The LAST Project: Polishing the Gold Standard

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The LAST Project

Lower Anogenital Squamous Terminology standardization project for histopathologic diagnoses of HPV-associated lesions of the lower anogenital tract
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”mi-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´menklā´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
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

LSIL (CIN1) & Productive infection
HSIL: 
HPV E6/E7 expression & risk of cancer
2-tiered system: LSIL & HSIL

Reflects HPV biology and clinical management

<table>
<thead>
<tr>
<th></th>
<th>Low-grade squamous intraepithelial lesion (LSIL)</th>
<th>High-grade squamous intraepithelial lesion (HSIL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condyloma</td>
<td>CIN/AIN grade 1</td>
<td>CIN/AIN grade 2</td>
</tr>
<tr>
<td></td>
<td>Very mild to mild dysplasia</td>
<td>Moderate dysplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe dysplasia</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>In Situ carcinoma</td>
</tr>
</tbody>
</table>

Infection & Precancer
2-tiered system: LSIL & HSIL

<table>
<thead>
<tr>
<th>Condition</th>
<th>LSIL</th>
<th>HSIL</th>
</tr>
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<tbody>
<tr>
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<td></td>
<td>In Situ carcinoma</td>
</tr>
<tr>
<td>CIN/AIN grade 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Infection & Precancer**
Diagnostic variation: What is your diagnosis?

1. Squamous metaplasia
2. Mild dysplasia (CIN1)
3. Moderate dysplasia (CIN2)
4. Severe dysplasia (CIN3)
Squamous metaplasia versus dysplasia?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous metaplasia</td>
<td>41%</td>
</tr>
<tr>
<td>Mild dysplasia (CIN 1)</td>
<td>21%</td>
</tr>
<tr>
<td>Moderate dysplasia (CIN 2)</td>
<td>28%</td>
</tr>
<tr>
<td>Severe dysplasia (CIN 3)</td>
<td>10%</td>
</tr>
</tbody>
</table>
CIN Grade?

1. CIN 1
2. CIN 2
3. CIN 3

- CIN 1: 49%
- CIN 2: 37%
- CIN 3: 15%
CIN Grade?

1. CIN 1
2. CIN 2
3. CIN 3

- CIN 1: 28%
- CIN 2: 36%
- CIN 3: 36%
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
What is -IN2?

• 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

Does not reflect our current understanding: infection vs. precancer

• A management safety net?
In a 3 grade system using H&E morphology, there is only poor agreement in the diagnosis of –IN2

- Benign  Kappa 0.52
- CIN1    Kappa 0.24
- CIN2    Kappa 0.20
- CIN3+   Kappa 0.61

Kappa values:
Strength of agreement
- ̸0.20 Poor
- 0.21 - 0.40 Fair
- 0.41 - 0.60 Moderate
- 0.61 - 0.80 Good
- 0.81 - 1.00 Very good

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.
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
Sampling and the “Gold Standard”
Unified morphology HPV-related Lesions: Infection and Precancer

- Penis
- Vulva
- Male – Anal
- Female – Cervix

High-grade SIL
Cutaneous or Mucosal

Low-grade SIL
? 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

Modified from Lehoux et al., Public Health Genomics, 2009
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).

Modified from Lehoux et al., Public Health Genomics, 2009
HPV hijacks the cell cycle

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

Modified from Lehoux et al., Public Health Genomics, 2009
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.

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.
p16 positive stain = “Block positive”

- Strong and diffuse staining
- Nucleus or nucleus plus cytoplasmic staining
- Of the basal cell layer with upward extension involving at least 1/3 of the epithelium
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 of HSIL

1. HSIL
2. Mimic of HSIL
DDx: HSIL vs. Mimic

p16 positive = HSIL

Anal biopsy
DDx: HSIL vs Reactive

1. HSIL
2. Reactive
DDx: HSIL vs Reactive

p16 negative = Reactive

Cervical Biopsy
DDx: HSIL vs. Atrophy

1. HSIL
2. Atrophy
DDx: HSIL vs. Atrophy

Transitional Cell Metaplasia

Cervical Biopsy

p16
Increasing Cancer Risk

HSIL vs. Mimic

p16 IHC

High-Grade SIL

Negative

LAST Terminology Diagnosis

Follow-up

Clinical Management

Histologic Interpretation

A

IHC

High-Grade SIL

Negative
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

1. LSIL
2. HSIL

70% and 30%
Query CIN 2

p16 negative = LSIL
1. LSIL
2. HSIL

Query AIN 2

LSIL: 43%
HSIL: 57%
Query AIN 2

HSIL (AIN2)

p16 +
**Histologic Interpretation**

- **IN1** 
- **IN2** 
- **IN3**

Increasing Cancer Risk

- **p16 IHC**

Follow-up

- **High-Grade SIL**
- **Low-Grade SIL**

Clinical Management

LAST Terminology Diagnosis

- **B**
Recommendation 4: Don’t use!

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

LSIL

**NO**
p16 stain

HSIL
ARE BIOMARKERS THE SOLUTION?

DATA ON ~1500 ADJUDICATED BIOPSIES

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

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
# HPV Biology: Infection vs. Precancer

## Schematic Representation of SIL

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## Biology & Management
Biomarkers – Add Objectivity: Reduce diagnostic variation

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**Biology & Management**
Biomarkers: p16
Surrogate for transforming infection

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Productive infection  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 for the cervix, vulva and vagina

- Revised edition
- Published April 2014
Pathologists Needed!
A real-life experience with LAST
Your participation will be important!

• We are conducting a large study of cervical histology adjudication using p16 in the New Mexico HPV Pap registry (NMHPVPR)
• The study will evaluate the real-life performance of LAST guidelines
• We are looking for pathologists interested in reading 100 slide sets (H&E first; H&E and p16 after washout phase)
• Participants will receive a unique opportunity to evaluate their cervical histology performance against an expert panel

Interested?
For more details, please complete the form below and email to: p16-nmhpvpr@salud.unm.edu
The LAST Project

Lower Anogenital Squamous Terminology Standardization Project