NEWER DEVELOPMENTS IN CYTOLOGY

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Metropolis Healthcare Ltd

2nd September 2012
Introductory Comments & Historical perspectives

“CERVICAL NEOPLASIA”
George Papanicolaou

- Arrived in New York – 1913
- Smears on Guinea pigs: Nasal speculum & Q-tips
- Late 1920: Extended the technique to human patients
  - Started with hormonal cytology & was extended to the pathological state
- Monastic ferocity: 1928-1950
  - Joined by Herbert Traut
- Hashime Murayama – painted the cells & using camera lucida
(cont)

• 1952: National Cancer Institute launched the largest clinical trial of secondary prevention in the history of cancer using his smearing technique.
First Screening Program

• 1950’s
• British Columbia, CA
• Introduced first organized screening program.
• Province-wide
• Incidence of cervical cancer had dropped 30% by 1960 and by 85% by the 1980’s
Dr. May Edward Chinn (1896-1980)

“Lady, have you been “Paptized”?

- New York Amsterdam News, on Pap smears, 1957
Birthplace of the Cervical Smear!

Coltea Hospital, Bucharest
AUREL BABEŞ | GEORGE PAPANICOLAOU

• Babeş (1886-1962)
• Papanicolaou (1883-1962)
• Rediscovery and translation 30 years ago by L. E. Douglass
• "BAB- PAP " TEST
Fringe Benefits of Vaginal Cytology

- Vaginal Cytology holds an unchallenged place among the all-too-few techniques for detecting carcinoma early enough to achieve a 100% cure
- Chiefly for screening, but allows an opportunity to comment on other findings
- All findings that are recorded allow for better patient management
Please submit!!

- Patient’s history/Age
- Clinical findings
- LMP
- Systemic or Topical hormones & additional information that aids in preparing a comprehensive report.
3 Steps to accurate Cyto-diagnosis

STEP 1:
• Time and effort in preparing a good smear sample
• **WHAT IS NOT ON THE SMEAR CANNOT BE DIAGNOSED!!**
• Spreading and uniformity
3 Steps to accurate Cyto-diagnosis

STEP 2:

• Screener

• **THERE IS NO SUBSTITUTE FOR CAREFUL SCREENING**

• An atypical cell not seen or misinterpreted is possibly the only chance of avoiding the development of a potentially fatal disease

• **NO DEFINITE WHITES & BLACKS**
3 Steps to accurate Cyto-diagnosis

STEP 3:

• *POSITIVE OR ATYPICAL SMEAR SHOULD BE FOLLOWED UP BY AN ADEQUATE CONFIRMATORY TEST*

• Good liaison & dialogue between the clinician & cytology department

• Follow up

• *CYTO-HPV- HISTO- CORRELATION*
Conventional Pap smear
The greatest challenge in cervical screening has been the tedious task of finding a needle in a haystack, where majority of haystacks have no needle!
The Goal of Pap Testing

THE GOAL OF PAP TESTING IS TO IDENTIFY PRECANCEROUS CELL CHANGES **BEFORE** THEY PROGRESS TO CANCER
Thin Prep Consumables
Thin Prep
Conventional Pap Smear
- Majority of cells not captured
- Non-representative transfer of cells
- Clumping and overlapping cells
- Obscuring material

ThinPrep Pap Test
- Virtually all of the sample is collected
- Randomized, representative transfer of cells
- Even distribution of cells
- Minimizing obscuring material
Specimen Reporting

• The BETHESDA System
  – A format for reporting cervicovaginal cytologic diagnoses that provides uniform terminology from laboratory to laboratory

• 3 Basic Elements
  – Specimen Adequacy
  – General Categorization
  – Interpretation/result
Specimen Adequacy

• Satisfactory for Evaluation

• Unsatisfactory for Evaluation...
  – insufficient squamous cells (5000 liquid based)
  – 75% obscured by blood, inflammation, etc.
  – Cells not adequately preserved
Mirror Image

• Over diagnosis versus under diagnosis

• Under diagnosis falsely reassures patients of their freedom from disease
MANAGEMENT OF WOMEN WITH ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE [ASC-US]

Repeat Cytology @ 6 & 12 mos

Both Tests Negative

> ASC (on either result)

Routine Screening

Colposcopy
Endocervical sampling preferred in women with no lesions, and those with unsatisfactory colposcopy

Approx. 55-60%

HPV DNA Testing*
Preferred if liquid-based cytology or co-collection available

HPV Negative
Repeat Cytology @ 12 mos

HPV Positive*
(Managed in same manner as women with LSIL)
LARGE, RETROSPECTIVE STUDY CONFIRMS SUPERIORITY OF THINPREP® PAP TEST

Two-period, retrospective analysis of approximately 2 million specimens showed with the ThinPrep Pap Test:

- Improvement was statistically significant after correcting for selection bias
- Significant decrease in false-negative proportion
- 233% improvement in the HSIL detection in high-risk cases
- Decrease in ASC-US to SIL ratio

SIL = squamous intraepithelial lesions.
MAJOR ADVANCES IN CERVICAL SCREENING

1941
Pap Smear
26,000 deaths

1996
ThinPrep® Pap Test
4,900 deaths

2003
ThinPrep® Imaging System
3,919 deaths

2009
Cervista™ HPV HR & 16/18 Tests

1999
Hybrid Capture® 2 HPV Test
4,204 deaths

YEAR
Advance
Deaths from cervical cancer

1941
Pap Smear
26,000 deaths

1996
ThinPrep® Pap Test
4,900 deaths

2003
ThinPrep® Imaging System
3,919 deaths

2009
Cervista™ HPV HR & 16/18 Tests

1999
Hybrid Capture® 2 HPV Test
4,204 deaths
HeLa cells in his lab tested positive for HPV-18 strain (1984)
Harald zur Hausen
GENETIC LEGACY OF THE VIRUS

• “Immortalized” cancer cells
• Relatively long time frame from acquisition of infection until development of target cervical cancer
• Infection often occurs between ages of 15 & 22
• Cervical cancer is most common between 40 & 45 years
• Virus is not the whole story / Co-factors
• THE VIRUS, THE HOST & THE STEM CELL
“I believe that these chronic diseases demand a persistent involvement from the scientific side”
Microscopy remains the cornerstone

Any new technique must provide information of prognostic or therapeutic significance beyond that provided by the current gold standard.

- Juan Rosai
Era of the molecular pap test

- Most critical is the interplay among sensitivity, specificity and disease prevalence
- Starting screening programs from scratch
Human papillomavirus (HPV)
Conventional cytology

• Grand synthesis of thousands of genes acting in concert and sometimes in opposition
• Practical stand point : VERY SOBERING
• Cancer genome atlas project USD 1.5 billion
• Lawrence Loeb : It is a wonderful study but there is no core of genes associated with a particular cancer.
Both Cytology and Molecular Tests are Possible from the ThinPrep® Vial

*ThinPrep is the only liquid-based Pap FDA-approved for use with molecular diagnostics tests*
Marriage between Morphology with Molecular Diagnosis

• DIAGNOSIS
• PROGNOSIS
• PREDICTION OF THERAPEUTIC RESPONSE
HPV Testing

- Early work: Southern Blot
- Hybrid Capture 2 (hc2)
  - Cervista
- PCR Messenger RNA
  - E6/E7 genes
- ISH
- Detects 14 HPV high risk types
  - 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- NucliSENS Easy q HPV
- Aptima
- Norchip
Human Papillomavirus (HPV)

Corden et al., J Clin Pathol: Mol Pathol 1999
Doorbar et al., Clinical Science 2006
Morris, Clin Chem Lab Med 2005
Pett and Coleman, J Pathol 2007
October 2007 issue of the American Journal of Obstetrics & Gynecology

- Specific guidelines governing what to do when a woman is found to have HPV were published.
- They were developed by 146 experts representing 29 organizations and professional societies.
- HPV testing is the preferred method of follow-up evaluation for women age 20-30 with inconclusive ("ASC-US") Pap results.
- If testing shows they do not have HPV, a repeat Pap is recommended in 12 months. If HPV is present, a colposcopy exam should be done.
- Routine HPV testing, along with the Pap, is beneficial for women 30 and older.

Centers for Disease Control and Prevention (CDC) in April 2007

- HPV vaccine be given to girls and young women age 11-26
- Ideally, the vaccine should be given before a girl becomes sexually active, and thus exposed to HPV. If given afterwards, the vaccine will only protect her against the HPV type(s) to which she has not yet been exposed.
- In any case, even after vaccination, screening for cervical cancer risk (see the next point) is still needed, since the protection offered by the HPV vaccine is incomplete.
- HPV testing along with the Pap for women age 30 and over.
New Cervical Cancer Screening Recommendations from the U.S. Preventive Services Task Force and the American Cancer Society/American Society for Colposcopy and Cervical Pathology/American Society for Clinical Pathology

March 15, 2012, issue of Annals of Internal Medicine

- What is new about these guidelines?

  1st time that USPSTF has recommended the combined use of cervical cytology and high-risk human papillomavirus (HPV) DNA testing (“co-testing”). The previous USPSTF guidelines had indicated that evidence was insufficient to make a recommendation regarding the use of co-testing. USPSTF now recommends that women age 30–65 years should be screened by either cytology every 3 years or co-testing every 5 years.

  HPV can take more than a decade to progress to cervical dysplasia or cancer

  In contrast, ACS/ASCCP/ASCP finds that co-testing every 5 years is preferred to cytology alone but that cytology alone every 3 years is an acceptable strategy. In choosing to make co-testing the preferred strategy, ACS/ASCCP/ASCP focused on evidence from multiple randomized trials showing that co-testing has improved performance compared with cytology alone.

  Specifically, co-testing has increased sensitivity for detecting cervical intraepithelial neoplasia grade 3 or greater (CIN3+), and women who have undergone co-testing have a lower risk of CIN3+ and invasive cancer after the first screening round.

  Because of this improved performance, co-testing can be used for screening at less frequent intervals than cytology alone. In addition, co-testing offers greater risk reduction than cytology alone for adenocarcinoma of the cervix and its precursors.
• Most surprising of these recommendations is that women under age 21 should not be tested. But it makes sense -- many sexually active women under 21 will develop a human papillomavirus infection, or HPV, which can lead to pre-cancerous lesions.

• And when doctors see those lesions on a Pap test, they want to treat them. Yet nearly all of those lesions will disappear on their own without residual effects. And those that do not are easily treated years later.

• Treating them as soon as they're spotted can lead to cervical incompetence and miscarriage down the road.

• Women who have a normal Pap result and a positive HPV test result should repeat both tests or receive a gene test called genotyping that determines if they have HPV 16 and 18. These types of HPVs are known to cause 70% of cervical cancers. There is no immediate need for a colposcopy.

• Mildly abnormal Pap result (called ASC-US) and a negative HPV test result should follow up with either HPV testing plus a Pap test, or HPV testing alone, at intervals of three years or longer.

• Women who have been vaccinated against HPV should begin cervical cancer screening at the same age as unvaccinated women.
### Distribution of Conventional & LBC Pap smear cases

<table>
<thead>
<tr>
<th>Year</th>
<th>Conventional pap</th>
<th>LBC pap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year 2009</td>
<td>3071</td>
<td>111</td>
</tr>
<tr>
<td>Year 2010</td>
<td>2739</td>
<td>801</td>
</tr>
<tr>
<td>Year 2011</td>
<td>3068</td>
<td>1203</td>
</tr>
<tr>
<td>Year 2012</td>
<td>1092</td>
<td>569</td>
</tr>
</tbody>
</table>
## Data summary of Cervical screening 2010 – Till date

<table>
<thead>
<tr>
<th></th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of pap</td>
<td>3553</td>
<td>4411</td>
<td>1718</td>
</tr>
<tr>
<td>Conventional pap</td>
<td>2739</td>
<td>3068</td>
<td>1092</td>
</tr>
<tr>
<td>% positive Conventional pap</td>
<td>2.3%</td>
<td>0.6%</td>
<td>0.6%</td>
</tr>
<tr>
<td>LBC</td>
<td>814</td>
<td>1343</td>
<td>626</td>
</tr>
<tr>
<td>% positive LBC</td>
<td>1.6%</td>
<td>1%</td>
<td>0.3%</td>
</tr>
<tr>
<td>HPV screening</td>
<td>43</td>
<td>899</td>
<td>415</td>
</tr>
<tr>
<td>% of HPV DNA detected</td>
<td>11.60%</td>
<td>11.01%</td>
<td>11.57%</td>
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</tbody>
</table>
% Distribution of abnormal cytology 2009-till date

<table>
<thead>
<tr>
<th></th>
<th>ASCUS</th>
<th>LSIL</th>
<th>HSIL</th>
<th>CA</th>
<th>AGUS</th>
<th>ASC-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>0.24%</td>
<td>0.60%</td>
<td>0.30%</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0</td>
</tr>
<tr>
<td>2010</td>
<td>0.80%</td>
<td>0.60%</td>
<td>0.50%</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0</td>
</tr>
<tr>
<td>2011</td>
<td>0.25%</td>
<td>0.1</td>
<td>0.20%</td>
<td>0</td>
<td>0.02%</td>
<td>0.05%</td>
</tr>
<tr>
<td>2012</td>
<td>0.60%</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0</td>
<td>0.20%</td>
</tr>
<tr>
<td>Turkey</td>
<td>1.9</td>
<td>0.5</td>
<td>0.1</td>
<td>0</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>USA</td>
<td>3.9</td>
<td>2.1</td>
<td>0.5</td>
<td>NA</td>
<td>0.2</td>
<td>NA</td>
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</table>
% of distribution of abnormal Conventional pap

<table>
<thead>
<tr>
<th></th>
<th>ASCUS</th>
<th>LSIL</th>
<th>HSIL</th>
<th>Carcinoma</th>
<th>AGUS</th>
<th>ASC-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>24.2%</td>
<td>42.4%</td>
<td>21.2%</td>
<td>6.1%</td>
<td>6.1%</td>
<td>0.0%</td>
</tr>
<tr>
<td>2010</td>
<td>41.3%</td>
<td>20.6%</td>
<td>27.0%</td>
<td>4.8%</td>
<td>6.3%</td>
<td>0.0%</td>
</tr>
<tr>
<td>2011</td>
<td>40.0%</td>
<td>30.0%</td>
<td>20.0%</td>
<td>0.0%</td>
<td>5.0%</td>
<td>5.0%</td>
</tr>
<tr>
<td>2012</td>
<td>14.3%</td>
<td>14.3%</td>
<td>14.3%</td>
<td>14.3%</td>
<td>0.0%</td>
<td>42.9%</td>
</tr>
<tr>
<td>Turkey(2006-10)</td>
<td>68.3%</td>
<td>16.33%</td>
<td>3.8%</td>
<td>0.3%</td>
<td>8.1%</td>
<td>3%</td>
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</table>

% of distribution of abnormal LBC cases

<table>
<thead>
<tr>
<th></th>
<th>ASCUS</th>
<th>LSIL</th>
<th>HSIL</th>
<th>Carcinoma</th>
<th>AGUS</th>
<th>ASC-H</th>
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<tbody>
<tr>
<td>2009</td>
<td>0</td>
<td>77.8%</td>
<td>22.2%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>2010</td>
<td>23.1%</td>
<td>77.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>2011</td>
<td>7.1%</td>
<td>64.3%</td>
<td>21.4%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>7.1%</td>
</tr>
<tr>
<td>2012</td>
<td>0.0%</td>
<td>50.0%</td>
<td>50.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>China(2006-08)</td>
<td>38.3%</td>
<td>43.4%</td>
<td>13.1%</td>
<td>2.1%</td>
<td>NA</td>
<td>3.2%</td>
</tr>
</tbody>
</table>
HPV Screening (PCR & Cervical cytology)

<table>
<thead>
<tr>
<th>Year</th>
<th>Total</th>
<th>PAP (NILM)</th>
<th>PAP(Abn)</th>
<th>HPV Detected</th>
</tr>
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<tbody>
<tr>
<td>2010</td>
<td>43</td>
<td>42</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2011</td>
<td>66</td>
<td>64</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>2012</td>
<td>57</td>
<td>55</td>
<td>2</td>
<td>3</td>
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HPV screening

% of case distribution

- PAP (NILM)
- PAP(Abn)
- HPV Detected
### HIGH RISK HPV (2011)

<table>
<thead>
<tr>
<th>TYPES</th>
<th>CASES DETECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>42</td>
</tr>
<tr>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>45</td>
<td>8</td>
</tr>
<tr>
<td>51</td>
<td>4</td>
</tr>
<tr>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>39</td>
<td>1</td>
</tr>
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<td>31</td>
<td>0</td>
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<td>52</td>
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<td>56</td>
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<td>59</td>
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<td>68</td>
<td>0</td>
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<tr>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
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### LOW RISK HPV (2011)

<table>
<thead>
<tr>
<th>TYPES</th>
<th>CASES DETECTED</th>
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<tbody>
<tr>
<td>6</td>
<td>4</td>
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<tr>
<td>11</td>
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<tr>
<td>40</td>
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<td>72</td>
<td>0</td>
</tr>
<tr>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>81</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
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</table>
### HIGH RISK HPV(2012)

<table>
<thead>
<tr>
<th>TYPES</th>
<th>CASES DETECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
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<td>35</td>
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<td>0</td>
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<tr>
<td>68</td>
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<tr>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>43</strong></td>
</tr>
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### LOW RISK HPV(2012)

<table>
<thead>
<tr>
<th>TYPES</th>
<th>CASES DETECTED</th>
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</thead>
<tbody>
<tr>
<td>6</td>
<td>3</td>
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<tr>
<td>11</td>
<td>2</td>
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<tr>
<td>40</td>
<td>0</td>
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<tr>
<td>42</td>
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<td>0</td>
</tr>
<tr>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>81</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>
CISH (Chromogenic in situ hybridization)

- Chromogen in situ hybridization (CISH) offers a real advantage by providing a cytomorphologic link in assessing HPV Positivity which is seen as either in or not in, the abnormal cells.

- It also provides additional information as to whether the virus is integrated or not in the host genome (discrete nuclear dots imply that virus is in the chromosomal DNA & now able to effect the genetic aberration almost always a marker of a high risk HPV).

- It is even possible to detect and localize single or very few HPV copies within infected nuclei.

- It can be tested real time.
Comparison between methods used for test validation

HPV IHC Genotype includes – 1, 6, 11, 16-16, 18 & 31
HPV CISH Genotype – 16, 18, 31, 33, 35, 45, 51, 82.
HPV DNA by PCR – **High Risk** : 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 69. **Low risk**: 6, 11, 40, 42, 43, 44, 53, 54, 61, 72, 73 and 81

<table>
<thead>
<tr>
<th>Parameter / test</th>
<th>CISH</th>
<th>PCR</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>97 %</td>
<td>*98%</td>
<td>40%</td>
</tr>
<tr>
<td>Specificity</td>
<td>86 – 89 %</td>
<td>44 – 95 %³</td>
<td>Variable according to genotype included.</td>
</tr>
</tbody>
</table>

The PPV and NPV of PCR are 41% and 97.5% respectively.

The PPV and NPV of CISH are 52% and 99% respectively.
INTERESTING CASES

CASE 1

ML G 2398/10
CASE 1: Clinical details

- 61 yr, female
- Colposcopy - Abnormal
- Thin Prep Pap smear
CASE 1: Cytology Diagnosis

HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESION
Significance of HSIL

- HSIL indicates moderate or severe dysplasia.

- Chance of a Pap smear showing HGSIL and of there being an invasive cancer of the cervix already present is less than 1%.

- In fact only about 1.5% of women with a HGSIL Pap smear will progress to having invasive cancer of the cervix within the next 24 months.

- The next step is to have a colposcopically directed biopsy of the cervix to see whether the changes on the cervix are the same as the Pap, worse than the Pap indicates (carcinoma in situ, or invasive cancer) or less severe (mild dysplasia, inflammation) than the Pap indicated.

- The biopsy result is the "gold standard"
CASE 1: Final diagnosis

CERVICAL INTRAEPITHELIAL NEOPLASIA, CIN III
CASE - 2

ML G – 2123/12
CASE 2: Final Diagnosis

LSIL

REFLEX TESTING DONE

HPV Genotype -16
CASE - 3

ML G - 797/12 & ML – 2935/12
ML G – 797/12 (Convention pap)
ML G – 797/12 (Convention pap)
CASE 3: Cytology Diagnosis

ASC-H
ML – 2935/12
CASE 3: Final Diagnosis

Cervical Intraepithelial Neoplasia Grade III (CIN-III)
CASE – 3(b)

ML G- 1285/12 & ML – 4633/12
ML G – 1285/12 Conventional Pap
Case 3(b): Final Diagnosis

HPV 16 DETECTED
HISTO CYTO NEGATIVE
Rationale For Co-testing

• Co-testing increases prevalently detected CIN3+ (in comparison to cytology) in the initial screening round and results in decreased CIN3+ in subsequent rounds.

• Co-testing significantly reduced the invasive cancer rate in the second screening round in one RCT.

• A negative co-test has a high negative predictive value for CIN3+ and cancer in subsequent 5 to 6 years.

• Screening at short intervals leads to unnecessary procedures and potentially harmful treatment of lesions destined to clear without intervention.
Management of Women with HPV-Positive, Cytology - Negative Co-tests

- “Women cotesting HPV positive, cytology negative should be followed with either (as noted in the interim ASCCP guidelines): 
  - Option 1, repeat cotesting in 12 months or 
  - Option 2, immediate HPV genotype-specific testing for HPV16 alone or for HPV16/18.
  - If cotesting is repeated at 12 months, women testing positive on either test (HPV positive or LSIL or more severe cytology) should be referred to colposcopy; women testing negative on both tests (HPV-negative and ASCUS or negative cytology) should return to routine screening.

  - If immediate HPV genotype-specific testing is used, women testing positive for HPV16 or HPV16/18 should be referred directly to colposcopy; women testing negative for HPV16 or HPV16/18 should be cotested in 12 months, with management of results as described in option 1.

- Women cotesting HPV positive, cytology negative should not be referred directly to colposcopy. Furthermore, they should not be tested for individual HPV genotypes other than HPV16 and HPV18.
CASE - 4

ML G - 922/12
LBC, Vault smear
LBC, Vault smear
CASE 4: Final Diagnosis

LSIL
Current Concepts in Cervical Pathology

Kay J. Park, MD and Robert A. Soslow, MD

SPECIAL SECTION—MEMORIAL SLOAN-KETTERING CANCER CENTER SURGICAL PATHOLOGY COURSE

- **HPV** infects cells in the cervical transformation zone, area of active cell turnover. Basal cells, which feature the HPV receptor, are a natural target for infection.

- The most common **diagnostic challenges** occur in distinguishing LSIL from benign reactive atypia, CIN 2 from CIN 3, and CIN 3 from atrophy & immature metaplasia (Do not have the mitotic activity, nuclear pleomorphism, loss of cell polarity, nuclear hyperchromasia, or clumped chromatin of HSIL).

- The distinction between CIN 2 and CIN 3 is not as crucial, because both lesions are in the high-grade category.

- Studies have shown that the subjective criteria used for separating the different grades and the fact that the thickness of the immature basaloid cells can vary within the same lesion lead to failures in reproducible diagnosis of CIN 2.
Ancillary Studies

- **p16INK4a** is a tumor suppressor gene that encodes a protein involved in cell cycle regulation. Diffuse, strong, cytoplasmic and/or nuclear staining in squamous and glandular lesions associated with high-risk HPV infection.
- Interpreting p16 immunostaining is complicated.

- **Mib – 1** : HSIL (CIN 2 and CIN 3) usually shows diffuse nuclear positivity scattered throughout all layers of the epithelium.

- **ProEx C** is a recently developed IHC assay that targets the expression of topoisomerase II-α and minichromosome maintenance protein-2.
- The assay is a nuclear stain that is positive in cervical dysplasia and has been validated in cytologic specimens for the detection of HSIL. Is comparable to p16 and Mib-1 in the detection of high-grade lesions in formalin-fixed tissue sections and in distinguishing them from benign mimics.

- **In situ hybridization (ISH)** is a direct signal detection assay that allows visualization of HPV DNA within infected cells. Episomal forms result in blocklike nuclear labeling, whereas integrated forms result in punctate, nuclear signals. Punctate signals are most frequently found in HSILs and invasive carcinomas.
I.H.C

- HPV
- P(16)p16\textsuperscript{ink4A} CINtec
- Ki-67 p53, p63
- 3p deletions, 3q amplification, cyclin E
- L1
- Minichromosome maintainence protein 2
- Topoisomerase II\textalpha
- ProExC
HPV CISH Signal Patterns

Diffuse Signals
(Episomal HPV)

Punctuated Signals
(Integrated HPV)
HPV CISH Signal Patterns

Diffuse Signals (Episomal HPV)

Punctuated Signals (Integrated HPV)
Screening Paradigm Has Changed Dramatically in the Past 12 Years

- Liquid-based cytology now accounts for ~90% of Pap testing in the United States
  - Nearly 90% of healthcare providers performing Paps use the ThinPrep® Pap Test

- Dual-review with the ThinPrep Imaging System is quickly becoming common lab practice
  - Over 70% of ThinPrep Pap Tests are now imaged

- Molecular testing from the ThinPrep vial is widely used to gather more information
  - HPV testing is recommended in specific populations to further assess risk
  - CT/NG screening is recommended for at-risk women

Data on File, Hologic.
References


* From the Department of Pathology, The Methodist Hospital, Houston, Texas
Sample Collection Video
THANK YOU