What’s new in cervical cytology and pathology?

The Bethesda System and the LAST Project

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Faculty Disclosure

• In the past 12 months...

• Hologic: Research supplies for anal cytology

• Roche: Honorarium and travel expenses
Objectives

- The Bethesda System for *Pap tests*
  - Bethesda 3 (2015): What’s new?

- Review the CAP-ASCCP LAST Project for *Biopsies*
  - Basic principles
  - Strengths & weaknesses of the “gold standard”
  - Recommendations for intraepithelial lesions
    - Terminology
    - Biomarker use
The Bethesda System: Atlases

The Bethesda System for Reporting Cervical and Vaginal Cytologic Diagnoses
Definitions, Criteria, and Explanatory Notes for Terminology and Specimen Adequacy

Diane Solomon, Ritu Nayar, Editors
Second Edition

TBS 1: 1991

TBS 2: 2001
The Bethesda System

- Negative for intraepithelial lesion or malignancy
  - Reactive changes, organisms
- Atypical squamous cells (ASC-US & ASC-H)
- LSIL
- HSIL
- Atypical glandular cells
  - NOS: endocervical, endometrial, glandular
  - Favor neoplastic: endocervical, glandular
  - AIS
- Cancer: squamous, glandular, other...
Why a 3rd Edition?

- Significant changes in practice of gynecologic cytology
  - Primary HPV screening with Pap as “diagnostic” triage
  - New screening and management guidelines
  - Changes in histopathology terminology
  - Increasing uptake of HPV vaccination

- New data and technology
  - Additional experience with LBP over last 10 yrs
  - Endometrial cells, Anal cytology, Biomarkers, Automation, Risk assessment
  - Still a need for Pap testing in low resource areas and for standardization of terminology for trials and research
Bethesda 3

- Publication: Spring 2015
  - Print & e-book formats

- Updates:
  - Greater page content
  - Updated recommendations
  - Increased background
    - Literature review
    - Data in support
    - Biological descriptions
  - Management issues for each entity

Few changes clinicians may notice
TBS: Possible Confusion?

- Bethesda 3 → Additional Guidance / Clarification
- Specimen adequacy: Lack of t-zone component
- LSIL + possible HSIL: how to report?
- Benign endometrial cells
  - Significance on Pap
  - Reporting issues
The Bethesda System: T-zone

- Definition of “adequate” endocervical cells or transformation zone component
- 10 well preserved cells
  - Endocervical or squamous metaplastic
  - Single cells or in clusters
- With atrophy
  - May not be able to tell atrophic T-zone from parabasal cells
  - TBS: “No identifiable t-zone component in an atrophic pattern sample”
- Quality indicator ≠ Unsatisfactory Pap
Quality Indicator: No t-zone on Pap

- No T-zone on approximately 10-20% of Paps
- More frequent in pregnant & older women

- Recent meta-analysis: Negative Pap →
  - Regardless +/- t-zone
  - Good specificity and NPV
- HPV test result independent of t-zone sampling

Zhao C. Gynecol Oncol 2007;107:231-5.
Bethesda 3: No t-zone

- TBS still recommends reporting the presence or absence of EC/TZ component as a quality indicator.
- Absence of an EC/TZ component should not lead to early repeat screening.
- Provides feedback to clinician.

- May provide valuable information in women with a history of atypical glandular cells, early adenocarcinoma, trachelectomy for early-stage cancer, or other high-risk processes.
Negative Pap, No t-zone

Cytology NILM but ECITZ Absent/Insufficient

Ages 21-29*

HPV negative

Routine screening

HPV testing (Preferred)

HPV unknown or

Repeat cytology in 3 years (Acceptable)

Age ≥30 years

HPV positive

Cytology+ HPV test in 1 year

Manage per ASCCP guideline

Genotyping

*HPV testing is unacceptable for managing women ages 21-29 years

© Copyright, 2013, American Society for Colposcopy and Cervical Pathology. All rights reserved. ASCCP

No early repeat needed*

*Unless HPV+
Bethesda 3: LSIL + ASC-H

- LSIL with some cells suggestive of HSIL
- Some labs report *modified* TBS
  - LSIL, cannot exclude HSIL
  - LSIL-H
- Risk for HSIL on biopsy intermediate between:
  - LSIL and HSIL on cytology
  - Risk similar to ASC-H
- No new category!
  - Management guidelines based on LSIL, ASC-H, HSIL
- Report as ASC-H + LSIL
  - Should be relatively uncommon interpretation
Bethesda: Benign Endometrial cells

- In post-menopausal women, exfoliated endometrial cells are abnormal.
  - Raise possibility of endometrial neoplasia

  - In US, average age is 51 years (but large variation)

- TBS 2: Report in all women ≥ 40 years
  - Status often unclear, inaccurate, or unknown to lab
  - Clinician to determine if further evaluation needed...
    - Confusion, especially among non-gynecologists
    - Led to unnecessary endometrial sampling in some women
Consequence of 2001 Bethesda

- Increased reporting of benign-appearing EMs
  - 0.17% to 0.49% of Paps (↑3x)
  - Decreased *predictive value* for hyperplasia and cancer with Bethesda 2

<table>
<thead>
<tr>
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<th>Pre-2001</th>
<th>Post-2001</th>
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<tbody>
<tr>
<td>Hyperplasia</td>
<td>12%</td>
<td>2%</td>
</tr>
<tr>
<td>Cancer</td>
<td>6%</td>
<td>1%</td>
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Bethesda 3: Reporting Benign Endometrial cells on Pap

- Endometrial cells are present in a woman \( \geq 45 \) years of age.
- Negative for squamous intraepithelial lesion.

Note: Endometrial cells in women 45 years and older may be associated with benign endometrium, hormonal alterations and less commonly, endometrial or uterine abnormalities. Endometrial evaluation is recommended in postmenopausal women.

Images: The Bethesda Atlas
Bethesda 3

- Risk assessment approach to cervical cancer screening
- Risk stratification
- Similar management for similar risk
Underlying Principle
Similar Management for Similar Risk

- Management threshold
- Risk stratification
- Population risk
- Risk among test-positives (PPV)
- Risk among test-negatives (cNPV)

Pre-test risk
Post-test risk

- Treatment
- Colposcopy
- Increased surveillance
- Repeat screen
- 100%
- 40%
- 2%
- 0%
Pap Test as **Benchmark**: Similar Management for Similar Risk

<table>
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**Pap Test as Benchmark:**
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Management options

- Repeat screen at regular intervals
- Increased surveillance
  - Shorter screening interval
- Colposcopy
- Treatment

Similar management for similar risk
Harmonizing Management According To Risk

- Treatment
- Colposcopy
- Increased Surveillance
- Screening
- Lower risk = ↑ interval

Cytology Screening → Colposcopy → Biopsy → Post-Colpo

HSIL & HPV+ & H-G Colpo
CIN3 Biopsy

Increased Risk of Precancer (CIN3)

HPV+/ASC-US
HPV+/Cyto-

HPV+/ASC-US
HPV+/Cyto-

HPV+/
Cyto-

HPV+/
Cyto-

HPV+/
Cyto-

HPV+/
Cyto-

Cervical Cancer Screening Options

• Rapid Evolution

• Advantage of screening and management recommendations based on *risk thresholds*:

• New assays can be integrated into current recommendations more easily based on risk equivalence studies
Underlying principles: Cervical Cancer Screening & Management

Benefits

Harms

Similar management for similar risk
The LAST Project

Lower Anogenital Squamous Terminology
standardization project for
histopathologic diagnoses of
HPV-associated squamous lesions
of the lower anogenital tract

CAP
ASCCP
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 laboratory 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 Principles

- There is unified epithelial biology to HPV-associated squamous neoplasia
- This biology is applicable to all sites in both sexes/genders
- Histopathologic classification & diagnosis:
  - The **Gold Standard** for clinical management
  - Subject to diagnostic variation
- Diagnostic variation can be improved by:
  - Limiting the number of tiers
  - The use of biologic markers
False Premises?

- Biopsy **may not be** a perfect representation and contain everything you need to know to manage the patient.

- All pathologists **do not** read a biopsy the same way.

- CIN2 is **not** a distinct biologically defined category.

- Interpretative variation **cannot** be eliminated through education on morphologic criteria alone.
LSIL:
Virion production & transient lesions

LSIL (CIN1)

LSIL

Productive infection
HSIL:
HPV E6/E7 expression & risk of cancer

HSIL (CIN3)

HSIL

Transforming infection
HPV-associated precancers: Unified morphology

CIN3

Mucosal

VIN3

Cutaneous

AIN3

PeIN3
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.
2-tiered system: LSIL & HSIL

Schematic Representation of SIL

Low-grade squamous intraepithelial lesion (LSIL)
- Condyloma
- Very mild to mild dysplasia

High-grade squamous intraepithelial lesion (HSIL)
- CIN/AIN grade 1
- CIN/AIN grade 2
- CIN/AIN grade 3
- Moderate dysplasia
- Severe dysplasia
- In Situ carcinoma

Reflects HPV biology and clinical management

Infection & Precancer
**Diagnostic Variation**

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Interobserver variability & Diagnostic (un)certainty
Diagnostic variation: What is your diagnosis?

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

- 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

UCSF CME May 2014:

CIN Grade?

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

49% 37% 15%
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
- A management safety net?

Does not reflect our current understanding: infection vs. precancer
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?
Art of Interpretation + Current Science

- 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.

**p16 and Normal cell cycle progression**

- Release of E2F from pRB results in cell cycle progression, mitotic replication, and low level expression of p16.
- p16 protein facilitates the re-binding of pRB to E2F, leading to cell cycle arrest.
Transforming HPV Infection: Oncogenesis

- Since pRb is deactivated by HPV’s E7 → p16 is overexpressed

- p16 screams STOP

- In cells with transforming HPV infections, HPV viral oncoprotein E7 impairs the function of pRB, disrupting its ability to bind to E2F
- This leads to deregulated cell proliferation, genetic instability and p16 over-expression detectible by immunohistochemistry staining
LAST: Use of p16

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

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

- Adds *objectivity* to subjective interpretation of H&E stained slide
The published literature indicates improved interobserver agreement of the diagnosis of CIN2+ with the conjunctive use of H&E morphology with p16\(^{\text{INK4a}}\) immunohistochemistry compared with H&E morphology alone.
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)
DDx: HSIL vs. Mimic

1. HSIL
2. Mimic of HSIL

CAP '14

17%  83%
DDx: HSIL vs. Mimic

p16 positive = HSIL
DDx: HSIL vs. Reactive

1. HSIL
2. Reactive

CAP '14

64%  36%
DDx: HSIL vs. Reactive

p16 negative = Reactive

Cervical Biopsy
When do we use p16?

LAST Recommendations

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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)
Query CIN 2

1. LSIL
2. HSIL
Query CIN 2

p16 negative = LSIL
Query AIN 2

1. LSIL
2. HSIL

CAP '14

[Image of histological section]

[Bar chart with percentages 40% and 60%]
Query AIN 2

HSIL (AIN2)

p16 +
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
Updates: WHO Blue Book

- Adopted the LAST Project’s terminology for the cervix, vulva and vagina
- 4th edition
- Published April 2014
The LAST Project

Lower Anogenital Squamous Terminology Standardization Project
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
...thank you...