HPV Genotyping: A New Dimension in Cervical Cancer Screening Tests

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The natural history of cervical cancer

99% association of cervical carcinoma with HPV
- STI and not genetic


CIN, cervical intraepithelial neoplasia (terminology used to
describe histology of squamous cell lesions).
Screening to prevent cervical cancer

- Screening with cervical cytology: “A triumph and a tragedy”*


George Papanicolaou developed Pap Smear in 1940s
- originally to detect early lung cancer
- but we want to detect “pre-malignant cells” before any s/x (ie. find stage 2);
- finding cervical cancer in early stage is still too late
- no difference between radiation and surgery for early stage cervical CA

New Cases, Deaths and 5-Year Relative Survival
SEER 9 Incidence & U.S. Mortality 1975-2010

In recent years cervical cancer incidence has leveled off, as cytology is not reducing incidence further


Not at 0, but should b/c it’s detectable so early
- average age of detection is 49yo

1976 discovered HPV and cervical cancer link
Screening history of women diagnosed with invasive cervical carcinoma (ICC)

<table>
<thead>
<tr>
<th>Cause, n (%)</th>
<th>Kaiser study(^1)</th>
<th>Swedish study(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No recent screen</td>
<td>464 (56%)</td>
<td>789 (64%)</td>
</tr>
<tr>
<td>Cytology detection failure</td>
<td>263 (32%)</td>
<td>300 (24%)</td>
</tr>
<tr>
<td>Failure of follow-up of abnormal cytology</td>
<td>106 (13%)</td>
<td>91 (7%)</td>
</tr>
</tbody>
</table>


Reasons for moving from cytology to HPV co-testing:

**Cytology is subjective, HPV testing is objective**
- The subjectivity of cytology decreases clinical confidence, increasing costs
- Highly variable results between laboratories

*Cytology has low sensitivity in detecting CIN2 or worse*

Wright TC et al. Inter-laboratory Variation in the Performance of Liquid-based Cytology: Insights from the ATHENA trial. 
**Conclusion:** The accuracy of cytology varies significantly even across good labs, whereas HPV testing does not provide significant detection between moderate to severe dysplasia with HPV test - all unanimously.


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**Cytology is Subjective:** Inter-laboratory variability in the ATHENA Trial

<table>
<thead>
<tr>
<th>Test</th>
<th>LAB A (n=12,294)</th>
<th>LAB B (n=4,218)</th>
<th>LAB C (n=16,979)</th>
<th>LAB D (n=12,442)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td>42.0</td>
<td>51.0</td>
<td>60.5</td>
<td>73.0</td>
</tr>
<tr>
<td>HPV testing</td>
<td>90.1</td>
<td>88.2</td>
<td>88.4</td>
<td>88.9</td>
</tr>
</tbody>
</table>

Sensitivity for CIN 2+(%)

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**Cytology has low sensitivity for detecting CIN2 or worse**

Sensitivity of cytology vs. HPV DNA for ≥CIN2

Average increase 35.7% (N=13,842)

Studies performed in developed countries in women 30 years and older.

Annual cytology is NOT better than less often HPV screening b/c it is such a good indicator of present and negative HPV

Results from two rounds of HPV DNA testing versus cytology screening:

94,000 women screened twice 3 years apart

<table>
<thead>
<tr>
<th>When found</th>
<th>HPV arm</th>
<th>Cytology arm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CIN3</td>
<td>Cancer</td>
</tr>
<tr>
<td>Round one</td>
<td>98</td>
<td>7</td>
</tr>
<tr>
<td>Round two</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>In total</td>
<td>106</td>
<td>7</td>
</tr>
</tbody>
</table>

Testing 3 years apart
- more CA px in cytology b/c these pxs were missed earlier
- the HPV arm were found earlier and tx to not develop into CA

HPV testing identified approximately double the number of ≥CIN3 cases compared to cytology at round one and ~40% more overall

Italian women aged 25-60 at recruitment.

The incidence of adenocarcinoma continues to rise despite cytology screening:

SCC and adenocarcinoma incidence rates

SCC incidence is decreasing while ADC incidence is increasing

HPV screen is best for identifying both SCC and adenocarcinoma
**Detection of CIN3, AIS, adenocarcinoma and SCC in the ATHENA Trial**

<table>
<thead>
<tr>
<th>Histology (number)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytology</td>
</tr>
<tr>
<td>CIN3 (254)</td>
<td>52% (132)</td>
</tr>
<tr>
<td>AIS (16)</td>
<td>63% (10)</td>
</tr>
<tr>
<td>Adenocarcinoma and AdenoSq Ca (1)</td>
<td>100% (1)</td>
</tr>
<tr>
<td>Squamous cell carcinoma (3)</td>
<td>100% (3)</td>
</tr>
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</table>

*a 25% difference


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**Trial design: The cobas HPV test was evaluated in the largest US screening trial: ATHENA**

**Baseline phase**
- Enrolled >47,000 women

**Follow-up phase (subset of ~8,000 women)**
- Follow-up Year 1
- Follow-up Year 2
- Follow-up Year 3

**Evaluating screening with cytology vs. cytology plus HPV (co-testing)**
- All women age 21-65 were screened with an HPV test with 16/18 genotyping (the Cobas 3 in 1 HPV test) and a Pap
- Any abnormal Pap (>ASC-US) or positive HPV test was referred to colposcopy
- >1000 women with a negative Pap and negative HPV test were also referred to colposcopy
- Everyone was biopsied: When no lesion was seen a random biopsy was required


ASC-US: atypical squamous cells of undetermined significance

*Biopsy even if there is no cell dysfunction - not even a trained colposcopist can detect*

*COBAS HPV TEST - same as hc2 to detect high risk HPV*
Absolute risk of ≥CIN2 stratified by hrHPV status in the ATHENA normal Pap population


Estimated absolute risk (%)

0 5 10 15 20

Normal Pap 14 hrHPV– 14 hrHPV+ 16+18+other 12 HPV

moderate dysplasia


cobas® HPV16 genotyping results identify a sub-population of women with negative cytology who are at the highest risk of ≥CIN2

ASC-US pap + one of the 14 HPV = SAME RISK of normal Pap with only HPV 16 +

**Comparative absolute risk in the ASC-US population pooled hrHPV+ and normal Pap population HPV16+**

<table>
<thead>
<tr>
<th>HPV16+</th>
<th>14 hrHPV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥21 years</td>
<td>14.0</td>
</tr>
<tr>
<td>≥30 years</td>
<td>13.6</td>
</tr>
</tbody>
</table>

ASC-US Pap⁴ NILM (normal Pap)²

Women with ASC-US who test pooled hrHPV positive have a risk for ≥CIN2 equivalent to that of HPV16 and/or HPV18 positive women with negative cytology.

If HPV 16+ will have 17.6% risk of getting cervical cancer
if HPV 18+ = 13%
- in 10 years

**ASCCP and ACOG 2012 Management Guidelines**

**Management of co-testing results:** women 30-65 years

- Pap & HPV
  - Normal Pap & HPV+
    - Immediate HPV 16/18 genotyping
      - HPV 16/18 neg
        - Cotest 3 years
      - HPV 16/18 +
        - Repeat cotest 12 mo
        - Colposcopy
  - Option 2
    - HPV 16/18 neg
    - HPV 16/18 +


ASC-US, atypical squamous cells of undetermined significance; NILM, negative for intraepithelial lesion or malignancies; * Estimated absolute risk shown for NILM. Note absolute risk measurements are estimates based on raw study data.
US Guidance on Primary HPV Screening
ASCUP & SGO (2015)

• Primary HPV testing can be considered as an alternative to current US cytology-based cervical cancer screening methods for women starting at age 25.

• Women with a negative primary HPV test result should not be retested again for at least three years.

• An HPV test positive for HPV 16 or 18 should be followed with colposcopy.

• A test that is positive for the 12 other high risk types should be followed with cytology testing.

• Clinicians should not use an FDA-approved test without a specific primary hrHPV screening indication.

Huh, W. et al. Gynecol Oncol. 2015; 136: 178-182

Guidelines shouldn’t be blindly followed

Primary Screening Algorithm

high risk (16/18-) cytology in 1 week
hrHPV=high risk HPV
Comparison to ASC-US Triage

<table>
<thead>
<tr>
<th>Strategy</th>
<th>2 CIN3</th>
<th>Relative Sensitivity</th>
<th>Relative Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASC-US Triage</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Co-testing† with 16/18 Genotyping</td>
<td>1.28*</td>
<td>0.99*</td>
<td></td>
</tr>
<tr>
<td>HPV with Genotyping and Reflex Cytology</td>
<td>1.40*</td>
<td>0.99*</td>
<td></td>
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</tbody>
</table>

Data from Medical Devices Advisory Committee Microbiology Panel Meeting: Sponsor Executive Summary

Adjusted results

†Relative to Cytology with Reflex HPV Strategy
*Statistically significant differences compared to Cytology with Reflex HPV Strategy
†Co-testing: Cytology with ASC-US triage in women 25-29. Co-testing with cytology and HPV in women 30+

HPV screen is most NB for pre-malignant screening, not to find cervical cancer

Summary

HPV16 and HPV18 genotyping identifies a subset of hrHPV-positive women with NILM cytology who would benefit from colposcopy†*

- >1 in 10 women who tested positive for HPV16 had ≥CIN3 at baseline despite having negative cytology
- HPV16-positive women are at high and immediate risk of cervical precancer
- HPV18-positive women appear to have lower baseline risk of having high-grade disease, but are at risk of developing precancer in the future²
- A negative HPV is far better predictor of reduced progression to advanced cervical dysplasia and malignancy than cytology

HPV DNA testing with integrated HPV16 and HPV18 genotyping adds medical value to adjunct screening programmes1-3


HPV should be introduced into all cytology screening when possible