Selected Dilemmas in Lower Genital Tract Pathology
Christopher P. Crum, MD

Topic 1: Unusual and difficult variants of VIN
Topic 2: Invasive vulvar carcinoma, yes or no?
Topic 3: CIN or immature metaplasia?
Topic 4: Where can I get into trouble and not even know it?

Topic 1: Unusual and difficult variants of VIN

Most VINs are classified into two categories; 1) classic or usual VINs, which exhibit full or near full-thickness atypia, are HPV positive and closely resemble high grade SIL of the cervix, and 2) differentiated VINs, which are classified by moderate basal atypia and abnormal keratinocyte differentiation, are HPV negative, and frequently harbor p53 mutations. A number of other variations on this theme can be encountered and include the following:

3) Classic VINs with superimposed lichen simplex chronicus. These lesions appear relatively normal in the upper third of the epithelium and may be confused with differentiated VIN. However, they typically exhibit more prominent atypia and are diffusely p16 positive.

4) Subtle classic VINs. These are similar to descriptions of bowenoid dysplasia. The atypia is full-thickness but is subtle. Thus the differential diagnosis includes lower grade lesions such as VIN1 etc.

5) Pagetoid VIN. This unusual variant is characterized by a poorly differentiated intraepithelial neoplasm that permeates the normal squamous epithelium analogous to more classical paget’s disease.

6) VIN with columnar differentiation. This is a rare variant.

7) Metastatic carcinomas growing on the vulvar mucosa. This can occur rarely with serous carcinomas of the upper genital tract and with urothelial carcinomas extending from the urinary tract.

8) VINs that are not really VINs. These include mucosal proliferations that display minimal atypia but increase risk of malignancy, such as LSC/LSA, VAAD and verruciform LSC. These are approached with caution and a recommendation for followup.
**Topic 2: Invasive vulvar carcinoma, yes or no?**

Excluding invasive carcinoma of the vulva is a paramount issue when evaluating vulvar biopsies. Pitfalls include the following:

1) Tangential sectioning of VIN. VINs are notoriously thick, tend to displace the underlying stroma with pushing margins and extend into appendages. It is the uniformity of the nesting pattern and preservation of cell polarity that aids in distinguishing this process from invasion.

2) Inflammation at the epithelial stromal interface in VIN. This can produce small discrete nests with vague appearing boundaries. The key to excluding cancer is the uniformity of the nests and the preservation of cell polarity.

3) Pseudo-epitheliomatous hyperplasia. This process takes two different forms. The most obvious is the fine interlaced strands of epithelium that are associated with inflammation. The second is the presence of discrete nests with mild alterations in squamous differentiation. The latter can be confused with invasive cancer.

4) Confusing entities such as keratoacanthoma. These lesions are problematic due to their uncertain risk of metastasis. We make this diagnosis rarely, to say the least.

**Topic 3. CIN or immature metaplasia?**

It is helpful to approach this problem with the following knowledge:

1) The association of a lesion with HPV16 increases as a function of inter-observer agreement for a diagnosis of CIN2 or higher. viii ix

2) The association between HPV16 (and cross-reacting HPVs) and high grade disease is such that nearly 85% of all CIN2-3 lesions are expected to disappear once the vaccines are established. x

3) While maturation is an important parameter in determining lesion grade, the emergence of HPV positive immature metaplasias with mild atypia requires attention to the fact that immaturity alone is not sufficient to warrant classification as HSIL.

4) Over 40% of CIN2s (by consensus) in women under age 25 will regress within 6 months.

5) The pathologist must be aware of five metaplastic patterns.
   a) Papillary immature metaplasia (PIM) or immature condyloma. xi
   b) Flat immature metaplasias with mild atypia.xii
   c) Flat reserve cell atypias with columnar differentiation and mild atypia.
Lesions in categories a-c can be followed with repeat cytology; categories d&e require cone biopsy. All but category a will be strongly positive for p16; categories a-c will generally have a lower Mib-1 index than d&e.

f) Biomarkers are helpful for ascertaining if a lesion is present, but not the grade of the lesion, with exception of SILs showing negative or patchy staining (eg exophytic condyloma). These latter examples will usually be associated with lower risk HPVs. Otherwise the distinction between a flat LSIL and a flat HSIL is principally morphologic.\textsuperscript{xv}

\textbf{Topic 4: Where can I get into trouble and not even know it?}

The following are situations in which the pathologist can be fooled when evaluating lower genital tract disease.

a) Giant condylomas of the cervix can be misclassified as malignancies in reproductive age women.

b) Multiple fragments of extraneous tissue (floater). These can be a problem in the setting of excluding ectopic pregnancy (mature villi), and endometrial carcinoma (floaters in curettings). They have a high medical legal import.

c) Biopsy artifacts in some settings will be mistaken as invasive cancer when juxtaposed to a co-existing squamous intraepithelial lesions.

d) Endometrial stromal tumors can mimic aglandular functionalis

e) Exceedingly subtle placental site trophoblastic tumors.

f) Spindle cell vaginal neoplasms: spindle cell epithelioma

g) Spindle cell or poorly differentiated vulvo/vaginal neoplasms: malignant melanoma

g) Clerical errors in reports. Most errors are picked up by one of the following individuals: the resident (in a training program), secretary, pathology attending and clinician. In rare instances all of these persons could miss an error. For this reason, virtually all climactically significant mistakes are the product of two or more detection failures.


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

HPV-Positive Usual (Classic) VIN

- Characterized by full or near full-thickness atypia
- Nuclear enlargement, multinucleation, abnormal mitoses
- HPV 16

Classic VIN (CIS)

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<sup>ink4</sup> 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.

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
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>
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<th>Genotype</th>
<th>Molecular Change</th>
<th>p53</th>
<th>p16</th>
</tr>
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<tr>
<td>dVIN 743G&gt;GA</td>
<td>R248RQ</td>
<td>P</td>
<td>N</td>
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- p53 staining is uniquely basal or immature cell-specific
Case 5

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.

P53 with Different Genetic Alterations

| A) 746G>GA | B) 746G>GA, 415A>AT | C) 746G>GA |

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

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

Vulvar Acanthosis with Altered Differentiation

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.

Nascimento et al
Pathogenesis of Vulvar Carcinoma

Vulvar epithelium

HPV 16 infection
Classic VIN
Differentiated (simplex) VIN
VAAD
Invasive Squamous Cell Ca
Verrucous carcinoma

Topic 2: 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

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

PEH
PEH with a differentiating nest
WD SCC

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 Systems and Management

1960s
- Mild
- Moderate
- Severe
- CIN
- Follow
- Cone/Hysterectomy

1970-80s
- Condyloma/CIN 1
- CIN 2
- CIN 3
- Cryotherapy
- Laser/Cone

1990s
- LSIL
- HSIL
- HSIL
- Follow
- LEEP

2007+
- LSIL
- HSIL
- HSIL
- Follow
- LEEP

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
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)

Categories of LSIL

- Cytologic 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

HSIL

- Parabasal nuclear enlargement, differences in size and staining with coarse chromatin, corresponding to higher grade cells on smear
  - Reflects fundamental change in the biology of the replicating cell population
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)

Mild atypias (SIL) in metaplastic epithelium (eosinophilic dysplasia)

Zheng et al, 2004

SIL in Reserve Cells (LSIL)
SIL in Microglandular Change (Reserve cell SIL)

SIL with Columnar Differentiation

Immature metaplastic Phenotype (HSIL)

SIL with Columnar Differentiation (SMILE)
Immature columnar 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.
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)
**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

**Defining CIN1**

**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

**HPV Status and Outcome**

<table>
<thead>
<tr>
<th>Viral parameter</th>
<th>HSIL</th>
<th>LSIL</th>
<th>Nl</th>
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<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>
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<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

Chen E, and Crum CP, unpublished

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

b) Extraneous tissues (floater).

Trouble Spots We have Seen
d) Endometrial stromal tumors vs. aglandular functionalis

Trouble Spots We have Seen
c) Biopsy artifacts.

Trouble Spots We have Seen
e) Exceedingly subtle PSTTs

Normal early gestation PSTT

PSTT
Trouble Spots We have Seen

f) Vaginal spindle cell tumors: exclude spindle cell epithelioma (benign mixed tumor)

g) Vulvo-vaginal spindle cell tumors: exclude malignant melanoma

Trouble Spots We have Seen

g) Clerical errors in reports.