THE PAP SMEAR

~

CURRENT CRITERIA AND CHANGING CONCEPTS

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Each year approximately 50 million women undergo Pap testing in the US. Of these approximately 3.5 million (7%) are diagnosed with a cytological abnormality requiring additional follow-up or re-evaluation.

* 2001 Bethesda System and guidelines for management, from JAMA with permission, p44
Features to evaluate in Pap smears

- adequacy
- presence of abnormal cells
- number and distribution of abnormal cells, and relationship between cells
- Cell size, shape
- Nuclear size and shape
- n:c ratio
- nuclear changes and nucleoli
- cytoplasmic features
- background or diathesis

Features of precancerous and cancerous cells

- Abnormality in size and shape of cells
- Variation in cell size and shape
- Increase in nuclear size
- Increase in nuclear membrane irregularity
- Hyperchromasia
- Prominence of nucleoli and irregularity in shape thereof
- Thickening of nuclear membrane
- Increase in n;c ratio
- cytoplasm scanty
- Mitosis, increased number and abnormal forms
- Non-cohesiveness
- Abnormal polarity
Epithelial cervical squamous cell types in Pap smear

<table>
<thead>
<tr>
<th>Features</th>
<th>superficial cells</th>
<th>intermediate</th>
<th>parabasal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>cells</td>
<td>singly</td>
<td>singly</td>
<td>singly/sheets</td>
</tr>
<tr>
<td>shape</td>
<td>polyhedral</td>
<td>polyhedral, oval</td>
<td>oval, round</td>
</tr>
<tr>
<td>cell diameter</td>
<td>&lt;25um,</td>
<td>~40um</td>
<td>~40um,</td>
</tr>
<tr>
<td>nucleus</td>
<td>&lt;12um, pyknotic</td>
<td>&lt;10um, vesicular</td>
<td>&lt;6um, vesicular</td>
</tr>
<tr>
<td>cytoplasm</td>
<td>transparent</td>
<td>transparent</td>
<td>opaque</td>
</tr>
<tr>
<td></td>
<td>granules</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>flat</td>
<td>folded, flat</td>
<td>flat</td>
</tr>
</tbody>
</table>


Comparative size of Pap smear cells \(\sim um^2\)

<table>
<thead>
<tr>
<th>Cell type</th>
<th>cell area</th>
<th>nuclear area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial</td>
<td>1500</td>
<td>20</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1500</td>
<td>35</td>
</tr>
<tr>
<td>Parabasal</td>
<td>300</td>
<td>50</td>
</tr>
<tr>
<td>Endocervical</td>
<td>200</td>
<td>50</td>
</tr>
<tr>
<td>Endometrial</td>
<td>175</td>
<td>30</td>
</tr>
<tr>
<td>Reserve cells</td>
<td>200</td>
<td>50</td>
</tr>
</tbody>
</table>

Causes of false-negative Pap smears

- atypical endocervical cells
- crowded cell aggregates
- cytolysis with isolated large nuclei
- intermediate cells with nuclear enlargement
- keratinized or metaplastic cells
- necrotic debris
- smears obscured by blood or inflammation
Causes of false-positive Pap smears
  • atrophic smear
  • atypical endocervical or endometrial cells
  • multinucleated cells
  • parakeratosis
  • perinuclear halo in non-koilocytes
  • pseudoparakeratosis
  • repair, squamous metaplasia
  • tubal metaplasia

Minimum number of cells in a Pap smear
  • conventional: 8,000-12,000, well-preserved, well-visualized.
  • liquid-based: 5,000, well-preserved, well-visualized.

>10 well-preserved endocervical/squamous metaplastic cells are required for a satisfactory specimen

Inflammation obscuring Pap smear cells
  • partially obscured: 50-75% of cells are obscured
  • unsatisfactory: >75% of cells are obscured

Iatrogenic changes in Pap smears
  • chemotherapy
  • cautery
  • irritation: IUD, diaphragm, pessary
  • laser
  • post-instrumentation: biopsy
  • radiation therapy

Representation of transformation-zone component for specimens determined by...
  • At least 10 well-preserved endocervical or squamous metaplastic cells, not necessarily in clusters—TBS 2001
Decidua in Pap smears

- abundant pale cytoplasm
- bland minute nucleoli (differentiates LSIL)
- central round nucleus
- polygonal, well-defined cell borders
- singly or in small groups
- x3 size of neutrophil
- only rare cells
- pale chromatin
- seen in pregnancy or immediately post-partum

2

ATROPHY

Atrophic vaginitis, findings

- blue blobs
- cellularity, variable
- chromatin, smudgy
- debris
- nuclei, dot-like, rare bare
- polygonal-round cells, slight pleomorphism

Differential diagnosis of atrophy

- keratinizing dysplasia  lower n/c ratio, and refractile dyskeratosis in atrophy
- HSIL  smudgy nuclei and lack of nuclear detail in atrophy
- LSIL  uniform nuclei and lack of nuclear detail in atrophy

Pap smear changes in pregnancy

- Arias-Stella reaction*
- Cytotrophoblasts, syncytiotrophoblasts
- decidua
- glycogenated, folded intermediate “navicular” cells
- hematoidin crystals
- inflammation
- low estrogen effect: atrophy
- reactive changes

*isolated cells, rarely in clusters, crowded, overlapping nuclei, n/c ratio variable, smudgy chromatin, inflammatory cells

**Hormonal effect on Pap smears**
- *atrophic smear*: ovarian dysgenesis, Turner’s syndrome, pituitary dysfunction, oophorectomy
- *high-estrogen effect*: estrogen-producing ovarian tumors,
- *variable hormonal patterns*: masculinizing tumors, Stein-Levinthal, ovarian dysfunction

**Small blue cells associated with tamoxifen therapy**
- present in 40% of Pap smears in women on tamoxifen
- small, tightly cohesive clusters of cells
- cells have scant to absent cytoplasm
- nuclei are similar to intermediate squamous cells with smooth nuclear membrane
- fine, hyperchromatic chromatin and indisintct, minute nucleoli
- non-neoplastic in nature
- could represent proliferative reserve cells of cervical-vaginal epithelium
- probably result from estrogenic effect of tamoxifen on cervical-vaginal epithelium

<table>
<thead>
<tr>
<th>Atrophy vs small cell ca</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atrophy</strong></td>
<td><strong>Small cell ca</strong></td>
</tr>
<tr>
<td>large sheets of cells</td>
<td>fewer cells</td>
</tr>
<tr>
<td>bland nuclei</td>
<td>hyperchromatic nuclei</td>
</tr>
<tr>
<td>regular shape of nucleus</td>
<td>irregular shape</td>
</tr>
<tr>
<td>coarse chromatin</td>
<td>bland chromatin</td>
</tr>
<tr>
<td>no necrosis</td>
<td>necrosis</td>
</tr>
</tbody>
</table>

**Differential diagnosis of small cells in Pap smears**

<table>
<thead>
<tr>
<th>Tamoxifen cells</th>
<th>Endometrial cells</th>
<th>Small cell ca</th>
<th>Met breast ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>architecture</td>
<td>tight clusters</td>
<td>tight clusters</td>
<td>crowded sheets</td>
</tr>
<tr>
<td>cells</td>
<td>small, bland</td>
<td>small, bland</td>
<td>small, ovoid</td>
</tr>
<tr>
<td>nuclei</td>
<td>dark, smooth</td>
<td>irregular</td>
<td>dark, irregular</td>
</tr>
<tr>
<td>nucleoli</td>
<td>+/-</td>
<td>+, minute</td>
<td>(-)</td>
</tr>
<tr>
<td>cytoplasm</td>
<td>minimal</td>
<td>minimal</td>
<td>scanty</td>
</tr>
<tr>
<td>necrosis</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>mitotic activity</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
</tbody>
</table>
Histiocytes in Pap smear

- single cells, variable size
- oval shape
- bean-shaped nucleus, snucleus, may be multinucleated
- large nucleolus
- well-defined cytoplasm, with phagocytized material
- cytoplasmic vacuoles, granular cytoplasm

Folic acid deficiency in Pap smear

- may occur in pregnancy or upon OC use
- increase cell size, Increase cytoplasm
- increase nuclear size, occasional binucleation
- hyperchromasia
- slightly increased nc ratio
- chromatin delicate and uniform

<table>
<thead>
<tr>
<th>Predominant cell types at various ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
</tr>
<tr>
<td>neonate</td>
</tr>
<tr>
<td>pre-pubertal</td>
</tr>
<tr>
<td>pubertal</td>
</tr>
<tr>
<td>menstrual</td>
</tr>
<tr>
<td>pre-ovulation</td>
</tr>
<tr>
<td>post-ovulation</td>
</tr>
<tr>
<td>pregnancy</td>
</tr>
<tr>
<td>post-partum</td>
</tr>
<tr>
<td>post-menopause</td>
</tr>
</tbody>
</table>
3 Repair & Regeneration

<table>
<thead>
<tr>
<th>Infections observed on Pap smears, cytological features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Actinomyces</strong></td>
</tr>
<tr>
<td><strong>Candida</strong></td>
</tr>
<tr>
<td><strong>Chlamydia</strong></td>
</tr>
<tr>
<td><strong>CMV</strong></td>
</tr>
<tr>
<td><strong>Gardnerella</strong></td>
</tr>
<tr>
<td><strong>Herpes</strong></td>
</tr>
<tr>
<td><strong>HIV</strong></td>
</tr>
<tr>
<td><strong>Leptothrix</strong></td>
</tr>
<tr>
<td><strong>Normal flora</strong></td>
</tr>
<tr>
<td><strong>Trichomonas</strong></td>
</tr>
</tbody>
</table>

Repair and regeneration, cytological features

- 2-dimensional sheets, single cells rare
- well-defined cell borders
- low n:c ratio,
- slightly enlarged nuclei
- nuclei rounded, smooth nuclear membrane
- fine chromatin
- multiple nucleoli
- mitoses (+)
- abundant cytoplasm
- neutrophils often present

Radiation changes in Pap smears

Acute changes

- large cell, with normal n:c ratio
- nuclear membrane irregular
- multinucleation common
- pleomorphism
- dirty background
- leukophagocytosis
- smudged nuclear chromatin
- cytoplasmic vacuoles

**Chronic changes**
- some changes of acute radiation effect persist
- pale smudged nuclei
- low n:c ratio
- biphasic (psychedelic) cytoplasmic staining
- changes of repair and regeneration

<table>
<thead>
<tr>
<th><strong>Glandular cells, status post-hysterectomy-possible origins:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>fistula, vaginal adenosis, endometriosis, prolapsed fallopian tube, supracervical hysterectomy, glandular metaplasia following radiation or chemotherapy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Obscuring Inflammation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>partially obscured by inflammation…</td>
</tr>
<tr>
<td>50-75% epithelial cells cannot be visualized—TBS, 2001</td>
</tr>
<tr>
<td>unsatisfactory due to inflammation…</td>
</tr>
<tr>
<td>&gt;75% epithelial cells cannot be visualized—TBS, 2001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Atypical repair</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>3D groups</td>
</tr>
<tr>
<td>slightly dyscohesive</td>
</tr>
<tr>
<td>piling-up of cells</td>
</tr>
<tr>
<td>prominent nucleoli</td>
</tr>
<tr>
<td>irregular chromatin,</td>
</tr>
<tr>
<td>mitosis +</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Benign parakeratosis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>miniature mature squamous cells</td>
</tr>
<tr>
<td>polygonal</td>
</tr>
<tr>
<td>small, round, pyknotic nuclei</td>
</tr>
<tr>
<td>eosinophilic cytoplasm</td>
</tr>
</tbody>
</table>
- associated with reactive, irritative, processes

**Atypical parakeratosis**
- miniature mature squamous cells
- irregular cell shapes
- spindled, elongated, nuclei
- hyperchromatic or pyknotic nuclei
- high n:c ratio
- deeply eosinophilic cytoplasm
- associated with SIL

### Reactive inflammatory atypia versus dysplasia

<table>
<thead>
<tr>
<th></th>
<th>atypia due to inflammation</th>
<th>dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>cells</td>
<td>single/loose clusters</td>
<td>same</td>
</tr>
<tr>
<td>nucleus</td>
<td>regular contour</td>
<td>irregular contour</td>
</tr>
<tr>
<td></td>
<td>chromatin non-crisp</td>
<td>chromatin crisp</td>
</tr>
<tr>
<td>cytoplasm</td>
<td>may have vacuoles</td>
<td>may be denser</td>
</tr>
<tr>
<td></td>
<td>cell membrane indistinct</td>
<td>cell membrane distinct</td>
</tr>
<tr>
<td>background</td>
<td>generally exudative</td>
<td>generally non-exudative</td>
</tr>
</tbody>
</table>

### Reactive inflammatory atypia versus significant glandular atypia

<table>
<thead>
<tr>
<th></th>
<th>atypia due to inflammation</th>
<th>glandular atypia</th>
</tr>
</thead>
<tbody>
<tr>
<td>cells</td>
<td>single/sheets</td>
<td>single/aggregates</td>
</tr>
<tr>
<td>shape of cells</td>
<td>variable</td>
<td>columnar</td>
</tr>
<tr>
<td>nucleus</td>
<td>macronucleolus</td>
<td>less prominent nucleolus</td>
</tr>
<tr>
<td>cytoplasm</td>
<td>granular</td>
<td>less granular</td>
</tr>
<tr>
<td>background</td>
<td>generally exudative</td>
<td>generally non-exudative</td>
</tr>
<tr>
<td>diathesis</td>
<td>non-tumoral</td>
<td>tumoral</td>
</tr>
</tbody>
</table>
Radiation atypia versus SIL

<table>
<thead>
<tr>
<th></th>
<th>Radiation</th>
<th>SIL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>cells</td>
<td>single/loose aggregates</td>
<td>single/aggregates</td>
</tr>
<tr>
<td>size of cells</td>
<td>large</td>
<td>-</td>
</tr>
<tr>
<td>n:c</td>
<td>normal</td>
<td>increased</td>
</tr>
<tr>
<td>nucleus</td>
<td>dark, vacuolated</td>
<td>hyperchromatic</td>
</tr>
<tr>
<td></td>
<td>enlarged, multiple</td>
<td>enlarged</td>
</tr>
<tr>
<td>cytoplasm</td>
<td>vacuolated</td>
<td>less granular</td>
</tr>
</tbody>
</table>

*post-radiation SIL shows features of dysplasia: high n:c ratio etc.

Squamous metaplasia versus SIL

<table>
<thead>
<tr>
<th></th>
<th>Squamous metaplasia</th>
<th>SIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>cells</td>
<td>flattened</td>
<td>isolated</td>
</tr>
<tr>
<td>nucleus</td>
<td>small</td>
<td>larger</td>
</tr>
<tr>
<td></td>
<td>&lt;nucleus of intermed cell</td>
<td>&gt;nucleus of intermed cell</td>
</tr>
<tr>
<td>fine chromatin</td>
<td>coarse chromatin</td>
<td></td>
</tr>
<tr>
<td>contour: smooth</td>
<td>contour: wrinkled, wavy</td>
<td></td>
</tr>
<tr>
<td>cytoplasm</td>
<td>more</td>
<td>less</td>
</tr>
</tbody>
</table>

Characteristic infectious inclusions

- chlamydia: variable vacuoles in metaplastic cells
- HPV: perinuclear cytoplasmic clearing
- Herpesvirus: intranuclear, ground-glass inclusions, haloed
- CMV: intranuclear: single, large, round; intracytoplasmic: multiple, small

Follicular cervicitis, cytological features

- polymorphous lymphocytes
- tingible body histiocytes
- inflammatory cells
- dirty background
- capillaries traversing lymphoid aggregates
- ~50% of patients have chlamydial infection
Mimics of ASCUS

- inflammation-associated changes
- parakeratosis
- atrophy
- radiation
- chemotherapy
- deciduas
- multinucleation
- LSIL
- air-drying artifact
- orangeophilia- over-staining artifact

HPV-effect, cytological features

- nuclear area >x3 normal intermediate cell nucleus
- increased n:c ratio
- mild hyperchromasia
- slightly darker nucleus with evenly distributed chromatin
- mature cytoplasm
- cave-like cytoplasmic vacuole ~ perinuclear halo
- sharp dense periphery and condensed cytoplasm

**Definition- ASC-US:** Cytological changes that are suggestive of SIL but lack criteria for a definitive interpretation. The category includes:

~a minority of cases formally classified as ASCUS, favor reactive
~most cases formally classified as ASCUS, NOS or ASCUS, favor SIL
**Definition- ASC-H:** Cytological changes that are suggestive of HSIL but lack criteria for a definitive interpretation. The association with underlying CIN2 and CIN3 for ASC-H is lower than for HSIL, but sufficiently higher than for ASC-US to warrant consideration of different management recommendations.

ASC-H: ASC, cannot exclude high-grade SIL:
- immature small cells
- slightly increased n:c ratio
- mild hyperchromasia
- mild focal nuclear membrane irregularity
- mimics of ASC-H: atrophy, squamous metaplasia

“The subjective perception of changes that are intermediate between benign abnormalities and carcinoma is a potential diagnostic nightmare.”

Koss and Gompel in *Introduction to Gynecological Cytopathology*
**SIL:** squamous intraepithelial lesion

### LSIL vs. HSIL, cytological features

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>LSIL</th>
<th>HSIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>nucleus</td>
<td>enlarged +</td>
<td>enlarged ++</td>
</tr>
<tr>
<td>chromatin</td>
<td>granular *</td>
<td>coarsely granular</td>
</tr>
<tr>
<td>nuclear membrane</td>
<td>irregular +/-</td>
<td>irregular+</td>
</tr>
<tr>
<td>mitosis</td>
<td>rare</td>
<td>frequent</td>
</tr>
<tr>
<td>n:c ratio</td>
<td>increased +</td>
<td>increased ++</td>
</tr>
<tr>
<td>cytoplasm</td>
<td>no abnormality**</td>
<td>oddly shaped, scanty</td>
</tr>
<tr>
<td>koilocytes</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

*Opaque in HPV, **perinuclear halo in HPV*

### Mimics of atypical parakeratosis
- degenerative changes
- marked atrophy

### Keratinizing dysplasia
- markedly increased n:c ratio
- nuclear and cytoplasmic pleomorphism
- nuclei dense and hyperchromatic
- tadpole-like shape
- associated with HSIL or invasive squamous carcinoma

### Microinvasive carcinoma on Pap smear
- in general, difficult to diagnose on Pap smears
- cells resemble those of HSIL or invasive carcinoma
- large number of atypical cells
- cells may be present in syncytial arrangement
- presence of nucleoli and parachromatin clearing favor microinvasion
### Invasive squamous cells
- 3D
- dyscohesive
- prominent nucleoli
- abnormal chromatin
- piling-up of cells
- nuclear size variation
- increased n:c ratio
- nucleoli +
- irregular chromatin
- tumor diathesis

### Cytological features of types of squamous cell neoplasia

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>CIS</th>
<th>Microinvasive*</th>
<th>Invasive Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>nucleus</td>
<td>enlarged +</td>
<td>enlarged +</td>
<td>enlarged ++</td>
</tr>
<tr>
<td>chromatin</td>
<td>irregular -</td>
<td>irregular +</td>
<td>irregular ++</td>
</tr>
<tr>
<td>nucleoli</td>
<td>prominent -</td>
<td>prominent +</td>
<td>prominent ++</td>
</tr>
<tr>
<td>diathesis</td>
<td>-</td>
<td>+/-</td>
<td>++</td>
</tr>
</tbody>
</table>

### Cytological features of types of squamous cell carcinoma

<table>
<thead>
<tr>
<th>Features</th>
<th>Keratinizing</th>
<th>Nonkeratinizing</th>
<th>Small Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>single cells</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>cell clusters</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>cell Shape</td>
<td>Bizarre/spindle</td>
<td>Round/polygonal</td>
<td>ovoid</td>
</tr>
<tr>
<td>cell variance</td>
<td>Pleomorphic</td>
<td>Uniform</td>
<td>Uniform</td>
</tr>
<tr>
<td>cytoplasm</td>
<td>Orangeophilic</td>
<td>Cyanophilic</td>
<td>Basophilic</td>
</tr>
<tr>
<td>nucleolus</td>
<td>+/-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>diathesis</td>
<td>+/-</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>
Relative chromatin pattern of various cells seen in Pap smears

<table>
<thead>
<tr>
<th>Chromatin</th>
<th>lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine, even</td>
<td>ASCUS, SIL</td>
</tr>
<tr>
<td>Fine, even, clumped</td>
<td>SIL</td>
</tr>
<tr>
<td>Fine, irregular, nucleoli+</td>
<td>HSIL</td>
</tr>
<tr>
<td>Coarse</td>
<td>HSIL</td>
</tr>
<tr>
<td>Dense and opaque</td>
<td>keratinizing SIL, carcinoma</td>
</tr>
</tbody>
</table>

Anogenital HPV

<table>
<thead>
<tr>
<th>Oncogenic Risk</th>
<th>HPV types</th>
<th>Associated lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>6, 11, 42-44, 53-55, 62, 66, 70</td>
<td>condyloma, LSIL</td>
</tr>
<tr>
<td>intermediate</td>
<td>31, 33, 35, 39, 51, 52, 54, 58, 59, 61, 66-68</td>
<td>LSIL, HSIL, carcinoma</td>
</tr>
<tr>
<td>high</td>
<td>16, 18, 45, 56</td>
<td>LSIL, HSIL, carcinoma</td>
</tr>
</tbody>
</table>

“Dark cell clusters”

- crowded
- increased n:c ratio
- immature cytoplasm
- anisonucleosis
- mitosis+
- difficult to determine whether squamous or glandular

Differential diagnosis of “dark cell clusters”-

- ASC-H,
- HSIL: *nuclear membrane irregular*
- Atrophy: *nuclear membrane smooth*
Estimated cervical HPV infections and HPV-related disease

<table>
<thead>
<tr>
<th>Condition</th>
<th>Estimated Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>$1.5 \times 10^4$</td>
</tr>
<tr>
<td>HSIL</td>
<td>$8-30 \times 10^4$</td>
</tr>
<tr>
<td>LSIL</td>
<td>$1-4 \times 10^6$</td>
</tr>
<tr>
<td>Cytologically normal, HPV+, non-amplified test</td>
<td>$8-11 \times 10^6$</td>
</tr>
<tr>
<td>Cytologically normal HPV+, PCR test</td>
<td>$20-40 \times 10^6$</td>
</tr>
</tbody>
</table>

6 GLANDULAR LESIONS

Endometrial versus endocervical cells

- Endometrial cells packed together, scant cytoplasm
- Endocervical cells form looser clusters, more abundant cytoplasm

Endometrial stromal cells

- Superficial type- resemble histiocytes and form loose aggregates
- Deep stromal cells- are round to spindle shaped with small oval nucleus and scant cytoplasm

Reactive vs. neoplastic endocervical cells

<table>
<thead>
<tr>
<th>Feature</th>
<th>Reactive</th>
<th>Neoplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architecture</td>
<td>Flat, 2-dimensional</td>
<td>Microacini, isolated cells or sheets</td>
</tr>
<tr>
<td></td>
<td>Minimal crowding</td>
<td>Crowding, papillary</td>
</tr>
<tr>
<td></td>
<td>Picket fence</td>
<td>Feathery</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Rounded contour</td>
<td>Oval, variable</td>
</tr>
<tr>
<td>Anisonucleosis</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Anisocytosis</td>
<td>Rare</td>
<td>Variable, + in higher grade tumors</td>
</tr>
<tr>
<td>Nuclear membrane</td>
<td>Smooth</td>
<td>Irregular</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Fine</td>
<td>Coarse, hyperchromatic</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>+/-, isolated</td>
<td>Usually +, prominent, multiple</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Cyanophilic, syncytial</td>
<td>Cyanophilic, poorly defined</td>
</tr>
<tr>
<td>Background</td>
<td>Clean</td>
<td>Usually tumor diathesis</td>
</tr>
<tr>
<td>Cilia</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mitotic activity</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Benign endometrial cells
- small clusters- hyperchromatic crowded groups
- degenerated small cells
- n:c ratio high
- ill-defined border
- single cells uncommon
- nucleus small(=size of intermediate cell nucleus)
- round single nucleoli, small and inconspicuous
- cytoplasm, scant, basophilic and vacuolated

Atypical endometrial epithelial cells
- small groups : <10 cells
- slightly enlarged nucleus
- hyperchromatic nucleus
- small nucleoli
- vacuolated cytoplasm
- nuclear atypia > benign reactive or regenerative cells

Cytological features of tubal metaplasia
- usually in tissue fragments, Cilia (+)
- palisading of cells, in strips
- cells variable in size
- nuclei round-oval and central
- nucleoli variably present
- cytoplasm variable and pale

Cytological features of AIS
- tissue fragments, crowded sheets
- arranged in rosettes, feathery edge, with palisades in gland-like structures
- scant cytoplasm,
- minimally enlarged, round-oval, nuclei that overlap
- granular cytoplasm,
- hyperchromatic nuclei
- slight anisonucleosis
- clean background
Differential diagnosis of AIS

- oversampling of endocervix,
- endometriosis
- reactive changes, e.g. post-cone, IUD,
- tubal (ciliated cell) metaplasia
- squamous cell carcinoma in situ
- microglandular hyperplasia
- colonic cells in rectovaginal cells
- endometrial cells from lower-uterine segment
- decidua
- reactive changes from prior procedure, eg: cone biopsy

Endometrial cells may be seen in...

dysfunctional bleeding, hormonal therapy, IUCD, endometriosis, endometritis, pregnancy, post-partal state, recent endometrial instrumentation

Endometrial cells on Pap smears-

- considered abnormal when...
  - shed in post-ovulatory phase
  - after menopause, and not on hormone therapy
- considered atypical when...
  - nucleus is enlarged (larger than nucleus of intermediate cells)
  - nucleoli are conspicuous

Atypical endometrial cells seen in...

- endometrial polyp
- endometritis,
- Arias-Stella
- menstrual smear
- hormone therapy in post-menopausal women
- endocervical sampling by brush
Cytoplasmic features of endocervical adenocarcinoma
- indistinct cell borders
- eosinophilia
- fine distinct granularity

Nuclear features of endocervical adenocarcinoma
- well-defined nuclear membrane +++
- round nucleolus +++
- oval nucleus ++
- round nucleus +
- irregular nuclear shape +/-
- multinucleation +/-

Differential diagnosis of endometrial carcinoma *
- endocervical repair
- endocervical cells under progesterone effect
- endocervical cells with tubal metaplasia
- endometrial cells with papillary syncytial metaplasia
- endometrial hyperplasia
- Arias-Stella reaction

*Pap smear should not be viewed as a method to screen for endometrial carcinoma

Endocervical vs. endometrial carcinoma on Pap smears*

<table>
<thead>
<tr>
<th>Feature</th>
<th>endocervical ca</th>
<th>endometrial ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>cellularity</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>diathesis</td>
<td>necrotic</td>
<td>watery-diathesis</td>
</tr>
<tr>
<td>cell size</td>
<td>large, ~190um(^2)</td>
<td>smaller, 140um(^2)</td>
</tr>
<tr>
<td>cell shape</td>
<td>columnar</td>
<td>cuboidal</td>
</tr>
<tr>
<td>nuclear size</td>
<td>large, ~90um(^2)</td>
<td>smaller, 60um(^2)</td>
</tr>
</tbody>
</table>
cytoplasm | amphophilic | cynaophilic
---|---|---
n:c ratio | lower | higher
nucleoli | ++ | +
cells | columnar | round-oval
cell arrangement | 2-dimensional | 3-dimensional
papillae | ++ | -
strips | + | -
apoptosis | + | +/-
associated SIL | ++ | -

| Squamous versus glandular carcinoma on Pap smears |
| --- | --- |
| squamous ca | glandular ca |
| nucleus pyknotic | vesicular |
| keratin in cytoplasm | vacuoles in cytoplasm |
| “pearl” formation | duct formation |
| tadpole cells | papillae |
| flattened cell aggregates | 3-d cell aggregates |
| crisper cell borders | less crisp borders |
| cells in isolation | cells in aggregates |

<table>
<thead>
<tr>
<th>Endocervical vs endometrial carcinoma on Pap smears, differentiating points</th>
</tr>
</thead>
<tbody>
<tr>
<td>o cells from endocervical ca shed more cells than endometrial ca.</td>
</tr>
<tr>
<td>o cells from endocervical ca are larger</td>
</tr>
<tr>
<td>o nuclei of endocervical ca cells are larger</td>
</tr>
<tr>
<td>o cells from endocervical ca are more cyanophilic than cells than endometrial ca.</td>
</tr>
<tr>
<td>o cells from endocervical ca more commonly have nucleoli, and are usually larger.</td>
</tr>
<tr>
<td>o endometrial ca cells are usually arranged in 3D clusters</td>
</tr>
<tr>
<td>o endocervical cells are usually arranged in 2D sheets</td>
</tr>
<tr>
<td>o endometrial cells usually have a watery background</td>
</tr>
<tr>
<td>o endocervical cells have necrotic diathesis</td>
</tr>
<tr>
<td>o apoptosis is more common in endocervical ca</td>
</tr>
</tbody>
</table>
Glandular cells, status-post hysterectomy, possible origins

- Fistula
- Vaginal adenosis
- Endometriosis
- Prolapsed fallopian tubes
- Supracervical hysterectomy
- Glandular meatplasia following radiation or chemotherapy

AIS on thin-layer preparation

- dark cellular groups and sheets with crowding, single cells common
- “continuous” depth of focus
- variability of nuclear size and shape
- stippled chromatin, irregular nuclear membrane
- nucleoli, strips
- feathering
- mitosis

“Of corn flakes & raisins”

Cytological appearances in Pap smears simulating common objects

<table>
<thead>
<tr>
<th>Compared to</th>
<th>Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ball-like</td>
<td>endometrial carcinoma</td>
</tr>
<tr>
<td>bamboo-like</td>
<td>geotrichium</td>
</tr>
<tr>
<td>bean-shaped</td>
<td>nucleus of histiocyte</td>
</tr>
<tr>
<td>blue balls</td>
<td>menstrual endometrium, contour of endometrial cells</td>
</tr>
<tr>
<td>blue blobs</td>
<td>atrophic vaginitis</td>
</tr>
<tr>
<td>cannonball</td>
<td>aggregates of neutrophils in candida and trichomonas</td>
</tr>
<tr>
<td>cartwheel</td>
<td>nuclear chromatin in plasma cells</td>
</tr>
<tr>
<td>cheesy discharge</td>
<td>candida vaginitis</td>
</tr>
<tr>
<td>clock-face</td>
<td>nuclear chromatin in plasma cells</td>
</tr>
<tr>
<td>clinging</td>
<td>diathesis in squamous cell carcinoma on thin-layer preparation</td>
</tr>
</tbody>
</table>
- clue cells: bacterial vaginosis
- cigar shape: nucleus of keratinizing squamous carcinoma
- cocklebur-like: hematoidin crystals
- corkscrew: Curschmann’s spirals in endocervical mucus
- corn flakes: trapped air under
- daisy cell: mesothelial hyperplasia in pelvic washings
- dirty background: menstrual cycle
- dust ball: actinomycetes
- exodus: endometrial cells and stroma and histiocytes, days 7-10.
- feathering: edges of endocervical carcinoma in situ
- ferning: estrogen-effect on mucus
- fiber cells: Suggestive of invasive squamous cells
- fibroid cells: invasive squamous carcinoma
- fishy smell: Gardnerella vaginalis infection, (+) “whiff” test
- ground-glass: Nuclei in herpes virus infection
- hair-like: leptothrix
- halo: HPV
- histiocytic shower: exodus, day 5-11 accompanied by endometrial cells
- hobnail: AIS of endocervix
- honeycomb: endocervical cells
- indian-filing: metastatic lobular carcinoma
- india-ink nuclei: HGD
- kidney-shaped: nucleus of histiocyte
- kite cells: HPV effect, soft sign
- koilocytes: koilo=hollow or cavity in HPV
- leaf-like: squamous metaplasia
- maltese cross: refractile starch contaminant
- mosaie-like: squamous metaplasia
- moth-eaten: trichomoniasis-affected cells
- navicular: boat-like (intermediate glycogenated) cells in pregnancy
- oat cell: small cell of cervix
- pear: *Trichomonas vaginalis*
- pearl: keratinized pearl of squamous cells
- pencil cells: endocervical polyp
- permissive cells: mature squamous cells that produce HPV virions
- pill effect: pseudoparakeratosis
- polka-dot cells: keratohyaline granules in HPV
- pomegranate: herpes virus molded nuclei
- raisin-like: HPV-effect
- ratty background: cytolysis due to bacteria
- scavenger cells: histiocytes in exodus
- school of fish: arrangement of regenerative cells
- small blue cells: tamoxifen related cells
- smudged cells: laser effect, smudging appears in nucleus and cytoplasm
- snake-like: elongate nuclei in atypical parakeratosis
- snowshoe: alternaria
- spaghetti & meatball: candida hyphae and spores
- spider-like: metaplastic cells pulled apart
- sticky histiocytes: superficial endometrial stromal cells
- strawberry cervicitis: colposcopic appearance of trichomonads
- strip formation: AIS
- streaming: cells in repair
- sulfur granule: actinomyces
- tadpole cells: nuclei of invasive squamous cells
- targetoid inclusions: chlamydia inclusions
- tingible-body: follicular cervicitis, macrophage with phagocytic activity
- watery diathesis: endometrial carcinoma
- wrinkled cells: progesterone effect in intermediate cells

**CONTAMINANTS**
organisms that it may be mistaken for
- contraceptive gels: candida spp
- cotton fibers: bacterial colonies
- fibrinous debris: leptothrix
- hematoidin: nocardia
- mucus: leptothrix
- sulfonamide crystals: lactobacilli

~

References


Hoda RS, Hoda SA. Yet more analogies in cytopathology. *Diagnostic Cytopathol*. 2004;30:133
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>acanthosis</td>
<td>thickening of epithelium, a protective hyperplastic phenomenon</td>
</tr>
<tr>
<td>acidophilia</td>
<td>staining reaction: pink or red with eosin dyes, aka eosinophilia</td>
</tr>
<tr>
<td>actinomyces</td>
<td>dark filamentous bacteria in clumps, common with IUD use</td>
</tr>
<tr>
<td>air-drying artifact</td>
<td>limits interpretation, effects nucleus and cytoplasm, usually account for higher ASCUS rate</td>
</tr>
<tr>
<td>anaplasia</td>
<td>loss of differentiation, organization and function in tumor cell</td>
</tr>
<tr>
<td>atrophic smears</td>
<td>smear characterized by dominance of parabasal cells</td>
</tr>
<tr>
<td>atypia</td>
<td>any deviation from normal cellular morphology</td>
</tr>
<tr>
<td>basophilia</td>
<td>aka cyanophilia, staining blue or purple with basic dyes</td>
</tr>
<tr>
<td>binucleation</td>
<td>may be seen with ASCUS or HPV, or various infections</td>
</tr>
<tr>
<td>“blue blobs”</td>
<td>characteristic of atrophy</td>
</tr>
<tr>
<td>brown artifact</td>
<td>“corn flakes”, air-trapping under coverslip causing brown cell artifact</td>
</tr>
<tr>
<td>brush artifact</td>
<td>glandular crowding due to aggressive sampling via endocervix brush</td>
</tr>
<tr>
<td>candida albicans</td>
<td>a variety of yeast observed in hyphae and conidia</td>
</tr>
<tr>
<td>caudate cell</td>
<td>tadpole-shaped cells, nucleus in the ‘head’</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>obligatory intracellular bacteria, cytological changes: non-diagnostic;</td>
</tr>
<tr>
<td>CMV-infected cell</td>
<td>may be associated with follicular cervicitis</td>
</tr>
<tr>
<td>“cockle-burrs”</td>
<td>hematoidin crystals</td>
</tr>
<tr>
<td>“corn flaking”</td>
<td>see brown artifact</td>
</tr>
<tr>
<td>cyanophilia</td>
<td>aka basophilia, staining blue or purple with basic dyes</td>
</tr>
<tr>
<td>cytolysis</td>
<td>dissolution of cytoplasm, usually with lactobacilli</td>
</tr>
</tbody>
</table>
decidual cells  endometrial stromal cells with progesterone effect

degenerative changes  may be nuclear (e.g. coarse granules) or cytoplasmic (e.g. vacuoles)
differentiation  morphological and functional specialization of cell
disordered honeycomb  adenocarcinoma in situ of endocervix on thinlayer preparations
Doderlein’s bacilli  heterogeneous group of lactobacilli
dyskeratosis  keratinization of cells below granular layer, premature
dyskaryosis  aka dysplasia
dysplasia  abnormal alteration of epithelia, characterized by increased primitive cells associated with variable surface maturation & abnormal differentiation
eosinophilia  property of staining pink or red with eosin dye
epithelial “pearl”  concentric structure with keratinized cells, nuclei are retained
estrogenic effect  proportion of superficial cells reflect estrogenic effect
“exodus”  histiocytes + endometrial epithelial and stromal cluster representing menstrual smear, common day 4-8 of menstrual cycle
“feathering”  characteristic of adenocarcinoma in situ of endocervix
ferning  arborizing palm-leaf like pattern of mucus at ovulation
folic acid deficiency  cytomegaly, nuclear enlargement; Binucleation, chromatin & cytoplasm are unchanged
follicular cervicitis  numerous lymphocytes and (tingible-body) histiocytes in Pap smear.
glandular grouping  usually 3D, poorly defined cell borders
halo  see perinuclear halo
herpesvirus-effected cell  ground-glass nuclei, multinucleated cells, pomegranate-like molding
histiocyte  cell with ill-defined border, finely vacuolated cytoplasm, may have engulfed material in cytoplasm, nucleus is rounded and reniform, often eccentrically located
hobnail cell  characteristic of clear-cell adenocarcinoma of endometrium
hyperchromatism  increase in intensity of nuclear staining, i.e. increased basophilia
hyperkeratosis  thickening of the stratum corneum layer of epithelium
hyperplasia  increase in number of cells
hypertrophy  increase in size of organ or tissue, by increase in size/number of cells
inclusions  contents of extraneous or abnormal intrinsic particles within cytoplasm or nucleus
inflammatory changes  reactive changes in cytoplasm (e.g. altered staining) and nucleus (e.g. blurred chromatin)
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>irradiation effect</td>
<td>cytomegaly, karyomegaly, vacuolation of cytoplasm and nucleus, etc. see Table earlier</td>
</tr>
<tr>
<td>karyolysis</td>
<td>degenerative change: nucleus swells, loses chromatin and disappears</td>
</tr>
<tr>
<td>karyorrhexis</td>
<td>nuclear fragmentation</td>
</tr>
<tr>
<td>keratinization</td>
<td>formation of anucleate squames</td>
</tr>
<tr>
<td>koilocytes</td>
<td>enlarged superficial or intermediate cells, raisin-like nuclei, cytoplasm is dense, empty perinuclear cavity with sharp borders, manifestation of HPV-effect ~ koilos: Greek for cavity</td>
</tr>
<tr>
<td>lactobacilli</td>
<td>aka Doderlein’s bacilli, thrive in low pH and high glycogen of vagina</td>
</tr>
<tr>
<td>leptothrix</td>
<td>filamentous microorganism, thrive with <em>trichomonas vaginalis</em></td>
</tr>
<tr>
<td>lubricating jelly</td>
<td>may contaminate Pap smears: amorphous material with blue tinge</td>
</tr>
<tr>
<td>metaplasia</td>
<td>transformation of one mature cell type to another mature cell type</td>
</tr>
<tr>
<td>moth-eaten cells</td>
<td>in trichomoniasis</td>
</tr>
<tr>
<td>multinucleated giant cells</td>
<td>could be epithelial, histiocytic, trophoblastic or malignant</td>
</tr>
<tr>
<td>multiple nucleoli</td>
<td>high-grade carcinoma, glandular repair, glandular neoplasia</td>
</tr>
<tr>
<td>navicular cell</td>
<td>boat-shaped cell, variant of intermediate cell, seen in pregnancy</td>
</tr>
<tr>
<td>necrosis</td>
<td>death of tissue, characterized by nuclear changes</td>
</tr>
<tr>
<td>nucleolus</td>
<td>round-oval intranuclear eosinophilic structure with RNA</td>
</tr>
<tr>
<td>nucleus</td>
<td>membrane-lined intracytoplasmic body, with chromosomal material</td>
</tr>
<tr>
<td>orangeophilia</td>
<td>stains orange with OG6 dye</td>
</tr>
<tr>
<td>parakeratotic cells</td>
<td>miniature superficial squamous cells</td>
</tr>
<tr>
<td>pearl</td>
<td>concentric structure with keratinized cells, nuclei are retained</td>
</tr>
<tr>
<td>perinuclear halo</td>
<td>vacuole around nucleus: HPV, trichomonas infection, folic acid def.</td>
</tr>
<tr>
<td>phagocytosis</td>
<td>presence of particles or fragments within another cell</td>
</tr>
<tr>
<td>plasma cell</td>
<td>eccentric clock-face nucleus, dense cytoplasm, perinuclear “hof”</td>
</tr>
<tr>
<td>prominent nucleoli</td>
<td>decidua, repair, squamous and glandular dysplasia and neoplasia</td>
</tr>
<tr>
<td>pseudokoiocytosis</td>
<td>may be due to glycogen content of cell, or inflammation</td>
</tr>
<tr>
<td>pseudoparakeratosis</td>
<td>glandular cells with orangeophilic cytoplasm with hormonal therapy, aka pill effect</td>
</tr>
<tr>
<td>psammoma bodies</td>
<td>may indicate papillary ovarian tumors, or mesothelial hyperplasia, etc</td>
</tr>
<tr>
<td>pyknosis</td>
<td>nuclear shrinkage, condensation of chromatin to structureless mass</td>
</tr>
<tr>
<td>repair</td>
<td>sheets or syncytial of cells, with nuclear enlargement, prominent nucleoli, chromatin remains even; mitosis are seen, but are normal</td>
</tr>
<tr>
<td>sheets</td>
<td>cell aggregate: monolayer, regularly arranged, distinct cell boundaries</td>
</tr>
<tr>
<td>sperm in Pap smear</td>
<td>In smears from HIV-infected, indicative of unprotected intercourse</td>
</tr>
</tbody>
</table>
spindle cells               rule out pleomorphic dysplasia and keratinizing squamous carcinoma
syncytium                 irregularly arranged cells with indistinct cell boundaries
torulopsis glabrata       may be symptomatic, difficult to distinguish from candida on Pap
transformation zone       most SIL arise in the transformation zone—an area of variable size
                          and extent wherein squamous metaplasia occurs. The t-zone recedes
                          with age and may lie within endocervical canal in older women
trichomonas vaginalis     ovoid protozoan, indistinct nucleus, cytoplasmic granules present
trophoblastic cells        syncytial: 20+ nuclei, amphophilic cytoplasm, uniformly coarse
                          irregular chromatin, nucleoli rare
                          cytotrophoblast: large cell, nucleus large and irregular, uniformly
                          amphophilic cytoplasm, nucleus lobulated may be vacuolated
tubal metaplasia          metaplastic process of endocervix, with tubal-type epithelium, more
                          common in women aged: 35+, aka ‘ciliated cell’ metaplasia
undifferentiated cells    cell lacking differentiation, organization or specialization
vacuole                   intracytoplasmic or intranuclear clear space with a sharp outline—in
                          IUD use, radiation, glycogen, glandular neoplasia
vaginal adenosis          glandular epithelium in vagina, usually follows DES exposure in utero
vesicular                 uniformly delicate nuclear chromatin
“watery” diathesis        usually seen in endometrial carcinoma

Acknowledgements:
Information in this material has been derived from references listed above and from the following textbooks.

- Bibbo, Comprehensive Cytopathology, Philadelphia, Saunders, 1997
- DeMay, Practical Principles of Cytopathology, Chicago, ASCP, 1999
- Ramzy, Clinical Cytopathology & Aspiration Biopsy, Appleton and Lange, 2000
- Gray and Mckee, Diagnostic Cytopathology, Elsevier, 2003
- Geisinger, Stanley, Raab, Silverman, Abati, Modern Cytopathology, Phila, Churchill-Living., 2004


10 Legends

Legends to CD-ROM Images

1 Normal squamous cells in Pap smear: Superficial cells are flattened, have abundant cytoplasm and pyknotic nuclei. Intermediate cells are folded, have less abundant cytoplasm and vesicular nuclei (thin-layer; Papanicolaou stain; 400X).

2 Normal endocervical cells appear en face as flattened honeycombed sheets of cells with finely vacuolated cytoplasm, round nuclei and small nucleoli. On-edge (inset) the cells are columnar with basal ovoid nuclei (thin-layer; Papanicolaou stain; 400X).

3 Squamous metaplasia: small parabasal-type cells with variable shape, distinct cell borders, and rounded regular nuclei (thin-layer; Papanicolaou stain; 400X).

4 Normal endometrial cells: epithelial cells of endometrium are usually in a 3-dimensional cluster. Nuclear size is comparable to intermediate cell nuclei, and cytoplasm is scant (thin-layer; Papanicolaou stain; 400X).

5 Lactobacilli induce cytolysis. Cytolytic changes in intermediate cells are typically due to peptolytic influences of Lactobacilli. Note the “free” nuclei, cellular debris, and rod-like bacilli (thin-layer; Papanicolaou stain; 400X).

6 Coccobacilli: partial obliteration of epithelial cells by small bacilli gives the cells a grainy, velvety (clue cell) appearance (thin-layer; Papanicolaou stain; 400X).

7 Candida spp: filamentous structures with pseudohyphae and spore forms (conventional smear; Papanicolaou stain; 400X).

8 Leptothrix with trichomonas vaginalis: Leptothrix are thin, curved filamentous organisms commonly found with trichomonas (conventional smear; Papanicolaou stain; 400X).

9 Trichomonas vaginalis: pear-shaped organisms with an eccentric faint nucleus and cytoplasmic granules (thin-layer; Papanicolaou stain; 400X).

10 Herpes simplex virus: multinucleate giant cell with homogenous, “ground-glass” appearance of nuclei with margination of chromatin. Note nuclear molding (thin-layer; Papanicolaou stain; 400X).
11 **Actinomyces** organisms occur as aggregates of filamentous, branching bacteria radiating from the darker center, usually seen with IUD use (conventional smear; Papanicolaou stain; 400X).

12 **Squamous cells with repair** are arranged in flat cohesive 2-dimesional sheets. Nuclear/cytoplasmic ratio is low. Nuclei show “streaming” with prominent nucleoli. Note intracellular neutrophils. Cytoplasm is usually cyanophilic (thin-layer; Papanicolaou stain; 400X).

13 **Reactive endocervical cells** are arranged in a honeycombed manner without cellular overlap. Cells are have rounded with hyperchromatic nuclei and prominent nucleoli (thin-layer; Papanicolaou stain; 400X).

14 **Radiation change** show enlarged cells with large nuclei and prominent nucleoli. Cytoplasm is vacuolated (conventional smear; Papanicolaou stain; 400X).

15 **IUD cell** are endometrial cells shed in a patient with intrauterine contraceptive device in place. These cells appear singly with high n:c ratio. Cytoplasm is finely vacuolated (conventional smear; Papanicolaou stain; 400X).

16 **Endometriosis**: Endometrial glandular and stromal cells in a patient with cervicovaginal endometriosis. Hemosiderin-laden macrophages may also be seen (thin-layer; Papanicolaou stain; 400X).

17 **Menstrual endometrium “exodus”**: This cluster of endometrial cells is seen towards the end of menstruation and comprise of glandular cells (at periphery) and stromal cells (at center) (conventional smear; Papanicolaou stain; 400X).

18 **Atrophy**: Sheets of parabasal cells with enlarged nuclei and bland smudgy chromatin. Background shows air-drying, inflammatory cells and mummified parabasal cells “blue blobs” (conventional smear; Papanicolaou stain; 400X).

19 **Atrophic vaginitis**: Advanced atrophy commonly shows parabasal cells associated with inflammatory cells (thin-layer; Papanicolaou stain; 400X).

20 **Follicular cervicitis**: Numerous lymphocytes derived from lymphoid follicle in cervix. Note macrophages with phagocytic activity (“tingible body” macrophage) (conventional smear; Papanicolaou stain; 400X).

21 **Hyperkeratosis** is a mucosal surface reaction characterized by anucleate squames and occurs in response to irritation (thin-layer; Papanicolaou stain; 400X).

22 **Parakeratosis** is another mucosal surface reaction characterized by presence of miniature squamous cells. These cells exfoliate either singly or in aggregates (thin-layer; Papanicolaou stain; 400X).
23 **Tubal metaplasia** is ciliated columnar endocervical cells that originate from upper endocervical canal (conventional smear; Papanicolaou stain; 400X).

24 **Endocervical polyp**: usually shows up in Pap smears as numerous columnar endocervical cells with tubal metaplasia. Nuclear atypia is minimal (conventional smear; Papanicolaou stain; 400X).

25 **Syncytiotrophoblast** is a large multinucleated cell and may be seen after an aborted pregnancy (conventional smear; Papanicolaou stain; 400X).

26 **Atypical squamous cells of undetermined significance** (ASC-US): Mature cells with moderately enlarged nuclei, minimal hyperchromasia, and smooth nuclear membranes. Binucleation may be seen (thin-layer; Papanicolaou stain; 400X).

27 **Low-grade squamous intraepithelial lesion (HPV effect)**: Mature squamous cells with classic human papillomavirus cytopathic effects- enlarged hyperchromatic, nuclei with irregular nuclear envelop. Binucleation is common. Cytoplasm has a sharply demarcated perinuclear halo (thin-layer; Papanicolaou stain; 400).

28 **Low-grade squamous intraepithelial lesion (mild dysplasia)**: Nuclei are enlarged, hyperchromatic with mild nuclear membrane irregularity. The cells have “mature” cytoplasm (thin-layer, Papanicolaou stain; 400X).

29 **Low-grade squamous intraepithelial lesion (mild dysplasia)**: Nuclei are enlarged, hyperchromatic with mild nuclear membrane irregularity. The cells have “mature” cytoplasm (conventional smear, Papanicolaou stain; 400X).

30 **Atypical squamous cells, cannot exclude HSIL (ASC-H)**: These atypical cells are of squamous metaplastic type. Nuclei are enlarged with membrane irregularity and finely granular chromatin. The n:c ratio is not as high as HSIL (thin-layer; Papanicolaou stain; 400X).

31 **High-grade squamous intraepithelial lesion (HSIL)**: Cells show high n:c ratio and enlarged, hyperchromatic nuclei with marked nuclear membrane irregularity (thin-layer, Papanicolaou stain; 400X).

32 **High-grade squamous intraepithelial lesion (HSIL)**: Cells show high n:c ratio and enlarged, hyperchromatic nuclei with marked nuclear membrane irregularity (thin-layer, Papanicolaou stain; 400X).

33 **HSIL (CIN 3/CIS) involving endocervical glands**: Large syncytial aggregate (ill-defined cell borders). Note loss of nuclear polarity (thin-layer; Papanicolaou stain; 400X).

34 **Atypical parakeratosis**: Sheet of elongated miniature squamous cells with atypical nuclei. Cytoplasm is eosinophilic to orangeophilic. Parakeratosis may be associated with LSIL (thin-layer; Papanicolaou stain; 400X).
35 **Keratinizing dysplasia**: High-grade keratinizing dysplasia showing both nuclear and cytoplasmic pleomorphism. Note orange cytoplasm and large, hyperchromatic nuclei (thin-layer; Papanicolaou stain; 400X).

36 **Keratinizing squamous cell carcinoma**: Dispersed cells of spindle to elongated and caudate forms with heavily keratinized cytoplasm. Nuclei are pleomorphic and hyperchromatic. Nucleoli are less conspicuous than the non-keratinizing type. These tumors generally lack tumor diathesis (conventional smear; Papanicolaou stain; 400X).

37 **Non-keratinizing squamous cell carcinoma**: Malignant polygonal cells are arranged in either loose clusters or crowded groups or singly. Nuclei are large, mostly round, and have coarse chromatin, and macronucleoli. Cytoplasm is dense with distinct cell borders. N:C ratio is moderate. Tumor diathesis is generally present (thin-layer; Papanicolaou stain; 400X).

38 **Atypical glandular cells** with pseudostratification. These cells may be of endocervical or endometrial in origin. Nuclei show minimal atypia. Differential diagnosis includes tubal metaplasia or glandular neoplasia (thin-layer, Papanicolaou stain; 400X).

39 **Atypical endocervical cells** with focal palisade arrangement (“feathering”), and enlarged hyperchromatic nuclei with small nucleoli (thin-layer; Papanicolaou stain; 400X).

40 **Atypical endometrial cells** in a 3-dimensional cluster. Nuclei are hyperchromatic with prominent nucleoli. (thin-layer; Papanicolaou stain; 400X).

41 **Atypical endocervical cells-favor neoplastic**: A dense cluster of endocervical cells with peripheral palisading and large hyperchromatic nuclei (thin-layer; Papanicolaou stain; 400X).

42 **Atypical glandular cells- favor neoplastic**: Strip of cells with large hyperchromatic nuclei. Nucleoli are not conspicuous (thin-layer; Papanicolaou stain; 400X).

43 **Endocervical adenocarcinoma in situ** seen as a cluster of hyperchromatic crowded group of cells with high n:c ratio and prominent nuclear protrusion. Nuclei are large, elongated and oval with coarse chromatin. Nucleoli are lacking. Background is clean. Inset shows a group of pseudostratified cells (thin-layer; Papanicolaou stain; 400X).

44 **Endocervical adenocarcinoma**: Well differentiated endocervical adenocarcinoma showing large group of cells in a predominantly 2-dimensional group. Cells show high n:c ratio, enlarged hyperchromatic nuclei and macronucleoli amid necrotic diathesis. Inset shows a rosette (conventional smear; Papanicolaou stain; 400X).

45 **Endometrial adenocarcinoma** seen as a papillary tuft of malignant cells from a case of papillary serous variant. The malignant cells show nuclear enlargement and pleomorphism (thin-layer; Papanicolaou stain; 400X).
Endometrial adenocarcinoma is seen as round cells with cyanophilic and vacuolated cytoplasm with intracytoplasmic neutrophils. Background shows the characteristic blue-tinged, finely granular “watery” diathesis {thin-layer (left) and conventional smear (right); Papanicolaou stain; 400X}.

Atypical histiocytes in endometrial adenocarcinoma: Large loosely structured cluster of cells with intracytoplasmic vacuoles containing numerous neutrophils. (conventional smear; Papanicolaou stain; 400X).

Small cell anaplastic carcinoma of cervix shows tight syncytial aggregate of small irregular tumor cells. Note scant cytoplasm and small nuclei. There is nuclear molding (thin-layer preparation; Papanicolaou stain; 400X).

Metastatic lobular carcinoma of breast shows a cluster of small cells with large irregular nuclei and scant cytoplasm. A clean background of the smear is often observed in metastatic tumors. Clinical history is important (thin-layer preparation; Papanicolaou stain; 400X).

Acknowledgements.

Contrary to popular belief, the purpose of the Pap smear is to screen for cervical cancer, not to diagnose them. Wang and Ducatman, in The Pap Smear

Supplement

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US&CAP short course

Pap smear: current criteria & changing concepts

Hoda & Hoda
normal squamous cells

- superficial cell
- intermediate cell
normal endocervical cells
squamous metaplastic cells
normal endometrial cells
lactobacilli inducing cytolysis
coccobacilli ~ bacterial vaginosis
candida spp
leptothrix and trichomonas vaginalis
trichomonas vaginalis
herpes simplex virus
actinomyces spp
squamous cells with repair
reactive endocervical cells
radiation changes
IUD cell
vaginal endometriosis
menstrual endometrium ‘exodus’
atrophy
atrophic vaginitis

parabasal cell
follicular cervicitis

tingible body macrophage
hyperkeratosis
parakeratosis
tubal metaplasia
endocervical polyp
syncytiotrophoblast
atypical squamous cells of undetermined significance, ASCUS
low grade squamous intraepithelial lesion, LSIL
LSIL
LSIL
atypical squamous cells cannot exclude HSIL, ASC-H
high grade squamous intraepithelial lesion, HSIL
HSIL involving endocervical glands
atypical parakeratosis
keratinizing dysplasia
keratinizing squamous ca
non-keratinizing squamous ca
atypical glandular cells
atypical endocervical cells
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endocervical adenocarcinoma *in situ* - AIS
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contributed invaluable time & effort in preparing these images

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