

HPV Testing as a Tool for Primary Tumor Identification

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Case Presentation

- 61-year-old female with chief complaint of painless bilateral neck swelling x1 month
- Review of systems:
 - Positive for dysphonia, left otalgia, fatigue
 - Negative for fevers, chills, unintentional weight loss, dysphagia
- Past medical history: CAD s/p stent placement, alcohol-related cirrhosis, HLD
- Social history: former smoker (33 pack-years), prior alcohol use disorder
- Physical examination: No intraoral lesions noted. Bilateral fixed, nontender neck masses noted at level 2A in the neck, extending down to level 3.
- Flexible laryngoscopy: Prominent normal asymmetric elevated lingual tonsillar tissue noted in the left tongue base. Examination of the vallecula was also normal. Both vocal cords move normally, and the subglottic area was free of pathology.

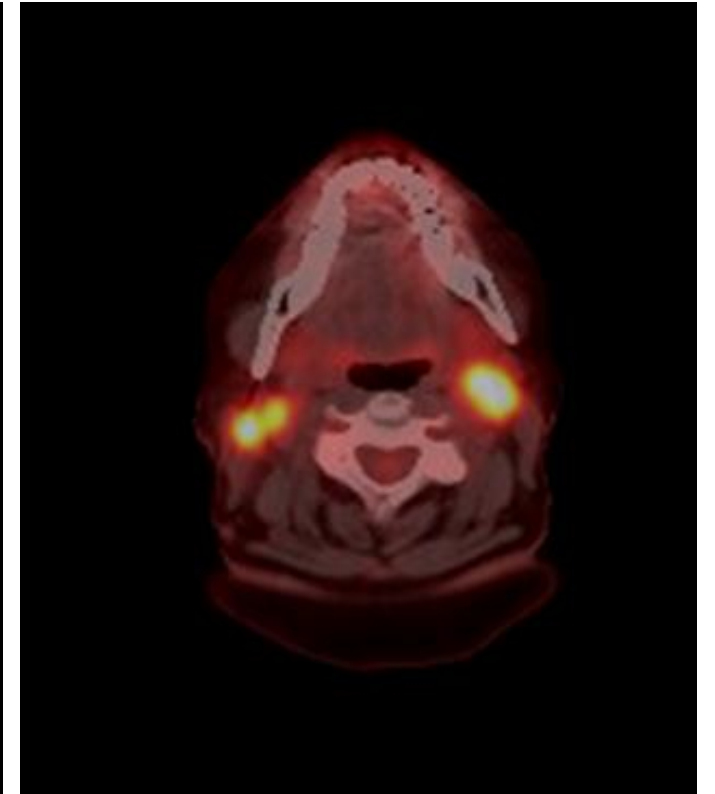
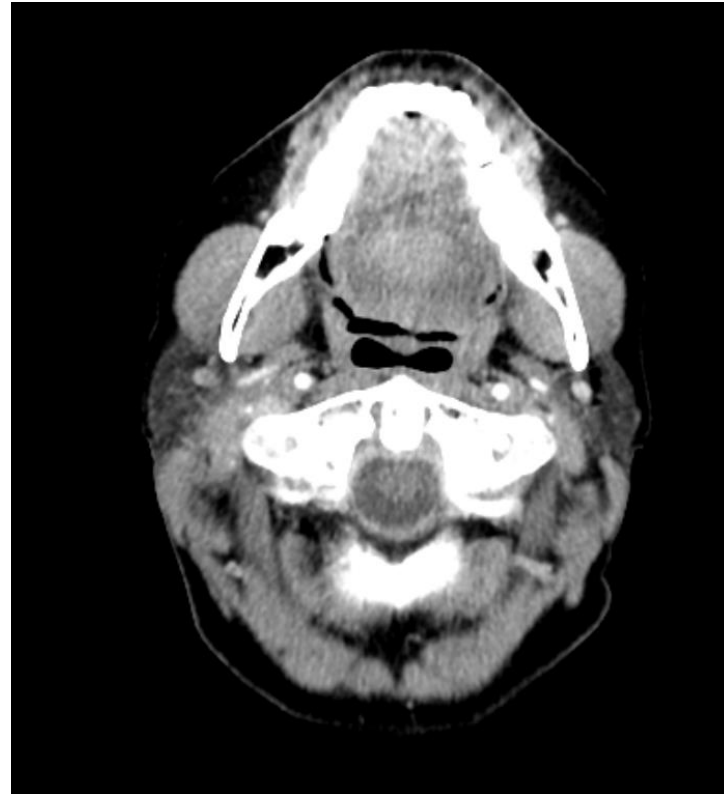
Radiographic Findings

CT of the neck soft tissues with IV contrast (L):

Bilateral adenopathy with enlarged lymph nodes, with representative node measuring 2.7 cm on the left and 3.1 cm on the right.

PET (R):

1. **Bilateral level 2A cervical hypermetabolic lymphadenopathy.**
2. **Mild asymmetric metabolic activity at the tongue base, more evident on the right**, which can be seen physiologically, but is of increased concern in the current clinical setting.



Pathology

- **Fine-needle aspiration of lymph node, right neck:** Metastatic nonkeratinizing squamous cell carcinoma.
- Immunohistochemistry: positive for p63; **negative for p16**. Given the morphology, HPV E6/E7 RNA ISH was ordered.
 - **HPV E6/E7 RNA ISH: positive.**
- **TransOral robotic surgical (TORS) resection of right base of tongue and right tonsil**
 - A) Right tongue base, excisional biopsy: Benign tongue tissue including squamous mucosa, underlying sublingual glands, and skeletal muscle.
 - B) Right tonsil, tonsillectomy: Benign tonsillar tissue with mild acute and chronic inflammation.
- **Diagnosis:** HPV-positive p16 negative HNSCC with occult primary, stage IVa (cT0 N2c M0)

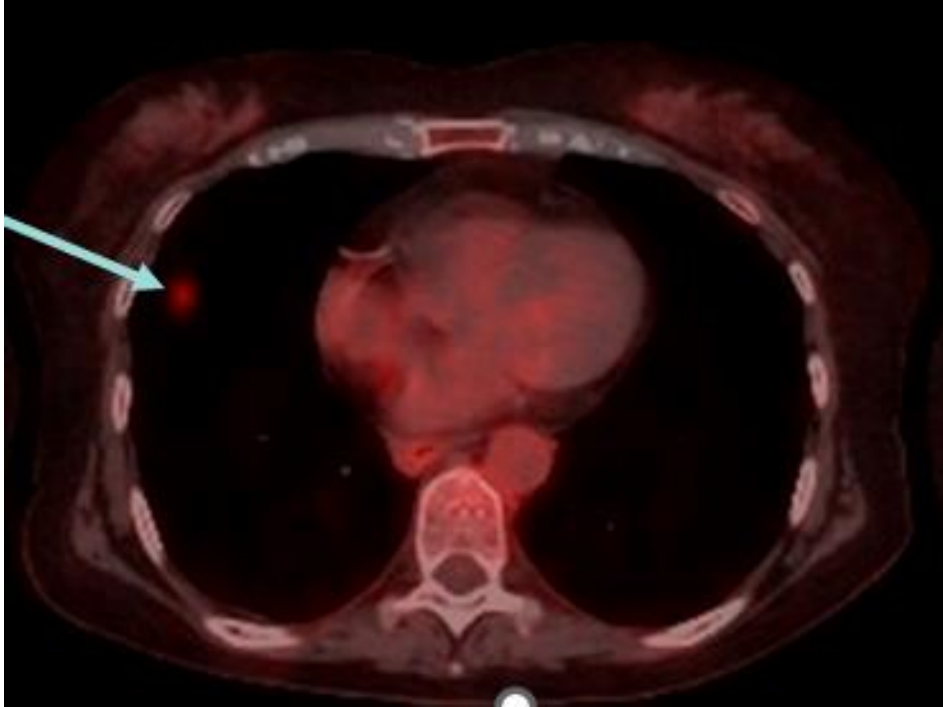
Treatment and Follow-up Evaluation

The patient completed concurrent cisplatin/radiation therapy.

CT and PET-CT were obtained at 3 months post treatment.

Surveillance Imaging Studies

3-MONTH POST-TREATMENT PET: NEW 8 MM HYPERMETABOLIC PULMONARY NODULE IN RIGHT MIDDLE LOBE IS SUSPICIOUS FOR PULMONARY METASTATIC DISEASE.



PET-CT 2 MONTHS LATER: INTERVAL ENLARGEMENT OF 18 X 23 MM IRREGULAR RIGHT MIDDLE LOBE NODULE.



Lung Pathology

- Biopsy, lung (right middle lobe): positive for malignancy. Reported as consistent with **metastatic squamous cell carcinoma**.
- IHC: positive for p63. Negative for p16.
- HPV DNA ISH:
 - HPV (family 6) ISH is **negative** for types 6 and 11.
 - HPV (family 16) ISH is **negative** for types 16, 18, 31, 33, and 51.
- HPV (E6/E7) RNA ISH: ISH is negative for types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82.
- Conclusion: patient **has second primary malignancy (lung SCC)**, not metastatic disease from head/neck primary!

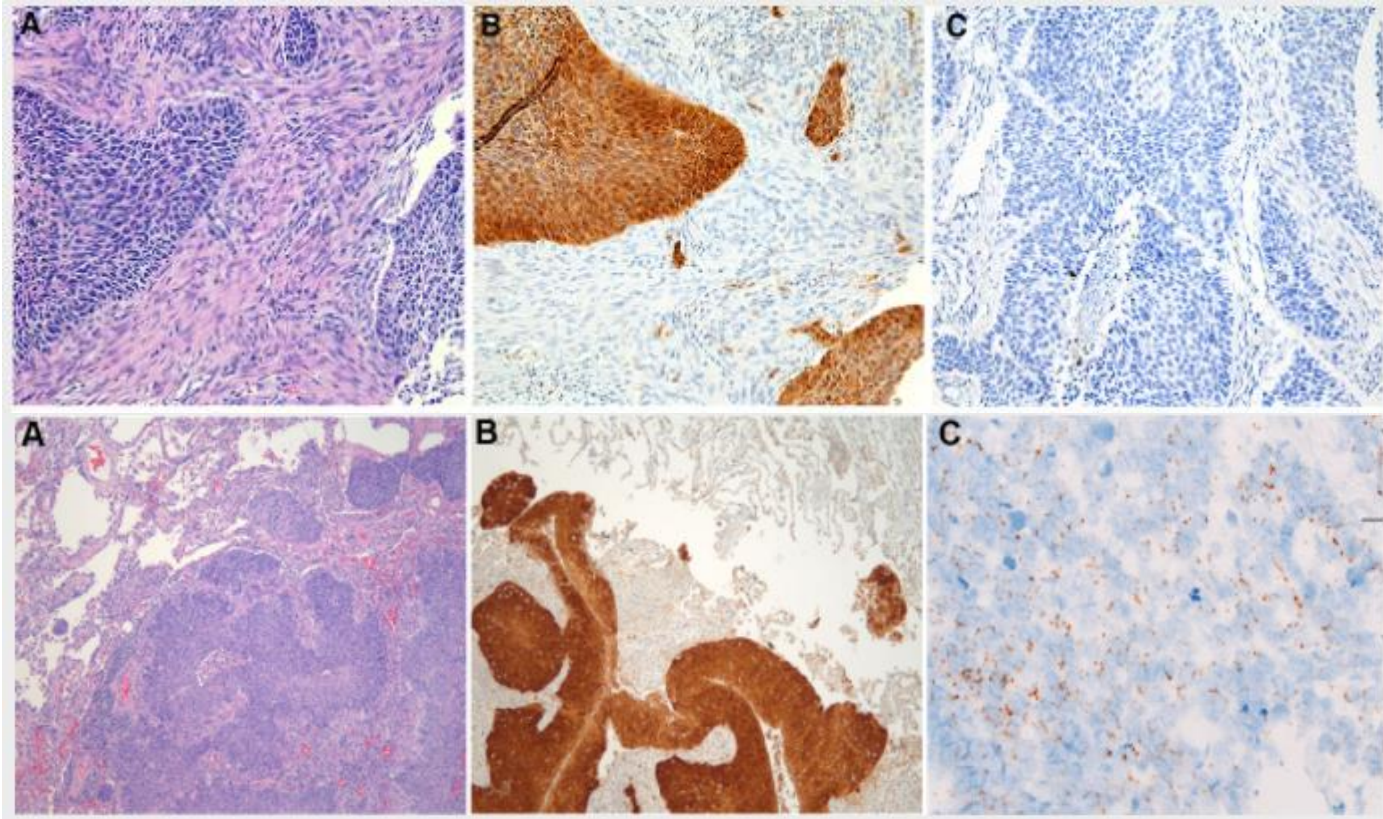
Question

Can HPV status or p16 expression help to differentiate metastatic HPV-associated head and neck squamous cell carcinoma from primary lung squamous cell carcinoma or metastatic cutaneous squamous cell carcinoma?

HPV and p16 Detection in NSCLC

- Chang et al. demonstrated that p16 was positive in 28 out of 196 patients with lung cancer; however, HPV DNA and RNA in situ hybridization did not detect HPV infection in any case of primary lung cancer.
- Lin et al. reported a p16 positive expression rate of 13.7% (57/415) in primary lung SCCs, but HPV DNA was not detected in any of the 57 primary lung SCC cases that positively expressed p16.

P16+ Lung Cancer



Proposed interpretation of p16 expression in pulmonary and oropharyngeal SCCs

Pulmonary SCC	Oropharyngeal SCC	Interpretation
Positive	Negative	Uncertain; may represent different tumors; HPV testing or advanced sequencing could be considered
Negative	Positive	Likely different tumors; confirmatory HPV testing could be considered
Positive	Positive	Likely metastasis; confirmatory HPV testing recommended
Negative	Negative	Uncertain; no HPV testing recommended; advanced sequencing could be considered

Non-keratinizing SCC (a, H&E) with strong nuclear and cytoplasmic expression of p16 in > 70% of tumor cells (b) and negative HPV RNA ISH (c, **top row**) vs positive RNA ISH (c, **bottom row**).

HPV and p16 Detection in Cutaneous Squamous Cell Carcinoma

Satgunaseelan et al. reported that nearly 32% of cutaneous SCCs of the head and neck, particularly poorly differentiated cases, show p16 expression.

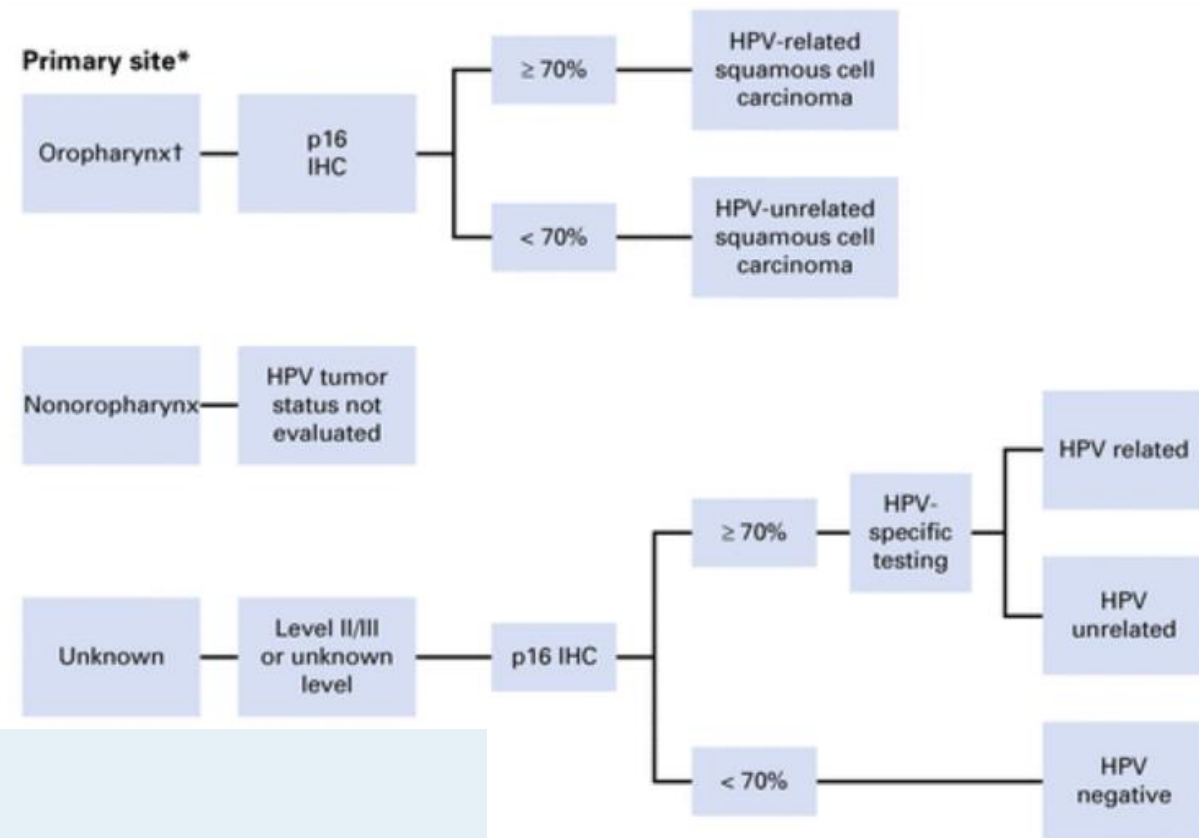
HPV ISH was negative in all cases.

ASCO SPECIAL ARTICLE

Human Papillomavirus Testing in Head and Neck Carcinomas: ASCO Clinical Practice Guideline Endorsement of the College of American Pathologists Guideline

Recommendation 11.

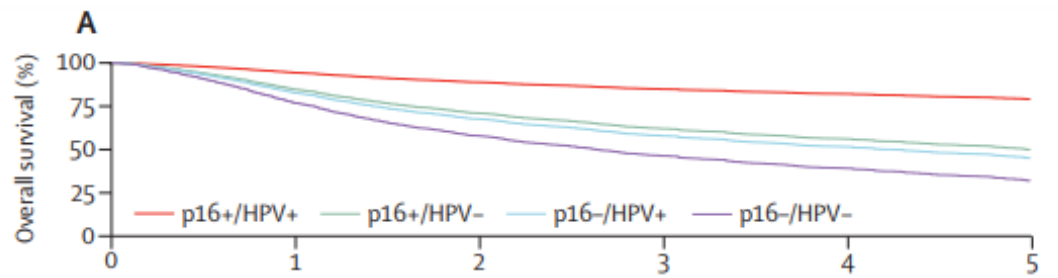
Pathologists should *not* routinely perform HR-HPV testing on patients with distant metastases if primary tumor HR-HPV status has been established. HPV testing may be performed on a case by-case basis for diagnostic purposes if there is uncertainty regarding whether the tumor in question is a metastasis or a new primary SCC.



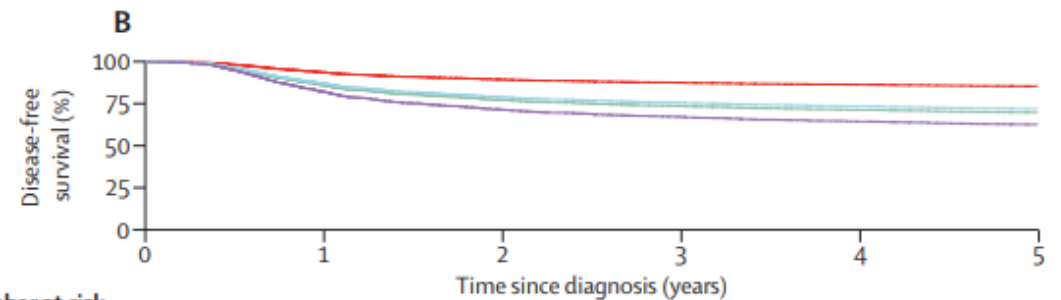
Question

What are the prognostic implications of discordant HPV status and p16 expression in head and neck squamous cell carcinoma?

Prognostic implications of p16 and HPV discordance in oropharyngeal cancer (HNCIG-EPIC-OPC): a multicentre, multinational, individual patient data analysis



	0	1	2	3	4	5
Number at risk (number censored)						
p16+/HPV+	3220 (1)	2927 (168)	2434 (517)	1966 (892)	1644 (1153)	1166 (2737)
p16+/HPV-	364 (0)	304 (17)	240 (43)	190 (68)	148 (91)	98 (226)
p16-/HPV+	240 (0)	184 (17)	138 (35)	108 (48)	92 (59)	59 (144)
p16-/HPV-	3058 (1)	2332 (108)	1702 (250)	1316 (392)	1057 (504)	804 (1424)



	0	1	2	3	4	5
Number at risk (number censored)						
p16+/HPV+	3120 (1)	2690 (244)	2205 (614)	1768 (996)	1466 (1269)	1009 (271)
p16+/HPV-	365 (0)	275 (41)	209 (80)	171 (111)	126 (146)	86 (270)
p16-/HPV+	226 (0)	165 (33)	127 (54)	100 (75)	86 (89)	54 (173)
p16-/HPV-	3054 (1)	2043 (479)	1490 (771)	1176 (993)	958 (1171)	736 (2104)

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