Squamous Precursor Lesions and Malignancies In Pap Test

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Conflict of Interest

- Dr. Mody has nothing to report

Cytology Report Format

- Specimen Adequacy
- General Categorization (optional)
- Interpretation/Result
- Educational notes and suggestions

For This lecture...

- LSIL: Patterns and Mimicks
- HSIL: Patterns and Mimicks
- Squamous Carcinomas: Types and Mimicks
- ASC-US and ASC-H
- Reporting rates
- Cytology-Histology correlations

PAP Classes (1954)

Class I, absence of atypical or abnormal cells
Class II, atypical cytology but no evidence of malignancy
Class III, suggestive of but not conclusive for malignancy
Class IV, strongly suggestive of malignancy
Class V, conclusive for malignancy

The Bethesda System and Other Classifications

<table>
<thead>
<tr>
<th>Bethesda</th>
<th>CIN</th>
<th>Dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low grade SIL</td>
<td>CINI</td>
<td>Mild</td>
</tr>
<tr>
<td>High Grade SIL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSIL</td>
<td>CIN II</td>
<td>Moderate</td>
</tr>
<tr>
<td>HSIL</td>
<td>CIN III</td>
<td>Severe</td>
</tr>
<tr>
<td>HSIL</td>
<td>CIN III</td>
<td>CIS</td>
</tr>
<tr>
<td>Sq CA</td>
<td>Microinv</td>
<td>Microinv</td>
</tr>
<tr>
<td>Sq Ca</td>
<td>Sq Ca</td>
<td>Sq Ca</td>
</tr>
</tbody>
</table>
Epithelial Cell Abnormalities

**Squamous Cell**
- Atypical Squamous cells
  - of undetermined significance (ASC-US)
  - Cannot exclude High grade SIL (ASC-H)

**Low Grade Squamous Intraepithelial Lesion**
- HPV and CIN I

**High Grade Squamous Intraepithelial Lesion**
- CIN II, CIN III, CIS, Susp for invasion

**Squamous Cell carcinoma**

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**LSIL Criteria**
- Changes limited to “Mature cells”
- Nuclear enlargement >3X nl int nucleus
- Variable hyperchromasia, (exception in liquid based) nu size, number, shape
- Slight nuclear membrane irregularity
- Koilocytosis
- Must have nuclear abnormalities to qualify
- Note differences in liquid based

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**LSIL with HPV**
HSIL Criteria

- Small less mature cells affected
- Single, sheets or syncytial-like aggregates
- Nuclear hyperchromasia, irregularity, variation in size and shape, occasional prominent folds
- Nucleoli generally absent except gland extension
- Cytoplasm may be immature/lacy, dense or rarely densely keratinized

Patterns of HSIL

- Dispersed
- Syncytial
- Endocervical Gland Involvement
- Hypochromatic
- Stripped nuclei
- Keratinizing
- Repair – like/stromal cells like
Atrophic Vaginitis and Bare Nuclei in Imager stained slides
Atypical Squamous Cells

ASC refers to cytologic changes suggestive of SIL, which are qualitatively and quantitatively insufficient for a definitive interpretation. 3 essential features

Squamous differentiation
Increased N:C ratio
Minimal hyperchromasia, clumping, irregularity, smudging or multinucleation

Note: Applies to entire specimen not individual cells

Atypical Squamous Cells- of Undetermined Significance (ASC-US)

Mature Cell type (superficial or intermediate)
Nuclei 2.5-3X the area of normal intermediate cell nucleus
Slightly increased N:C ratio
Minimal nuclear hyperchromasia, irregularity in chromatin distribution or shape
Nu abnormality with dense orangeophilic cytoplasm (atypical parakeratosis)

Note: Applies to entire specimen not individual cells
PAP interpretations preceding HSIL Biopsies

- ASCUS 39%
- HSIL 31%
- LSIL 20%
- AGUS 10%

Atypical Squamous Cells, Cannot exclude HSIL(ASC-H)

Immature Cell types
Single cells or small fragments of <10 cells
Small cells with high N:C ratios (Atypical immature metaplasia)
Metaplastic cells with nu 1.5-2.5 X normal N:C ratio closer to HSIL but other nuclear abnormalities fall short
In liquid based, cells small and 2-3X neutrophil nuclei

HPV Reporting Rates

<table>
<thead>
<tr>
<th>Category</th>
<th>5th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>90th</th>
<th>95th</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASC-US</td>
<td>4</td>
<td>26</td>
<td>37</td>
<td>48</td>
<td>53</td>
<td>62.2</td>
</tr>
<tr>
<td>ASC-H</td>
<td>0</td>
<td>1.8</td>
<td>50</td>
<td>71</td>
<td>89</td>
<td>98.7</td>
</tr>
<tr>
<td>&gt;30scrs</td>
<td>0</td>
<td>1.9</td>
<td>4</td>
<td>11</td>
<td>25</td>
<td>26</td>
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</table>


Atypical Squamous Cells, Cannot exclude HSIL(ASC-H)

Immature Cell types
Single cells or small fragments of <10 cells
Small cells with high N:C ratios (Atypical immature metaplasia)
Metaplastic cells with nu 1.5-2.5 X normal N:C ratio closer to HSIL but other nuclear abnormalities fall short
In liquid based, cells small and 2-3X neutrophil nuclei

ASC-H with HR HPV and Histologic Follow-up (%)

<table>
<thead>
<tr>
<th>Age</th>
<th>HR-HPV+</th>
<th>HR-HPV-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CIN2 +</td>
<td>CIN1 +</td>
</tr>
<tr>
<td>20-29</td>
<td>33.9</td>
<td>31.4</td>
</tr>
<tr>
<td>30-39</td>
<td>46.3</td>
<td>26.9</td>
</tr>
<tr>
<td>40-49</td>
<td>24.2</td>
<td>30.3</td>
</tr>
<tr>
<td>50-59</td>
<td>25</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>32.7</td>
<td>29.2</td>
</tr>
</tbody>
</table>

*Statistically significant

Arch Pathol Lab Med 132:1874-1881 2008
Squamous Cell Carcinoma

- Non Keratinizing and Keratinizing types
- Features and diathesis vary by preparation type
- Cellularity also variable
- Diathesis usually subtle in liquid based

Keratinizing Squamous Cell Carcinoma

- Isolated cells or in aggregates
- Variable size, shape, tadpoles, spindles
- Variation in nuclear size, shape, hyperchromasia, granularity
- Macronucleoli uncommon
- Diathesis less than in non keratinizing types, clinging diathesis in liquid based

Non Keratinizing Squamous Cell Carcinoma

- Syncytia with ill defined cell borders
- Features of HSIL but cells usually smaller
- Variation in nuclear size, shape, hyperchromasia, granularity
- Macronucleoli and basophilic cytoplasm in large cell variant
- Diathesis more obvious, clinging diathesis in liquid based

Keratinizing Squamous Cell Carcinoma

- Beware of the Bloody Unsat!
- Dilute/Lyse and reprep the case!
Squamous Cell Carcinoma Pitfalls

Cytology Histology Correlations
- In the US, mandated for HSIL and carcinomas (CLIA88)
- Good QA practice
- Good patient care
- Different ways and timelines for doing the correlations
- Varies by institutions and practice settings

Cytology Histology Correlations
- Probes study by CAP of 22,439 correlations in 348 labs
- 94.3% of US labs
- 2.3% Canada, 3.4% Australia, Belgium, UK and others

Participant Practices Regarding Cytology Histology Correlations
Correlations performed and documented at
- At time of biopsy signout 60.7%
- After biopsy is reported 22.4%
- Both of above 17%
- Correlation documented in Bx report 42%
- Discrepancy routinely documented in Bx report 66%


Etiologies for Non-Correlating Cervical Cytologies and Biopsies

What is considered a positive correlation?
- Cytology and tissue diagnosis match or are within one grade of each other
- Reasons for non correlation
  - Tissue(colpo sampling)
  - Tissue Interpretive
  - Cytology Sampling
  - Cytology Screening
  - Cytology Interpretive
  - Other/technical

Cervical Biopsy–Cytology Correlation, a CAP Q probes study of 22439 correlations in 348 labs

<table>
<thead>
<tr>
<th>Biopsy Dx</th>
<th>Neg</th>
<th>ASC/G</th>
<th>I</th>
<th>II+</th>
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<tbody>
<tr>
<td>NILM</td>
<td>67</td>
<td>37</td>
<td>21</td>
<td>5</td>
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<tr>
<td>LSIL</td>
<td>26</td>
<td>44</td>
<td>60</td>
<td>7</td>
</tr>
<tr>
<td>HSIL+</td>
<td>7</td>
<td>14</td>
<td>18</td>
<td>88</td>
</tr>
</tbody>
</table>

Factors Influencing Accuracy of Colposcopy Guided Biopsy

- Severity of Referral Pap
- Patient age and menopausal status
- Visibility of Squamo columnar junction
- Lesion Size
- Endocervical extension
- Training and experience of colposcopist
- Type of clinician

Number of colposcopically Directed Biopsies and Outcomes (ALTS)

<table>
<thead>
<tr>
<th>Bx result</th>
<th>one Bx</th>
<th>2Bxs</th>
<th>3 or &gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN3+</td>
<td>52%</td>
<td>63%</td>
<td>57%</td>
</tr>
<tr>
<td>CIN2+</td>
<td>68%*</td>
<td>82%*</td>
<td>83%</td>
</tr>
<tr>
<td>Aty+CINI</td>
<td>13%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>Cum</td>
<td>81.3%*</td>
<td>91.7%*</td>
<td>93%*</td>
</tr>
</tbody>
</table>

* P<0.01 between one Bx Vs >1 Bx

Interobserver Diagnostic Agreement for Colpo QC using Digitized Colposcopic Images (ALTS)

<table>
<thead>
<tr>
<th>QC reviewers</th>
<th>Colpo agreement</th>
<th>kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&amp;2(NL)</td>
<td>63%</td>
<td>0.21</td>
</tr>
<tr>
<td>1&amp;3</td>
<td>57%</td>
<td>0.23*</td>
</tr>
<tr>
<td>2&amp;3</td>
<td>63%</td>
<td>0.35*</td>
</tr>
<tr>
<td>1&amp;2(CIN2&gt;)</td>
<td>51%</td>
<td>0.32</td>
</tr>
<tr>
<td>1&amp;3</td>
<td>55%</td>
<td>0.39</td>
</tr>
<tr>
<td>2&amp;3</td>
<td>55%</td>
<td>0.38*</td>
</tr>
</tbody>
</table>

*statistically significant, (NL) histologic diagnosis normal, (CIN2>) histologic diagnosis of CIN 2 or worse

Intraobserver Variability Colposcopy

<table>
<thead>
<tr>
<th>First Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(%)</td>
</tr>
<tr>
<td>CINI</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Second session</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(%)</td>
</tr>
<tr>
<td>CINI</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
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