The Biology of HPV Infection and Cervical Cancer

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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
Cervix Uteri Cancer
By Race/Ethnicity
All Ages

The Pap test: Morphology

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

Low grade Squamous Intraepithelial Lesion (LSIL)
High grade Squamous Intraepithelial Lesion (HSIL)

Invasive Squamous Cell Carcinoma
Atypical Squamous Cells of Undetermined Significance (ASCUS)
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://www.cdc.gov/hpv/hcp/for-hcp-tipsheet-hpv.pdf)
• [https://wicancer.org/action-plans/hpv-vaccination-rates/](https://wicancer.org/action-plans/hpv-vaccination-rates/)
Gardasil 9 vaccine

• L1 protein virus-like particles
• Protective againsts:
  • 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
Human papillomavirus (HPV) is thought to access the basal cells through the basement membrane, which is supported by the dermis.

Overexpression of E6 and E7 oncogene expression. LCR, long control region.

Integration of the HPV genome into the host chromosomes can lead to the development of cervical intraepithelial neoplasia (CIN) and eventually invasive cancer.

Cervical intraepithelial neoplasia

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low grade</td>
</tr>
<tr>
<td>2</td>
<td>High grade</td>
</tr>
<tr>
<td>3</td>
<td>Advanced</td>
</tr>
</tbody>
</table>

Invasive cancer

Infectious viral particles

Normal cervix

Squamous intraepithelial lesion

Low grade

High grade

Episome

Discussion

Inversely related to quality score.

Study quality was inversely related to progression at 6 and 24 months. For ASCUS and low-grade SIL combined, regression to normal was seen in about 53% of cases (95% CI 42%, 63%). Although reviews on the natural history of untreated cervical dysplasia have been published previously, ours is the first to our knowledge, based on our literature review, to apply the techniques of meta-analysis to process of cervical dysplasia.

The following variables were included: perforation, duration of follow-up, percentage of subjects lost to follow-up, mean or median age, and performance of biopsy. The progression rate for invasive cancer at 24 months was highest for high-grade SIL (1.44%) and lowest for low-grade SIL (0.15%). Regression rates were highest for ASCUS (68.19%) and lowest for high-grade SIL (35.03%). These rates are shown with their 95% confidence intervals in Figure 3. All estimates are based on random effects models, except progression to cancer used to determine whether study level variables contributed significantly to between-study variability in between 7.13% for ASCUS and 20.81% for low-grade SIL. The progression rate for invasive cancer at 24 months was highest for high-grade SIL (1.44% (95% CI 1.00%), 3.95%). These rates are shown with their 95% confidence intervals in Figure 3. All estimates are based on random effects models, except progression to cancer used to determine whether study level variables contributed significantly to between-study variability in regression rates were not related to time in our analysis, progression to higher-grade lesions and to invasive cancer occurred more frequently after longer times.
grade SIL combined at 6 months, 0.04% (95% CI 0%, 0.59%); both combined at 24 months, 0.15% (95% CI 0%, 0.70%); high-grade SIL at 6 months, 0.15% (95% CI 0%, 1.00%); and high-grade SIL at 24 months, 1.44% (95% CI 0%, 3.95%). These rates are shown with their 95% confidence intervals in Figure 3. All estimates are based on random effects models, except progression to cancer for ASCUS and low-grade SIL, in which between-study variance was negative, and fixed effects models were used.

Estimated rates for progression at 24 months were between 7.13% for ASCUS and 20.81% for low-grade SIL. The progression rate for invasive cancer at 24 months was highest for high-grade SIL (1.44%) and lowest for low-grade SIL (0.15%). Regression rates were highest for ASCUS (68.19%) and lowest for high-grade SIL (35.03%).

Single factor random effects regression analysis was used to determine whether study level variables contributed significantly to between-study variability in rates of regression to normal, progression, and invasive cancer. The following variables were included: performance of biopsy, duration of follow-up, percentage of subjects lost to follow-up, mean or median age, and quality score. None of these variables accounted for variation in rates of progression or regression of cervical dysplasia in the analysis, except for study quality score. For high-grade SIL, study quality was inversely related to progression at 6 and 24 months. For ASCUS and low-grade SIL combined, regression to normal was inversely related to quality score.

Discussion

Although reviews on the natural history of untreated cervical dysplasia have been published previously, ours is the first to our knowledge, based on our literature review, to apply the techniques of meta-analysis to the English language literature on this topic. Studies of women whose cervical smears showed squamous atypia or worse and who were observed for a minimum of 6 months were identified by a search of MEDLINE from 1966 to 1996, Current Contents, the Federal Research in Progress database, and references of review articles and identified studies, and by experts in the field. We found that high-grade lesions were less likely to regress to normal and more likely to progress to invasive cancer, consistent with biologic theory on the process of cervical dysplasia.
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-3c1c06cf0e66/635912171989730000/asccp-guidelines-pdf*
Management of Women with Atypical Squamous Cells of Undetermined Significance (ASC-US) on Cytology*

**Repeat Cytology**
@ 1 year
Acceptable

- Negative
- > ASC

**Routine Screening†**

**HPV Testing**
Preferred

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

**Repeat Cotesting**
@ 3 years

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

- **Repeat Cotesting @ 1 year Acceptable**
  - Cytology Negative and HPV Negative
    - Repeat Cotesting @ 3 years

- **HPV DNA Typing Acceptable**
  - ≥ ASC or HPV Positive
    - HPV 16 or 18 Positive
      - Repeat Cotesting @ 1 year
    - HPV 16 and 18 Negative
      - Manage per ASCCP Guideline

Manage per ASCCP Guideline

http://www.asccp.org/asccp-guidelines
<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 Assay</td>
<td>L1</td>
<td>DNA</td>
<td>Beta globin</td>
<td>PCR</td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</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>16, 18/45, 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68</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>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</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>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</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>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68</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>16, 18, 31, 45, 51, 52, and 59, (33, 56, 58, 66), (35, 39, 68)</td>
<td>SurePath</td>
</tr>
</tbody>
</table>
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
  • Presumed loss of HPV viral DNA in the tumor after acquisition of other mutations, such as DNA repair defects
  • L1 gene may be lost

Of the 387 reviewed HSIL cases, 104 had subsequent biopsy results.

The 68 cases with biopsy confirmed CIN 2 were HPV 16/18 negative.

One-third (35 of 104) were positive for HPV 16 on initial testing, which are likely attributable to false-negative results due to analytic sensitivity issues. Fifty-seven percent (59 of 104) were positive for HPV 16 on secondary testing that were previously negative.

Secondary genetic typing for 205 HSIL cases of found to be either totally negative for HPV or positive for other high-risk HPV genotypes other than HPV 16/18 on initial testing.

Table 2  Secondary genetic typing for 205 HSIL cases of found to be either totally negative for HPV or positive for other high-risk HPV genotypes other than HPV 16/18 on initial testing.

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

Negative | 76 | 18 | 37.1 | 26.5 | 1 | 0 | 0.5 | 0.0 |

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?

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