Cytology/Biopsy/Leep Gynecologic Correlation: Practical Considerations and Approaches.


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).

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.

**CAP: Q1 - Gynecologic Cytologic-Histologic Correlation:**
- 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


**The Accuracy of Colposcopic Biopsy**
- “Colposcopy can easily determine the location and extent of 90% of cervical intraepithelial neoplasia (CIN) lesions.” Morrow and Townsend's textbook
- The system of triaging cytological and histological abnormalities is frequently challenged, the actual “sensitivity” of the colposcopic exam is less frequently challenged.
- Colposcopic findings a colposcopically directed biopsy have not been shown to correlate strongly with the severity of the CIN2+
- 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%


**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.
• 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.


ALTS: Size of the CIN3 Lesion and Preceding Cytologic Interpretation
• CIN3 lesions found in ALTS were generally small (median distal-proximal length was 6.5 mm/median replacement of total epithelium = 5%)
• CIN3 lesions detected at the exit visit that were associated with cytological interpretations of HSIL were larger consistent with increasing severity of cytological interpretation with greater extent of CIN3.
• Aggressive follow-up of ASCUS and LSIL leads mainly to detection of CIN3 lesions that are smaller than those typically associated with invasion, especially when HPV testing is used.
• The identification of extensive CIN3 in a patient with ASCUS or LSIL is exceptional and should prompt a quality assurance review if practical to determine a possible role for sampling, screening, or interpretive errors.
• Colposcopic impressions of high-grade disease were associated with the largest cases, and lesions associated with a negative HPV test at the exit visit were slightly smaller than those that tested positive.
• Cytology and colposcopy particularly underestimate the prevalence of small CIN3 lesions, which also have a slightly higher risk of a false negative HPV test.


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.
When the analysis was repeated using biopsy of cervical quadrants with colposcopic impressions of CIN 2, CIN 3, or cancer, the sensitivity of directed biopsy was significantly lower (36.9%) than biopsy of cervical quadrants with colposcopic impressions of HPV, CIN, or cancer (63.5%).


Thin CIN: Difficult to Visualize

- The thickness and nuclear density of squamous epithelium of 261 selected cervical biopsies (CIN 2/CIN 3, N=144; Normal/CIN 1, N=117) were measured.
- Average epithelial thickness was defined as the thinnest area plus the thickest area divided by two. Average nuclear density was defined as the number of nuclei in a 2,500 microm(2) grid at the junction of the superficial and intermediate zones plus that at the junction of the intermediate and parabasal zones divided by two.
- Mean average epithelial thickness for 33 biopsies of CIN 2/CIN 3 from cervical quadrants with colposcopic impression of normal (184 microm) was less than that of 111 biopsies of CIN 2/CIN 3 from quadrants with colposcopic impressions of low, high, or cancer (321 microm, p<.001). CIN 2/CIN 3 had higher mean average nuclear density and was thinner than normal/CIN 1.


Non-Correlating HSL and CIN1 or Less Biopsy sampling error

- 108 HSIL Paps and cervical biopsies < HSIL: 47 had a subsequent procedure, either cone/LEEP, cervical biopsy, or repeat Pap test.
- HSIL Pap test followed by cervical biopsy with or without subsequent cone/LEEP, there was a discordant cervical biopsy rate for HSIL of 43%.
- HSIL by Pap test followed up by cervical biopsy and subsequent cone/LEEP or repeat cervical biopsy, the proportion of women with negative colposcopic cervical biopsy and subsequent histology-proven HGCIN was 56%.

- These figures justify sampling error as a valid cause of non-correlation in women with HSIL followed up by cervical biopsy alone.


Number of Cervical Biopsies and Sensitivity of Colposcopy

- The sensitivity was significantly greater when the colposcopists took two or more biopsies instead of one, a pattern observed across all types of colposcopists.
- Because the biopsies can be submitted in one vial, multiple sampling would not need to increase the cost of pathology.

Multiple Lesion-Directed Biopsies

- 690 women: Up to four directed biopsies were taken from distinct acetowhite lesions and ranked by colposcopic impression. A nondirected biopsy of a normal-appearing area was added if fewer than four directed biopsies were taken.
- Sensitivities for detecting HSIL increased from 60.6% from a single biopsy to 85.6% after two biopsies and to 95.6% after three biopsies.
- The highest increase in yield of HSIL was observed for women with a HG colposcopic impression, HSIL cytology, and HPV type 16 positivity. Only 2% of all HSILs diagnosed in the participants were detected by biopsies of normal-appearing transformation zone.
- Taking additional biopsies when multiple lesions are present should become the standard practice of colposcopic biopsy.


“Random Biopsies”

- We advise up to 4 "random" biopsies at the SCJ in cervical quadrants without visible lesions: 25.7% of the CIN 3+ and 9.7% of the invasive cancers in this series were diagnosed by "random" biopsy.
- The incremental yield of CIN 3+ per colposcopy of the "random" biopsies decreased as the number of "random" biopsies increased, there was still a significant increase in yield of CIN 3+ per colposcopy with the fourth "random" biopsy.
- The importance of obtaining the cervical biopsies with a forceps that obtains small (2 or 3 mm) biopsies cannot be overestimated. These biopsy forceps are not sharp. The biopsies were obtained by grasping the tissue and jerking the tissue from the cervix. For vaginal biopsy, a cotton-tipped swab can be used to provide counter traction adjacent to the biopsy site.
- These small biopsies are less painful than those obtained with traditional colposcopic biopsy instruments.


ATHENA trial: Random Biopsy and Negative Colposcopy: Genotyping

- ATHENA trial: 47,000 women with cytology and H-HPV genotyping. Colposcopy was performed in all women with abnormal cytology or positive HPV. A single random biopsy was taken at the SCJ if colposcopy was adequate/no lesion.
- The random biopsy diagnosed 20.9% and 18.9% of the total CIN2+ or CIN3+ 3, respectively. This additional disease was detected in both HPV 16 or 18+ and for 12 other high-risk HPV+ women.
- For HPV 16 or 18, the absolute risk for detection of CIN 2+non random biopsy in the overall population was 13.1% and 8.2% for CIN 3+. By contrast, the absolute risk for 12 other high-risk HPV+ women was 3.5% and 1.7% for CIN 2+ and CIN 3+
- Supports performing a random biopsy in women undergoing colposcopy without visible lesions, particularly in those positive for HPV 16 or 18 (AIDS? Larger lesions)
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 colposcopically-directed biopsy, at least 5 levels (a priori or in recuts) are required to ensure a 100% diagnostic accuracy for CIN 2-3.

ALTS: Inter-observer Viability of CIN1

- An interpretation of CIN 1 by the CC was corroborated by the QC group in only 42.6% of 887 biopsies.
- Equal proportion of originally diagnosed CIN 1 biopsies (41.0%) were interpreted as negative by the QC group.
- Biopsies diagnosed as negative or CIN2+ had 90.8% and 76.9% concordance.
- CIN 1 diagnosed on a colposcopically directed biopsy who undergo a LEEP have identified CIN 2,3 in 23–55% of the excised specimens.
- The poor reproducibility of the histologic diagnosis of CIN 1, as well as the uncertain biological potential of lesions that are classified based on their histologic appearance as CIN 1, makes management of these women problematic.
- Using either cytologic or histologic methods alone, it is impossible to determine whether a CIN 1 that appears to be persistent is persistent lesion or a new lesion.

The Interpretive Variability of Cervical Biopsies

- 6272 biopsies, extrapolated to 21,297 biopsies read by CP
- Panel agreement with the community diagnosis:
  - CIN1: 38.2% (Fewer CIN1 and more negative diagnoses in the P review but similar proportions of CIN2/3.
  - CIN2: 38.0% (Significant variability in the CP and P diagnoses)
  - CIN3: 68.0%
  - Cancer 70.6%
• HPV16 and hr-HPV positivity increased with disease severity, but P review did not improve the correlation of higher-grade disease with these objective measures.

• New biomarkers are needed to more accurately stratify precursor lesions according to their malignant potential.

• The use of antecedent cytology results influenced the diagnostic process in a limited way, but more definitive markers are needed.


Do Colposcopically Directed Biopsy and Endocervical Curettage Serve to Induce Regression of Cervical Intraepithelial Neoplasia?
• 555 patients with abnormal screening and underwent colposcopy followed by cervical excision procedures for CIN2+.
  • Median interval from colposcopy to excision was 48 days.
  • Neither demographics nor colposcopic findings influenced the probability of regression.
  • Patients with shorter intervals between colposcopy biopsy and excision exhibited a higher rate of regression.
• Regression was less likely with longer latency from colposcopy to excision:
  • Emergence and documentation of persistent occult neoplasia.
  • Lesion is incompletely excised.
  • Minute occult lesions escape discovery.
• Biopsy may not affect immune recognition allowing the natural history to unfold, and the neoplasia is at risk of progression during this latency interval.


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

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. Examination of sources of diagnostic error leading to cervical cone biopsies with no evidence of dysplasia. Am J Clin Pathol 2013, 139;422-427

**Endocervical Curettages (ECC)**

• ECC: appears to be more regression. This is especially true when there is no visible lesion at the time of colposcopy, and curettage may theoretically remove an isolated occult endocervical lesion completely.
• Serves to excavate an area of the endocervix large enough to remove neoplasia completely, or whether it causes an inflammatory reaction leading to increased immune recognition.
• Do not support the routine ECC in all patients.
• However, among postmenopausal women, those with HSIL, large lesions, or unsatisfactory examination results, ECC remains crucial.
• ECC detected 5.4% - 9.3% of CIN2+ cases missed by biopsy.


**Factors that may influence Cytologic-Histologic Features**

• Interval to colposcopy/biopsy.
• Sensitivity of the colposcopy.
• Number of biopsies taken.
• Inter-observer variability.
• Knowledge of prior HPV status.
• Performing deeper levels.
• Ancillary studies-P16