Cervical Cancer Screening in the Era of Prophylactic HPV Vaccination

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Even in the Era of HPV Vaccines:

- Cervical carcinogenesis is the same process as before.
- Vaccines are prophylactic, not therapeutic.
Incidence and Mortality

Incidence of Cervix uteri cancer: ASR (World) (All ages)

<table>
<thead>
<tr>
<th>Incident cases in 2008</th>
<th>Worldwide</th>
<th>530,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deaths in 2008</td>
<td>Worldwide</td>
<td>275,000</td>
</tr>
</tbody>
</table>

Future Prospects

- If nothing further is done to prevent cervical cancer, there will be one million women to develop the disease annually by 2050.
- The poorest parts of the world will be the worst affected.
- The translation of scientific knowledge into effective control measures is absolutely mandatory.

Peter Boyle, Director of IARC: EUROGIN 2004, Nice
Measures for Global Control of Cervical Cancer

**Primary Prevention:**
- Change of Sexual Habits
- Vaccination against HPV

**Secondary Prevention:**
- Health Education
- Improved Diagnosis
- More Effective Therapy
- Down-Staging

**Screening of Precursors:**
- PAP Smear
- Colposcopy
- Cervicography
- Polar Probe
- Visual Inspection (VIA, VILI)

**HPV Testing:**
- PCR
- Hybrid Capture II
- Amplicor HPV Test
- RNA Proofer
IMPACT OF ORGANISED SCREENING

- Declining trends in incidence and mortality in the developed countries are attributable to implementation of organized screening programs based on Pap smear.

- The best examples are the Nordic Countries, where up to 80% reduction in cervical cancer incidence has achieved since the early 1960's (e.g. Finland).


Cervical Cancer in Finland

- A rapid increase in incidence since 1992 by 25-30%

- Particularly among younger (25-39-y-o) women
ORGANISED SCREENING: PRIVILEGE OF RARE COUNTRIES

- Organized screening programs exist in few countries only, and the prospects for effective Pap smear screening in the majority of developing countries seem gloomy, if not entirely pessimistic, in the foreseeable future.

- The necessity to develop optional diagnostic tools for cervical cancer screening particularly in the low-resource settings is widely recognized.


COMPARING OPTIONAL SCREENING TOOLS

Direct one-to-one transfer of a screening program that works well in one country to a completely different setting in another country is not a realistic mode of action.

The only viable option to establish a cost-effective screening program in a low-resource setting is through extensive comparison of the optional screening tools under field conditions.


Prospects of cervical cancer prevention by prophylactic HPV vaccines
### Prospects of cervical cancer prevention by prophylactic HPV vaccines

<table>
<thead>
<tr>
<th>Stage of Development</th>
<th>Time (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective vaccines available</td>
<td>0</td>
</tr>
<tr>
<td>Phase I and II trials</td>
<td>1</td>
</tr>
<tr>
<td>Phase III trials</td>
<td>2-3</td>
</tr>
<tr>
<td>Short-term effects: (Condyloma)</td>
<td>5-6</td>
</tr>
<tr>
<td>Middle to long-term effects: (CIN2+)</td>
<td>15-25</td>
</tr>
<tr>
<td>Global implementation</td>
<td>30-40</td>
</tr>
<tr>
<td>Global incidence of CC declining</td>
<td>50-60</td>
</tr>
</tbody>
</table>
Human Papillomavirus (HPV)

- At least 120 known types
- Usually infect either skin or mucosal squamous cell epithelia
- Within these groups:
  - Low risk types: benign lesions only
  - High risk types: associated with cancers and their precursor lesions

What are prophylactic HPV vaccines?
Structure of Papillomavirus

HPV vaccines are sub-unit vaccines made of virus like particles: VLPs

Making VLPs: molecular “cut and paste”

“cut” the L1 gene from the virus DNA
“paste” into the DNA of another microbe such as yeast or baculovirus

grow the recombinant microbe in large amounts
   - as it grows it makes the L1 protein

The chemistry of this protein is such that it self assembles into a virus like particle
   - an empty protein shell without DNA

The VLP is morphologically and immunologically identical to the HPV virus particle
- Quadrivalent HPV (Types 6, 11, 16, 18) L1 virus-like particle (VLP) vaccine
- VLPs manufactured in *Saccharomyces cerevisiae*
- Aluminum adjuvant 225 µg per dose
- 0.5 mL injection volume
Bivalent HPV (Types 16, 18) L1 virus-like particle (VLP) vaccine
- VLPs manufactured in *Baculovirus*
- AsO4 adjuvant 500 µg per dose
- 0.5 mL injection volume
How do HPV vaccines work?
Immune Response Evoked by HPV Vaccines: Implicated Mechanism$^{1-4}$

How to measure vaccine efficacy?
Measures of vaccine effect:

- Prophylactic Vaccine Efficacy (VE)
- Population Impact (PI)
For the evaluation of Prophylactic Vaccine Efficacy (VE), women in the vaccine and placebo groups must NOT have previous exposure to vaccine HPV-types.
**Prophylactic Vaccine Efficacy (VE)**

**EXAMPLE in PPE / GHN / RMITT2 population**

<table>
<thead>
<tr>
<th></th>
<th>Prevalent (Old) Disease</th>
<th>Incident (New) Disease</th>
<th>Total Cases</th>
<th>Prophylactic Vaccine Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine Group (N = 100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>$1 - \left[\frac{(0/100)}{(20/100)}\right] \times 100$</td>
</tr>
<tr>
<td>Placebo Group (N = 100)</td>
<td>0</td>
<td>20</td>
<td>20</td>
<td>$100%$</td>
</tr>
</tbody>
</table>

Prophylactic Efficacy

$= (1 - \text{Incidence}_{\text{Placebo}} / \text{Incidence}_{\text{Vaccine group}}) \times 100\%$

PPE, per protocol; GHN, generally HPV naïve (RMITT2)
Population Impact (PI) is dependent on the level of previous exposure and the mix of new vaccine- and non-vaccine HPV-type exposure.
Population Impact (PI)

**Example in ITT / MITT3 / FAS population**

<table>
<thead>
<tr>
<th></th>
<th>Prevalent (Old) Disease</th>
<th>Incident (New) Disease</th>
<th>Total Cases</th>
<th>Prophylactic Vaccine Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine Group (N = 100)</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>$1- \frac{[(30/100)/\frac{(50/100)}]}{*100}$</td>
</tr>
<tr>
<td>Placebo Group (N = 100)</td>
<td>30</td>
<td>20</td>
<td>50</td>
<td>$= 40%$</td>
</tr>
</tbody>
</table>

**Population Impact = Observed Disease Reduction**

$$= 1 - \left( \frac{\text{Incidence due to (New + Previous Exposure)}_{\text{Placebo}}}{\text{Incidence due to (New + Previous Exposure)}_{\text{Vaccine group}}} \right) \times 100\%$$
Population Impact (PI)

More Previous Exposure = Less Population Impact Observed
Less Previous Exposure = More Population Impact Observed

Less Disease due to Vaccine HPV types = Less Population Impact Observed
More Disease due to Vaccine HPV types = More Population Impact Observed

Population Impact = Observed Disease Reduction
= 1 – (Incidence due to (New + Previous Exposure)_{Placebo} / Incidence due to (New + Previous Exposure)_{Vaccine group}) \times 100\%
Efficacy of Cervarix® and Gardasil® cannot be directly compared

<table>
<thead>
<tr>
<th>REGION</th>
<th>PATRICIA Study % Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>34</td>
</tr>
<tr>
<td>North America</td>
<td>16</td>
</tr>
<tr>
<td>Europe</td>
<td>35</td>
</tr>
<tr>
<td>Latin America</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>REGION</th>
<th>FUTURE II Study % Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>3</td>
</tr>
<tr>
<td>North America</td>
<td>25</td>
</tr>
<tr>
<td>Europe</td>
<td>44</td>
</tr>
<tr>
<td>Latin America</td>
<td>27</td>
</tr>
</tbody>
</table>

IMPORTANT

"Differences among the efficacy trials of the quadrivalent and bivalent vaccines in terms of choice of placebo recipients or control subjects, immunological assays and populations analyzed preclude direct comparison of results for the 2 vaccines."

WHO Weekly Epidemiological Record 8415, April 2009
http://www.who.int/wer
HPV eradication: Do we need to vaccinate everybody?
Basic Reproductive Rate ($R_0$):

$$R_0 = \beta \times k \times D$$

$\beta$ = the risk of transmission per contact (attack rate);

$k$ = average number of contacts per person per time unit (month, year);

$D$ = the average duration of infection
Basic Formula for Protection by Vaccination:

\[ p > \frac{R_0 - 1}{R_0} = 1 - \frac{1}{R_0} \]

\( p \) = the proportion of the population that must be vaccinated

### Example: data from the NIS Cohort:

18-year-old: *HPV+ 60.3%

<table>
<thead>
<tr>
<th>$\beta$</th>
<th>$k/(y)$</th>
<th>$D(y)$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>1</td>
<td>1.1</td>
<td>-908.0%</td>
</tr>
<tr>
<td>0.01</td>
<td>2</td>
<td>1.1</td>
<td>-3.54%</td>
</tr>
<tr>
<td>0.1</td>
<td>3</td>
<td>1.1</td>
<td>-2.0%</td>
</tr>
<tr>
<td>0.45</td>
<td>4</td>
<td>1.1</td>
<td>50.0%</td>
</tr>
<tr>
<td>*0.6</td>
<td>5</td>
<td>1.1</td>
<td>70.0%</td>
</tr>
<tr>
<td>0.6</td>
<td>2</td>
<td>1.1</td>
<td>24.2%</td>
</tr>
<tr>
<td>0.6</td>
<td>1</td>
<td>1.1</td>
<td>-0.51%</td>
</tr>
<tr>
<td>0.8</td>
<td>6</td>
<td>1.1</td>
<td>81.0%</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>1.1</td>
<td>91.0%</td>
</tr>
</tbody>
</table>
How to monitor vaccine efficacy?

- HPV testing (HR & LR)
- HPV genotyping (VT, nVT)
- Screening by Pap/LBC
- Colposcopy, biopsy, etc.

VT, vaccine HPV-types; nVT, non-vaccine HPV-types
In the Era of HPV Vaccination:

*Cervical Cancer Screening by Cytology is Still Mandatory*
Both current and new generation HPV vaccines are prophylactic, not therapeutic

- HPV16/18 vaccine coverage reaches 70% of cervical carcinomas

- With cross-protection against HPV31,33 & 45, coverage increases up to 80% (max)
Why is cytology screening needed?

With the 2nd generation 9-valent vaccines, coverage might increase up to 90%, but never to 100%

- Possibility of HPV-type replacement (not confirmed yet)
- Duration of vaccine protection not known (follow-up too short as yet)
Why is cytology screening needed?

Women who have been vaccinated by current HPV vaccines or 2nd generation HPV vaccines need to be monitored for vaccine efficacy by:

- HPV testing
- HPV genotyping

Women testing HR-HPV-positive need to be monitored for true disease by:

- Pap test/LBC and colposcopy
Compared with HPV assays, Pap/LBC is less sensitive but more specific test.

Both tests are compromised by transient HPV infections (test+/equivocal smears) among young women.

Both tests perform better among older women, i.e., SE/SP balance (by AUC) is better.
Pap test performance in screening setting

Should a screening test be different in young and older women?
Test performance depends on age

Syrjänen, K., et al. Value of conventional Pap smear, Liquid Based Cytology, visual inspection (VIA) and Human papillomavirus (HPV) testing as optional screening tools among Latin American women below and above 35 years of age. Experience from the LAMS Study. Acta Cytol. 2008;52:641-653
Both Pap and HC2 perform remarkably better among the older women.

Of the single tests, the best performance is obtained for HC2 among the older women.

If only the PPV is considered, there is no test superior to the conventional Pap test in both young and older women.

In older women, the best balance in SE/SP is obtained when HC2 is combined with the Pap test.

Syrjänen, K., et al. Value of conventional Pap smear, Liquid Based Cytology, visual inspection (VIA) and Human papillomavirus (HPV) testing as optional screening tools among Latin American women below and above 35 years of age. Experience from the LAMS Study. Acta Cytol. 2008;52:641-653
In younger women, such a combination does not give any added value to HC2 as the stand-alone test (AUC=0.728 and 0.721, respectively), and

 Adds very little to the conventional Pap test (AUC=0.662 for HSIL and AUC=0.684 for LSIL).

The choice of optimal screening test for young and older women depends on whether the highest PPV (Pap test) or the best SE/SP balance (HC2) is used as the selection criteria.

Syrjänen, K., et al. Value of conventional Pap smear, Liquid Based Cytology, visual inspection (VIA) and Human papillomavirus (HPV) testing as optional screening tools among Latin American women below and above 35 years of age. Experience from the LAMS Study. Acta Cytol. 2008;52:641-653
According to Cancer Epidemiology Textbooks*, an optimal screening test should be the one with the highest PPV, because it would detect only true positives, without wasting resources in detection of false positives, which necessitates the use of other tests…

What will happen to Pap/LBC performance in populations with high vaccine uptake?

- CC and its precursor lesions become more rare
- PPV of Pap test will deteriorate (depends on Prev)
- Performance of Pap test will degenerate (decrease in signal-to-noise ratio)
- Adversely affects the subjective interpretation of Pap smear

What will happen to Pap test performance in populations with high vaccine uptake?

- Lower prevalence of clinically significant lesions will result in:
  - **Loss of sensitivity** (decreased familiarity of recognizing true abnormalities)
  - And potentially:
    - **Loss of specificity** (a fear of missing a significant disease could lead to overcalls of benign abnormalities)

**Pap test performance:**

**ASC-US+ cut-off, prevalence 10%**

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
<th>Tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>600</td>
<td>180</td>
<td>780</td>
</tr>
<tr>
<td>-</td>
<td>400</td>
<td>8820</td>
<td>9220</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>9000</td>
<td>10000</td>
</tr>
</tbody>
</table>

SE 60.0%; SP 98.0%; PPV 76.9%; NPV 95.7%; AUC= 0.790

**ASC-US+ cut-off, prevalence 5%**

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
<th>Tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>250</td>
<td>190</td>
<td>440</td>
</tr>
<tr>
<td>-</td>
<td>250</td>
<td>9310</td>
<td>9560</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>9500</td>
<td>10000</td>
</tr>
</tbody>
</table>

SE 50.0%; SP 98.0%; PPV 56.8%; NPV 97.4%; AUC= 0.740
Pap test performance:

ASC-US+ cut-off, prevalence 2.5%:

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
<th>Tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>100</td>
<td>975</td>
<td>1075</td>
</tr>
<tr>
<td>-</td>
<td>150</td>
<td>8775</td>
<td>8925</td>
</tr>
<tr>
<td>250</td>
<td>9750</td>
<td>10000</td>
<td></td>
</tr>
</tbody>
</table>

SE 40.0%; SP 90.0%; PPV 9.3%; NPV 98.3%; AUC = 0.650

ASC-US+ cut-off, prevalence 1%:

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
<th>Tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>40</td>
<td>1980</td>
<td>2020</td>
</tr>
<tr>
<td>-</td>
<td>60</td>
<td>7920</td>
<td>7980</td>
</tr>
<tr>
<td>100</td>
<td>9900</td>
<td>10000</td>
<td></td>
</tr>
</tbody>
</table>

SE 40.0%; SP 80.0%; PPV 1.9%; NPV 99.2%; AUC = 0.600
Pap test performance:

Impact of changes in SE and SP due to declining CIN1+ on PPV of Pap test

Franco E & Cuzick J. Vaccine 2008;26S:16-23
What will happen to Pap test performance in populations with high vaccine uptake?

- **Loss of PPV:**
  - Inevitable (PPV depends on disease prevalence)

- **Loss of sensitivity:**
  - Not inevitable (human performance)

- **Loss of specificity:**
  - Not inevitable (human performance)
What are the potential counteractive measures?

Loss of PPV:
- None (affects equally also the HPV tests)

Loss of sensitivity:
- Improve human performance

Loss of specificity:
- Improve human performance

How to improve human performance?

**Centralization:**
- Does not affect PPV itself
- Possibility of viewing enough cases to:
  - Offset loss of sensitivity
  - Offset loss of specificity

**Cytology automation:**
- Possibly offsets impaired Se & Sp?

- Intensify training and quality control to:
  - Offset loss of sensitivity
  - Offset loss of specificity
Laboratory prevalence?

In the era of HPV vaccination:

- it is essential to keep the prevalence of true abnormalities (ASC-US+ or LSIL+) IN YOUR LABORATORY at the same level as before to:
  - avoid the adverse impact of decreasing prevalence on PPV, and the consequent
    - Loss of sensitivity
  - Loss of specificity
True prevalence vs. laboratory prevalence?

- Current level of vaccine uptake (40-50%) does not affect CIN prevalence for several years!

- Calculate your current performance and ASC-US+ (LSIL+) prevalence at baseline

- Keep monitoring ASC-US+ (LSIL+) prevalence on regular basis (with histology control for CIN1+)

- When prevalence starts declining, compensate it on daily basis

- Add adequate number of verified (ASC-US+/LSIL+) cases among daily routine samples
Laboratory performance of Pap test:

**ASC-US+ cut-off, true prevalence 2.5%**

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
<th>Tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>125</td>
<td>1950</td>
<td>2075</td>
</tr>
<tr>
<td>-</td>
<td>125</td>
<td>7800</td>
<td>7925</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>9750</td>
<td>10000</td>
</tr>
</tbody>
</table>

SE 50.0%; SP 80.0%; PPV 6.0%; NPV 98.4%; AUC= 0.650

**ASC-US+ cut-off, laboratory prevalence 10%**

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
<th>Tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>600</td>
<td>900</td>
<td>1500</td>
</tr>
<tr>
<td>-</td>
<td>400</td>
<td>8100</td>
<td>8500</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>9000</td>
<td>10000</td>
</tr>
</tbody>
</table>

SE 60.0%; SP 90.0%; PPV 40.0%; NPV 95.3%; AUC= 0.750
What should be the optimal screening strategy?

### Test Performance

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP Conventional</td>
<td>52.0 (49.4-54.6)</td>
<td>98.9 (98.2-99.6)</td>
<td>85.7 (83.9-87.5)</td>
<td>94.5 (93.3-95.7)</td>
</tr>
<tr>
<td>AutocytePREP</td>
<td>25.0 (11.1-54.5)</td>
<td>100 (100-100)</td>
<td>100 (100-100)</td>
<td>83.3 (63.1-100)</td>
</tr>
<tr>
<td>DNACitoliq</td>
<td>40.0 (15.2-64.8)</td>
<td>96.4 (92.9-99.8)</td>
<td>60.0 (29.6-90.4)</td>
<td>92.2 (87.4-97.1)</td>
</tr>
<tr>
<td>Screening Colpo</td>
<td>90.7 (84.2-97.3)</td>
<td>53.4 (49.8-57.1)</td>
<td>17.2 (13.5-20.9)</td>
<td>98.2 (96.9-99.5)</td>
</tr>
<tr>
<td>VIA (abnormal)</td>
<td>47.7 (40.2-55.1)</td>
<td>60.9 (58.3-63.5)</td>
<td>13.4 (10.7-16.1)</td>
<td>90.2 (88.3-92.2)</td>
</tr>
<tr>
<td>VILI (abnormal)</td>
<td>53.8 (38.2-69.5)</td>
<td>12.1 (7.9-16.3)</td>
<td>9.1 (5.4-12.8)</td>
<td>61.7 (47.8-75.6)</td>
</tr>
<tr>
<td>VIA (suggests ca)</td>
<td>10.5 (5.9-15.0)</td>
<td>99.7 (99.4-100)</td>
<td>81.8 (65.7-97.9)</td>
<td>89.8 (88.2-91.3)</td>
</tr>
<tr>
<td>VILI (suggests ca)</td>
<td>7.8 (0.6-16.1)</td>
<td>99.6 (98.7-100)</td>
<td>75.0 (32.6-100)</td>
<td>86.9 (82.9-90.9)</td>
</tr>
<tr>
<td>HCII conventional</td>
<td>90.4 (88.2-92.6)</td>
<td>69.0 (65.6-72.4)</td>
<td>31.6 (28.1-35.1)</td>
<td>97.9 (96.4-99.2)</td>
</tr>
<tr>
<td>HCII self-sample</td>
<td>100 (100-100)</td>
<td>66.7 (47.8-85.5)</td>
<td>27.3 (9.5-53.6)</td>
<td>100 (100-100)</td>
</tr>
</tbody>
</table>

**CIN2+ Cut-Off:**

RECOMMENDATION:
HPV Testing Followed by Pap Smear

Women who have sex with HPV-infected men
(within weeks to months some will develop)

HR-HPV infection
(within months some will develop)

Persistent HR-HPV infection
(within months to years some will develop)

HG cervical lesions
(within years some will develop)

Cervical cancer

Pap Cytology
Detected with low sensitivity

HPV Testing
Detected with high sensitivity

Detected with moderate sensitivity

Detected with high sensitivity

Perceived as cause of low specificity

Franco E & Cuzick J. Vaccine 2008;26S:16-23
### Combined use of PAP and HC2 in detecting CIN2+ lesions among women below and above 35 years

<table>
<thead>
<tr>
<th>Sub-cohort/HC2-Pap cut-off*</th>
<th>Sensitivity-% (95%CI)</th>
<th>Specificity-% (95%CI)</th>
<th>Positive Predictive Value-% (95%CI)</th>
<th>Negative Predictive Value-% (95%CI)</th>
<th>Area under ROC curve (95%CI)</th>
<th>Significance p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women &lt;35 yrs:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncorrected (HSIL)</td>
<td>83.0 (70.2-91.9)</td>
<td>61.2 (55.9-66.3)</td>
<td>24.3 (18.3-31.2)</td>
<td>96.0 (92.5-98.2)</td>
<td>0.721 (0.664-0.778)</td>
<td>p=0.0001</td>
</tr>
<tr>
<td><strong>Corrected for Verification Bias</strong></td>
<td>65.9 (58.7-72.8)</td>
<td>79.8 (77.9-81.5)</td>
<td>24.3 (18.3-31.2)</td>
<td>96.0 (92.5-98.2)</td>
<td>0.728 (0.692-0.764)</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Uncorrected (LSIL)</td>
<td>83.0 (70.2-91.9)</td>
<td>59.2 (53.9-64.4)</td>
<td>23.4 (17.6-30.1)</td>
<td>95.9 (92.3-98.1)</td>
<td>0.711 (0.654-0.768)</td>
<td>p=0.0003</td>
</tr>
<tr>
<td><strong>Corrected for Verification Bias</strong></td>
<td>65.2 (57.8-71.9)</td>
<td>79.0 (77.1-81.0)</td>
<td>23.4 (17.6-30.1)</td>
<td>95.9 (92.3-98.1)</td>
<td>0.721 (0.685-0.757)</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Women &gt;35 yrs:</td>
<td></td>
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<tr>
<td>Uncorrected (HSIL)</td>
<td>97.8 (88.5-99.9)</td>
<td>74.1 (68.3-79.4)</td>
<td>40.0 (31.0-49.9)</td>
<td>99.5 (97.1-100.0)</td>
<td>0.860 (0.826-0.894)</td>
<td>p=0.0001</td>
</tr>
<tr>
<td><strong>Corrected for Verification Bias</strong></td>
<td>92.5 (88.4-96.1)</td>
<td>91.2 (90.1-92.3)</td>
<td>40.0 (31.0-49.9)</td>
<td>99.5 (97.1-100.0)</td>
<td>0.919 (0.898-0.940)</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Uncorrected (LSIL)</td>
<td>97.6 (87.4-99.9)</td>
<td>71.0 (65.1-76.5)</td>
<td>35.3 (26.7-44.8)</td>
<td>99.5 (97.0-100.0)</td>
<td>0.843 (0.807-0.880)</td>
<td>p=0.0003</td>
</tr>
<tr>
<td><strong>Corrected for Verification Bias</strong></td>
<td>91.8 (87.5-95.7)</td>
<td>90.4 (89.3-91.6)</td>
<td>35.3 (26.7-44.8)</td>
<td>99.5 (97.0-100.0)</td>
<td>0.911 (0.889-0.933)</td>
<td>p=0.0001</td>
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</tbody>
</table>

*HC2 cut-off=1pg/ml RLU/CO; HSIL, Pap smear cut-off HSIL; LSIL, Pap smear cut-off LSIL; **Corrected for verification bias according to Reichenheim et al (ref 36); 1) 95%CI derived by bootstrapping (1,000 simulations); 2) Difference between AUC (area under ROC curve) for HSIL cut-off; 3) Difference between AUC for LSIL cut-off; 4) Difference between AUC for HSIL (corrected) cut-off; 5) Difference between AUC for LSIL (corrected) cut-off.


Combined Pap Smear and HPV test: What do we get?

**Compared with Pap stand-alone (LSIL):**

*Gain in sensitivity:*
- 45.3% to 83.0% (younger)
- 72.8% to 97.6% (older)

*Drop in specificity:*
- 91.4% to 59.2% (younger)
- 92.6% to 71.0% (older)

*Drop in PPV:*
- 34.8% to 23.4% (younger)
- 64.4% to 35.3% (older)
Compared with HC2 stand-alone (1µg/ml):

- Slight gain in sensitivity:
  - 81.5% to 83.0% (younger)
  - 95.7% to 97.6% (older)

- Slight drop in specificity:
  - 62.3% to 59.2% (younger)
  - 74.5% to 71.0% (older)

- Slight drop in PPV:
  - 24.9% to 23.4% (younger)
  - 40.0% to 35.3% (older)
HPV test followed by Pap: performance indicators

Combined NIS-LAMS cohort: n=15,301; HR-HPV+ n=1,852; CIN2+ lesions n=194; LSIL cut-off

<table>
<thead>
<tr>
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<tr>
<td>+</td>
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<tr>
<td>-</td>
<td>69</td>
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<td>430</td>
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<tr>
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<td>194</td>
<td>476</td>
<td>670</td>
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</tbody>
</table>

SE 64.4%; SP 75.8%; PPV 52.1%; NPV 84.0%; AUC= 0.701

**HPV test followed by Pap: performance indicators**

**Combined NIS-LAMS cohort:** n=15,301; HR-HPV+ n=1,852; CIN2+ lesions n=194; HSIL cut-off

<table>
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<tr>
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<td>115</td>
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<tr>
<td></td>
<td>194</td>
<td>476</td>
<td>670</td>
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</table>

SE 40.7%; SP 97.1%; PPV 84.9%; NPV 80.1%; AUC= 0.689

### HPV test followed by Pap: performance indicators

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<tr>
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<td>175</td>
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<td>1867</td>
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<tr>
<td></td>
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<td>2020</td>
</tr>
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</table>

SE 42.1%; SP 98.5%; PPV 83.0%; NPV 90.6%; AUC = 0.703

**Combined NIS-LAMS cohort:** n=15,301; ALL; CIN2+ lesions n=302; HSIL cut-off

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HPV test followed by Pap: performance indicators

Combined NIS-LAMS cohort: n=15,301; HR-HPV+ n=1,852; CIN3+ lesions n=119; HSIL cut-off

<table>
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<tr>
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<tr>
<td></td>
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<td>551</td>
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</tbody>
</table>

SE 52.1%; SP 94.4%; PPV 66.7%; NPV 90.1%; AUC= 0.732

HPV test followed by Pap: performance indicators

Combined NIS-LAMS cohort: n=15,301; ALL; CIN3+ lesions n=192; HSIL cut-off

<table>
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<tr>
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<td>192</td>
<td>1828</td>
<td>2020</td>
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</table>

SE 53.6%; SP 97.3%; PPV 67.3%; NPV 95.2%; AUC = 0.755

HPV test followed by Pap: Longitudinal predictive values

Combined NIS-LAMS cohort: n=15,301; HR-HPV+ n=1,105; Incident CIN/SIL lesions n=164 (*Baseline Pap available from 140)

<table>
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<tr>
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<tbody>
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<td>72</td>
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</tr>
<tr>
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<td>793</td>
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<tr>
<td>140*</td>
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<td></td>
<td>943</td>
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</tbody>
</table>

LSE 55.7%; LSP 91.0%; LPPV 52.0%; LNPV 92.2%; AUC= 0.734

Screening interval be extended?

Which algorithm for vaccinated women and at which age?

Different algorithms for vaccinated and non-vaccinated?

Different screening intervals for vaccinated and non-vaccinated?

Different strategies for young and older women?

Frequency of HPV testing for HR-HPV+/Pap-women?

How soon after negative HR-HPV test be returned to regular screening?

The value of adjunct tests: E6/E7 mRNA, HPV genotype, p16 and other biomarkers?
Conclusions:

We will definitely need:

🌟 Modified screening strategies, based on
  🌟 Pap test (LBC)
  🌟 HPV testing / genotyping
  🌟 Something else??

🌟 Counteractive measures to offset the deterioration of Pap test performance