HPV Testing in Head and Neck Cancer

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Head & Neck Pathology
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Think before you order p16

Disclosures

I have nothing to disclose

The WHO Head and Neck 2017

Oropharyngeal squamous cell carcinoma:

- HPV-positive
- HPV-negative
**Why?**

Despite reductions in smoking...

the incidence of cancer of the oropharynx has *increased*

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**SEER DATA**

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Average annual no.</th>
<th>Attributable to any HPV type No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical</td>
<td>11,771</td>
<td>10,700 (90.6%)</td>
</tr>
<tr>
<td>All oropharyngeal cancers</td>
<td>15,738</td>
<td>11,000 (70.1%)</td>
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**Histomorphology**

Morphological spectrum of HPV+ oropharyngeal SCC:
- Basaloid
- Papillary
- Lymphoepithelioma-like
- Sarcomatoid
As a group, patients with HPV+ oropharynx SCC have improved clinical outcomes compared to patients with conventional, HPV-negative SCC.

Given significant implications on patient prognosis, HPV status will be integrated into the American Joint Committee on Cancer (AJCC) staging in new 8th Ed.

Many HPV+ patients may be cured by single modality therapy (surgery or radiation alone).

NCCN Treatment Guidelines are currently the same for both HPV+ and HPV- tumors, but many clinicians will take a less aggressive approach in HPV+ cases.

While patients with HPV+ disease have a good prognosis, the side effects from multi-modality therapy are significant.
Clinical utility

If metastatic SCC of unknown primary involves and groin node and is HPV+
  ➢ Look in the anogenital tract

If metastatic SCC of unknown primary involves an upper cervical lymph node and is HPV+
  ➢ Look in the oropharynx

Where is the oropharynx?

How should we test?

- Need a technically practical, reproducible, and easily interpreted marker for high risk HPV within tumor cells
- Widely available, inexpensive, standardized
- Utilization and interpretation should follow best practice guidelines and consensus statements for consistent reporting

What HPV test is the “best”?  

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P16: High sensitivity, lower specificity

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**DNA ISH: Lower sensitivity, better specificity, technically challenging**


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<tr>
<td>hrHPV RNA ISH*</td>
<td>97%</td>
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**mRNA ISH detects transcriptionally active HPV oncogenes E6/E7 with high sensitivity and specificity.... But technically challenging**


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<td>DNA qPCR</td>
<td>91%</td>
<td>87%</td>
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**DNA PCR: Lower sensitivity/specificity Cannot distinguish passenger virus from driver**


**High risk HPV RNA in situ**


**SCHOOL OF MEDICINE * UNIVERSITY OF CALIFORNIA, SAN FRANCISCO**
**hrHPV RNA in situ**
- High sensitivity, high specificity
- New protocols and reagents for automated platforms
- Standardization will be challenging

**Reconsidering P16**
- The correlation between hrHPV RNA ISH and p16 IHC is very high.*
- An endogenous cell cycle protein overexpressed in tumor cells with transcriptionally-active high risk HPV.
- E6H4, MTM Lab, ER 1 20’ (BOND) Predilute
- Widely available, technically practical, reproducible, and easily interpreted


**P16 Immunohistochemistry**

**What is positive?**
- P16 IHC is positive in tissue specimens (non-cytology) when there is at least 70% nuclear and cytoplasmic expression with at least moderate to strong intensity.
- Staining must be both nuclear and cytoplasmic to be considered positive.
P16 interpretation

What is positive?

- With these criteria, sensitivity of p16 approaches 100%.
- The specificity of p16 in the oropharynx is ~85-95%.
- Excellent inter-rater agreement (κ = .97)

Keratinizing HPV+ SCC

CAP guidelines coming soon...

1. Pathologists should use p16 IHC as a surrogate for hrHPV on all new oropharynx cancers.
2. Additional HPV-specific testing is at the discretion of the pathologist.
CAP guidelines coming soon...

3. Pathologists should not routinely perform low-risk HPV testing on patients with head and neck carcinomas.

4. HPV status is not a reliable marker for aggressive behavior in non-SCC. *Don’t test other tumor types.*

What about other head and neck sites?

But I’ve heard that HPV DNA is present in 25% of laryngeal and sinonasal carcinomas?

- Using RNA-based HPV detection methods HPV may be an etiologic agent in 2% of SCC outside the oropharynx.
- The prognostic significance is unknown.
- P16 status is misleading.

What about other head and neck sites?

- The PPV of p16 IHC for HPV in non-oropharyngeal SCC is low (25-50%).
- There is no proven prognostic difference based on HPV status outside of the oropharynx.
  - **DO NOT** routinely test non-oropharyngeal SCC.

What about recurrences?

Recurrence can occur outside of the boundaries of the oropharynx.

- P16 status can be helpful to distinguish between a new primary tumor and recurrence.
What about metastases?

Two scenarios:

1. New diagnosis of cancer. Primary site has not been established
2. Diagnosis has been established and patient has a new metastatic lesion.

1. New diagnosis:
   HPV testing is indicated in patients with metastatic SCC of unknown primary in a cervical upper or mid jugular chain lymph node (levels 2 and 3).
   - Note that p16 status is suggestive of oropharyngeal origin.
   - 20 to 30% of aggressive head and neck cutaneous SCCs overexpress p16 unrelated to high risk HPV.

2. New metastasis:
   High risk HPV testing is indicated for patients with oropharyngeal SCC and a new metastatic lesion:
   - When the original tumor was not tested.
   - When there is diagnostic uncertainty regarding recurrence vs. new primary.
   P16 is positive in a ≈25% lung SCC. HPV specific testing is indicated if p16 is positive.

Testing FNAs

1. FFPE cell block from FNA
   - P16 IHC
   - hrHPV in situ

2. Liquid based specimens
   - Published sensitivities and specificities >90%
     - Roche cobas (Roche Molecular Systems)
     - Cervista HR and HPV16/18 (Hologic)
     - Hybrid-Capture 2 (Qiagen)
How should p16 be interpreted on cell block specimens?

Recent studies suggest that thresholds as low as 10-15% positive cells may be valid for cell blocks.


Conclusions

1. Perform hrHPV testing on all new oropharyngeal SCC by p16 IHC.
   - Reporting p16 status for non-oropharyngeal SCC may be misleading and is not recommended.
2. The cutoff for positive p16 on tissue is 70%.
**P16+ Oropharyngeal SCC**

- Histological grading or subtyping is not currently advocated.
- The diagnosis should include “HPV positive” or “P16 positive.”

*Let’s eat!*