Glandular Lesions in Cervicovaginal Cytology: Patterns, Pitfalls and Bethesda Updates

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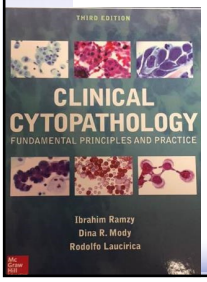
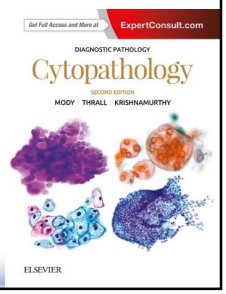
Conflict of Interest

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





Bethesda 3: What's new in Glandulars?

Chapter 6: Glandular Epithelial Abnormalities
David C. Wilbur, David C. Chhieng, Barbara Guidos and Dina R Mody

- Basic reporting terminology stays the same
- More images and differentials
- Expanded on mimics
- Expanded on variants of adenocarcinomas
- Tables illustrating differences in criteria for quick reference
- Notes about HPV testing and reporting rates
- Endometrials reporting

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

Other

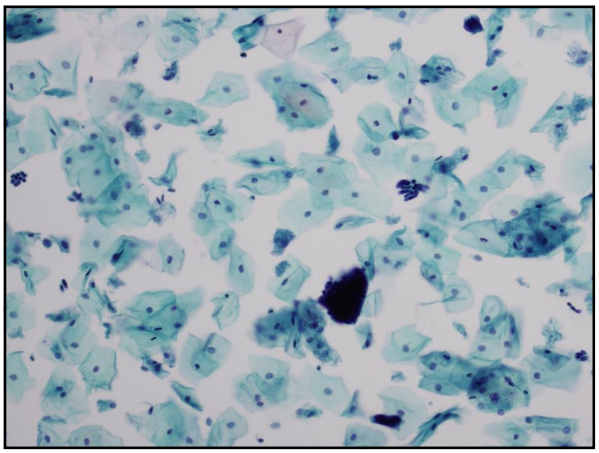
Atypical Glandular Cells(TBS 2001 and 2014)

Endocervical	unqualified(NOS)
	probably neoplastic/AIS/CA
Endometrial Glandular	NOS
	probably neoplastic

Adenocarcinoma In Situ(TBS 2001 and 2014)

Adenocarcinoma In Situ free standing entity(if all criteria) met and not under AGC (Atypical glandular cells)

If all criteria not met then under Atypical Endocervicals, probably AIS/neoplastic

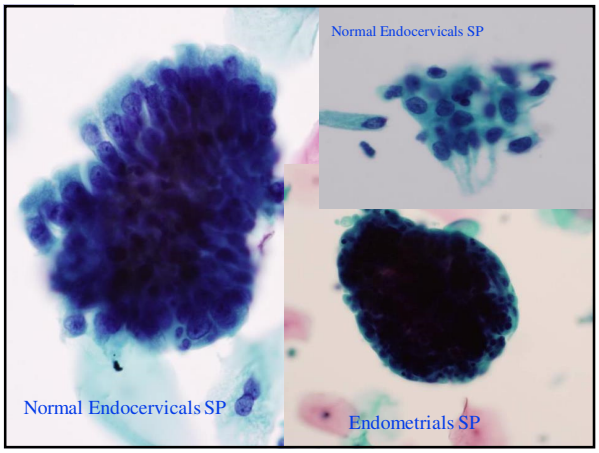


Hyperchromatic Crowded Groups (HCGs)

- AIS
- HSIL
- ACA Cx
- Sq ca cx
- ACA endometrium
- Other Carcinomas
- Exodus ball
- Aggressive endocervical sampl
- Follicular cervicitis
- LUS endometrium
- Tubal metaplasia
- MGH
- Atrophy

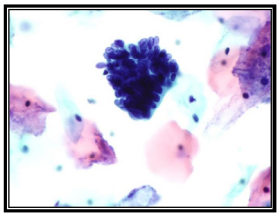
Endocervical Vs Endometrial cells

Features	EndoCx	Endometrials
Configuration	Sheets, strips	Cell balls, acini
Size	Large	Small
Mitosis	Absent	May be present
Mucin	Present	Absent
Stroma	Absent	Present



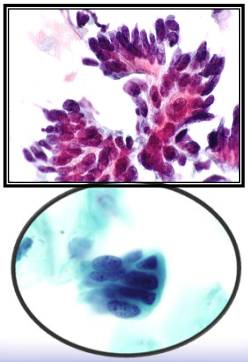
Adenocarcinoma In Situ of the Cervix

- Precursor lesion of most endocervical adenocarcinomas
- HPV positive
- Most associated with SIL
- Cellular specimens 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



AIS Conventional Vs Thin Prep Vs SurePath

Features	Convent	TP	SP
Large sheets+	+	+/-	-
3D groups	-	+	+
Feathering	+	subtle	+
Rosettes	+	subtle	sub
Strips	+	+/-	++
Single cells	-	+	+

AIS Conventional Vs Thin Prep Vs SurePath

Features	Convent	TP	SP
Nuclear shape	oval	round	Ov
Nu crowd	+	+	+
Chromatin	Hyper even	Hyper open	Hyper
Nucleoli	Incons	+	+/-
Cellularity	+++	Variable	Variable

AIS of cervix…other issues

- HPV positivity rates
  - Should be 100%
  - Reality ~ 90%
- Diagnostic Accuracy Rates
  - Major reason for litigation due to missed diagnosis
- Other

ENDOCERVICAL ADENOCARCINOMA IN SITU, VARIANTS, AND MIMICS

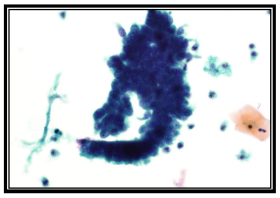
Comparison Between Conventional Pap Smear and Liquid-Based Technologies

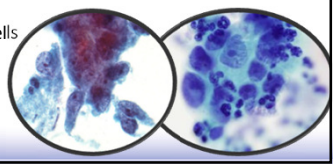
Cytologic Criteria	Conventional Smear	ThinPrep	SurePath
Cellularity	Cellular	Variable	Variable
Sheets	Large	Smaller	Small, dispersed
Strips	Present, few	Present, few	Present, many
Feathering	Present, low-power recognition	Present but can be subtle	Present but can be subtle
Rosettes	Present	Present but 3D	Present but subtle
Mitosis/apoptosis	With or without	Difficult to visualize	Difficult to visualize
3D groups	Usually flat	Mostly rounded/3D	Present
Single AIS cells	Unusual	Present, few	Present, many
Nuclear shape	Oval/cigar-shaped	Oval/rounded	Oval
Nucleoli	Absent	Chromocenters/rare	Chromocenters/rare
Background	Clean or intact RBCs	Clean	Clean

From Diagnostic Pathology: Cytopathology Mody D Amirsys Publishing, 2014 and 2018 (Elsevier)

Endocervical Adenocarcinomas

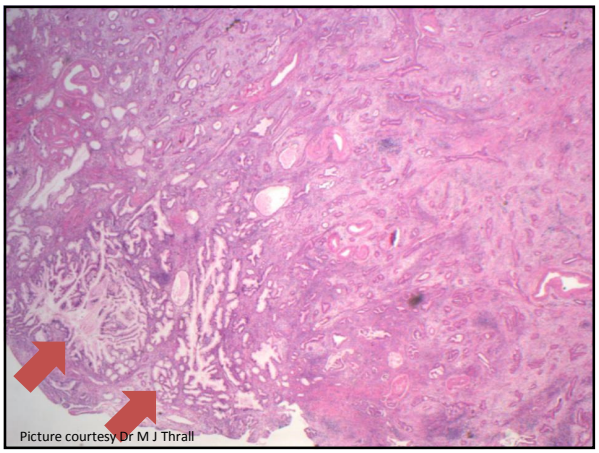
- Many features of AIS in early invasive ACAs
- Nuclear pleomorphism and irregularity
- Chromatinic clearing
- Nucleoli
- Loss of polarization
- Three dimensional/Acinar groupings
- Single intact malignant cells
- Mitosis
- Tumor Diathesis





LEGH Features

- Glands typically have small bland basal nuclei and abundant apical cytoplasm
- Positive for HIK 1083, negative for ER/PR, negative for p16
- Can have foci of intestinal metaplasia
- Atypical areas can be seen
- Associated with invasive carcinoma
- Chromosome gains of 3q and losses of 1p are associated with atypia/malignancy


Picture courtesy Dr M J Thrall

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

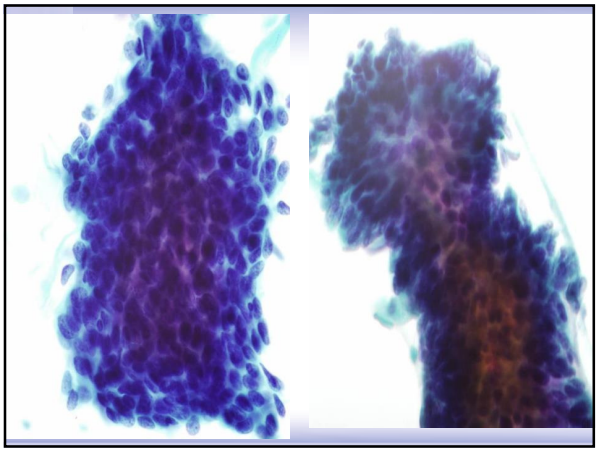
Adenocarcinoma In Situ and Mimics

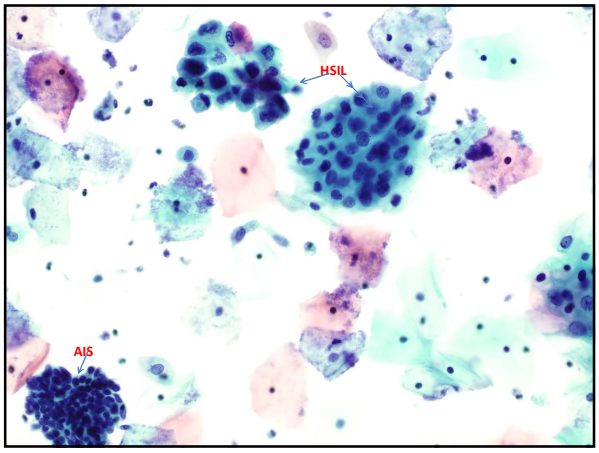
Cytologic Criteria	AIS	HSIL	Repair	Tubal Metaplasia	Directly Sampled Endometrium
Cellularity	Cellular	Usually cellular	Rare fragments	Rare event	Few groups
Hyperchromatic crowded groups	Many	Can be many	Not present	Rare event	Present
Sheets	Many with feathering	Syncytia	Flat sheets	Absent/rare/small	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,Elsevier 2018

HSIL Vs AIS

Features	HSIL	AIS
Strips & Rosettes	Absent	Present
Gland forms	Absent	Present
Feathering	Absent	Present
Polarity	Lost	Maintain
Nuclear Shape	Round/irreg	Oval/cigar
Chromatin	Coarse	Even
Cytoplasm	Dense	Even
Background	Isolated cells	Rare/abs
Frequency	0.2-0.4%	<.02%





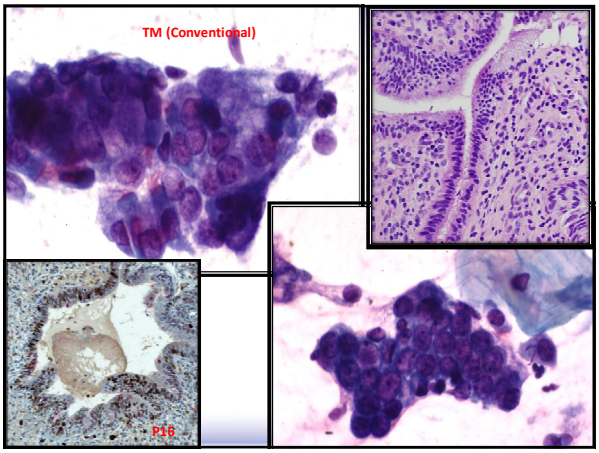
Squamous vs Adenocarcinomas(Cervical)

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

Features	Tubal metaplasia	AIS
Cellularity	Scant	Cellular
Honeycombing	Many	Rare
Feathering	Rare/absent	Common
Strips	Rare	Common
Single cells	Many	Rare
T.Bars/cilia	Present	Absent
Nuclei	Round/oval	Oval/cigar
Chromatin	Normochromic	Hyperchromatic

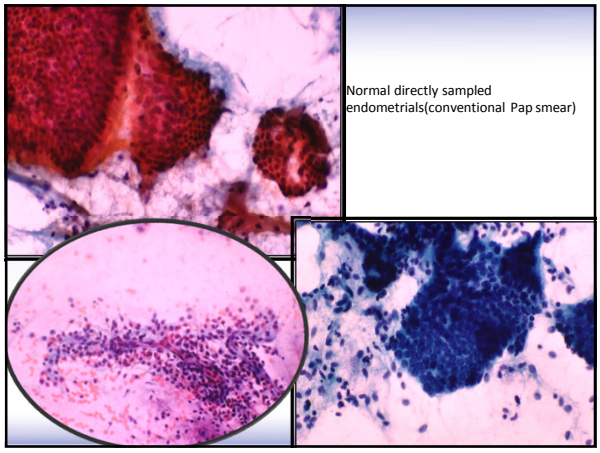


Scenarios Where Normal Endometrials are seen on Paps

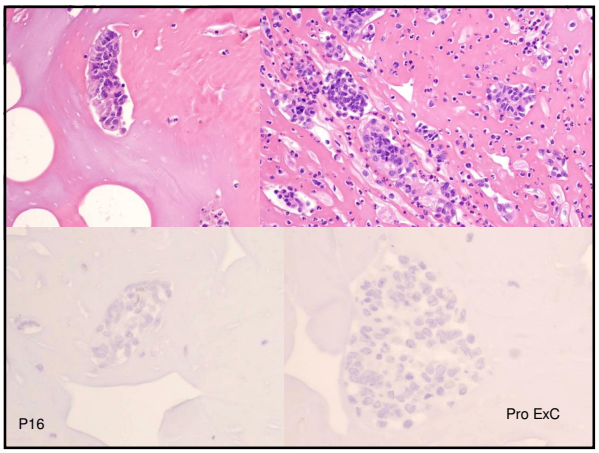
- Aggressive sampling
- S/P Cone or LEEP
- Endometriosis in vaginal vault
- Post Trachelectomy
- Menstrual pattern

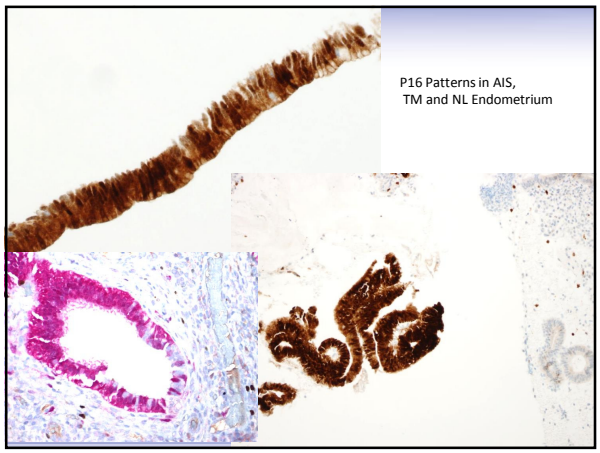
Directly Sampled Lower Uterine segment Endometrium or Endometriosis

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



Normal directly sampled endometrials(conventional Pap smear)





Gynecologic Cytopathology

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p16^{INK4a} and ProEx C Immunostains Facilitate Differential Diagnosis of Hyperchromatic Crowded Groups in Liquid-Based Papanicolaou Tests with Menstrual Contamination

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Key Words
Cervical cancer · Hyperchromatic crowded group · Menstrual contamination · Papanicolaou tests · Squamous intraepithelial lesion

Patchy/mosaic staining may be seen in benign endometrial tissue with tubal metaplasia, but strong, diffuse staining likely indicates HSIL or carcinoma. These findings can be helpful in interpreting hyperchromatic crowded groups in menstrual Pap specimens. Further study may be prudent, being aware of the small study group.

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Endometrial Cells in Liquid-Based Cervical Cytology: A Diagnostic Pitfall Solved by Preparing Cytohistology from the Residual Thin Layer Sample

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Key Words
Liquid-based cervical cytology · Cytohistology · Immunohistochemistry · Normal endometrium

Abstract
Objective: It was our aim to assess the usefulness of cytohistology in cervical thin layer brush samples with problems in rangement is sometimes suggestive of AIS or AC. In the 8 cases presented, we could avoid a false-positive diagnosis of AIS or AC through cytohistology/immunohistochemistry, and in consequence, unnecessary colposcopical/histological examination.

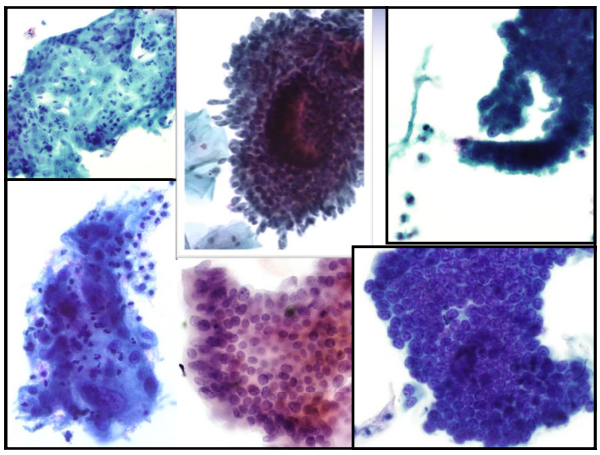
Atypical Repair Vs AIS or Adenocarcinoma

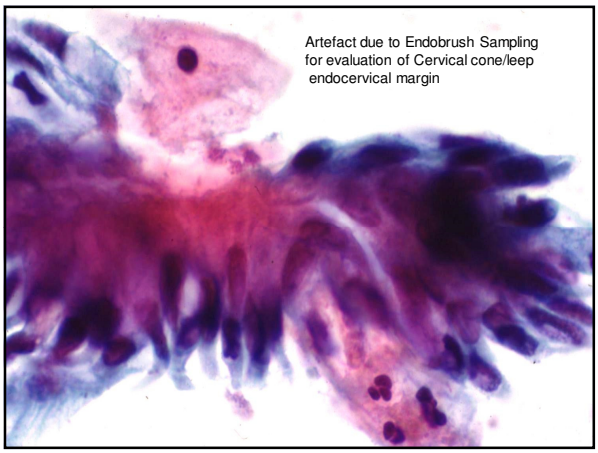
Atypical repair

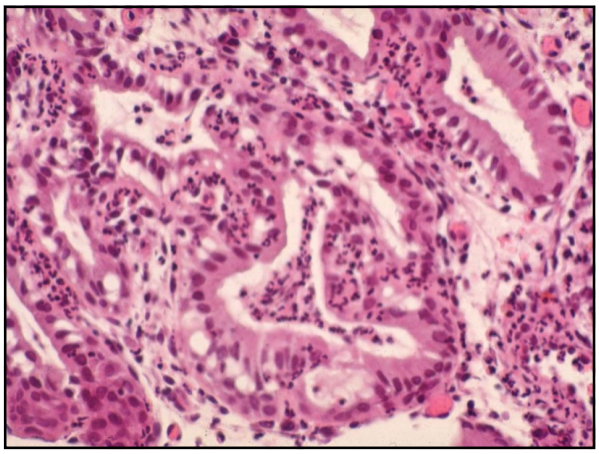
- Flat sheets with some disorganization/overlap
- "School of fish" arrangement and polarization
- Minimal nuclear crowding, no overlapping and usually hypochromasia
- Smooth nuclear contours
- No feathering, rosettes
- Bland vesicular chromatin with nucleoli

AIS/Adenocarcinoma Cervix

- Usually 3 D
- Nuclear polariztion 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







MICROGLANDULAR HYPERPLASIA

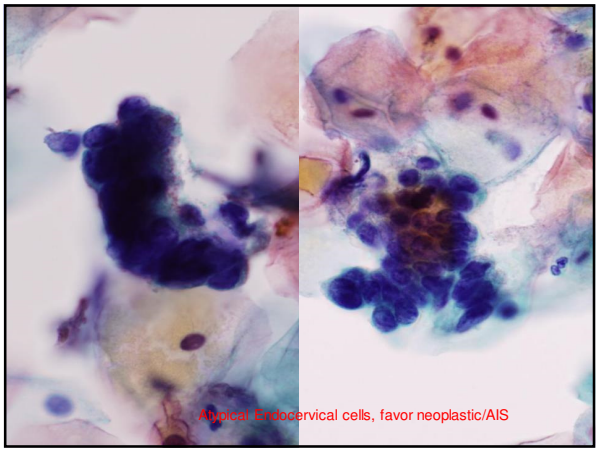
- Most look like normal endocervicals
- Repair like configuration
- Full spectrum of cell sizes within group
- Nuclei nl or increased in size. Nucleoli may be present and prominent. No mitosis
- Normo or slight hyperchromasia. Rarely pyknosis
- Cytoplasm abundant, fine, vacuolated.
- Pseudoparakeratosis

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

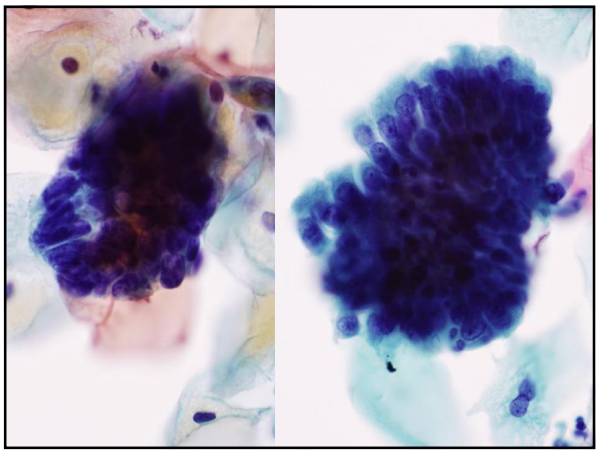
ATYPICAL ENDOCERVICAL CELLS, favor neoplastic

- ***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
- Nuclear crowding, overlap, Increased N/C ratio
- Ill-defined cell borders
- Palisading, Feathering, stratification
- Hyperchromasia with even chromatin
- Nucleoli Inconspicuous, Mitosis
- Clean or slightly bloody background



AGC (Endometrial)

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

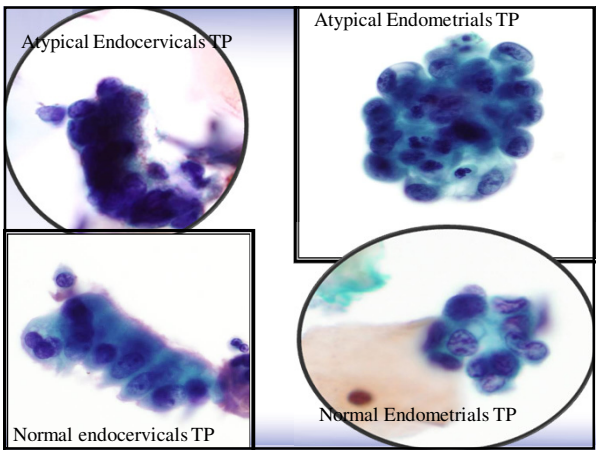
Normal vs Atypical Endometrial Cells

Normal (exfoliated)

- Nuclei ≤ than intermediate cell nucleus (35µm2)
- Chromatin dense, heterogenous, apoptotis
- Nucleoli small or absent
- Scant cytoplasm, dense or vacuolated
- Menstrual endometrium may look worse on liquid based preps(pleomorphism of nuclear size and shape)

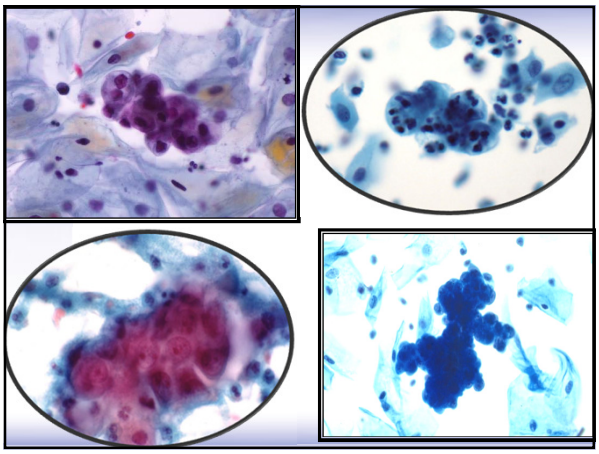
Atypical

- 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

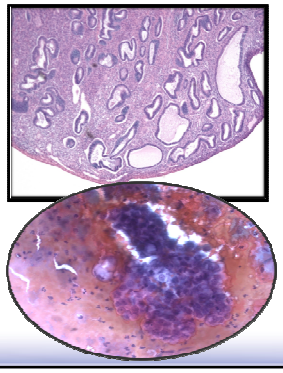


Mimics of Endometrial Adenocarcinoma/Hyperplasia

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

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

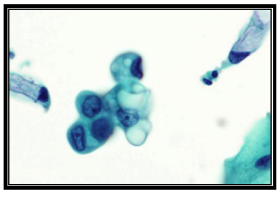


Arias Stella

- Young women, pregnant
- Rare cells
- Vacuolated
- May show some degeneration
- Disappear after pregnancy

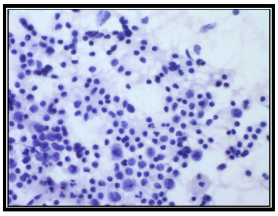
IUD Changes

- H/O IUD
- Scant cells
- Vacuolated cytoplasm
- Bubble gum cytoplasm
- Reactive nuclear features
- Variation in cell and nuclear size
- Regular nucleoli



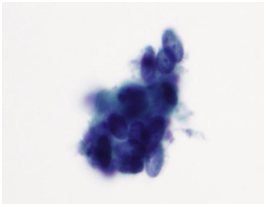
Follicular Cervicitis

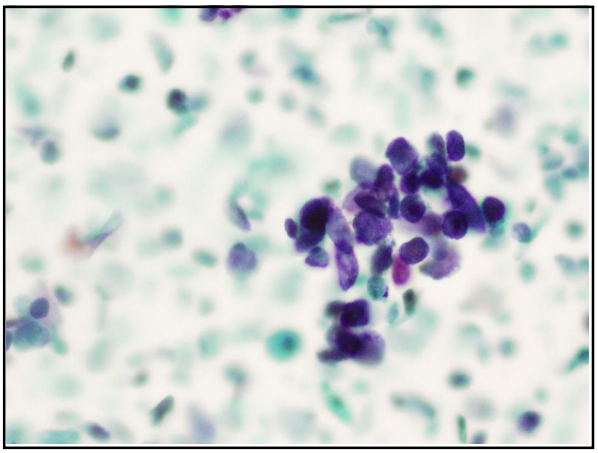
- Few cells/cell groups
- Small cells(lymphocytes)
- Variation in cell sixes
- Tingible body macrophages helpful if recognized



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 Tests

- 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%

Chapter 3: Endometrial cells: The How and When of Reporting

Edmund S. Cibas, David Chelmow, Alan Waxman* and Ann T Moriarty (* Gynecologists)*

- Exfoliated endometrial cells in post menopausal women are considered abnormal and raise possibility of endometrial hyperplasia or carcinoma
- Often menopausal status is unclear or inaccurate
- Median age in US is 51 yrs but varies greatly
- TBS 1988 recommended reporting *benign exfoliated endometrial cells in post menopausal women*
- *TBS 2001 recommended that this be done in all women ≥ 40 yrs*

Consequences of TBS 2001

- Increased reporting of benign-appearing endometrial cells: (0.17%-0.49% of Paps(3X)
- Decreased predictive value for hyperplasia and cancer

	Risk Associated With Endometrial Cells	
	Pre TBS 2001	Post TBS 2001
Hyperplasia	12%	2%
Carcinoma	6%	1%

For Bethesda 2014…..

- Category re evaluated
- Recommended to raise reporting age to 45 years
- 2 cytopathologists (Cibas, Moriarty) and 2 gynecologists (Chelmow, Waxman) on the committee
- Evidence based and should increase the predictive value of this finding and reduce unnecessary biopsies
- Support for change during public comment period
- ASCCP 2012 management guidelines already recommended histologic evaluation in postmenopausal women

From Presentation by E. Cibas at ASC 62nd annual meeting in Dallas, Nov 16th 2014

Education Note for Endometrial cells

- Specifies endometrial evaluation only in post menopausal women

Sample Report:
Endometrial cells present in a woman ≥ 45 years of age
Negative for Squamous Intraepithelial Lesion or Malignancy
Or
Endometrial cells correlate with menstrual history provided

Note: Endometrial cells in a woman 45 years or older may be associated with benign endometrium, hormonal alterations and less commonly, endometrial or uterine abnormalities. *Endometrial evaluation is recommended in postmenopausal women*