The LAST Project: Polishing the Gold Standard

Teresa M. Darragh, MD
UCSF
Departments of Pathology and Obstetrics, Gynecology & Reproductive Sciences

Disclosures: Teresa M. Darragh, MD

- The CAP-ASCCP LAST Project
  - Steering Committee Co-Chair
- College of American Pathologists (CAP)
  - Former member, Cytopathology Committee
- ASCCP: Immediate Past President
- Hologic:
  - Research supplies for anal cytology
- Advisory Boards:
  - OncoHealth: Stock options
  - Roche: Honorarium paid to UCSF

Objectives

- Review: The LAST Project
  - Basic principles
  - Strengths & weaknesses of the “gold standard”
  - Recommendations for squamous intraepithelial lesions
- Overview: HPV-related squamous disease
  - Pathogenesis, in brief
  - Low-grade HPV infection
  - High-grade HPV-associated precancer
Focus: words...

Terminology /ter-mi-nol-"o-gy/ (ter"mī-nol′ah-je)
• 1. the vocabulary of an art or science.
• 2. the science which deals with the investigation, arrangement, and construction of terms.

Medicine = Art + Science

Nomenclature (nō′menkl′ā′chur):
• the formally adopted terminology of a science, art, or discipline;
• the system of names or terms used in a particular branch of science.

LAST Project Work Groups

• WG 1 – Historical Review of Lower Anogenital Tract Terminology Across Disciplines
• WG2 – Terminology for Intraepithelial Lesions, Integrating Morphology, Biology, and Clinical Management
• WG3 - Terminology for Minimally Invasive Cancers, Integrating Morphology, Biology, and Clinical Management
• WG4 – Molecular Markers for Histopathology
• WG5 – Implications and Implementation of Standardized Terminology

The Bethesda System: A Historical Perspective

Terminology: 3 fundamental principles
1. Communicate clinically relevant information from the laboratory to the patient’s health care provider.
2. Uniform and reasonably reproducible across different pathologists and laboratories and also flexible enough to be adapted in a wide variety of lab settings and geographic locations
3. Reflect the most current understanding of the disease process

These principles were adopted by the LAST Project

Robert J. Kurman, MD  Forward to the Bethesda Atlas, 2nd edition

Underlying Premises [1]

• There is unified epithelial biology to HPV-associated squamous neoplasia
• This biology is applicable to all sites in both sexes/genders
• Histopathologic classification is subject to diagnostic variation
• But diagnostic variation can be improved by:
  • Limiting the number of tiers
  • The use of biologic markers
Underlying Premises [2]

- To understand the biology we are dependent upon samples from patients
- Each patient sample is only a statistical representation of the patient’s true biology
- The more samples or data points available, the closer you get to the patient’s “true” biology
- Our understanding of the biology allows us to designate “risk” for cancer at the current time and to a lesser extent “risk” over time

The LAST Project: Intraepithelial Lesions Recommendations

1. A unified histopathological nomenclature with a single set of diagnostic terms is recommended for all HPV-associated preinvasive squamous lesions of the lower anogenital tract (LAT).
   - Regardless of anatomic site.
   - Regardless of sex/gender.

The LAST Project: Intraepithelial Lesions Recommendations

2. A 2-tiered nomenclature is recommended for non-invasive HPV-associated squamous proliferations of the LAT which may be further qualified with the appropriate –IN terminology.
   - IN refers to the generic intraepithelial neoplasia terminology, without specifying the location. For a specific location, the appropriate complete term should be used. Thus for an –IN 3 lesion: cervix = CIN 3, vagina = VaIN 3, vulva = VIN 3, anus = AIN 3, perianus = PAIN 3, and penis = PeIN 3

The LAST Project: Intraepithelial Lesions Recommendations

3. The recommended terminology for HPV-associated squamous lesions of the LAT is:
   - Low-grade squamous intraepithelial lesion (LSIL) and
   - High-grade squamous intraepithelial lesion (HSIL)

May be further classified by the applicable –IN subcategorization.
LSIL: Virion production & transient lesions

Productive infection

HSIL: HPV E6/E7 expression & risk of cancer

Transforming infection

2-tiered system: LSIL & HSIL

Reflects HPV biology and clinical management
Diagnostic variation: What is your diagnosis?

A. Squamous metaplasia
B. Mild dysplasia (CIN1)
C. Moderate dysplasia (CIN2)
D. Severe dysplasia (CIN3)

Cervical biopsy

Squamous metaplasia versus dysplasia?

1. Squamous metaplasia 41%
2. Mild dysplasia (CIN1) 21%
3. Moderate dysplasia (CIN2) 28%
4. Severe dysplasia (CIN3) 10%

CIN Grade?

A. CIN 1
B. CIN 2
C. CIN 3
What is -IN2?

“CIN2” is a poorly recognized “intermediate risk” lesion

<table>
<thead>
<tr>
<th></th>
<th>Regression (%)</th>
<th>Persistence (%)</th>
<th>Progression to CIN 3 (%)</th>
<th>Progression to Invasive Cancer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 1</td>
<td>60</td>
<td>30</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>CIN 2</td>
<td>40</td>
<td>40</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>CIN 3</td>
<td>33</td>
<td>55</td>
<td>N/A</td>
<td>&gt; 12</td>
</tr>
</tbody>
</table>

Ostor AG. Int J Gynecol Pathol 1993; 12: 186-92

In a 3 grade system using H&E morphology, there is only poor agreement in the diagnosis of –IN2

<table>
<thead>
<tr>
<th></th>
<th>Kappa</th>
<th>Strength of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>0.52</td>
<td>Poor</td>
</tr>
<tr>
<td>CIN1</td>
<td>0.24</td>
<td>Fair</td>
</tr>
<tr>
<td>CIN2</td>
<td>0.20</td>
<td>Moderate</td>
</tr>
<tr>
<td>CIN3+</td>
<td>0.61</td>
<td>Very good</td>
</tr>
</tbody>
</table>

Kappa values:


Rationale for Recommendations

There is evidence that a 2-tiered system for cervical disease is more reproducible (with higher kappa statistics).

• For 2 tiers: Kappa statistics ranged from .30 to .71.
  • Studies are case series or cross sectional with low numbers other than one study from the ALTS trial which has high numbers and is a blinded study comparing 2 expert panel groups.

• For 3 tiers: Kappa statistics ranged from .12 to .58.
  • All studies are case series or cross sectional and have low numbers.
  • CIN2 has the lowest reproducibility of the 3 tiers.

• A Distinct Biologic Stage?
• Ugly Looking -IN1?
• Not So Ugly -IN3?
• An equivocation that is NOT reproducible
• A representation of incomplete sampling
• ~2/3 HSIL; ~1/3 LSIL
• A management safety net?
Each patient sample is only a statistical representation of the patient’s true biology

- **Sampling**: A sample from one area does not necessarily represent the most significant disease
- **Colposcopy**
  - *Not a gold standard* – significant variability in accuracy and sensitivity based on size, location and physical characteristics of the lesion and the skill and experience of the colposcopist
- **Biopsy**
  - *Not a gold standard* – significant variation in diagnosis based on terminology used and training

**The “Gold Standard” and Diagnostic Error**

- 17% of all cones = “Negative”


**Unified morphology HPV-related Lesions: Infection and Precancer**

- **Penis**
- **Vulva**
- **Male – Anal**
- **Female – Cervix**
**False Premises**

- Biopsy is perfect representation and contains everything you need to know to manage the patient
- CIN2 is a distinct biologically defined category
- All pathologists read a biopsy the same way
- Interpretative variation can be eliminated through education on morphologic criteria alone

**Morphologic interpretation = Art**

Can the *science* of medicine make the *art* of medicine more reliable?

Can we use our knowledge of HPV biology to make histopathologic diagnoses more objective?

---

**Normal Cell Cycle**

- E2F family of transcription factors
- Form transcription activating complexes (E2F-DP)
- Push a cell into S phase

**The Cell Cycle and pRb**

- pRb = tumor suppressor protein
- pRb binds and inhibits transcription factors of the E2F family
- pRb prevents the cell from replicating damaged DNA by preventing its progression along the cell cycle through G1 (first gap phase) into S (synthesis phase).
HPV hijacks the cell cycle

- HPV E7 preferentially binds to pRB
- E7 of oncogenic HPVs, especially HPV 16 the strongest
- p53 → apoptosis

Effect of HPV on the cell cycle

- Combined effect of E6 and E7
  - Maintain damaged cells in a hyper-proliferative state
  - Immortalize cells with un-repaired DNA damage

Art of Interpretation + Current Science Hypotheses

- Diagnostic variation can be improved by:
  - Limiting the number of tiers
  - The use of biologic markers, such as:
    - p16
    - Ki-67
    - ProEx C
- *Add objectivity to the art...*

What is p16?

It is a tumor suppressor protein that is a biomarker for *transforming HPV infection* and can be used as a *surrogate marker* of HPV-associated precancer

- Negative feedback loop on pRb to inhibit cell cycle
- Since pRb is deactivated by HPV’s E7 → p16 is overexpressed
Use of p16

- In the largest prospective adjudicated study and other supporting studies, diffuse strong (block positive) staining with p16 showed similar accuracy for high grade disease when compared to an adjudicated histology standard.

- p16 immunohistochemistry improves the accuracy of a single pathologist’s interpretation of high grade vs. low grade disease relative to an adjudicated pathology panel of experts.

- Addition of a p16 result leads to a more accurate prediction of the patient’s risk for high grade disease.

When do we use p16?

LAST Recommendations

1. HSIL vs. Mimic
2. Query -IN2
3. Difference in opinion
4. NOT for obvious –IN1 or –IN3

4a. “a priori”: When no histologic HSIL is found on biopsy in “high-risk” situations – prior Pap with HSIL, ASC-H, HPV16+ ASC-US, AGC (NOS)

LAST: Biomarkers Recommendations

1. p16 IHC is recommended when the H&E morphologic differential diagnosis is between precancer (–IN2 or –IN3) and a mimic of precancer (e.g., processes known to be not related to neoplastic risk such as immature squamous metaplasia, atrophy, reparative epithelial changes, tangential cutting).

- Strong and diffuse block-positive p16 results support a categorization of precancerous disease.
DDx: HSIL vs. Mimic

A. HSIL
B. Mimic of HSIL

p16 positive = HSIL

DDx: HSIL vs Reactive

A. HSIL
B. Reactive

p16 negative = Reactive
DDx: HSIL vs. Atrophy

A. HSIL

B. Atrophy

Cervical Biopsy

Transitional Cell Metaplasia

LAST: Biomarkers Recommendations

2. If the pathologist is entertaining an H&E morphologic interpretation of –IN 2 (under the old terminology, which is a biologically equivocal lesion falling between the morphologic changes of HPV infection [low-grade lesion] and precancer), p16 IHC is recommended to help clarify the situation.

- Strong and diffuse block positive p16 results support a categorization of precancer. Negative or non-block-positive staining strongly favors an interpretation of low-grade disease or a non-HPV associated pathology.
Query CIN 2

A. LSIL
B. HSIL

p16 negative = LSIL

Query CIN 2

A. LSIL
B. HSIL

p16 +

Query AIN 2

A. LSIL
B. HSIL

HSIL (AIN2)
Recommendation 4: Don’t use!

If BIOPSY is morphologically unequivocal:
- Negative
  - IN 1
  - IN 3

NO
p16 stain

...A priori...
31 y.o. G2P2 with persistent HSIL on Pap

- Repeat colposcopy
- Colpo satisfactory
- Small area of AWE on anterior lip with mosaic pattern
- Repeat biopsy
  - Scant

- p16 +
- Dx = HSIL

**ARE BIOMARKERS THE SOLUTION?**

DATA ON ~1500 ADJUDICATED BIOPSIES

- NIL 5%
- CIN1 39%
- CIN2 77%
- CIN3 99%

HPV Biology: Infection vs. Precancer

Biomarkers – Add Objectivity: Reduce diagnostic variation

Biomarkers: p16 Surrogate for transforming infection

The LAST Project:

The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: Background and Consensus Recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology.


- Int J Gynecol Pathol. 2013 Jan;32(1):76-115
Specimens that are positive for squamous intraepithelial lesions should be reported using a 2-tiered nomenclature. The recommended terminology is Low Grade Squamous Intraepithelial Lesion and High Grade Squamous Intraepithelial Lesion (LSIL, HSIL).

Updates: WHO Blue Book
- IARC
- Lyon, June 2013
- Adopted the LAST Project’s terminology
- Revised edition
- ~June 2014