Terminology, Patterns and Pitfalls in Gynecologic Cytology

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Conflict of Interest

• None with vendors of cytology equipment or HPV testing
• Amirsys (now Elsevier) and McGraw Hill
  – (Book publishers/Royalties)

Goals of this talk...

• Discuss patterns of Squamous intraepithelial lesions and benign conditions that may be overcalled as SIL
• Discuss patterns and major pitfalls encountered in high grade glandular lesions and malignancies of the cervix
• Discuss and demonstrate reasons and patterns that may result is a benign diagnosis of Malignancy or High grade squamous or glandular lesion
• Present benchmarking data on lab and individual performances where available/appropriate
For this talk I will discuss....

- Normal
- Repair and atypical repair
- Radiation
- Pregnancy and Provera related pitfalls
- Mimics of LSIL
- Mimics of HSIL
- Mimics of ASC-US and ASC-H
- Recognizing Diathesis in various preparations
- Pitfalls in Squamous cell carcinoma diagnosis

For this talk....

- Major mimics of Adenocarcinoma in situ and Adenocarcinoma of the cervix
- Under diagnosis of adenocarcinomas of the cervix
- Problems with normal endometrial cells on paps(exfoliated or directly sampled)
- Issues with diagnosis of endometrial carcinoma on Cervicovaginal cytology
- Extra uterine carcinomas, presentations on Paps...can we really tell the difference

Lax Laboratories
The Pap Test Misses Much Cervical Cancer Through Labs’ Errors
Cut-Rate ‘Pap Mills’ Process Slides Using Screeners With Incentives to Rush
Misplaced Sense of Security?

BY WALT ROGANEK
Staff Reporter of THE WALL STREET JOURNAL
Evolution of Cervicovaginal Cytology Reporting Terminology

- Negative for Intraepithelial Lesion or Malignancy (NILM)
- Epithelial Cell Abnormality
  - Squamous (ASC-US, ASC-H, LSIL, HSIL, CA)
  - Glandular (AGC, AIS, Adenocarcinomas)
  - Other
  - Other
NORMAL PAP TEST

Normal Epithelial Cell Sizes and Morphology

Superficial
Intermediate
Parabasal
Endocervical
Endometrial

A flow chart for ThinPrep specimen processing shows the equipment needed to collect the concentrated specimen, concentrate cells from the specimen to a disk, and perform image-assisted scoring.

From chapter by M.J. Thrall in Diagnostic Pathology: Cytopathology, Mody: Amirsys/Elsevier 2014, 2018

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AUTOMATED SCREENING IN GYNECOLOGIC CYTOLOGY

Satisfying processing is similar to ThinPrep, but uses the smaller size of slides for the slide and the technology for visual analysis of image fields of view. The slide scanner can also accommodate conventional slides.

From chapter by M.J. Thrall in Diagnostic Pathology: Cytopathology, Mody: Amirsys/Elsevier 2014, 2018

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Normal

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For this talk I will discuss....

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• Radiation
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• Mimics of LSIL
• Mimics of HSIL
• Recognizing Diathesis in various preparations
• Pitfalls in Squamous cell carcinoma diagnosis

Repair Criteria

- Flat sheets with distinct cellular outlines, non-overlapping nuclei
- Streaming pattern, PMNs
- Smooth, round nuclear outlines, slight nuclear enlargement
- Normo or hypochromic, rarely mild hyperchromasia
- Regular nucleoli
- Rounding on LBPs
- Bi and multinucleation
Radiation

- Increased cell size
  without change in N:C ratio
- Bizarre shapes
- Degenerative changes, vacuoles in nu/cytopl
- Mild hyperchromasia, variable nucleoli
- Polychromatic staining

Atypical Repair

- Many features of repair
- Large nucleoli
- Nuclear features and overlap brings carcinoma in differential
- Often interpreted as atypical glandulars

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- Mimics of HSIL
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- Pitfalls in Squamous cell carcinoma diagnosis
Examples of LSIL

**LSIL Criteria**

- Changes limited to “Mature cells”
- Nuclear enlargement >3X normal intermediate cell nucleus
- Variable hyperchromasia, (exception in liquid based) nu size, number, shape
- Slight nuclear **membrane irregularity**
- **Koilocytosis**
- Must have nuclear abnormalities to qualify
- Note differences in liquid based

**Mimics of LSIL**

- Pseudokoilocytosis
- Radiation
- Herpes
- Hyperkeratosis
- Tight halos
Mimics of LSIL

Navicular cells/Pseudokoilocytosis
- Nuclear features of LSIL are not present
- Glycogenation/yellow tinge
- No distinct condensation
- Tight halos may also be seen

Mimics of LSIL

Tight Halos of Reactive changes
- Small tight halo usually due to organisms
- No peripheral condensation of cytoplasm
- Equal distance between edge of nucleus and halo rim (unlike LSIL)
- Lack of nuclear features of LSIL

Mimics of LSIL

Radiation
- Increased cell size without change in N:C ratio
- Bizzare shapes
- Degenerative changes, vacuoles in nu/cytoplasm
- Mild hyperchromasia, variable nucleoli
- Polychromatic staining
Mimics of LSIL

Herpes
- Multinucleation, Molding and margination of the chromatin
- These changes in mature cells, if not well developed may be mistaken for LSIL
- Pay attention to other cells for classic features of herpes
- Both can co-exist

Mimics of LSIL

Hyperkeratosis
- Anucleate unremarkable polygonal mature squamous cells
- Tight halos/empty spaces or “ghost” nuclei
- Often associated with mature squamous cells showing keratohyaline granules

HSIL Criteria
- Small less mature cells affected
- Single, sheets or syncytial-like aggregates
- Nuclear hyperchromasia, irregularity, variation in size and shape, occasional prominent folds
- Nucleoli generally absent except gland extension
- Cytoplasm may be immature/lacy, dense or rarely densely keratinized
Patterns of HSIL

- In mucin streaks (conventional smears)
- Dispersed (liquid based)
- Syncytial
- Endocervical Gland Involvement
- Hypochromatic (Thinprep)
- Stripped nuclei
- Keratinizing
- Repair-like/ stromal cells like
- AND...unique to the USA...litigation cells
Mimics of HSIL

- Isolated epithelial cells
  - Reserve cells, Parabasal cells, immature metaplasia
- IUD cells
- Isolated cells with herpes
- Exfoliated endometrial cells
- Endometrial stromal cells
- Histiocytes
- Isolated bizarre cells with atrophy
- Hyper chromatic crowded groups of benign cells
- Uncommon malignancies
Mimics of HSIL

Transitional Metaplasia
- Postmenopausal women
- Atrophic background
- Few groups
- Fine even chromatin
- Linear/longitudinal grooves
- P16 and HPV negative

Mimics of HSIL

Benign Hyperchromatic Crowded Groups (HCGs)
- Follicular cervicitis
- Atrophy
- Histiocytes
Atypical Squamous Cells of Undetermined Significance (ASC-US)

- Mature Cell type (superficial or intermediate)
- Nuclei 2.5-3X the area of normal intermediate cell nucleus
- Slightly increased N:C ratio
- Minimal nuclear hyperchromasia, irregularity in chromatin distribution or shape
- Nuclear abnormality with dense orangeophilic cytoplasm (atypical parakeratosis)

Note: Applies to entire specimen not individual cells
Decidual Cells on Pap

- Pregnancy, Postpartum or high Provera
- Cells single or rarely in clusters
- Abundant, vacuolated or granular cytoplasm+/_ processes
- Nuclei 35-50 cubic microns, generally smooth contours, rarely multinucleation, fine chromatin, normo or hyperchromic

Peri/Post menopausal atypia

- Atrophic or intermediate cell pattern with occasional cell showing atypia
- Often called ASC-US or ASC-H if atrophic
- HPV negative
- Negative follow up

Atypical Squamous Cells, Cannot exclude HSIL(ASC-H)

- Immature Cell types
  - Single cells or small fragments of <10 cells
  - Small cells with high N:C ratios (Atypical immature metaplasia)
- Metaplastic cells with nu 1.5-2.5 X normal
- N:C ratio closer to HSIL but other nuclear abnormalities fall short
- In liquid based, cells small and 2-3X neutrophil nuclei
**Misclassified ASC-H**

- Isolated endocervical cells
- Endometrial cells
- Histiocytes
- IUD cells
- Decidual cells
- Artefacts
- ASC-H/HSIL may be under called in atrophic cases

**ASC-H/HSIL with Atrophy**

- Hyperchromasia of nuclei compared to benign atrophic/parabasal cells
- Nuclear contour irregularities compared to benign parabasal cells
- Nuclear overlap in syncytial fragments within a single plane
For this talk I will discuss:

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• Mimics of LSIL
• Mimics of HSIL
• Recognizing Diathesis in various preparations
• Pitfalls in Squamous cell carcinoma diagnosis
Squamous Cell Carcinoma

- Non Keratinizing and Keratinizing types
- Features and diathesis vary by preparation type
- Cellularity also variable
- Diathesis usually subtle in liquid based
Keratinizing Squamous Cell Carcinoma

- Isolated cells or in aggregates
- Variable size, shape, tadpoles, spindles
- Variation in nuclear size, shape, hyperchromasia, granularity
- Macronucleoli uncommon
- Diathesis less than in non keratinizing types, clinging diathesis in liquid based

Non Keratinizing Squamous Cell Carcinoma

- Syncytia with ill defined cell borders
- Features of HSIL but cells usually smaller
- Variation in nuclear size, shape, hyperchromasia, granularity
- Macronucleoli and basophilic cytoplasm in large cell variant
- Diathesis more obvious, clinging diathesis in liquid based

Overcalling Squamous cell carcinomas

- Pseudo diathesis of atrophic vaginitis
- Irritated and ulcerated endocervical Polyps
- Lubricant simulating diathesis
Squamous Cell Carcinoma Pitfalls

- Low cellularity
- Obscuring inflammation or blood
- Repair like features
Beware of the Bloody Unsat! Dilute/Lyse and reprep the case!

Comparison of Two Recent Real World Publications From the USA

Figure 1. False Negative Rates by Test Method from Two Real World Studies

Mody D R comparison in lay press (clpmag.com June 14 2016) Quest data (Blatt et al) based largely on conventional smears and HCII platform, Bio data (Zhou H et al) on imaged liquid based and Cobas platform.


Budapest Photograph By Amos Chapple (taken by camera from a Drone)
TBS 2001 and 2014

Negative for Intraepithelial Lesion or Malignancy (NILM)

Epithelial Cell Abnormality

- Squamous (ASC-US, ASC-H, LSIL, HSIL, CA)
- Glandular (AGC, AIS, Adenocarcinomas)
- Other

Adenocarcinoma In Situ of the Cervix

- Precursor lesion of most endocervical adenocarcinomas
- HPV positive
- Most associated with SIL
- Cellular specimens, HCGs on low mag, presenting as sheets, strips with nuclear crowding and overlapping
- Peripheral feathering, nuclear palisading

Adenocarcinoma In Situ of the Cervix

- Nuclei oval, elongated, hyperchromatic, with coarse but evenly distributed chromatin
- Increased N:C ratios
- Apoptosis, mitosis
- Clean background
- “strips/birdtails on SP
- Feathering more subtle on liquid based
Endocervical Adenocarcinomas

- Many features of AIS in early invasive ACAs
- Nuclear pleomorphism and irregularity
- Chromatinic clearing
- Nucleoli
- Loss of polarization
- Three dimensional/Acinar groupings
- Single intact malignant cells
- Mitosis
- Tumor Diathesis

ATYPICAL GLANDULAR CELLS

- **Definition**
  - Cells showing either endometrial or endocervical differentiation displaying nuclear atypia that exceeds obvious reactive or reparative changes but lacks unequivocal features of invasive adenocarcinoma or adenocarcinoma in situ
ATYPICAL ENDOCERVICAL CELLS, favor neoplastic

- **Definition**
- Cells showing endocervical differentiation that QUALITATIVELY OR QUANTITATIVELY fall short of an interpretation of invasive endocervical adenocarcinoma or adenocarcinoma in situ
ATYPICAL ENDOCERVICAL CELLS (Probably Neoplastic)

- Sheets, strips, rosettes
- Nu crowding, overlap, Incr N/C ratio
- Ill-defined cell borders
- Palisading, feathering, stratification
- Hyperchromasia with even chromatin
- Nucleoli Inconspicuous, Mitosis
- Clean or slightly bloody background

Mimics of Endocervical Adenocarcinoma and AIS

- High grade Squamous Intraepithelial Lesion and Squamous carcinoma
- Tubal Metaplasia
- Endometrium, directly sampled or shed
- Aggressive endobrush sampling
- Repair, Polyps, Hormonal effects
- Dark staining of cells/Imager staining

From Diagnostic Pathology: Cytopathology Mody D Aminyo/Elsevier Publishing, 2014 and 2018
<table>
<thead>
<tr>
<th>Features</th>
<th>HSIL</th>
<th>AIS</th>
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<tbody>
<tr>
<td>Strips &amp; Rosettes</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Gland forms</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Feathering</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Polarity</td>
<td>Lost</td>
<td>Maintain</td>
</tr>
<tr>
<td>Nu Shape</td>
<td>Round/irreg</td>
<td>Oval/cigar</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Coarse</td>
<td>Even</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Dense</td>
<td>Even</td>
</tr>
<tr>
<td>Background</td>
<td>Isolated cells</td>
<td>Rare/abs</td>
</tr>
<tr>
<td>Frequency</td>
<td>0.2-0.4%</td>
<td>&lt;.02%</td>
</tr>
</tbody>
</table>
Squamous vs Adenocarcinomas (Cervical)

**Squamous**
- Keratinization (if present)
- Dense cytoplasm
- Syncytial arrangement
- Features of HSIL
- Cell block from Liquid based
- P40 IHC positive

**Adenocarcinoma**
- Mucin or delicate cytoplasm
- Columnar configuration
- Organoid architectural features
- Nuclear polarization
- Cell block
- P40 negative
### TUBAL METAPLASIA Vs AIS

<table>
<thead>
<tr>
<th>Features</th>
<th>Tubal meta</th>
<th>AIS</th>
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<tbody>
<tr>
<td>Cellularity</td>
<td>Scant</td>
<td>Cellular</td>
</tr>
<tr>
<td>Honeycombing</td>
<td>Many</td>
<td>Rare</td>
</tr>
<tr>
<td>Feathering</td>
<td>Rare/absent</td>
<td>Common</td>
</tr>
<tr>
<td>Strips</td>
<td>Rare</td>
<td>Common</td>
</tr>
<tr>
<td>Single cells</td>
<td>Many</td>
<td>Rare</td>
</tr>
<tr>
<td>T.Bars/cilia</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Round/oval</td>
<td>Oval/cigar</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Normochromic</td>
<td>Hyperchromatic</td>
</tr>
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Scenarios Where Normal Endometrials are seen on Paps

- Aggressive sampling
- S/P Cone or LEEP
- Endometriosis in vaginal vault
- Post Trachelectomy
- Menstrual pattern

Directly Sampled Lower Uterine segment
Endometrium or Endometriosis

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Tissue fragments, sheets, +/- gland openings. Stromal cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td>Small, 2.5 X Int nucleus or nucleus=int nucleus</td>
</tr>
<tr>
<td>Sheets</td>
<td>Appear crowded with minimal to no nuclear overlap in plane of focus. Tubular gland openings may be seen</td>
</tr>
<tr>
<td>Feathering</td>
<td>Absent</td>
</tr>
<tr>
<td>Palisading</td>
<td>Absent</td>
</tr>
<tr>
<td>Mitosis</td>
<td>May be present</td>
</tr>
<tr>
<td>Mucin</td>
<td>Absent</td>
</tr>
</tbody>
</table>
What about Undercalling cancer as Endometrials

Look at Cell Size, configuration and Nuclear details, background dysplasia
Cancer patients do bleed
or be in menstrual cycle!
The two can co-exist!!
Atypical Repair Vs AIS or Adenocarcinoma

**Atypical repair**
- Flat sheets with some disorganization/overlap
- “School of fish” arrangement and polarization
- Minimal nuclear crowding, no overlapping and usually hypochromasia
- Smooth nuclear contours
- No feathering, rosettes
- Bland vesicular chromatin with nucleoli

**AIS/Adenocarcinoma Cervix**
- Usually 3 D
- Nuclear polarization perpendicular to lumen
- Nuclear crowding, overlapping and hyperchromasia
- Subtle nuclear contour irregularities
- Peripheral feathering, rosettes
- Dispersed or vesicular chromatin, hyperchromasia, chromocenters or irregular nucleoli once invasive
What about Undercalling Cancer as Repair?

- Configuration...more single cells in cancer compared to repair
- Nuclear contour irregularities and N:C ratios
- Chromatinic characteristics
- Don't be fooled by the “taffy pull” cytoplasm

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Imager stain

Artefact due to Endobrush Sampling for evaluation of Cervical cone/leep endocervical margin

Photograph By Amos Chapple (taken by camera from a Drone)
AGC (Endometrial)

- Small groups of 5-10 cells
- Slight nuclear enlargement, small nucleoli
- Slight hyperchromasia
- Ill-defined cell borders,
- Scant cytoplasm, vacuoles+/−

Normal vs Atypical Endometrial Cells

**Normal (exfoliated)**
- Nuclei slightly larger than intermediate cell nucleus (35μm²)
- Chromatin dense, heterogenous, apoptotic
- Nucleoli small or absent
- Scant cytoplasm, dense or vacuolated
- Menstrual endometrium may look worse on liquid based prep (pleomorphism of nuclear size and shape)

**Atypical**
- Nuclei slightly larger compared to normal
- Mild hyperchromasia
- Occasional nucleoli
- Scant vacuolated cytoplasm
## Endometrial Carcinoma

<table>
<thead>
<tr>
<th><strong>Age</strong></th>
<th>Peri &amp; post menopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellularity</strong></td>
<td>Low</td>
</tr>
<tr>
<td><strong>Configuration</strong></td>
<td>Loose cell groups, acini, papillae</td>
</tr>
<tr>
<td><strong>Nuclei</strong></td>
<td>Round, vesicular</td>
</tr>
<tr>
<td><strong>Nucleoli</strong></td>
<td>Multi/macro</td>
</tr>
<tr>
<td><strong>Cytoplasm</strong></td>
<td>Scant, cyanophilic</td>
</tr>
<tr>
<td><strong>Background</strong></td>
<td>Diathesis</td>
</tr>
</tbody>
</table>
Mimics of Endometrial Adenocarcinoma/Hyperplasia

- Endometrial & Endocervical polyps
- Arias Stella Reaction & Pregnancy
- IUD changes
- Cervical Small cell carcinoma
- Post menopausal atrophy and bare nuclei
- Radiation changes
- Fixation & staining artifacts
- AND menstrual endometrium

In other words, what else will shed normal or atypical endometrial cells usually in peri and post menopausal women?

Polyps

- Can be endocervical or endometrial
- Irritation causes repair like changes
- Can shed normal or atypical endometrial cells
- If directly sampled then glands and stromal cells
- Bleeding can cause diathesis like background
Arias Stella

- Young women, pregnant or high hormones
- Glandular cells singly or in clusters, rare cells
- N:C ratio variable but often high
- Nuclei large, hyperchromatic with contour irregularities, INCI, grooves, degeneration
- Prominent nucleoli, multiple
- Vacuolated cytoplasm, leukophagocytosis
- May show some degeneration
- Disappear shortly after pregnancy

IUD Changes

- H/O IUD
- Scant cells
- Vacuolated cytoplasm
- Bubble gum cytoplasm
- Reactive nuclear features
- Variation in cell and nuclear size
- Single rare HSIL like cell
- Nucleoli

Small Cell Carcinoma

- Usually young women
- Overwhelming cellularity of malignant cells
- Apoptosis, necrosis, mitosis, diathesis
- Small cells 2Xlymphocytes
- Characteristic chromatin
- Scant cytoplasm
- IHC on cell blocks + for Chromo, Synapto, CD56
Follicular Cervicitis

- Few cells/cell groups
- Small cells (lymphocytes)
- Variation in cell sizes
- Tingible body macrophages helpful if recognized
- Better seen on conventional smears

Postmenopausal Atrophy with Bare Nuclei

- Postmenopausal
- Usually deep atrophy
- Bare nuclei
- Smooth nuclear contours
- Small cells in groups/clusters
- Normochromic, no nucleoli
- Smooth nuclear contours
Extrauterine Carcinomas on PAP smears

- Origin of primary
- Location and extent of spread
- Patency of fallopian tubes
- Ascites

Tumor Diathesis

Endometrial Ca...........92.5%
Endocervical ca..........85%
Extrauterine Ca...........19.7%
### Cervical Adenocarcinoma and Preceding HPV Negative Vs Cytology Negative

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number</th>
<th>% HPV neg</th>
<th>% cytology neg</th>
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<tbody>
<tr>
<td>Farnsworth A. Acta Cytol. 2011;55:307-12</td>
<td>5</td>
<td>80</td>
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<tr>
<td>Zaibol J. J Am J Clin Dermatol 2015</td>
<td>n/a</td>
<td>n/a</td>
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<tr>
<td>Katki H.A. Lancet Oncol. 2011;12(7)663-672</td>
<td>27</td>
<td>22</td>
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<tr>
<td>Questl Jean A et al Cancer Cytopath PMID 3054832</td>
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<td>Zheng 2015 Cancer Cytopath PMID 2554842</td>
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<tr>
<td>Conrad et al 2018 Cancer Cytopath PMID 20151473</td>
<td>45</td>
<td>22.2</td>
<td>4.4 neg, 2.2 unsat, 4.4 ASC-US</td>
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</tbody>
</table>
HPV testing and Glandulars: Notes....

- Atypical Glandulars and above are high risk lesions
- Associated HPV negative results should NOT alter the initial management
- 25% of AGC cases will test + for HPV
- 50% of AGC cases which are HPV+ are found to have significant cervical lesions on follow-up (HSIL/AIS/Ca)
- <5% HPV negative AGC have significant HPV associated lesions
- HPV negative AGC more likely to have endometrial pathology
- Please refer to ASCCP.org/guidelines for the most current management guidelines