

Patterns and Pitfalls in Gynecologic Cytology

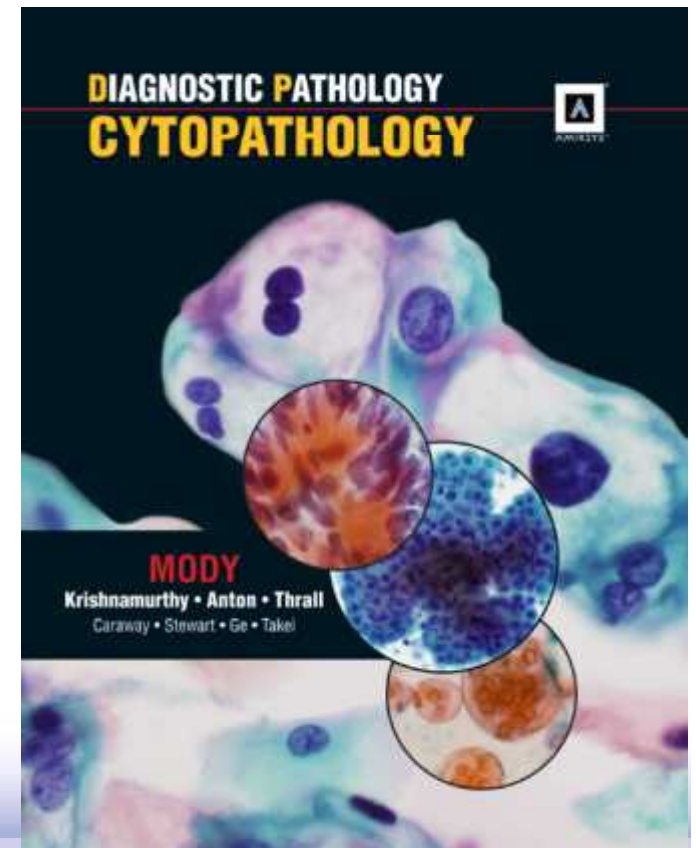
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Conflict of Interest

- None with vendors of cytology equipment or HPV testing
- Amirsys (now Elsevier)



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 in 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....

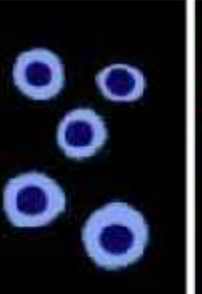
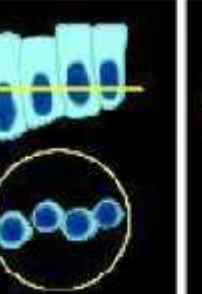
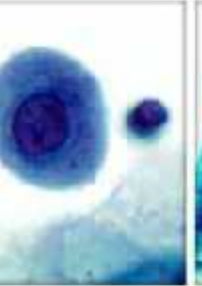
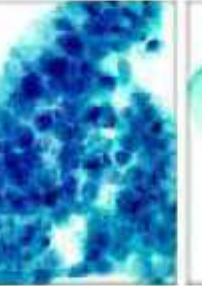
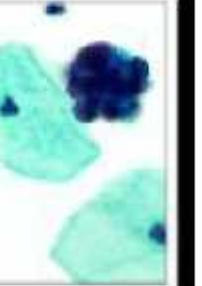
- Normal
- Repair and atypical repair
- Radiation
- Pregnancy and Provera related pitfalls
- Mimics of LSIL
- Mimics of HSIL
- Mimics of ASC-US and ASC-H
- Recognizing Diathesis in various preparations
- Pitfalls in Squamous cell carcinoma diagnosis

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

NORMAL PAP TEST

Normal Epithelial Cell Sizes and Morphology

Superficial	Intermediate	Parabasal	Endocervical	Endometrial
 Cells: $\pm 50 \mu\text{m}$ Nuclei: $\pm 5-8 \mu\text{m}$	 Cells: $\pm 30-40 \mu\text{m}$ Nuclei: $\pm 10-12 \mu\text{m}$	 Cells: $\pm 20 \mu\text{m}$ Nuclei: $\pm 10-14 \mu\text{m}$	 Cells: Height: $\pm 25-30 \mu\text{m}$ Diameter: $\pm 8-10 \mu\text{m}$ Nuclei: Diameter: $\pm 8-10 \mu\text{m}$	 Cells: Height: $\pm 15-20 \mu\text{m}$ Diameter: $\pm 6-9 \mu\text{m}$ Nuclei: Diameter: $\pm 7-8 \mu\text{m}$
				

Schematic representation and images depict the various normal cellular components seen on a Pap smear/ test. The cell sizes (and dimensions) and how they relate to each other are shown in the drawings. Actual pictures of superficial, intermediate, parabasal, endocervical, and endometrial cells are shown below the drawings. The pictures are not of the same magnification.

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

Repair Criteria

Repair

- **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
- Bizzare 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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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

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/cytopl
- Mild hyperchromasia, variable nucleoli
- Polychromatic staining

Mimics of LSIL

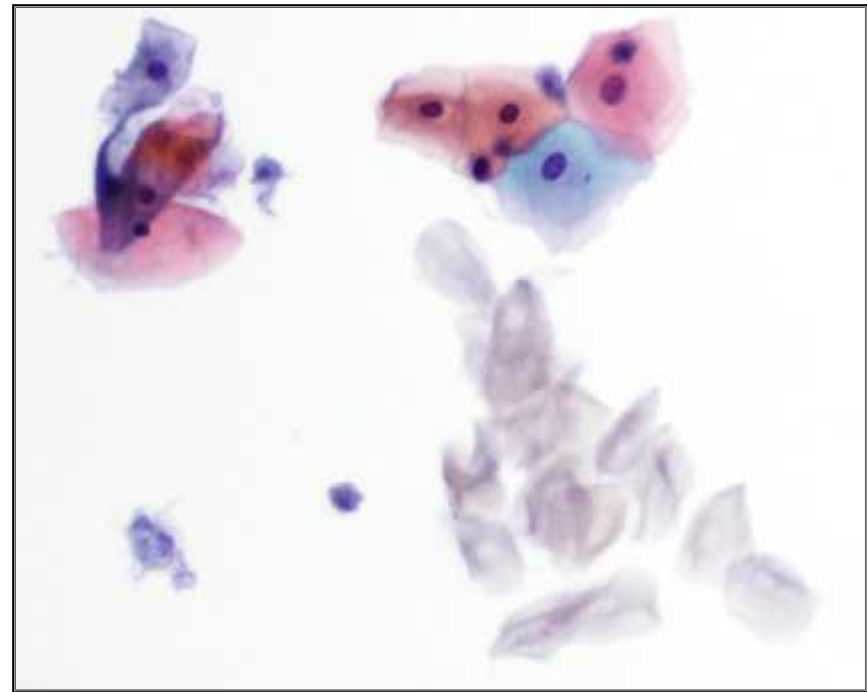
Herpes

- 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

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

Common Patterns Classified as ASC-US

- Atypical parakeratosis
- Atypical repair
- Atypia in Postmenopausal women with atrophy
- Decidua
- Trophoblastic cells

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)

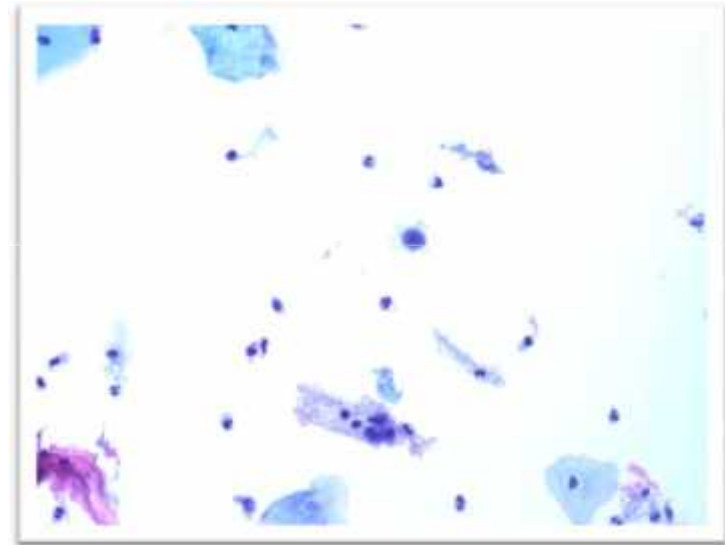
Metaplastic cells with nu 1.5-2.5 X normal

N:C ratio closer to HSIL but other nuclear abnormalities fall short

In liquid based, cells small and 2-3X neutrophil nuclei

Mis classified ASC-H

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



Squamous Cell Carcinoma

- Non Keratinizing and Keratinizing types
- Features and diathesis vary by preparation type
- Cellularity also variable
- Diathesis usually subtle 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

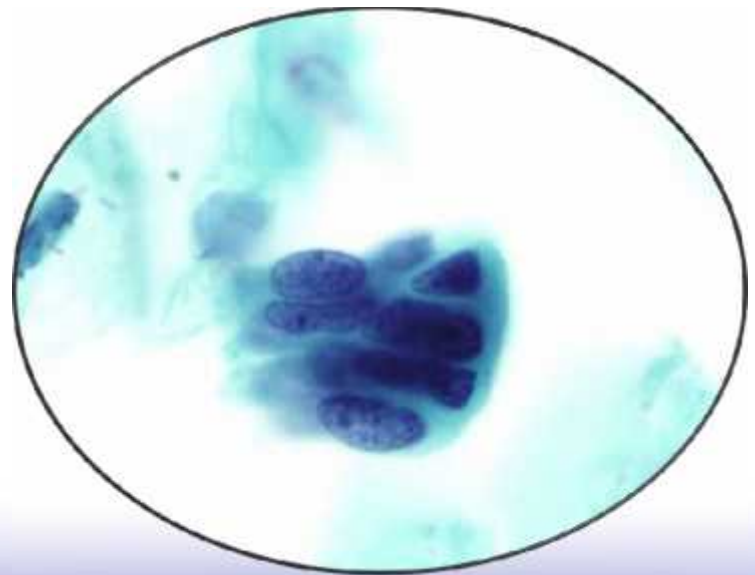
- Pseudo diathesis of atrophic vaginitis
- Irritated and ulcerated endocervical Polyps
- Lubricant simulating diathesis

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

Adenocarcinoma In Situ and Mimics

Cytologic Criteria	AIS	HSIL	Repair	Tubal Metaplasia	Directly Sampled Endometrium
Cellularity	Cellular	Usually cellular	Rare fragments	Rare event	Few groups
Hyperchromatic crowded groups	Many	Can be many	Not present	Rare event	Present
Sheets	Many with feathering	Syncytia	Flat sheets	Absent/rare/small	Present, 3D
Nuclear crowding/overlap	Present	Present	Absent	Present but mild	Present
Nuclear polarization perpendicular	Present	Absent	Absent	Present	Can be present
Hyperchromasia	Present	Present	Absent	Mild	Mild
Nuclear shape	Oval/elongated	Round	Round	Oval/cigar-shaped	Oval/cigar-shaped
Peripheral feathering	Present	Absent/focal	Absent	Rare	Absent
Strips	Present	Absent	Absent	Present	Present
Rosettes	Present	Absent	Absent	Absent	May be present
Terminal bars/cilia	Absent	Absent	Absent	Present/diagnostic	May be present
Spindled stroma	Absent	Absent	Absent	Absent	Present
Mitosis/apoptosis	With or without	May be seen	Rare	Absent	May be present
P16	Block-positive pattern	Block-positive pattern	Negative	Patchy positive	Patchy/focal/rare cells positive

From Diagnostic Pathology: Cytopathology Mody D Amirsys Publishing, 2014

Directly Sampled Lower Uterine segment Endometrium or Endometriosis

Configuration

**Tissue fragments, sheets, +/-
gland openings. Stromal cells***

Cell size

**Small, 2.5 X Int nucleus or nucleus=int
nucleus**

Sheets

**Appear crowded with minimal to
no nuclear overlap in plane
of focus. Tubular gland openings may be
seen**

Feathering

Absent

Palisading

Absent

Mitosis

May be present

Mucin

Absent

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

AGC (Endometrial)

- **Small groups of 5-10 cells**
- **Nu slight enlarged, small nucleoli**
- **Slight hyperchromasia**
- **Ill-defined cell borders,**
- **Scant cytoplasm, vacuoles+/_**

Normal vs Atypical Endometrial Cells

Normal (exfoliated)

- Nuclei $\leq$ than intermediate cell nucleus ($35\mu\text{m}^2$)
- Chromatin dense, heterogenous, apoptotic
- Nucleoli small or absent
- Scant cytoplasm, dense or vacuolated
- Menstrual endometrium may look worse on liquid based preps (pleomorphism of nuclear size and shape)

Atypical

- Nuclei slightly larger compared to normal
- Mild hyperchromasia
- Occasional nucleoli
- Scant vacuolated cytoplasm

Endometrial Carcinoma

Age	Peri & post menopausal
Cellularity	Low
Configuration	Loose cell groups, acini, papillae
Nuclei	Round, vesicular
Nucleoli	Multi/macro
Cytoplasm	Scant, cyanophilic
Background	Diathesis

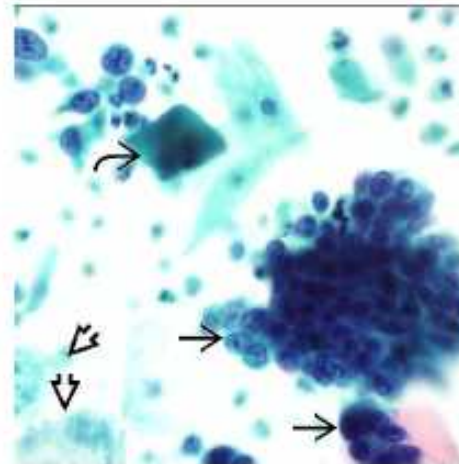
Mimics of Endometrial Adenocarcinoma/Hyperplasia

- 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

EXTRAUTERINE CARCINOMAS AND PRESENTATIONS IN CERVICOVAGINAL CYTOLOGY



Conventional Pap shows a 3D papillary cluster of ovarian serous papillary carcinoma with psammoma bodies with a clean background.



SurePath Pap shows rectal carcinoma and fecal material from a rectovaginal fistula (directly sampled). Note the cancer cells with rosettes, tumor diathesis, and skeletal muscle fibers.

TERMINOLOGY

Abbreviations

- Extrauterine carcinoma (ExUCa)

Definitions

- Involvement of vagina (&/or very rarely, cervix) by secondary malignant neoplasm via metastasis, direct extension, or passing through fallopian tube, endometrial cavity, & cervix without actual metastasis

CLINICAL ISSUES

Presentation

- Incidental finding
 - Vaginal bleeding, symptoms related to mass/metastasis
- Carcinomas more frequent than sarcomas and other metastatic tumors
- Metastatic tumors constitute 90% of all adenocarcinomas in vagina
- In most cases, primary is known

CYTOPATHOLOGY

- Patients may have ascites as well
- Diathesis if direct extension or actual metastasis to vagina or (rarely) cervix or endometrium
- Diathesis if direct extension from adjacent organ or fistula

Cells

- Varies by primary site

Cell Block Findings

- Cell blocks from liquid-based specimen can be helpful for immunohistochemical work-up of unknown primary

MICROSCOPIC PATHOLOGY

Histologic Features

- Resembles primary tumor with respect to architectural and cytologic features

ANCILLARY TESTS

Immunohistochemistry

- Immunostains can be helpful in confirming diagnosis for tumors originating outside female genital tract

Extrauterine Carcinomas on PAP smears

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

Key Facts

Cytopathology

- Low cellularity if extrauterine carcinoma spreads/floats through fallopian tube and endometrium without metastasis
- Highly cellular if metastasis to vagina or direct extension from rectum or bladder
- Cytologic features depend on primary tumor, extent of spread, ascites, and patency of fallopian tubes

- Clean background if spread via fallopian tube without metastasis; patients may have ascites as well
- Diathesis if direct extension or actual metastasis to vagina, endometrium, or cervix due to stromal response
- Morphologically, cells do not belong in environment

Ancillary Tests

- Immunostains are often of no use when suspected primary is in female genital tract

Presentation of Extrauterine Malignancies in Cervicovaginal Cytology

Primary Site	Mechanism of Presentation	Cytology	Background	Notes
Ovary	Passing through fallopian tube/endometrium; rarely, metastasis to vagina	Papillae ± psammoma bodies and large nucleoli	Usually clean unless metastasis to cervix or vagina	Rare primary presentation on Pap test
Fallopian tube	Passing through endometrium and cervix	Papillae and high nuclear grade, ± psammoma bodies	Usually clean or watery	Rare primary presentation on Pap test
Bladder	Direct extension or metastasis	Transitional or carcinoma cells ± squamous or glandular differentiation	Usually tumor diathesis	p16 and high-risk HPV (-) helps in differentiation from cervical primary
Colon	Direct extension through rectovaginal septum or fistula or metastasis	Tall columnar cells ± mucin and cigar-shaped nuclei with palisading	Tumor diathesis; fecal material if rectovaginal fistula	CK20 and CDX-2 positive; pax-8 negative, helpful in diagnosis
Breast	Ascites, rarely metastasis	Signet rings, intracytoplasmic targetoid mucin, single files	Usually clean	pax-8(-), ER/PR(+)
Stomach	Ascites, passing through tube, rarely metastasis	Signet rings, single files	Usually clean	Usually known history; differential diagnosis is breast carcinoma
Kidney	Metastasis; history usually known	Abundant fragile cytoplasm, large nuclei with nucleoli	Diathesis if metastasis to cervix and/or vagina	pax-8(+), RCC(+), and CD10(+)
Melanoma	Usually metastasis, known history; rarely, primary	Cells can look epithelial, spindle, pleomorphic, amelanotic	Diathesis or clean	S100/HMB45(+)
Lymphomas	Involvement by metastasis; rarely, ascites	Dispersed lymphoid population	Diathesis or clean if ascites	Usually known history
Sarcomas	Usually metastasis	Varies by primary type	Tumor diathesis	Primary type usually known