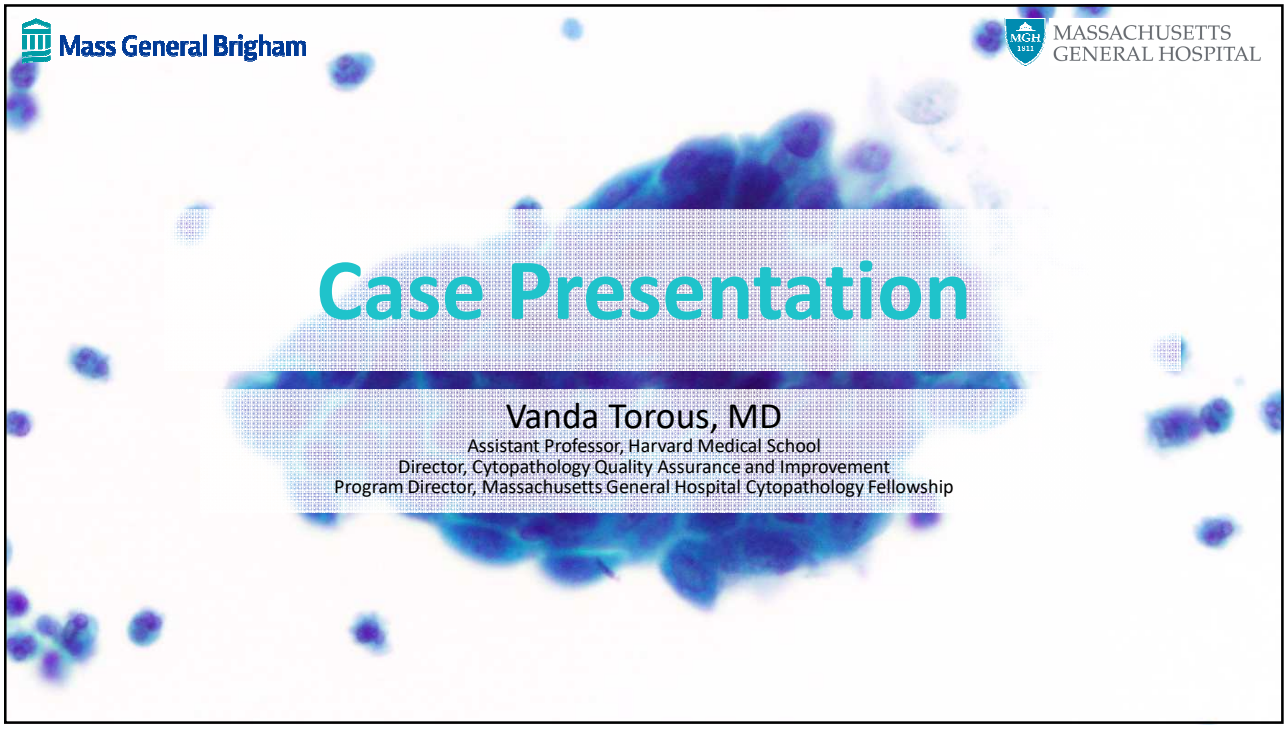
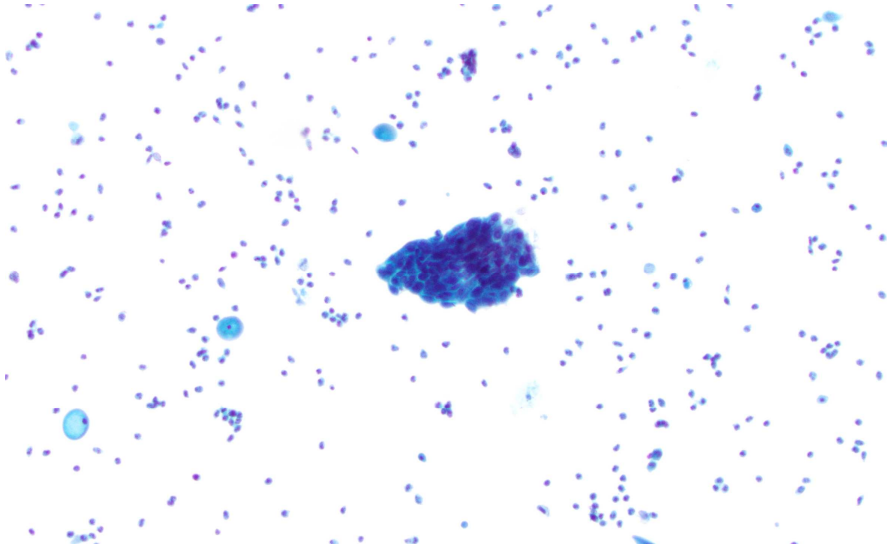
Mass General Brigham

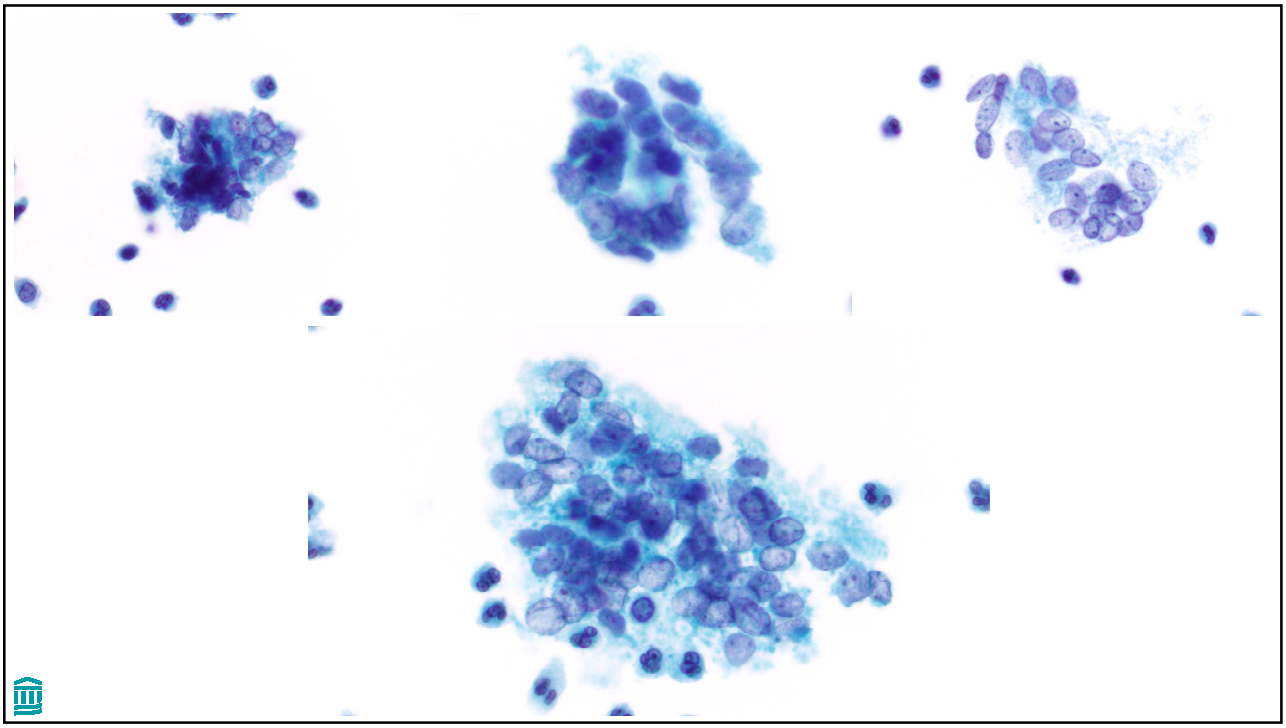
Challenging Cases and Lessons Learned Virtual Microscopy 1

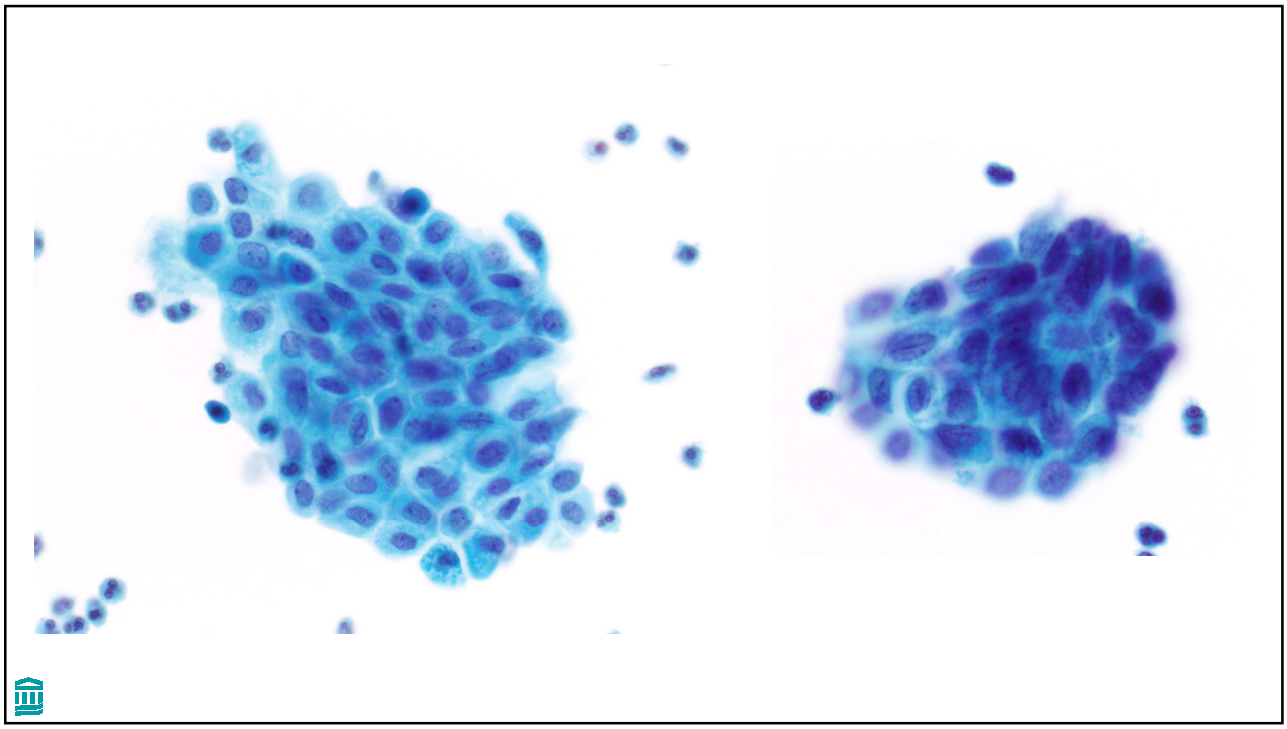
Moderator: Emilio Madrigal, DO
Vanda Torous, MD, Caroline Hilburn, MD, and Jeffrey Mito, MD

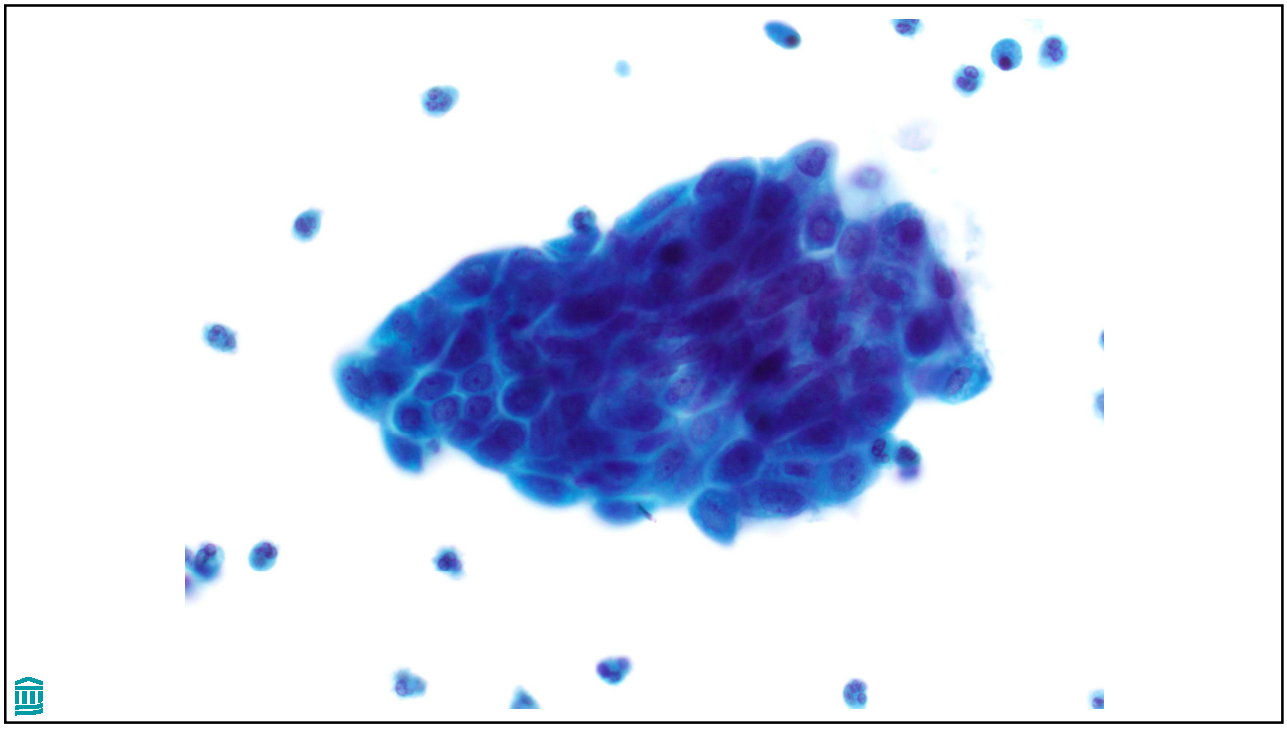


33 yo cervical Pap test (SurePath) HPV16+









33 yo cervical Pap test (SurePath) HPV16+

- Hyperchromatic clusters of cells
  - Somewhat crowded but well organized overall
  - No overt nuclear atypia (regular nuclear contours, evenly distributed chromatin)
- Clusters of stripped nuclei
- Hypocellular
- Atrophy
  - Hormonal changes? Causes?

➢ **Female-to-male transgender patient on testosterone hormone therapy**

Transgender Population

- Over 1.6 million individuals identify as transgender or gender diverse in the US
- The development of self-identity is a unique experience
  - Gender-affirming hormone therapy
  - Gender-affirming surgeries
- Only a minority of transgender men undergo hysterectomy
- Any anatomical structure present that warrants screening should be screened regardless of gender identity

Table 1. Percent of each age group that identifies as transgender in the U.S.

	PERCENT	NUMBER
13 to 17	1.4%	300,100
18 to 24	1.3%	398,900
25 to 64	0.5%	766,500
65 and older	0.3%	171,700
13 and older	0.6%	1,637,200

UCLA School of Law Williams Institute

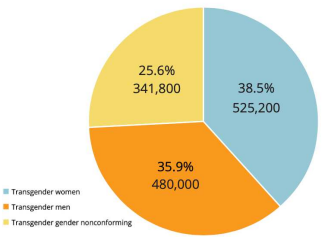
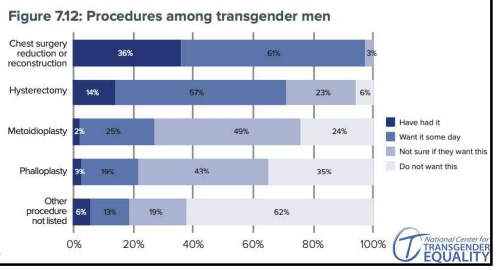
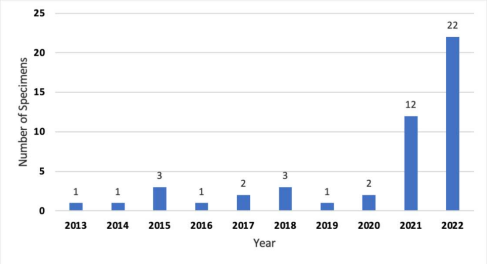


Figure 1. Gender identity of adults who identify as transgender in the U.S.



Laboratory Considerations

- Laboratories are increasingly receiving specimens from this patient population
- Best practices have yet to be established
- There are important clinical and morphologic considerations



Challenges to Screening

- Despite recommendations, there are disparities in the rates of cervical cancer screening in transgender men relative to cisgender women
  - Misconceptions about rate of dysplasia -> reduced screening
  - Psychological and physical discomfort
    - Gender dysphoria
    - Fear of discrimination
    - Prior negative experiences or distrust of healthcare providers
    - Prior trauma and/or posttraumatic stress disorder
    - Issues with insurance coverage
    - Lack of trans-inclusivity
    - Physical discomfort due to atrophy

Self Collection

- Combination of psychosocial and physical barriers may lead to preference of self collection
  - May trigger less emotional distress and gender dissonance
- Self-collected cervical cytology testing currently not FDA approved
  - Important preanalytic, analytic, and postanalytic considerations
- Questions remain:
  - Can it reduce the underscreened population?
  - Can it reliably sample the transformation zone?
  - How does it affect adequacy rates?
  - How does it affect cancer rates?
  - Do screening intervals need modification?

Adequacy

- Sampling adequacy is a significant issue in this patient population
  - Association between inadequate Pap tests and increased likelihood of developing cancer in cisgender women
  - Transgender men found to have a lower likelihood of following up within the recommended timeframe after an inadequate sample
- Majority of patients on testosterone therapy -> atrophy
- Unsatisfactory rate varies by study (0% to 16%)
- Adjust adequacy requirements as do for other atrophic paps?

Abnormality and HPV Rates

- Abnormality rates 5.7% to 29.2%
- HR-HPV+ rates 8% to 33%
- Performance of HR-HPV testing varies by study 29.4% to 59.8%; MGH 76.5%

Abnormality rate corresponds to the HR-HPV+ rate

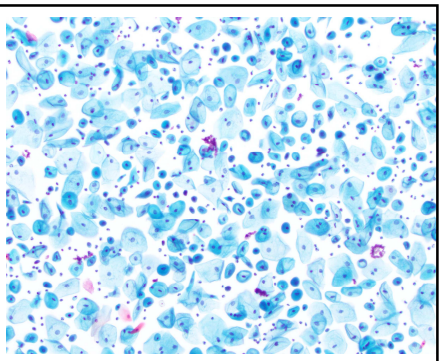
	Present Study (original review)	Adkins et al (2018)	Williams et al (2020)	Plummer et al (2021)	Lin et al (2022)	Davis et al (2022)	Moatamed et al (2023) (original review)
Number of patients	43	11	14	71	61	89	111
Number of cases	51	24	17	77	65	89	122
Mean age (years)	31	N/A	42.5	28	28	31.3	N/A
Pap categorization							
UNSAT	3.9% (2/51)*	13% (3/24)	5.9% (1/17)	23.4% (18/77)	16% (10/65)	0% (0/89)	9.8% (12/122)
NILM	90.2% (46/51)	58% (14/24)	82% (14/17)	68.8% (53/77)	74% (48/65)	94.4% (84/89)	84.4% (103/122)
ASCUS	5.9% (3/51)	13% (3/24)	5.9% (1/17)	5.2% (4/77)	6% (4/65)	0% (0/89)	3.3% (4/122)
ASCH	0% (0/51)	13% (3/24)	0% (0/17)	0% (0/77)	0% (0/65)	0% (0/89)	1.6% (2/122)
LSIL	0% (0/51)	0% (0/24)	5.9% (1/17)	1.3% (1/77)	1% (1/65)	4.5% (4/89)	0.8% (1/122)
HSIL	0% (0/51)	4% (1/24)	0% (0/17)	0% (0/77)	3% (2/65)	1.1% (1/89)	0% (0/122)
AGC	0% (0/51)	0% (0/24)	0% (0/17)	1.3% (1/77)	0% (0/65)	0% (0/89)	0% (0/122)
Cancer	0% (0/51)	0% (0/24)	0% (0/17)	0% (0/77)	0% (0/65)	0% (0/89)	0% (0/122)
Abnormal total	5.9% (3/51)	29.2% (7/24)	11.8% (2/17)	7.8% (6/77)	10.8% (7/65)	5.6% (5/89)	5.7% (7/122)*
Transgender unsat rate vs control cohort	NS	Higher	NS	Higher	Higher	NS	Higher
Transgender abnormal rate vs control cohort	NS	Higher	NS	NS	Higher (HSIL)	NS	NS
HPV Performed	76.5% (39/51)	50% (12/24)	29.4% (5/17)	35% (27/77)	49% (32/65)	56.2% (50/89)	59.8% (73/122)
HPV+ Rate	12.8% (5/39)	33% (4/12)	20% (1/5)	18.5% (5/27)	19% (6/32)	8% (4/50)	16.4% (12/73)
Transgender HPV+ rate vs control cohort	NS	Higher	N/A	NS	N/A	NS	N/A

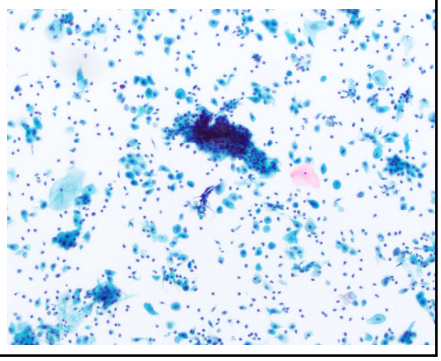
Torous, Cancer Cytopathol, *in press*

Morphologic Alternations

Atrophy

- Physiologic response resulting from decreased estrogen stimulation leading to thinned immature cervicovaginal squamous epithelium consisting of parabasal and basal cells
- Challenging due to variability in appearance
- Found in 62% to 93% of transgender Pap tests; 92% MGH

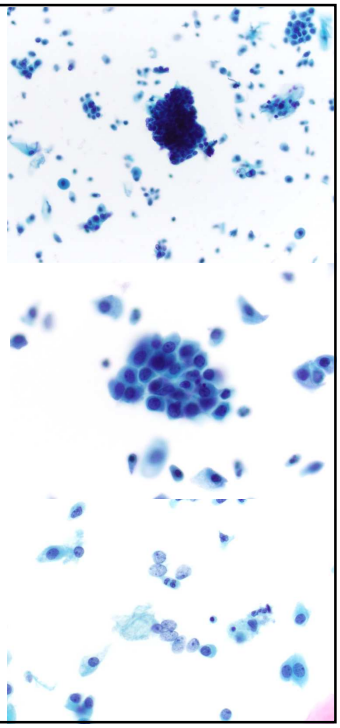




Morphologic Alternations

Small blue cells

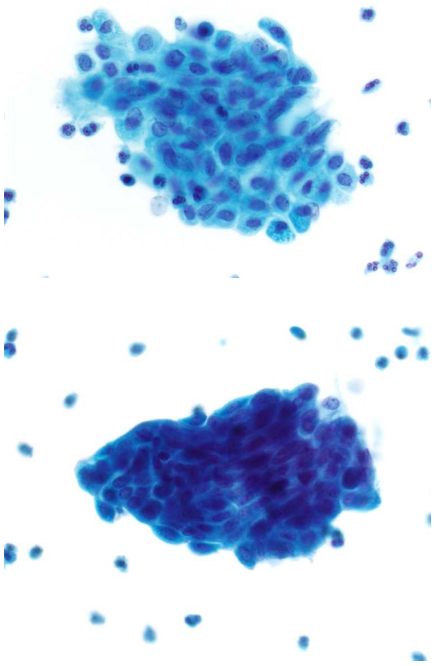
- Small cells demonstrating scant to absent cytoplasm arranged in grapelike clusters which occurred
- Parabasal and basal squamous cells
- Potential to mistake for ASCH/HSIL or endometrial cells
- Up to 82% in published literature; MGH 53% (15.7% with naked blue cells)



Morphologic Alternations

Transitional cell metaplasia

- An infrequent but not rare finding in cytology and surgical specimens that is likely underrecognized
- Characterized by streaming groups with spindled-ovoid nuclei and longitudinal grooves
- Considered a variant or type of atrophy
- Traditionally, described in peri or postmenopausal women; now, known to occur frequently in those on androgen / testosterone therapy
  - Up to 88% of transgender men Pap tests; MGH 43%



Pitfalls in Cytomorphologic Evaluation

Retrospective reviews have demonstrated the importance of clinical information on diagnostic outcome

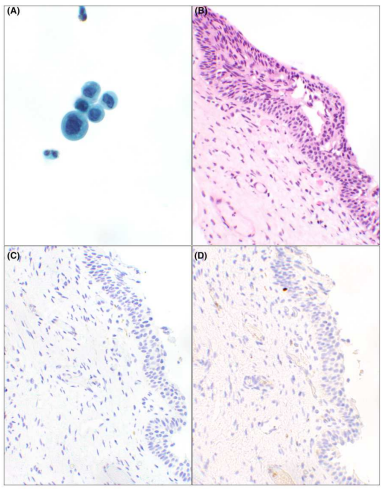


FIGURE 2 "Original" cytological diagnosis downgraded from atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion (ASC-H) to atypical squamous cells of undetermined significance (ASCUS). Photomicrograph of the cervical cells from a 23-year-old female-to-male transgender patient (case# 108, Table S1). (A) Cells on Papanicolaou test slide showing dense cytoplasm, high nuclear to cytoplasmic ratios, and hyperchromasia, with wrinkled, enlarged nuclei which were "originally" interpreted as ASC-H (x60 magnification). With the clinical information regarding gender identity and testosterone therapy status, the cytological diagnosis became ASCUS at the "retrospective" review, which might have been interpreted as negative for intraepithelial lesion or malignancy (NILM) by some cytologists. (B) The follow-up cervical biopsy revealed thinning of the squamous epithelial cell layers composed of parabasal cells on a haematoxylin & eosin-stained section (x20 magnification). (C) Negativep16 as opposed to diffuse positive block pattern and (D) negative Ki67 immuno-stain were observed (x20 magnification)

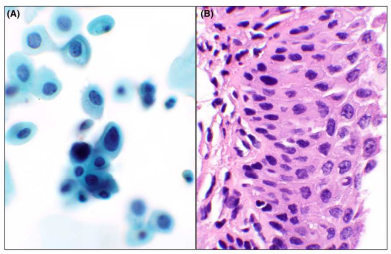


FIGURE 3 "Original" cytological diagnosis downgraded from atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion to atypical squamous cells of undetermined significance (ASCUS). Photomicrograph of the cervical cells from a 21-year-old female-to-male transgender patient (case# 107, Table S1) with unknown human papillomavirus status. (A) Cells on Papanicolaou test slide showing hyperchromasia, high nuclear to cytoplasmic ratios, and dense cytoplasm, which were interpreted as ASC-H "originally." At the "retrospective" diagnostic stage, the diagnosis became ASCUS (x60 magnification). (B) Following hysterectomy, the cervical tissue showed atrophic ectocervix composed of parabasal cells on a haematoxylin & eosin-stained section (x40 magnification). As observed in the case shown in Figure 2, p16 and Ki67 immunostains were negative

Moatamed et al, Cytopathology, 2023

Pitfalls in Cytomorphologic Evaluation

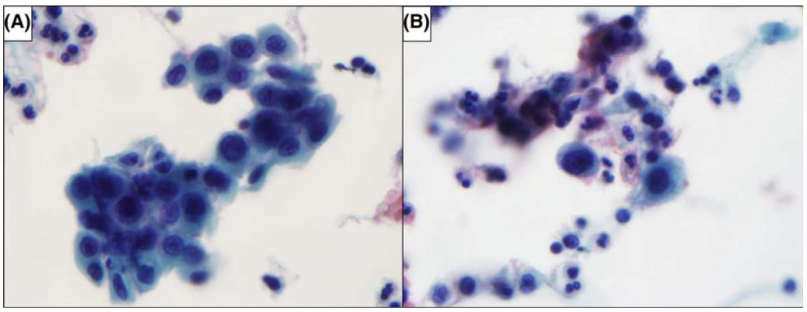


FIGURE 2 Inflammatory changes in Papanicolaou tests of female-to-male transgender patients on androgen therapy: (A) cluster of cells with enlarged nuclei but smooth, regular chromatin, and prominent nucleoli (100×); (B) Cells with enlarged nuclei, irregular nuclear borders, and prominent nucleoli (100×)

Adkins et al, Cytopathology, 2018

Summary

- There are important considerations we should keep in mind regarding Pap tests from transgender patients including the psychosocial and physical barriers that may cause disparities in care
- Knowledge of the transgender status is important as it may affect both interpretation and adequacy
- There is still a need to refine how we handle and manage these samples in order to optimize patient care

Further Reading and Additional Resources

- Adkins BD, Barlow AB, Jack A, Schultenover SJ, Desouki MM, Coogan AC, Weiss VL. Characteristic findings of cervical Papanicolaou tests from transgender patients on androgen therapy: Challenges in detecting dysplasia. Cytopathology. 2018 Jun;29(3):281-287.
- Williams MPA, Kukkar V, Stemmer MN, Khurana KK. Cytomorphologic findings of cervical Pap smears from female-to-male transgender patients on testosterone therapy. Cancer Cytopathol. 2020 Jul;128(7):491-498.
- Plummer RM, Kelting S, Madan R, O'Neil M, Dennis K, Fan F. Cervical Papanicolaou tests in the female-to-male transgender population: should the adequacy criteria be revised in this population? An Institutional Experience. J Am Soc Cytopathol. 2021 May-Jun;10(3):255-260.
- Lin LH, Zhou F, Elishaev E, Khader S, Hernandez A, Marcus A, Adler E. Cervicovaginal cytology, HPV testing and vaginal flora in transmasculine persons receiving testosterone. Diagn Cytopathol. 2022 Nov;50(11):518-524.
- Moatamed NA, Barco AD, Yang SE, Ying Y, Zhang S, Rodriguez EF. Clinical history of female-to-male transgender patients is needed to avoid misinterpretation of cervical Papanicolaou tests. Cytopathology. 2023 Mar;34(2):120-129.
- Torous VT. Cervicovaginal Papanicolaou tests in transgender men: cytomorphologic alterations, interpretation considerations, and clinical implications. Cancer Cytopathol. 2023 *in press*
- Peitzmeier SM, Reisner SL, Harigopal P, Potter J. Female-to-male patients have high prevalence of unsatisfactory Paps compared to non-transgender females: implications for cervical cancer screening. J Gen Intern Med. 2014 May;29(5):778-84.
- Compton ML, Taylor SS, Weeks AG, Weiss VL, Hogan MM, Wang H, Ely KA. Cytology and LGBT+ health: establishing inclusive cancer screening programs. J Am Soc Cytopathol. 2022 Sep-Oct;11(5):241-252.
- The Report of the US Transgender Survey https://transequality.org/sites/default/files/docs/usts/USTS-Full-Report-Dec17.pdf (new survey results expected in 2023)
- UCLA Williams Institute Transgender Report https://williamsinstitute.law.ucla.edu/wp-content/uploads/Trans-Pop-Update-Jun-2022.pdf

Mass General Brigham

Challenging Cases and Lessons Learned - Virtual Microscopy 1

Caroline Hilburn, MD
Fellow in Cytopathology, MGH

Case Presentation

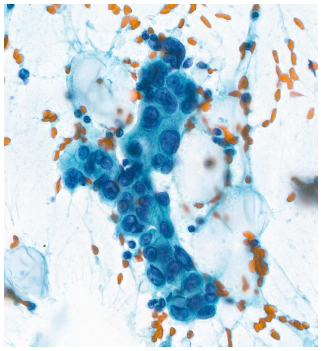
71-year-old female with a history of pancreatic adenocarcinoma status post neoadjuvant chemotherapy and total pancreatectomy. Found to have a new perigastric nodule radiologically concerning for metastasis.

Additional imaging findings:

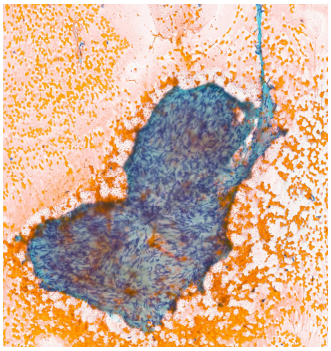
- Hepatic abscess
- Post-surgical changes

Differential diagnosis based on presentation & imaging

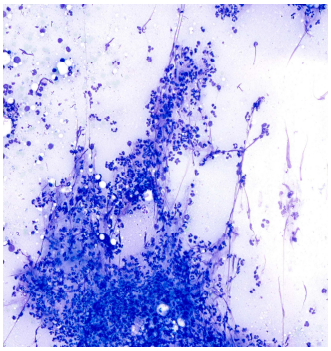
Metastasis from pancreatic adenocarcinoma



Gastrointestinal Stromal Tumor (GIST)



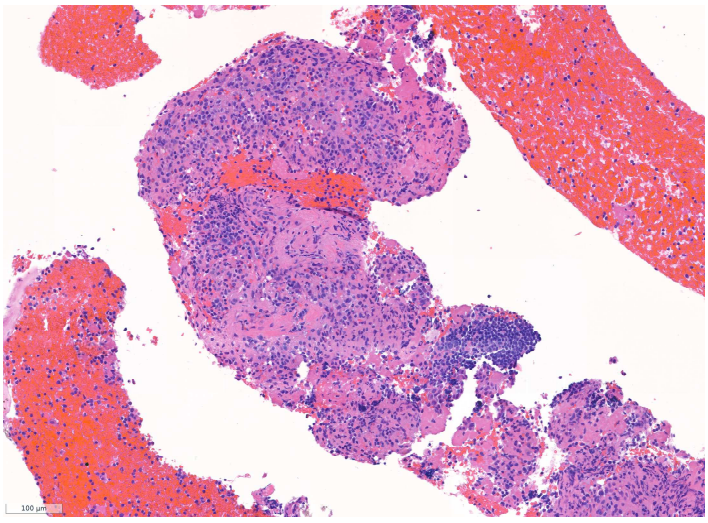
Inflammatory process or Abscess



> “1 cm, round, hypoechoic, well-circumscribed lesion adjacent to the gastric wall”

–Interventional Radiologist

What should we do next?



1. Sign it out: Non-Diagnostic
2. Sign it out: Reactive changes
3. Sign it out: Neoplastic
4. Order ancillary studies
5. Obtain a consult

Consult to Hematopathology

Consult question

"71 F with pancreatic adenocarcinoma s/p resection. Now with hepatic lesion concerning for abscess and perigastric nodule. Do you think this is a heme lesion? We are considering IMT, LCH, EMH, others."

Hematopathology response

"Unusual appearance. Not sure what this is. IHC we did:

- CD45
- CD68
- CD138
- Kappa
- Lambda
- CD20
- CD3
- MNF116"

“Could the underlying tissue possibly be spleen? (Could get a CD8)”

–Hemepath

Final Report

A. FINE NEEDLE ASPIRATION BIOPSY, PERIGASTRIC NODULE:
SPECIMEN ADEQUACY:
Satisfactory for evaluation.
INTERPRETATION:
No malignant cells identified
DIAGNOSIS:
Abundant histiocytes and mixed chronic inflammation with crush artifact.

B. PERIGASTRIC NODULE FNB FORMALIN:
Splenic tissue consistent with splenule or splenosis.

Challenges & Lessons Learned

- Splenic tissue can be challenging to identify on cytologic specimens.
  - Cytomorphologic appearance: Mixed chronic inflammation with vascular tissue fragments and lymphoid aggregates
- Types of splenic tissue encountered on biopsy:
  - Splenule
    - Developmental anomaly common in the general population
    - Remnant of fetal splenic tissue which forms encapsulated nodules
  - Splenosis
    - Autotransplantation of splenic tissue within the abdomen following trauma or splenectomy
- Both splenules and splenosis functionally and cytomorphologically recapitulate normal splenic tissue and IHC staining

Teaching Points

1

Splenules/splenosis can radiologically mimic pancreatic neoplasia

2

Splenic tissue can expand quickly following splenectomy

3

CD8 IHC highlights endothelial cells in splenic tissue

4

According to the Papanicolau Society reporting guidelines for pancreaticobiliary cytology, splenules belong in category II (negative for malignancy)

Acknowledgements

Martha B. Pitman, MD
Vanda F. Torous, MD
Jeffrey Mito, MD, PhD

ADVANCES IN CYTOLOGY AND SMALL BIOPSIES – VIRTUAL MICROSCOPY SESSION

Case #3:
71 y/o postmenopausal woman, routine screening

Jeffrey Mito MD PhD
6/12/2023

History

- Recent “abnormal” pap at an outside hospital

Diagnosis?

- A) NILM-R
- B) Atypical glandular cells, NOS
- C) Atypical glandular cells, favor neoplastic
- D) Adenocarcinoma, NOS
- E) Other

Final Cytologic Diagnosis

Satisfactory for evaluation; transformation zone present.

EPITHELIAL CELL ABNORMALITY - GLANDULAR.
Atypical glandular cells, favor neoplastic.

The Bethesda System: Glandular Abnormalities

- Atypical glandular cells, not otherwise specified (NOS)
  - If possible, specify endocervical or endometrial
- Atypical glandular cells, favor neoplastic
  - Specify if endocervical
- Endocervical adenocarcinoma *in situ* (AIS)
- Adenocarcinoma
  - Endocervical, endometrial, extrauterine, or not otherwise specified

Jones R, et al. JASC (2020) **9**: 137

Atypical Glandular Cell – Favor Neoplastic

- Cell morphology either quantitatively or qualitatively falls short of a diagnosis of *in situ* or invasive adenocarcinoma
- Higher rate of invasive cancer and significant pathology vs AGC-NOS: 55-65% vs 24-32%
- In one study, most predictive of glandular neoplasia on histologic follow-up (21 of 26, 81%)

Chatchotikawong U, et al. Int. J. Gyn. Obst. (2012) **119**: 30
Pradhan D., et al. Can. Cyto. (2016) **124:** 589

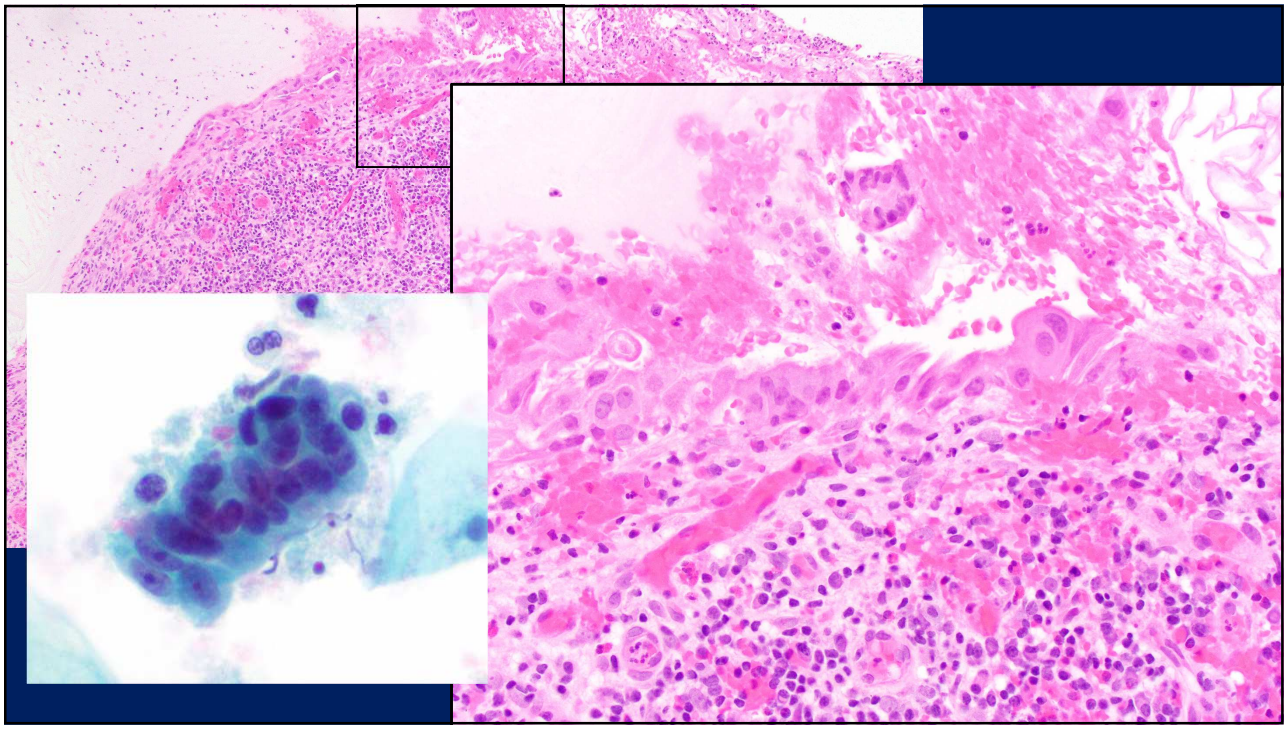
ASCCP Management Guidelines

- All subcategories of “Atypical glandular cells” (except ”Atypical endometrial cells”) should be managed with colposcopy and endometrial sampling (if ≥ 35 years of age or age <35 and at risk for endometrial neoplasia)
  - Abnormal uterine bleeding
  - Obesity
  - Chronic anovulation
  - Family history or genetic predisposition

Perkins RB, et al. J. Low. Gen. Tract. Dis. (2020) **24**: 102

Follow-up

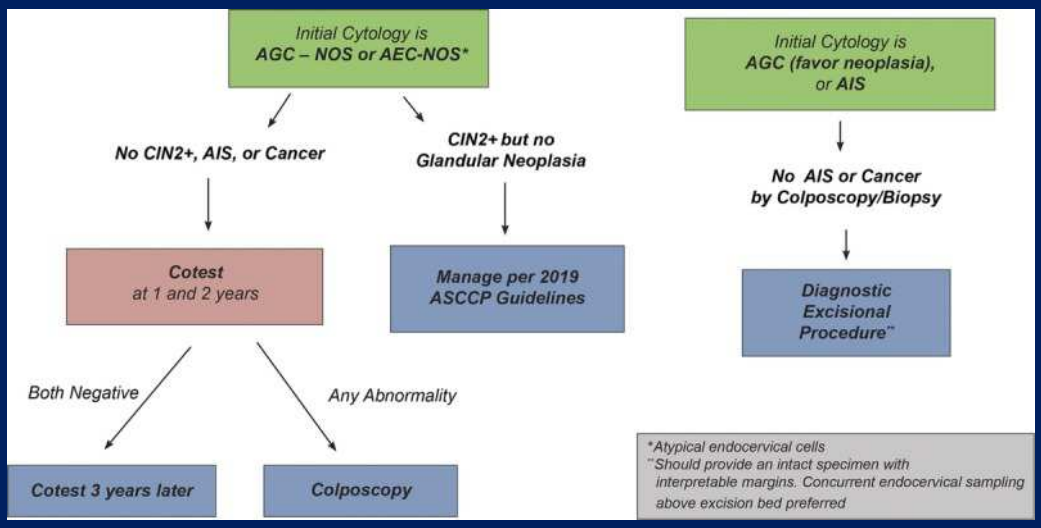
- Follow-up endometrial sampling was benign



Endocervical Polyps

- Similarities to adenocarcinoma:
  - Nuclear enlargement
  - Prominent nucleoli
  - Mitotic activity
- Lack of necrotic debris
- Lack of isolated single cells
- Correlation with clinical exam

ASCCP Management Guidelines



Perkins RB, et al. J. Low. Gen. Tract. Dis. (2020) **24**: 102

Follow-up

- Follow-up endocervical sampling, pap test, and HR-HPV testing were all negative for the subsequent two years
- Patient is alive and well 10+ years later

Take Home Points

- Reparative changes can mimic invasive cancer
- History and clinical exam can refine the differential of an AGC diagnosis
- Specifying endometrial or endocervical atypia is important for subsequent patient management

Questions?

ADVANCES IN CYTOLOGY AND SMALL BIOPSIES – VIRTUAL MICROSCOPY SESSION

Case #4:
71 y/o postmenopausal woman,
routine screening

Jeffrey Mito MD PhD
6/12/2023

History

- No prior abnormal pap tests
- No screening in the last 20 years
- Normal exam

Diagnosis?

- A) HSIL
- B) Squamous cell carcinoma
- C) Atypical glandular cells, NOS/favor neoplastic
- D) Adenocarcinoma *in situ*
- E) Other

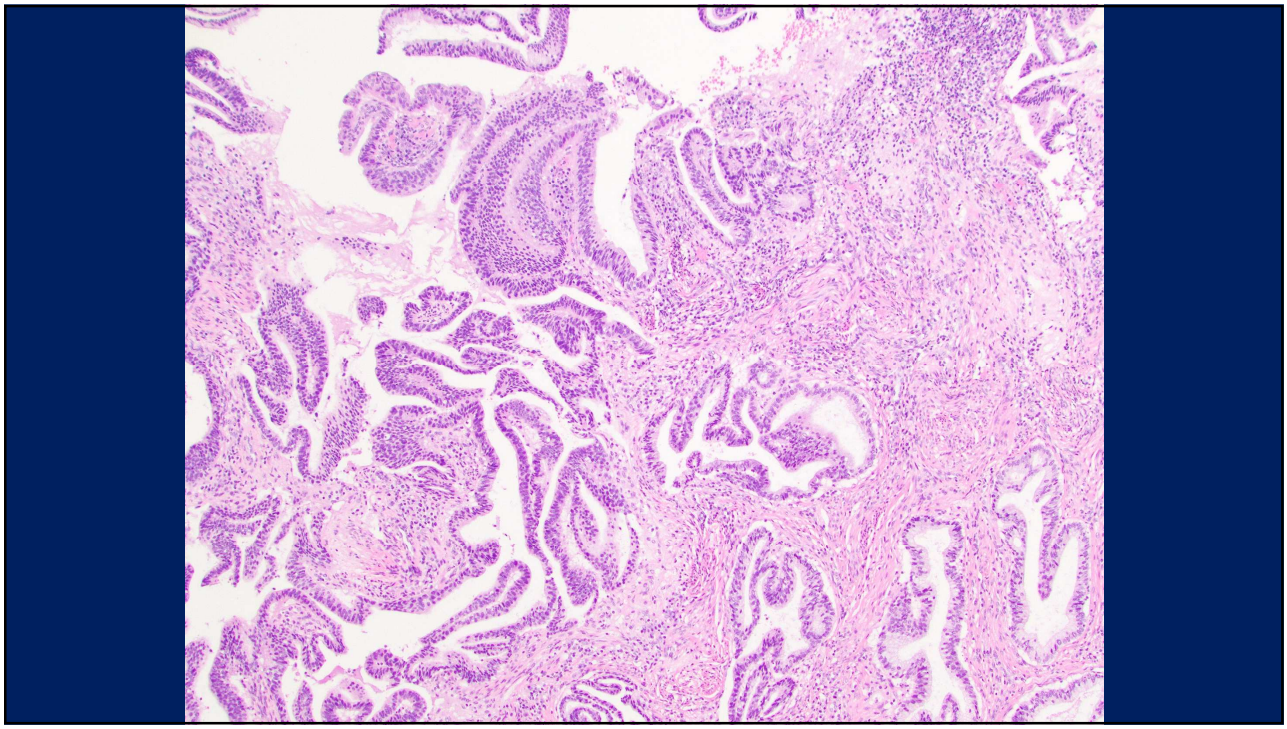
Final Cytologic Diagnosis

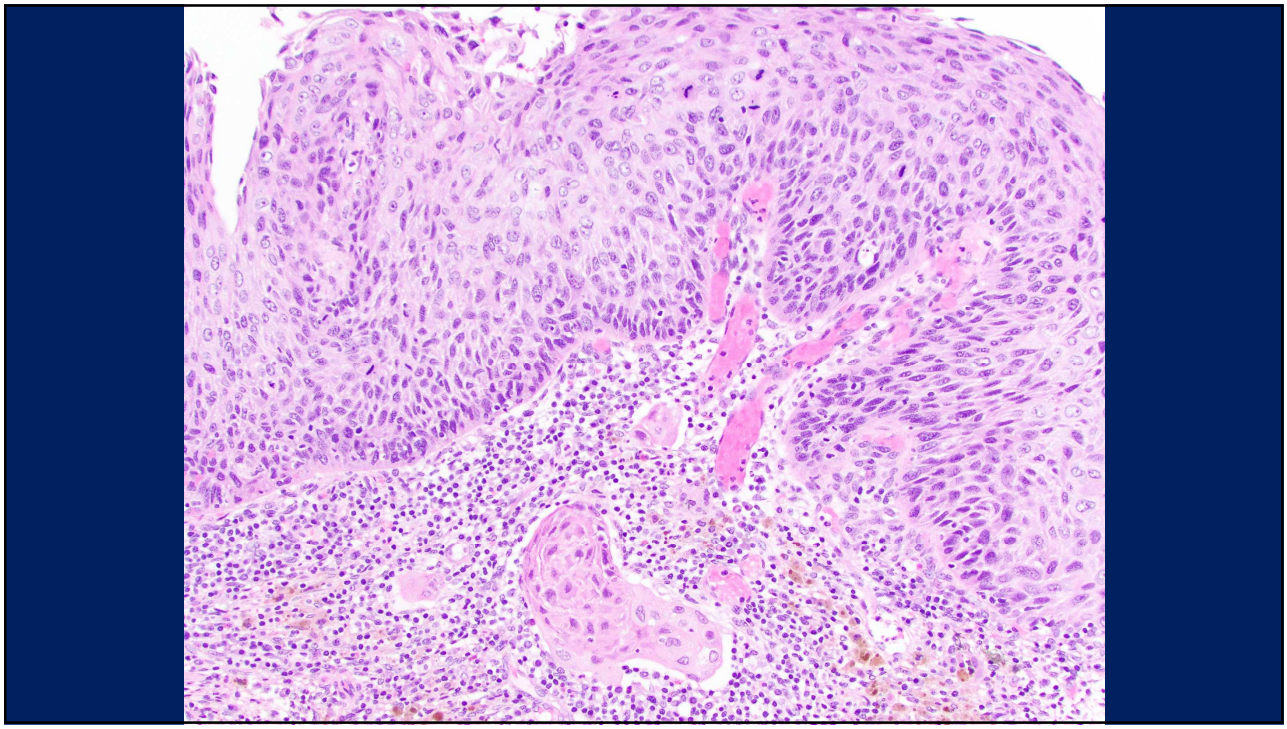
Satisfactory for evaluation; transformation zone present.

EPITHELIAL CELL ABNORMALITY - GLANDULAR.
Adenocarcinoma, not otherwise specified.

EPITHELIAL CELL ABNORMALITY - SQUAMOUS.
High grade squamous intraepithelial lesion (see NOTE).

NOTE: The presence of a more significant lesion cannot be excluded.





Final Pathologic Diagnosis

INVASIVE ENDOCERVICAL ADENOCARCINOMA, MUCINOUS (HPV-associated) TYPE

HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESION (CIN3) involving endocervical crypts, with one focus representing superficial invasion (<1 mm)

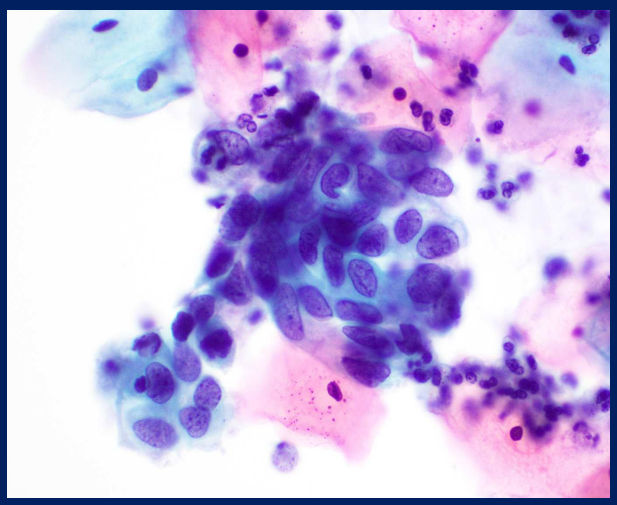
Squamous and Glandular Abnormalities on Pap Test

- Identifying BOTH squamous and glandular abnormalities on Pap test is infrequent:
  - <0.1% (230 of 361,953) of Pap tests
- Histologic follow-up:
  - Squamous lesions: 52-53%
  - Glandular lesions: 6-7%
  - Both squamous and glandular lesions: 3-4% (AIS+SIL)
- The majority are positive for HR-HPV (57-66%)

Yan Khor L, et al. Can. Cyto. (2014) **122**: 620
Harbhajanka A, et al. Diag. Cyto. (2019) **47**: **88**

HSIL with Gland Involvement

- Features:
  - Cells with centrally whorled or spindled cells and peripheral flattening
  - Clusters or sheets of atypical cells with pseudostratification and peripheral palisading (mimicking AIS)



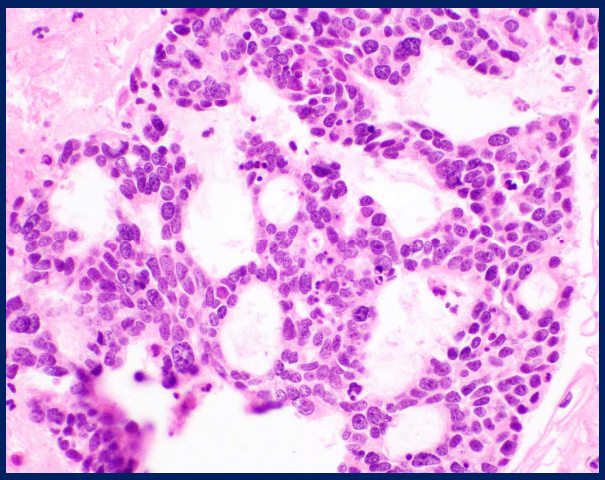
HSIL with Gland Involvement

- Interobserver agreement of HSIL with gland involvement on pap test is poor
- Histologic identification of HSIL with endocervical gland involvement has been linked to increased rates of detection of HSIL (32 vs 25%) or ASC-H (17 vs 12%) on pap test
- Features favoring HSIL over AIS
  - Limited feathering, rosettes or columnar cells
  - Denser cytoplasm
  - Single atypical squamous cells in the background

Jones R, et al. JASC (2020) **9**: 137

The Utility of Cell Blocks

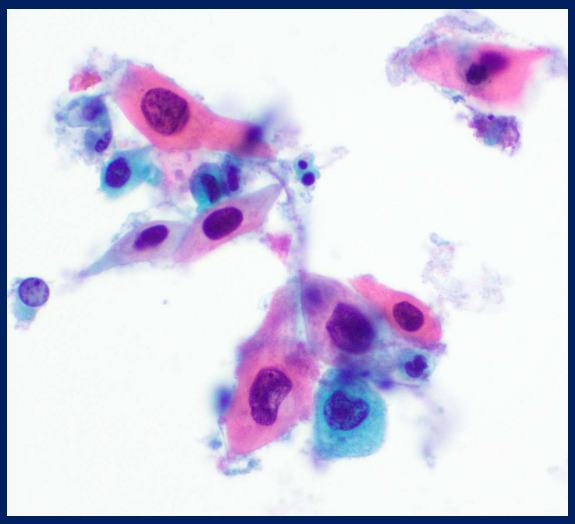
- Cell blocks can be useful in the differential diagnosis of Atypical glandular cells:
  - Xing, et al.: 148 patiens with pap tests screened as AGC
    - 31 of 68 (46%) samples screened as AGC reclassified as NILM/LSIL
    - 7 of 68 (10%) were given a specific diagnosis: HSIL, AIS, or invasive ACA



Xing W, et al. Can. Cyto. (2014) **122**: 8

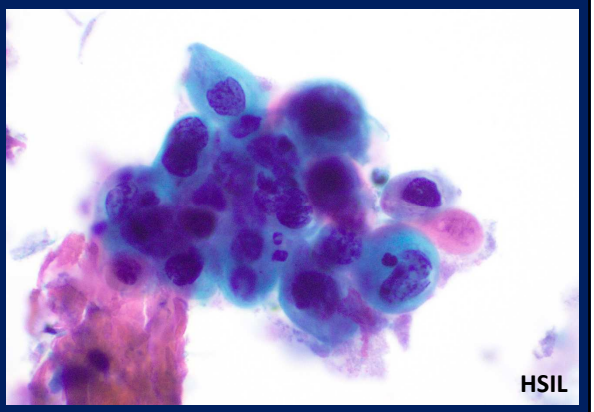
Cytologic Criteria for Squamous Cell Carcinoma

- Cells occur singly or in groups with poorly defined cell borders
- Keratinizing SQC: typically isolated single cells or aggregates of markedly variable cell size and shape
- Display most of the features of HSIL
- Nuclei with markedly irregular clumped or coarse chromatin
- Nucleoli may be prominent
- Tumor diathesis



HSIL vs Invasive Squamous Cell Carcinoma

- Challenging to differentiate:
  - Not all invasive cancers will have a tumor diathesis – especially true with liquid based cytology
  - Tumor diathesis can be seen in benign conditions and HSIL
  - Keratinizing SILs can have highly abnormal keratinized cells
  - Prominent nucleoli can be seen in HSIL
- False positives often caused by a constellation of these findings


HSIL

Take Home Points

- The diagnosis of both a squamous and glandular abnormality on Pap test is rare - most of these cases will represent HSIL
- The distinction between HSIL and squamous cell carcinoma can be challenging
- Cell blocks can be helpful to further characterize hyperchromatic crowded groups and refine a diagnosis of AGC