Goals

1. Appreciate the changing epidemiology of glandular lesions of the cervix.

2. Discuss the pitfall changes of the cervix that mimic neoplastic lesions of the cervix.

3. Review the histologic clues and patterns of invasive and early invasive glandular lesions of the cervix.
Cervical Cancer Incidence & Mortality

modified from American Cancer Society Statistics, 1991
Invasive Cervical Cancer by Histological Subtype

- Squamous Cell
- Adenocarcinoma

Percent of Total

adapted from SEER data in J Womens Health 21(10): 1031, 2011
Etiology of Cervical Adenocarcinoma

- HPV infection can alter squamous cells, glandular cells, or both

<table>
<thead>
<tr>
<th>HPV Type</th>
<th>SCC</th>
<th>Adeno</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 16</td>
<td>50-60%</td>
<td>30%</td>
</tr>
<tr>
<td>HPV 18</td>
<td>10-20%</td>
<td>40-60%</td>
</tr>
</tbody>
</table>

- HPV & adenocarcinoma: type 18 > type 16
- Concurrent squamous HSIL: 46-72% (~50%)
Normal Cervical Microscopic Anatomy
"Typical" Presentations of Glandular Cervical Lesions: The Pathologist's Role

- incidental, non-neoplastic findings
  - polyps
  - microglandular hyperplasia
- cancer screening
  - Pap negative, high-risk HPV positive (type 18)
  - atypical glandular cells
- following colposcopy
  - biopsy, curettage, LEEP or cone biopsy evaluation
- following hysterectomy
Cancer after PAP-/HPV+: what is the risk?

N=46,647 Pap Neg/HPV +

> 1 Pap neg/HR-HPV pos

- 44 women with cervix cancer (Kaiser Permanente)
  - one PAP neg/HPV pos: 26
  - two PAP neg/HPV pos: 15
  - three PAP neg/HPV pos: 3

Histology
- adenocarcinoma 24*
- squamous 16
- adenosqamous 2
- adeno & squamous 1
- small cell 1

Cancer - Pap pos/HR-HPV neg

- n=21
  - Histology
    - adenocarcinoma 7
    - squamous 12
    - neuroendocrine 1
    - not specified 1

Pap Nomenclature for Glandular Abnormalities

Bethesda 2001

- Atypical Glandular Cells (AGC)
  - Atypical endocervical cells
  - Atypical endometrial cells
  - Atypical glandular cells not otherwise specified (NOS)

- Atypical Glandular Cells, favor neoplasia
  - Atypical endocervical cells
  - Atypical glandular cells

- Endocx Adenocarcinoma In Situ (AIS)

- Adenocarcinoma

*AGC replaces prior terminology, “AGUS”*

Solomon D et al. JAMA 2002; 287: 2114-9
AGC Cytology: What does it imply?

- < 0.5% of all cervical cytology

- Histologic diagnosis after AGC Cytology
  - *Squamous intraepithelial lesion (any)*
    - Most common pathology with AGC
    - Often coexist with glandular lesions

- *Endocervical Adenocarcinoma In Situ (AIS)*

- *Adenocarcinoma*
  - Cervix, endometrium, tube, ovary, metastatic
  - Reactive, reparative, polyps
  - Microglandular hyperplasia from OCPs
  - Adenosis from DES
Significance of Atypical Glandular Cells
Schnatz et al Obstet Gynecol 2006;107:701-8

Meta-analysis of 3,890 AGC cytology with follow-up data:

Follow-up diagnosis of AGC, NOS
- HSIL 11.1%
- AIS 2.9%
- Endometrial hyperplasia 1.4%
- Malignancy 5.2%

AGC favor neoplasia
- AIS 13%
- Malignancy 21%

Cancers found: Endometrium, endocervix, squamous cervix, ovary, fallopian tube, colon, breast

AGC cytology has higher association with cancer and pre-cancer than ASC-US
Most likely disease with AGC cytology is squamous. Cancer may be squamous or glandular. Endometrial cancer not related to HPV status and more common in older women.
Glandular Neoplasia of the Cervix: Diagnostic Classification

- **U.S.**
  - glandular atypia or endocervical glandular dysplasia (EGD)
  - adenocarcinoma in situ (ACIS)
  - invasive adenocarcinoma

- **W.H.O.**
  - ACIS (or CGIN*)
    - cervical glandular intraepithelial neoplasia
  - adenocarcinoma

**key points:**

- L.A.S.T doesn’t apply (lower anogenital squamous terminology)
  - terminology

- classic AIS is caused by HPV
  - some adenocarcinomas may not

- AIS is a precursor of invasive adenocarcinoma
  - AIS precursor lesion not well defined
Colposcopy of Glandular Neoplasia

- Most lesions lie within T-zone or close to SCJ
  - When glandular and squamous disease coexist, *squamous component is more likely to be visible*

- Lesions may be within the endocervical canal
  - “Skip” lesions may exist
Cervical Adenocarcinoma In Situ (AIS)

- average age: 35.8 yr
  - range: 29-46 years
  - 10-18 years younger than adenocarcinoma

- 10% multifocal skip lesions

- difficult to detect
  - may be missed on Pap
  - *does not obey usual colposcopy “rules”*
    - doesn’t arise from the squamo-columnar junction
Challenges in Atypical Glandular Lesions of the Cervix (occult lesions)

- #1: making the clinical diagnosis
  - very difficult to “see” the lesion

- #2: making the pathology diagnosis of AIS
  - threshold criteria?
  - is there a “precursor” lesion?

- #3: not making the diagnosis of AIS
  - recognizing lesions that mimic AIS

- #4: “When” is invasion present?
Lesion overlying columnar epithelium not contiguous with the SC junction

Coalescing Papillae

Variegated red and white lesions

Papillary lesions

densely acetowhite glandular neoplasia

squamous neoplasia
29 year old with AGC cytology
Ectropion or glandular lesion?

Photo: Richard Lieberman, MD
Atypical coalescence of papillae
Cone Biopsy

Photo: Richard Lieberman, MD
AIS: Typical Histology

- complex glands with preservation of “normal” distribution (so-called lobular)
  - cellular changes
    - loss of polarity
    - enlarged nuclei
      - +/- nucleoli
    - stratification
      - pseudostratification
    - mitoses (& atypical mits)
    - apoptosis
    - reduced apical mucin
  - strong p16 and MIB-1 staining
Cervical Glandular Neoplasia: Histological Classification

- endocervical, usual or classic type
- mucinous, nos
  - gastric
  - intestinal
  - signet-ring
- villoglandular
- endometrioid
- clear cell
- serous
- mesonephric
- neuroendocrine
SMILE (stratified mucinous intraepithelial lesion-endocx: AdSq in situ)

endocervical, usual

intestina, goblet cells & signet ring

endometrioid
Endocervical (mucinous) adenocarcinoma, gastric type
Adenocarcinoma in-situ: What we do know…

1. **AIS is a recognizable precursor of invasive adenocarcinoma with distinct histologic subtypes**

2. **AIS is multifocal or involves multiple quadrants in about half the cases**

3. **AIS can be cured by simple hysterectomy and in many cases treated effectively with cone biopsy**

4. **Endocervical glandular dysplasia (EGD) is not a reproducibly recognizable lesion**, and its behavior and existence are undefined

*Int J Gynecol Pathol 21(4):314-326, 2002*
Challenge: AIS or not AIS ≈ EGD?

**EGD Criteria (less than AIS)**

1. glands lined by cells with slightly atypical nuclei and fewer mitotic figures than AIS
2. the presence of only one gland with histologic AIS
3. nuclear atypia, apoptotic bodies ≤ 2 mitoses per gland
4. moderate nuclear enlargement, hyperchromasia, atypia
5. architectural and cytologic features short of AIS

**AIS Criteria**

- complex glands with preservation of “normal” distribution (so-called lobular)
- cellular changes
  - loss of polarity
  - enlarged nuclei
    - +/- nucleoli
  - stratification
    - pseudostratification
  - mitoses (& atypical mits)
  - apoptosis
  - reduced apical mucin
    - **strong p16 and MIB-1 staining**

*Int J Gynecol Pathol 21(4):314-326, 2002*
Is there a “precursor” lesion to ACIS?

Ioffe & Silverberg

- Proposed a scoring system for the diagnosis of noninvasive endocervical glandular lesions, AIS & EGD

**Results:** There scoring system improved observer Kappa statistic from 0.565 to 0.705.

**Conclusions:**
- The schema for its accurate distinction between AIS and lesser lesions.
- The use of this scheme will result in more consistency of data allowing uniformity of diagnosis of AIS.

**Long-term follow-up:**
- the biologic and clinical significance of EGD remains to be determined
- not in general use...

**TABLE 1. Scoring from distributed to the participants of the study in the second round review**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratification</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td>Up to the luminal surface</td>
<td>3</td>
</tr>
<tr>
<td><strong>Nuclear atypia</strong></td>
<td></td>
</tr>
<tr>
<td>As normal</td>
<td>0</td>
</tr>
<tr>
<td>Small (size of normal) or slightly enlarged uniform nuclei, minimal hyperchromasia, little dispolarity, no nucleoli</td>
<td>1</td>
</tr>
<tr>
<td>Nuclear enlargement (up to 3 x normal), moderate anisocytosis, moderate hyperchromasia, moderate dispolarity, occasional small nucleoli</td>
<td>2</td>
</tr>
<tr>
<td>Large nuclei (&gt;3 x normal), marked anisocytosis, marked hyperchromasia, severe dispolarity, frequent prominent nucleoli</td>
<td>3</td>
</tr>
<tr>
<td><strong>Mitoses and apoptoses</strong> in two most active glands, number per gland (average between two glands)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Less than 0.5 per gland</td>
<td>1</td>
</tr>
<tr>
<td>0.6–3.0 per gland</td>
<td>2</td>
</tr>
<tr>
<td>&gt;3.0 per gland</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total score</strong></td>
<td></td>
</tr>
<tr>
<td>0–3 = benign</td>
<td></td>
</tr>
<tr>
<td>4–5 = endocervical glandular dysplasia (EGD)</td>
<td></td>
</tr>
<tr>
<td>6–9 = adenocarcinoma in situ (AIS)</td>
<td></td>
</tr>
</tbody>
</table>
Immunohistochemical tests to aid in the diagnosis: p16Ink (most data)

**Pros**
- p16 is a surrogate marker for integrated HR-HPV (neoplastic transformation)
- best utilized for confirming or excluding a diagnosis (i.e. AIS v. Immature metaplasia)

**Cons**
- Unlike squamous lesions, not all glandular lesions are related to HPV infection (i.e. clear cell adenocarcinoma)
- Some HPV negative neoplasms and metaplasias can overexpress p16
p16 normally results in cell cycle arrest, but is “modified” by E7 (HPV) protein leading to:
1) unsuppressed cell growth (neoplasia), and
2) accumulation of p16 (not degraded by interaction with retinoblastoma protein = pRB)

Figure 2. Scheme of HPV E7 effects on p16^{INK4a} expression levels and cell cycle regulation (black arrows). E7 mediates p16^{INK4a} overexpression via transcriptional activation. High p16^{INK4a} levels would normally result in cell cycle arrest (grey arrow and text in figure). However, at the same time E7 disrupts pRB and thereby cell cycle progression is triggered (lower part of the figure) despite high p16^{INK4a} levels.
Adjunctive Testing With p16

- "Positive" ≈ expression in nucleus, cytoplasm, or both

- "Block positive" ≈ immunoreactivity of EVERY CELL involving full thickness, or almost full thickness
AIS: Block Positive, confirmatory
AIS?
TEM: tuboendometrial metaplasia
Challenge #2: Mimics of AIS

Common
- inflammatory atypia
- TEM: tuboendometrial metaplasia
- microglandular hyperplasia
- endometriosis
- tunnel clusters
- cautery artifact
- mesonephric remnants and hyperplasia

Uncommon
- LEGH-lobular endocervical glandular hyperplasia, and alas
- diffuse laminar endocervical glandular hyperplasia
- deep cervical glands
- adenomyoma
- radiation changes
- Arias-Stella effect
Reactive Atypia – Endocervical Glandular Stratification

p16 negative
TEM: “mosaic” pattern p16lnk
\(\approx\) *NEGATIVE* (*non – integrated*)
TEM: tuboendometrial metaplasia
TEM: tuboendometrial metaplasia

- tubal metaplasia
- tubo-endometrial metaplasia

- found in 30-60% of cervices

- most common benign mimic of AIS

- IHC
  - MIB-1 low
  - p16 mosaic pattern
  - bcl-2 positive
Post-Cone, Premenstrual and Post-Coital Bleeding
Cervical Endometriosis

- mid-cycle, post-coital spotting
- asymptomatic

- cause
  - probable trauma based
  - post-LEEP, cone, laser, fulguration
Cervical Endometriosis
Histology Cervical Endometriosis: Menstrual

Lieberman collection
MGH: microglandular hyperplasia
MGH: microglandular hyperplasia
MGH: microglandular hyperplasia
MGH: microglandular hyperplasia
MGH: microglandular hyperplasia
Deep Endocervical Glands and Lobular EC Gland Hyperplasia
Deep Endocervical Glands and Lobular EC Gland Hyperplasia
Stratified Mucinous Intraepithelial Lesions: S.M.I.L.E.
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Stratified Mucinous Intraepithelial Lesions: S.M.I.L.E.
Case

- 59 yo G0 post-menopausal with vaginal atrophy and cervical stenosis. PAP shows “AGC”
  - Referred for evaluation, Consult related no visible lesions
  - ECC positive for at least ACIS, No EMBx
  - Colposcopy…
Dx: Endocervical Adenocarcinoma, villoglandular type
Challenge #3: When “is” invasion?

...in the absence of an “obvious” gross lesion. i.e. an occult adenocarcinoma

- more difficult to recognize than squamous

- glandular lesions *may* have minimal stromal response when it invades

- relationship to adjacent thick walled vessels
  - glands “molded” around thick-walled vessel suggest invasion
  - glands surround a vascular structure

- Villoglandular lesions can be *either* in-situ or superficially invasive.
AIS vs. Invasive Adenocarcinoma

Unequivocal Invasion
- individual or fragmented cells or incomplete glands lined by malignant epithelium at a stromal interface
- malignant glands with a desmoplastic response (obvious infiltrative growth)

Less Convincing, but...
- confluent growth of complex, branching, irregular or small glands i.e. too many glands/unit area
- cribriforming or “maze-like” labyrinthine architecture
- DEEP deep glands away from the gland-stromal interface

Kurman *Int J Gynecol Pathol* 24:125-130, 2005
Fragmented Cells & Incomplete Glands
Fragmented Cells (minimal stromal response)
Desmoplastic Response
Glands Deeper than “base”
Close Approximation to Thick-walled vessels
Coalescing papillae in variegated red-white (inside SCJ)
Challenge #3: Occult (early) invasive adenocarcinoma

- Also referred to as minimally invasive adenocarcinoma
  - “microinvasive” carcinoma terminology is no longer utilized

Depth of invasion:
- Measure from the base of the epithelium, either surface or glandular, from which it originates.
- The depth of invasion is defined as the measurement of the tumor from the epithelial and stromal junction of the adjacent most superficial papillary to the deepest point of invasion.
- Vascular space involvement does not affect the classification.
Recommendations for measuring glandular lesions

- Always report with volumetric assessment

- Contiguous lesions:
  - Provide an axial length, depth as noted previously, and lateral extent.
  - In a cone biopsy, lateral extent assessment may be difficult. One method of reporting involves sectioning the cone biopsy based on quadrants and reporting what proportion of each quadrant contains in situ and/or invasive disease.

- Example: Invasive adenocarcinoma is confined to 3 of 5 sections of the 12 to 3 o'clock quadrant while adenocarcinoma in situ is present in 3 of 4 quadrants, not involving the 6 to 9 o'clock quadrant
Summary

- Glandular lesions of the cervix are becoming more frequent clinically, providing new challenges to the clinician & pathologist.

- Be aware of the “mimics” – benign lesions looking like variants of AIS and adenocarcinoma.

- Minimally invasive lesions occur in younger women and can be treated conservatively.
Thank you!