An Algorithm to Evaluate Suspected Lung Metastases in Patients with HPV-Associated Oropharyngeal Cancer

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract
Distinguishing between distantly metastatic and metachronous lung primary carcinoma is challenging for patients with a history of head and neck cancer. There are implications for registry data, prognosis and related counseling, and management options, including eligibility for precision oncology trials. Patients with human papillomavirus (HPV)–associated oropharyngeal squamous cell carcinoma who were treated under a uniform clinical protocol and achieved a complete response were identified in a single-institution prospective head and neck cancer epidemiology database (n = 205). Fifteen patients presented with pulmonary nodule(s) after completion of therapy. We describe our algorithm for the evaluation of these patients, including histopathology, p16 immunohistochemistry, and HPV in situ hybridization.

Keywords
oropharyngeal cancer, human papillomavirus, lung nodule, pulmonary metastases, head and neck cancer

Received May 24, 2017; revised July 19, 2017; accepted September 6, 2017.

In patients with oropharyngeal squamous cell carcinoma (OPSCC), the lung is both the most common site of distant metastasis and the most likely site of a metachronous primary to arise outside of the head and neck region.1,2 In the human papillomavirus (HPV) era, distant failure is unpredictable, occurring in atypical locations and at longer time intervals than HPV-negative head and neck squamous cell carcinoma (HNSCC).3 The treatment algorithm for a pulmonary nodule(s) in this setting—including eligibility for clinical trials—varies widely and is influenced by its distinction as a metastasis rather than a second primary.4 As such, a reliable method for distinguishing between the two is critical. Although p16 overexpression is widely accepted as a surrogate marker for the presence of HPV in OPSCC, the use of p16 immunohistochemistry and HPV in situ hybridization (ISH) in combination improves sensitivity to 90% and specificity to 88%.5 However, there is not a parallel paradigm in lung squamous cell carcinoma (SCC). Ten percent to 20% of lung SCCs stain positive for p16, but this does not signify the presence of HPV.4,6-8 Herein, we describe our approach and present a simple algorithm to evaluation of a lung nodule in patients with a history of OPSCC.

Materials and Methods
Patients with HPV-associated OPSCC treated under a uniform clinical protocol consisting of weekly carboplatin and paclitaxel with intensity-modulated radiation therapy (IMRT) between 2003 and 2010 were identified in a single-institution prospective head and neck cancer epidemiology database (n = 205). Patients presenting with subsequent lung nodule(s) were identified. The study was approval by the University of Michigan Medical School Institutional Review Board (IRBMED).

Demographics, treatment modality, recurrence pattern, and outcomes were collected. p16 detection was performed on untreated primary tumors and on metastatic lesions as

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previously described, as well as on metastatic lesions using ISH for HPV DNA using a cocktail directed against a subset of high-risk HPV genotypes, including 16, 18, 33, 35, 39, 45, 51, 56, and 66 (INFORM HPV ISH assay; Ventana Medical Systems, Tucson, Arizona). In a subset of patients for whom enough DNA could be isolated from the biopsy specimens, MultiPlex HPV PCR-MassArray was performed. Descriptive statistics were tabulated using Excel 2013 (Microsoft Corporation, Redmond, Washington).

**Results**

Fifteen patients treated under this uniform clinical protocol developed pulmonary lesion(s). Fourteen patients were male with a mean age of 52 years. The primary tumor arose from either the tonsil (53%) or base of tongue (47%). All primary tumors were p16 positive. All patients had pulmonary lesions that were biopsied or resected. One hundred percent of the lung lesions were p16 positive (positive defined as >20% staining). Thirteen of the 15 metastatic lesions were HPV positive by ISH, and 2 of 15 were ISH negative (Table 1). The mean time from start of treatment to recurrence was 16 months (range, 6-41). At last known follow-up, 73% (11/15) of patients died of disease, 13% (2/15) of patients were alive with no evidence of disease, and 13% (2/15) were lost to follow-up with unknown outcomes. Of those who were p16 and ISH positive, 10 of 13 (77%) died of disease, while 1 of 2 (50.0%) p16-positive and ISH-negative patients died of disease at follow-up.

**Discussion**

Distinguishing between distant relapse and metachronous lung primary in patients with a history of OPSCC has implications for maintaining accurate registry data, determining prognosis, and potentially altering management options, including eligibility for oncology trials. Initial diagnostic assessment includes standard histologic evaluation. While OPSCC is classically characterized as “nonkeratinizing” or “basaloid” (Figure 1), this morphologic feature can overlap with poorly differentiated primary lung SCC and is not sufficient for clarification.

If the lung lesion is suspected to be SCC among patients treated for p16-positive OPSCC, we suggest utilization of a simple algorithm for workup (Figure 2). Immunohistochemical staining for p16 should be performed. If the lung nodule is p16 negative, it can be concluded that this likely represents a lung primary and there is no need to proceed with ISH. However, if there is p16 positivity in the lung nodules, ISH is necessary to determine whether this represents a lung primary with p16 expression vs a OPSCC metastatic lesion. While it would simplify the algorithm to eliminate p16 testing of lung lesions and perform HPV ISH alone, p16 is a well-established, inexpensive, and easily interpreted test that can be performed at most pathology laboratories. This simple step can prevent the need for ISH testing in some patients.

In our cohort, we identified patients with a history of advanced stage OPSCC who subsequently presented with pulmonary nodule(s). Thirteen of these patients were correctly identified as having distant relapse using this diagnostic algorithm. Of the 2 discordant patients (p16 positive, ISH negative), 1 died of disease at follow-up. Our group has previously shown polymerase chain reaction (PCR) to be more a sensitive test to determine HPV status in virally related OPSCC. In this study, we did attempt to perform PCR on the lung specimens, but only 8 of 15 patients had sufficient tissue available, highlighting a practical challenge of PCR testing with small biopsy samples. Of the 8 patients with PCR available, ISH and PCR concurred in 7 of 8 cases, confirming our previous findings. Due to practical challenges with PCR availability and implementation, we believe ISH should be used in the algorithm in the workup of these patients. In the future, the challenges associated with this multistep process may be circumvented by next-generation sequencing techniques.

These data are limited by the relatively low number and uniform treatment paradigm, thereby limiting generalizability. However, the use of a robust, “clean” prospectively maintained data set with standardized treatment and surveillance is also a strength. The dearth of many p16-negative lung lesions reflects our patient population; cohorts with higher tobacco use histories may contain a higher percentage of second primary lung malignancies that can further “test” this algorithm.

We introduce a pragmatic system for categorizing lung lesions in patients with a history of OPSCC. A standardized methodology for evaluating these patients will ensure patients are directed to appropriate management, given accurate prognostic information, and stratified appropriately.

### Table 1. p16/HPV Status of Pulmonary Lesion(s) in Patients with Oropharyngeal Squamous Cell Carcinoma.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>p16</th>
<th>HPV ISH</th>
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<td>1</td>
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Abbreviations: HPV, human papillomavirus; ISH, in situ hybridization; +, positive; –, negative.

*Testing only performed on pulmonary metastasis.*
Figure 1. (A) Hematoxylin and eosin (H&E) stain of primary tumor. (B) Positive p16 immunohistochemistry stain of primary tumor. (C) H&E stain of pulmonary metastasis. (D) Positive p16 immunohistochemistry stain of pulmonary metastasis. (E) Positive human papillomavirus in situ hybridization of pulmonary metastasis.

Figure 2. Evaluation of lung nodule(s) in patients with a history of p16-positive oropharyngeal squamous cell carcinoma (OPSCC). ISH, in situ hybridization.

Author Contributions
Kyle K. VanKoevering, project initiation and data acquisition, drafting the manuscript and final approval thereof, agreement for accountability; Emily Marchiano, data acquisition and interpretation, drafting the manuscript and final approval thereof, agreement for accountability; Heather M. Walline, data acquisition and interpretation, critically editing the manuscript and final approval.
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Disclosures

Competing interests: None.

Sponsorships: None.

Funding source: Supported by grant P50CA097248 from the National Institutes of Health–National Cancer Institute (NIH-NCI) Head and Neck SPORE (Specialized Programs of Research Excellence) and NIH grant U01DE025184.

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