The Biology of HPV Infection and Cervical Cancer

Kaitlin Sundling, M.D., Ph.D.
Clinical Instructor
Faculty Director, Cytotechnology Program
Wisconsin State Laboratory of Hygiene and
University of Wisconsin School of Medicine and Public Health

ksundling@wisc.edu
@kaitlinsundling on Twitter

Learning Objectives

• Describe the key molecular events in HPV oncogenesis.
• Relate transient and persistent HPV infection to patient clinical history and cytomorphologic findings.
• Explain the integration of HPV testing and cytologic findings in cervical cancer screening follow up guidelines.
• Troubleshoot pitfalls in HPV testing.
The Pap test: A minimally invasive test for cancer and pre-cancer

- Originally developed by Dr. George Papanicolaou, immigrant to the US from Greece
- Early scientific work used vaginal smears to study the reproductive cycles of guinea pigs
- Developed a staining method that allowed identification of benign and malignant cells under the microscope
- Original papers were published in late 1910’s-1920’s
- Pap test widely adopted in the 1940’s
The Pap test: Morphology

The Netter Collection of Medical Illustrations: The Reproductive System

Normal superficial and intermediate squamous cells
Negative for Intraepithelial Lesion or Malignancy

Low grade Squamous Intraepithelial Lesion (LSIL)

https://bethesda.soc.wisc.edu
High grade Squamous Intraepithelial Lesion (HSIL)

Invasive Squamous Cell Carcinoma

Atypical Squamous Cells of Undetermined Significance (ASCUS)

https://bethesda.soc.wisc.edu
The Pap test: A crucial component of cervical cancer prevention

• Primary prevention
  • HPV vaccination
  • Condoms (But what about areas not covered by condoms? How likely will patients be to use a condom for all contact, every time?)
  • Limiting sexual partners (But what about the partner’s partners?)

• Secondary prevention
  • Pap test
  • Appropriate treatment and follow-up of dysplasia (precancerous lesions)

Human papillomavirus

• Non-enveloped, circular dsDNA virus
• Early genes E6 and E7 bind p53 and Rb
• Late gene L1 makes the major coat protein
• HPV types infect birds and mammals
• Infection is ubiquitous

HPV-related disease

- Anogenital tract skin and mucosa – penile, vulvar, vaginal, cervical, and anal
- Oropharynx – tonsils and base of tongue
- Skin – most commonly low risk types, causing warts
- Papillomas of the respiratory tract and conjunctiva – usually low risk types

Low risk vs. high risk HPV infection

- Low risk HPV types can cause koilocytosis and condylomas, unlikely to cause cancer
- High risk HPV types can cause koilocytosis and condylomas, may progress to HSIL (high grade SIL) and cancer
- HPV testing almost uniformly refers to testing for high risk HPV types
HPV vaccination

• Most effective at preventing infection when given prior to first exposure
• When given later, may still be effective in preventing infection by new HPV types

HPV vaccination

• Risks of HPV Vaccination: Allergic response to vaccine components, minor localized or febrile (fever) vaccine reactions
• Benefits of HPV Vaccination:
  • Boys: Reduced risk of genital warts, reduced risk of penile cancer, reduced risk of anal cancer, reduced risk of oropharyngeal cancer
  • Girls: Reduced risk of genital warts, reduced risk of cervical, vaginal, vulvar, and anal cancer, and reduced risk of oropharyngeal cancer
  • General public: Herd immunity
• https://www.cdc.gov/hpv/hcp/for-hcp-tipsheet-hpv.pdf
• https://wicancer.org/action-plans/hpv-vaccination-rates/
Gardasil 9 vaccine

- L1 protein virus-like particles
- Protective against:
  - 6, 11 – low risk, causing genital warts
  - 16, 18, 31, 33, 45, 52 and 58 – high risk
- Recommended for boys and girls ages 11 or 12
- May begin as early as age 9, catch up recommended up to age 26 for women, 21 for men
- FDA approval recently extended to upper limit of age 45

Transient HPV infection
• Usually LSIL/koilocytic changes
• Common in women in their 20s
• Regresses

Persistent HPV infection
• HSIL, koilocytes less likely
• More common in women in their 30s and up
• May lead to cancer

Routine Cervical Cancer Screening Guidelines

<table>
<thead>
<tr>
<th>Age group</th>
<th>ASCCP 2012</th>
<th>USPSTF 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 21</td>
<td>No screening</td>
<td>No screening</td>
</tr>
<tr>
<td>Age 21-29</td>
<td>Cytology alone every 3 years</td>
<td>Cytology alone every 3 years</td>
</tr>
<tr>
<td>Age 30-65</td>
<td>Cytology alone every 3 years OR Cotesting with cytology and HPV testing every 5 years</td>
<td>Cytology alone every 3 years OR Cotesting with cytology and HPV testing every 5 years OR Primary screening with HPV testing along every 5 years</td>
</tr>
<tr>
<td>Over 65</td>
<td>Discontinue screening if adequately screening and not at high risk for cervical cancer</td>
<td>Discontinue screening if adequately screening and not at high risk for cervical cancer</td>
</tr>
</tbody>
</table>

http://www.asccp.org/Assets/fcd6fdab-0325-466b-a5cd-3c1c06f0e66/635912171989730000/asccp-guidelines-pdf
Management of Women with Atypical Squamous Cells of Undetermined Significance (ASC-US) on Cytology*

- **Repeat Cytology**
  - @ 1 year
  - Acceptable

  - Negative
  - ≥ ASC

- **HPV Testing**
  - Preferred

  - HPV Positive
    - (managed the same as women with LSIL)
  - HPV Negative

- **Colposcopy**
  - Endocervical sampling preferred in women with no lesions, and those with inadequate colposcopy; it is acceptable for others

- Manage per ASCCP Guideline

* Management options may vary if the woman is pregnant or ages 21-24
† Cytology at 3 year intervals

http://www.asccp.org/asccp-guidelines

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Management of Women ≥ Age 30, who are Cytology Negative, but HPV Positive

- **Repeat Cotesting**
  - @ 1 year
  - Acceptable

  - Cytology Negative and HPV Negative

- **HPV DNA Typing**
  - Acceptable

  - ≥ ASC or HPV Positive
  - HPV 16 or 18 Positive
  - HPV 16 and 18 Negative

- **Colposcopy**

- Manage per ASCCP Guideline

- Repeat Cotesting
  - @ 1 year

http://www.asccp.org/asccp-guidelines
### Test Target Gene(s) Target Biomolecule Internal Control Technology HPV Types Detected Fixative

<table>
<thead>
<tr>
<th>Test</th>
<th>Target Gene(s)</th>
<th>Target Biomolecule</th>
<th>Internal Control</th>
<th>Technology</th>
<th>HPV Types Detected</th>
<th>Fixative</th>
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<tbody>
<tr>
<td>Abbott RealTime High Risk HPV</td>
<td>L1</td>
<td>DNA</td>
<td>Beta globin</td>
<td>PCR</td>
<td><strong>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</strong></td>
<td>ThinPrep</td>
</tr>
<tr>
<td>APTIMA</td>
<td>E6 and E7</td>
<td>mRNA</td>
<td>Spiked in</td>
<td>Transcription mediated amplification</td>
<td><strong>16, 18/45, 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68</strong></td>
<td>ThinPrep</td>
</tr>
<tr>
<td>Cervista</td>
<td>L1</td>
<td>DNA</td>
<td>HIST2H2BE</td>
<td>Isothermal DNA amplification, Invader FRET</td>
<td><strong>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</strong></td>
<td>ThinPrep</td>
</tr>
<tr>
<td>Cobas 4800 HPV Test</td>
<td>L1</td>
<td>DNA</td>
<td>Beta globin</td>
<td>PCR</td>
<td><strong>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</strong></td>
<td>ThinPrep</td>
</tr>
<tr>
<td>Hybrid Capture 2</td>
<td>L1</td>
<td>DNA</td>
<td>None</td>
<td>RNA probes and antibody detection of RNA:DNA hybrids</td>
<td><strong>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</strong></td>
<td>ThinPrep</td>
</tr>
<tr>
<td>BD Onclarity</td>
<td>E6 and E7</td>
<td>DNA</td>
<td>Beta globin</td>
<td>PCR</td>
<td><strong>16, 18, 31, 45, 51, 52, and 59, (33, 56, 58, 66), (35, 39, 68)</strong></td>
<td>SurePath</td>
</tr>
</tbody>
</table>

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**Risks of screening**

- **False positives**
  - Unnecessary biopsies and loop electrosurgical excision procedures
    - May lead to shortened cervix or cervical stenosis
    - Reduced fertility or incompetent cervix
- **False negatives**
  - Lost opportunity for early treatment
  - Lesions may present when already invasive or even metastatic, requiring more invasive treatment, impact of quality of life and survival
- **Direct costs to patients**
- **Public health impacts**
- **Utilization of healthcare resources**
Potential biological pitfalls of HPV testing

• 10% of invasive carcinomas may be HPV negative\(^1\)
  • Presumed loss of HPV viral DNA in the tumor after acquisition of other mutations, such as DNA repair defects
  • L1 gene may be lost\(^2\)


<table>
<thead>
<tr>
<th>HPV type</th>
<th>Total</th>
<th>Single</th>
<th>Mixed</th>
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<tbody>
<tr>
<td></td>
<td>N Bx Conf % HSIL cases Bx Conf</td>
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</tr>
<tr>
<td>31</td>
<td>30 12 14.6 17.6</td>
<td>23 10 11.2 14.7</td>
<td>7 2 3.4 2.9</td>
</tr>
<tr>
<td>52</td>
<td>13  5  6.3  7.4</td>
<td>10  5  4.9  7.4</td>
<td>3  0  1.5  0.0</td>
</tr>
<tr>
<td>58</td>
<td>13  6  6.3  8.8</td>
<td>11  4  5.4  5.9</td>
<td>2  2  1.0  2.9</td>
</tr>
<tr>
<td>35</td>
<td>9  7  4.4 10.3</td>
<td>8  6  3.9  8.8</td>
<td>1  1  0.5  1.5</td>
</tr>
<tr>
<td>45</td>
<td>7  3  3.4  4.4</td>
<td>1  1  0.5  1.5</td>
<td>6  2  2.9  2.9</td>
</tr>
<tr>
<td>33</td>
<td>6  3  2.9  4.4</td>
<td>4  2  2.0  2.9</td>
<td>2  1  1.0  1.5</td>
</tr>
<tr>
<td>59</td>
<td>6  2  2.9  2.9</td>
<td>4  2  2.0  2.9</td>
<td>2  0  1.0  0.0</td>
</tr>
<tr>
<td>16</td>
<td>3  1  1.5  1.5</td>
<td>1  1  0.5  1.5</td>
<td>2  0  1.0  0.0</td>
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<tr>
<td>56</td>
<td>1  0  0.5  0.0</td>
<td>1  0  0.5  0.0</td>
<td>0  0  0.0  0.0</td>
</tr>
<tr>
<td>Negative</td>
<td>76 18 37.1 26.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Potential biological pitfalls of HPV testing

• Endogenous flora (coccobacilli including *Gardnerella* sp., lactobacilli) and cytolysis may lead to interference

• Shifts in high risk HPV types with increasing HPV vaccination
  • Gardasil 9: 16, 18, 31, 33, 45, 52, 58
  • Most HPV tests: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68

• Selective pressure on the L1 gene due to HPV vaccination

https://bethesda.soc.wisc.edu

Technical pitfalls in HPV testing

• SurePath vs. ThinPrep
  • SurePath vials should contain the collection device, while ThinPrep should not
  • SurePath fixative contains a small amount of formaldehyde
  • Some laboratories have validated a boiling pre-processing step

• Alternate collection methods/sources
  • Vaginal self-collection
  • Urine
On the horizon

- HPV primary screening
  - Australia and the Netherlands
  - Potential reflex to cytology
  - Concerns: PPV, NPV, colposcopy infrastructure
- HPV testing combined with other tests to improve specificity for precancerous lesions
  - DNA methylation
  - Gene expression
  - IHC staining
- CDC Grand Rounds: Preventing Cervical Cancer in the 21st century

Thank you!

Questions, comments, suggestions, or potential collaborations?

ksundling@wisc.edu
@kaitlinsundling on Twitter