Head and Neck Cytology Specimens: HPV Testing and p16 Staining
Two different pathways to HN cancer: tobacco-associated vs. HPV-related

**Direct damage vs. indirect alteration**

Most oral cancers develop from direct damage to DNA caused by **tobacco-associated mutations** or indirect alteration of DNA caused by **viral proteins**.

**In infected cells**, HPV produces viral proteins E6 and E7 that bind to and inactivate p53 and Rb so that DNA replication proceeds unchecked.

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HPV “Epidemic” in HNSCC

HPV-Associated Head and Neck Cancer: A Virus-Related Cancer Epidemic

Trends in Head and Neck Cancer Incidence in Relation to Smoking Prevalence

An Emerging Epidemic of Human Papillomavirus-Associated Cancers?

• Reflex testing for HR-HPV is indicated for certain HN cancers:
  • Diagnosis
  • Prognosis
  • Guide Management

• Guidelines are needed to establish:
  • When should reflex testing be performed?
  • Which testing method(s) should be used?
  • How should HPV testing be applied to Cytology?
THE HPV-HNSCC EPIDEMIC

225% increase in HPV-positive SCC vs 50% decrease in HPV-negative SCC

Chaturvedi, SEER data from 1984-2004
Clinical presentation of HPV-positive HNSCC is different than smoking-related cancer. This pertains especially to the oropharynx.

More likely to be younger, male, married, and college educated.

>3:1-8:1 M:F

Typically lack a significant history of tobacco or alcohol abuse.

Sexual risk factors for oral or genital HPV exposure.

Low T and high N stage tumors.
Role of HR-HPV in Head and Neck Cancer at Various Sites

- Association between HR-HPV and cancer at various HN sites:
  - Oropharynx: 80-90%
  - Sinonasal Cavity: 20-25%
  - Oral Cavity: 3-6%
  - Larynx: <5%
The oropharynx is the only HN site where there is strong evidence-based information linking HPV-positivity and improved outcome.
1) Patient Prognosis and Etiology Counseling

2) UICC/AJCC Staging

Specific, Separate Staging System for p16 Positive OPSCC
What is the Role of FNA?
Nodal metastases are present at presentation in approx 80-85% of all HPV-positive OP SCC.

HPV-Positive Oropharyngeal SCC:
Often first detected and diagnosed by FNA!

FNA is a key method used to detect these metastatic cancers.
Given the role of FNA in the initial detection of these cancers, appropriate HPV testing modalities for cytology are needed....
Why Should We Test for HR-HPV in HNSCC?

• Improved prognosis among most patients
• Different staging for HPV+ vs HPV- OPSCC
• Identify primary site of metastatic SCC (CUP)
• Distinguish HPV- from EBV-related carcinomas
• Determine patient eligibility for clinical trials/de-escalation therapy
The CAP EBG HPV Testing Committee was formed to address these questions.

Human Papillomavirus Testing in Head and Neck Carcinomas

Guideline from the College of American Pathologists

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CAP EBG HPV Testing Committee:
Co-Chairs- Drs. Faquin and Lewis
Summary of 14 CAP Recommendations for HPV Testing in Head and Neck Cancer

General Overview:
- The tumors of all patients presenting with oropharyngeal SCC should be tested for HR-HPV.
- Neck nodal tissue from all patients with metastatic SCC of unknown primary should be tested for HR-HPV.
- Staining with p16 can be used as the sole initial screening method but confirmatory testing may be necessary in selected cases.
- HR-HPV Testing of FNA specimens is recommended.
Methods for determining HR-HPV status in HNSCC: 
Vary in cost, sensitivity, specificity, technical difficulty

- **IHC for p16**  
  Reduced specificity, esp outside OP

- **PCR for HPV DNA**  
  High sensitivity but low specificity

- **ISH for HPV DNA**  
  High specificity; reduced sensitivity at low viral load

- **RT-PCR E6/E7 mRNA**  
  Needs fresh frozen tissue

- **ISH for HPV RNA**  
  Excellent sensitivity and specificity

- **IHC for E6/E7**  
  Low sensitivity/poor performance

- **Cytology Test Platforms**  
  Validation studies needed; automated
HPV in Oropharyngeal SCC: p16 Immunohistochemistry

- E6H4 Ab is most commonly used
- **Sensitivity approaches 100%**
- Specificity is high in OP (>90%) but low outside OP (79-82%)
- In the OP, p16 is sufficient
- In mets to level II/III and NK morphology, p16 is sufficient
p16 Immunohistochemistry
>70% positivity for FFPE Tissue

Negative

Positive (extensive)
How should HR-HPV testing be done in FNA specimens?
HR-HPV in FNAs of HNSCC

- FNA specimens can be used (CBs, smears, LB):
  - P16 IHC, DNA or RNA ISH, PCR
- Caveats for p16 and cell blocks:
  - No consensus for interpreting p16 staining
  - Criteria for percentage of stained cells less defined
  - P16 testing alone restricted to certain conditions for FNA of CUP
  - CB fixative can affect p16 IHC results
  - Branchial cleft cysts can be p16 positive
P16 and Cytology Specimens

- **p16 and cell blocks:**
  - Agreement between CB and FFPE tissue staining with p16 is generally high
  - False positive cases are rare
  - Biggest problem is **false negative** cases
    - Hypocellularity/cyst contents
    - Fixation
    - Degenerated and necrotic cells

  - 15% cutoff = 98% concordance with tissue p16 staining

- Xu et al (2016)
  - 1-10% cutoff had good correlation with tissue p16

- **Negative p16 staining in CB should be repeated in tissue if it becomes available**
Summary of P16 in CB versus Tissue (Jalaly et al., 2018)
High Specificity but Variable Sensitivity

Table 1. Correlation of p16 staining in cell blocks (CB) and tissue specimens

<table>
<thead>
<tr>
<th>Author, year</th>
<th>p16 clone/cutoff for p16 positivity in CB</th>
<th>Cases, total n</th>
<th>CB+/tissue+</th>
<th>CB-/tissue+</th>
<th>CB+/tissue−</th>
<th>CB−/tissue+</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cohen’s κ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Begum et al. [15], 2007</td>
<td>MTM Laboratories, Heidelberg, Germany/any staining</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>66.7</td>
<td>100</td>
<td>0.6</td>
</tr>
<tr>
<td>Holmes et al. [16], 2015a</td>
<td>MTM Laboratories, Heidelberg, Germany/any staining</td>
<td>59</td>
<td>40</td>
<td>18</td>
<td>0</td>
<td>1</td>
<td>97.6</td>
<td>100</td>
<td>0.9</td>
</tr>
<tr>
<td>Jalaly et al. [17], 2015</td>
<td>E6H4 MTM Laboratories, Heidelberg, Germany/15%</td>
<td>48</td>
<td>35</td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>97.2</td>
<td>100</td>
<td>0.9</td>
</tr>
<tr>
<td>Xu et al. [18], 2016</td>
<td>E6H4 MTM Laboratories/10%</td>
<td>36</td>
<td>25</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>83.3</td>
<td>100</td>
<td>0.6</td>
</tr>
<tr>
<td>Allison [19], 2016a</td>
<td>MTM Laboratories, Heidelberg, Germany/70%</td>
<td>29</td>
<td>24</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>100.0</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Segura et al. [20], 2018b</td>
<td>Unknown/any staining</td>
<td>52</td>
<td>24</td>
<td>17</td>
<td>0</td>
<td>11</td>
<td>68.6</td>
<td>100</td>
<td>0.6</td>
</tr>
</tbody>
</table>

a Cases included CB and small core biopsies (CB constituted approximately 50% of cases). b Abstract only.
ROC curve showed 50% cutoff to be best

Sensitivity = 74%

Specificity = 100%

A cutoff of 70% decreased sensitivity to 45%
The **GOLD STANDARD** is the demonstration of transcriptionally active **HR-HPV**
ISH for HPV E6/E7 mRNA:

Potential to apply ISH for E6/E7 HPV mRNA to cytologic preparations.

Performance of a Branch Chain RNA In Situ Hybridization Assay for the Detection of High-risk Human Papillomavirus in Head and Neck Squamous Cell Carcinoma

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• P16 staining is heterogeneous in CBs
• **Any p16 positivity** should prompt confirmatory HPV studies
• RNA ISH has high sensitivity and may be a better first-line HPV test
HR-HPV in FNAs of HNSCC: What about PCR-Based Liquid-Phase Testing?

• Liquid-phase testing is effective:
  – Advantages over cell block (FFPE)
  – Objective result with clear-cut scoring
  – Can be automated

Several tests have been validated:
  » Digene Hybrid Capture II
  » CervistaTM HPV HR
  » CervistaTM HPV 16/18
  » Roche cobas® HPV test
  » APTIMA® HPV Assay
  » OncoE6 Test
RC Test for HR-HPV in FNA

- PCR-based analysis
- >90% concordance with ISH and p16 IHC
- RC sensitivity = 100%
- RC specificity = 86%
- Automated + objective result
SUMMARY

- HPV-positive HNSCC represents a **distinct disease** from traditional smoking-related HNSCC.
- Reflex testing for HR-HPV is needed for **histologic and cytologic specimens**
- Many testing options/scenarios for FNA
  - P16, RNA ISH, PCR-based
  - CB, smears, LB
- For FNA, caution is needed when using p16 alone
  - Lower threshold for positive result
- RNA ISH on CB or PCR-based on LB may be preferable first-line tests
THANK YOU