-positive vs p16-negative tumors (Figure 1). Of the 11 patients in the database who died during follow-up, 5 died without any evidence of recurrence. Five patients had a recurrence at the primary site (19%) (3 were p16-negative; 2 were p16-positive); 2 of these underwent salvage laryngectomy (1 was p16-positive; 1 was p16-negative); and the remaining 3 did not undergo surgery owing to medical comorbidities or death shortly after radiation therapy. As seen in Table 2 and Figure 2, only T stage was significantly predictive of OS, LRC, DFS, and LEDFS. Overall stage group, N stage, and p16 status were not significant predictors of OS, LRC, DFS, or LEDFS.

Six of the p16-positive tumors had enough tissue to perform ISH for HPV, and only 1 of those tumors was HPV-positive. All 13 of the p16-negative tumors with tissue available for ISH were found to be HPV-negative. Thus, 1 of 19 tumors with tissue available for ISH was HPV-positive, giving an overall HPV positivity rate of 5.3% (95% CI, 0.1%-26.0%). The only patient with an HPV-positive tumor was alive at last follow-up with no evidence of recurrence after 85.0 months.

As seen in Table 3, when p16 was used as a test for HPV, p16 had a sensitivity of 100%, a specificity of 72%,
a positive predictive value of 17%, and a negative predictive value of 100%.

**COMMENT**

In the present study, we investigated the correlation between p16 and HPV and the prognostic significance of p16 in patients with HPSCC who were treated with definitive IMRT. Our results are in stark contrast to what is known about p16 and HPV in OPSCC. Over 86% of HPV-positive OPSCC tumors have been found to overexpress p16, while only 3% of HPV-negative OPSCC tumors overexpress p16. The correlation between p16 and HPV in OPSCC has been so close that p16 has been used as a surrogate immunohistochemical marker for HPV. The expression of p16, or lack thereof, has been found to be a superior prognostic biomarker for OPSCC. Our institutional experience has been similar; our research group previously reported an approximately 23% improvement in outcomes for p16-positive vs p16-negative OPSCC tumors.

Compared with patients with p16-negative tumors, patients with p16-positive tumors had a significantly improved 3-year locoregional progression-free survival (97.8% vs 73.5%) (P = .006) and 3-year DFS (88.2% vs 61.4%) (P = .004). In addition, when patients with OPSCC treated with definitive radiation therapy underwent postradiation neck dissection, patients with p16-positive tumors were significantly less likely to have residual viable tumor (18% vs 50% for p16-negative tumors) (P = .02), regardless of appearance on imaging.

The prognostic significance of HPV in subsites outside of the oropharynx is unclear. While data from multiple studies indicate that HPV-associated tumors account for 38% to 64% of OPSCC, a far lower percentage (13%-29%) of HPSCCs are reported to be HPV-positive. It is difficult to interpret the prognostic significance of HPV in the studies that have included primary tumors at multiple head and neck subsites and patients treated with various combinations of surgery, radiation, and/or chemoradiation.
The retrospective study by Clayman et al\(^8\) of 65 patients with laryngeal and hypopharyngeal carcinoma was derived from a database of patients who were treated with surgery alone, radiotherapy alone, or a combination of surgery and postoperative irradiation. They reported an HPV prevalence of 46% for the entire cohort and found HPV to be a negative prognostic indicator for LRC and OS. Of note, there were only 6 HPSCCs in their study population, and all 6 were reported to be HPV-positive (100%).\(^8\) In contrast, we found a low rate (5%) of HPV-associated hypopharyngeal cancers, similar to subset analyses from other series that have reported HPV-positive rates of 13% to 29% in the hypopharynx.\(^12,13\) There were insufficient patients with HPV-positive tumors in our series to comment on the prognostic significance of HPV. Thus, while most evidence suggests that there is a low prevalence of HPV, the true prevalence and prognostic significance of HPV in HPSCC remain unclear. One potential anatomic mechanism of HPV association with HPSCC could be tumor initiation in the lymphoid tissue of the inferior pole of a tonsil followed by tumor progression inferiorly along the lateral pharyngeal wall into the hypopharynx.

In the present analysis, 9 of 27 patients had p16-positive tumors (33%), which is consistent with subset analyses from other series that demonstrated p16-positive rates in the hypopharynx of 11% to 28%.\(^14,15\) Most importantly, in contrast to OPSCC, patients with p16-positive HPSCC did not have better outcomes than patients with p16-negative tumors. We did find T stage to be predictive of recurrence and survival, which is consistent with other studies.\(^16,17\)

When used as a test for HPV in the oropharynx, p16 has a sensitivity of nearly 100% and a specificity of approximately 79%.\(^18\) As seen in Table 3, in the present series we found p16 to have a sensitivity of 100% and a specificity of 72% when used as a test for HPV in the hypopharynx. Though p16 has a similar sensitivity and specificity in our series to that of the oropharynx, the prevalence of HPV in the hypopharynx (5%) in our se-

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**Figure 1.** Overall survival, locoregional control, disease-free survival, and laryngoesophageal dysfunction–free survival stratified by p16.

**Table 2. Survival Outcomes Stratified by Various Predictors**\(^a\)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>3-Year LRC</th>
<th>P Value(^a)</th>
<th>3-Year DFS</th>
<th>P Value(^a)</th>
<th>3-Year OS</th>
<th>P Value(^a)</th>
<th>3-Year LEDFS</th>
<th>P Value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients ((n = 27))</td>
<td>83.5</td>
<td>NA</td>
<td>59.2</td>
<td>NA</td>
<td>65.3</td>
<td>NA</td>
<td>61.3</td>
<td>NA</td>
</tr>
<tr>
<td>p16-positive ((n = 9))</td>
<td>74.1</td>
<td>.52</td>
<td>43.2</td>
<td>.60</td>
<td>64.3</td>
<td>.88</td>
<td>49.4</td>
<td>.97</td>
</tr>
<tr>
<td>p16-negative ((n = 18))</td>
<td>87.7</td>
<td>65.4</td>
<td>64.6</td>
<td>65.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall stage I-II ((n = 7))</td>
<td>100.0</td>
<td>.53</td>
<td>71.4</td>
<td>.58</td>
<td>71.4</td>
<td>.49</td>
<td>71.4</td>
<td>.69</td>
</tr>
<tr>
<td>Overall stage III-IV ((n = 20))</td>
<td>76.6</td>
<td>54.3</td>
<td>63.0</td>
<td>57.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T stage 1-2 ((n = 15))</td>
<td>83.3</td>
<td>.03</td>
<td>77.0</td>
<td>.03</td>
<td>82.5</td>
<td>.01</td>
<td>82.5</td>
<td>.01</td>
</tr>
<tr>
<td>T stage 3-4 ((n = 12))</td>
<td>62.5</td>
<td>25.0</td>
<td>38.8</td>
<td>25.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N stage 0-1 ((n = 15))</td>
<td>86.7</td>
<td>.94</td>
<td>62.0</td>
<td>.47</td>
<td>69.1</td>
<td>.36</td>
<td>62.0</td>
<td>.68</td>
</tr>
<tr>
<td>N stage 2-3 ((n = 12))</td>
<td>78.8</td>
<td>54.1</td>
<td>57.1</td>
<td>58.3</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Abbreviations: DFS, disease-free survival; LEDFS, laryngoesophageal dysfunction–free survival; LRC, locoregional control; OS, overall survival.*

\(^a\)Unless otherwise indicated, data are reported as Kaplan-Meier probabilities of the respective outcomes at 3 years.

\(^b\)Log-rank test
demonstrated for patients with OPSCC will hold at other subsites other than the oropharynx. It is clear from the present study that it cannot be assumed that the favorable prognostic significance of HPV and p16 that has been demonstrated for patients with OPSCC will hold at other subsites. For patients with HPSCC, p16 is not a useful biomarker, and, while HPV status may still be of prognostic value, few patients with HPSCC appear to have HPV-associated tumors.

In conclusion, in contrast to OPSCC, p16 expression in patients with HPSCC had a low positive-predictive value for HPV and did not predict improved OS, LRC, DFS, or LEDFS. Thus, for HPSCC, p16 is not a prognostic biomarker. Caution must be taken when extrapolating the prognostic significance of p16 expression in patients with OPSCC to patients with head and neck SCC of other mucosal subsites with low HPV infection rates.

### Table 3. Using p16 as a Test for HPV in the Hypopharynx

<table>
<thead>
<tr>
<th>p16 Status</th>
<th>Patients, No.</th>
<th>HPV-Positive (Sensitivity 100%)</th>
<th>HPV-Negative (Specificity 72%)</th>
<th>Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>PPV, 17%</td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td>NPV, 100%</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>1</td>
<td>18</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Abbreviations: HPV, human papillomavirus; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value.

** Nineteen of the 27 samples had sufficient tissue to test for HPV DNA testing with in situ hybridization.

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**Author Contributions:** David Wilson had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Wilson, Jameson, and Read. Acquisition of data: Wilson, Rahimi, Saylor, Stelow, Jameson, Reibel, and Levine. Analysis and interpretation of data: Wilson, Saylor, Stelow, Jameson, Shonka, and Levine. Drafting of the manuscript: Wilson and Rahimi. Critical revision of the manuscript for important intellectual content: Wilson, Saylor, Stelow, Jameson, Shonka, Reibel, Levine, and Read. Statistical analysis: Wilson and Jameson. Administrative, technical, and material support: Wilson, Saylor, Stelow, and Read. Study supervision: Rahimi, Jameson, Shonka, Reibel, Levine, and Read.

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