The Effectiveness of Molecular Assays and Sampling Methods: An Application on HPV Detection in Oropharyngeal and Oral Cavity Cancers

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Objectives

At the completion of this presentation the learner will be able to:

1. Identify precursors and possible warning signs of oropharyngeal (OP) cancer.

2. Describe challenges faced by and the impact on quality of life for individuals diagnosed with quality of life OP cancer.

3. Compare current screening methods used to detect persistent HPV infection and diagnose anogenital – vs. non-anogenital HPV-related cancers.
HPV-ASSOCIATED DISEASE & CANCER
Epidemiology
Infectious Agents cause 17-20% of Cancer Worldwide: Higher in Developing World

- **Bacterium: H. pylori**
  - Stomach

- **Virus: HPV**
  - Cervix, others

- **Viruses: HBV/HCV**
  - Liver

- **Virus: EBV**
  - Nasopharynx, lymphoma (gastric cancer)

- **Virus: HHV8**
  - Kaposi’s sarcoma

- **Other agents**
  - Several tumor types

Cervical Cancer accounts for >90% of HPV-associated cancer in Developing World

- ~85% of global cervical cancer occurs in developing world; ~88% of deaths
- ~1% of women in developing world will die of cervical cancer before they are 75 (Ferlay et al, Int J Cancer, 2015)

USA: HPV-associated Non-cervical Cancers Affect Both Genders and are as Common as Cervical Cancer

- Pap screening has reduced cervical cancer incidence by ~80%
- No approved screening tests for other HPV-associated cancers
- Incidence of HPV-positive oropharynx cancer 1988-2004 increased >3-fold

Cervical Cancer is Attributable to Multiple HPV Types; HPV16 Predominates

Adapted from Munoz et al, Int J Cancer 111: 278-85, 2004
CERVICAL CANCER SCREENING

HPV Testing
Natural History of Cervical Cancer

HPV Infection

CIN1, 2

Average 6-24 Months

CIN2, 3

Average of 10 Years

Invasive Cancer

HPV Clearance

Primary & Secondary Prevention

Primary Prevention: Vaccination

Secondary Prevention: Screening

Treatment (tertiary prevention)

Normal → Pre-cancer → Cancer
Cervical Cancer Screening: From Pattern Recognition to Molecular Diagnosis

- Pap smear screening and other cytology-based methods are based on pattern recognition.

- They have reduced cervical cancer incidence and mortality (~80% in the USA since 1940’s).

- 2014: Primary cervical cancer screening by HPV-based testing approved by FDA.

- HPV-based screening is etiology-based. It is more sensitive and has higher negative predictive value.
Declining cervical cancer rates: attributable to decreased squamous cell cancer, but not adenocarcinoma

Squamous cell: blacks
Squamous cell: whites

Adenocarcinoma: whites
Adenocarcinoma: blacks
Adenosquamous: blacks & whites
HPV testing can prevent more cervical cancers, especially adenocarcinomas, than cytology.

Pooled cervical cancer incidence from 4 randomized controlled trials of cytology (control arm) vs. HPV testing (experimental arm).

Pooled rate ratio*(95% CI)

<table>
<thead>
<tr>
<th>Morphology</th>
<th>0.78 (0.49–1.25)</th>
<th>0.31 (0.14–0.69)</th>
<th>0.34 (0.12–0.90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous-cell carcinoma</td>
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<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td></td>
<td></td>
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<tr>
<td>Adenocarcinoma vs squamous-cell</td>
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</table>

*Ratio of incidence with HPV testing vs. incidence with cytology

OROPHARYNGEAL SQUAMOUS CELL CARCINOMA (OPSCC)

Clinical Presentation
Risk Factors

- Tobacco use
- Excessive alcohol use
- Gender
- Age
- Race
- **Human Papillomavirus (HPV)**
Warning Signs: Precursors

- Leukoplakia (white lesion)
- Erythroplakia (red lesion)
- Erythroleukoplakia (red-white lesion)

**Any white or red lesion that does not resolve itself in two weeks should be reevaluated and considered for biopsy to obtain a definitive diagnosis.**
Other Possible Warning Signs

- A sore, irritation, lump, or thick patch in the mouth, on the lip, or throat
- A white or red patch in the mouth
- A feeling that something is caught in your throat
- Any difficulty chewing or swallowing
- Any difficulty moving the tongue or jaw
- Any numbness of the tongue or other parts of the mouth
- Any swelling of the jaw that causes dentures to fit poorly or become uncomfortable
- Any pain in one ear with or without hearing loss
TREATMENT & MANAGEMENT
Impact/Prevention
There is no profit in curing the body if in the process we destroy the soul.
Oropharyngeal Squamous Cell Carcinoma (OPSCC)

- Devastating diagnosis for an individual and their loved ones
- The treatment is brutal and life altering
  - Facial disfigurement
  - Speech dysfunction
  - Chewing & swallowing difficulties
  - Depression
  - Social Isolation
- Side effects cause many months/years of physical and mental distress
RESEARCH
An Investigation of the Utility of Molecular Assays & Sampling Methods for the Detection of Oropharyngeal HPV Infection
Screening

Currently have no FDA approved assay for the detection of HPV infection of the oropharynx.
To identify a screening test that could be used to test a large group of individuals at-risk for human papillomavirus (HPV) infection of the oropharynx (mouth and tonsils).
Aims

1. To identify differences in the sensitivity, specificity, and positive and negative predictive values across various combinations of biologic samples, analytic methods and collection kits.

2. To investigate how well the various combinations of biologic samples, analytic methods and collection kits predicted the presence of HPV infection in the oropharynx.
Methods

• Cross-sectional, exploratory pilot study
• 40 individuals
• Explored sensitivity and sensitivity of various combinations of biologic samples, and analytic and collection methods
  • Histologic and cytological evaluation
  • Molecular genomic interrogation
    • DNA isolation
    • Third Wave Invader Technology
    • Immunohistochemistry
INCLUSION CRITERIA

• 18 years of age or older
• English speaking
• Primary lesion
• Sore, irritation, lump, and/or thick, red or white patch noted in their mouth and/or on their tonsils
• No history of prior chemo radiation therapy
## METHODS

<table>
<thead>
<tr>
<th>Sexual History &amp; Social Behavior Questionnaire</th>
<th>Demographic &amp; Descriptive Data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saliva, Exfoliated cells, and Biopsy Tissue</strong></td>
<td><strong>1. Gold Standard: Biopsy tissue</strong></td>
</tr>
<tr>
<td></td>
<td>a. Histology: Routine Evaluation &amp; Diagnosis</td>
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<tr>
<td></td>
<td>b. DNA Analysis for HR HPV Status</td>
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<td>c. p16 IHC Staining for HPV Detection</td>
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<td><strong>2. Combinations (Biologic Sample, Collection Container, and Assay)</strong></td>
</tr>
<tr>
<td></td>
<td>a. HPV Positive &amp; Negative Saliva and Exfoliated Cells</td>
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<tr>
<td></td>
<td>i. DNA/Saliva Container &amp; Cytology</td>
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<tr>
<td></td>
<td>ii. DNA/Saliva Container &amp; Third Wave Invader Chemistry-DNA for HR HPV</td>
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<td>iii. Non-GYN Liquid Based Container &amp; Saliva &amp; Cytology</td>
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<td>iv. Non-GYN Liquid Based Container &amp; Saliva &amp; Third Wave Invader Chemistry-DNA for HR HPV</td>
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</tbody>
</table>
Combination #1: Saliva, Third Wave Invader Technology HR HPV Assay
### Table 1: Sensitivity, Specificity, PPV, and NPV.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity, % (95% CI)</td>
<td>100% (75.9-100)</td>
</tr>
<tr>
<td>Specificity, % (95% CI)</td>
<td>20% (5.3-48.6)</td>
</tr>
<tr>
<td>Positive Predictive Value (PPV), (95% CI)</td>
<td>57.1% (37.4-74.9)</td>
</tr>
<tr>
<td>Negative Predictive Value (NPV), (95% CI)</td>
<td>100% (30.9-100)</td>
</tr>
</tbody>
</table>

Kostas-Polston et al. (2015), unpublished data.
Combination #2: Saliva, Non-GYN, Third Wave Invader Technology HR HPV Assay
Combination #2

Table 1: Sensitivity, Specificity, PPV, and NPV.

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<td>Sensitivity, % (95% CI)</td>
<td>56.2% (30.5-79.2)</td>
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<tr>
<td>Specificity, % (95% CI)</td>
<td>66.7% (38.7-87)</td>
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<tr>
<td>Positive Predictive Value (PPV), (95% CI)</td>
<td>64.3% (35.6-86)</td>
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<tr>
<td>Negative Predictive Value (NPV), (95% CI)</td>
<td>58.8% (33.4-80.5)</td>
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Kostas-Polston et. al. (2015), unpublished data.
Combination #3: Oral Scraping, Non-GYN, Cytology
Combination #3

<table>
<thead>
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<th>Table 1: Sensitivity, Specificity, PPV, and NPV.</th>
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<tr>
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Kostas-Polston et. al. (2015), unpublished data.
Combination #4: Saliva, Tube, Cytology
### Table 1: Sensitivity, Specificity, PPV, and NPV.

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Kostas-Polston et. al. (2015), unpublished data.
Combination #5: Oral Scraping, Non-GYN, Third Wave Invader Technology HR HPV Assay
## Table 1: Sensitivity, Specificity, PPV, and NPV.

<table>
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<tr>
<th>Measure</th>
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<tbody>
<tr>
<td>Sensitivity, % (95% CI)</td>
<td>78.5% (48.8-94.2)</td>
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<tr>
<td>Specificity, % (95% CI)</td>
<td>78.5% (48.8-94.2)</td>
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Kostas-Polston et al. (2015), unpublished data.
Combination #6: Biopsy Tissue and p16 Immunohistochemistry (IHC)
Table 1: Sensitivity, Specificity, PPV, and NPV.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value (95% CI)</th>
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<tbody>
<tr>
<td>Sensitivity, % (95% CI)</td>
<td>87.5% (60.4-97.8)</td>
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<tr>
<td>Specificity, % (95% CI)</td>
<td>100% (73.2-100)</td>
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Kostas-Polston et. al. (2015), unpublished data.
HPV Status, Lifestyle Habits & Clinical Parameters

- Gender
  - 1:5 (males : females)
  - 1 AA
- All OP lesions → tumors
  - 16 BOT, 14 Tonsil
- Average age 53.7 years
- Marital Status
  - 60% Married
  - 28% Divorced/Separated
- 82% Ever Smokers
  - Average age of onset 17.8 years
  - Average number of years smoking 26.4 years
- ETOH
  - 60% Non-drinkers
  - 40% 4 drinks/week
HPV Status, Lifestyle Habits & Clinical Parameters (cont’d).

- First Sexual Activity = 17.4 years
- Total # of Partners = 14 (Range 1-75)
- 9% Self-Reported History of STI
- 9% Self-Reported Partner with History of STI
- 24% Anal Sex
- 67% Oral Sex
- 83% Never Used Condoms
- 38% No Condoms with Anal Sex
- 71% Steady Partner
HPV Status, Lifestyle Habits & Clinical Parameters (cont’d).

- 100% OPSCC (histological diagnosis)
  - 50% HPV Positive
  - All but one presented with advanced stage disease/distant metastasis (Tumor Stages III-IVA)

- p16 (Gold Standard)
  - All but one HR HPV status correlated with p16 status
  - + p16/- HR HPV
    - Third Wave Invader Technology HR HPV Assay
Implications

1. Findings expand understanding of natural history of OP HPV infection.
   - Initial infection $\rightarrow$ clearance $-$vs. persistence $\rightarrow$ carcinogenesis.

2. Establishing the best biological sample type & method, collection kit and laboratory assay may lead to the identification of an accurate, non-invasive test to be used in clinical settings for the purpose of early detection of persistent HPV-related OP infection.
PARTNERING

Mentors, Colleagues and Friends…
REFERENCES


REFERENCES (cont’d.)


REFERENCES (cont’d.)