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Objectives/Hypothesis: From the 1950s through the 1960s, an unknown number of oropharyngeal squamous cell carcinomas (SCCs) presented with metastases to cervical lymph nodes from an unknown primary (SCCUP) and were not recognized as oropharyngeal in origin. At present, pathologic evaluation of SCCUP for human papillomavirus (HPV) improves discovery of occult oropharyngeal SCC and may partially explain increased incidence of HPV-positive oropharyngeal SCC.

Study Design: Retrospective cohort study.

Methods: A retrospective study of 13 cases of SCCUP diagnosed from 1956 to 1969 was performed. The probability of these cases of metastatic SCC to originate from the oropharynx was assessed by characterizing their morphology (keratinizing vs. nonkeratinizing) and HPV status by in situ hybridization and p16 immunostaining.

Results: Two cases of nonkeratinizing SCC positive for HPV by in situ hybridization and p16 immunohistochemistry were identified. These cases were most likely of oropharyngeal origin.

Conclusions: These two cases can be added to the other 15 cases of HPV-positive primary oropharyngeal SCC identified in our department from 1956 to 1969. When determining the incidence of HPV-positive oropharyngeal SCC before the 1970s, a correction factor of about $+13\% (2/15)$ accounting for modern pathologic workup of SCCUP during the last couple of decades may be appropriate.

Key Words: Human papillomavirus, oropharyngeal, unknown primary, nonkeratinizing, p16.

Level of Evidence: 2.
follows: 1) lack of a known history of malignancy; 2) primary carcinoma could not be identified by physical examination or biopsies at the time and after neck mass biopsy or excision; and 3) histologic confirmation of metastatic SCC. Unfortunately, medical records prior to 1981 have been discarded, and clinical information was therefore extracted from the pathology reports.

The following information was indexed: age, sex, surgical procedure, histologic appearance, and diagnostic workup. Original hematoxylin and eosin slides or re-cuts from tissue blocks were reviewed. The tumor was classified according to the extent of keratinization (nonkeratinizing, keratinizing, and mixed).6

This study was approved by the University of Pittsburgh Institutional Review Board (IRB# PRO10100585).

### Immunohistochemistry

Original tissue blocks from 1956 to 1969 were re-embedded to allow cutting with modern microtomes. Five-micrometer sections were deparaffinized. Heat-induced epitope retrieval was performed in citrate buffer. Immunohistochemistry for p16 (G175-405; BD Pharmingen, San Diego, CA), as a surrogate marker for HPV,7 was performed as per the manufacturer’s protocol. Cases were considered positive if >80% of tumor cells showed diffuse strong cytoplasmic and nuclear positivity staining.4 Immunostaining for p63 (Thermo Scientific, Rockford, IL) was performed in one case as per manufacturer’s protocol to confirm the diagnosis of nonkeratinizing SCC.

### HPV Detection

HPV detection was performed by in situ hybridization using probes targeting a wide spectrum of HPV strains including 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, and 52 (Y1404; Dako, Carpinteria, CA). Five-micrometer tissue sections were deparaffinized and digested with proteinase K (Roche Diagnostics, Indianapolis, IN). Cases with punctate nuclear signal were considered positive.5

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As previously described by us,6 cases of cervical SCC were chosen as positive control. It is expected that HPV DNA is identifiable in a great majority of cervical carcinomas.8 Seven random cases of cervical SCC were identified from 1956 to 1969. P16 immunostaining and HPV in situ hybridization were

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### Table I. Clinicopathologic Features of Squamous Cell Carcinomas With Unknown Primary Site 50 Years Ago.

<table>
<thead>
<tr>
<th>1956–1969 (n = 13)</th>
<th>Male:female</th>
<th>Median age, yr</th>
<th>Management of clinically positive lymph node</th>
<th>Histologic type</th>
<th>Extranodal extension</th>
<th>P16 immunohistochemistry positive</th>
<th>HPV hybridization in situ positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11:2</td>
<td>53*</td>
<td>Biopsy 4</td>
<td>Nonkeratinizing 3</td>
<td>10/12†</td>
<td>2/13 (15%)</td>
<td>3/13 (23%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Excision 5</td>
<td>Keratinizing 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neck dissection 4</td>
<td>Mixed 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diagnostic workup† 2∥/13</td>
<td></td>
<td>Extranal extension 10/12∥</td>
<td></td>
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</tr>
</tbody>
</table>

*Age at the time of diagnosis was not available in two cases.
†Diagnostic workup as indicated on pathology report.
∥One patient had nasopharyngeal biopsy and another patient had laryngeal biopsy.
‡In one case, biopsy was too small to evaluate for extranodal extension.
§In one case, biopsy was too small to evaluate for extranodal extension.

Both p16 positive cases were also positive for human papillomavirus by in situ hybridization. HPV = human papillomavirus.

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**Fig. 1.** Metastatic squamous cell carcinoma with unknown primary site in a 21-year-old male. Node excision was performed in 1958. (A) Cystic predominantly nonkeratinizing squamous cell carcinoma, hematoxylin and eosin, 100×. (B) Strong diffuse positivity for p16, a surrogate marker for human papillomavirus, immunohistochemistry, 100×. (C) Nuclear punctate signals indicating the presence of human papillomavirus, in situ hybridization, 600×. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
performed in six cases with available tissue blocks. All cases were positive for p16, and three of six were positive for HPV, comparable to the previously reported HPV prevalence in cervical SCC, from 1950 to 1960 (8 of 13 cases, 62%).

RESULTS
Clinicopathologic features of patients diagnosed with SCCUP 50 years ago are summarized in Table I. Clinical workup at the time of neck mass management included only one “blind” biopsy of the nasopharynx. One laryngeal biopsy was done; however, it most likely was not random, as it revealed a vocal cord nodule. No diagnostic tonsillectomies were performed. Possible primary sites were suggested by pathologists in three of 13 cases: lung in two cases and nasopharynx in one case. The possibility of a “branchial cleft carcinoma” was raised in additional two cases. The lack of a primary carcinoma after physical examination was explicitly indicated by clinicians on the pathology requisition form in two of 13 cases. In one case, a 53-year-old woman developed contralateral neck metastases 3 years after the initial presentation without revealing the site of the primary carcinoma.

Two of 13 cases were positive for p16 by immunohistochemistry and HPV by in situ hybridization (1956–1969); the clinicopathologic features of two cases are described.

Case 1. A 21-year-old man presented in March 1958 with “swelling of the right neck.” Excision of the neck mass revealed a 4.2-cm cystic predominantly non-keratinizing SCC (Fig. 1A) with minimal extranodal extension. Both p16 immunostaining (Fig. 1B) and in situ hybridization for HPV were positive (Fig. 1C).

Case 2. A 52-year-old man with a smoking history (2–3 packs per day for 20 years) presented in April of 1960 with a 1.5-cm right scalene node. Lymph node biopsy was diagnostic of nonkeratinizing basaloid SCC. Based on morphology and HPV positivity, the oropharynx is the most common site of origin of this SCC. However, the remote possibility of a hypopharyngeal or sinonasal primary SCC cannot be entirely excluded.

One case showed keratinizing SCC that was positive for HPV by in situ hybridization and negative for p16. Detection of HPV in a keratinizing p16-negative SCC has an unclear significance in pinpointing the site of tumor origin.

DISCUSSION
The increasing incidence of tonsillar SCC in the United States has been documented by epidemiologic studies. The increase in proportion of HPV-positive OSCC is also apparent from assessment of tumor samples for HPV DNA. Change in sexual norms is believed to explain this trend. Because there are few, if any, convincing reports of branchial cleft carcinoma, the impact of this diagnosis on the incidence of OSCC is most likely negligible.

The impact of the improved clinical and surgical diagnostic workup of SCCUP remains unknown. Only in the early 1970s was it recommended that random pharyngeal biopsies be performed. More recently it has been shown that detection of an occult tonsillar SCC can be improved by diagnostic tonsillectomy.

HPV testing of 43 OSCCs diagnosed in our department from 1956 to 1969 revealed 15 HPV-positive SCCs. In the current study, we have asked how many additional HPV-positive cases of potentially oropharyngeal origin could be uncovered among SCCUP by using modern pathologic assessment. Retrospective search for SCCUP of possible oropharyngeal origin was made possible by advances in pathologic evaluation. It has been shown that 87% of HPV-positive cystic nonkeratinizing SCCs metastatic to the neck originate from the oropharynx. Applying these currently standard means of

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**TABLE II.**

<table>
<thead>
<tr>
<th>OSCC With Neck Metastasis as the Only Presenting Complaint, % (No. of Cases)</th>
<th>Period Studied</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>1947–1949</td>
<td>Martin et al. 18</td>
</tr>
<tr>
<td>20 (21/104)</td>
<td>1955–1974</td>
<td>Givens et al. 19</td>
</tr>
<tr>
<td>13 (9/70)</td>
<td>1960–1968</td>
<td>Whicker et al. 20</td>
</tr>
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</table>

OSCC = oropharyngeal squamous cell carcinoma.

Today, based on morphology and HPV positivity, this metastatic cystic SCC is almost definitely of oropharyngeal origin. In 1958, the pathologist raised the possibility of a branchial cleft carcinoma.

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**TABLE III.**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Martin et al. 17</th>
<th>Martin et al. 18</th>
<th>Jesse et al. 14</th>
<th>Barrie et al. 22</th>
<th>Acquarelli et al. 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of SCCs with unknown primary source</td>
<td>218</td>
<td>122</td>
<td>35</td>
<td>104</td>
<td>26</td>
</tr>
<tr>
<td>OSCC as an established primary, % (actual number of OSCC cases/total number of cases with identified primary)</td>
<td>At least 23% 1 (35/152)</td>
<td>46% (56/122)</td>
<td>23% (8/35)</td>
<td>29% (9/31) 2</td>
<td>7.6% (2/26)</td>
</tr>
</tbody>
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1 Carcinomas of thyroid and those of infraclavicular origin were excluded from this summary.
2 The distinction between base of tongue and mobile tongue was made only in the 7th revision of International Classification of Disease in 1955.
3 In up to 70% of squamous cell carcinomas with unknown primary, the site of primary carcinoma was not identified. 22
4 SCC = squamous cell carcinoma; OSCC = oropharyngeal squamous cell carcinoma.
pathologic evaluation to our historic cohort of 13 SCCUP cases, we identified two additional cases that are most likely of oropharyngeal origin. These two cases can be added to the other 15 HPV-positive OSCCs identified in our department from 1956 to 1969. Therefore, modern pathologic evaluation uncovered an additional 13% of HPV-positive cases of OSCC. These are cases that are routinely assigned to oropharyngeal primary sites today and were most likely “missed” 4 to 5 decades ago.

Our findings are consistent with review of the literature on SCCUP from the 1930s to the 1960s (Tables II and III). The overall pool of potential SCCUPs is limited to patients presenting with an asymptomatic oropharyngeal SCC before the 1970s, a correction factor of about +13% accounting for modern pathologic workup of SCCUP during the last couple of decades may be appropriate. This “proof of principle study” shows that part of the apparent “HPV epidemic” may be explained by the progress in the diagnostic workup of head and neck cancer.

CONCLUSION
When determining the incidence of HPV-positive oropharyngeal SCC before the 1970s, a correction factor of about +13% accounting for modern pathologic workup of SCCUP during the last couple of decades may be appropriate. This “proof of principle study” shows that part of the apparent “HPV epidemic” may be explained by the progress in the diagnostic workup of head and neck cancer.

BIBLIOGRAPHY