

What's new in cervical cytology
and pathology?

The Bethesda System and the
LAST Project

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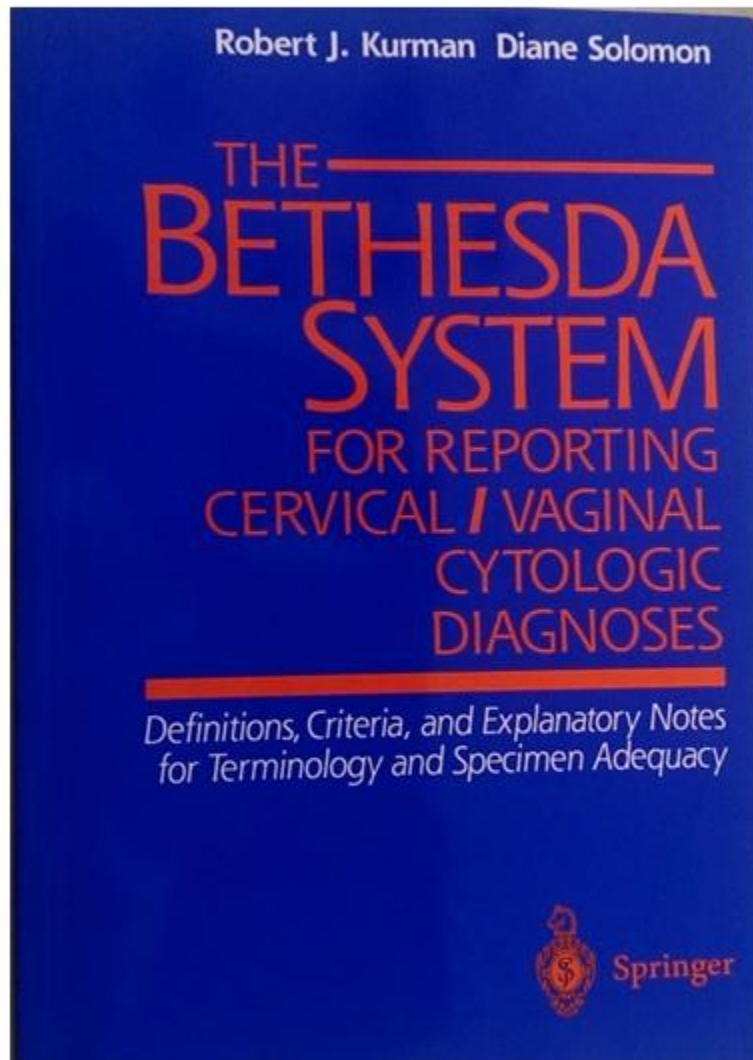
Faculty Disclosure

- In the past 12 months...
- Hologic: Research supplies for anal cytology
- Roche: Honorarium and travel expenses

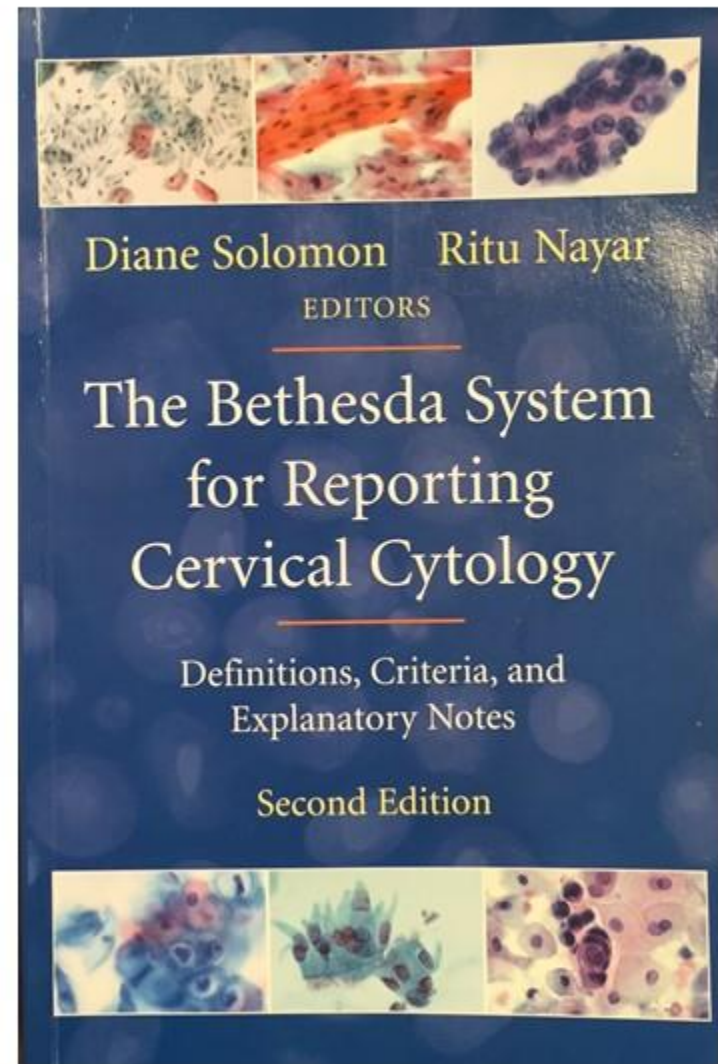
Objectives

- 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



TBS 1: 1991



TBS 2: 2001

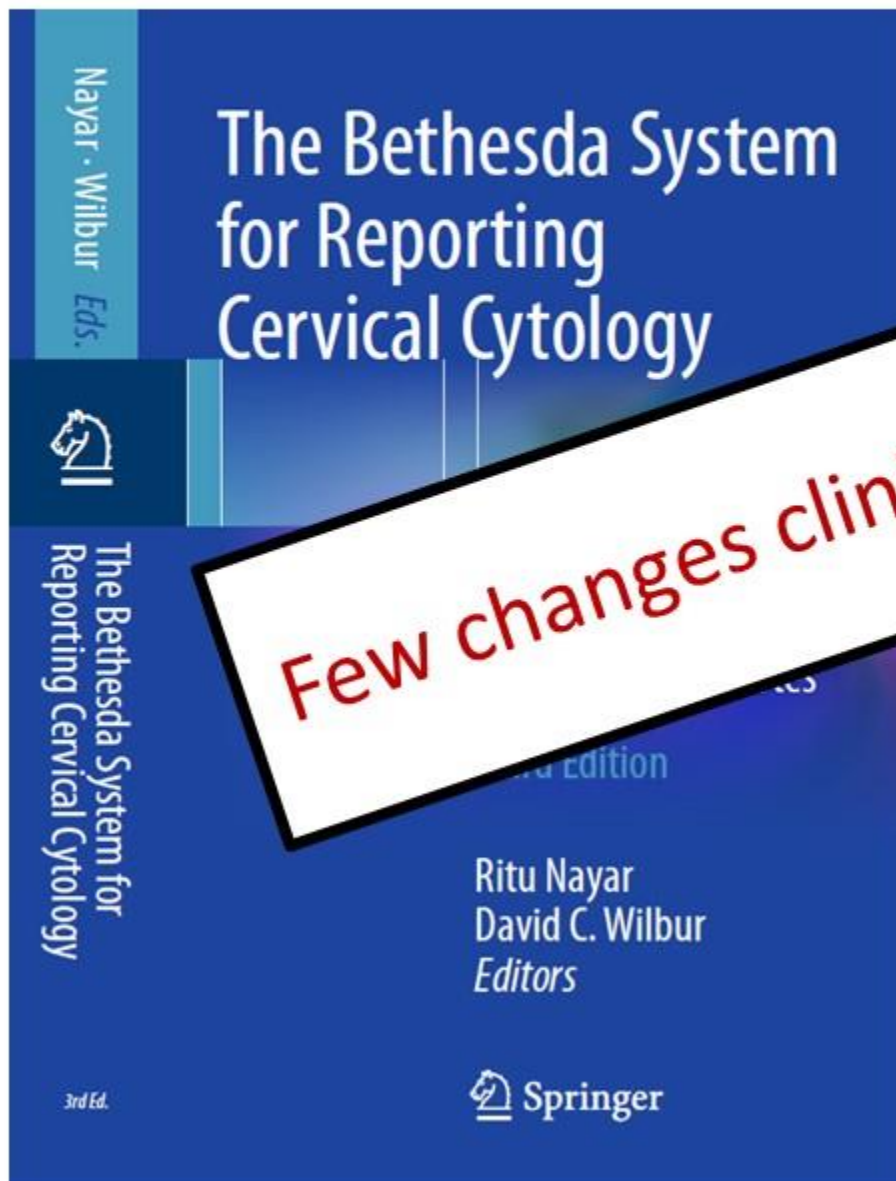
The Bethesda System

- 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

Few changes clinicians may notice

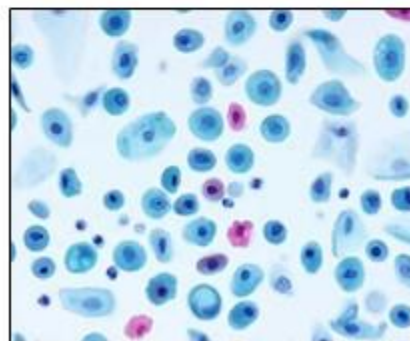
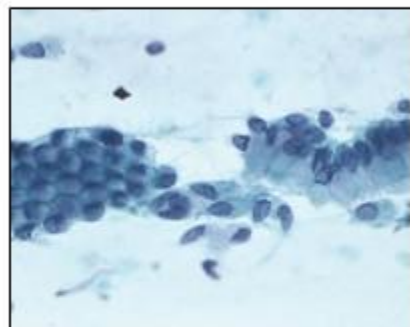
- Greater page content
- Updated recommendations
- Increased background
 - Literature review
 - Data in support
 - Biological descriptions
- Management issues for each entity

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 \neq Unsatisfactory Pap**



Quality Indicator: No t-zone on Pap

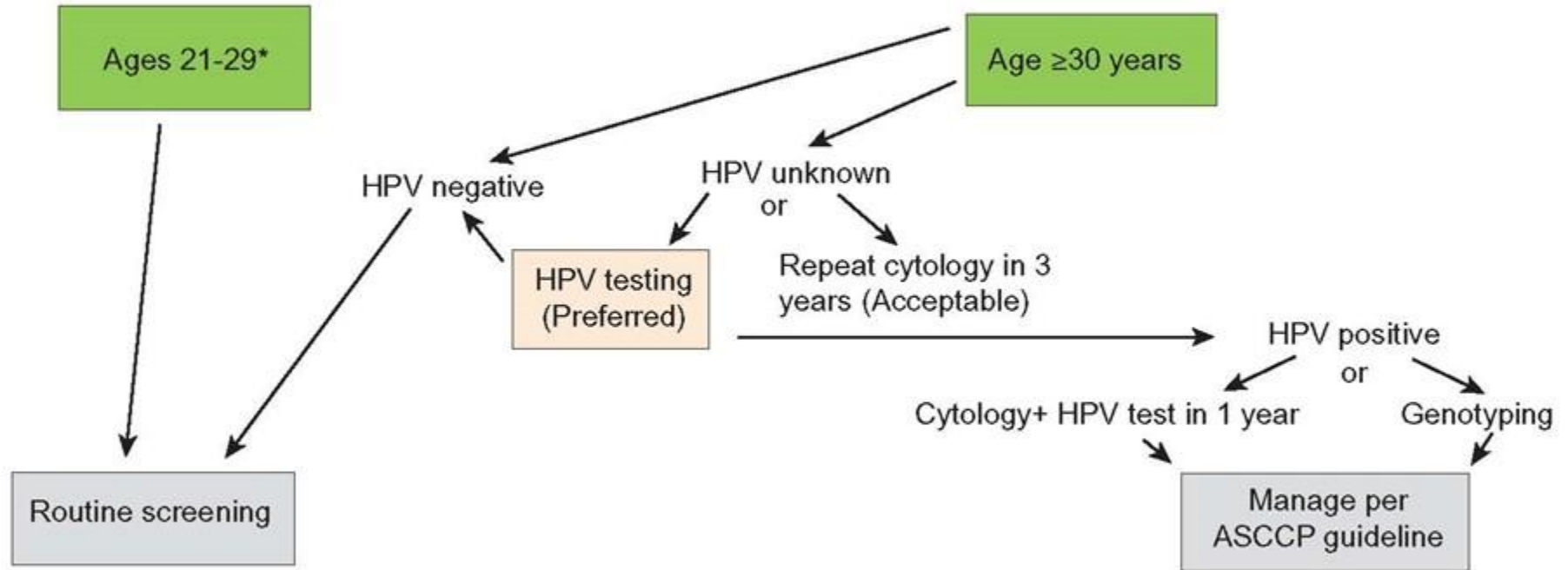
- No T-zone on approximately 10-20% of Paps
- More frequent in pregnant & older women
- Recent meta-analysis: Negative Pap →
 - Regardless +/- t-zone
 - Good specificity and NPV
- HPV test result independent of t-zone sampling

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 EC/TZ Absent/Insufficient



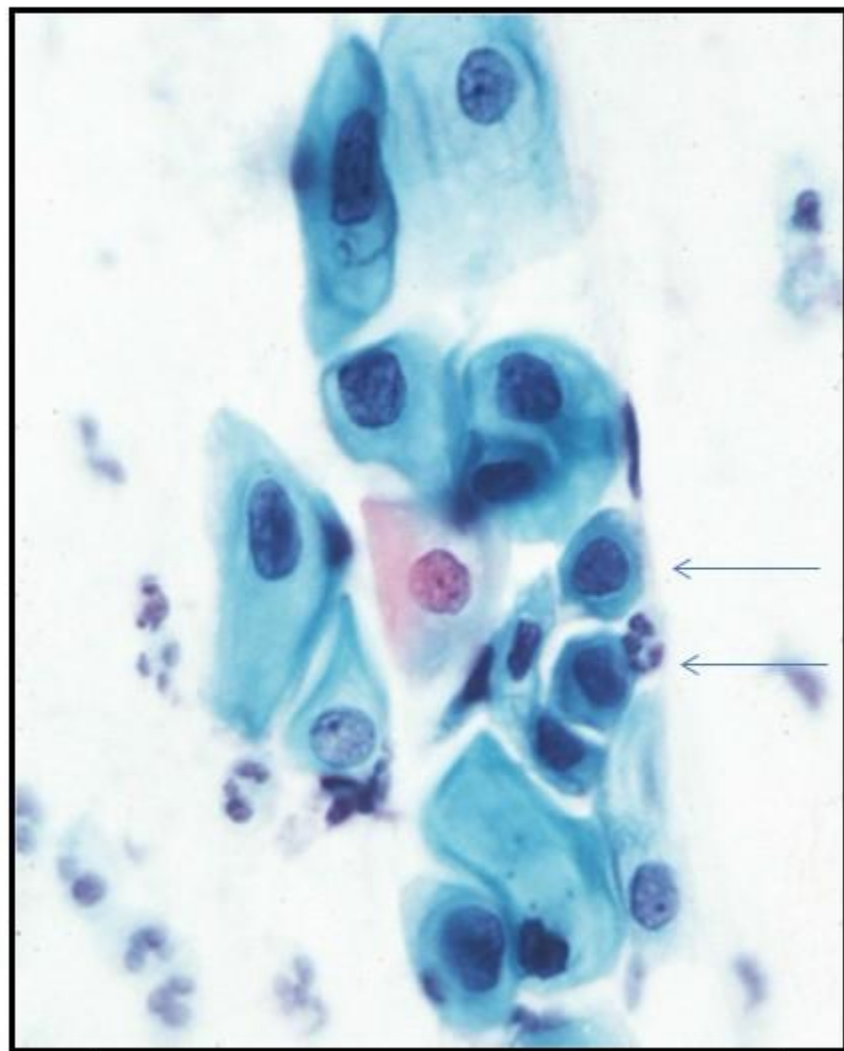
*HPV testing is unacceptable for managing women ages 21-29 years

© 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 concurrent HSIL

- 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
- TBS 1: Report benign EMs in post-menopause.
 - 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

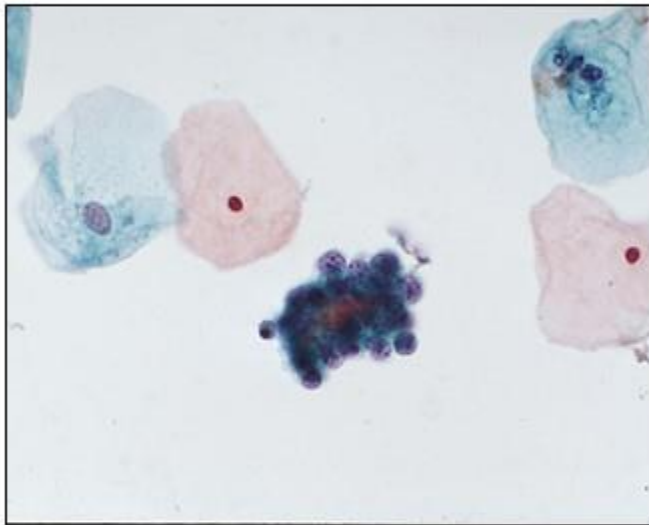
Consequence of 2001 Bethesda

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

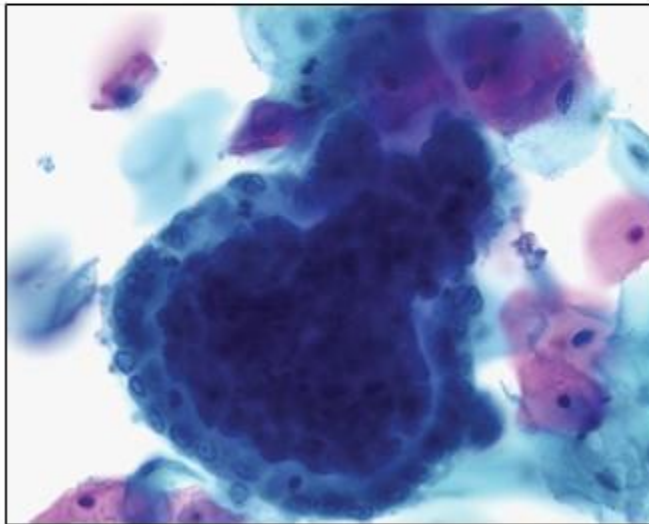
Risk Associated with Benign-appearing Endometrial cells on Pap

	Pre-2001	Post-2001
Hyperplasia	12%	2%
Cancer	6%	1%

Bethesda 3: Reporting Benign Endometrial cells on Pap



- Endometrial cells are present in a woman ≥ 45 years of age.
- Negative for squamous intraepithelial lesion.



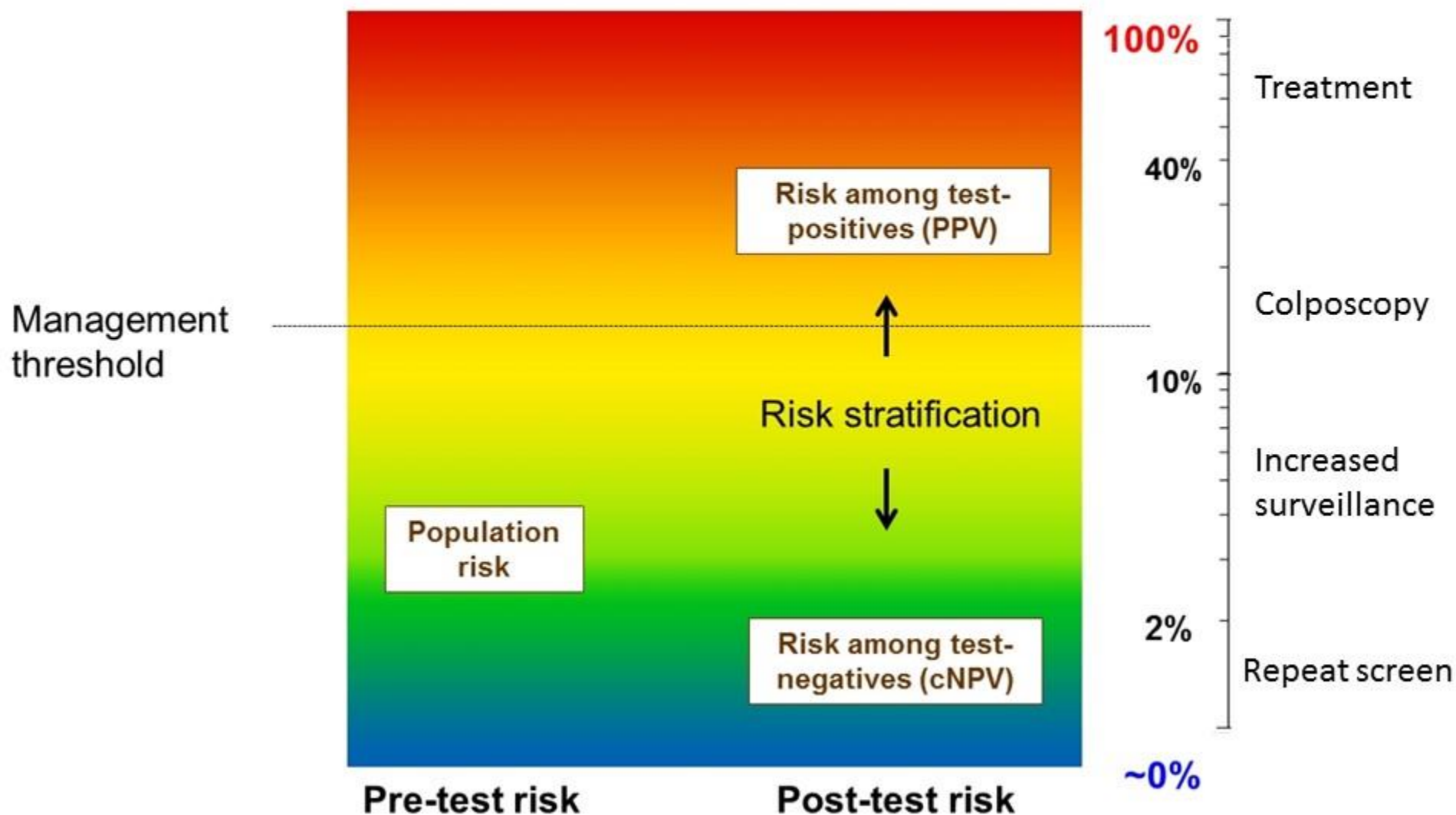
*Note: Endometrial cells in women 45 years and older may be associated with benign endometrium, hormonal alterations and less commonly, endometrial or uterine abnormalities. **Endometrial evaluation is recommended in postmenopausal women.***

Bethesda 3

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

Underlying Principle

Similar Management for Similar Risk



Pap Test as Benchmark: Similar Management for Similar Risk

Immediate colposcopy	SCC	HPV+/HSIL
	HSIL	HPV+/AGC
	ASC-H	HPV-/HSIL
	AGC	HPV+/ASC-H
6-12 month return	LSIL	HPV-/ASC-H
		HPV-/AGC
3-year return		HPV+/ASC-US
		HPV+/LSIL
5-year return	ASC-US	HPV+/NILM
		HPV-/LSIL
	NILM	HPV-/ASC-US
		HPV-/NILM
	Cytology result	Co-testing result

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Pap Test as **Benchmark**: Similar Management for Similar Risk

Immediate colposcopy	SCC	
	HSIL	HPV+/HSIL HPV+/AGC HPV-/HSIL HPV+/ASC-H
	ASC-H AGC	HPV-/ASC-H HPV-/AGC HPV+/ASC-US HPV+/LSIL
	LSIL	
6-12 month return	ASC-US	HPV+/NILM HPV-/LSIL
3-year return	NILM	HPV-/ASC-US
5-year return		HPV-/NILM
Cytology result		Co-testing result

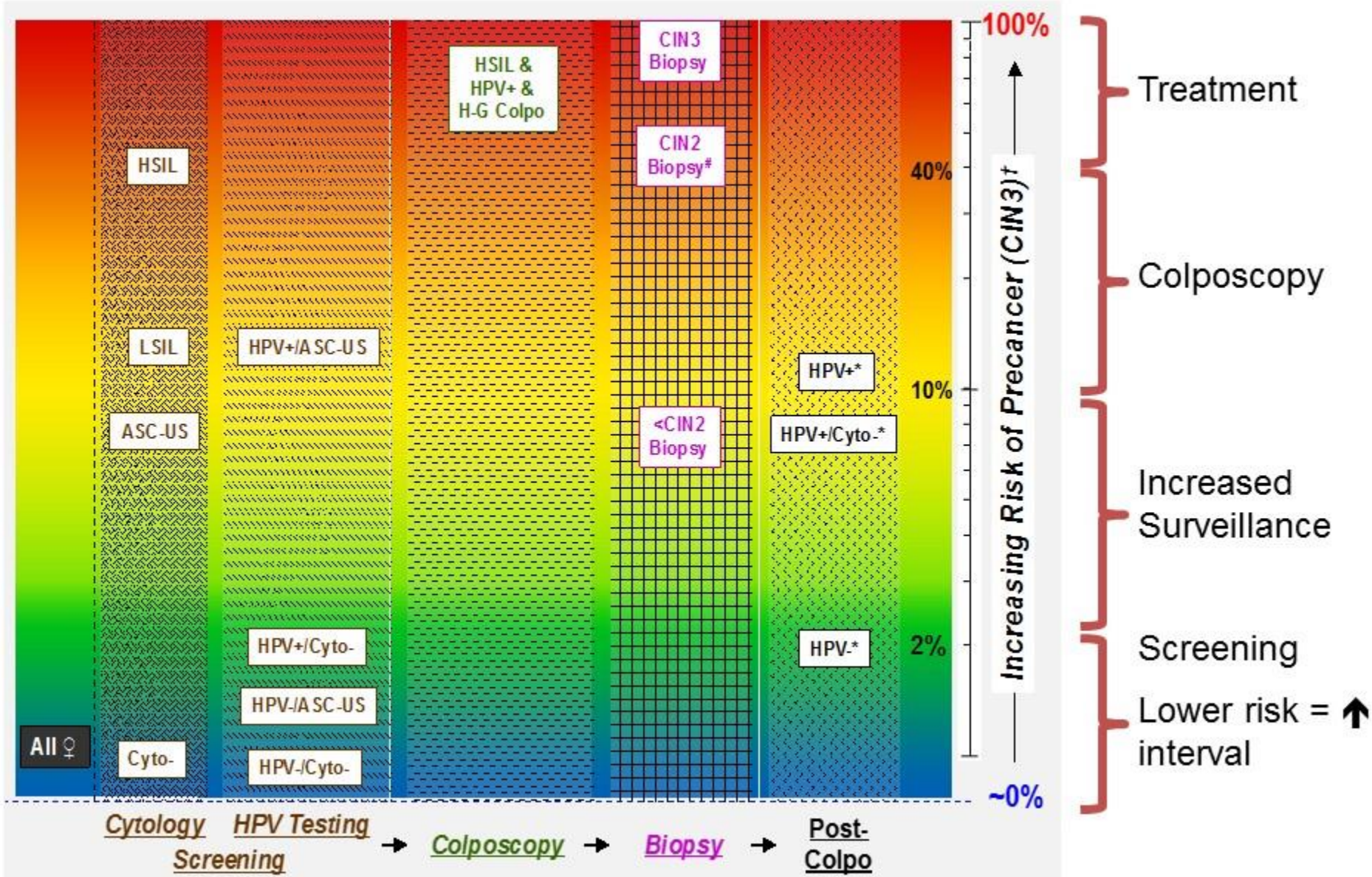
Management options



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

Similar management for similar risk

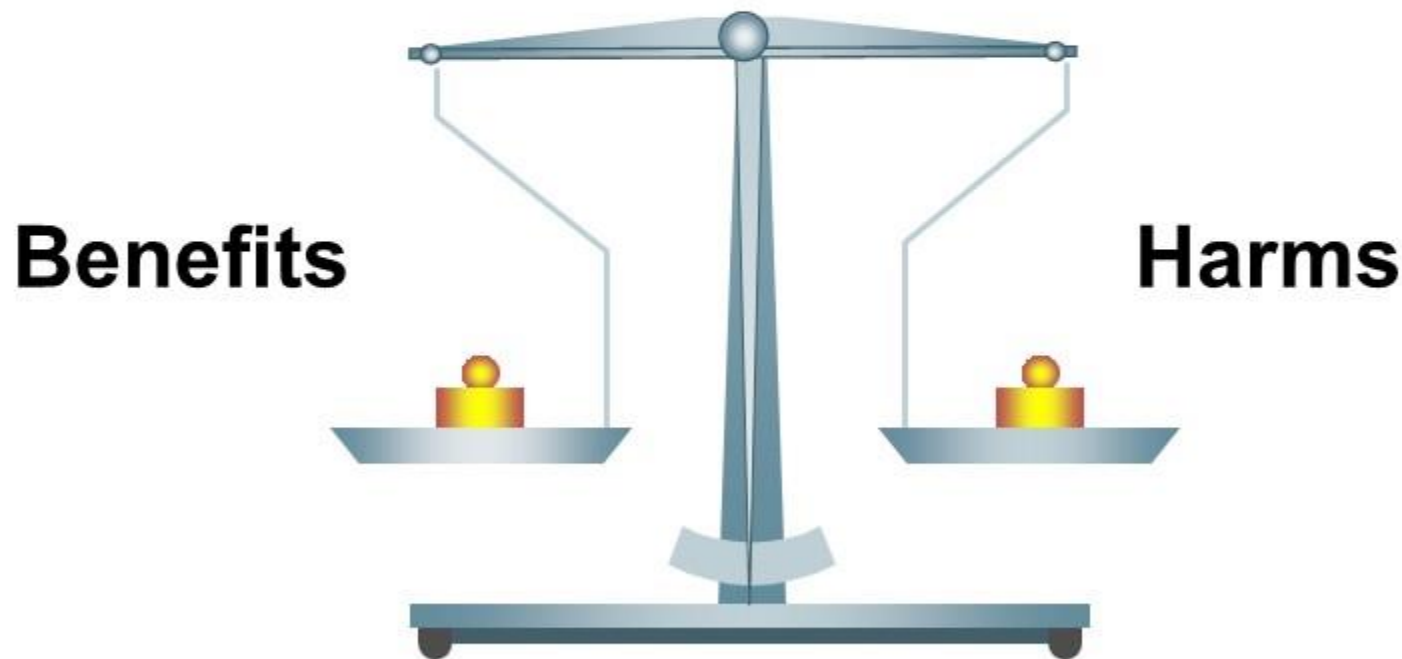
Harmonizing Management According To Risk



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



Similar management for similar risk

The LAST Project

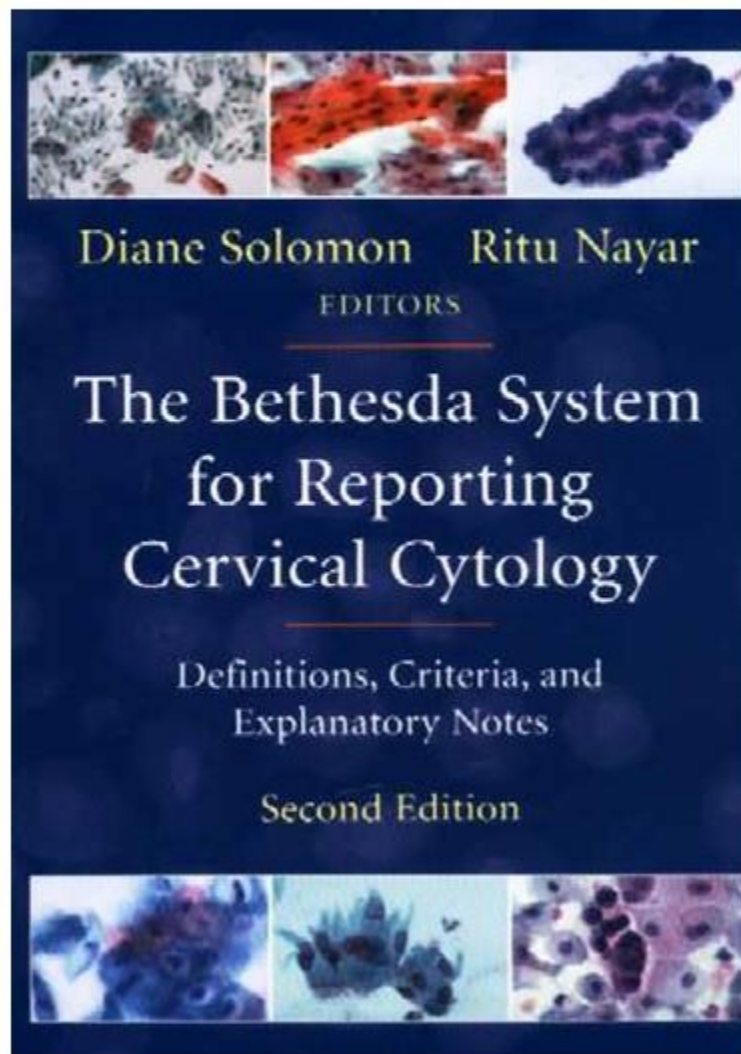
Lower Anogenital Squamous Terminology
standardization project for
histopathologic diagnoses of
HPV-associated squamous lesions
of the lower anogenital tract



cap



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 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

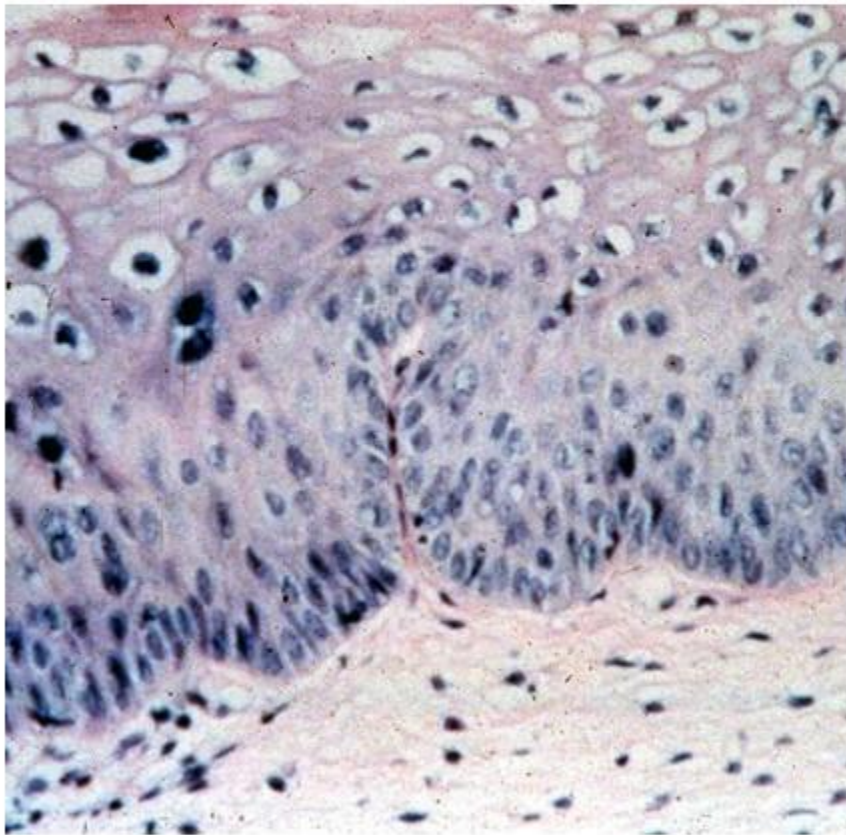
? False Premises ?

- 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.

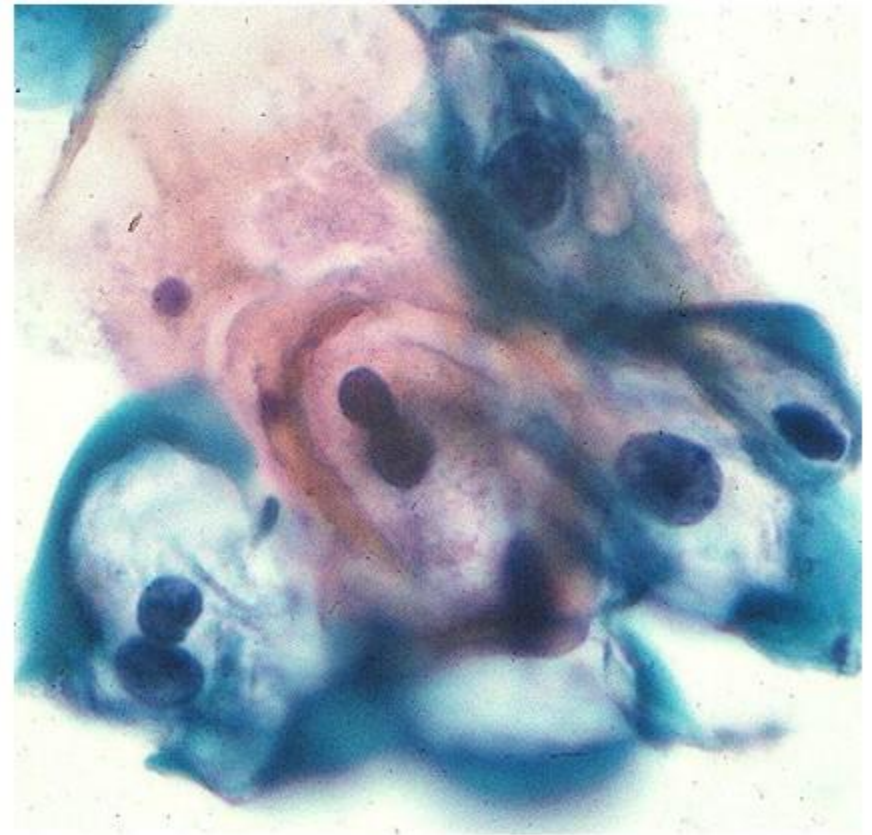
LSIL:

Virion production & transient lesions

LSIL (CIN1)



LSIL

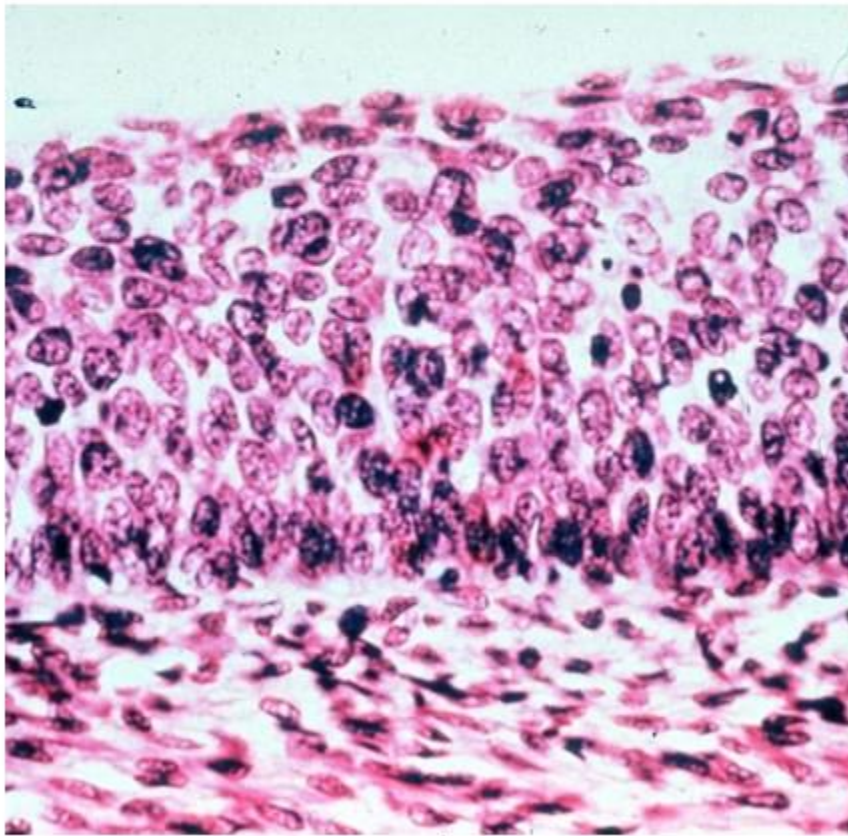


Productive infection

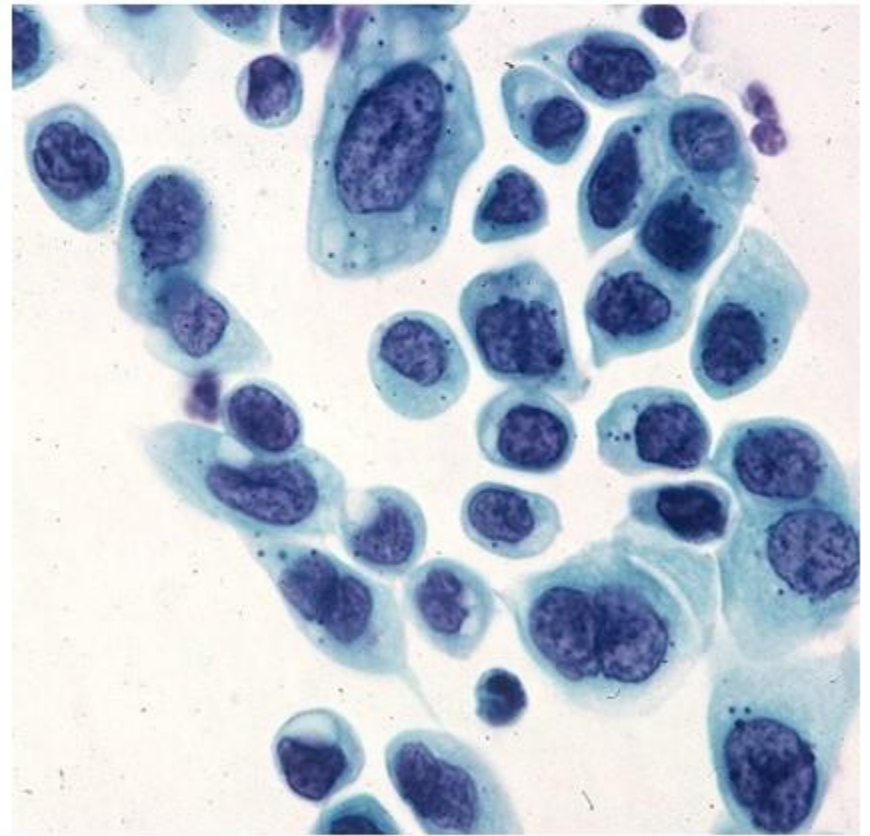
HSIL:

HPV E6/E7 expression & risk of cancer

HSIL (CIN3)



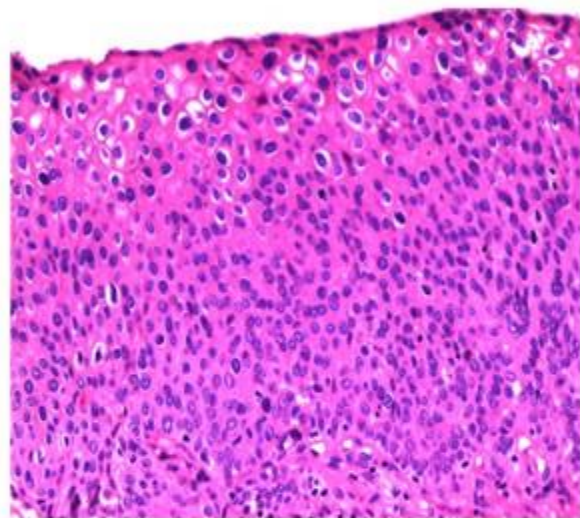
HSIL



Transforming infection

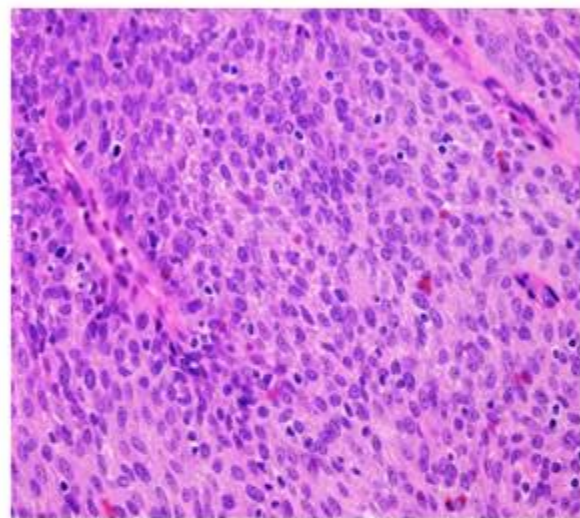
HPV-associated precancers: Unified morphology

CIN3

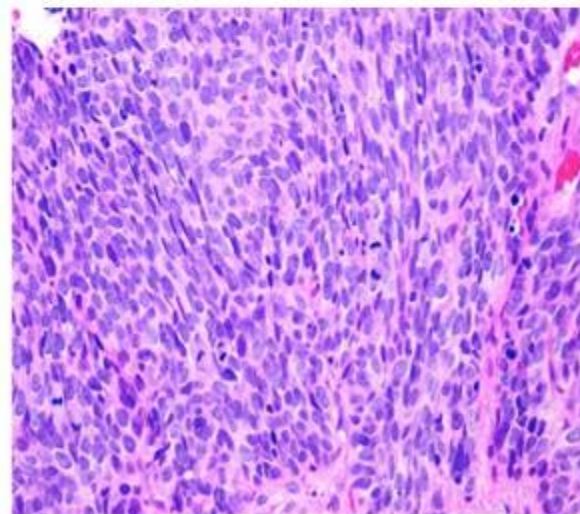


Mucosal

AIN3

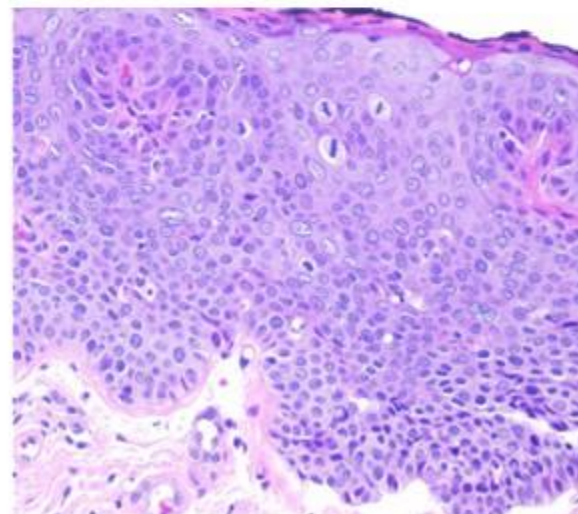


VIN3



Cutaneous

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

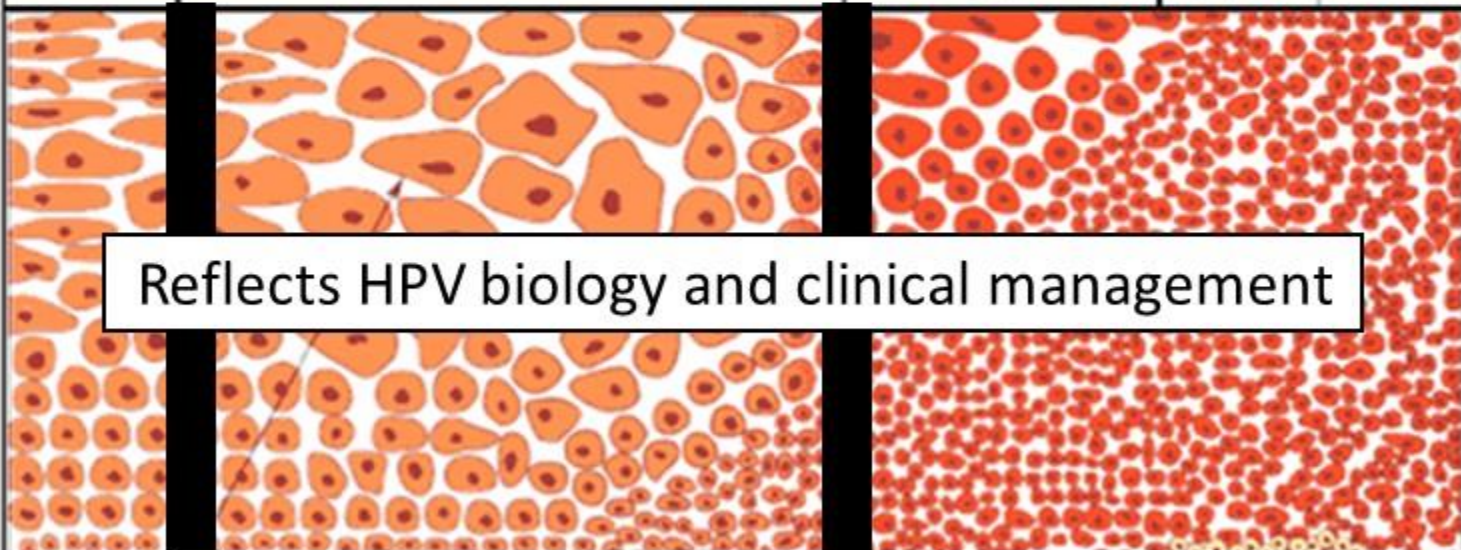
The LAST Project:

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)***



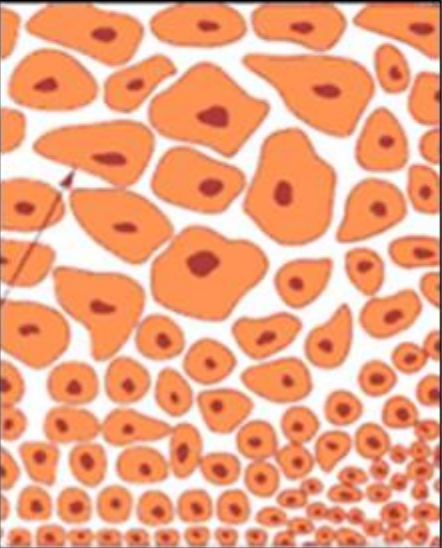



May be further classified by the applicable
–IN subcategorization.

2-tiered system: LSIL & HSIL

Schematic Representation of SIL				
Normal	Low-grade squamous intraepithelial lesion (LSIL)		High-grade squamous intraepithelial lesion (HSIL)	
	Condyloma	CIN/AIN grade 1	CIN/AIN grade 2	CIN/AIN grade 3
	Very mild to mild dysplasia		Moderate dysplasia	Severe dysplasia
				
Koilocytes				
Infection &			Precancer	

Diagnostic Variation

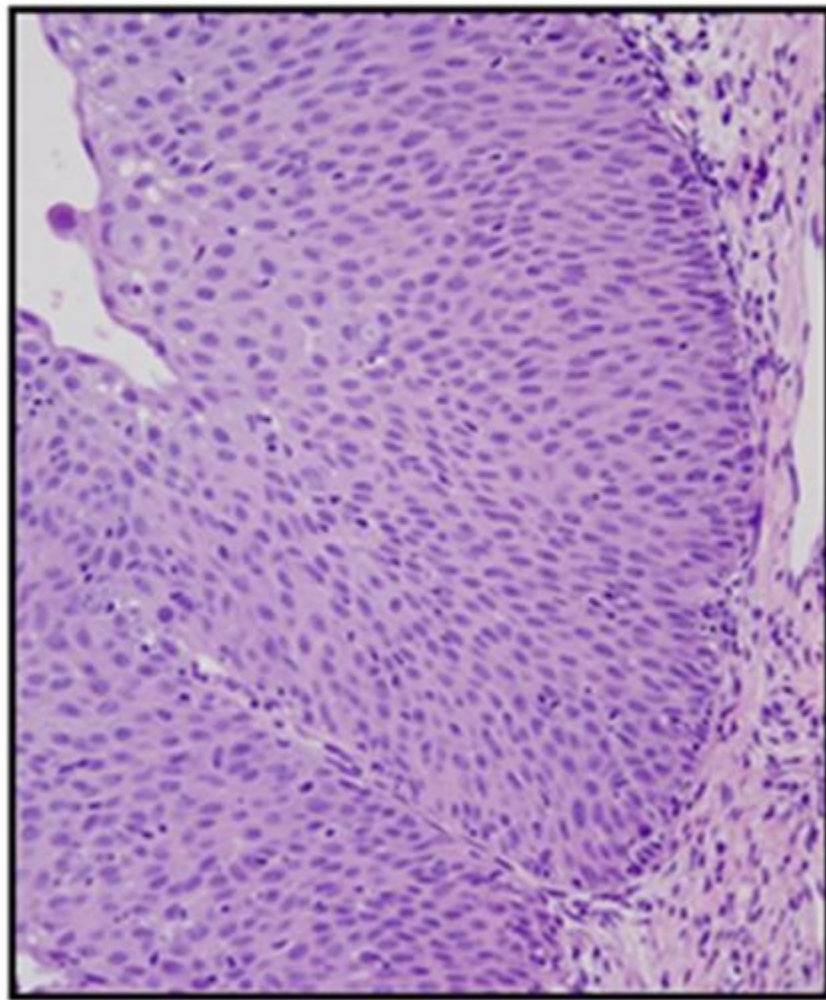
Schematic Representation of SIL

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	Condyloma	CIN/AIN grade 1	CIN/AIN grade 2	CIN/AIN grade 3	
	Very mild to mild dysplasia		Moderate dysplasia	Severe dysplasia	<i>In Situ</i> carcinoma
					

Interobserver variability & Diagnostic (un)certainty

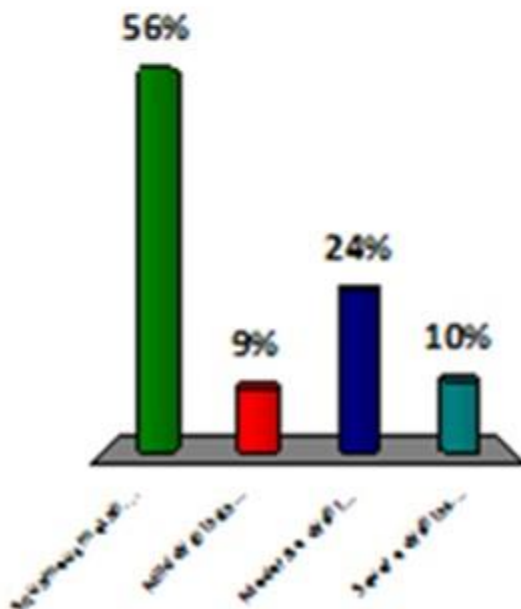
UCSF CME May 2014

Diagnostic variation: What is your diagnosis?



Cervical biopsy

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:

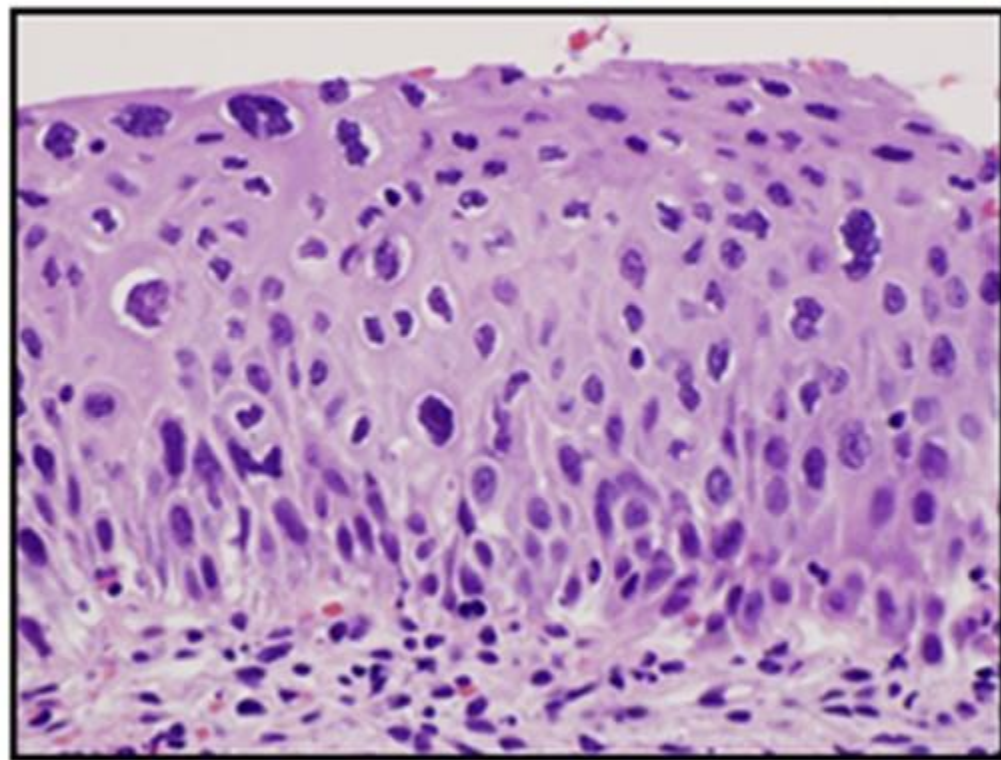
Strength of agreement

- **< 0.20 Poor**
- 0.21 - 0.40 Fair
- 0.41 - 0.60 Moderate
- 0.61 - 0.80 Good
- 0.81 - 1.00 Very good

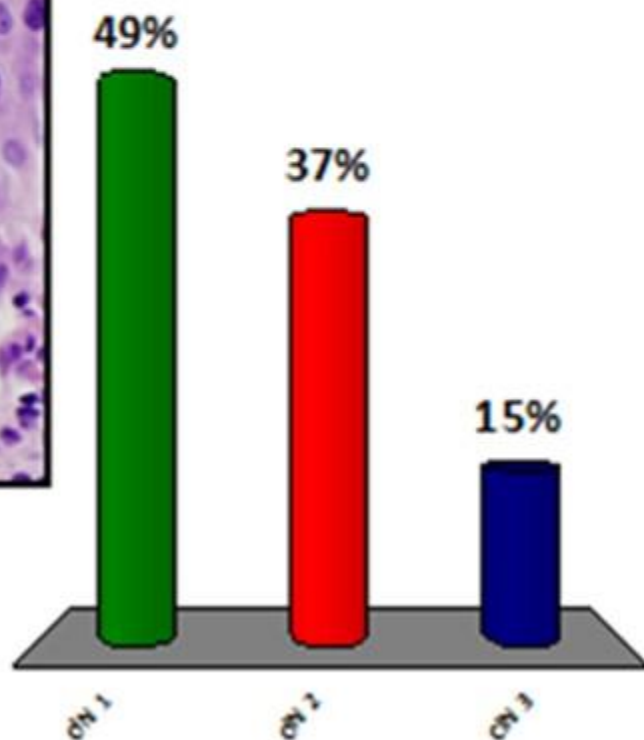
*Observer variability in histopathological reporting
of cervical biopsy specimens. J Clin Pathol 1989;42:231-8.
Robertson AJ, Anderson JM, Beck JS, et al.*

UCSF CME May 2014:

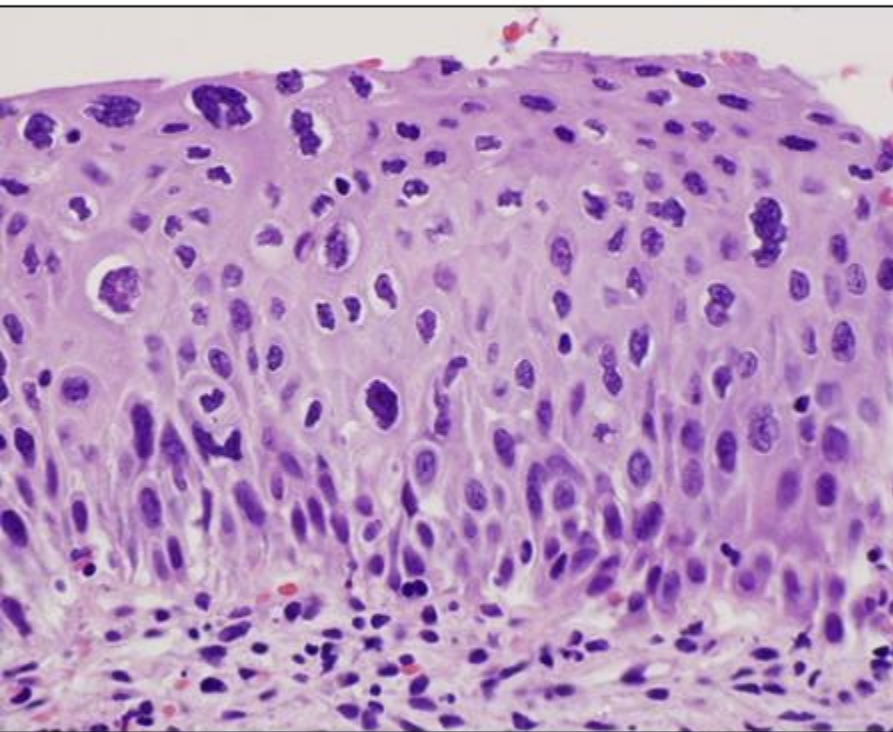
CIN Grade?



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



What is -IN2?



Does not reflect our
current understanding:
infection vs. precancer

- A Distinct Biologic Stage?
- Ugly Looking -IN1?
- Not So Ugly -IN3?
- An equivocation that is NOT reproducible
- A representation of incomplete sampling
- $\sim 2/3$ HSIL; $\sim 1/3$ LSIL
- A management safety net?

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

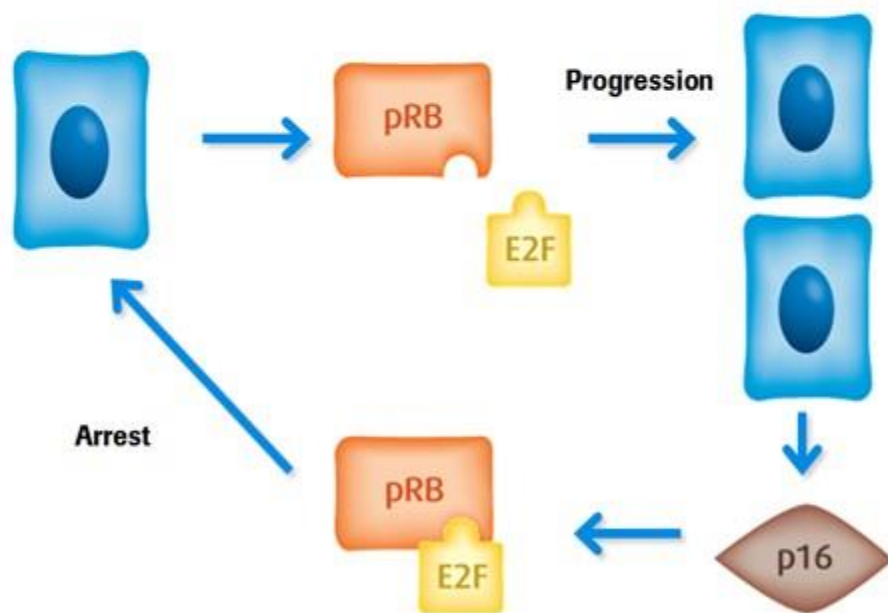
- Diagnostic variation can be improved by:
 - Limiting the number of tiers
 - The use of biologic markers, such as:
 - **p16**
 - Ki-67
 - ProEx C
- *Add objectivity to the art..*



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

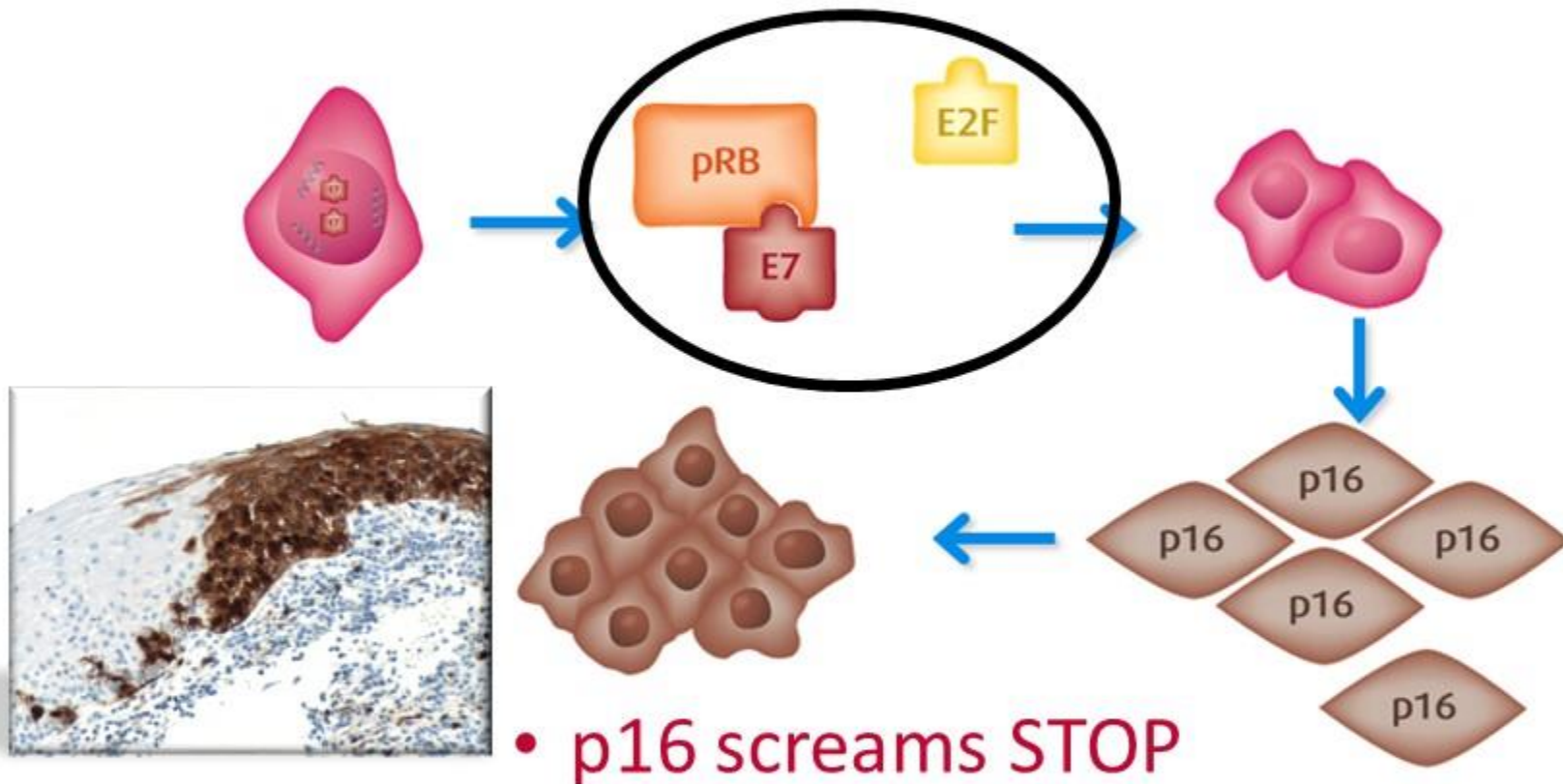
p16 and Normal cell cycle progression



- 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



- 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 detectable 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

p16^{INK4a} Immunohistochemistry in Cervical Biopsy Specimens

A Systematic Review and Meta-Analysis of the Interobserver Agreement

Miriam Reuschenbach, MD,¹ Nicolas Wentzensen, MD,² Maaike G. Dijkstra, MD,³
Magnus von Knebel Doeberitz, MD,¹ and Marc Arbyn, MD⁴

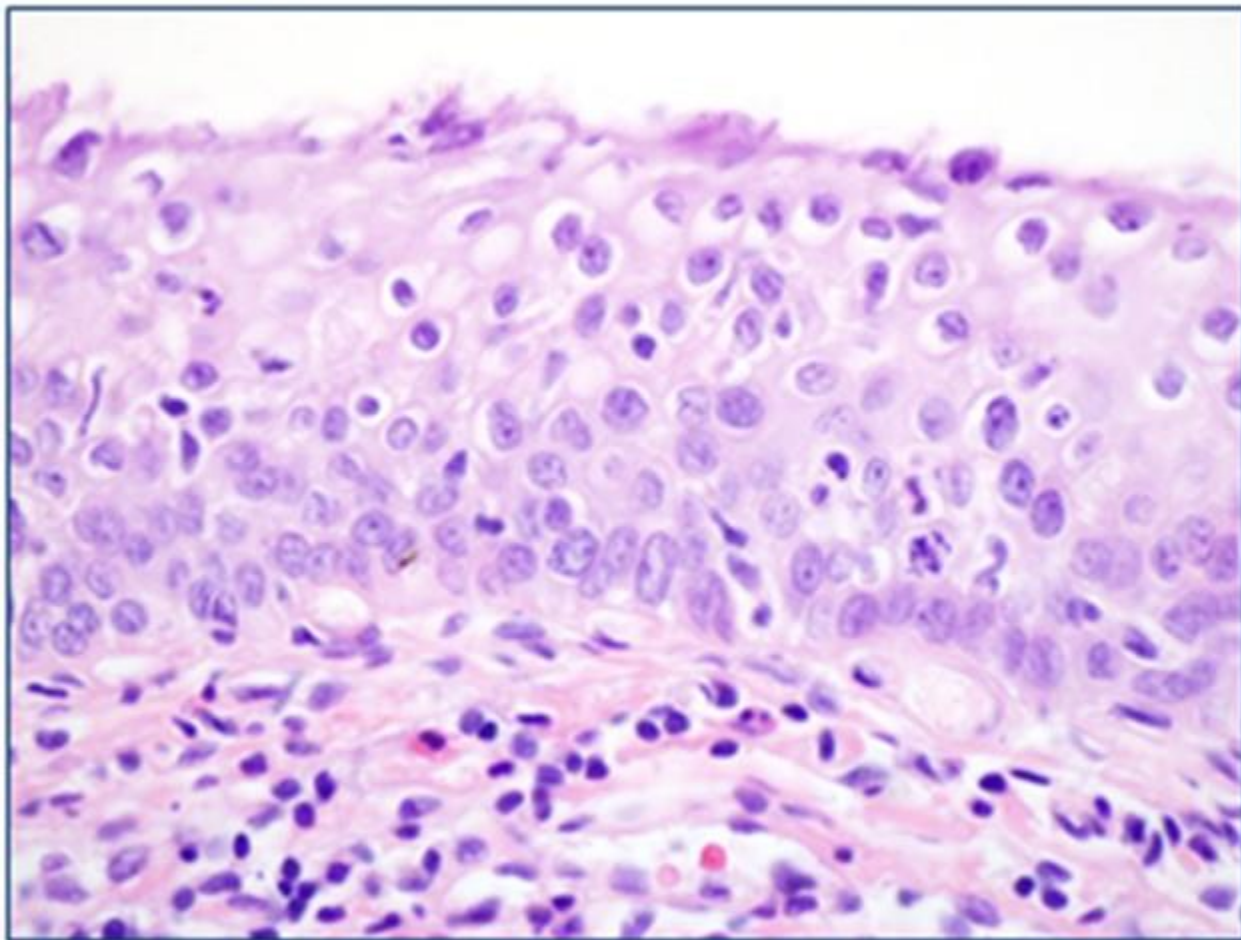
The published literature indicates ***improved interobserver agreement*** of the diagnosis of CIN2+ with the conjunctive use of H&E morphology with p16^{INK4a} immunohistochemistry compared with H&E morphology alone.

When do we use p16?

LAST Recommendations

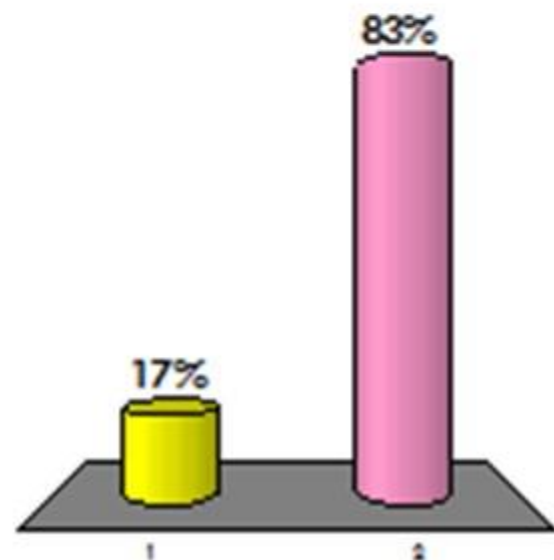
1. HSIL vs. Mimic
2. Query -IN2
3. Difference in opinion
4. NOT for obvious -IN1 or -IN3
 - 4a. “*a priori*”: When no histologic HSIL is found on biopsy in “high-risk” situations – prior Pap with HSIL, ASC-H, HPV16+ ASC-US, AGC (NOS)

DDx: HSIL vs. Mimic

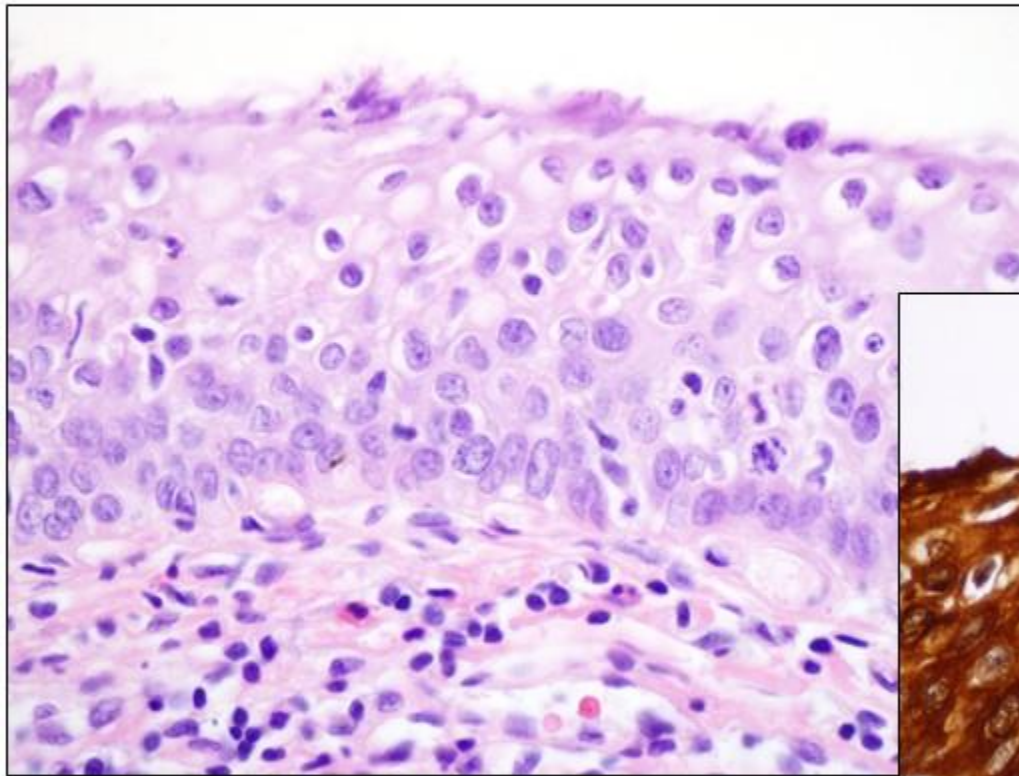


CAP '14

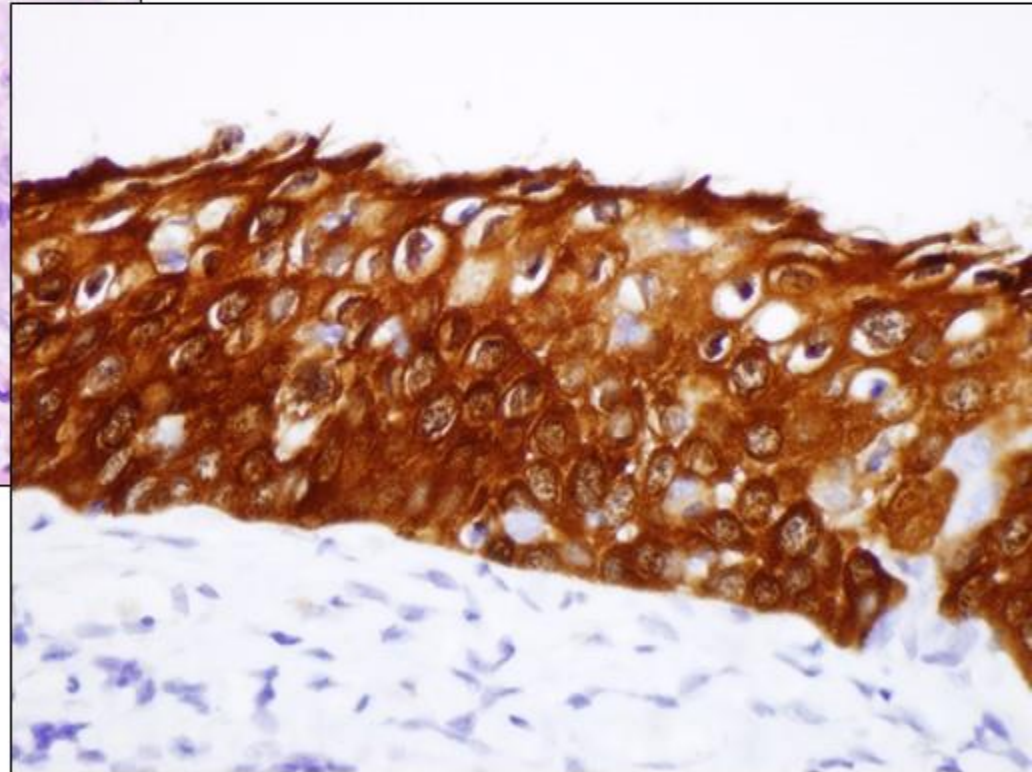
1. HSIL
2. Mimic of HSIL



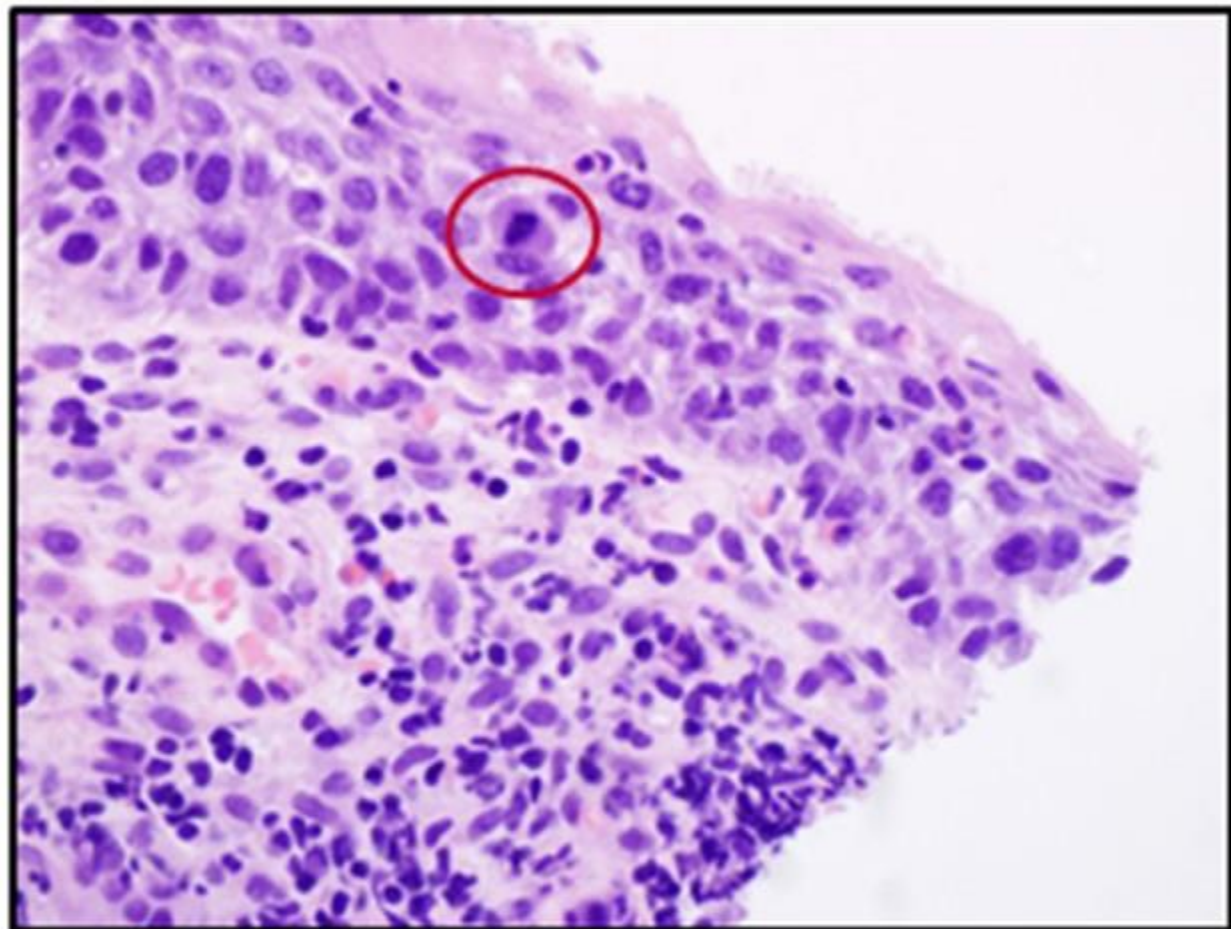
DDx: HSIL vs. Mimic



p16 positive = HSIL

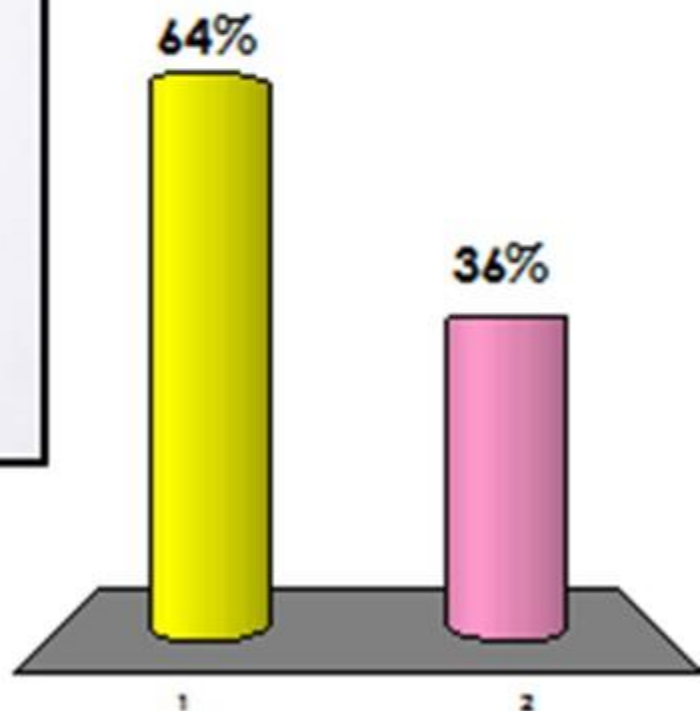


DDx: HSIL vs. Reactive



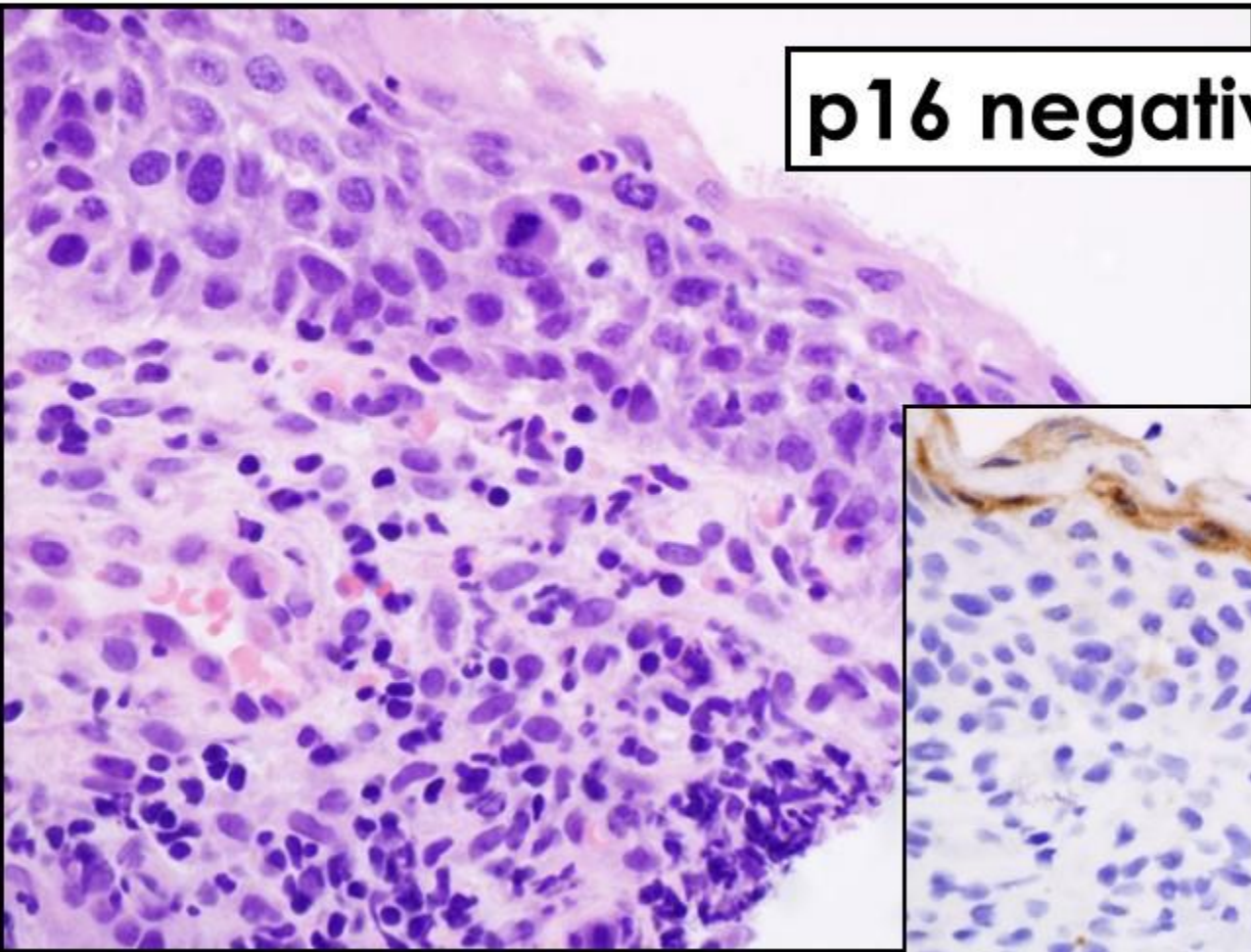
CAP '14

1. HSIL
2. Reactive

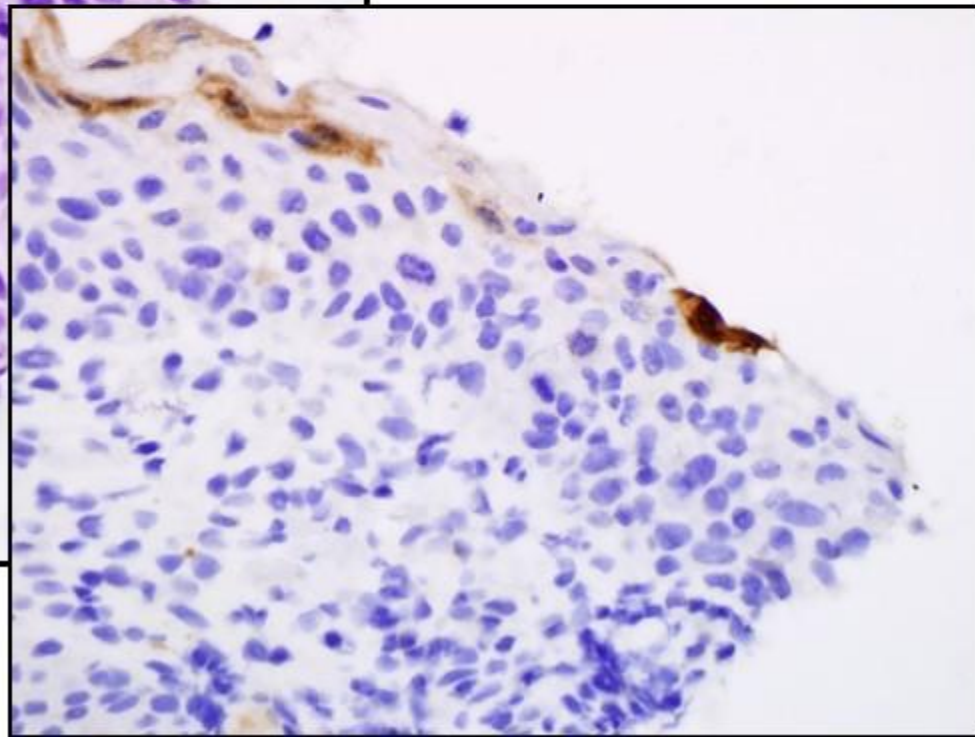


DDx: HSIL vs. Reactive

p16 negative = Reactive



Cervical Biopsy

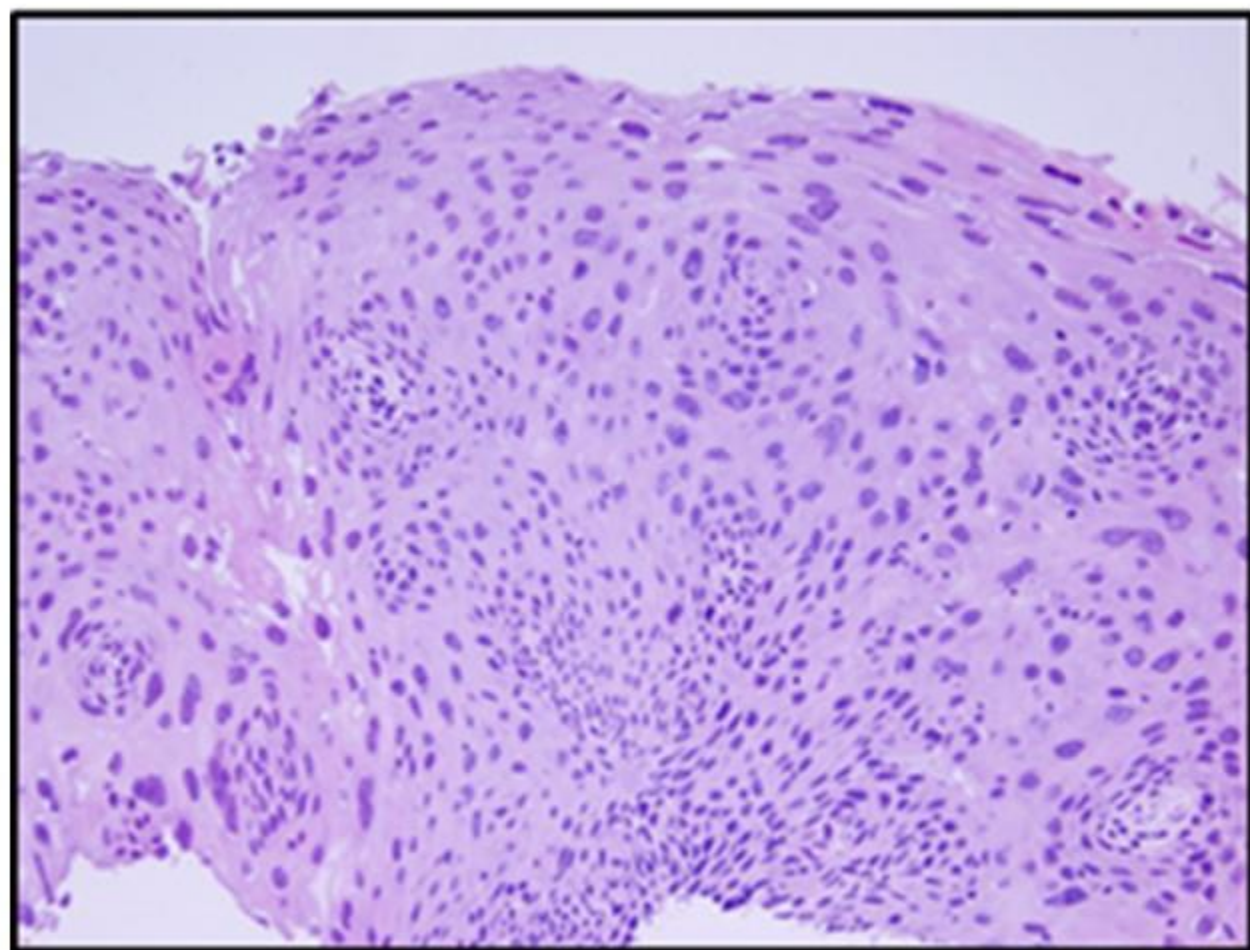


When do we use p16?

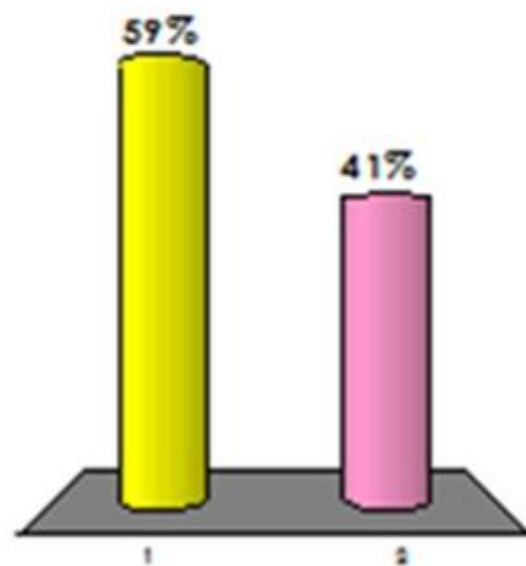
LAST Recommendations

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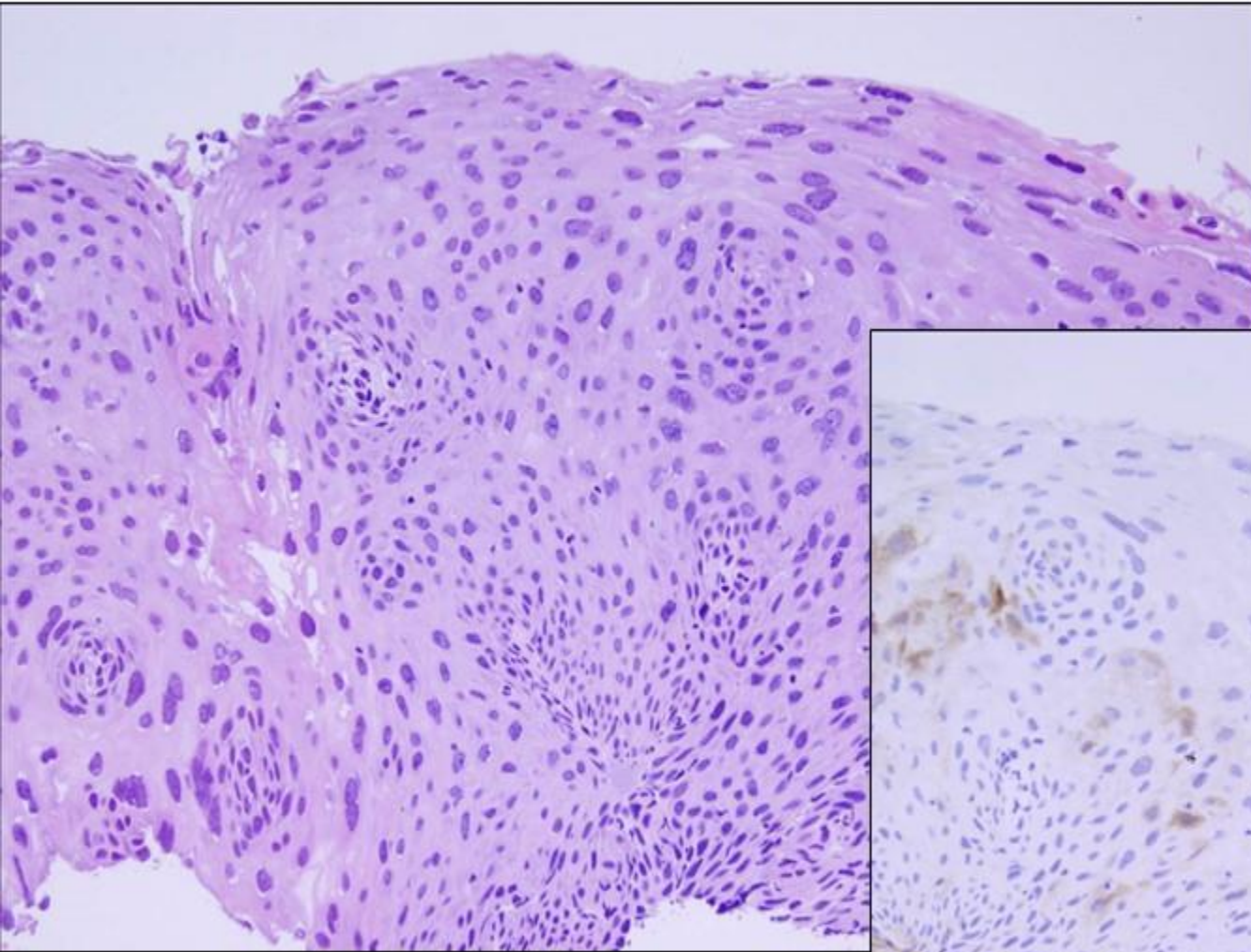
Query CIN 2



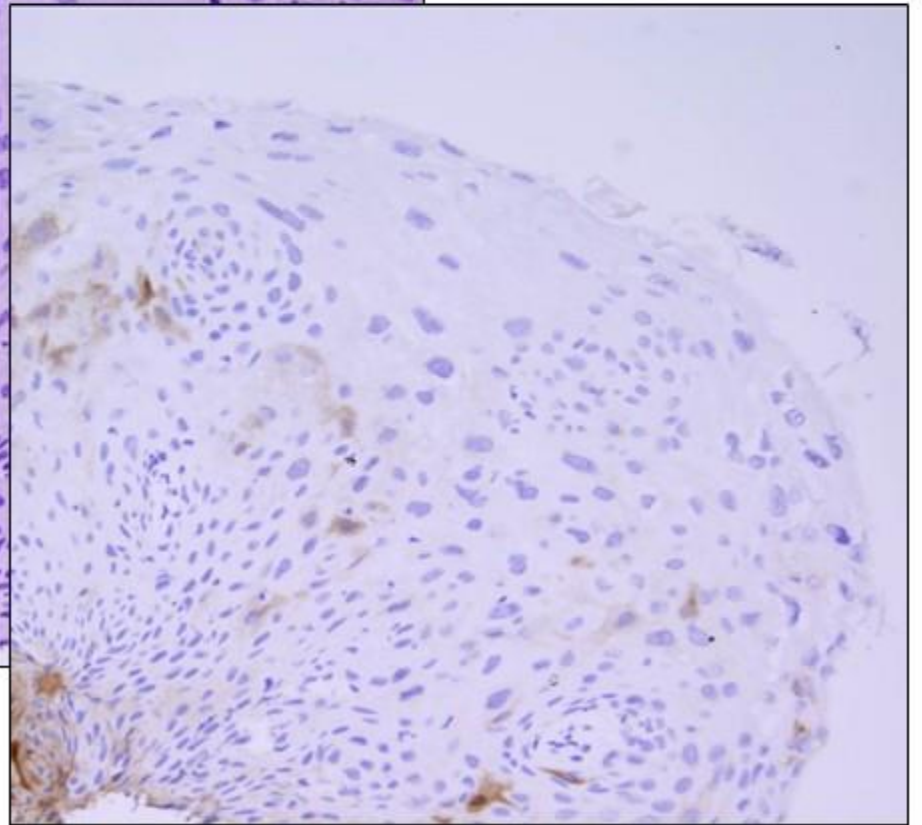
1. LSIL
2. HSIL



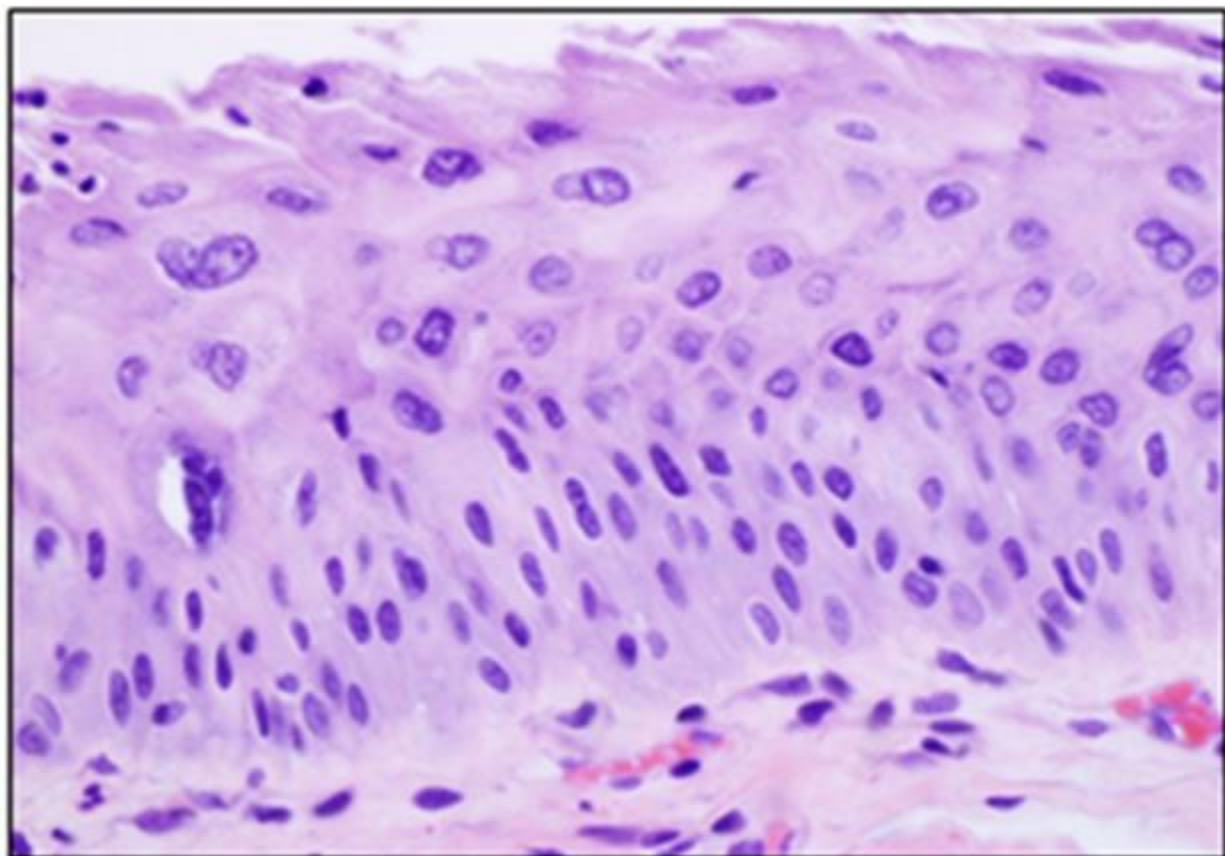
Query CIN 2



p16 negative
= LSIL

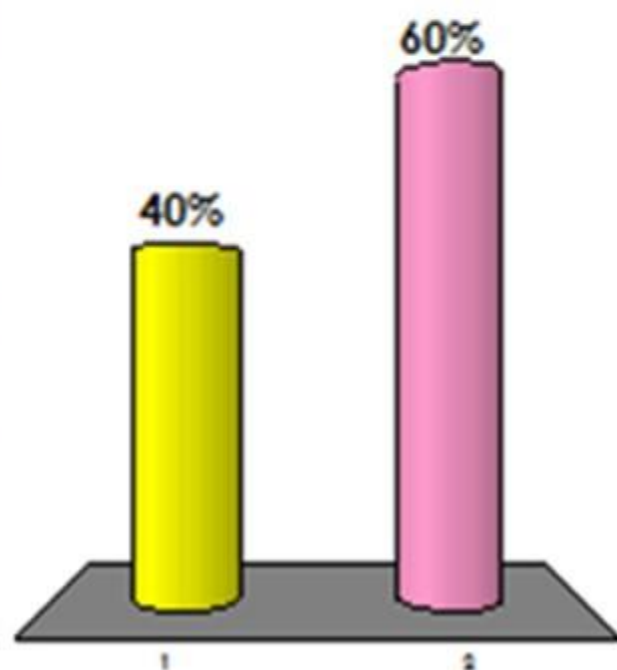


Query AIN 2

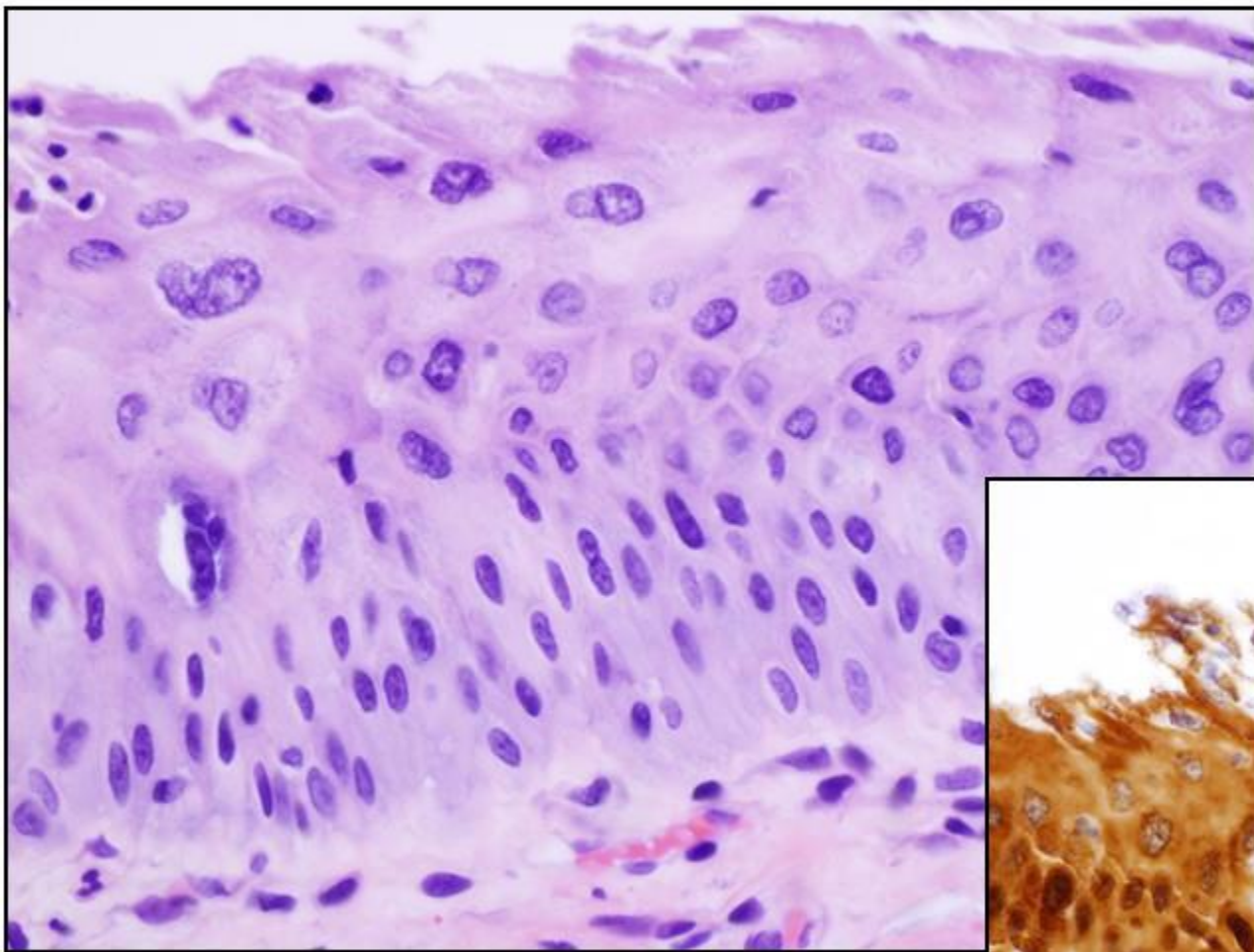


1. LSIL
2. HSIL

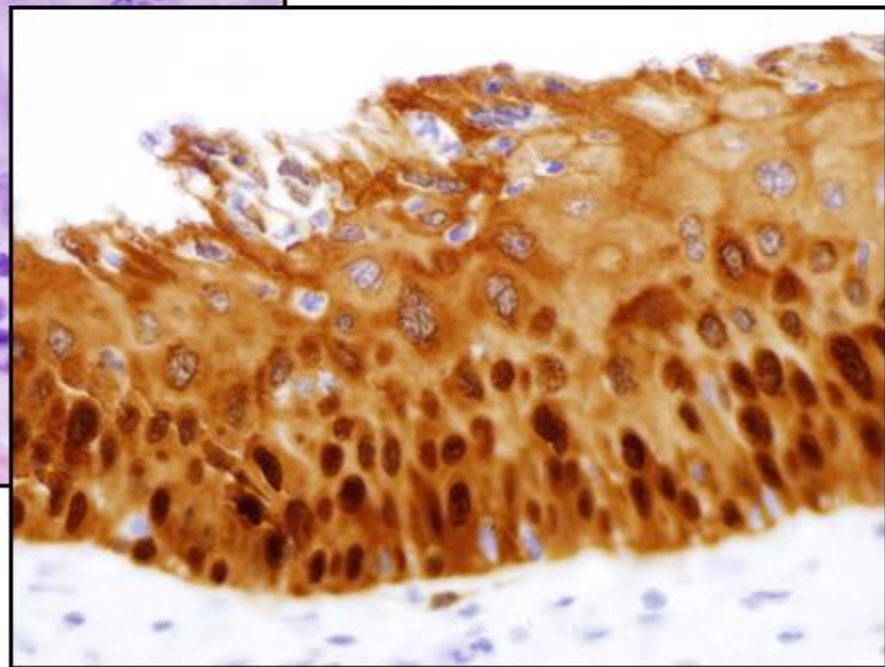
CAP '14



Query AIN 2



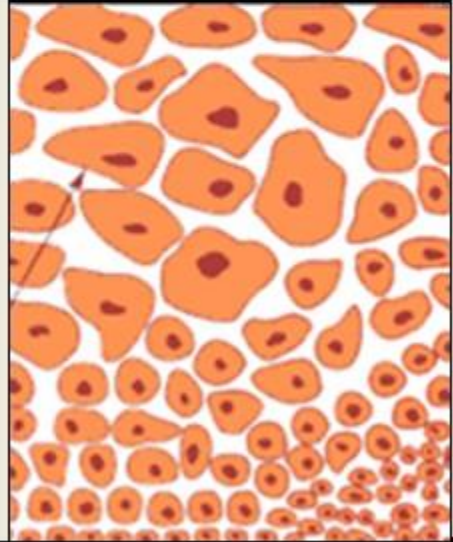




p16 +




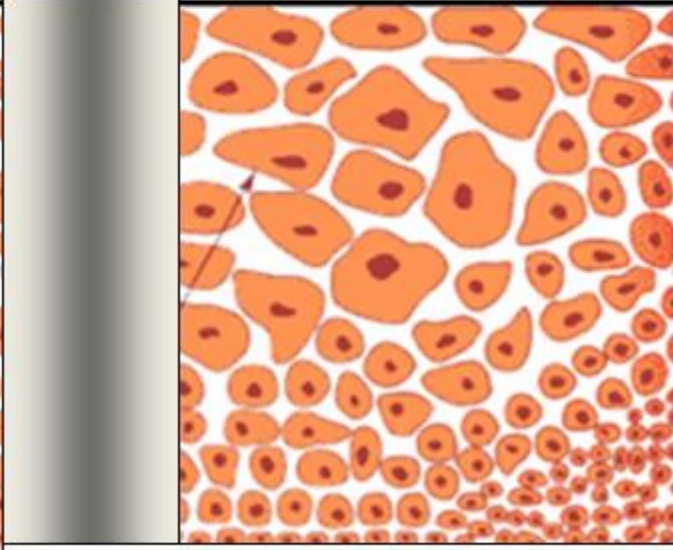
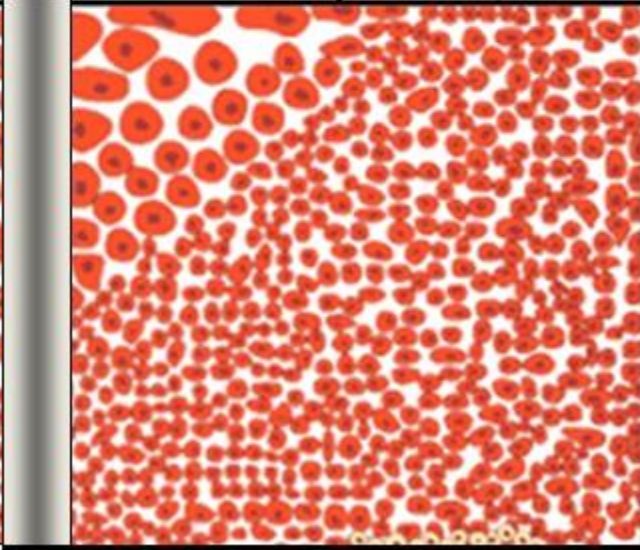
HSIL (AIN2)

HPV Biology: Infection vs. Precancer

Schematic Representation of SIL				
Normal	Low-grade squamous intraepithelial lesion (LSIL)		High-grade squamous intraepithelial lesion (HSIL)	
	Condyloma	CIN/AIN grade 1	CIN/AIN grade 2	CIN/AIN grade 3
	Very mild to mild dysplasia		Moderate dysplasia	Severe dysplasia <i>In Situ</i> carcinoma
				
Koilocyt	Biology &		Management	

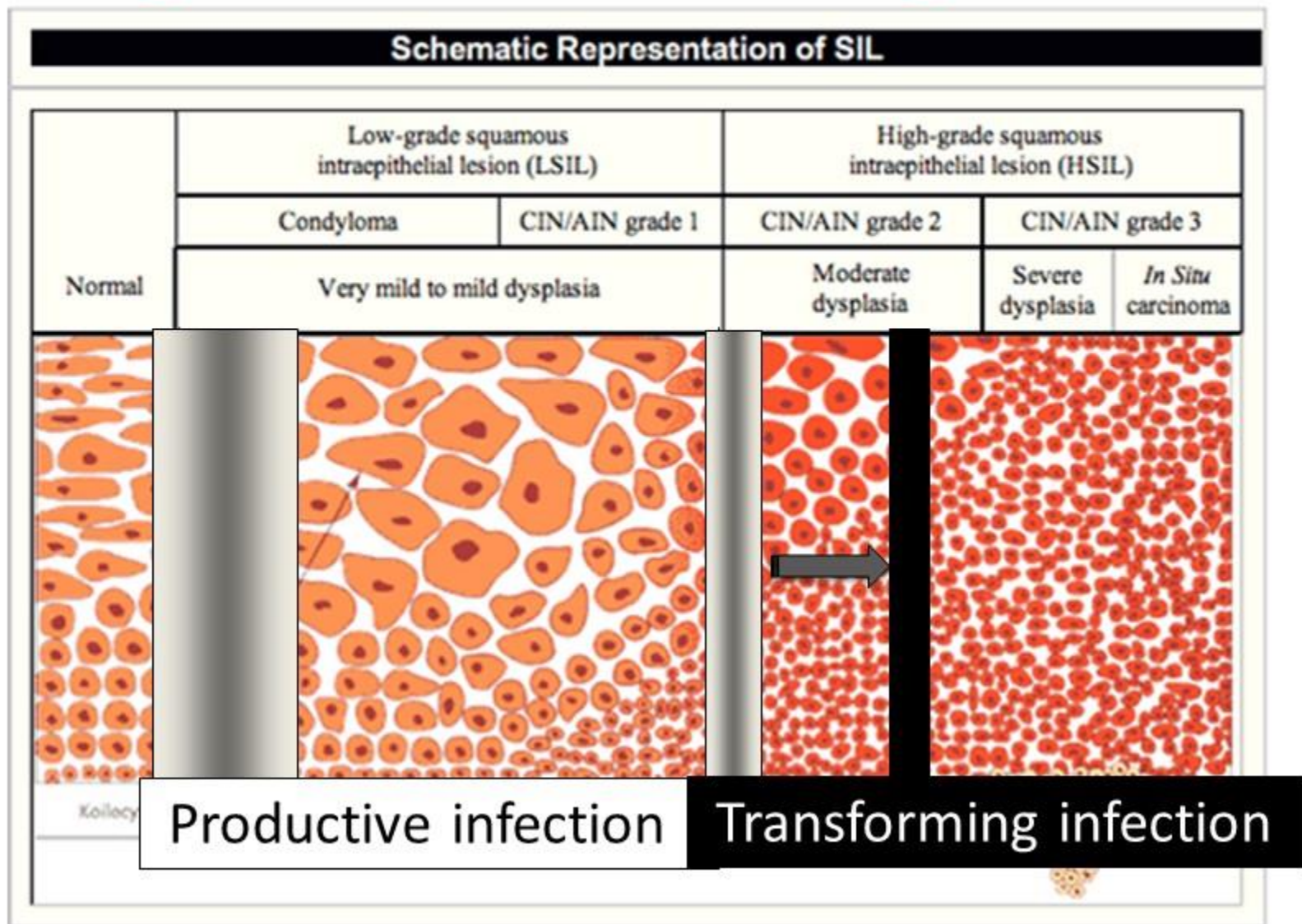
Biomarkers – Add Objectivity: Reduce diagnostic variation

Schematic Representation of SIL

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Koilocyte	Biology &		Management		

Biomarkers: p16

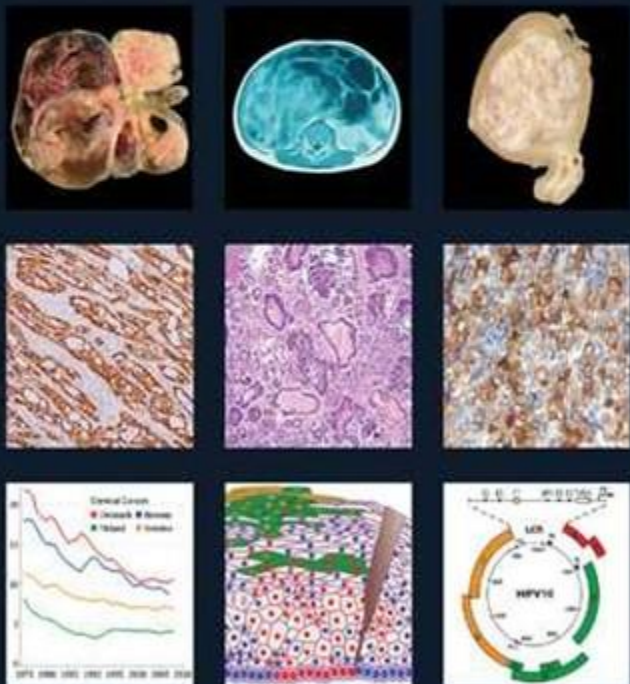
Surrogate for transforming infection



Updates: WHO Blue Book

WHO Classification of Tumours of Female Reproductive Organs

Edited by Robert J. Kurman, Maria-Luisa Carcangiu, C. Simon Herrington, Robert H. Young



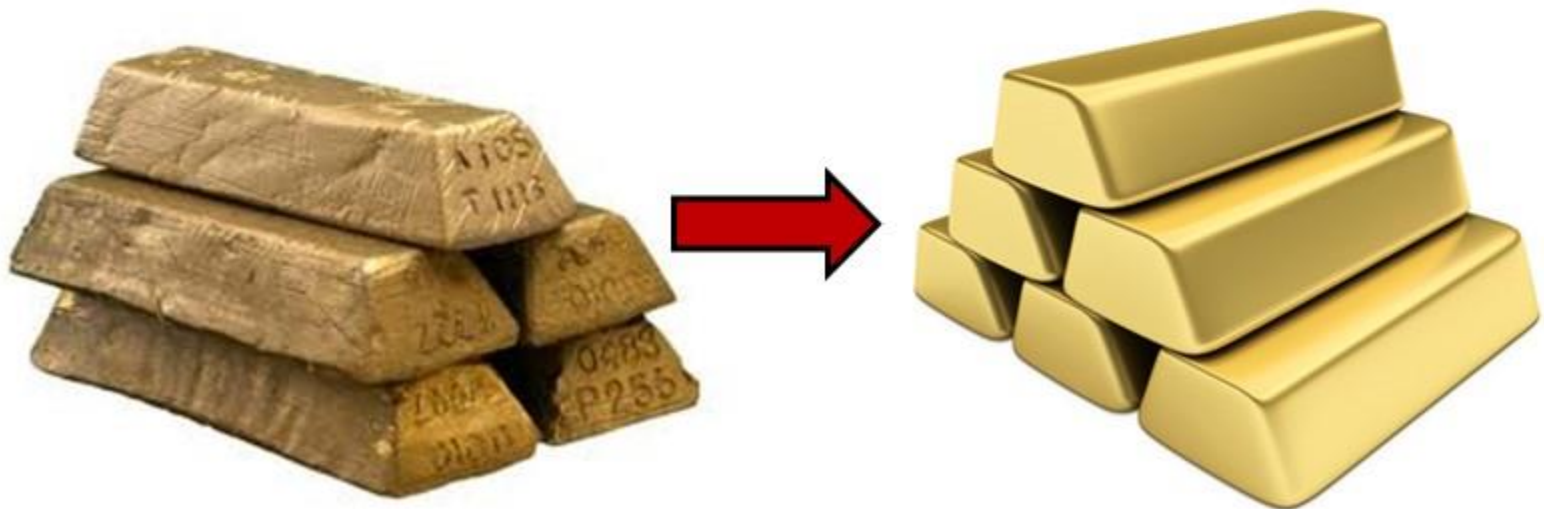
- Adopted the LAST Project's terminology for the cervix, vulva and vagina
- 4th edition
- Published April 2014

The LAST Project

Lower Anogenital Squamous Terminology Standardization Project



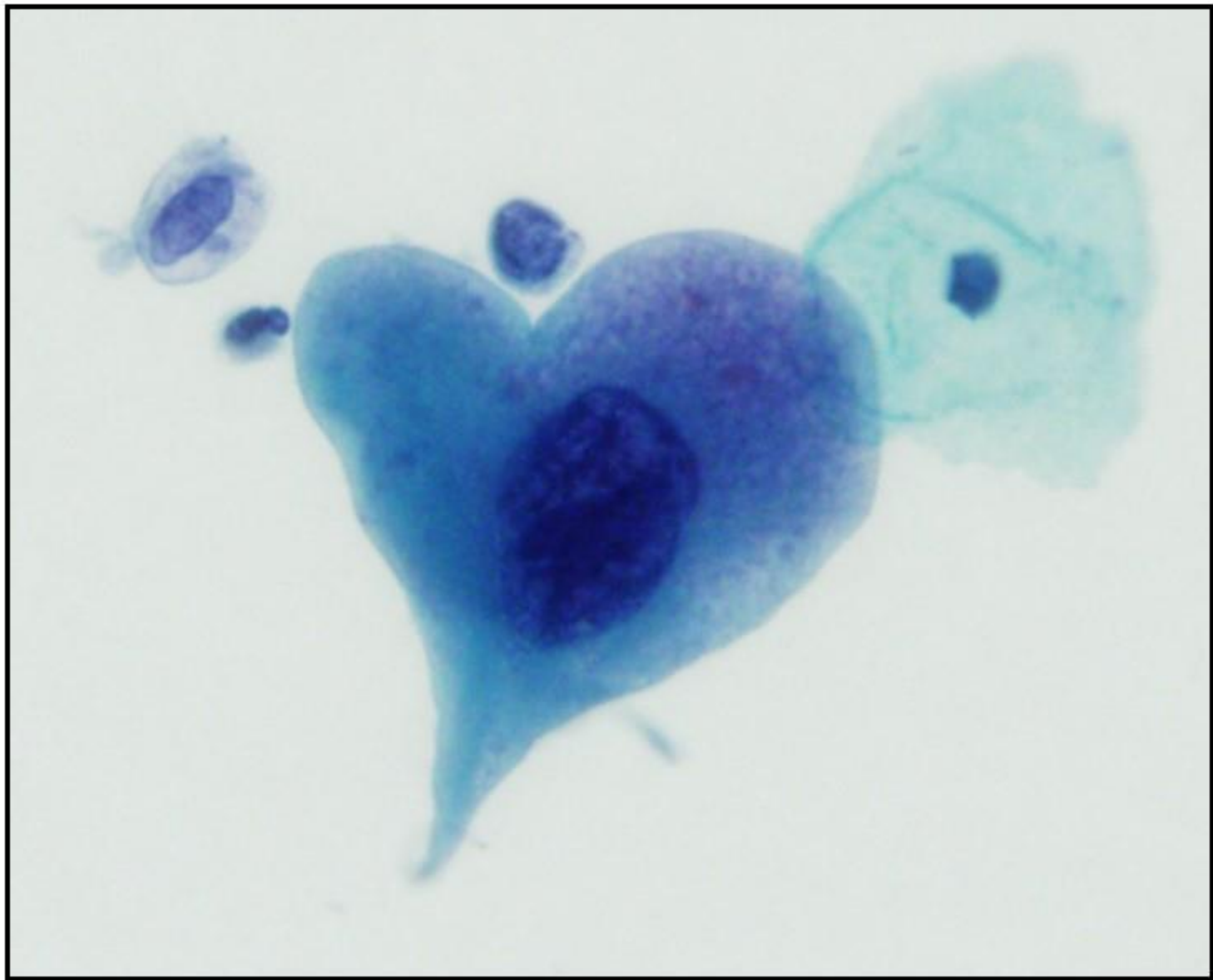
cap



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.

- Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, McCalmont T, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ, Zaino RJ, Wilbur DC; Members of LAST Project Work Groups.
- J Low Genit Tract Dis. 2012 Jul;16(3):205-42.
- Arch Pathol Lab Med. 2012 Oct;136(10):1266-97. Epub 2012 Jun 28.
- Int J Gynecol Pathol. 2013 Jan;32(1):76-115



...thank you...