Disclosure

• **Speaker Disclosures**
  
  *No Disclosures to make.*

  
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CAP EBG HPV Testing Committee: Scope

- The clinical role of human papillomavirus (HPV) testing in head and neck squamous cell carcinomas and target populations has previously been established by other studies (Cancer Care Ontario). Our review will inform recommendations for methods of high-risk HPV (HR-HPV) testing in both histologic and cytologic specimens of head and neck carcinomas in the clinical setting, including the performance, interpretation, and reporting of results from those tests.

THE HPV-HNSCC EPIDEMIC

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

Incidence of OPSCC in the USA: Has surpassed cervical carcinoma

Chaturvedi, SEER data from 1984-2004
Worldwide Epidemiology


- 23 countries in:
  - Asia, Australia, Europe, North, South, and Central America
- Increased incidence of OPC in 9 countries:
  - Australia, Brazil, Canada, Denmark, Japan, Netherlands, Slovakia, USA, UK
- Increase almost exclusively in economically developed countries:
  - Related to sexual behaviors
  - Findings supported by global studies on HPV in OPSCC tumors

Clinical presentation of HPV-related 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.

OROPHARYNGEAL CARCINOMA

HPV-Positive Non-Keratinizing SCC of the Oropharynx – Subepithelial Cancer

Lobular growth but invasive
Why test for HR-HPV in HNSCC?

- Prognostic implications
- Identify likely primary site in pts with metastatic SCC of unknown primary in cervical nodes (CUP)
- Determine pt. eligibility for clinical trials of de-escalation therapy
- Distinguish metastatic SCC from branchial cleft cyst or other benign squamous cysts
- Distinguish HPV-related carcinoma from EBV-related carcinoma

Key Questions

**Should patients with newly diagnosed [oropharyngeal squamous cell carcinoma (OP SCC), non-oropharyngeal squamous cell carcinoma (non-OP SCC), oropharyngeal non-squamous cell carcinoma (non-OP non-SCC), non-oropharyngeal non-squamous cell carcinoma (non-OP non-SCC) and cervical nodal metastatic carcinomas of unknown and/or known primary] be routinely tested for HR-HPV?**

- Reflex testing for OP SCC and cervical LN with CUP, but not for other sites or types of HN cancer

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%
  - Nasopharynx: 7%
  - Others (periocular etc)
Survival in HPV+ Oropharyngeal SCC

- Retrospective analyses of clinical trials: better survival for patients with HPV positive oropharyngeal SCC.
- Most comprehensive meta-analysis to date, reports a 53% better overall and 74% better disease-specific survival for HPV-positive OPSCC
- Subset of patients who have aggressive disease
- Smokers with HPV+ OPSCC have intermediate or worse prognosis

HPV+ Small Cell Carcinoma

- HPV types 16, 18, 33
- High grade features
- Aggressive clinical behavior

HPV+ Papillary SCC

Schneiderian Carcinoma of Sinonasal Cavity: Inverted Growth Pattern
HPV in Oral Cavity SCC

- HR-HPV DNA in 5-50% (average 33%)
  - DNA PCR and ISH testing
  - No survival benefit for patients with HPV DNA positive carcinomas
- Transcriptionally-active HPV uncommon (3-5%)
- No clear association with morphology or patient outcome

Ukpo et al. *Histopathol* 2012; 60:982.

HPV + SCC in Larynx & Hypopharynx

- HR-HPV DNA in 8 – 60% (average 33%)
  - DNA PCR and ISH testing
- Transcriptionally-active HPV uncommon
  - p16+DNA or mRNA = approx. 4%
  - RNA ISH and RT-PCR studies
- No difference in prognosis for HPV positive vs HPV negative SCC

Ukpo et al. *Histopathol* 2012; 60:982.

HPV in Nasopharyngeal SCC

- WHO Nonkeratinizing Type
  - Strongly related to EBV
- HPV+:
  - 7% of all NPC; 39% of EBV- cases
  - No mixed EBV/HPV infections
  - p16 correlates with HPV+/EBV-

Lo et al. *Laryngoscope* 2010; 120 S4: S185
Maxwell et al. *Head Neck* 2010; 32: 562
Robinson et al. *Inf Agents Cancer* 2013; 8: 30

Nasopharyngeal Carcinoma

- Prognosis by EBV status
  - EBV+ = improved treatment response and survival
- Prognosis by HPV status
  - Three small studies (22/325 pts)
    - No difference in outcome HPV+/EBV- versus HPV-/EBV-

Dogan et al. *Head Neck* 2014; 36: 511
Lin et al. *Head Neck* 2014; 36: 709
Robinson et al. *Inf Agents Cancer* 2013; 8: 30
Key Questions

**Do relevant clinical outcomes differ based on the test used?**

- A variety of test(s) and test combinations are acceptable, including p16 alone for primary OP SCC with a standard non-keratinizing morphology.
- Confirmatory testing for CUP.

Methods for determining HR-HPV status in HNSCC

- **IHC for p16**
  - High sensitivity, 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**
  - Reduced sensitivity at low viral load
- **IHC for E6/E7**
  - Low sensitivity, poor performance
- **Cytology Test Platforms**
  - Validation studies needed

OROPHARYNGEAL CARCINOMA AND HPV:
Common algorithm is p16 IHC followed by confirmation with ISH or PCR using HR-HPV/16/18 Cocktail

IHC for p16

ISH HR-HPV Cocktail

Positive: nuclear & cytoplasmic in >70% of tumor cells

High sensitivity but low specificity outside of oropharynx

Punctate nuclear dot-like signals which may be very focal

Low sensitivity but high specificity

PCR for HR-HPV DNA

- Very sensitive (>95%)…May be too sensitive
- When used alone, may not be able to distinguish presence of passenger virus, latent infection or viral cross-contamination from biologically and clinically relevant HPV which integrated into host genome
The **GOLD STANDARD** should be the demonstration of transcriptionally active HR-HPV.

**HPV testing in HNSCC:**
Detection of HPV E6/E7 mRNA transcripts

**Reverse transcriptase (RT) PCR**
- Generally requires fresh frozen tissue
- Commercial test for using on FFPE tissue being developed – technically challenging

**RNA in situ hybridization (ISH)**
- Probes complementary to E6/E7 mRNA allow direct visualization in tissue, including FFPE
- RNA ISH View RNA (Affymetrix)
- RNAscope HPV kit (Advanced Cell Dx’tics)

**ISH for HPV E6/E7 mRNA:**
*Collaborative study MGH-BWH*

**Key Questions**

**For patients with oropharyngeal, non-oropharyngeal, and cervical nodal metastatic squamous cell carcinoma, what is the optimal method of reporting HPV test results to best inform patients and clinicians about the clinical significance of the results (including considerations about uncertainty)?**

- HPV-related non-keratinizing squamous cell carcinoma
- Do not grade.
HPV in Oropharyngeal SCC

- Non-keratinizing or partially keratinizing
- Basaloid appearance
- Do not grade
- 90-95% are due to HPV type 16
- Small subset due to HPV 18 and other HR-HPV types (31, 33, 53 etc)

Key Questions

**Do relevant clinical outcomes of specific tests or testing algorithms for HR-HPV differ based on:

a. Features of the specimen.

-None were identified.

b. For IHC p16 testing: specific antibodies, dilution, testing conditions, and definition of a positive test?

- A majority of data for only one p16 IHC clone (E6H4).

b. For ISH and PCR, testing conditions, criteria/definition for a “positive test”, types of probes?

- Important to use a cocktail which covers a range (6 or more) of HR-HPV types (16, 18, 31, 33, 52 etc)

P16 Immunohistochemistry for HR-HPV:
Most labs use E6H4 clone but no evidence favoring one over another
**HPV Testing in Oropharyngeal SCC**

- **p16 Immunohistochemistry**
  - Sensitivity approaches 100%
  - Lower specificity: 79-82%
  - Lewis et al (AJSP 2010): p16+ HPV but DNA neg by ISH or PCR associated with good prognosis
    - Other studies mixed findings
  - Low viral load and rare HR-HPV types may explain some cases with favorable prognosis
  - p16 negative/HPV PCR+ is likely not HPV-related ca
    - May represent passenger HPV or viral contamination

**Nodal Metastases in HPV-Related OPC**


Nodal metastases are present at presentation in approx 80-85% of all HPV-related OPSCC.

FNA is a key method used to detect these metastatic cancers.

**Key Questions**

- Do relevant clinical outcomes differ if the diagnosis is based on fine needle aspiration (FNA) rather than biopsy?
  - Which test(s) should be performed?
  - Does testing FNA specimens vary based on the cytologic appearance of the metastatic head and neck SCC/non-SCC?
  - Do specific HR-HPV tests differ based on the FNA sample or preparation?
    - FNA is effective for obtaining samples of CUP.
    - A variety of testing methods can be used.

**HR-HPV in FNAs of HNSCC**

- Cell blocks can be used
- Caveats for p16 and cell blocks:
  - P16 alone can be used for FNA of mets, but confirmatory testing should be considered
  - Criteria for percentage of stained cells less defined for cell blocks (Jalaly et al, 2015)
  - Branchial cleft cysts and metastatic cutaneous SCC can be p16 positive
Liquid-phase testing:
- May be more efficient than cell block (FFPE)
- Objective result with clear-cut scoring
- Can be automated

Several have already been validated for FNA of CUP:
- Hybrid Capture II
- CervistaTM HPV HR
- CervistaTM HPV 16/18
- Roche cobas® HPV test
- APTIMA® HPV Assay

Roche Cobas® HPV Test:
Validated for Use in HN FNA Samples
- Real-time PCR amplification and detection
- Automated
- Specifically identifies type 16 and 18 as well as “other HR-HPV” (types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68)
- Concordance 91% (n=30) between cobas® and ISH for HR-HPV status
- Performance of Roche cobas® (n=34)
  - Sensitivity 100%
  - Specificity 86%

Kerr D et al. Cancer Cytopathol 2014
Key Questions

**Should patients with recurrent/persistent oropharyngeal, non-oropharyngeal, and cervical nodal metastatic squamous cell carcinoma be routinely tested for HR-HPV?**

**Should patients with distant metastatic disease be tested?**

- While clinicians are sometimes requesting HPV testing in these cases, it is not clear (other than for diagnostic purposes) that there is a role for “repeat” reflex testing.

SUMMARY

- HPV-related HNSCC distinct disease
- Many HN sites and tumor types, but OP is only primary site with implications for management
  - Reflex testing for HR-HPV in OP SCC and nodal metastatic SCC with unknown HN primary
- p16 IHC alone, +/- ISH or PCR for HR-HPV confirmation
- Gold standard: demonstrate transcriptionally active HR-HPV (mRNA ISH)
- Evidence-based CAP guidelines should help clarify some of the complex issues

Thank You!