HPV Testing in Head and Neck Cancer: Evidence-Based Guidelines and Challenges

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Marked decreased incidence in smoking-related head and neck cancers in US and elsewhere over past few decades, paralleling decreased smoking rates.
Oropharyngeal Squamous Cell Ca (SCC)

- Significant increase in incidence of oropharyngeal SCC over past 30-40 years, esp. in US and northern Europe
- Association with high risk HPV
- “Epidemic of viral-induced carcinoma”
- 70% of oropharyngeal SCC in US are HPV positive
HPV-positive oropharyngeal carcinoma

- Oropharyngeal squamous cell carcinomas will likely comprise 47% of all head and neck cancers by 2030
  
  *Chaturvedi AK et al. JCO 2011; 29:4294-43*

- Had been projected that incidence of HPV positive oropharyngeal ca in US will exceed that of cervical ca by 2020
But the lines have already crossed for HPV-attributable cervical ca and oropharyngeal ca

![Diagram showing estimated number of cancer cases attributable to HPV by sex, cancer type, and HPV type.]

HPV IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS (HNSCC)

- Most HPV-positive HNSCC are oropharyngeal
- Associated with better prognosis in oropharynx
- Lower prevalence of high risk HPV in non-oropharyngeal HNSCC
- Wide ranges reported, depending in part on methodology
- Detected HPV not associated with proven better prognosis at non-oropharyngeal sites
- 20-30% sinonasal carcinoma are HPV positive.
  Significance not clear – evolving data


HPV-POSITIVE OROPHARYNGEAL SCC

- 90-95% caused by HPV 16
- Next most common HPV 18
- Uncommonly other HR-HPV types
- HPV-driven – detection of integrated and transcriptionally active virus
- Not passenger HPV
HPV-POSITIVE SQUAMOUS CELL CARCINOMA (SCC)

- Different demographics
- Different genetic profile
- Better prognosis than HPV negative squamous cell carcinoma
Classic patient with oral or oropharyngeal squamous carcinoma

- Older male
- Smoked cigarettes for many years
- Drank alcohol for many years
- Poor oral hygiene, diet

Credit: Konstantin Chernenko
HPV-POSITIVE OROPHARYNGEAL SCC

- Younger, 40-70 years
- Mainly in men
- Caucasian>>other groups
- Many never smoked
- Most never drank much alcohol
- Higher socio-economic status
- College-educated
HPV-positive oropharyngeal SCC: Risk factors

- Sexual behavior
- **Lifetime number of oral sex partners** most strongly and consistently associated with oropharyngeal SCC
- **Oral HPV infection** = main risk factor for HPV positive oropharyngeal SCC
- Most oral HPV infections sexually acquired: oral-genital, oral-anal, oral-oral
- Higher transmission rate HPV from F to M
HPV vaccine

- Currently only Gardasil 9 (Merck), HPV-9 valent recombinant vaccine available in US
- CDC’s ACIP’s recommendations: 2 doses HPV-9 for boys and girls ages 9-15. “Catch-up” vaccine = 3 doses for F ages 15-27 and M ages 15-21 not previously vaccinated. M ages 22-26 may be vaccinated (men who have sex with men, immunocompromised)
- On 10-05-18, FDA approved Gardasil-9 vaccine for use in adults ages 27 to 45
HPV vaccination

- Many barriers to vaccination: parental fear of promiscuity, concerns about safety, lack of knowledge, not routinely recommended by MDs and other health care providers
- Up to date HPV vaccination: 48.6% for US teens, only 39.7% for Texas teens
  *MMWR Aug 24, 2018 67(33): 909-917*
- TX ranks 42nd out of 50 states for HPV vaccination rates of 13-17 year olds
- Advocate for vaccination!
HPV-POSITIVE OROPHARYNGEAL SQUAMOUS CARCINOMAS (OPSCC)

- Patients typically present with small primary (T1-2) and large neck nodes (N2-3)
- Nodal metastases at presentation in 80-85% of patients with HPV-positive OPSCC. Nodal metastases often cystic
- Overall better prognosis than patients with advanced HPV-negative HNSCC
Multiple phase II and III clinical trials of locally advanced HNSCC have demonstrated that HPV status is an independent predictor of improved survival.

HPV positive status in oropharyngeal SCC associated with better overall survival, disease-specific survival and disease-free survival.
HPV-POSITIVE OROPHARYNGEAL
SQUAMOUS CELL CARCINOMA

- 5 year survival for patients with HPV positive SCC greater than 80% vs. 40% for patients with HPV-negative HNSCC, for stage III-IV cancers
- Smokers with HPV-positive OPSCC have intermediate prognosis (RTOG 0129)
- About 20% of patients with HPV positive OPSCC have bad prognosis—need to be able to identify this population
HPV-positive oropharyngeal SCC (OPSCC)
Impact of increasing age

- Mean age of pts with OPSCC increasing
- From 2010 to 2014, % of OPSCC which are HPV positive increased for all age groups, including from 45 to 60% for those ≥70 yrs
- Survival benefit of HPV tumor status in older pts significantly attenuated compared to younger pts with HPV positive OPSCC
- Gender and ethnicity not associated with survival difference in HPV positive OPSCC

Lu et al. Eur J Cancer 2018; 103:195-204
Rettig EM et al. Oral Oncol 2018; 83:147-153
Multimodality therapy often associated with considerable morbidity, including persistent severe swallowing dysfunction and aspiration.

Because these patients survive longer, more likely to have chronic therapy-induced morbidity.

Since these patients have better prognosis, can the therapy be de-escalated?
Identification of HPV Status in Head and Neck Squamous Cell Carcinoma

Needed for:

- Clinical practice - for prognosis
- Identification of likely primary in nodal metastases with carcinoma of unknown primary (CUP)
- Clinical trials – to determine if therapy can be de-escalated in HPV-positive oropharyngeal squamous cell carcinoma
Testing for HPV status in head and neck squamous cell carcinoma

- p16 immunohistochemical stain
- PCR for HPV DNA
- HPV DNA in situ hybridization
- RT-PCR for E6/E7 mRNA
- HPV E6/E7 mRNA in situ hybridization
- Liquid-based assays

**Gold standard**: demonstration of transcriptionally active virus
p16

- Usually absent in smoking-related HNSCC, as gene usually inactivated

- Integration of HPV DNA into host genome → increased transcription HPV E6 and E7 mRNA → inactivation Rb protein → p16 upregulation → overexpression of nuclear and cytoplasmic p16

- Strong diffuse IHC staining for p16 sensitive surrogate marker for transcriptionally active HPV in HPV-positive oropharyngeal SCC
IHC for p16

- Positive in tissue: moderate to strong nuclear and cytoplasmic staining in at least 70% of cells
- No consensus on what % positive required in cell blocks. Some reports as low as 10-15%
- Caveat: other pathways may lead to p16 positivity
HPV E6/E7 mRNA in situ hybridization

- Probes complementary to E6/E7 mRNA allow direct visualization in routine tissue sections
- Detects integrated transcriptionally active HPV
- RNA ISH ViewRNA (ThermoFisher Scientific)
- RNAscope HPV kit (Advanced Cell Diagnostics)
  Can be automated with Leica Bond or Ventana Ultra stainers
Controversies: Which tumors should be tested? What is the best method for detection of HPV?

Do relevant clinical outcomes differ based on testing modality? If dx based on FNA vs tissue?

What is the optimal method of reporting HPV test results to best inform about the clinical significance of the results?
Expert Panel
Beth Beadle MD, PhD
Justin A. Bishop, MD
Rebecca D. Chernock, MD
William C. Faquin, MD, PhD
James S. Lewis, Jr., MD
Joel T. Moncur, MD, PhD
James W. Rocco, MD, PhD
Mary R. Schwartz, MD
Raja R. Seethala, MD
William H. Westra, MD

Advisory Panel
Maura Gillison MD, PhD
Bryan Hill, patient advocate
Amy Lynn, MD
Dina R. Mody, MD
Bert Noojin, JD, patient advocate
Cherie Paquette, MD
Michael Prystowsky, MD, PhD
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Staff
Nicole E. Thomas, MPH, CT(ASCP), Guideline Development Manager
Christina Lacchetti, MHSc, Contracted Methodologist
Carol Colasacco, SCT(ASC), Medical Librarian
CAP Center Project

- Multidisciplinary group systematically reviewed the literature, assessed the evidence and developed evidence-based consensus guidelines
- Title and abstract screening: 2803 studies
- Full text review: 919 papers
- Data extraction: 503 papers which met inclusion criteria
- Draft recommendations of evidence-based guidelines developed
CAP Center Project: Development of Evidence-Based Guidelines

- Open comment period, 2016 on CAP web site
- Revision of 7 of 14 draft recommendations based on review of public comments
- Independent review panel, masked to expert panel and vetted through COI process provided final approval on behalf of CAP Council on Scientific Affairs
- Guidelines reviewed by ASCO’s Head and Neck Guideline Advisory Group and ASCO’s Clinical Practice Guideline Committee
Human Papillomavirus Testing in Head and Neck Carcinomas

Guideline From the College of American Pathologists

James S. Lewis Jr, MD; Beth Beadle, MD, PhD; Justin A. Bishop, MD; Rebecca D. Chernock, MD; Carol Colasacco, MLIS, SCT(ASCP); Christina Lacchetti, MHSc; Joel Todd Moncur, MD, PhD; James W. Rocco, MD, PhD; Mary R. Schwartz, MD; Raja R. Seethala, MD; Nicole E. Thomas, MPH, CT(ASCP)CM; William H. Westra, MD; William C. Faquin, MD, PhD

Arch Pathol Lab Med 2018; 142(5):559-597
Human Papillomavirus Testing in Head and Neck Carcinomas: ASCO Clinical Practice Guideline Endorsement of the College of American Pathologists Guideline

Carole Fakhry, Christina Lacchetti, Lisa M. Rooper, Richard C. Jordan, Danny Rischin, Erich M. Sturgis, Diana Bell, Mark W. Lingen, Seema Harichand-Herdt, John Thibo, Jose Zevallos, and Bayardo Perez-Ordonez

Results
The ASCO Expert Panel determined that the recommendations from the HPV Testing in Head and Neck Carcinomas guideline, published in 2018, are clear, thorough, and based upon the most relevant scientific evidence. ASCO endorsed the guideline and added minor qualifying statements.

Pathologists should perform HR-HPV testing on all patients with newly diagnosed oropharyngeal squamous cell ca (OPSCC) including all histologic subtypes. This testing may be performed on the primary tumor or on a regional lymph node metastasis when the clinical findings are consistent with an oropharyngeal primary.

*Strength of recommendation: Strong recommendation*
Oropharynx vs Oral Cavity

**Oropharynx:**
- Palatine tonsils
- Lingual tonsils
- Soft palate
- Base of tongue
- Lateral and posterior pharyngeal walls

**Oral cavity:**
- Lips, gingiva, retromolar trigone, hard palate, buccal mucosa, floor of mouth, anterior tongue

For oropharyngeal tissue specimens (i.e., non-cytology), pathologists should perform HR-HPV testing by surrogate marker p16 immunohistochemistry (IHC). Additional HPV-specific testing may be done at the discretion of the pathologist and/or treating clinician, or in the context of a clinical trial.

*Strength of recommendation: Recommendation*
Abundant literature on p16 IHC as an independent predictor of prognosis in oropharyngeal squamous cell carcinoma

IHC for p16 used in retrospective analysis of major clinical trials and in ongoing prospective de-escalation trials

Need for test that best stratifies patient survival outcomes, while also practical and inexpensive: p16 IHC widely available, reproducible, ease of testing
A small fraction of oropharyngeal tumors are not driven by HPV yet overexpress p16. Pathologists should be experienced with and have available confirmatory HPV testing for use at their discretion.
Pathologists should not routinely perform HR-HPV testing on patients with nonsquamous carcinomas of the oropharynx.

*Strength of recommendation: Expert consensus opinion*
When oropharyngeal tumors are poorly differentiated and there is uncertainty that the carcinoma is nonsquamous, e.g. neuroendocrine tumors, HPV-specific testing is warranted.
HPV-associated Neuroendocrine Carcinomas

- Small cell, large cell or mixed types
- Often present with nodal metastases
- Often p16 positive (small cell ca freq p16+)
- Unlike HPV positive OPSCC, aggressive clinical course and poor outcome
- High rate of distant metastases
- HPV status does not predict prognosis for these tumors
Oropharyngeal NE carcinoma

- p16
- CD56
Pathologists should **not** routinely perform HR-HPV testing on patients with non-oropharyngeal primary tumors of the head and neck

*Strength of recommendation: Recommendation*
HPV in Non-Oropharyngeal HN Squamous Cell Carcinomas (SCC)

- HR-HPV DNA in 0-95+% oral cavity and laryngeal SCC by HPV DNA PCR or ISH
  
  Transcriptionally active HPV in less than 5%

- HR-HPV in 20-30% of sinonasal SCC – data on clinical significance evolving

- No proven prognostic or therapeutic significance of HPV status in non-oropharyngeal primaries (possible exception some sinonasal carcinomas)
Cervical lymph nodes with metastatic squamous cell ca of unknown primary (CUP)

- 5-10% of pts presenting with met SCC to neck
- Generally comprehensive radiotherapy to neck and all possible primary sites, encompassing oral cavity, all of pharynx and larynx
- Greater morbidity with more extensive radiotherapy
Pathologists should routinely perform HR-HPV testing on patients with metastatic SCC of unknown primary in a cervical upper- or mid-jugular chain lymph node. An explanatory note on the significance of a positive HPV result is recommended.

Strength of recommendation: Recommendation
HPV Testing Guideline Statement #6

For tissue specimens (i.e., noncytology) from patients presenting with metastatic SCC of unknown primary in a cervical upper- or mid-jugular chain lymph node (levels II and III), pathologists should perform p16 IHC.

Strength of recommendation: Expert consensus opinion
Note: Additional HPV-specific testing on p16-positive cases should be performed for tumors located outside of level II or III in the neck and/or for tumors with keratinizing morphology.
Pathologists should perform HR-HPV testing on head and neck FNA SCC samples from all patients with known oropharyngeal SCC not previously tested for HR-HPV, with suspected oropharyngeal SCC, or with metastatic SCC of unknown primary.

*Strength of recommendation: Expert consensus opinion*
Note: No recommendation is made for or against any specific testing methodology for HR-HPV testing in FNA samples. If the result of HR-HPV testing on the FNA sample is negative, testing should be performed on tissue if it becomes available. If pathologists use cytology samples for p16 IHC testing, they should validate the criteria (i.e., cut-off) for a positive result.
IHC alone may not be sufficient in this scenario, especially with cytology material.

Additional confirmatory testing should be performed at discretion of pathologists and/or clinicians.

ASCO recommends 70% cutoff for p16 IHC and use restricted to patients with known OPSCC.
HR-HPV testing in FNAs of cervical lymph nodes with metastatic squamous cell ca

- p16 – on cell blocks
- HPV DNA in situ hybridization
- PCR
- Hybrid Capture 2 (Qiagen)
- Cervista HPV HR
- Cervista HPV 16/18
- Cobas HPV test (Roche)
- Aptima (Hologic) – detects HPV E6/E7 mRNA
- mRNA in situ hybridization