Cyto-histology Correlation

- **Cytologic-Histologic Discrepancies in Pathology of the Uterine Cervix: Analysis of the Clinical and Pathologic Factors**

- **Fadi W. Abdul-Karim, MD, MEd and Bin Yang, MD, PhD. Adv Anat Pathol 2017;24:304–309**

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Gynecologic Cyto- Histo Correlation

Cytology	HPV Testing	5 yr Risk CIN2+	Recommend Management
Negative	Negative	0.27	Repeat testing in 5 yrs
Negative	Positive	10	Repeat testing in 6-12 months
ASC	Negative	1.1	Repeat testing in 3 yrs
ASC	Positive	18	Immediate colposcopy if >25
LSIL	Negative	5.1	Repeat testing in 6-12 months
LSIL	Positive	19	Immediate colposcopy if >25
ASC-H	Negative	12	Immediate colposcopy
ASC-H	Positive	45	Immediate colposcopy
HSIL	Negative	49	Immediate colposcopy
HSIL	Positive	71	Immediate colposcopy
AGC	Negative	2.2	Immediate colposcopy
AGC	Positive	45	Immediate colposcopy

Schiffman M and Solomon D. Cervical Screening with HPV and Cytologic Cotesting.New Engl J Med. 2013; 369:2324,-31

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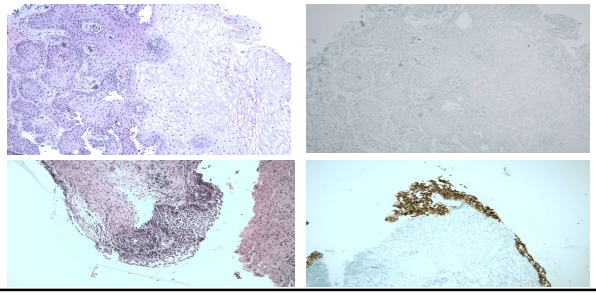
4. Post Analytic:
E. Gynecologic Cytologic- Histologic Correlation
Pap HSIL: Biopsy results



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Gynecologic Cytologic- Histologic Correlation

The Clinical Laboratory Improvement Amendments of 1988 (CLIA) requires "Laboratory comparison of clinical information, when available, with cytology reports and comparison of all gynecologic cytology reports with a diagnosis of high-grade squamous intraepithelial lesion (HSIL), adenocarcinoma, or other malignant neoplasms with the histopathology report, if available in the laboratory (either on-site or in storage), and determination of the causes of any discrepancies." This requirement is generally referred to as cytologic-histologic correlation (CHC).

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4. Post Analytic:
E. Gynecologic Cytologic- Histologic Correlation; CAP: Q I

- Cytologic-histologic correlations can be performed retrospectively, during initial case review or both.
- As a minimum, all available slides should be reviewed for a HSIL Pap test with negative biopsies.
- The preferred monitor for correlations is the positive predictive value of a Pap test.
- Laboratories should design cytologic-histologic correlation programs to explore existing or perceived quality deficiencies.

Crothers BA. **Quality improvement opportunities in gynecologic cytologic-histologic correlations: findings from the College of American Pathologists.** Arch Pathol Lab Med. 2013 Feb;137(2):199-213

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CAP: Quality Improvement

Monitor Papanicolaou test characteristics

- **Record variables adversely affecting interpretation such as:**
  - **Stain quality**
  - **Thickness of preparation**
  - **Obscuring factors**
  - **Atrophic changes**
  - **Did not have subsequently detected cells marked (screening variance)**
  - **Demonstrate difficult patterns of detection**

Crothers BA. **Quality improvement opportunities in gynecologic cytologic-histologic correlations: findings from the College of American Pathologists.** Arch Pathol Lab Med. 2013 Feb;137(2):199-213

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CAP: Quality Improvement

Educational Group Microscopic Review of Select Cases

Optimize biopsies

- **Reorient tissue in block**
- **Obtain additional levels from the block**
- **Perform ancillary studies**
- **Record the presence or absence of the transformation zone in the report**
- **Develop laboratory policies for the number of routine serial sections and/or levels**
- **Coordinate with clinical colleagues to improve biopsy sampling**
- **Provide caregiver statistics on biopsy adequacy**

Monitor biopsy characteristics

- **Record the number of events where subsequent actions are necessary for discrepant biopsies**
- **Record negative cervical biopsies that:**
  - **Lack transformation zone**
  - **Are <2 mm**
  - **Are not associated with additional biopsies or endocervical curettage**
  - **Are poorly oriented**
  - **Require additional levels**

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Cytology-Histology Correlation

- **Although the practitioner may expect the biopsy to explain the abnormality on the smear, approximately 70%, 45% and 20% of cytologic interpretations of ASCUS, LSIL and HSIL, respectively, will not be verified on biopsy.**
- Colposcopic impression of HSIL identified only 56% of CIN2+ and the sensitivity for CIN 2+ of biopsy of colposcopically abnormal cervical epithelium is between 43.4% and 74.7% .
- The sensitivity of the first directed biopsy for CIN is around 52%.

Apgar BS et al **Gynecologic procedures: colposcopy, treatments for cervical intraepithelial neoplasia and endometrial assessment.** Am Fam Physician. 2013 Jun 15;87(12):836-43

ACOG. Management of abnormal cervical cytology and histology. ACOG practice bulletin no. 99. Obstet Gynecol 2008;112:1419-44

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4. Post Analytic:
E. Gynecologic Cytologic- Histologic Correlation

- Accuracy of cytology interpretation
- Colposcopy evaluation
- Clinical sampling error
- HPV type
- Number of biopsies taken + Endocervical sample
- Diagnostic imprecision of biopsies.
- Deeper levels obtained.
- Consultation
- Ancillary studies- IHC- P16

Crum, Cibas, Rose and Peters, Chapter 13 of Crum, Nucci, Lee, Diagnostic Gynecologic and Obstetric Pathology, 2011.

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Colposcopy

- Based on:
  - opacity
  - Margins
  - Contour
  - Vasculature
  - Staining reactions

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Agreement Between Colposcopically Directed Biopsies and the Definitive Excisional Specimens (Three clinical trials)

- 737 women (16–45y): Cervical biopsy taken within 6 months before their definitive therapy.
- The overall agreement between the biopsies and the definitive therapy diagnoses was 42%. The overall underestimation of CIN2-3/AIS and CIN3/AIS was 26 and 42%, respectively.
- Accuracy improved when CIN2 and CIN3/AIS were grouped together: HG
- Colposcopy functioned well when allowed a one-degree difference between the biopsy and the surgical histologic interpretations , as done in clinical practice: 92% overall agreement for CIN2-3/AIS

Stoler MH et al **The accuracy of colposcopic biopsy: analyses from the placebo arm of the Gardasil clinical trials.** Int J Cancer. 2011 Mar 15;128(6):1354-62.

11

Agreement Between Colposcopically Directed Biopsies and the Definitive Excisional Specimens (Three clinical trials)

- There were significant associations in the agreement between biopsies and excisional specimen diagnoses:
  - Patients were stratified by age: Lesions in older patients may be larger.
  - Lesion size
  - Number of biopsies.
  - Presence of human papillomavirus (HPV)16/18.
  - Region (no difference).
- that instead of a one-size-fits-all approach to colposcopy, the procedures undertaken during the colposcopy visit should be modified based on the underlying risk

Stoler MH et al **The accuracy of colposcopic biopsy: analyses from the placebo arm of the Gardasil clinical trials.** Int J Cancer. 2011 Mar 15;128(6):1354-62

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Colposcopy

- Several studies have shown that taking a single biopsy from the cervix may miss up to 40% of prevalent precancers.
- Multiple-biopsy protocols have been proposed and implemented mainly in research studies and clinical trials.
- The role of random biopsies is controversial: some studies have reported
- increased detection of cervical precancers by random biopsy sampling, whereas others have shown no benefit of adding random biopsies to multiple targeted biopsies.
- There is currently a wide variety of colposcopy practice in the US, ranging from single targeted biopsy to 4-quadrant random biopsy protocols.

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Colposcopy at a crossroads

- The accuracy and reproducibility of colposcopy-directed biopsy are limited.
- Factors that may contribute to these limitations:

(1) Lack of standardized terminology and

(2) Lack of recommendations for colposcopy practice and procedures.

(3) Lack of quality assurance measures.

- ASCCP Colposcopy Standards: Risk-Based Colposcopy Practice. J Low Genit Tract Dis 2017;21: 230–234

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Adapting Colposcopy Practice to Previous Risk and Colposcopy Impression

Recommendation:

- Colposcopy practice may be modified based on the risk level (which can be viewed as the probability of finding precancer/ cancer at the time of the procedure), based on reason for referral and colposcopy impression.

- For example, when the risk of precancer is very high, immediate treatment may be recommended to minimize costs and avoid loss to follow-up across multiple visits. Conversely, if the risk is very low, expectant management with serial cytology and HPV testing but no biopsy may be warranted. For intermediate risks, multiple biopsies of acetowhite lesions lead to increased detection of precancer.

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Number and Type of Biopsies Taken at Colposcopy

- **Recommendation**
- Multiple biopsies targeting all areas with acetowhitening, metaplasia, or higher abnormalities are recommended.
- Usually, at least 2 and up to 4 targeted biopsies from distinct acetowhite lesions should be taken.

- A single biopsy targeting the worst-appearing lesion may miss a third and up to half of prevalent precancers. In all studies, there was a substantial increase moving from 1 to 2 targeted biopsies. In the National Cancer Institute Biopsy Study, which used a very low threshold of colposcopic abnormality (any acetowhitening), the yield of precancer increased substantially from the first to second and from second to third biopsies. A fourth targeted biopsy, or an additional non-targeted biopsy (random biopsy), provided only a minimal increase in disease yield. Targeted biopsies should be taken from women with any degree of acetowhitening.

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Biopsy Practice in Women With Low Risk of Precancer

Recommendation

- Non-targeted biopsies are not recommended for women referred to colposcopy at the lowest end of risk, that is, those with less than HSIL cytology, no evidence for HPV16/18, and a completely normal colposcopic impression (i.e., no acetowhitening, metaplasia, or other visible abnormality).

- "random biopsies": More appropriate to differentiate targeted biopsies, that is, biopsies targeting any visible change, including acetowhitening, metaplasia, and other changes within the normal and abnormal spectrum, from completely non-targeted biopsies. Studies that have systematically evaluated the incremental yield of non-targeted biopsies in addition to targeted biopsies have shown very limited additional benefit for detection of precancer.

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Biopsy Practice in Women With Very High Risk of Precancer

- **Recommendation**
- In nonpregnant women 25 years and older with very high risk of precancer (at least 2 of the following: HSIL cytology, HPV 16 and/or HPV 18 positive, high-grade colposcopy impression), either immediate excisional treatment without biopsy confirmation or colposcopy with multiple targeted biopsies is acceptable.

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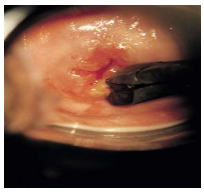
Pathology Requisition Form

- SPECIMEN SUBMITTED.
  A: Cervix, Biopsy 2:00
  B: Cervix, Biopsy 4:00
  C: Cervix, Biopsy 7:00
  D: Cervix, Biopsy 10:00
  E: Endocervical, Curettings
- SPECIMEN SUBMITTED:
  A: Cervix, Biopsy 3,6,9,12
  B: Endocervical, Curettings



Cervical Biopsy, Endocervical Curettage, and Cervical Biopsy During Pregnancy. Baggish, Michael S., Atlas of Pelvic Anatomy and Gynecologic Surgery, Chapter 45, 499-504. Elsevier

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Size of CIN3 and Colposcopic Sensitivity

- **Using a logistic regression model; the most important predictor of increasing sensitivity of colposcopically directed biopsy was increasing size of CIN 3+.**
- Once corrected for increasing size of CIN 3+, there was no added predictive value of cervical cytology of cancer or HSIL, **suggesting that cervical cytology of cancer or HSIL is a marker for large CIN 3+ rather than an independent predictor of higher sensitivity of colposcopically directed biopsy.**

Pretorius RG. **Regardless of skill, performing more biopsies increases the sensitivity of colposcopy.** J Low Genit Tract Dis. 2011 Jul;15(3):180-8.

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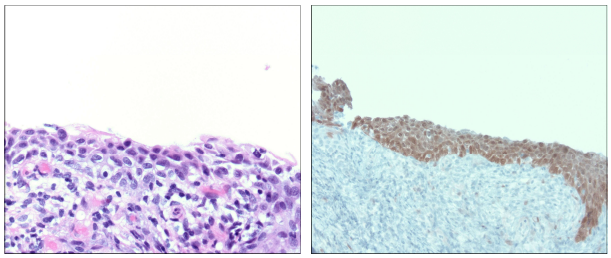
Thin CIN: The inability of expert colposcopists to visualize some CIN 2/CIN 3 is associated with thinner epithelium.



Yang B. **False negative colposcopy is associated with thinner cervical intraepithelial neoplasia 2 and 3.** Gynecol Oncol. 2008 Jul;110(1):32-6.

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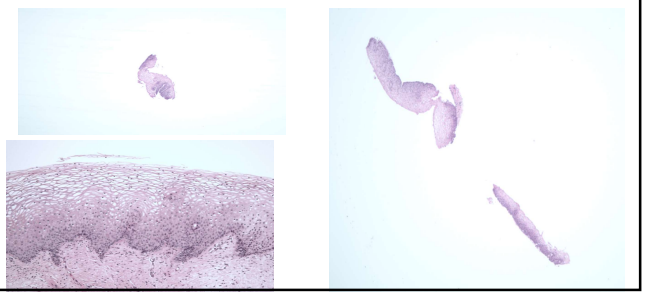
ASC-H HPV +/Normal Biopsy



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Additional tissue levels

- Lack of correlation between dysplastic cervicovaginal Papanicolaou (Pap) tests and subsequent cervical biopsies raises the concern that a significant squamous intraepithelial lesion (SIL) may go unconfirmed.
- Complete step sectioning of paraffin blocks was undertaken on 111 non-correlating biopsy specimens from 95 patients and selected slides were reviewed for the presence of SIL.
- 27 biopsies (24.3%) demonstrated the presence of a SIL in deeper levels. The presence of squamous atypia was significantly associated with the presence of dysplasia deeper in the block. Acute and chronic cervicitis was seen roughly equally.
- **Additional tissue levels are a productive way of confirming SILs, and squamous atypia allows a refined selection of negative cervical biopsies most likely to reveal an SIL on review of deeper levels.**

Jaste NK. **Noncorrelating Pap tests and cervical biopsies: histological predictors of subsequent correlation.** Diagn Cytopathol. 2005 May;32(5):310-4.

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Deeper Levels: How many

- There continues to be a 10% to 20% discordance rate between the colposcopic findings and the histological diagnoses on the resultant biopsies.
- 600 consecutive biopsies from 404 patients were reviewed.
- If sectioning were limited to 3 levels, 17.5% (105/600) of all dysplastic lesions would have been missed, including 19.6% (100/511) of CIN 1 and 5.6% (5/89) of CIN 2-3.
- Because not more than 3 levels are routinely evaluated in most laboratories, our findings suggest that sampling error is indeed at least 1 significant factor contributing to colposcopic/histological discrepancies.
- **Using our clinical efficacy standard, when no pathologic findings are initially identified in a colposcopic-directed biopsy, at least 5 levels (a priori or in recuts) are required to ensure a 100% diagnostic accuracy for CIN 2-3**.

Fadare O[1], Rodriguez B. **Squamous dysplasia of the uterine cervix: tissue sampling-related diagnostic considerations in 600 consecutive biopsies.** Int J Gynecol Pathol. 2007 Oct;26(4):469-74.

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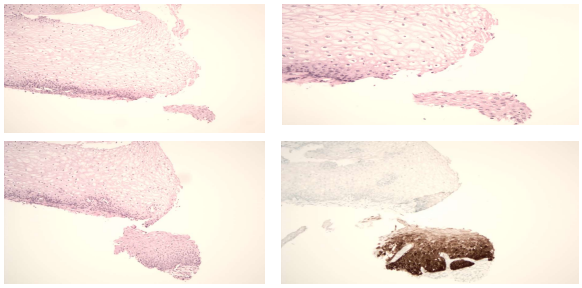
Deeper Levels



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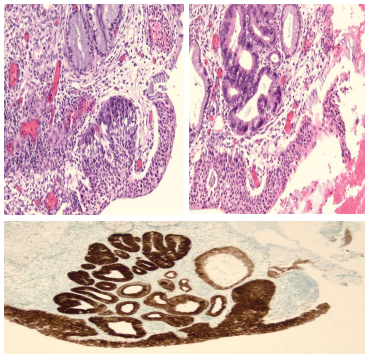

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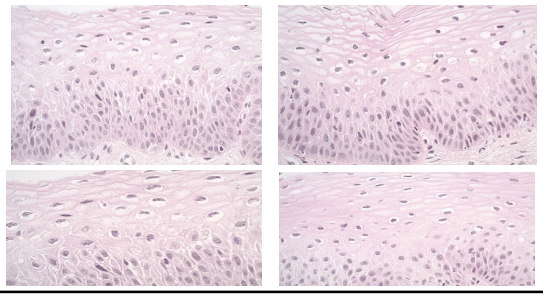
Interobserver variability: LSIL ?



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Cytology and Biopsy Interpretation: ALTS trial Interobserver reproducibility

Pap

1473 ASC-US: 43% concurred by QC and rest normal, 38.6 Negative

433 HSIL: 47.1% carcinoma, rest 27% LSIL and 22.6% ASC-US

Biopsy

887 CIN 1: 42.6% concordance, 41.0% Negative

481 > CIN 2: 90.8% concordance

685 Negative 76/9% concordance

LEEP

CIN 1 diagnosed on a colposcopically directed biopsy who undergo a LEEP have identified CIN 2,3 in 23–55% of the excised specimens.

Interobserver Reproducibility of cervical cytologics and histologic interpretations. Realistic estimates from ALTS Stoler MH, Schiffman M. JAMA (2001); 285: 1500-1505

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ALTS: Is the Biopsy Neg. or LSIL: 2 year Follow Up

- 897 cases of LSIL and 1193 cases of ASCUS HPV+: CIN1 or less were followed up for 2 years: Cumulative risk of CIN 2-3 was equivalent for LSIL (27.6%) and ASCUS HPV+(26.7%).
- After excluding the women with a diagnosis of CIN2/3 at initial colposcopy and biopsy (17.9%), the remaining were at nearly identical risk for subsequent CIN 2/3 regardless of initial colposcopy result.
- LSIL and ASCUS HPV+ are clinically equivalent. Initial colposcopic detection of obviously prevalent CIN2/3 reduces risk. However, for the remaining women who have CIN1or less on colposcopy and biopsy, the risk for subsequent CIN 2/3 is approximately 12% over 2 years. This risk does not vary meaningfully by initial distinction of histologic CIN grade 1 from negative colposcopy and biopsy.
- **Most CIN 3 cases diagnosed within the 2-year time frame were prevalent cases, and most incident CIN 3 cases followed a prevalently detected HPV infection.**

Castle PE. A descriptive analysis of prevalent vs incident cervical intraepithelial neoplasia grade 3 following minor cytologic abnormalities. Am J Clin Pathol. 2012 Aug;138(2):241-6.

Cox JT. Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. Am J Obstet Gynecol. 2003 Jun;188(6):1406-12.

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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, for Members of the Last Project Work Groups.

•Archives of Pathology and Laboratory Medicine: October 2012 – Volume 135 – p 1266-1297.

•Journal of Lower Genital Tract Diseases. July 2012 – Volume 16 – p 205-242

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p16 in Cervical Squamous Lesions

- Tumor-suppressor p16 is overexpressed in cervical carcinomas
- p16 expression is altered by the effect of HPV on the retinoblastoma protein
- IHC staining for p 16 has become standard practice in the evaluation of cervical lesions
- Although considered a surrogate marker for HPV infection *in the appropriate setting,* p16 does not, in general act, as a surrogate marker for HPV infection

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p16 Immunohistochemistry

Negative p16

- Cytoplasmic only staining
- Focal or patchy staining
- Discontinuous staining of basal layer
- Staining of upper layers but nor basal layer

Positive stain

- Diffuse (>80%) strong block positive nuclear or nuclear and cytoplasmic staining of basal layer and extending up at least 1/3 of epithelial thickness:
  - Correlates with presence of HR-HPV and diagnosis of dysplasia
  - Grading of dysplasia MUST be based on histology

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P16 is not to be used

- A negative p16 is of greater significance than positive.
- Do not use to separate CIN1/ 2 or 2/ 3.
- HSIL HPV negative probably metaplasia but can use CIN undetermined or atypical squamous epithelium cannot exclude SIL
- P16 mainly needed for young women CIN2 to assure diagnosis so not over treat

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False positive P16

- Squamous morules from endometrium that are detached and they are CDX2 positive.
- Papillary syncytial metaplasia of endometrium and can be a pitfall with serous carcinoma of endometrium
- Small cell carcinomas of cervix (not just squamous cell carcinoma)
- Can be strong positive in leiomyosarcoma
- Do not use on conventional LSIL ?/ significance?
- Cytoplasmic p16 and discontinuous are negative.
- Hybrid/non HPV vulvar lesions
- Glandular lesions: Need significant tissue. Focal staining in tubal metaplasia. Focal strong in Endometrial carcinoma, and extensive staining in serous carcinoma. Minimal deviation ca and mesonephric ca are negative for p16
- Cervical endometriosis can have one gland positive but next to it a negative endometrial gland.

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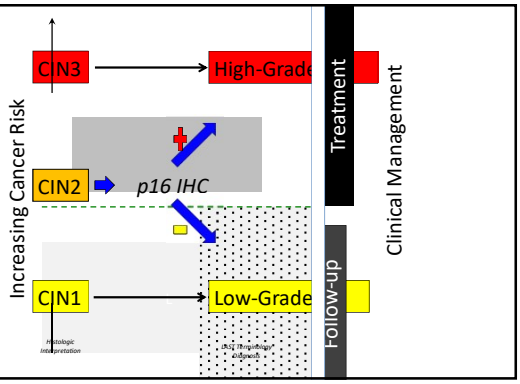

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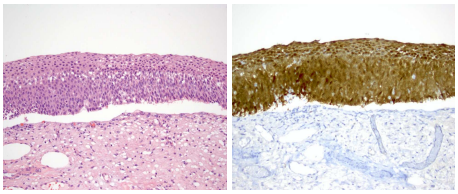
CIN2: P16



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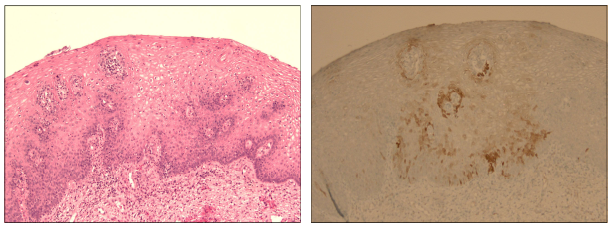
LSIL and P16



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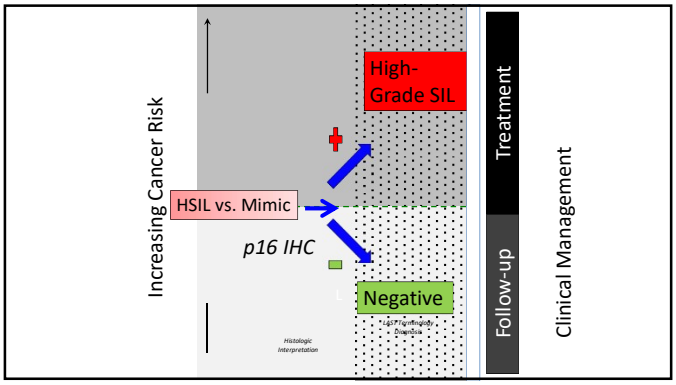

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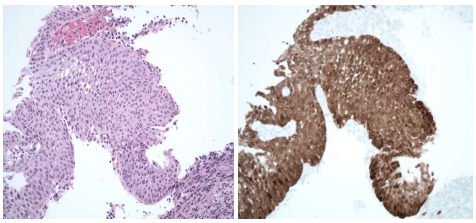
Immature squamous metaplasia vs. HSIL



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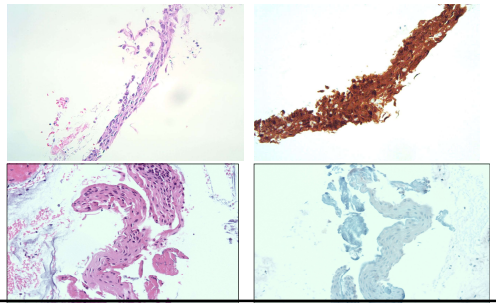
Atrophy vs CIN3



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LAST p16: Recommendation No. 4a

- *Special Circumstance.*—**p16 IHC is recommended as an adjunct to morphologic assessment for biopsy specimens interpreted as ≤–IN 1 that are at high risk for missed high-grade disease, which is defined as a prior cytologic interpretation of HSIL, ASC-H, ASC-US/HPV-16 +, or AGC (NOS).**
- **Any identified p16-positive area must meet** H&E **morphologic criteria for a high-grade lesion to be reinterpreted as such**.

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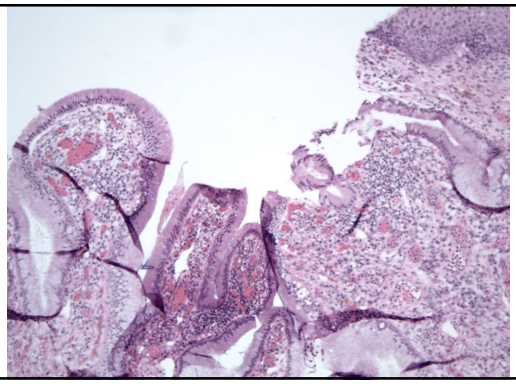

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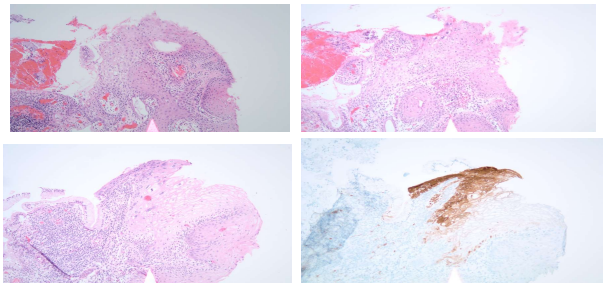
CASE INITIAL BIOPSY LEVEL FOLLOWING HSIL NEGATIVE DEEPER AND p16 is CIN2



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Routine Use of Adjunctive p16 Immunohistochemistry Improves Diagnostic Agreement of Cervical Biopsy Interpretation Results From the CERTAIN Study

Adjunctive use of p16 IHC provides more accurate and reproducible diagnostic results in the interpretation of cervical biopsies, ensuring that more patients are treated correctly without treating more patients.

Reduces false -ve interpretations of CIN2+

Reduces false +ve interpretations of CIN2+

Refs: Am J Surg Pathol 2016;42:1001–1009

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Examination of Sources of Diagnostic Error Leading to Cervical Cone Biopsies with No Evidence of Dysplasia

- 53 cone biopsies initially reported as negative for dysplasia or malignancy (17% of all cone biopsy specimens).
- Each negative cone biopsy specimen was examined with at least 3 deeper levels. If dysplasia not identified on deeper levels, p16 stain was performed on the most atypical level.
- Additional review by 3 pathologists for consensus diagnosis
  - 14 cases (26.4%) showed dysplasia to be present by at least 1 of the additional modalities (6 LSIL, 5 HSIL, 3 SIL)
  - 4 cases (7.5%) were identified by **additional level** sections (two-dimensional sampling of a 3-dimentional specimen)
  - 7 cases (13.2%) were identified by additional levels and **p16**
  - 3 cases (5.7%) were found by **consensus review**

Carrigg A, et al. *Am J Clin Pathol* 2013, 139:422-427.

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Examination of Sources of Diagnostic Error Leading to Cervical Cone Biopsies with No Evidence of Dysplasia

- Remaining 39 cases that remained negative with additional workup:
  - 15 cases (28.3%) were attributed to over interpretations on pre-surgical specimens.
  - 24 patients had confirmed HSIL on pre-surgical specimens but negative cone biopsy specimens, and 6 of 20 of these patients (11.3% of the total) with follow-up had confirmed dysplasia or carcinoma on subsequent specimens.
  - Therefore, the overall false-negative rate for cone biopsy specimens, when the fourth category of under-sampling was added, was 21%, a hardly insignificant proportion.

Carrigg A, et al. *Am J Clin Pathol* 2013, 139:422-427.

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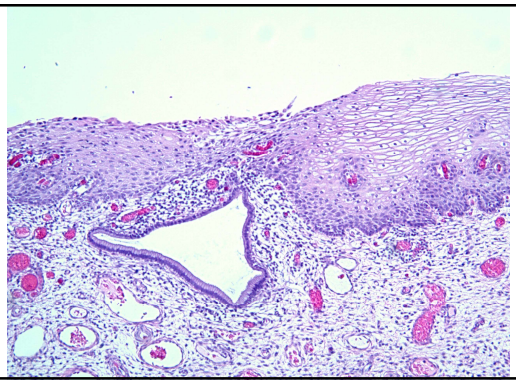

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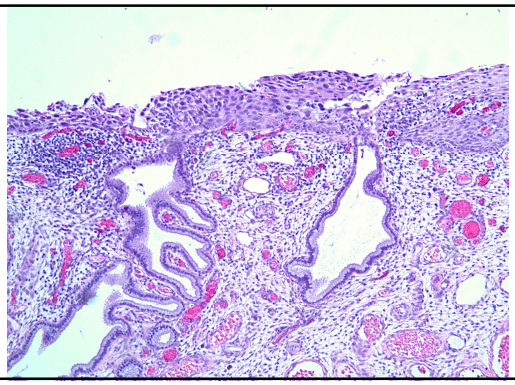

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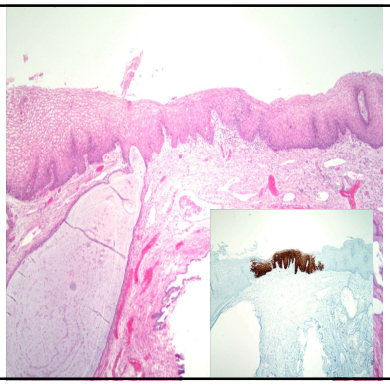

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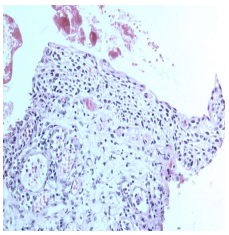
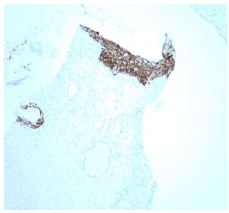
LEEP: P16

LEEP: Difficult to see HSIL



P16 positive for HSIL



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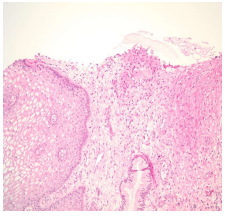

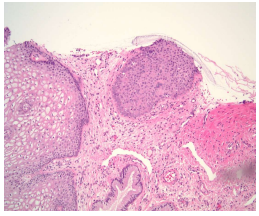

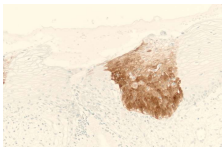

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Cytologic- Histologic Correlation: Preanalytical Factors:

- The Sensitivity of colposcopy.
- Characteristics of the squamous intraepithelial lesion:
  - Size of the LSL
  - Thickness and border
  - Distribution
  - Vascularture
- Factors affect of the sensitivity of colposcopy and accuracy of biopsy:
  - Colposcopically directed biopsy based on acetic white vs random biopsy
  - Number of Biopsies taken
  - Visibility of SCJ transformational zone
  - Performance of endocervical curetting
  - Patient's age and location of lesions
  - Physician's colposcopic experience

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Cytologic- Histologic Correlation: Enhancing Cytologic-Histologic Correlation in Surgical Pathology

- Specificity of cytologic interpretation
- Inter-Observer Reproducibility of Cervical Cytology
- Accuracy of Histopathologic diagnosis of SIL
- HPV testing and Genotyping
- Quality of Cytopathology and Histology laboratory
- Sampling issues and Obtaining deeper levels from tissue block
- Immunohistochemistry for p16 for HSIL diagnosis
- Consensus pathology reviewing with colleagues

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Sample Report Prior H- Pap and CIN1 or Less on Biopsy

- 1. Endocervical, curettings (A):
  - Fragments of endocervical epithelium with no significant pathologic findings.
- 2. Ectocervix, biopsy at 4 o'clock (B):
  Low-grade squamous intraepithelial lesion (CIN I). See comment.

Comment:

Deeper levels were obtained. Immunohistochemical stain for P16 shows done for prior HSIL. Pap test shows focal patchy staining supporting the above interpretation. The prior Pap test C16-33880 was reviewed and the interpretation of HSIL is reaffirmed (Staff consultant: Dr. …….).

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4. Post Analytic: QA
E. Gyn/Non-GYN Cytologic- Histologic Correlation: Summary

- High grade (GYN) and malignant cytology (GYN and Non-GYN) cases have to be correlated with histology
- Determine cause of discrepancies (i.e. sampling, locator errors, interpretation).
- No standard method.
- Findings should be documented and categorized as sampling or interpretation (major/minor) discrepancies.
- Amended report indicated only if current patient care impacted. \
- Annual report should be made

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