Selected Dilemmas in Lower Genital Tract Pathology

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Topic 1: Unusual and difficult variants of VIN

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VULVAR CARCINOMA MODEL

40%
35-65
STD
HPV

Classic VIN
Moderate to Poorly Diff SCC

60%
55-85
Lichen Sclerosus and LSC
p53 mutations

Differentiated VIN
Keratinizing SCC

Crum CP. Carcinoma of the vulva: Epidemiology and pathogenesis.
Obstet Gynecol 79:448-454, 1992

HPV-Positive Usual (Classic) VIN

- Characterized by full or near full-thickness atypia
- Nuclear enlargement, multinucleation, abnormal mitoses
- HPV 16
Classic VIN (CIS)

Classic VIN with Lichen Simplex Chronicus-Like Change

Subtle Classic HIVIL ( Bowenoid dysplasia)
Biomarker Staining for Classic VIN

- P16 – relatively specific for classic VIN
- Caveats
  - Some cases demonstrate predominately cytoplasmic staining
  - Heterogeneity

Riethdorf et al
Medeiros et al

Classic/Usual VIN

- Full or near full-thickness atypia
- Diffuse horizontal p16\textsuperscript{ink4a} positive
- P16 staining parallels the level of differentiation
Utility of p16 Staining

- Will be weak or negative in all variants of exophytic Low grade lesions
- May be strong in flat condylomata (VIN 1)
- Is occasionally helpful in evaluating margins with subtle atypias.
- Resolving problematic atypias.

Subtle Lesions

Margins
Differentiated VIN (HPV negative)

- Less well defined category
- Exhibits one or more of the following features
  - Atypia confined to the first 2-3 cell layers (basal atypia)
  - Discrete basal/parabasal cellularity/hyperchromasia
  - Acantholysis
  - Abnormal cell maturation with abnormal keratinization

Differentiated VIN

HPV negative VSCC

Neither precursor nor invasive cancer may exhibit surface atypia
p53 MUTATIONS IN DVIN AND VULVAR SCC

- Is not well understood due to molecular heterogeneity in both cancers and precursors
- Is implied in some cases by strong co-staining with p53 but the role of p53 mutations is unknown
- Has not been established by p53 mutation analysis


Results

Differentiated VINs contain p53 mutations

Laser capture microdissected DNAs from both lower and upper epithelial layers contain the mutations

<table>
<thead>
<tr>
<th>Case</th>
<th>Directed tissue (L to U)</th>
<th>Nucleotide Change</th>
<th>p53</th>
<th>p16</th>
<th>HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dVIN</td>
<td>743G&gt;GA</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>dVIN (1)</td>
<td>743G&gt;GA</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
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<td>dVIN (2)</td>
<td>743G&gt;GA</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>dVIN</td>
<td>743G&gt;GA</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>dVIN (1)</td>
<td>743G&gt;GA</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>dVIN (2)</td>
<td>743G&gt;GA</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

P53 staining is uniquely basal or immature cell-specific

dVIN 743G>GA R248RQ
dVIN 743G>GA R248RQ
Case 5

There is a link between p53 mutations and a subset of p53 immunopositive dVINs described by Yang and Hart.

However, p53 staining will not identify all dVINs with p53 mutations.

Because of the frequency (and sometimes multiplicity) of p53 mutations in the vulvar epithelium, the predictive value of a given p53 mutation for a SCC outcome is unclear.

P53 is normally expressed in the basal cells of squamous epithelium and decays with maturation. This pattern of expression is identical to p63, which is a marker of keratinocyte basal cells. In both benign and neoplastic squamous epithelium, p53 and p63 are concordant in their distribution. Thus, in squamous epithelium (unlike glandular neoplasms), mutant p53 accumulates in the basal (type) cell only and is degraded as function of maturation.

Other Alterations that may Confer Increased Risk

- LSA with superimposed LSC
- Verruciform lichen simplex chronicus (non-specific)
- Verruciform acanthosis with altered differentiation
Verruciform Lichen Simplex Chronicus

Vulvar Acanthosis with Altered Differentiation
- Often seen adjacent to verrucous carcinoma
- Five components
  - Verruciform architecture (variable)
  - Non-invasive
  - Minimal nuclear atypia
  - Multilayered-parakeratosis
  - Superficial epithelial cell pallor

Nascimento et al

VAAD
- Multi-layered parakeratotic sheets
- Cytoplasmic “pallor” with loss of keratohyaline granules

VAAD is distinct from differentiated (simplex) VIN
- Abnormal keratinization
- Basal layer atypia
- Differentiated VIN
- VAAD

Nascimento et al
VAAD is distinct from classic VIN

VAAD is distinct from verrucous carcinoma

Pathogenesis of Vulvar Carcinoma

HPV 16 infection

Vulvar epithelium

Inflammatory dermatoses

Differentiated (simplex) VIN

Invasive Squamous Cell Ca

Invasive Squamous Cell Ca

Verrucous carcinoma

DAO is distinct from verrucous carcinoma

Invasive vulvar carcinoma, yes or no?
Patterns of Invasion

- PD infiltrative
- PD nested
- WD infiltrative
- WD nested
- WD blunt
- VC blunt

Mimics

- PD nested or infiltrative
  - Tangentially sectioned epithelium
  - Inflammatory artifacts in VIN
- WD infiltrative
  - Pseudoepitheliomatous hyperplasia
  - Isolated foci of dysmature epithelium
- WD nested
  - Severe reactive inflammatory

Mimics

- PD nested or infiltrative
  - Tangentially sectioned epithelium

Non-invasive

Invasive
Mimics

- PD nested or infiltrative
  - Inflammatory artifacts in VIN

Mimics

- WD nested or infiltrative
  - Tangentially sectioned epithelium

Mimics

- WD nested or infiltrative
  - Tangentially sectioned epithelium

Mimics

- WD infiltrative
  - Pseudoepitheliomatous hyperplasia

Mimics

- Other problems
  - Are we there yet?

NO  Definitely

Topic 3: CIN or immature metaplasia?

Issues

- The spectrum of CIN
- Accuracy of grading
- Dynamics of HPV infection over time
- How frequent is “true” progression from LSIL to HSIL?

Understanding Early Cervical Neoplasia

- Our job is to:
  - Determine what is and what is not a precursor lesion
  - Grade the lesion to guide management
    - HSIL = LEEP
    - LSIL = Follow
  - Avoid the over-diagnosis of HSIL
  - Manage the more recently described “metaplastic spectrum” of CIN
**Important Points**

- CIN has been re-defined over the years, largely as a function of therapeutics
- Classic diagrams of CIN are a simplification
- The transformation zone has an important impact on the presentation and interpretation of CIN
Classification

- **LSIL**
  - Corresponds to those lesions with milder forms of atypia and can be followed

- **HSIL**
  - Corresponds to those lesions with specific patterns of atypia that reflect the biologic effects of viral oncogenes

**Categories of LSIL**

- **Cytopathic effect**
  - Variations in Size/Staining
  - Correspond to LSIL in the cytologic smear

- **Mild atypia**
  - in the lower third of the epithelium
  - Indicates that the parabasal cells have not undergone a significant morphologic transformation
What do all SILs have (generally) in common?

- Nuclear atypia – variations in nuclear size and staining
- Increased nuclear density in the upper epithelial cell layers – very helpful.

Non-classic SILs

- SILs highlighting the metaplastic-columnar transition
  - Immature metaplastic LSIL – Immature condyloma
  - Immature metaplastic HSIL
  - Partially mature metaplastic SIL (“Eosinophilic dysplasia”)
  - Microglandular SIL
  - Stratified mucin-producing intraepithelial lesions (SMILE)
**Immature Condyloma**

- Imagine infecting immature epithelium with a low risk HPV
- Resembles condyloma with papillary architecture
- Koilocytosis is not obvious because the cells cannot mature
- Regular nuclear spacing with nucleoli
- Low Ki-67 index

**Papillary Immature Metaplasia (LSIL)**

[Images of histological sections are shown]
Mild atypias (SIL) in metaplastic epithelium (eosinophilic dysplasia)

Zheng et al, 2004

SIL in Reserve Cells (LSIL)

SIL in Microglandular Change (Reserve cell SIL)

<table>
<thead>
<tr>
<th>Mature</th>
<th>Immature</th>
<th>Reserve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Immature metaplastic Phenotype (HSIL)

SIL with Columnar Differentiation

SIL with Columnar Differentiation (SMILE)
Immature columnar cells
  ↓
Reserve Cells
  ↓
Immature metaplastic cells
  ↓
Mature metaplastic cells

HPV can transform epithelial cells at any point in this spectrum of differentiation. For this reason, you can expect a wide range of histologic patterns. The distinction of low from high grade lesions is based on distribution and severity of atypia.

Biomarker Staining

- P16 – Particularly useful for immature epithelia in reproductive age women
- MiB-1 – Atrophic background
- We use neither when the differential diagnosis is LSIL vs Normal
- P16 immunostaining will not discriminate LSIL from HSIL.
MiB1 p16

Reactive

MiB1 p16

HSIL (immature met phenotype)

MiB1 p16

LSIL (immature met phenotype)

Eosinophilic Dysplasia

Zheng et al, 2004
Eosinophilic Dysplasia

Zheng et al, 2004

Ascertaining Outcome Risk

- Most high risk HPVs will not result in an HSIL (CIN3) outcome (Kahn)
- 40% or more of confirmed CIN2 biopsies will be followed by regression in women under age 25 (Crum, unpublished)
- The risk of HSIL in women with mild abnormalities and negative colpo or a biopsy of CIN1 is 11% (Cox)

Prospective Risk of ≥CIN3 (1)

Follow-up time (years)

Cumulative incidence rate (%)
Progression

• Must be defined in the context of
  – The transition in question:
    • LSIL - HSIL
    • HSIL - Malignancy
  – How LSIL or CIN1 is defined
  – How many HPVs are involved
  – The reliability of the pathologic interpretation

Possible Outcomes

• Lesion disappears following biopsy
• Lesion transiently persists then disappears
• Lesion persists until cone biopsy
• More than one lesion is present, with persistence/regression of one or more
• New infections develop during follow-up and may or may not contribute to pathology

Defining CIN1

HPV Status and Outcome

<table>
<thead>
<tr>
<th>Viral parameter</th>
<th>HSIL</th>
<th>LSIL</th>
<th>NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistence of one</td>
<td>62</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>Additional types</td>
<td>27</td>
<td>78</td>
<td>34</td>
</tr>
<tr>
<td>Replacement types</td>
<td>6</td>
<td>39</td>
<td>7</td>
</tr>
<tr>
<td>Clearing of all</td>
<td>3</td>
<td>0</td>
<td>50</td>
</tr>
</tbody>
</table>
Summary

• Persistence of a single HPV type significantly influenced risk of HSIL
  – 62% of HSILs had a single HPV type throughout the study
• The presence of new or replacement HPV types correlates significantly with a CIN 1 outcome
  – 78% of CIN1 outcomes were associated with one or more additional HPVs during the study

Frequency of True Progression

• 12% of LSILs are followed by an HSIL at 2 years (Cox et al).
• 1% of LSILs (CIN1) progress to carcinomas (Östor’s review)
• What percent are true progressions from LSIL to HSIL (CIN2)?

Assessing Progression

• Ascertain percentage of biopsy proven LSIL resulting in an HSIL outcome
• Rate of HSIL outcome verification by biopsy review
• Compare to rate of HSIL outcome verification in a random sample

Results

• 29/264 LSIL biopsies followed by HSIL outcome on report (11%).
• 22/24 reviewed confirmed initial LSIL
• 5/17 with outcome review (30%) confirmed the diagnosis of CIN2 or higher
• 42/50 (84%) randomly reviewed cases confirmed the original diagnosis

Chen E, and Crum CP, unpublished
CONCLUSION

1. The most likely explanations for “progression” from LSIL to HSIL, based on record and histologic review, are, in decreasing frequency:

   a) Over-diagnosis of HSIL on subsequent biopsy/cone (especially when the outcome diagnosis is CIN2).

   b) Change in HPV type over time (based on p16 stain discrepancy)

   c) Under-diagnosis of HSIL on initial biopsy

2. Studies that use progression from LSIL to HSIL as an endpoint must take the above into account and any study that claims differences in progression rates must be viewed critically with the above possibilities in mind.

CONCLUSION

3. A diagnosis of HSIL on a follow-up biopsy following an initial biopsy diagnosis of LSIL is more likely to represent a misclassification than a routine diagnosis of HSIL.

   i.e. Such a diagnosis should be subject to quality assurance review.

   Agreement by two or more observers on a diagnosis of CIN2 is recommended prior to proceeding to LEEP.

Topic 4: Where can I get into trouble and not even know it?
Trouble Spots We have Seen

- a) Giant condylomas of the cervix misclassified as malignancies.
- b) Extraneous tissues (floater).
- c) Biopsy artifacts.
- d) Endometrial stromal tumors vs. aglandular functionalis
- e) Exceedingly subtle PSTTs
- f) Poorly diff vaginal tumor-r/o melanoma
- g) Clerical errors in reports.
Trouble Spots We have Seen

d) Endometrial stromal tumors vs. aglandular functionalis

Trouble Spots We have Seen
e) Exceedingly subtle PSTTs

Normal early gestation PSTT

Trouble Spots We have Seen
f) Vaginal spindle cell tumors: exclude spindle cell epithelioma (benign mixed tumor)

Trouble Spots We have Seen
g) Vulvo-vaginal spindle cell tumors: exclude malignant melanoma
Trouble Spots We have Seen

g) Clerical errors in reports.

CIN or Immature Metaplasia?

Cervical Biopsy

1. Squamous metaplasia
2. LSIL
3. SIL, not amenable to precise grading (CIN1-2)
4. HSIL
5. Atrophy
Diagnosis: HSIL

Cervical Biopsy

1. Squamous metaplasia
2. LSIL
3. SIL, not amenable to precise grading (CIN1-2)
4. HSIL
5. Atrophy
**Diagnosis:** SIL, not amenable to precise grading (CIN1-2)

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**Cervical Biopsy**

1. Squamous metaplasia
2. LSIL
3. SIL, not amenable to precise grading (CIN1-2)
4. HSIL
5. Atrophy
Diagnosis: HSIL (CIN2)

Cervical Biopsy

1. Squamous metaplasia
2. LSIL
3. SIL, not amenable to precise grading (CIN1-2)
4. HSIL
5. Atrophy
Diagnosis: SIL in metaplastic epithelium, favor LSIL

Cervical Biopsy

1. Squamous metaplasia
2. LSIL
3. SIL, not amenable to precise grading (CIN1-2)
4. HSIL
5. Atrophy

Diagnosis: “Differentiated” SIL
Vaginal lesion
1. Benign
2. Malignant

Diagnosis: Squames trapped in granulation tissue (benign)

1. HSIL
2. Implantation site
3. PSTT
4. Squamous carcinoma
<table>
<thead>
<tr>
<th>Diagnosis: PSTT involving cervix</th>
</tr>
</thead>
<tbody>
<tr>
<td>• This case was initially diagnosed as a SIL on smear and biopsy</td>
</tr>
</tbody>
</table>