“Not Your Grandmother’s Pap Test”

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DISCLOSURES:

• SPEAKER’S BUREAUS:
  – Hologic, Qiagen, Roche, Merck, Glaxo

• CONSULTANTSHIPS:
  – FDA, Shared Medical Resources, ClearPath

• RESEARCH SUPPORT:
  – PATH (Bill and Melinda Gates Foundation)
  – NIH
Human Papillomavirus (HPV) Is a Cause of Cervical Cancer

• HPV is highly prevalent in young women; up to 60% of certain age groups can be positive for HPV.
• HPV alone is a limited predictor of disease.
• Of the 100 HPV types, only “high-risk” types are important.

Natural history of cervical cancer

HPV infection → CIN 1,2 → Avg. 6-24 mo → HPV disappearance → CIN 2,3 → Avg. 6-9 mo → HPV disappearance → CIN 2,3 → Avg. 10 yrs → Invasive CA

**HPV INFECTION: Classification of Histological Findings: CIN**

- **CIN (Cervical Intraepithelial Neoplasia)**
  - CIN 1: Mild dysplasia
  - CIN 2: Moderate dysplasia
  - CIN 3: Severe dysplasia; includes carcinoma in situ (CIS)

<table>
<thead>
<tr>
<th>CIN&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Normal</th>
<th>CIN 1 (condyloma)</th>
<th>CIN 1 (mild dysplasia)</th>
<th>CIN 2 (moderate dysplasia)</th>
<th>CIN 3 (severe dysplasia/CIS)</th>
<th>Invasive Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology of squamous cervical epithelium&lt;sup&gt;1&lt;/sup&gt;</strong></td>
<td>![Histology Image]</td>
<td>![Histology Image]</td>
<td>![Histology Image]</td>
<td>![Histology Image]</td>
<td>![Histology Image]</td>
<td>![Histology Image]</td>
</tr>
</tbody>
</table>

Basal cell ➔
Basal membrane ➔

Cervical Cancer Screening: The Pap

• Since the introduction and widespread use of the Pap Test in the USA:
  – There has been a 70% reduction in cervical cancers

• Half of all Cervical Cancers occur in unscreened or underscreened women:
  – Reaching unscreened women is difficult
  – Using the “best test available” improves prevention

• Pap screening has been linked to an annual well woman exam:
  – Culture is difficult to change!
Cervical screening and abnormal Pap management have been changing. Why?

• Poor sensitivity of cervical cytology has driven annual screening.

• Even with lifetime annual Pap tests the estimated risk of getting cervical cancer is approximately 216/100,000.
  *IARC 1986*

• HPV is present in virtually all cervical cancers, estimated at 99.7%  *J MM Walboomers et al., Journal of Pathology 189:12-19, 1999*

• “Cervical cancer may be the first human cancer identified to have a single necessary cause.”  *IARC 1999*
### Pap Smear Performance

#### Meta-analyses:

<table>
<thead>
<tr>
<th>Author</th>
<th>Biopsy proven CIN 2,3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sens</td>
</tr>
<tr>
<td>Fahey</td>
<td>49%</td>
</tr>
<tr>
<td>Follen-Mitchell</td>
<td>67%</td>
</tr>
<tr>
<td>Nanda</td>
<td>51%</td>
</tr>
</tbody>
</table>


Sensitivity of cervical cytology for CIN 3:

In recent well controlled trials

• Conventional Pap smears sensitivity: 70-80%

• Even liquid-based Pap tests may miss 15% of CIN 3

• This carries important medical implications and has stimulated interest in the development of more sensitive screening tests.

ACOG Practice Bulletin. 2005;61:3
How Far Have We Come and What Does the Future Hold?

• Significant changes in the past 10 years
  – Liquid-based Paps
  – HPV reflex testing for ASC-US

• HPV vaccine
  – First FDA-approved vaccine
  – Milestone advance in cervical cancer prevention

• Cost-effectiveness
  – Best clinical outcomes
  – Underserved in Title X and Planned Parenthood clinics

• Future of cervical screening
  – Pap testing
  – HPV testing
  – Molecular technology
### Relative Risk of Specific HPV Types

<table>
<thead>
<tr>
<th>HPV Type</th>
<th>Cancer</th>
<th>Controls</th>
<th>RR.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 16</td>
<td>53%</td>
<td>3.0%</td>
<td>434</td>
</tr>
<tr>
<td>HPV 18</td>
<td>11%</td>
<td>1.0%</td>
<td>248</td>
</tr>
<tr>
<td>HPV 45</td>
<td>4%</td>
<td>0.5%</td>
<td>197</td>
</tr>
<tr>
<td>HPV 31</td>
<td>3%</td>
<td>0.6%</td>
<td>123</td>
</tr>
<tr>
<td>HPV 52</td>
<td>2%</td>
<td>0.2%</td>
<td>200</td>
</tr>
<tr>
<td>HPV 33</td>
<td>2%</td>
<td>0.2%</td>
<td>373</td>
</tr>
</tbody>
</table>

*Munoz et al. (2003) NEJM*
Kaiser Portland NCI Study 1990-1999:

Sherman ME et al JNCI 2003;95:46-52.
HPV Prevalence and Cervical Cancer
- Incidence by Age $^{1,2}$

## 2012 Cervical Cancer Screening Guidelines

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Screening Recommendations</th>
</tr>
</thead>
</table>
| < 21 Years | - No routine speculum exam or cytology regardless of age of onset of intercourse or other risk factors.  
- STD testing and counseling on safe sex and contraception as needed. |
| 21-29 Years | - Annual pelvic exam  
- Screening with cytology alone every 3 years is recommended |
| 30-65 Years | - Annual pelvic exam AND screening with:  
  - Cytology and HPV testing (“co-testing”) every 5 years (preferred per ASCCP)  
  OR  
  - Cytology alone every 3 years (acceptable) is recommended. |
| > 65 Years | - Discontinue screening after age 65 following adequate prior screening  
Comment: Women with a history of CIN2 or a more severe diagnosis should continue screening for at least 20 years. |

Cotesting: Pap plus HPV
Rationale: Why consider HPV testing in primary cervical screening?

- “..persistent, high-risk HPV infection is necessary for the development of cervical CA

- “..an obvious corollary is that the absence of HPV means that the risk of cervical cancer is negligible.

Wright TC, Schiffman MH NEJM 2003;348:489-90
HPV Testing for Screening

A risk stratifier

• A normal Pap and a negative HPV test give a 99-100% assurance that cervical cancer is not present and will not likely occur in the next few years.

  Allowing less frequent screening.

• A positive HPV test and a normal Pap reflects increased risk for either missed disease or for the subsequent development of CIN 2/3 and cancer.

  Requiring increased surveillance.
CIN3+ Risk Following a Negative Screening Test

Cumulative incidence of CIN3+ (per 10,000)

Time since initial testing (mos.)

Dillner et al., BMJ, 2008
Risk Stratification with HPV Types 16 and 18 in Women ≥ 30 Years of Age with Negative Cytology

In women ≥ 30 years of age, 10-year cumulative incidence of ≥ CIN 3 was 20% and 18% for HPV 16/18, respectively

Follow-up Time (Years)

Cumulative Incidence Rate of ≥ CIN 3 (%)
PRIMARY HPV SCREENING?
Roche cobas HPV FDA (proposed) indications (ATHENA Trial)

- As a primary screening test in women ≥25 years to detect high risk HPV, including genotyping for 16 and 18

- Women testing positive for HPV 16 or 18 should be referred to colposcopy.

- Women testing high risk HPV positive but 16/18 negative should be reflexed to cytology to determine the need for referral to colposcopy

- Women testing negative for high risk HPV should be followed up in accordance with the physician’s assessment of screening and medical history, other risk factors, and professional guidelines.
Roche HPV Primary Submission (ATHENA Trial)

• Algorithm proposed\(^1\)
  – Proposed age cutoff of >25
  – HPV primary compared to cytology only – statistically improved sensitivity
  – HPV primary compared to co-testing – statistically lower sensitivity

• Cancer cases
  – 8 cases of invasive cervical cancer in ATHENA Trial\(^2\)
  – 19 additional cases obtained from HPV Cytology Registry
  – Sensitivity of cobas HPV primary = 88.5% (23/26*)
  – Sensitivity of co-testing = 92.6% (25/27)
  – 2 cases of AIS missed by cobas, but cytology positive
  – Need more data

*1 case invalid and excluded from the analysis

1. FDA Microbiology Devices Panel Meeting March 12, 2014.
Primary HPV Screening?

• FDA approved (Roche Cobas test only)
• Women between ages 25 and 65
• New algorithm of follow-up
• More effective at reducing CIN 3+ than Pap plus HPV, when used at recommended intervals (3 yrs for HPV, 5 yrs for Pap+HPV 1)

Cumulative risk of cervical intraepithelial neoplasia grade 2, grade 3 or cervical cancer among women 30 to 64 years at Kaiser Permanente Northern California
How will HPV primary screening impact false negative rates?
### Kaiser Data

<table>
<thead>
<tr>
<th></th>
<th>All women</th>
<th>No biopsy or &lt;CIN2</th>
<th>CIN2</th>
<th>CIN3/AIS</th>
<th>AIS</th>
<th>Squamous carcinoma</th>
<th>Adenocarcinoma</th>
<th>Total cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>331,818</td>
<td>329,508</td>
<td>14,766</td>
<td>747</td>
<td>7</td>
<td>49 (100%)</td>
<td>27 (100%)</td>
<td>87 (100%)</td>
</tr>
<tr>
<td>Baseline HPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>315,061</td>
<td>314,589</td>
<td>322 (22%)</td>
<td>123 (16%)</td>
<td>14 (20%)</td>
<td>18 (37%)</td>
<td>6 (22%)</td>
<td>27 (31%)</td>
</tr>
<tr>
<td>Positive</td>
<td>16,757 (5.1%)</td>
<td>14,919 (4.5%)</td>
<td>1,154 (7.8%)</td>
<td>624 (8.4%)</td>
<td>56 (8.0%)</td>
<td>31 (53%)</td>
<td>21 (78%)</td>
<td>60 (69%)</td>
</tr>
<tr>
<td>Baseline Pap</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pap negative</td>
<td>319,177 (96.2%)</td>
<td>318,093 (96.5%)</td>
<td>687 (47%)</td>
<td>354 (47%)</td>
<td>42 (60%)</td>
<td>15 (31%)</td>
<td>23 (85%)</td>
<td>43 (49%)</td>
</tr>
<tr>
<td>Total Pap positive</td>
<td>12,641 (3.8%)</td>
<td>11,415 (3.5%)</td>
<td>789 (53%)</td>
<td>393 (53%)</td>
<td>28 (40%)</td>
<td>34 (69%)</td>
<td>4 (15%)</td>
<td>44 (51%)</td>
</tr>
<tr>
<td>ASC-US</td>
<td>8,517 (2.6%)</td>
<td>8,106 (2.5%)</td>
<td>283 (19%)</td>
<td>123 (16%)</td>
<td>12 (17%)</td>
<td>4 (8%)</td>
<td>1 (4%)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>LSIL</td>
<td>2,527 (0.76%)</td>
<td>2,208 (0.67%)</td>
<td>253 (17%)</td>
<td>61 (8%)</td>
<td>1 (1%)</td>
<td>4 (8%)</td>
<td>0 (0%)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>AGUS/NOS</td>
<td>764 (0.23%)</td>
<td>705 (0.21%)</td>
<td>26 (2%)</td>
<td>27 (4%)</td>
<td>7 (10%)</td>
<td>1 (2%)</td>
<td>2 (7%)</td>
<td>6 (7%)</td>
</tr>
<tr>
<td>ASC-H/HSIL/SCC</td>
<td>633 (0.25%)</td>
<td>396 (0.12%)</td>
<td>227 (15%)</td>
<td>182 (24%)</td>
<td>8 (11%)</td>
<td>25 (31%)</td>
<td>1 (4%)</td>
<td>32 (32%)</td>
</tr>
<tr>
<td>Baseline HPV/Pap</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV negative/Pap negative</td>
<td>306,969 (92.5%)</td>
<td>306,597 (93.0%)</td>
<td>258 (17%)</td>
<td>96 (13%)</td>
<td>11 (16%)</td>
<td>10 (20%)</td>
<td>6 (22%)</td>
<td>18 (21%)</td>
</tr>
<tr>
<td>HPV positive/Pap negative</td>
<td>12,208 (3.7%)</td>
<td>11,496 (3.5%)</td>
<td>429 (29%)</td>
<td>258 (35%)</td>
<td>31 (44%)</td>
<td>5 (10%)</td>
<td>17 (63%)</td>
<td>25 (29%)</td>
</tr>
<tr>
<td>HPV positive/Pap positive</td>
<td>4,549 (1.4%)</td>
<td>3,423 (1.0%)</td>
<td>725 (49%)</td>
<td>366 (49%)</td>
<td>26 (36%)</td>
<td>7 (16%)</td>
<td>4 (15%)</td>
<td>35 (40%)</td>
</tr>
</tbody>
</table>

Total cancers includes squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, and cervical cancer of unknown histology. CIN3/AIS includes 13 histologies that were either CIN3 or AIS, but precisely which is unknown. Pap positive is ASC-US or worse cytology. CIN2=cervical intraepithelial neoplasia grade 2. CIN3/AIS=cervical intraepithelial neoplasia grade 3 or adenocarcinoma in situ. HPV=human papillomavirus test. ASC-US=atypical squamous cells of undetermined significance. LSIL=low-grade squamous intraepithelial lesion. AGUS/NOS=atypical glandular cells of undetermined significance or not otherwise specified. ASC-H=atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion. HSIL=high-grade squamous intraepithelial lesion. SCC=squamous cell carcinoma.

Table 1: Distribution of worst histological diagnosis by enrolment HPV test and Pap smear

## ATHENA Data

<table>
<thead>
<tr>
<th></th>
<th>LBC+</th>
<th>HPV+</th>
<th>LBC- and HPV-</th>
<th>LBC- and HPV+</th>
<th>LBC+ and HPV-</th>
<th>LBC+ and HPV+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No colposcopy</strong> (n=32 834)</td>
<td>375  (1%)</td>
<td>665  (2%)</td>
<td>31 937 (97%)</td>
<td>522 (2%)*</td>
<td>232 (1%)</td>
<td>143 (&lt;1%)</td>
</tr>
<tr>
<td><strong>Out of time window</strong> (n=60)†</td>
<td>18   (30%)</td>
<td>27   (45%)</td>
<td>23  (38%)</td>
<td>19 (32%)</td>
<td>10  (17%)</td>
<td>8  (13%)</td>
</tr>
<tr>
<td><strong>Colposcopy or no biopsy</strong> (n=184)‡</td>
<td>38   (21%)</td>
<td>81   (44%)</td>
<td>83   (45%)</td>
<td>63 (34%)*</td>
<td>20  (11%)</td>
<td>18  (10%)</td>
</tr>
<tr>
<td><strong>Negative biopsy</strong> (n=6802)</td>
<td>1704 (25%)</td>
<td>2756 (41%)</td>
<td>2922 (43%)</td>
<td>2176 (32%)*</td>
<td>1124 (17%)</td>
<td>580 (9%)</td>
</tr>
<tr>
<td><strong>CIN1</strong> (n=590)</td>
<td>260 (44%)</td>
<td>366 (62%)</td>
<td>124 (21%)</td>
<td>206 (35%)*</td>
<td>100 (17%)</td>
<td>160 (27%)</td>
</tr>
<tr>
<td><strong>CIN2</strong> (n=157)</td>
<td>76  (48%)</td>
<td>128 (82%)</td>
<td>20  (13%)</td>
<td>61 (39%)*</td>
<td>9  (6%)</td>
<td>67 (43%)</td>
</tr>
<tr>
<td><strong>CIN3</strong> (n=254)</td>
<td>132 (52%)</td>
<td>234 (92%)</td>
<td>9  (4%)</td>
<td>113 (44%)*</td>
<td>11 (4%)</td>
<td>121 (48%)</td>
</tr>
<tr>
<td><strong>Adenocarcinoma in situ</strong> (n=16)</td>
<td>10 (63%)</td>
<td>14 (88%)</td>
<td>0</td>
<td>6 (38%)</td>
<td>2 (13%)</td>
<td>8 (50%)</td>
</tr>
<tr>
<td><strong>Squamous cell carcinoma</strong> (n=3)</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (100%)</td>
</tr>
<tr>
<td><strong>ADC or ASC</strong> (n=1)</td>
<td>1  (100%)</td>
<td>1 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (100%)</td>
</tr>
<tr>
<td><strong>Total</strong> (n=40 901)</td>
<td>2617 (6%)</td>
<td>4275 (10%)</td>
<td>35 118 (86%)</td>
<td>3166 (8%)</td>
<td>1508 (4%)</td>
<td>1109 (3%)</td>
</tr>
</tbody>
</table>

LBC=liquid-based cytology. + = positive. HPV=human papillomavirus. - = negative. CIN1, CIN2, or CIN3 = cervical intraepithelial neoplasia grade 1, 2, or 3. ADC=adenocarcinoma. ASC=adenosquamous carcinoma. *p<0.05 for LBC vs HPV with McNemar’s χ² test. †Colposcopy visit happened after at least 12 months. ‡Includes inadequate biopsy for diagnosis and no biopsy taken for various reasons. §Includes 2609 women selected for visit 2, minus 6 who had invalid cobas HPV test results, plus 14 who exited the study because of an error in randomisation.

**Table 2:** Diagnosis of biopsy by central pathology review panel, stratified by liquid-based cytology and HPV test results

L1-based HPV Primary Screening for Cervical Cancer: What’s the risk?
HPV Detection Strategies

**APTIMA® HPV**¹

- HPV Viral mRNA
- APTIMA HPV Probes

**Cervista® HPV**²

- HPV Viral DNA
- Cervista HPV Probes

**cobas® HPV**³

- HPV Viral DNA
- Roche HPV Primers

**Hybrid Capture 2®⁴**

- HPV Viral DNA
- hc2 Probes

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1. APTIMA HPV Assay package insert #502170 Rev A 2011
2. Cervista HPV HR package insert #15-3053 2010
3. cobas 4800 HPV Test US Package Insert #05641268001-01EN Rev 1.0 2011
4. Hybrid Capture 2 High-Risk HPV DNA Test® package insert #L00665 Rev. 2 2007
HPV DNA levels decrease as disease progresses toward cancer. How will this impact detection of the most severe disease cases?
HPV DNA Detection

Percentage of integration increases from CIN to invasive

- Benign HPV lesions and CIN 1: NO integrated or episomal HPV DNA
- HPV 16 cancers: up to 55 - 80%
- HPV 18 cancers: up to 83%
- HPV 45 cancers: 92 - 100%\(^1\)

What collateral benefits do we forfeit by abandoning the pap in screening strategies?
Collateral Benefits of Cytology

Ability to Identify non-HPV Related Malignancies with Pap

- Endometrial
- Ovarian
- Fallopian Tube

HPV Primary: Are we there yet?

• HPV primary vs. co-testing vs. cytology alone
  – Do we have enough data?

• To date, there is only one test indicated for HPV primary
  – Is an L1 DNA based test sufficient?

• There is not yet guidance regarding what will follow an HPV+ primary test (ie: PAP, colpo, repeat testing)
  – How do we adapt to changing guidelines?

• Co-testing intervals are supported by the data
  – What will the intervals be with primary screening?

• The pap has proven benefits
  – Which malignancies would be missed by eliminating the pap? (endometrial & non HPV pre cancers)
Projected number of cervical cancer cases in developed countries,

Plummer M, Franceschi S Vir Research 2002;89:285-93
Questions?