

# URINE CYTOLOGY

**Richard Doughty**  
**Avd for patologi**  
**Ahus**

# The main purpose of urine cytology is



To detect High Grade Urothelial Carcinoma

LOONEY TUNES



*"That's all Folks!"*

# Outline



- Urine – the basics
- A little on the history of classification systems.
- What is the goal of urine cytology?
- Why to standardize, why Paris?
- What is the guiding principle?
- What are diagnostic categories?
- What are the criteria?
- What adjuvant studies?
- What are future clinical and research needs?

# Bladder cancer - current status

**TABLE 1. Risk Factors for Bladder Cancer**

- Smoking
- Occupational carcinogen exposure
- Arsenic ingestion
- Genetic predisposition: mutations in *GNT*, *NAT*, *Rb1*, and *PTEN*
- Personal/family history of bladder cancer
- Age >55 years
- Male sex
- White race
- Chronic bladder infections
- Lynch syndrome

Nielsen ME et al. Trends in stage-specific incidence rates for urothelial carcinoma of the bladder in the United States: 1998 to 2006. *Cancer* 2014;120:86

# In Norway...

- 2015: 1731 new cases of cancer in the urinary bladder, ureters or urethra
  - ▣ 1262 men
  - ▣ 469 women



### 6.2.3 *Urinundersøkelser*

#### **Bakteriologisk undersøkelse**

Denne undersøkelse er nødvendig bare hvis klinikken gir mistanke om assosiert infeksjon.

#### **Urincytologi**

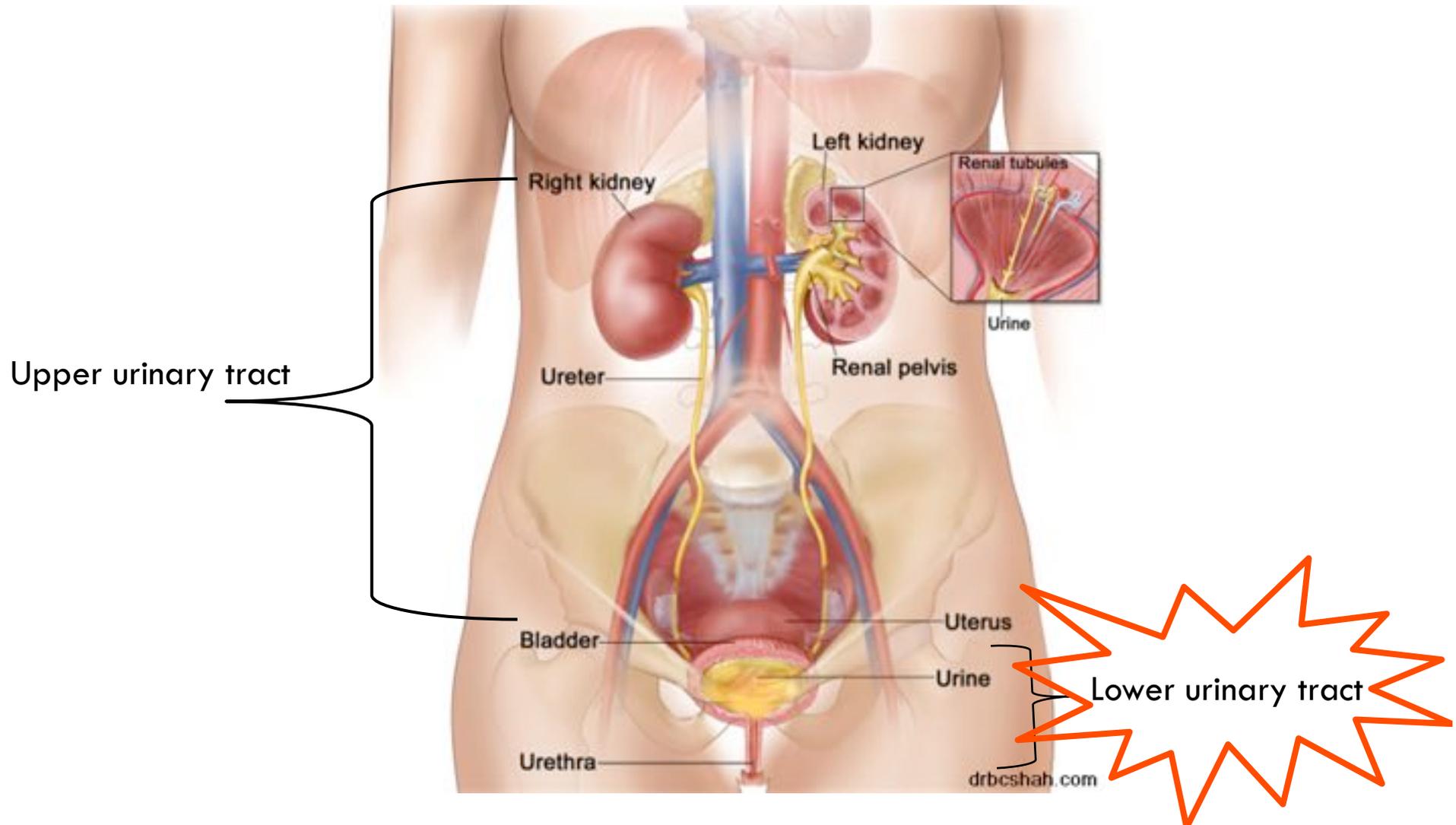
Cytologisk undersøkelse av eksfolierte cancerceller i urin kan være nyttig i følgende tilfelle:

- Hematuri uten positive funn ved cystoskopi og bildeundersøkelse av øvre urinveier
- Usikkert cystoskopifunn
- Negativ cystoskopi hos pasient med suspekke urinveissymptomer (CIS?)
- Negativ cystoskopi og mulig svulst i øvre urinveier ved bildeundersøkelse
- Oppfølging av enkelte pasienter (for eksempel CIS) (se Kap 8 om oppfølging)

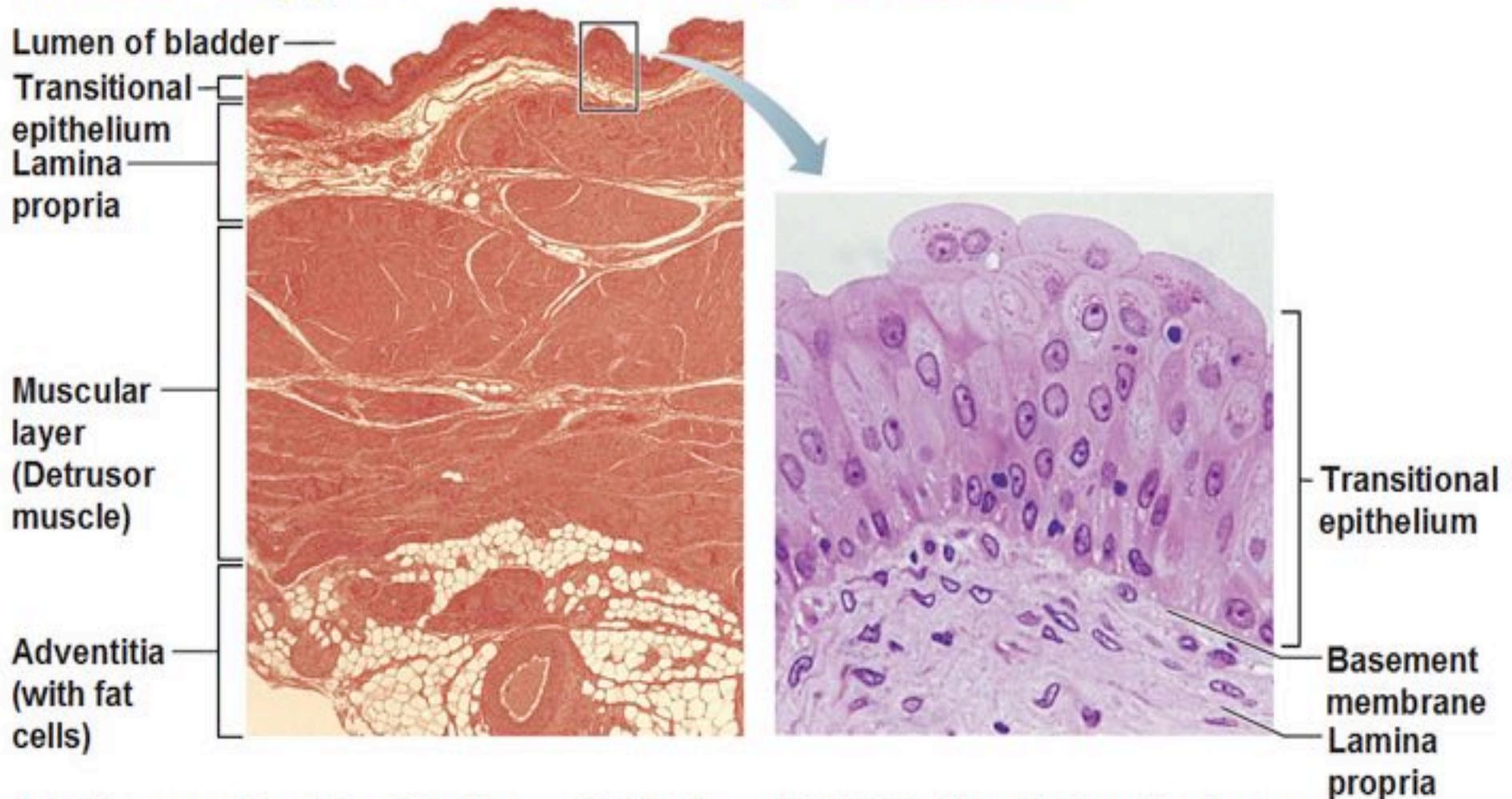
Undersøkelsen kan gjøres i spontanurin eller i væske fra blæreskylling. Det anbefales at spontanurinen ikke skal være den første morgenurinen, men heller fra en tid på døgnet da pasienten er godt hydrert for å sikre flest mulig bevarte celler i urinen (unngå cytolyse). En prøve tatt under pågående makroskopisk hematuri kan være vanskelig å tolke.

Urincytologi er mest pålitelig for påvisning av svulster av høy malignitetsgrad og CIS. Svulster med lav malignitetsgrad gir positiv urincytologi i langt færre tilfelle. Tolkning av det cytologiske preparat kan være problematisk av flere grunner: Lavt celletall, degenerative/irritative forandringer (for eksempel steinsykdom) og terapiinduserte forandringer (BCG, stråleterapi). Det er derfor viktig at man anvender et laboratorium med erfaring i cytologisk vurdering og gir adekvate kliniske opplysninger til patologen(70) (evidensgrad C).

# The urinary tract – the basics



# Histology of the Urinary Bladder



**(a) Micrograph of the bladder wall (17X)**

**(b) Epithelium lining the lumen of the bladder (360X)**

Benign nuclei: oval, with nuclear groove, point towards top (normal polarity) and not hyperchromatic

Umbrella cells

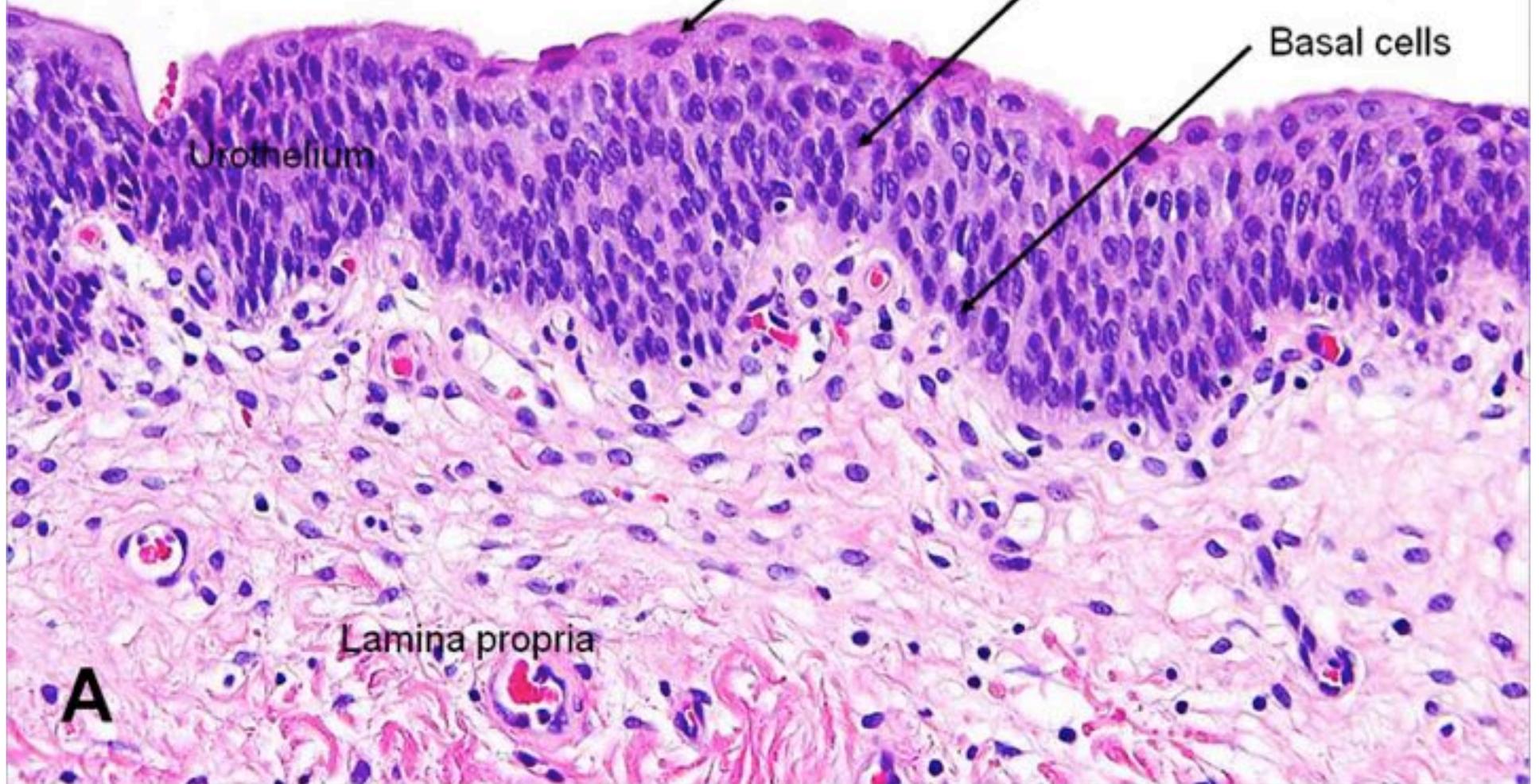
Intermediate cells

Basal cells

Urothelium

Lamina propria

**A**





What is urine?

# Urine – a definition

- Urine is a liquid by-product of the metabolism in humans and in many animals. Urine flows from the kidneys through the ureters to the urinary bladder.



# Urine composition

Relative composition of plasma and urine in normal men

	plasma g/100 ml	urine g/100 ml	concentration in urine
water	90–93	95	—
protein	7–8.5	—	—

**Not the perfect cell  
presevation medium!!**

potassium	0.02	0.15	×7
calcium	0.01	0.015	×1.5
magnesium	0.0025	0.01	×4
chloride	0.37	0.6	×2
phosphate	0.003	0.12	×40
sulfate	0.003	0.18	×60
ammonia	0.0001	0.05	×500



## **WARNING**

# **Cigarettes cause bladder cancer.**

**Toxic chemicals in tobacco smoke damage the lining of the bladder causing cancer. The most common sign is blood in the urine.**

**You have the will. There is a way.**



**1-866-366-3667**

**[gosmokefree.gc.ca/quit](http://gosmokefree.gc.ca/quit)**

**Health Canada**



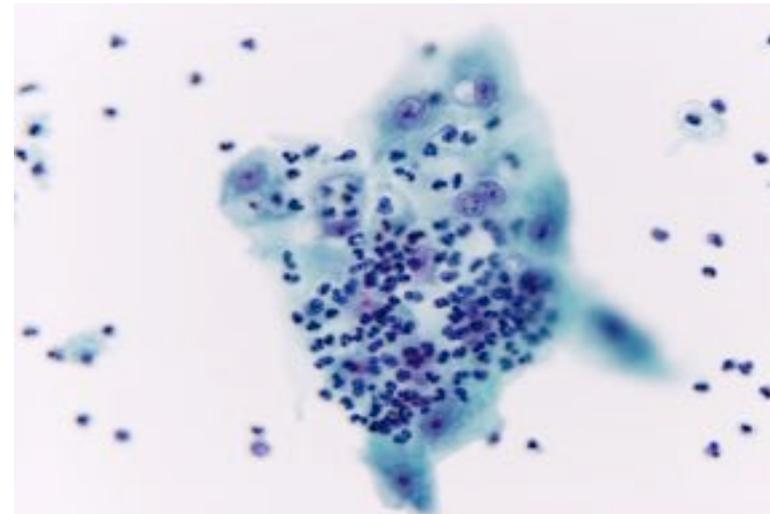
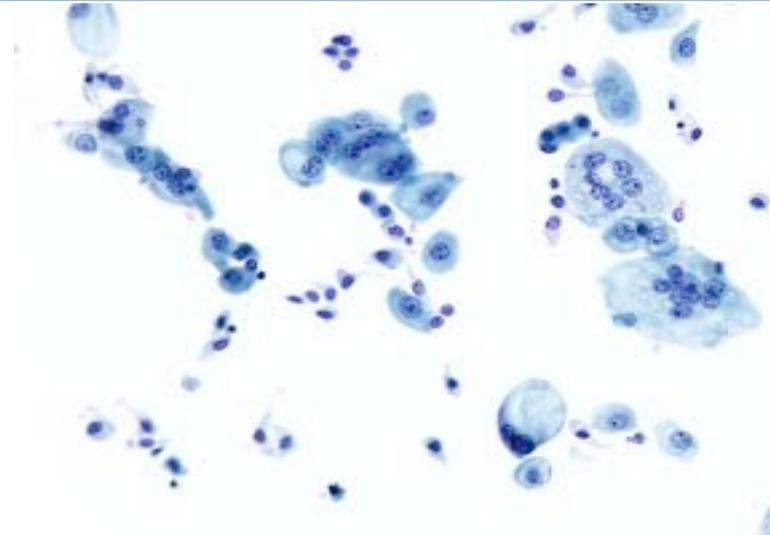
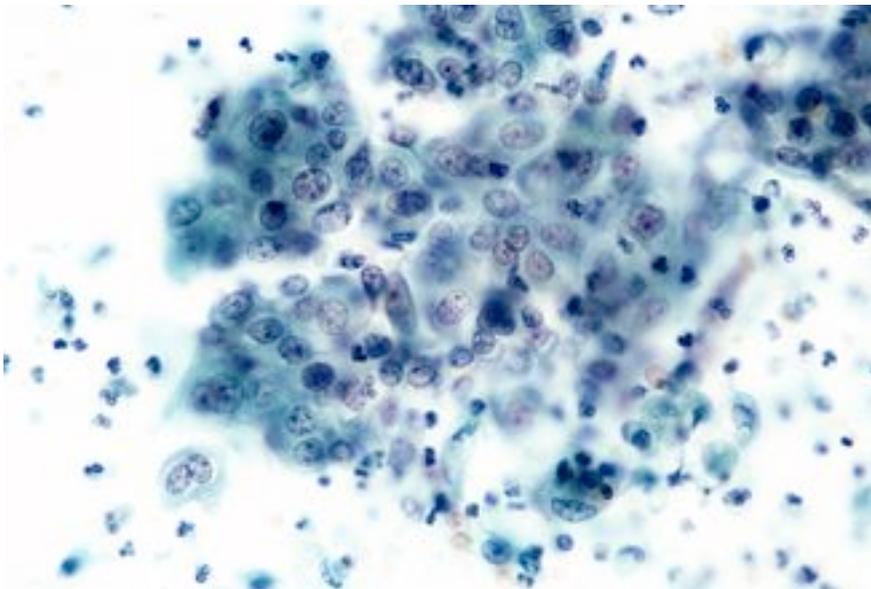
What can you expect to find in a normal urine?

# Normal Urinary Elements

- ❑ Urothelial cells
  - ❑ Intermediate and superficial (umbrella) cells (voided urine)
  - ❑ Intermediate, superficial and basal cells (catheterized urine, washing)
- ❑ Squamous cells
- ❑ Miscellaneous findings
  - ❑ Prostate and seminal vesicle epithelial cells
  - ❑ Renal tubular cells and casts
  - ❑ Corpora amylacea
  - ❑ Crystals
  - ❑ Inflammatory cells
- ❑ Degenerated intestinal epithelial cells (ileal conduit)

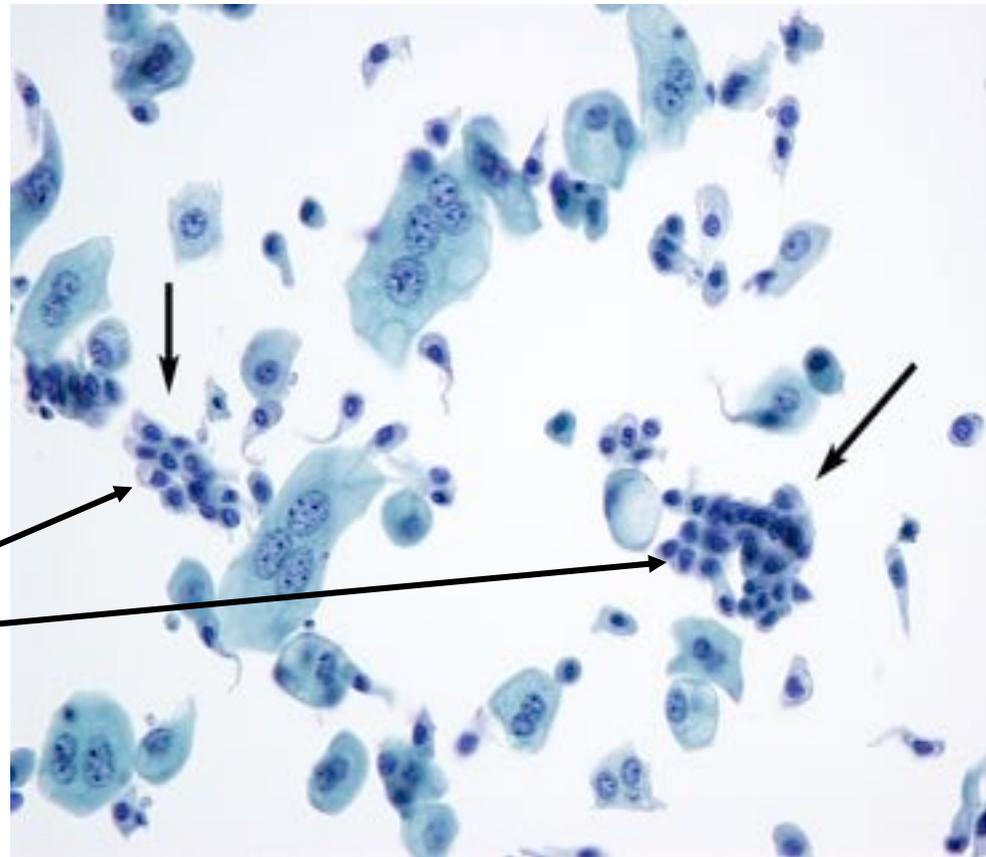
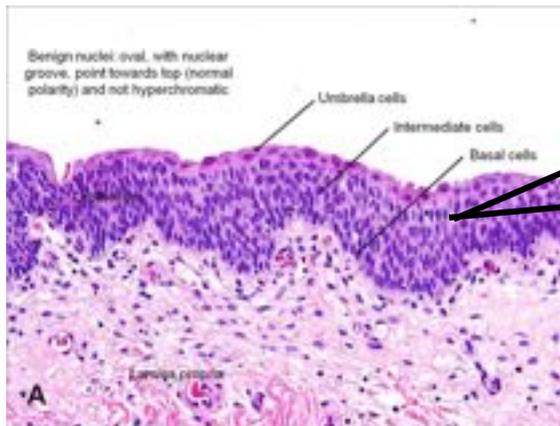
# Umbrella cells

- Low N/C ratio
- Pale finely granular chromatin
- Smooth nuclear shapes
- Multinucleation common
- Cytoplasm transparent

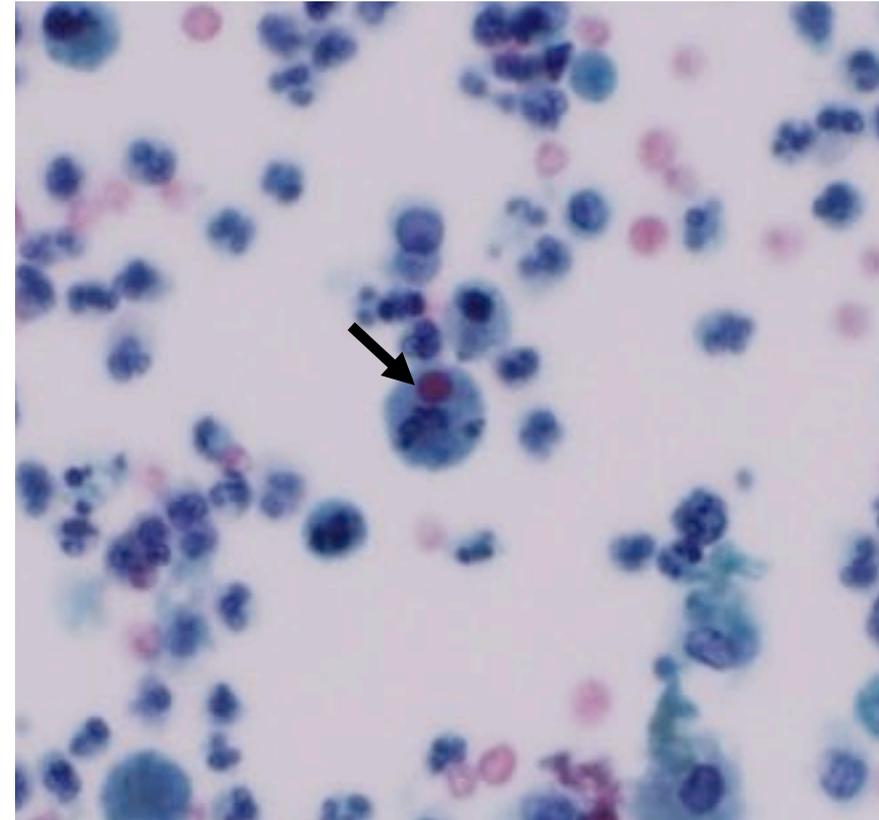
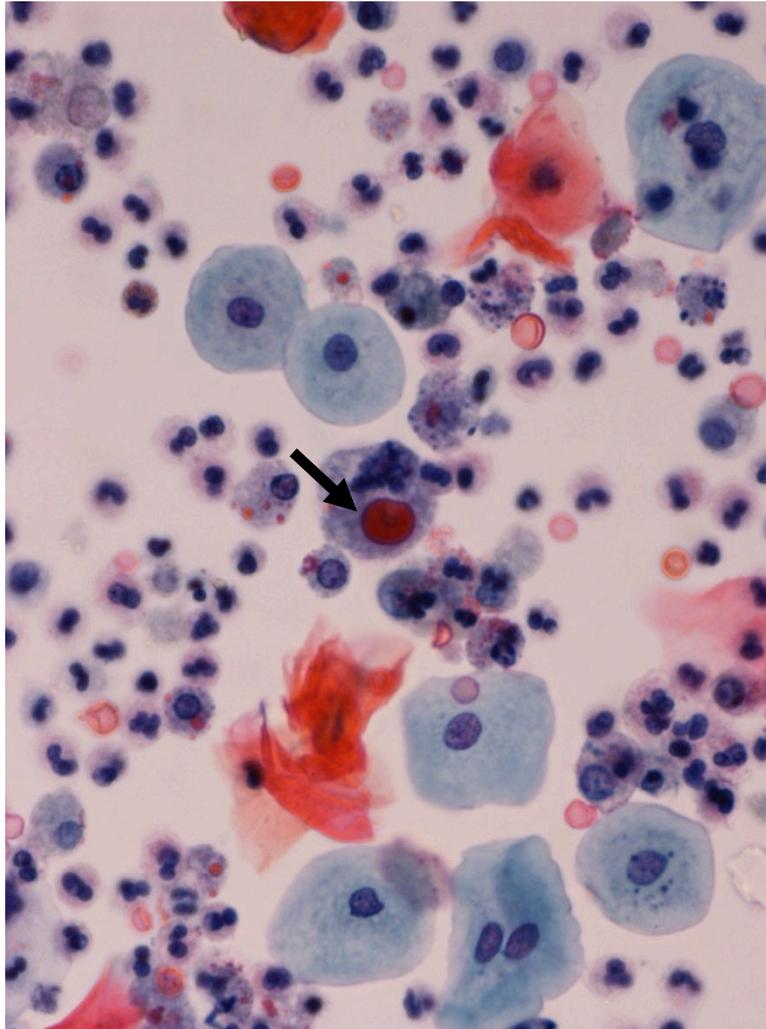


# Intermediate and basal cells

- High N/C ratio
- Chromatin darker than superficial cells
- Nuclei smaller than superficial cells
- Nuclear shape round
- Even nuclear spacing



# Normal Urinary Elements



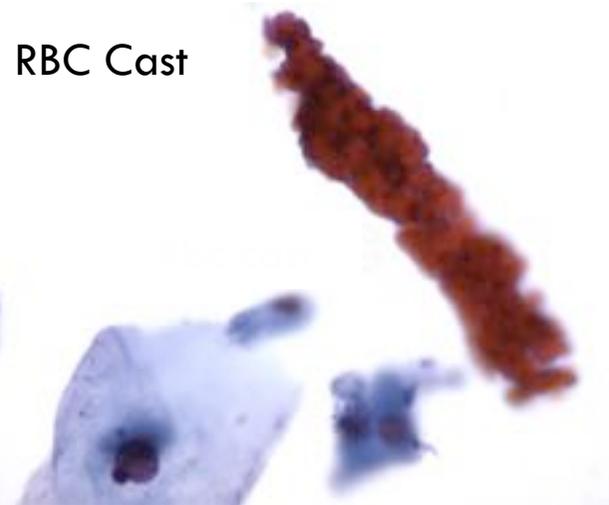
Melamed-Wolinska Bodies

# Casts

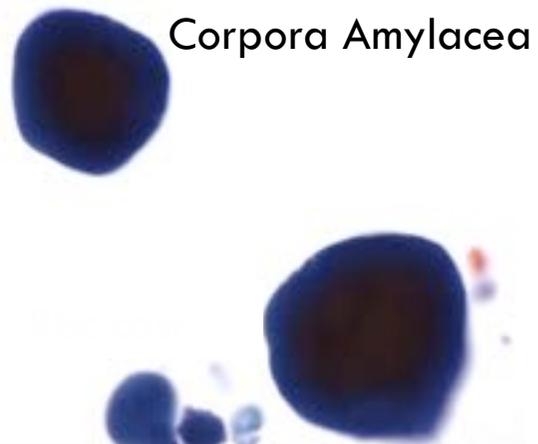


- ❑ Renal Diseases:
  - ❑ RBC casts: Glomerular diseases
  - ❑ WBC casts: Tubulointerstitial diseases and transplant rejection
  - ❑ Renal tubular casts: Renal parenchymal diseases
  - ❑ Fatty casts: Nephrotic syndrome
- ❑ Physiologic:
  - ❑ Hyaline and granular casts: Secondary to dehydration, fever, exercise etc

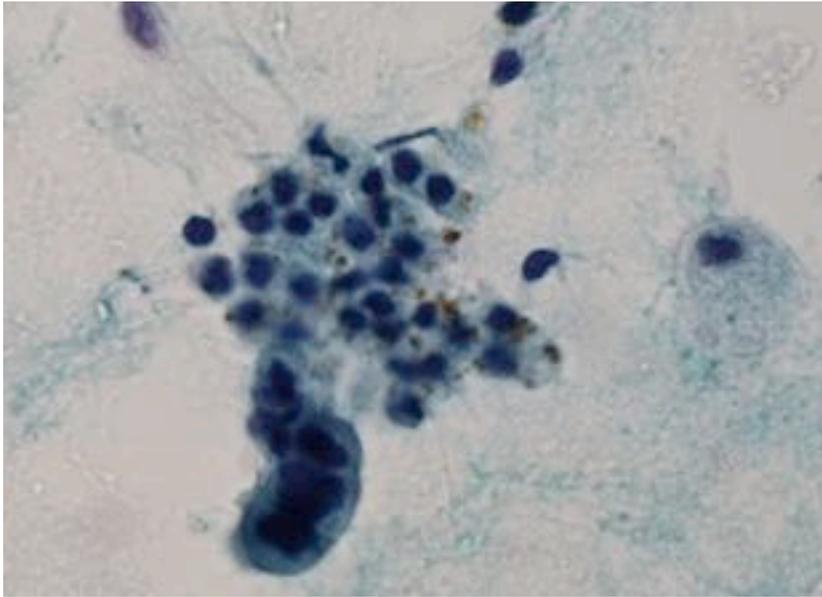
# Normal Urinary Elements



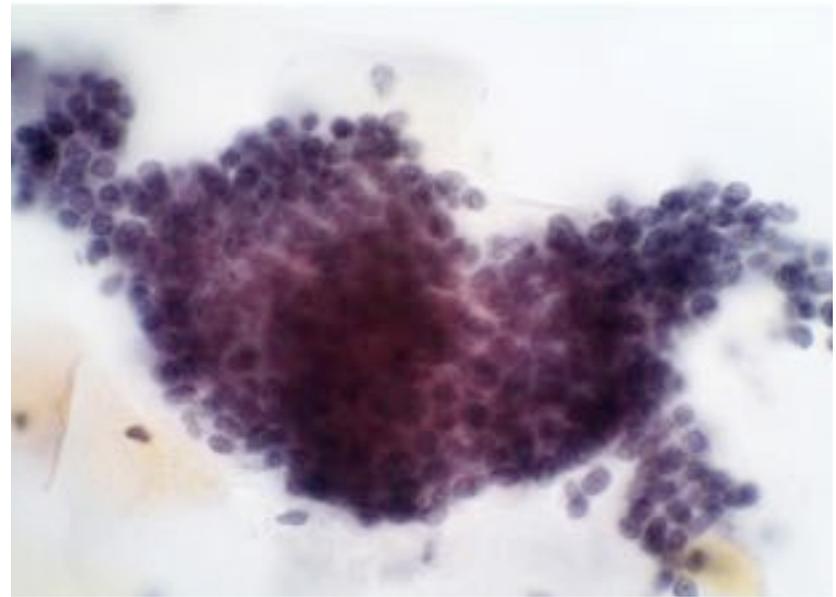
Renal Tubular Cast



# Non-Urinary Elements

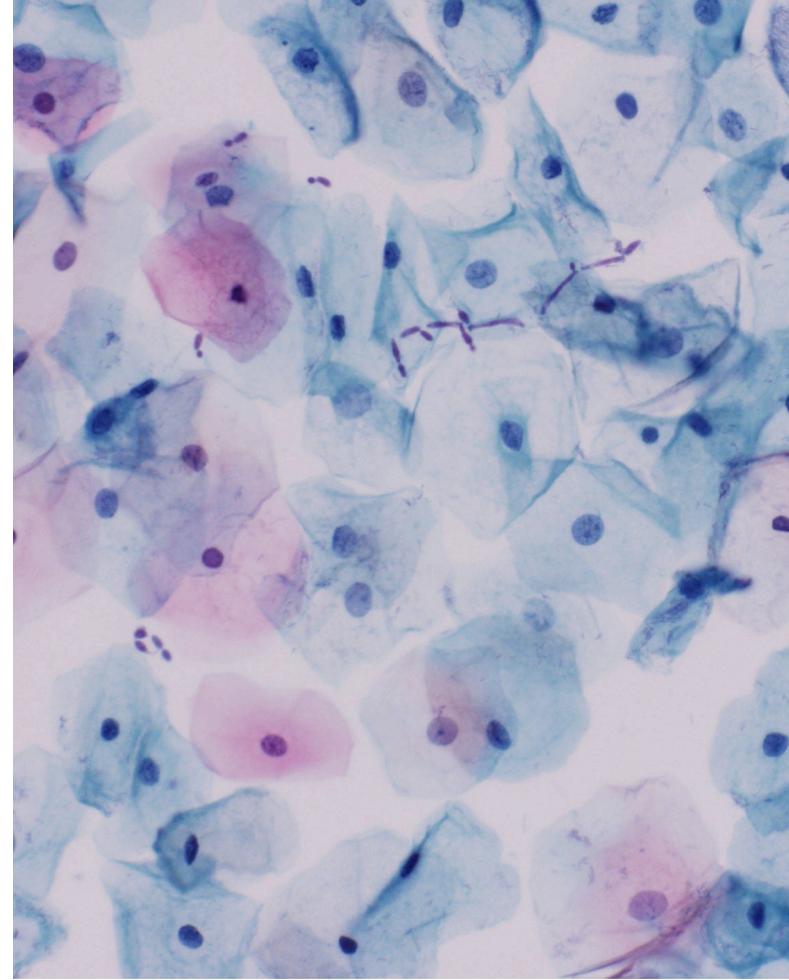
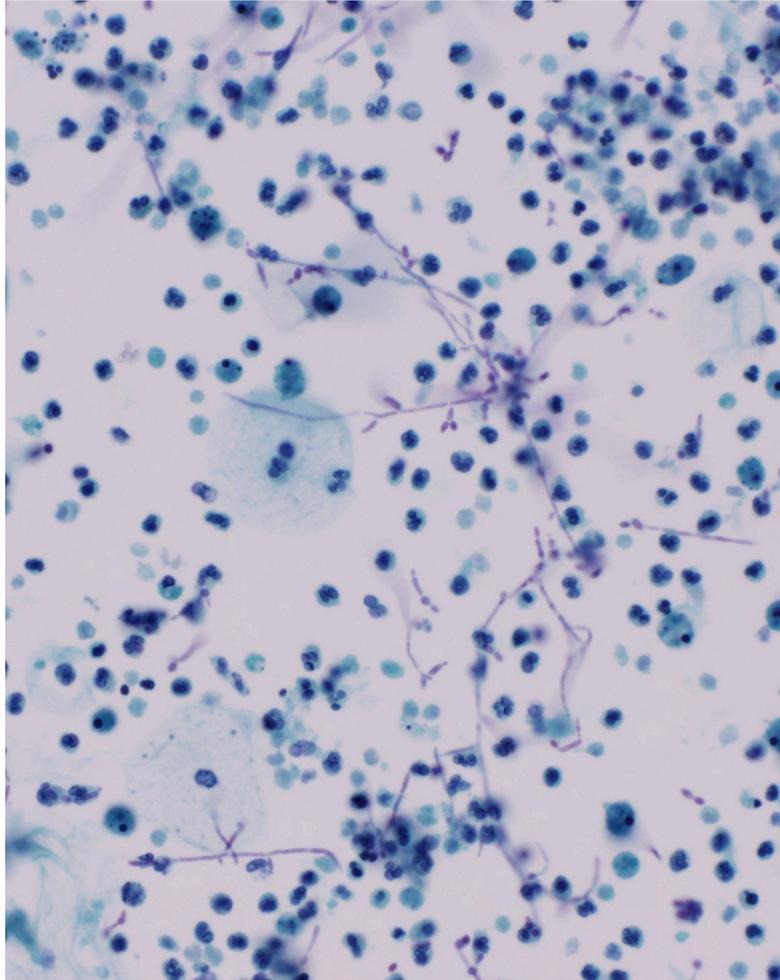


Seminal Vesicle Cells



Endometrial Cells

# Infections -Fungal



# Crystals

- ❑ Common finding, no clinical significance in most cases
- ❑ Crystals analysis part of routine urinalysis rather than urine cytology
- ❑ Uric acid: most common, variable shape
- ❑ Triple-phosphate: prism shaped and resemble coffin lids
- ❑ Ammonium biurate: “Thorn apples”
- ❑ Calcium Oxalate: Oval, dumbbell shaped
- ❑ Pathologic crystals: much less common, bilirubin (brown granules and needles), cholesterol , cysteine (hexagonal plates), leucine (spheres with radiating striations) and tyrosine (slender needles)

# Types of Urinary Specimens



- ❑ Voided Urine
- ❑ Catheterized Urine
- ❑ Bladder Washings
- ❑ Upper Tract Washings and Brushings
- ❑ Ileal Conduit Samples

Urine samples is a relatively easy sample to obtain....maybe

# Physicians have it easy...



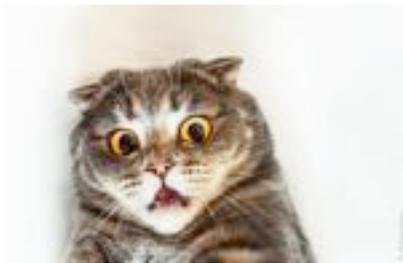
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# Voided Urine



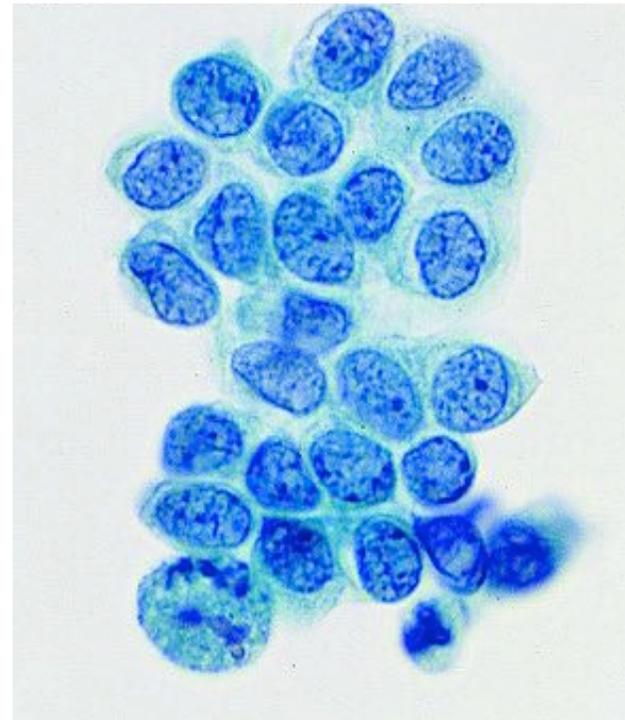
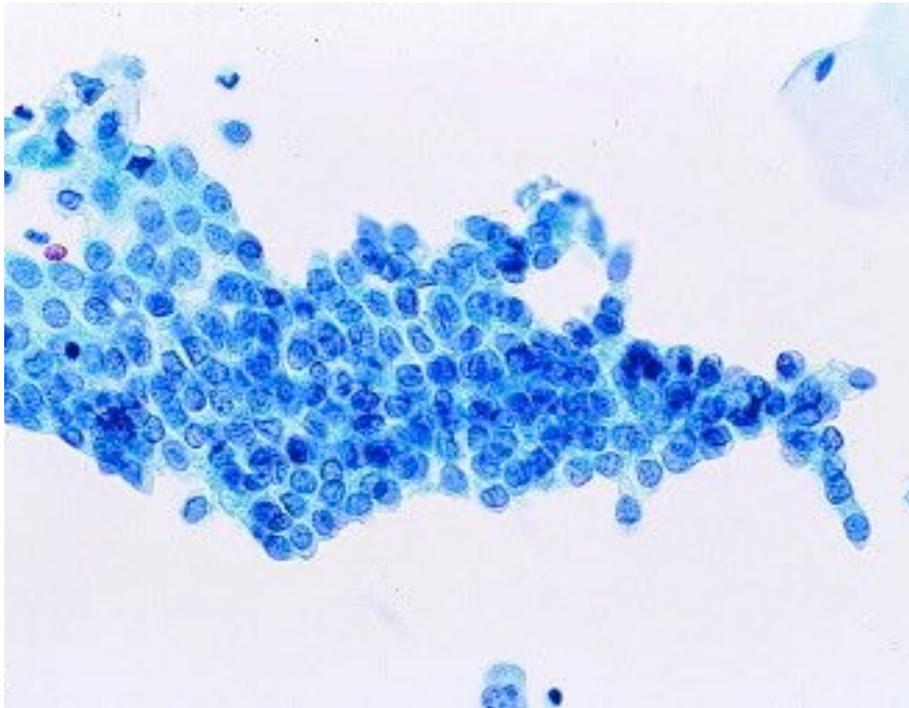
- ❑ Collected 3-4hrs after the last void (100-300ml)
- ❑ Sparse cellularity, superficial and intermediate cells
- ❑ Degenerative changes
- ❑ Squamous cells common
  - ❑ Trigone or genital tract contamination in women
  - ❑ Inflammation or irritation
- ❑ Non-cellular constituents such as crystals, casts, corpora amylacea
- ❑ Non-invasive technique and no instrumentation effect

# Catheterized Urine



- ❑ Moderate to highly cellular
- ❑ Superficial, intermediate and basal cells
- ❑ Poor preservation with pronounced degenerative changes in pooled specimens
- ❑ Urethra not sampled
- ❑ Instrumentation artifacts: Urothelial clusters can mimic low-grade urothelial carcinoma
- ❑ Risk of infection

# Catheterized Urine



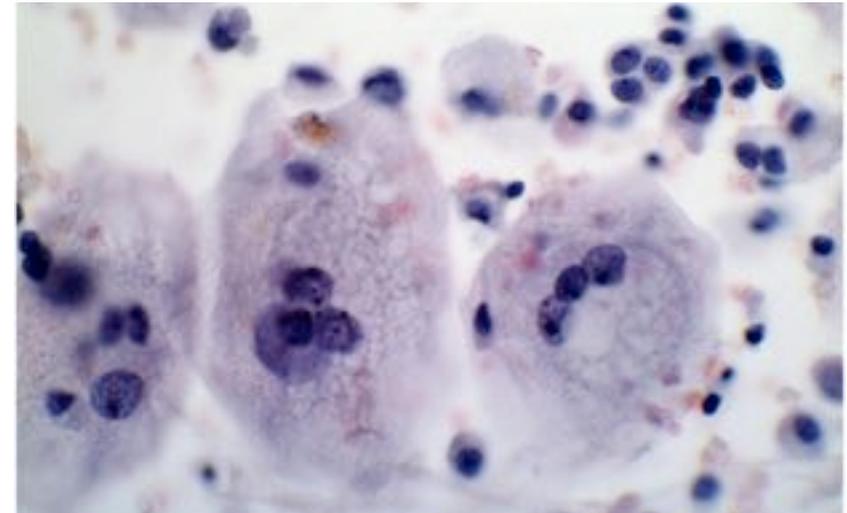
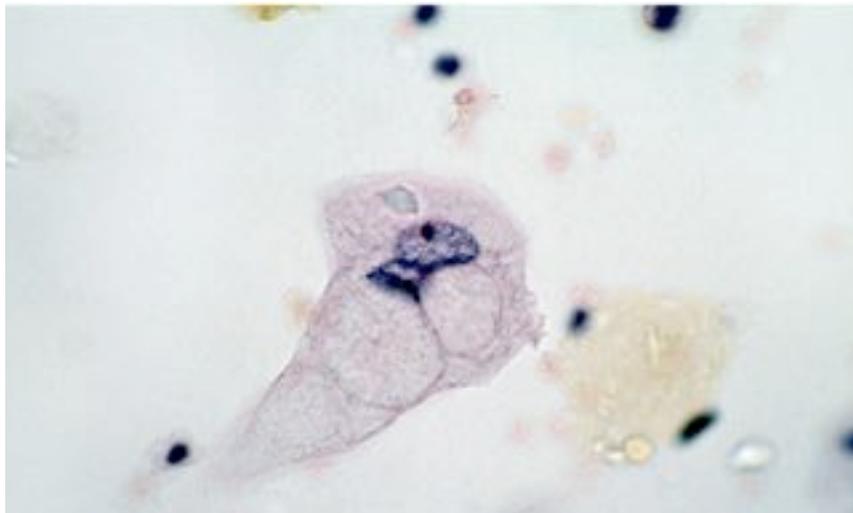
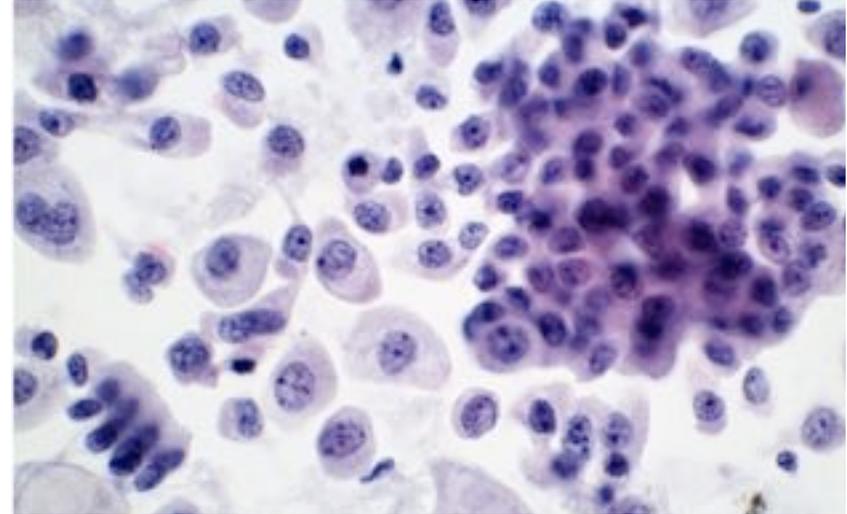
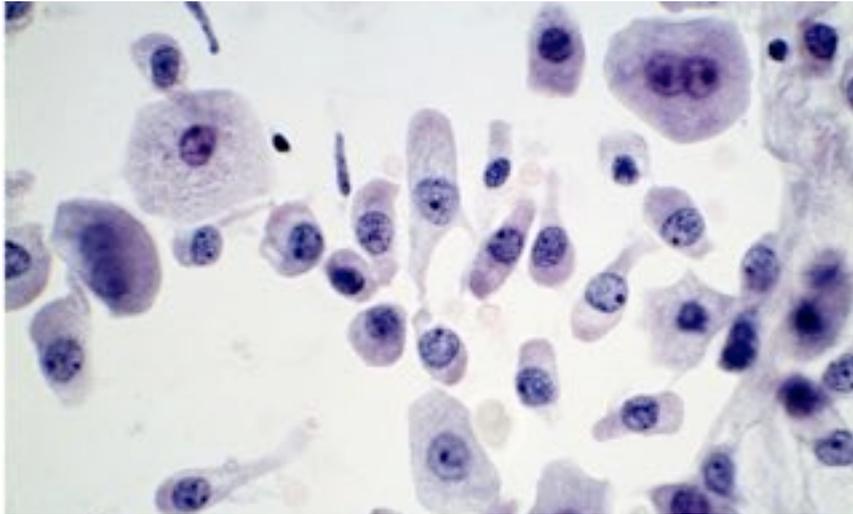
Basal Urothelial Cells in Catheterized Urine Specimen

# Bladder Washings



- ❑ Obtained through a catheter by irrigating the bladder with 5-10 pulses of 50 ml sterile saline
- ❑ Better cellularity and preservation
- ❑ Less contamination by background debris
- ❑ Increase sensitivity (66%-77%)
- ❑ Only bladder epithelium represented – upper tract not sampled
- ❑ Quality of sample dependent on the skill of urologist

# Bladder Washings



# Upper Tract Washings / Brushings

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- ❑ Comparable sensitivity to other type of urinary specimens
- ❑ Technically and morphologically challenging
- ❑ Prone to false positive results – marked cellularity
- ❑ Comparison of bilateral specimens (normal vs lesional) helpful in making diagnosis
- ❑ Cytological diagnosis with conservative approach
  - ❑ Ureterectomy or nephrectomy



Right kidney

Left kidney

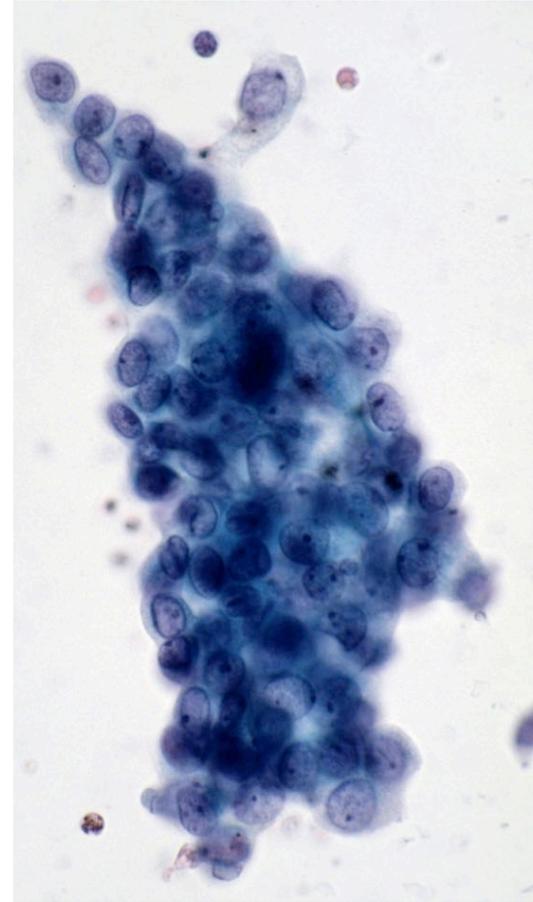
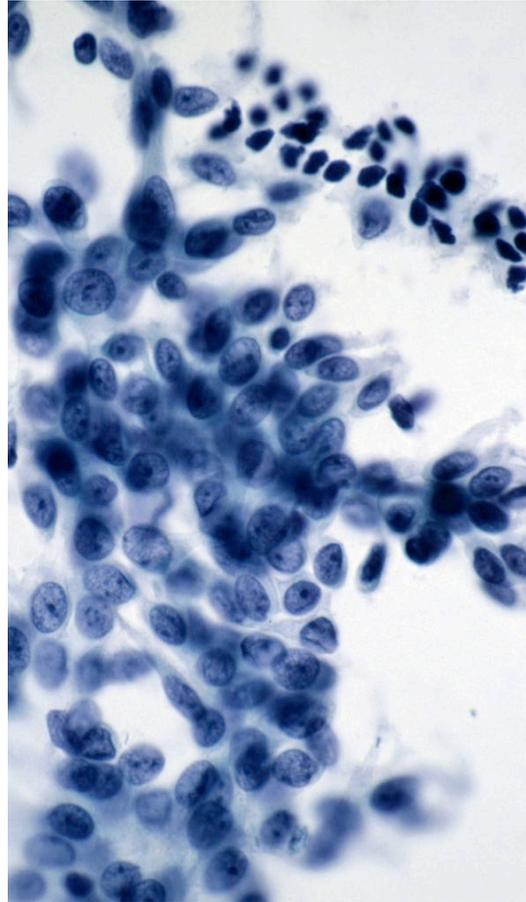
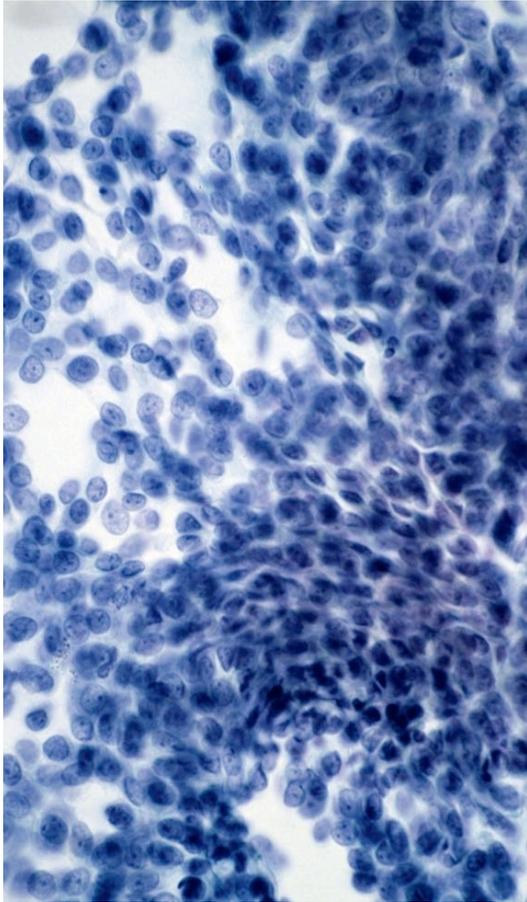
Ureter

Ureteroscope

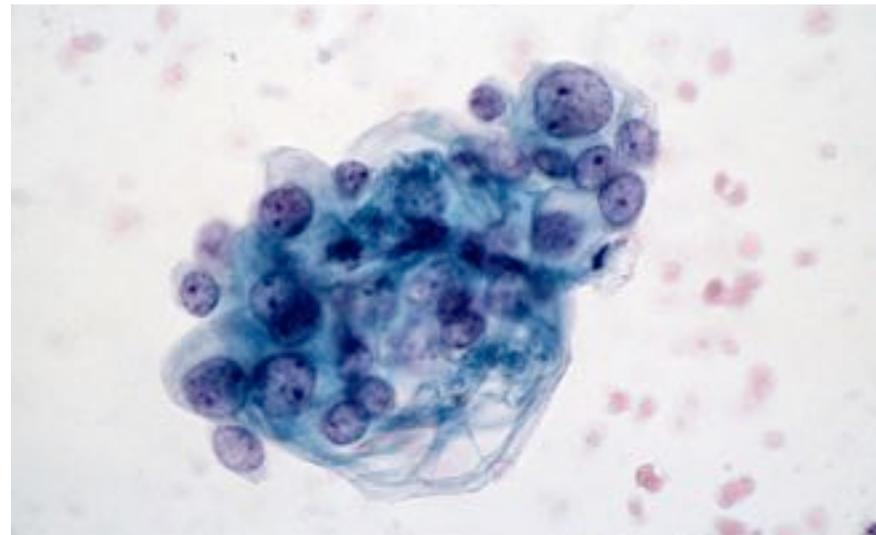
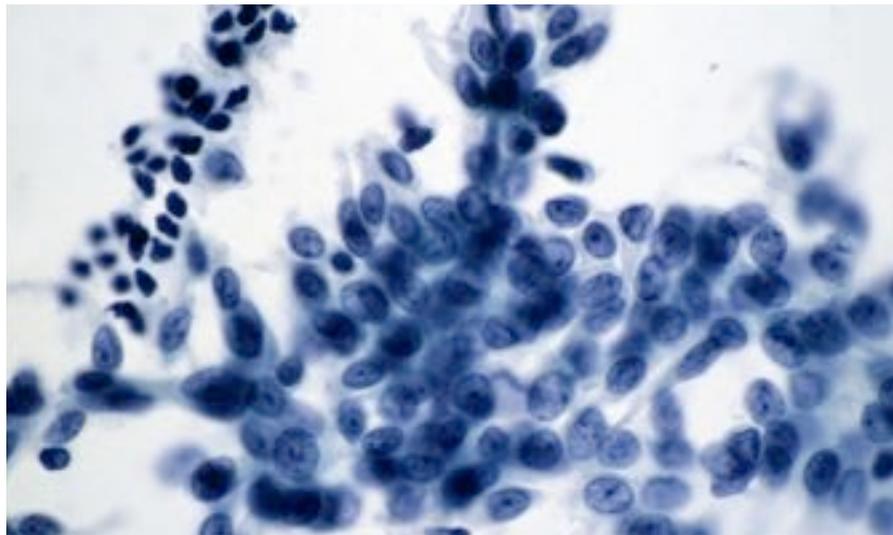
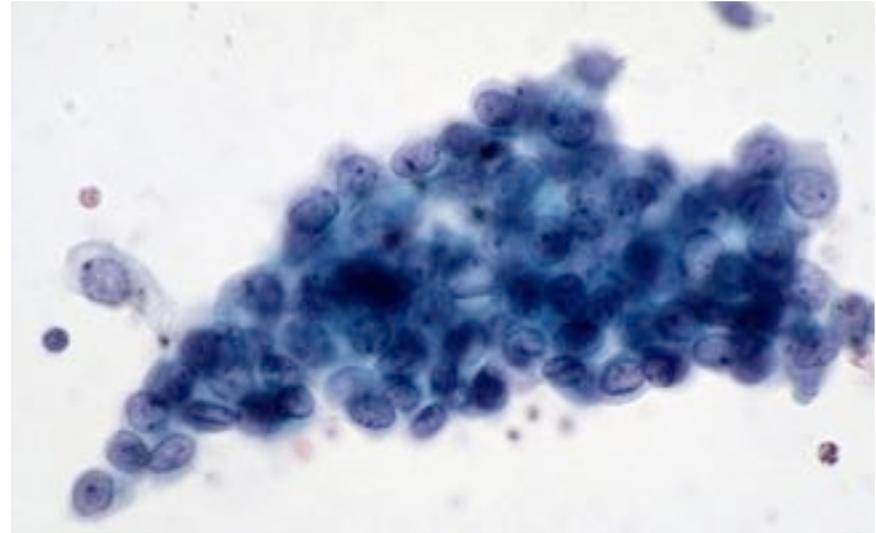
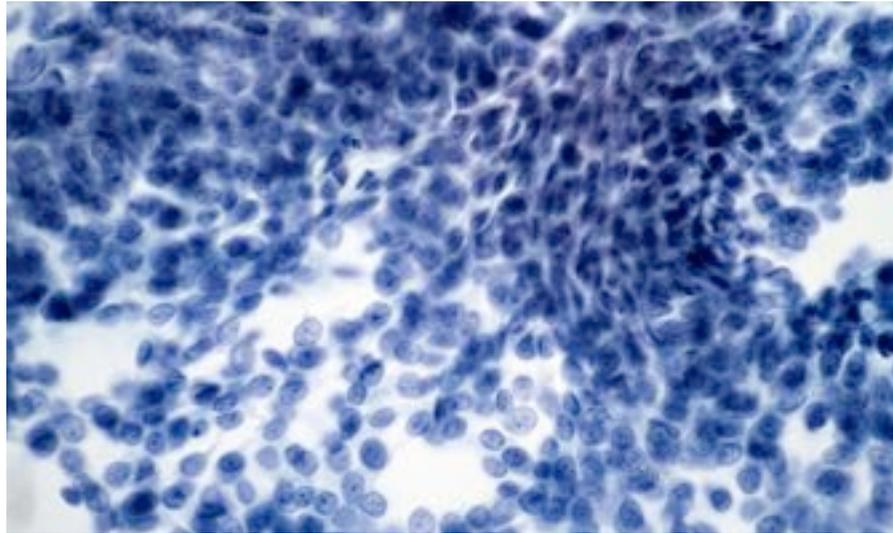
Bladder

Urethra

# Urethral Brushings

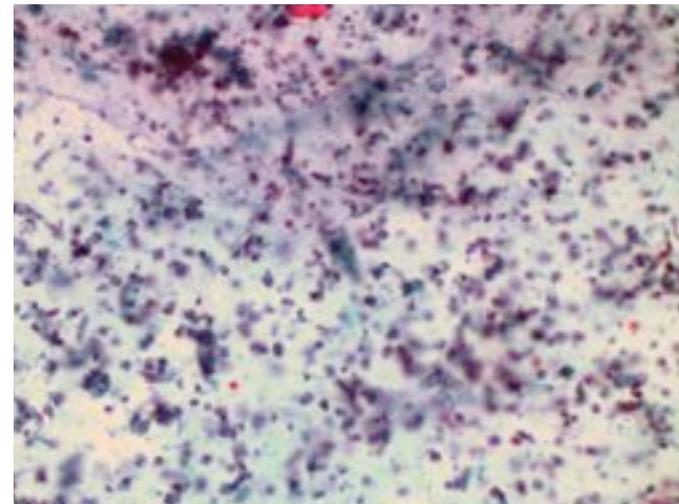
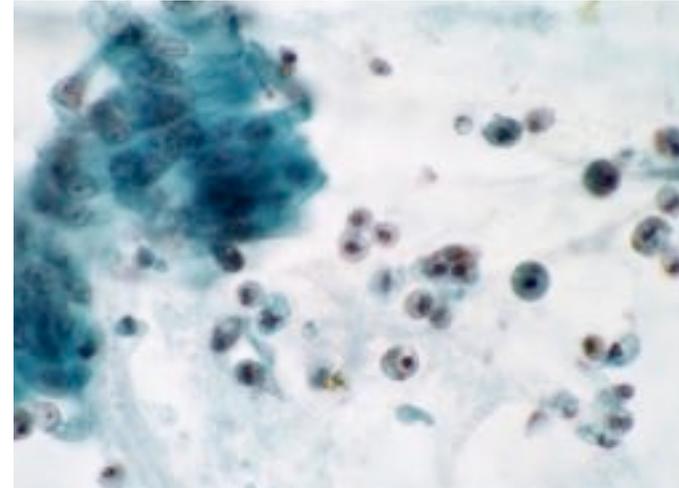


# Urethral Brushings



# Ileal Conduit

- ❑ Surveillance of ureters and renal pelvis post cystectomy
- ❑ Cellular specimen with large amount of degenerated intestinal epithelial cells and background debris
- ❑ Malignant cells may be obscured



# Take home from sampling:



- There is a balance between the invasiveness of the sampling method and the cellularity obtained



# The history of systems for reporting urine samples

# Olfactory detection of human bladder cancer by dogs: proof of principle study

Carolyn M Willis, Susannah M Church, Claire M Guest, W Andrew Cook, Noel McCarthy, Anthea J Bransbury, Martin R T Church, John C T Church

## Abstract

**Objective** To determine if dogs can be trained to distinguish patients with bladder cancer on the basis of urine odour more successfully than would be expected by chance alone. This suggests that tumour related volatile compounds are present in urine, imparting a characteristic odour signature distinct from those associated with secondary effects of the tumour, such as bleeding, inflammation, and infection.

**Design** Experimental study. Dogs were trained to distinguish between urine samples from patients with bladder cancer and controls and then tested on a series of one bladder cancer urine sample and five control urine samples.

**Participants** 30 patients (15 with bladder carcinoma of the bladder, 15 with carcinoma of the prostate) and 30 healthy, age related controls were used for testing.

**Main outcome measures** The dogs achieved a success rate of 1 in 7 (14%) during the training phase. This gave a mean success rate of 23% to 58% using a bootstrap method. Multivariate analysis did not recognise a characteristic odour independent of that detected by urinalysis.

**Conclusions** Dogs can be trained to distinguish patients with bladder cancer on the basis of urine odour more successfully than would be expected by chance alone. This suggests that tumour related volatile compounds are present in urine, imparting a characteristic odour signature distinct from those associated with secondary effects of the tumour, such as bleeding, inflammation, and infection.



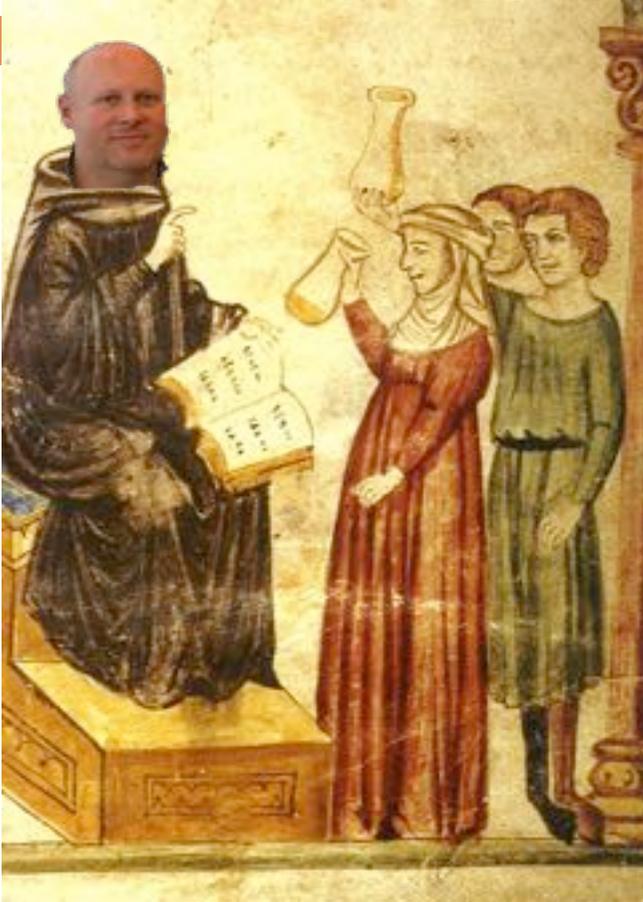
© MARK LARGE

Although these anecdotal events remain unsupported by formal testing, they suggest that dogs may be able to detect bladder cancer through their sense of smell. We chose to release the results of this study to dogs in order to determine if dogs can be trained to distinguish between urine samples from patients with bladder cancer and controls and then tested on a series of one bladder cancer urine sample and five control urine samples.

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We should emphasise that our objective at this stage was to conduct a simple, yet stringent, "proof of principle" study to answer the question, "Can dogs be trained to detect bladder cancer more successfully than would be expected by chance alone?" This was not an attempt to assess or predict the clinical usefulness of this hypothesised capability of dogs.

Th



# From urine analysis to urine cytology

- Rise of modern light microscopes – 1600s
- No mention of cellular elements in urine until 1800s



Alfred Donne 1801 - 1878

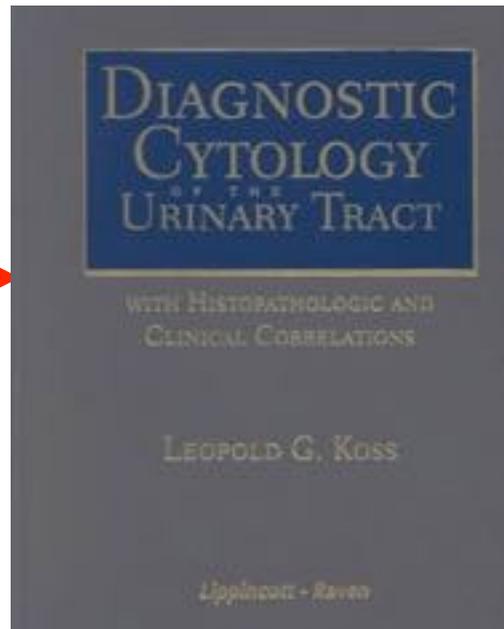


Hermann Lebert 1813 - 1878

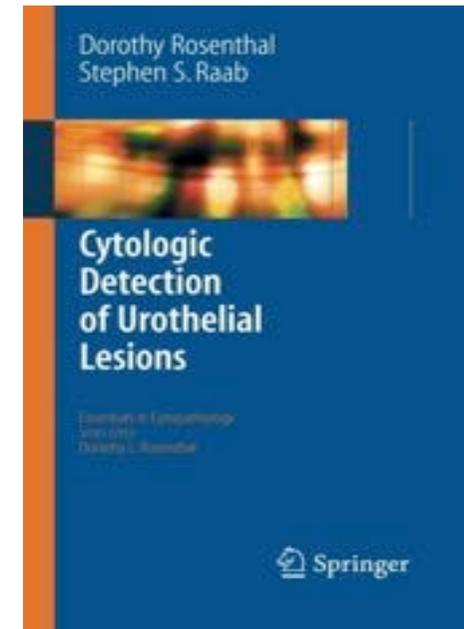
# Modern times....



**1928**  
**Georgios Papanikolaou**  
**Pap smear**



**1940s - onwards**  
**Leopold Koss**  
**'Father of urine**  
**cytology'**

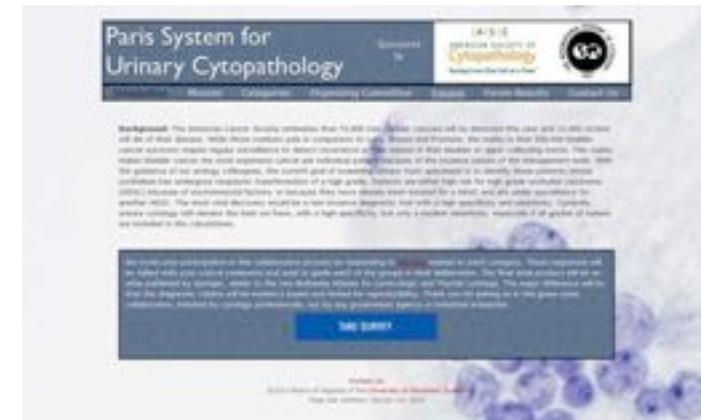


**Dorothy Rosenthal**  
**The Johns Hopkins**  
**Hospital template**  
**for urologic cytology samples**

# Onwards to Paris!

18th International Congress of Cytology, Paris, May, 2013

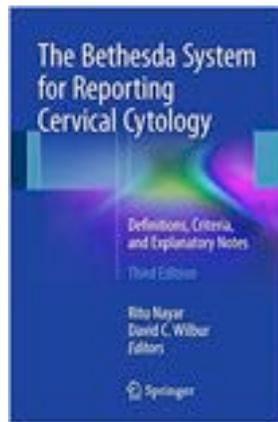
- “Paris Group” – all participants of two Urine Cytology Symposia
- Outline of the Paris System for Reporting Urinary Cytopathology
- Ultimate goal – detection of HGUC
- Sponsorship by the ASC and IAC
- Contract with Springer
- Numerous face-to-face meetings



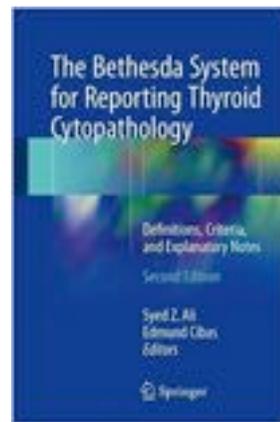
## 2. The goal of urine cytology is to detect clinically significant high grade lesions (HGUC).

#	Answer	Bar	Response	%
1	I agree with this statement		127	85%
2	I disagree with this statement		22	15%
3	Comments/Suggestions:		18	12%

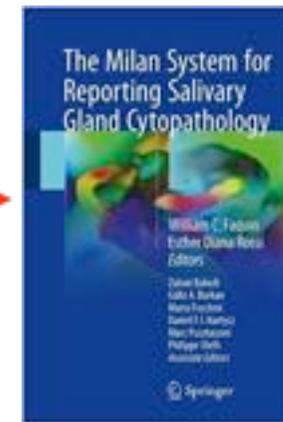
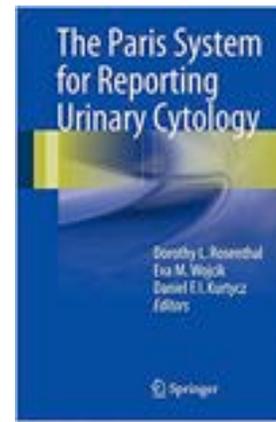
# The move to standardise...



2001



2007



# The Paris System for Reporting Urinary Cytology

The Paris Working Group consisted of 49 members, 28 from 12 US states, and 21 from 9 countries including Canada, France, Italy, Japan, Korea, Luxembourg, Slovenia, Switzerland, and the United Kingdom.



# Why to standardize reporting of urinary cytology?

- Reproducibility
- Improvement of communication
- Atypical cells
  - ▣ Wide intraobserver variability
- Nationally rates of atypical vary among institution
  - ▣ Range from 2% to 30% (51% atypical + suspicious)

For ex

Irregulære  
betydning

kker



# The Paris System

1. Pathogenesis of Urothelial Carcinoma
2. Adequacy
3. Negative for High Grade Urothelial Carcinoma
4. Atypical Urothelial Cells
5. Suspicious for High Grade Urothelial Carcinoma
6. High Grade Urothelial Carcinoma
7. Low Grade Urothelial Neoplasm
8. Other malignancies, both primary and secondary
9. Ancillary Studies
10. Clinical management
11. Preparatory techniques relative to Urinary Tract samples

# System has to be build based on:



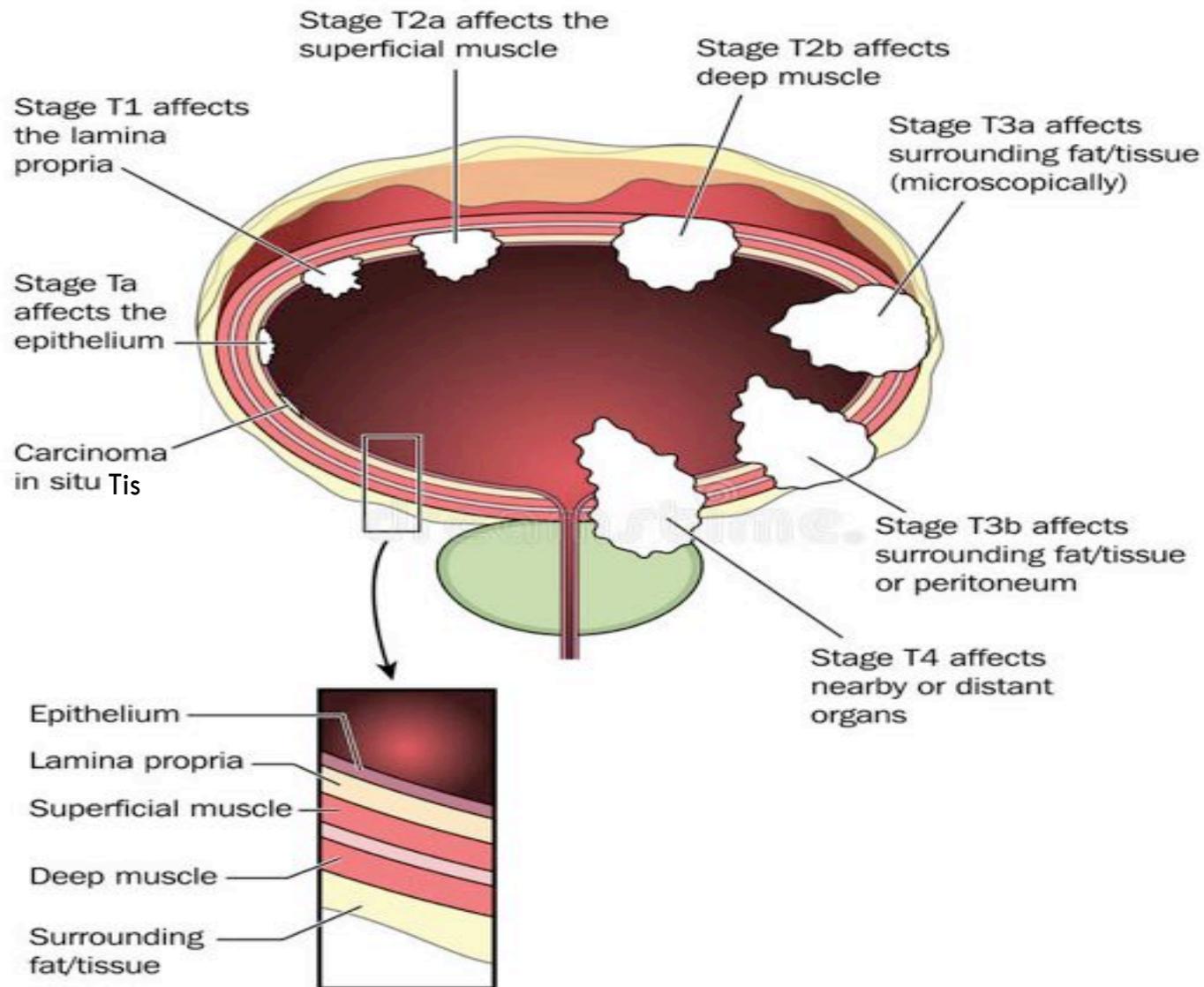
- Consensus
- Evidence
- Inclusion
- Acceptance
- Understanding

# A little on grading and staging



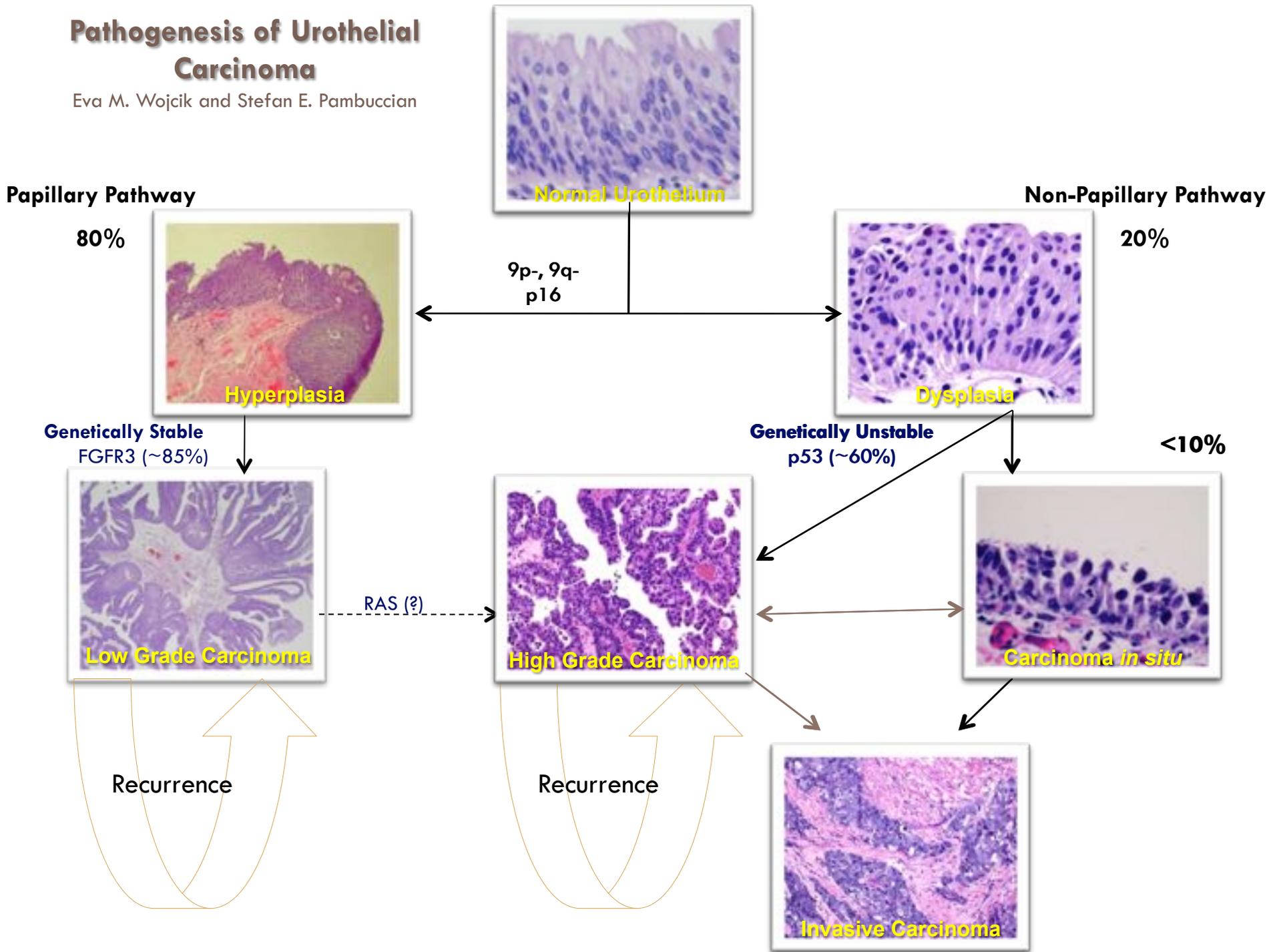
- Grading
  - ▣ Histological appearance
    - Low grade
    - High grade
- Staging
  - ▣ Non muscle invasive bladder cancer (NMIBC)
    - Tis, Ta, T1
  - ▣ Muscle invasive bladder cancer (MIBC)
    - >T1

# TNM classification for bladder cancer

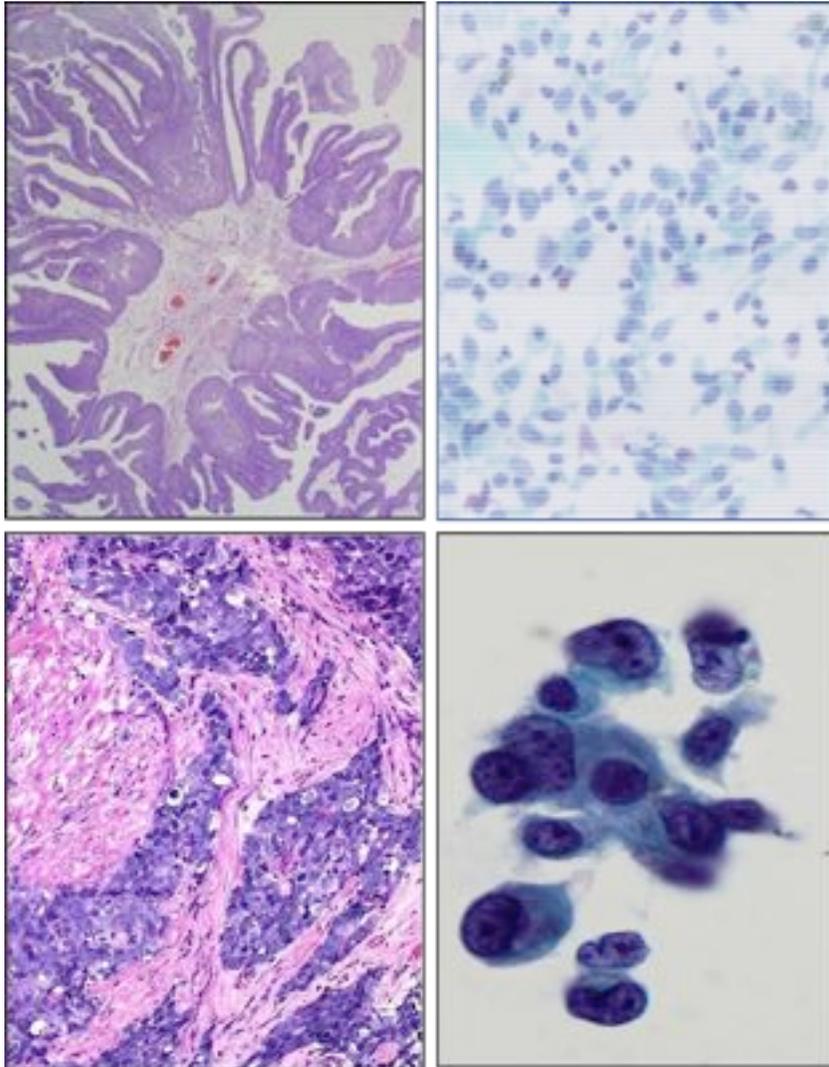


# Pathogenesis of Urothelial Carcinoma

Eva M. Wojcik and Stefan E. Pambuccian



# Bladder cancer – more than one disease?



- ~ 75 % Non-Muscle-Invasive (Ta/T1)
- Good prognosis
- Recurrence
- 10%-15% progression (LG Ta <1%)\*
  
- ~ 25 % Muscle-Invasive (> T2)
- >60% overall survival

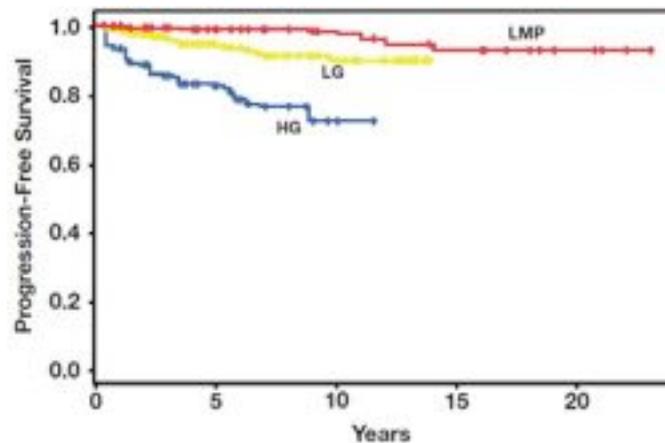
# A Review of Outcomes for Stage Ta Bladder Tumors

Robin T. Vollmer, MD

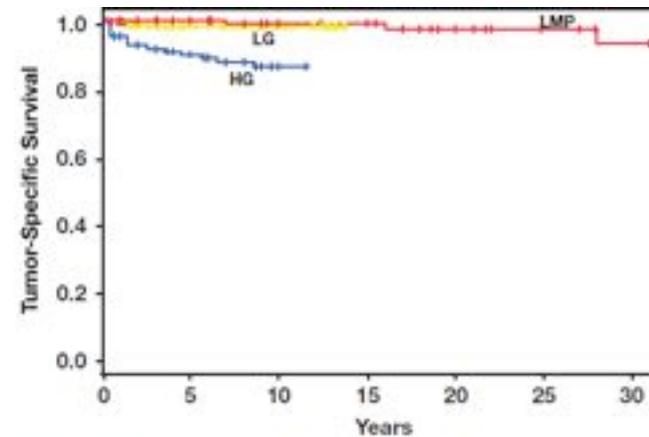
From the VA and Duke University Medical Centers, Durham, NC.

**Key Words:** Urothelial tumors; Tumor grade; Outcomes; Evidence based; Survival times

*Am J Clin Pathol* August 2016;146:215-220



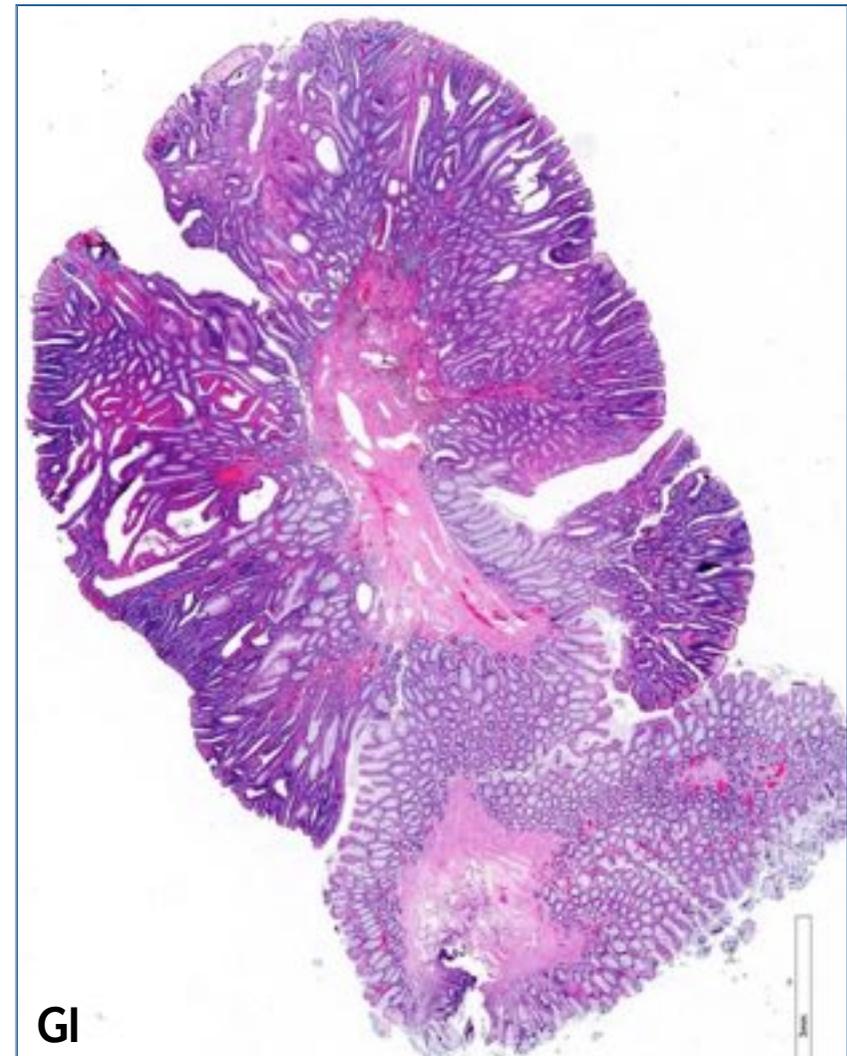
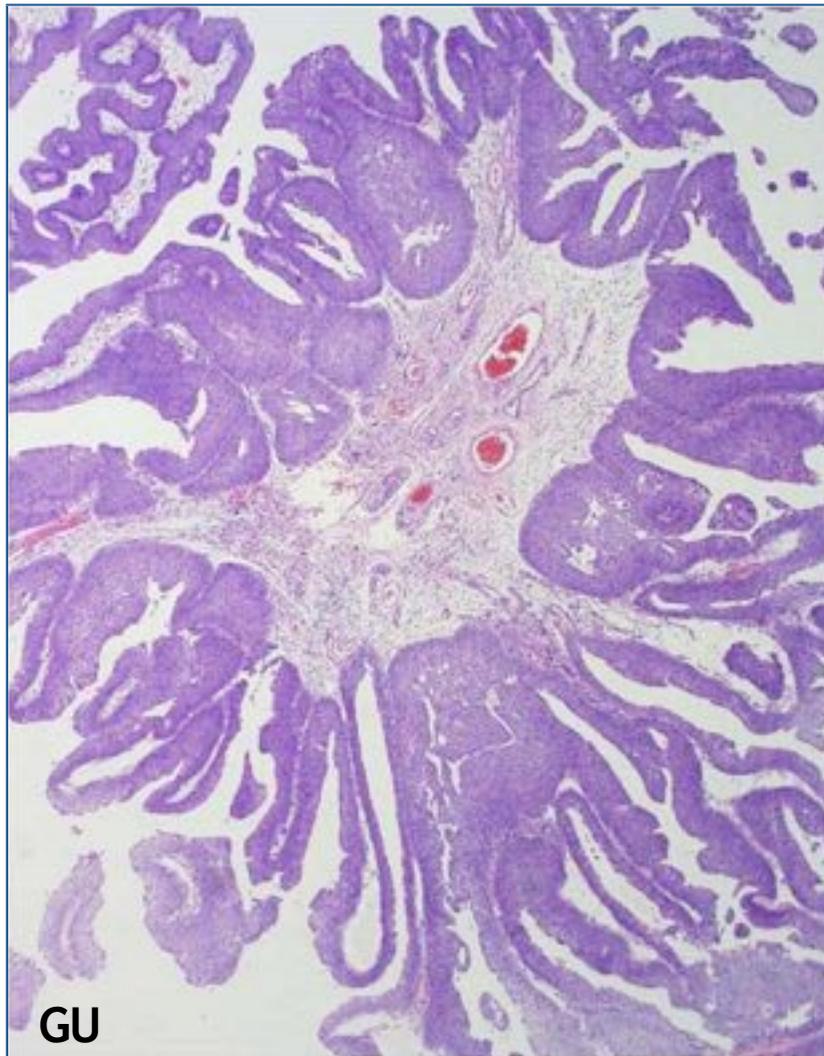
**Figure 5** Kaplan-Meier plots of the probability of being free of invasive tumor vs years of follow-up stratified according to the 2004 World Health Organization grades. HG, high grade; LG, low grade; LMP, papillary tumors of low malignant potential.



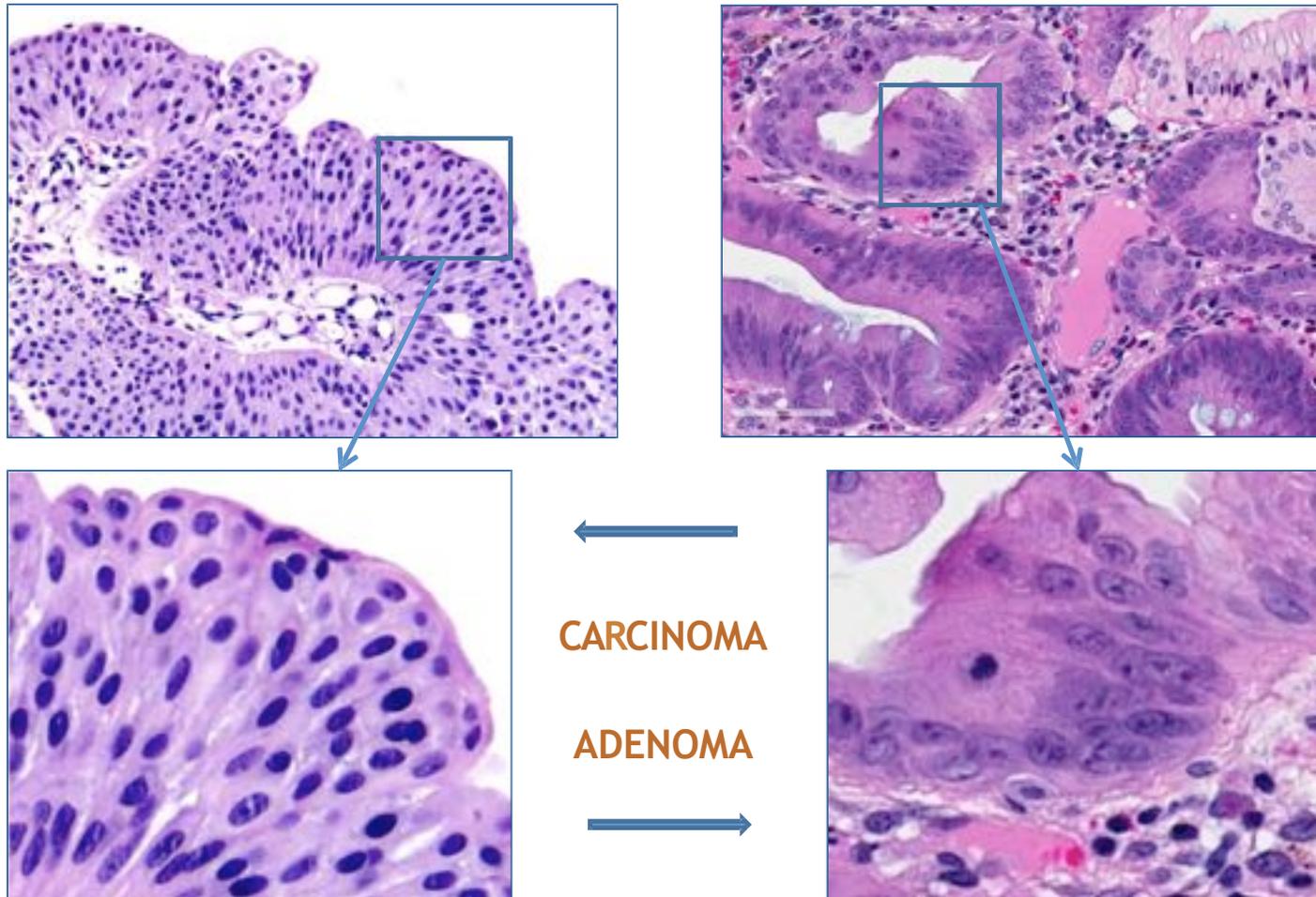
**Figure 7** Kaplan-Meier plots of the probability of tumor-specific survival vs years of follow-up stratified according to the 2004 World Health Organization grades. HG, high grade; LG, low grade; LMP, papillary tumors of low malignant potential.

“Approximately 80% (of Ta bladder tumors) appear to follow a benign course without developing invasive tumors or dying of bladder cancer”

# Question... “Carcinoma”?

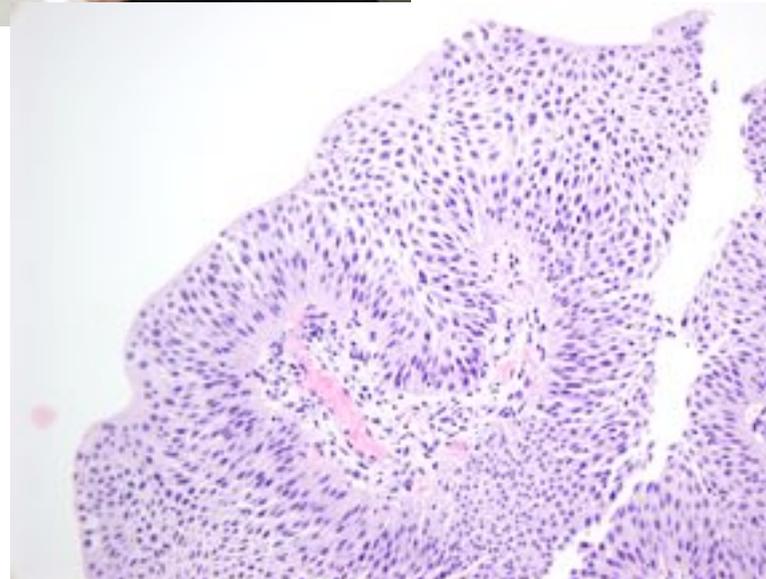
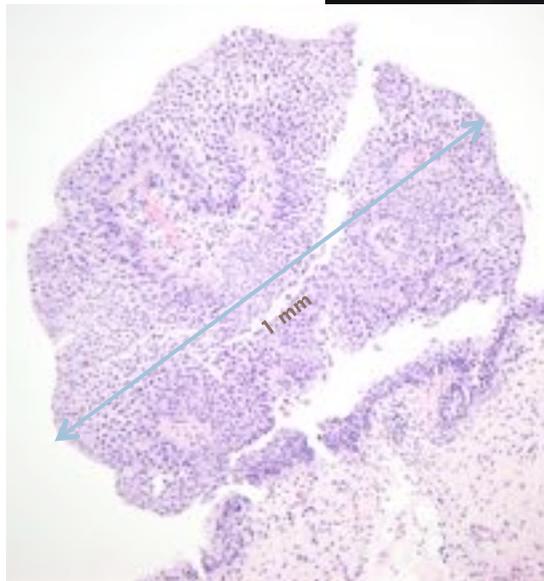


# Question... “Carcinoma”?

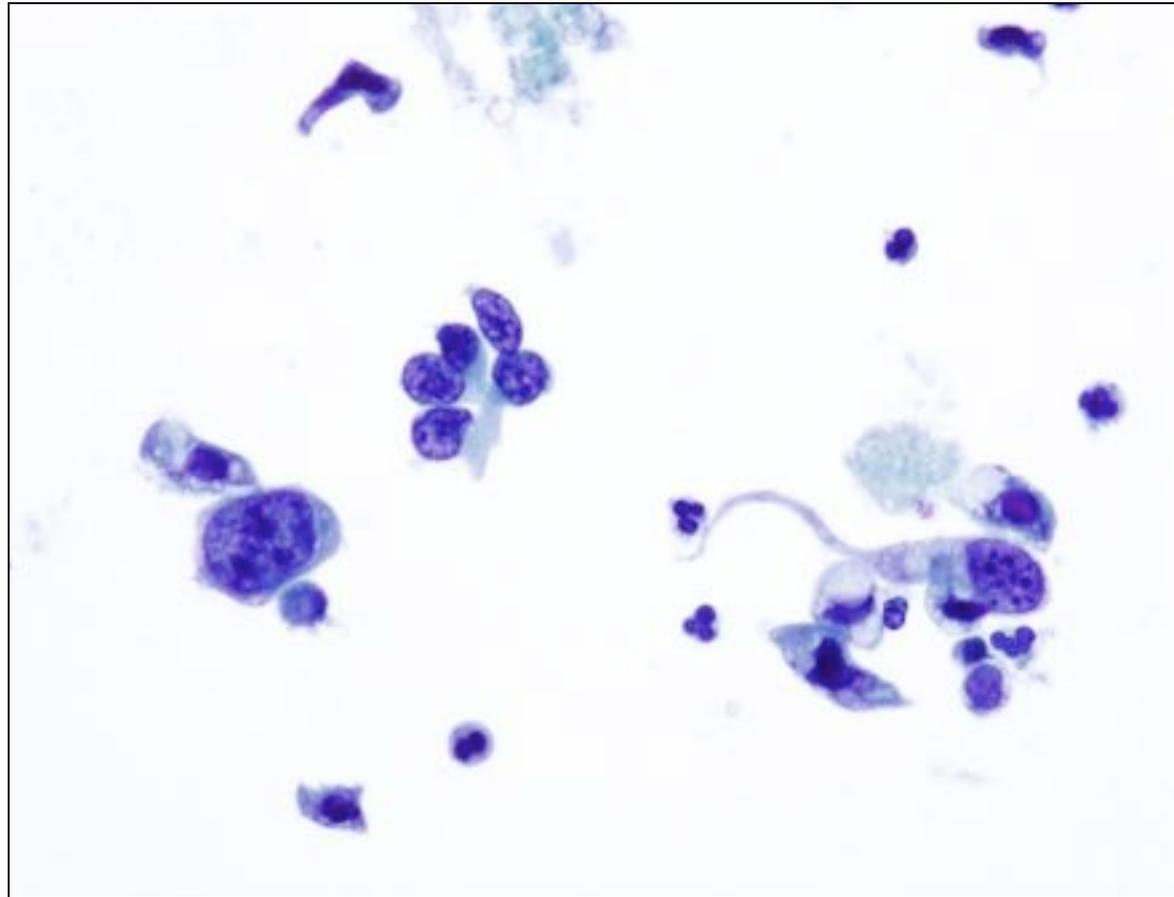


# Hansen

~~Mr. Smith~~ - You have a bladder cancer



# What really matters?



High Grade Urothelial Carcinoma



NaturalNews.com

# Classifications

WHO 1973

Papilloma	Grade I	Grade II	Grade III
Papilloma	PUNLMP	Low Grade	High Grade

WHO/ISUP 2004



URINE CYTOLOGY SENSITIVITY



Very high probability that we are going to be wrong

# Evolution of the Classification

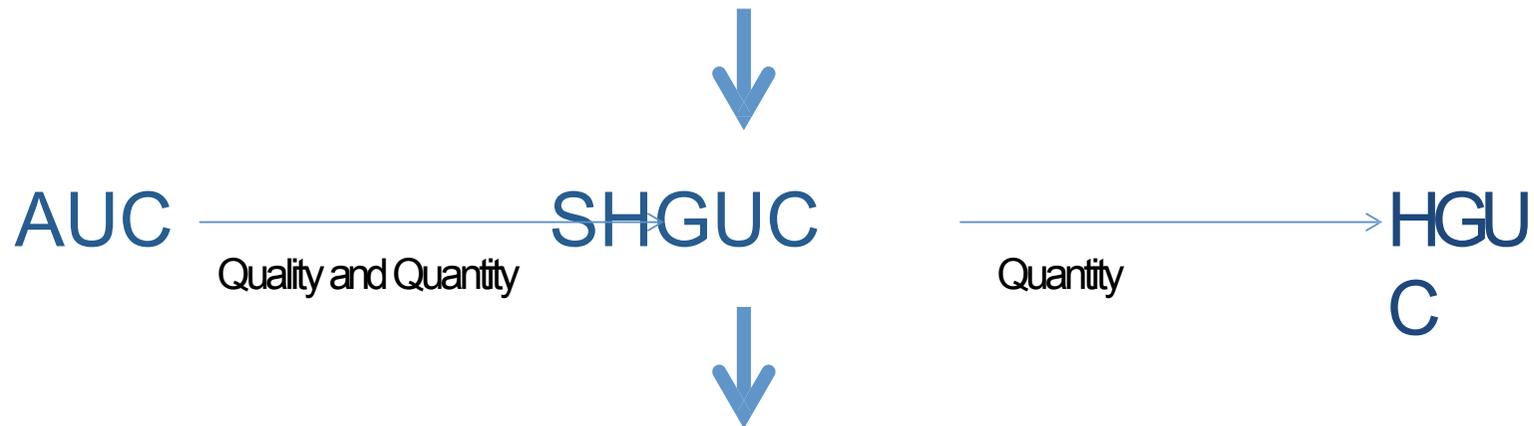
	Cytologic Classification					Histologic Classification		
	Papanicolaou 1947 <sup>5</sup> (Papanicolaou Classification System)	Koss 1985 <sup>10</sup>	Murphy 1984 <sup>11</sup>	Ooms & Veldhuizen 1993 <sup>12</sup>	Layfield et al 2004 <sup>13</sup> (Papanicolaou Society of Cytopathology)	Hopkins Template*	Mostofi & Torloni 1973 <sup>9</sup> (WHO <sup>11</sup> )	Epstein 1998 <sup>14</sup> (WHO/ISUP)
I								
II	Benign cells, ATY 1 cells, few clusters		Negative	Negative	Negative	NUAM	Papilloma TCC, grade 1	Papilloma PUNLMP LGUC
III	Clusters, nuclear elongation, few ATY 2 cells	Dysplastic cells	Atypical, significance uncertain	Atypical urothelial cells	AUC-US		TCC, grade 2	
IV		Suspicious	Suspicious		AUC-H			
V	Malignant tumor cells, many ATY 2 cells	Malignant cells	Neoplastic cells present	Urothelial carcinoma	Urothelial carcinoma			HGUC
							TCC, grade 3	



Abbreviations: ATY 1, atypical cells with hyperchromasia and predominantly round or oval contours; ATY 2, cells with hyperchromasia and nuclear membrane abnormalities; AUC-H, atypical urothelial cells cannot exclude high-grade urothelial carcinoma; AUC-US, atypical urothelial cells of uncertain significance; HGUC, high-grade papillary urothelial carcinoma; ISUP, International Society of Urological Pathology; LGUC, low-grade papillary urothelial carcinoma; NUAM, no urothelial atypia or dysplasia identified; PUNLMP, papillary urothelial malignancy of uncertain malignant potential; TCC, transitional cell carcinoma; WHO, World Health Organization. See Table 7.

# NEW paradigm

- It is all about High Grade Urothelial Carcinoma
- Negative for High Grade Urothelial Carcinoma



- LGUN – Low Grade Urothelial Neoplasm

# But first adequacy



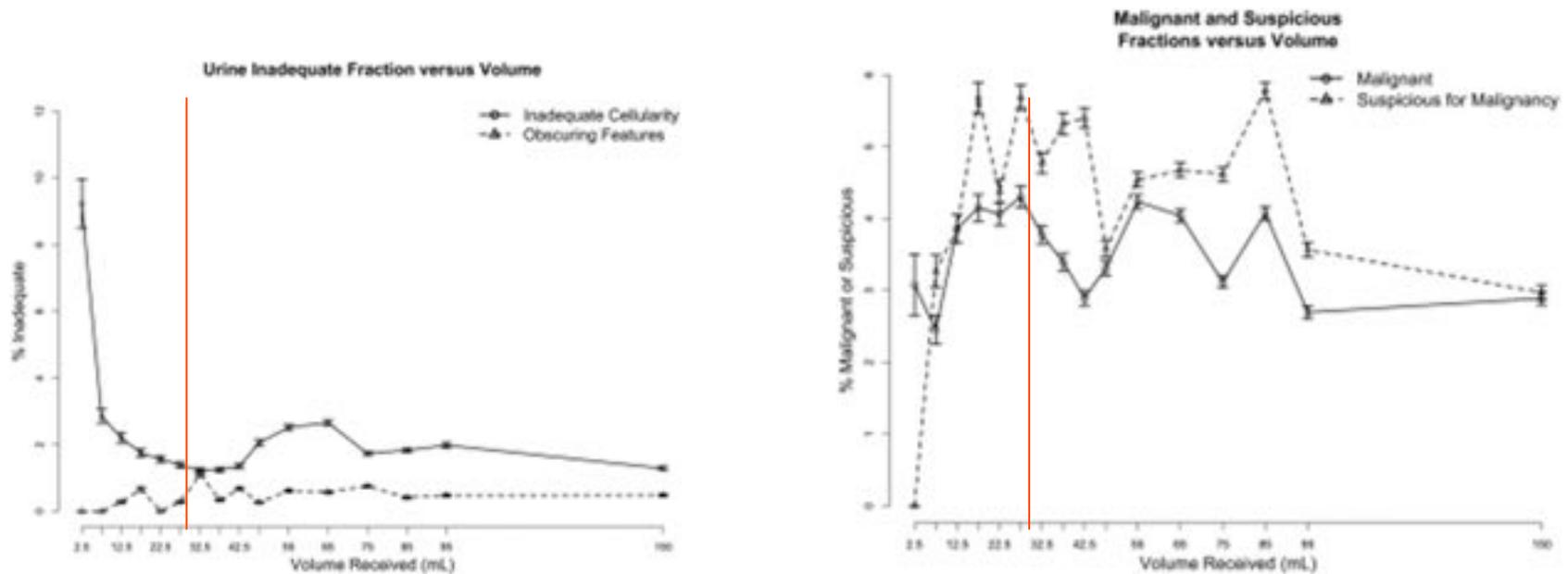
- Surprisingly little data...
  - ▣ Prather J, Arville B, Chatt G, et al. Evidence-based adequacy criteria for urinary bladder barbotage cytology. *Journal of the American Society of Cytopathology*.4: 57-62.
  - ▣ VandenBussche CJ, Rosenthal DL, Olson MT. Adequacy in voided urine cytology specimens: The role of volume and a repeat void upon predictive values for high-grade urothelial carcinoma. *Cancer Cytopathol*. 2015.

# Prather J, Arville B, Chatt G, et al.

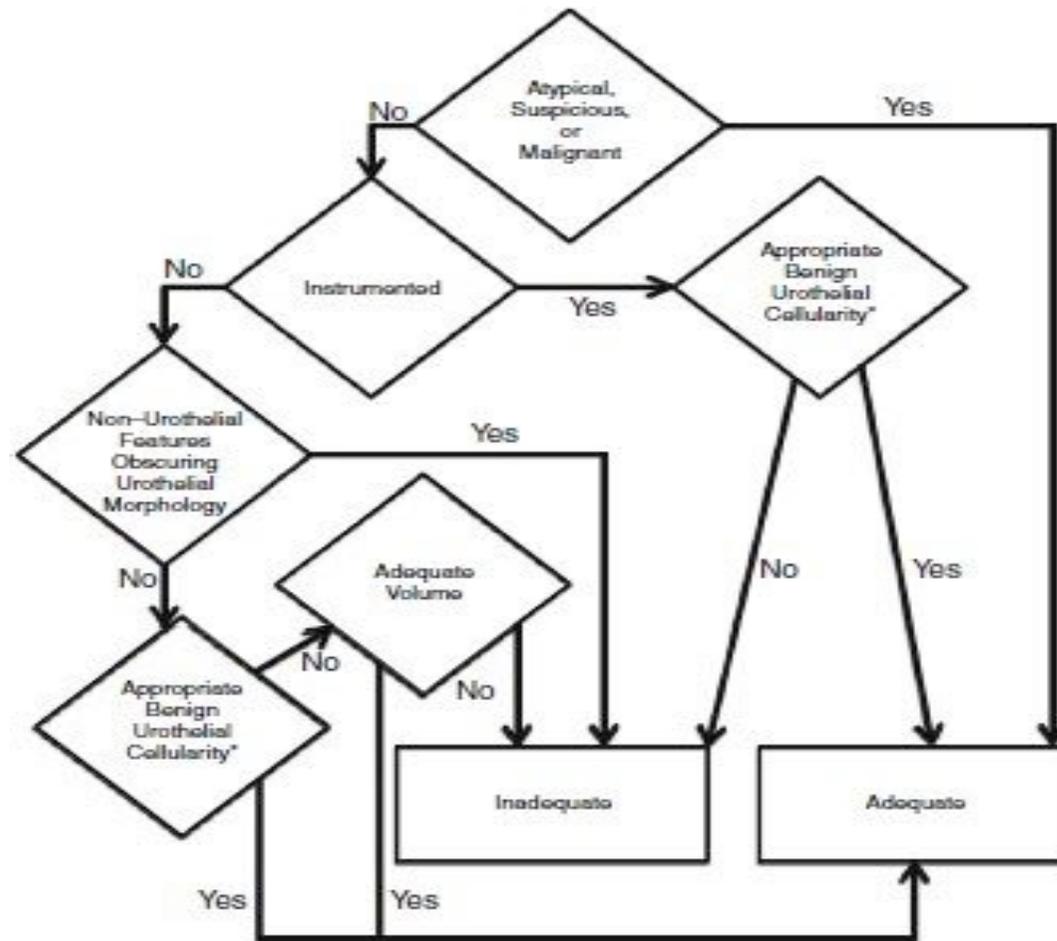
Table 1. Prospective study.

Cellularity	Sensitivities	
	AUC+	HGUC
<10 per 10 hpfs	60.5	37.2
≥10 per 10 hpfs	95.2	76.2
<i>P</i> value	0.0001	0.0004
<20 per 10 hpfs	68.3	43.3
≥20 per 10 hpfs	100.0	88.0
<i>P</i> value	0.001	0.0001

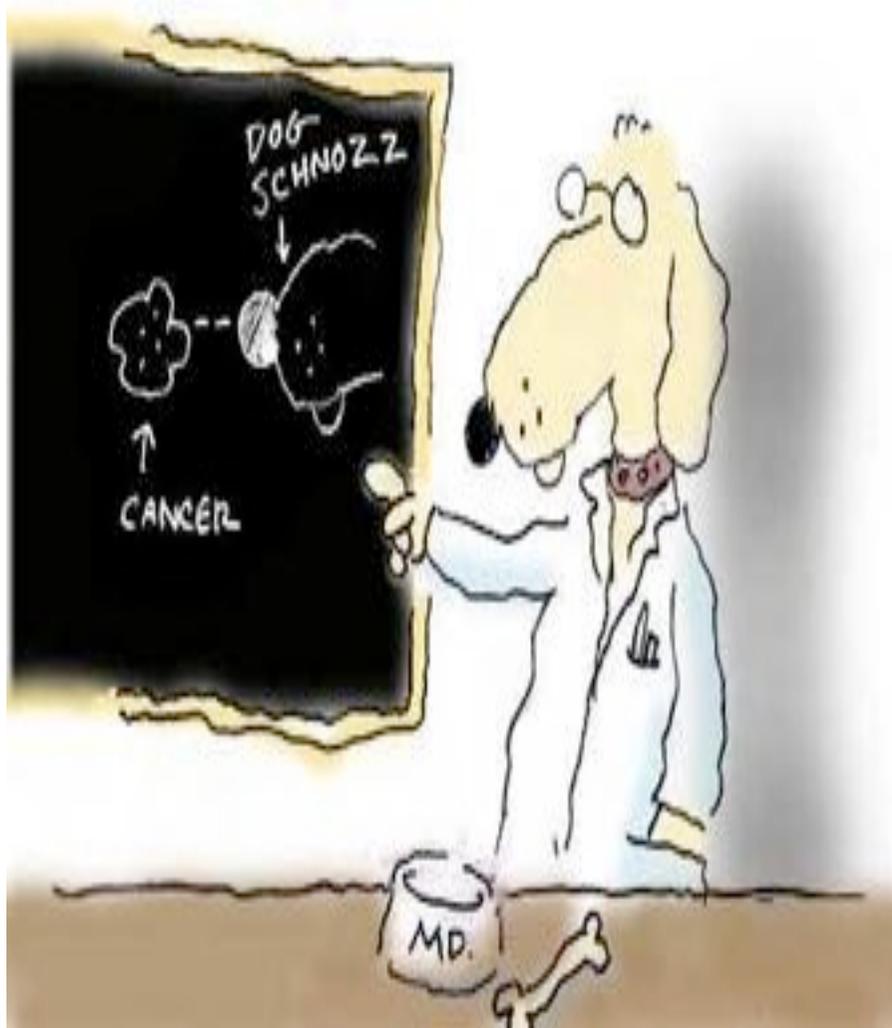
# Volume is important...



# Adequacy of Urine Specimens (Adequacy)



# Lets take a break!

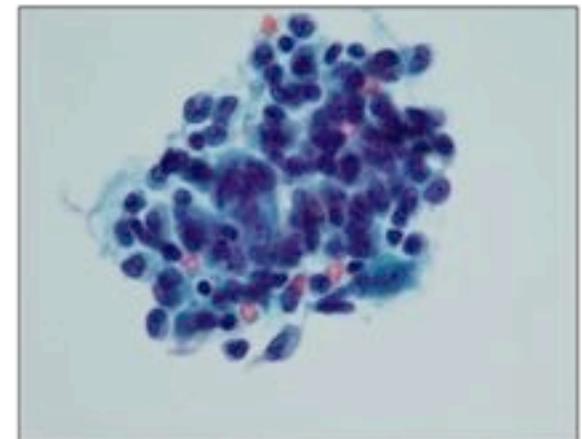
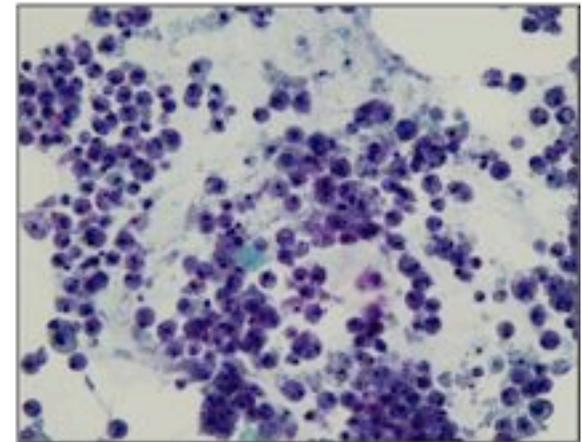
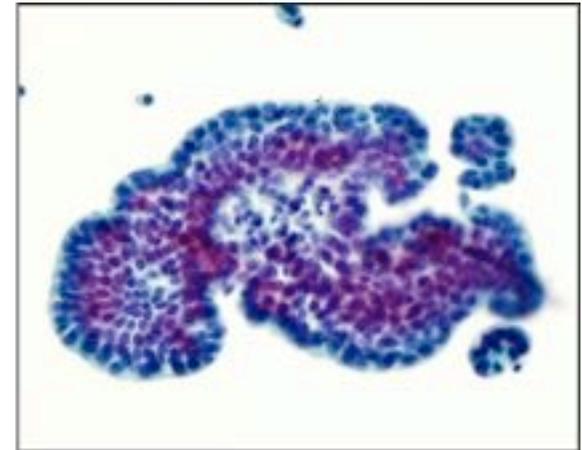
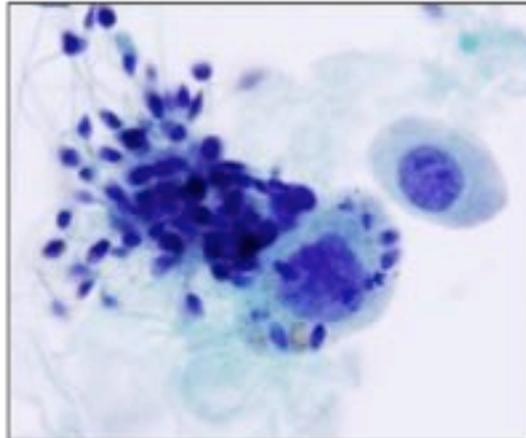
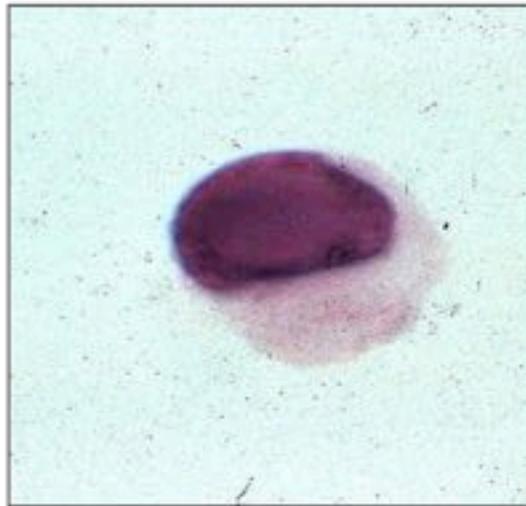
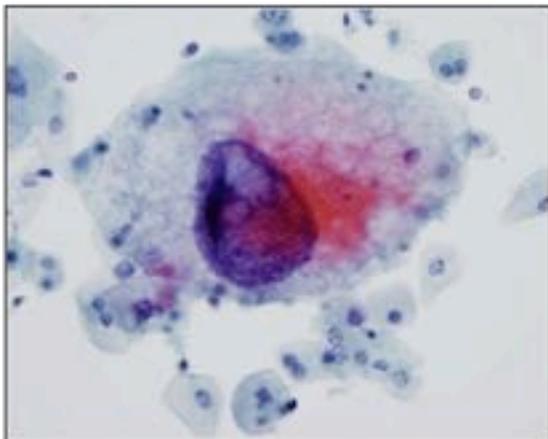


# Diagnostic categories



1. Negative for High Grade Urothelial Carcinoma
2. Atypical Urothelial Cells
3. Suspicious for High Grade Urothelial Carcinoma
4. High Grade Urothelial Carcinoma
5. Low Grade Urothelial Neoplasm
6. Other malignancies, both primary and secondary

# “Negative, NOT atypia”



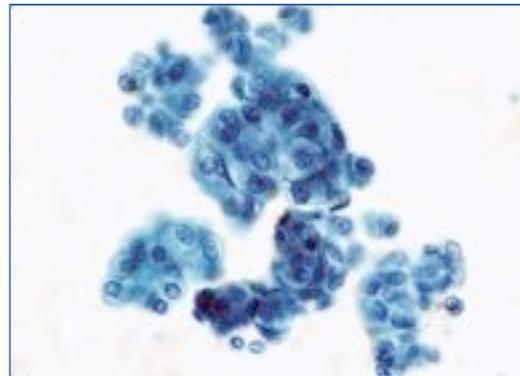
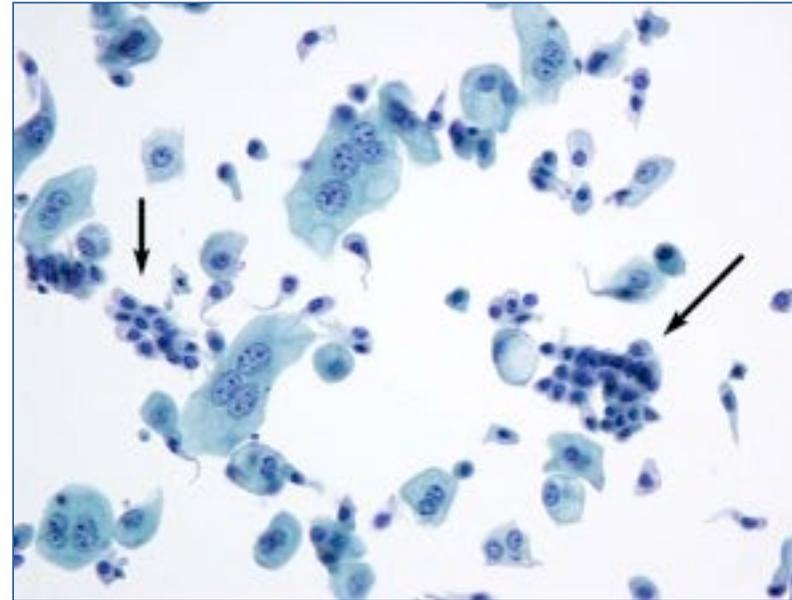
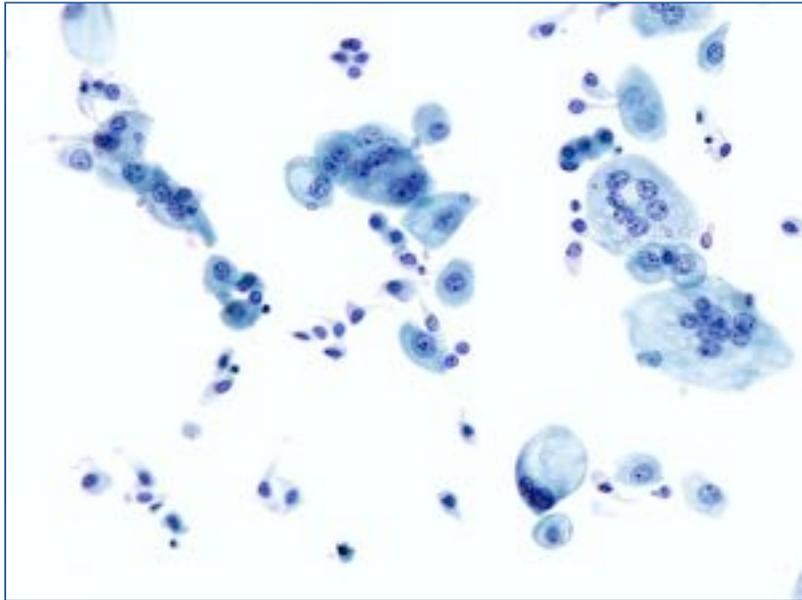
Wojcik EM: What should not be reported as atypia  
in urine cytology: JASC 2015;4;3;30-36

# Negative for High-Grade Urothelial Carcinoma (Negative)

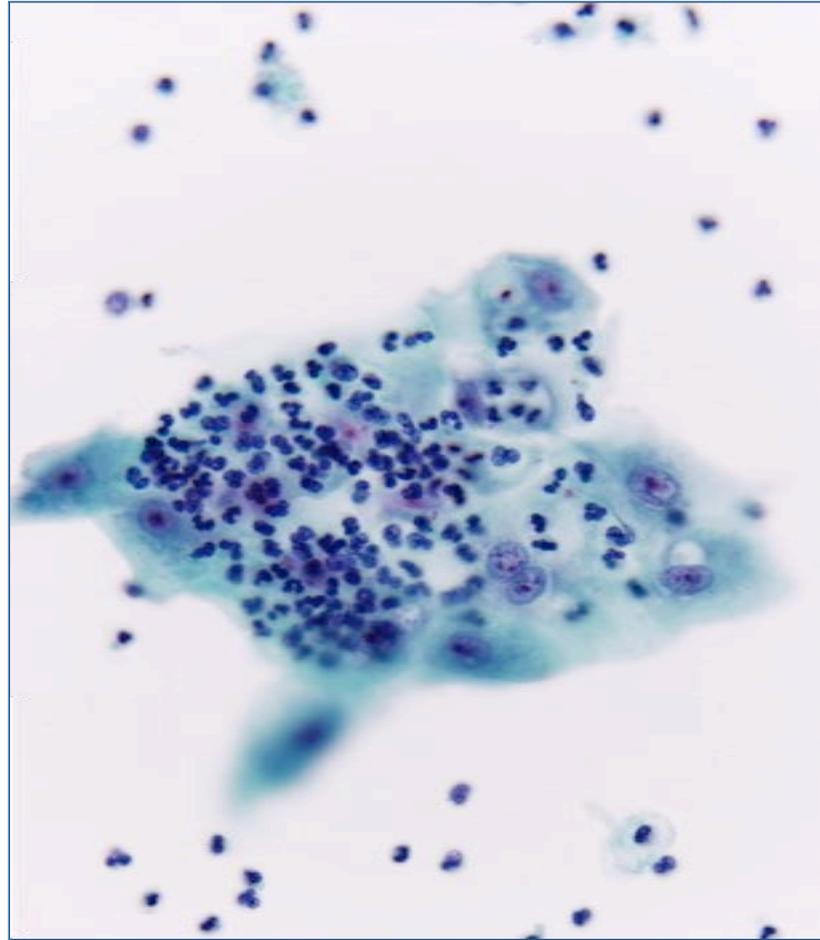
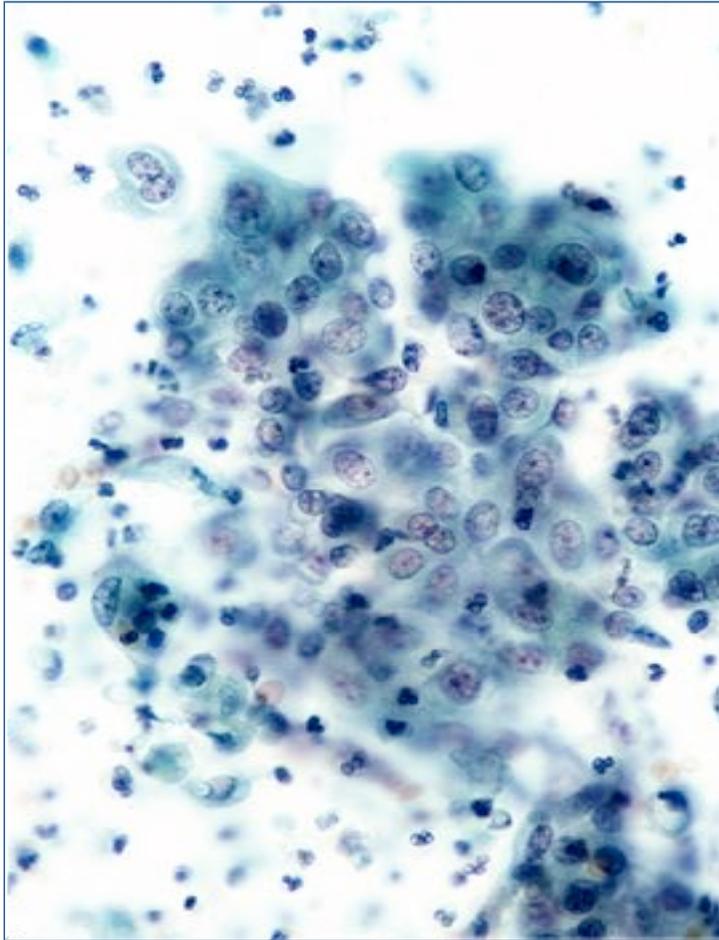
## **Definition of Negative for High-Grade Urothelial Carcinoma**

- A sample of urine, either voided or instrumented, may be considered benign, i.e., NHGUC, if any of the following components are present in the specimen:
  - Benign urothelial, glandular, and squamous cells
  - Benign urothelial tissue fragments (BUTF) and urothelial sheets or clusters
  - Changes associated with lithiasis
  - Viral cytopathic effect; polyoma virus (BK virus—decoy cells)
  - Post-therapy effect, including epithelial cells from urinary diversions

# Benign Superficial (Umbrella) Urothelial Cells

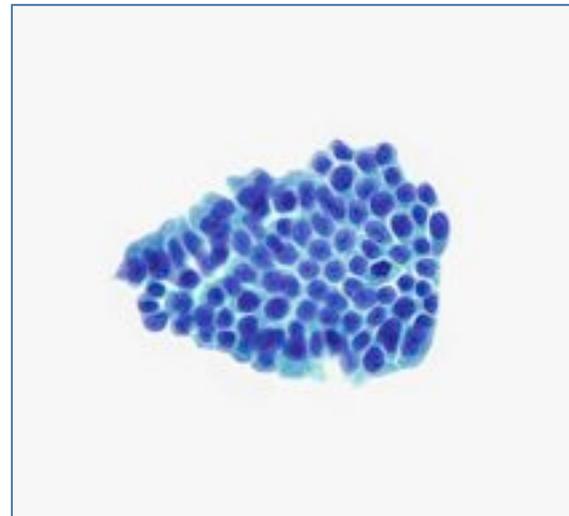
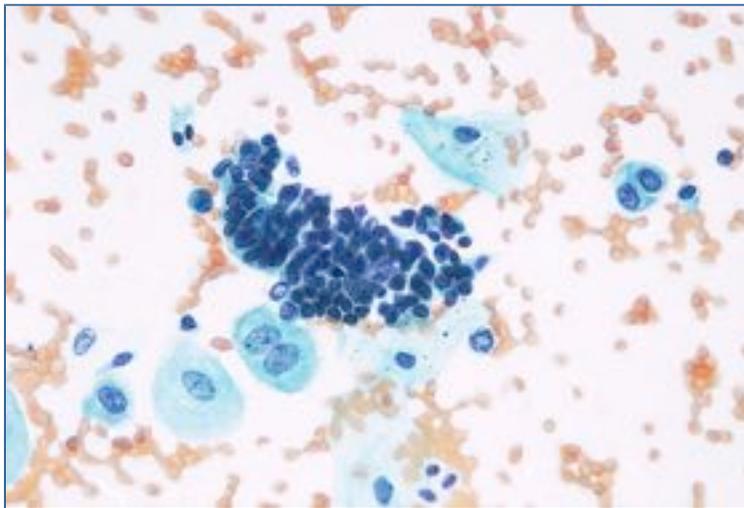


# “Atypical” Umbrella Cells

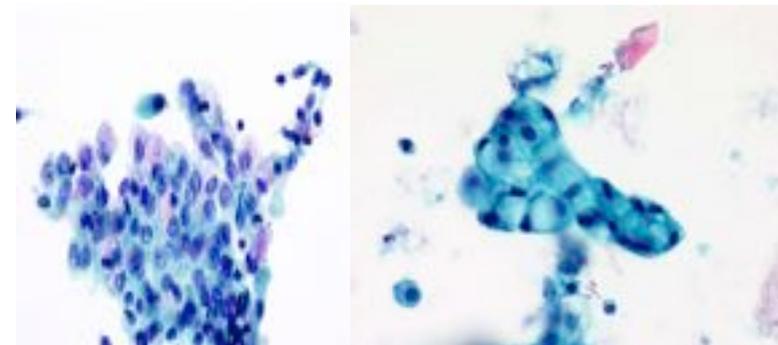
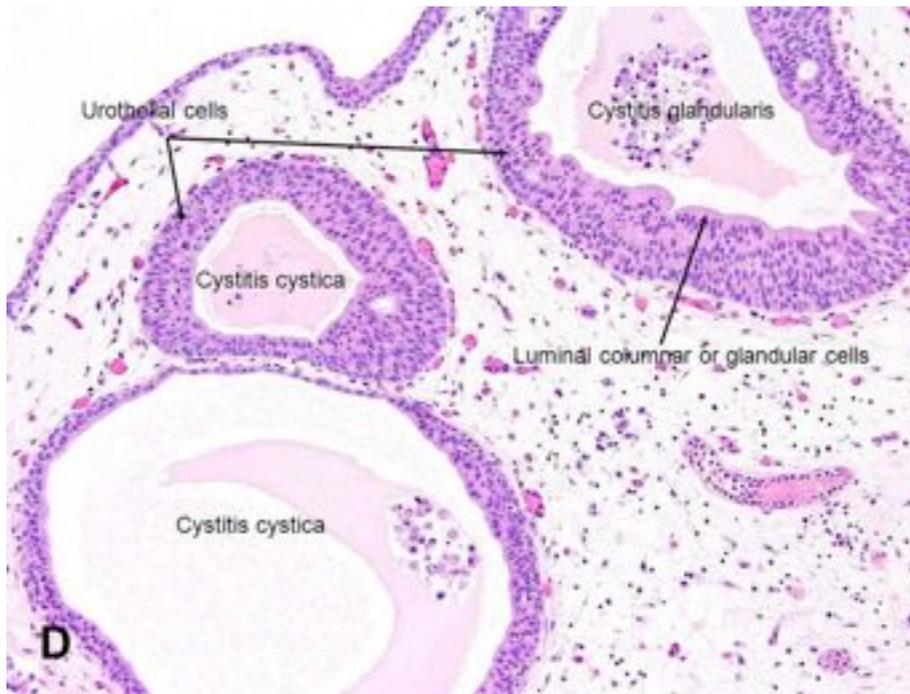


# Glandular Cells

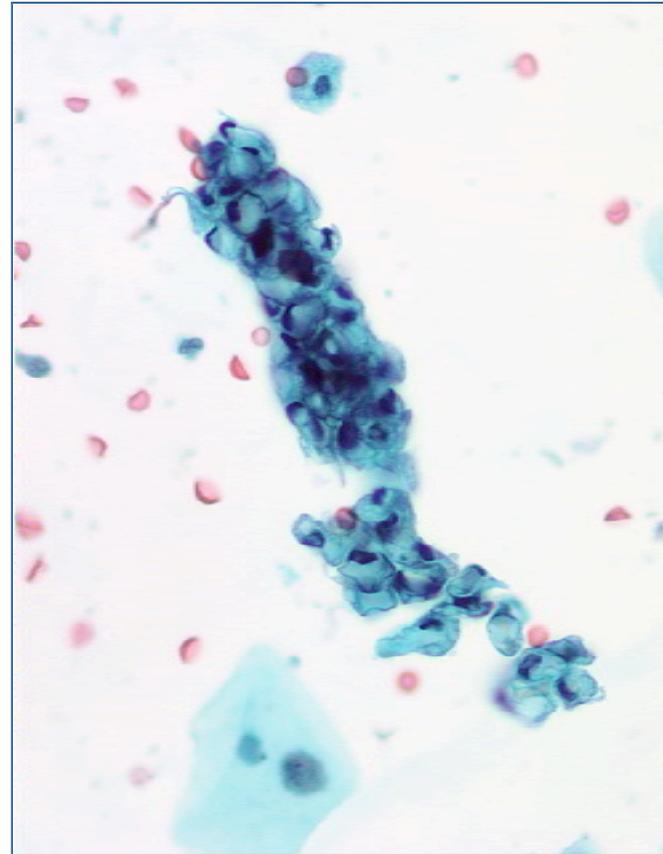
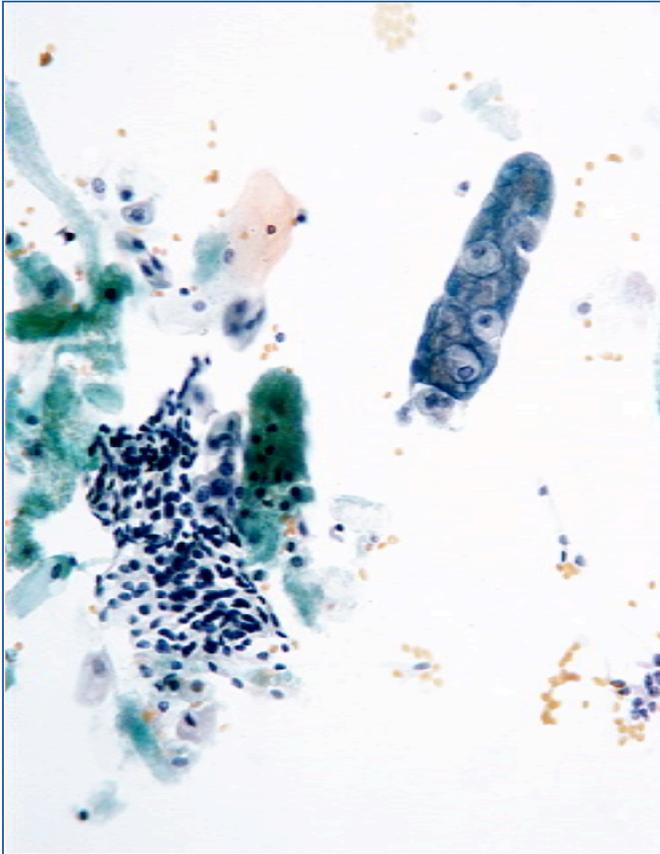
- Sources: endometrium, prostate, kidneys, urachal remnants, metaplasia



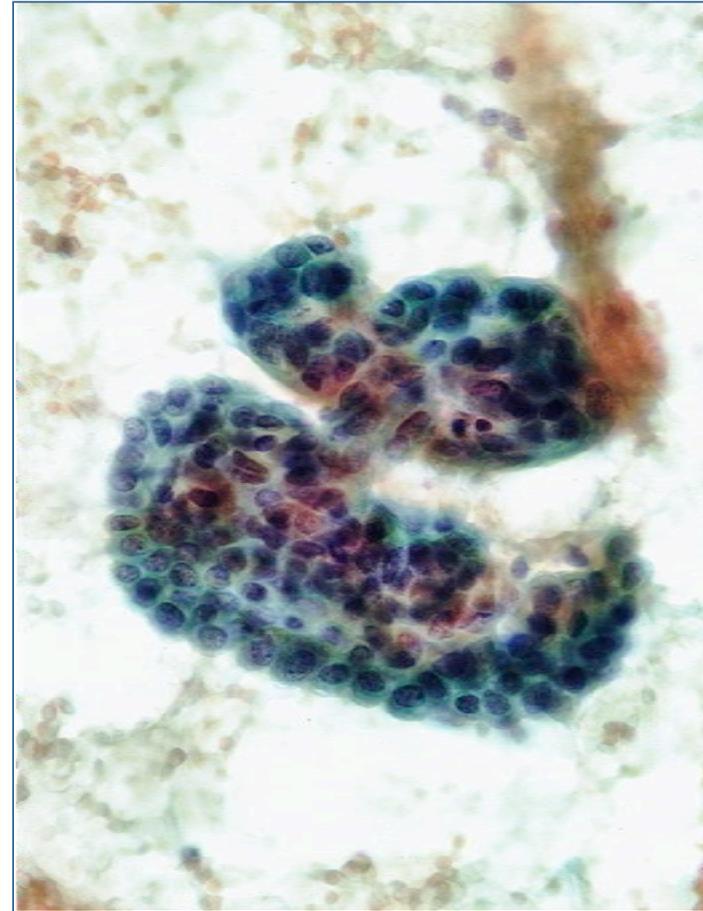
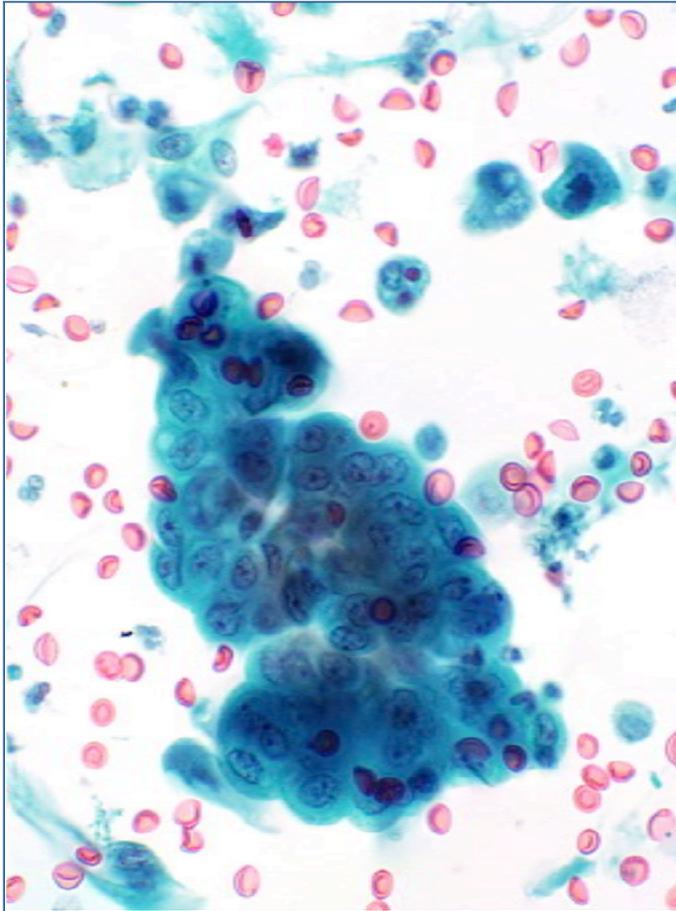
# Cystitis cystica/glandularis



# Renal Tubular Epithelial Cells

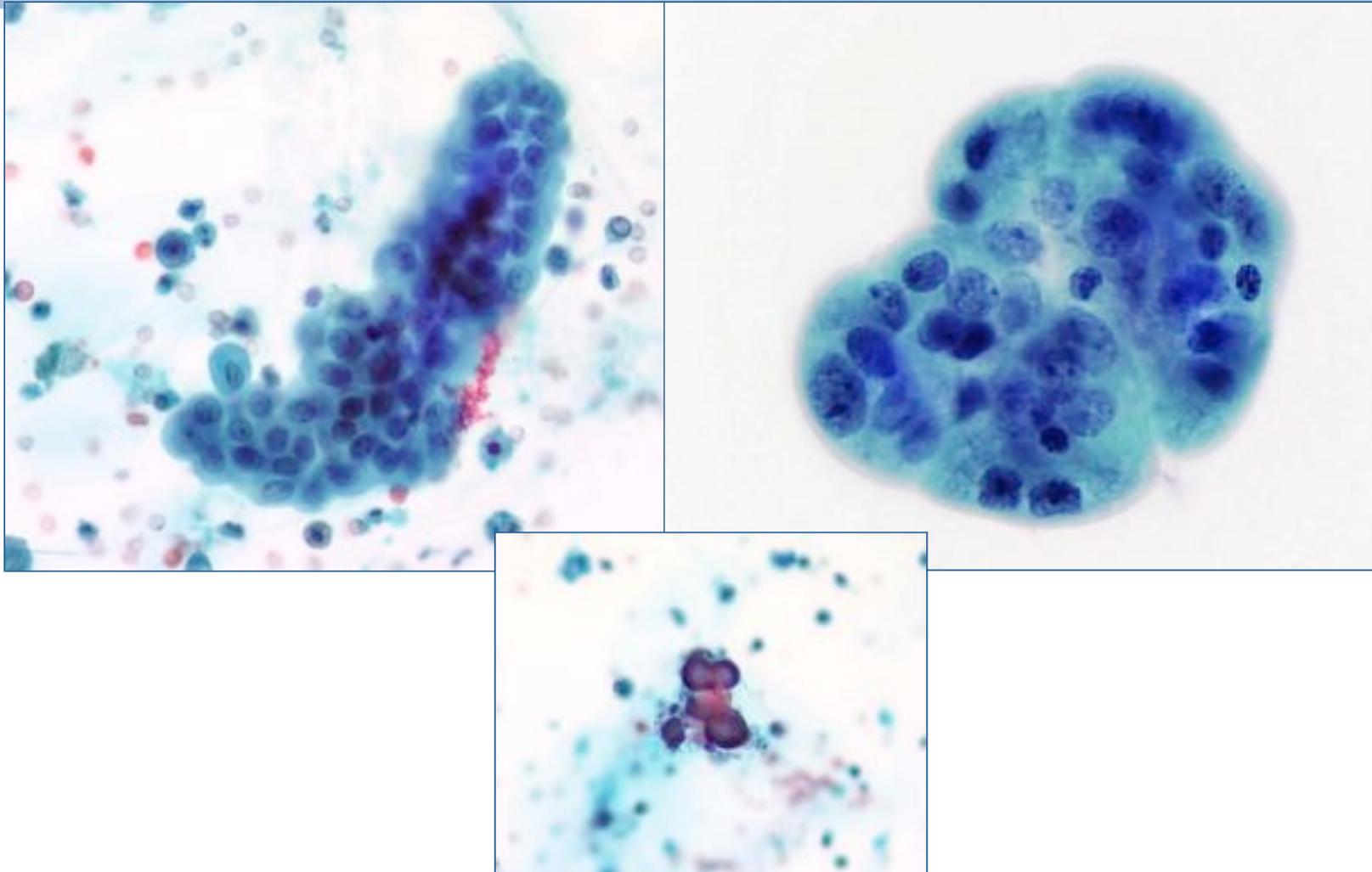


# Benign Urothelial Tissue Fragments - BUTF

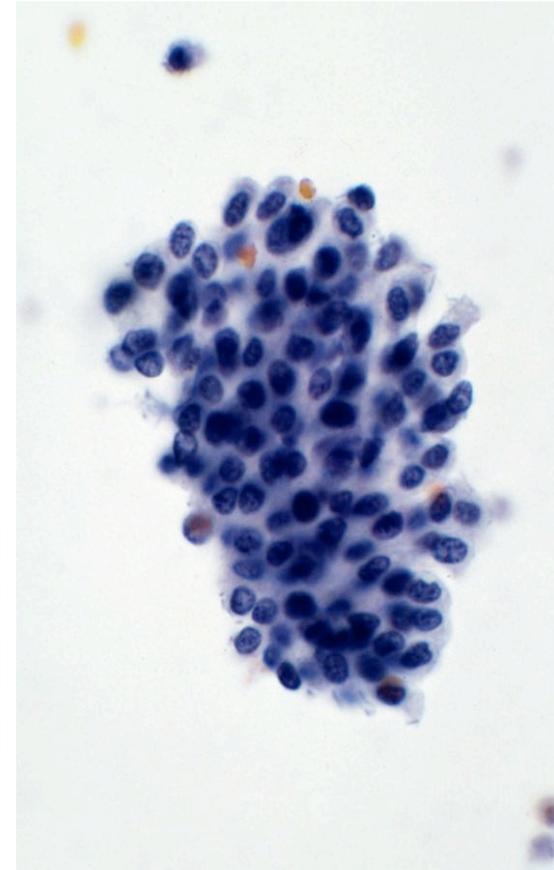
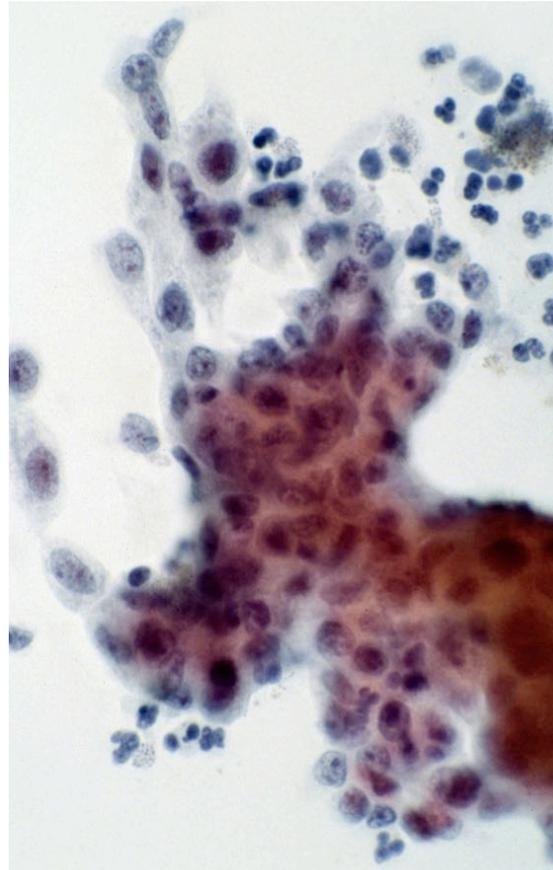


Onur, I., Rosenthal, D. L., & VandenBussche, C. J. (2015). Benign-appearing urothelial tissue fragments in noninstrumented voided urine specimens are associated with low rates of urothelial neoplasia. *Cancer cytopathology*, 123(3), 180-185.

# Nephrolithiasis – 3D fragments

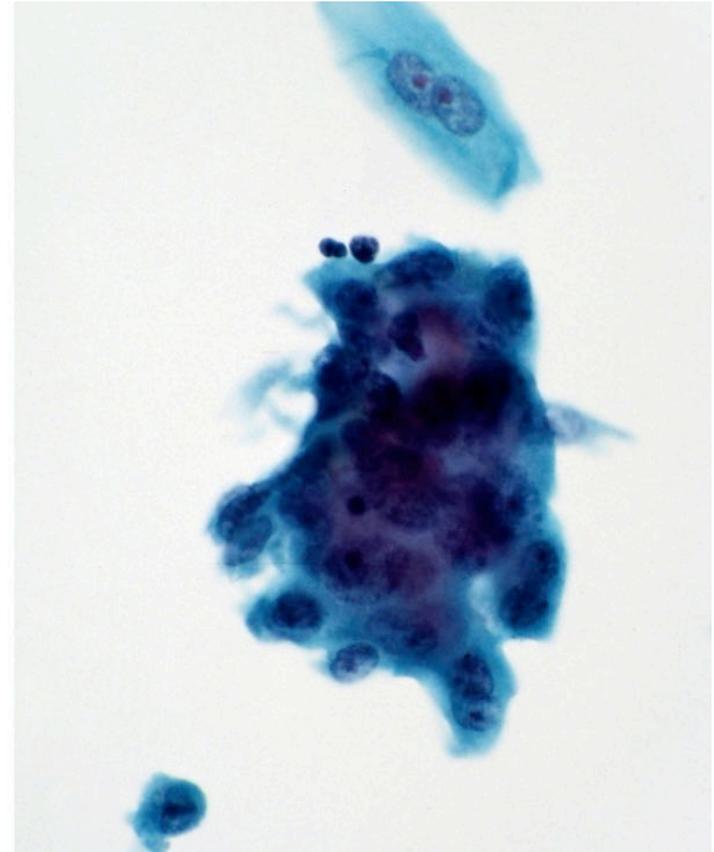


# Stone Atypia

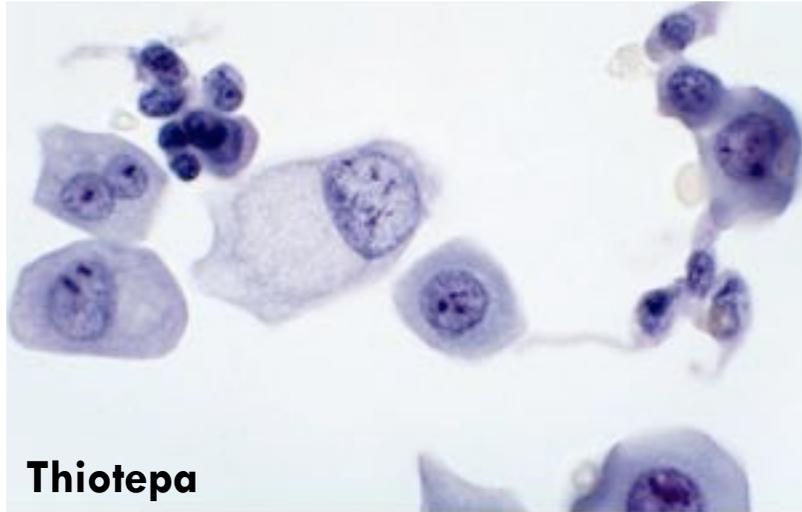


# Systemic Chemotherapy Changes

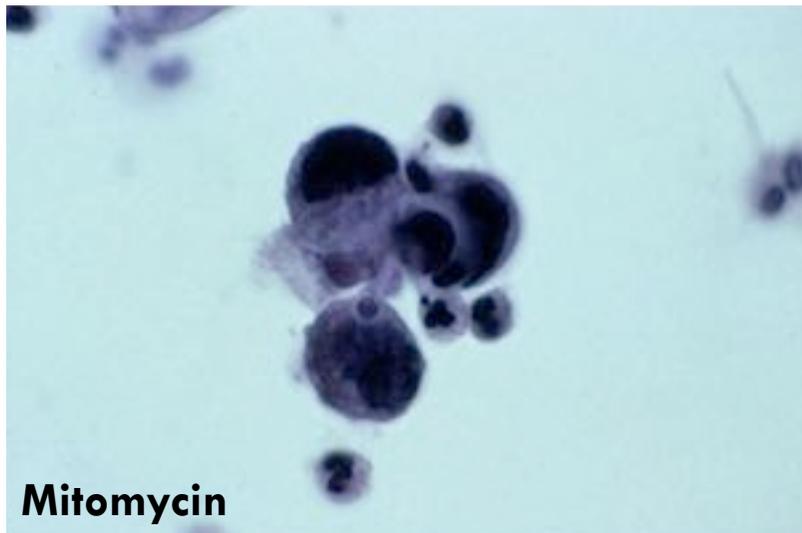
- ❑ Degenerative changes with frayed cell borders
- ❑ Enlarged hyperchromatic but smudgy nuclei
- ❑ Vacuolated cytoplasm
- ❑ Irregular dark nucleoli
- ❑ Multinucleation



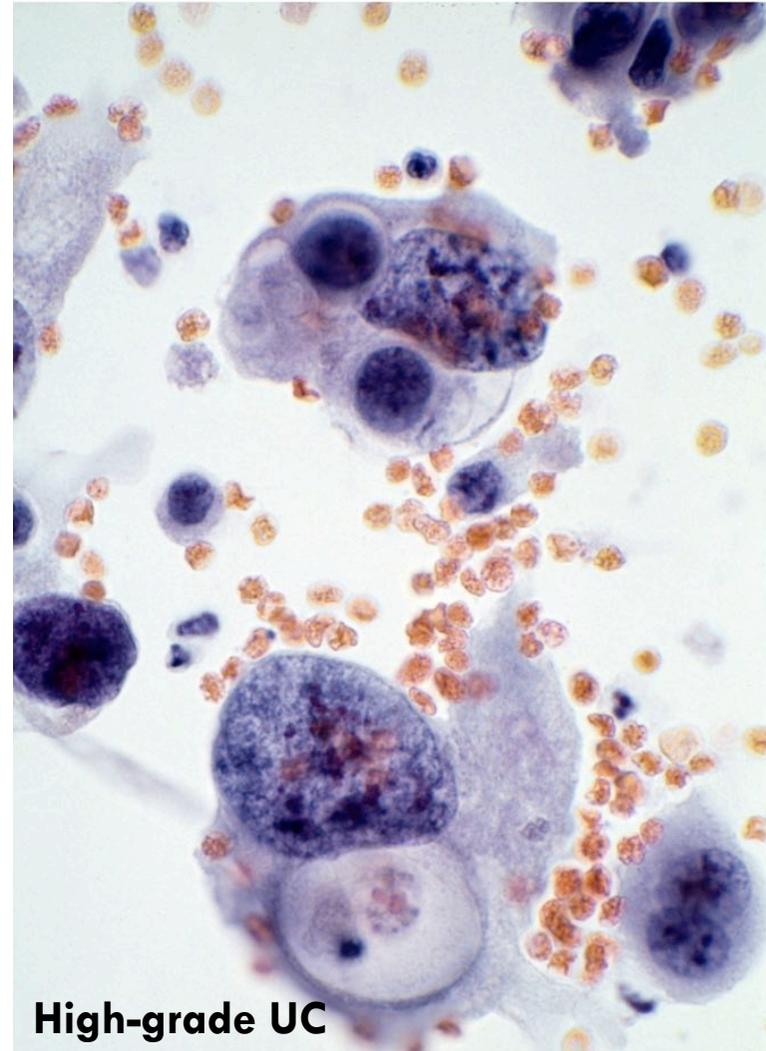
# Chemotherapy Changes



**Thiotepa**



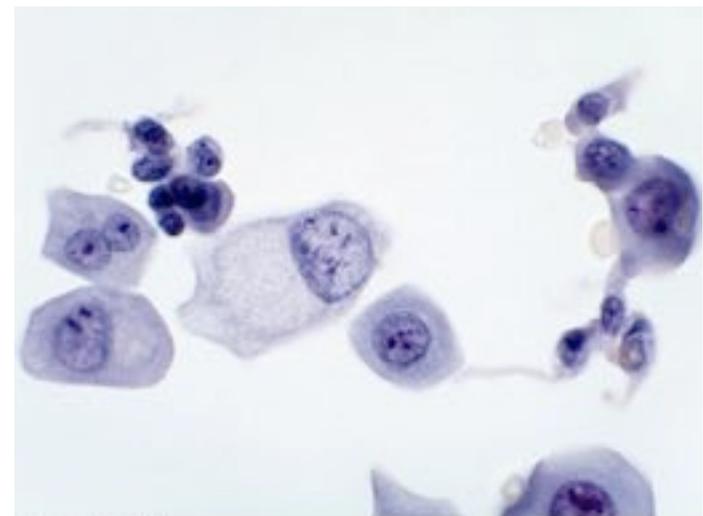
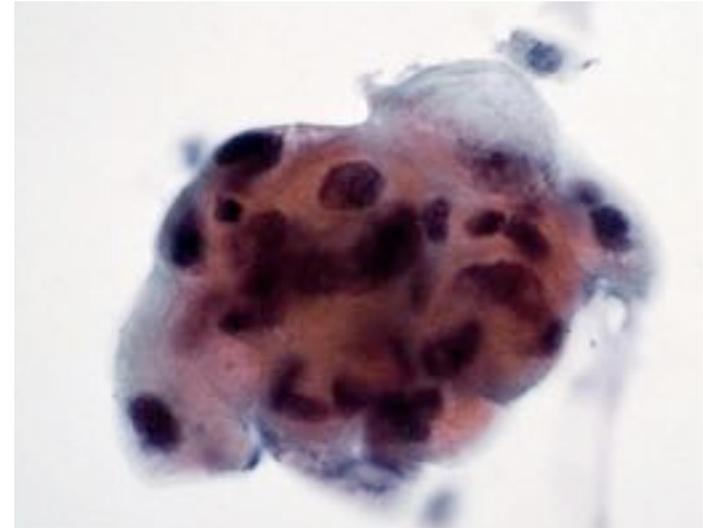
**Mitomycin**



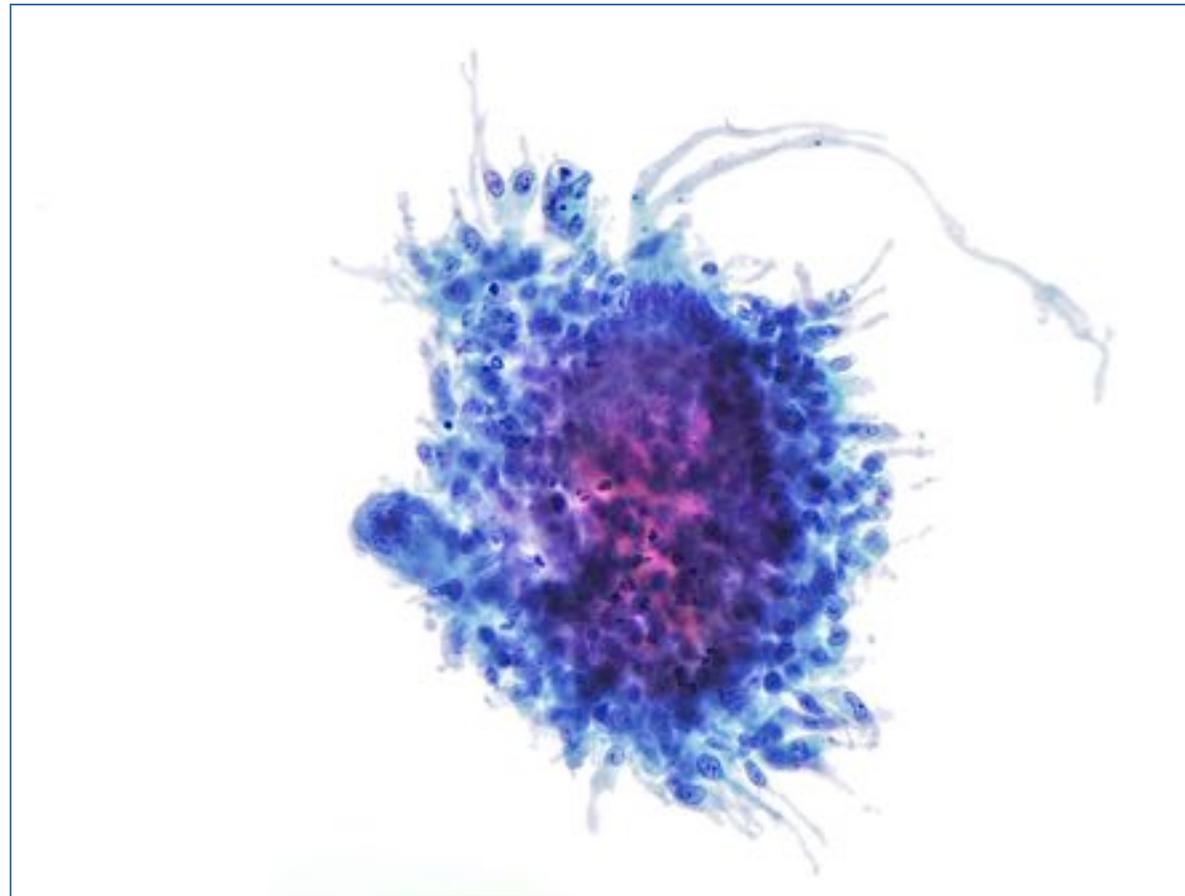
**High-grade UC**

# Intravesical Chemotherapy Changes

- ❑ Predominantly effect the superficial cells
- ❑ Marked cytomegaly with abundant vacuolated cytoplasm and one or more nuclei
- ❑ Nuclear chromatin chunky, clumped, deeply staining or structureless and smudgy with smooth borders
- ❑ Prominent nucleoli
- ❑ Frayed borders
- ❑ No significant effect on neoplastic cells



# Immunotherapy

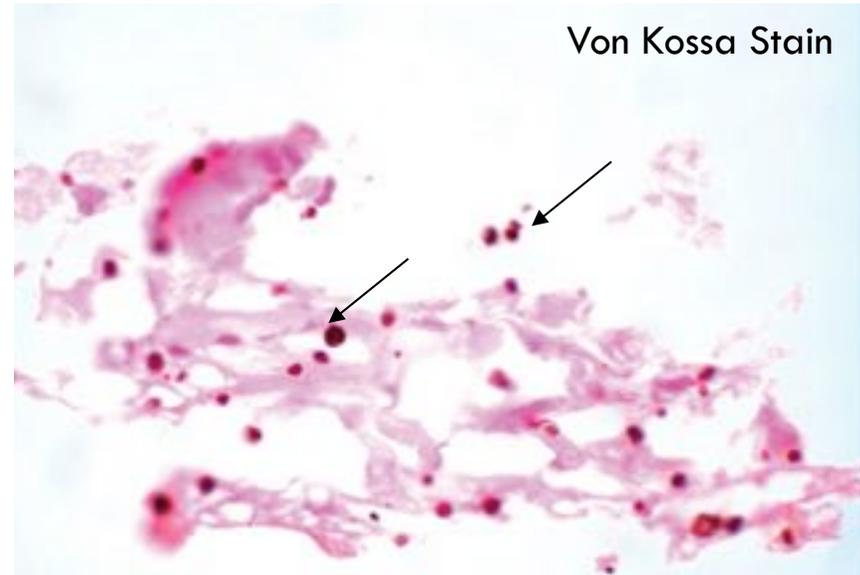
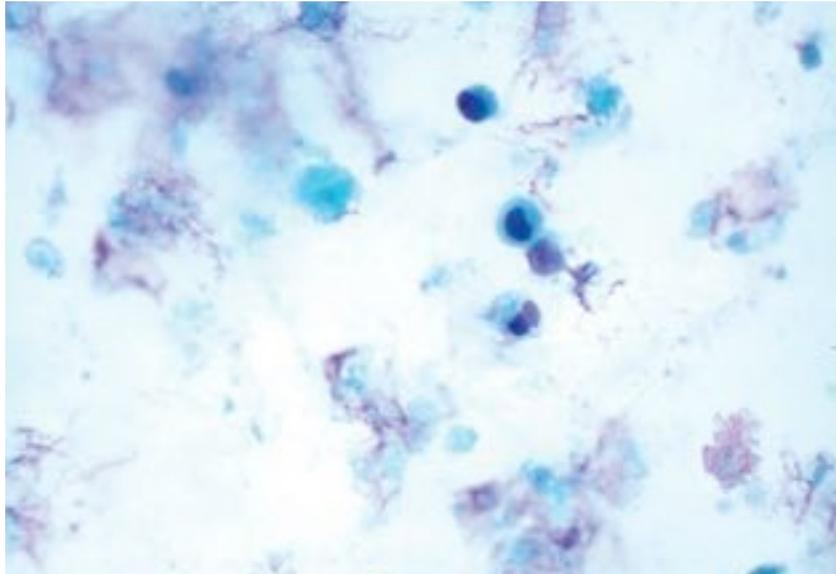


# Radiation Changes

- Cytomegaly with binucleation or multinucleation
- Enlarged nuclei without significant increase in N/C ratio
- Smudgy chromatin
- Nucleoli
- Cytoplasmic polychromasia and vacuolation



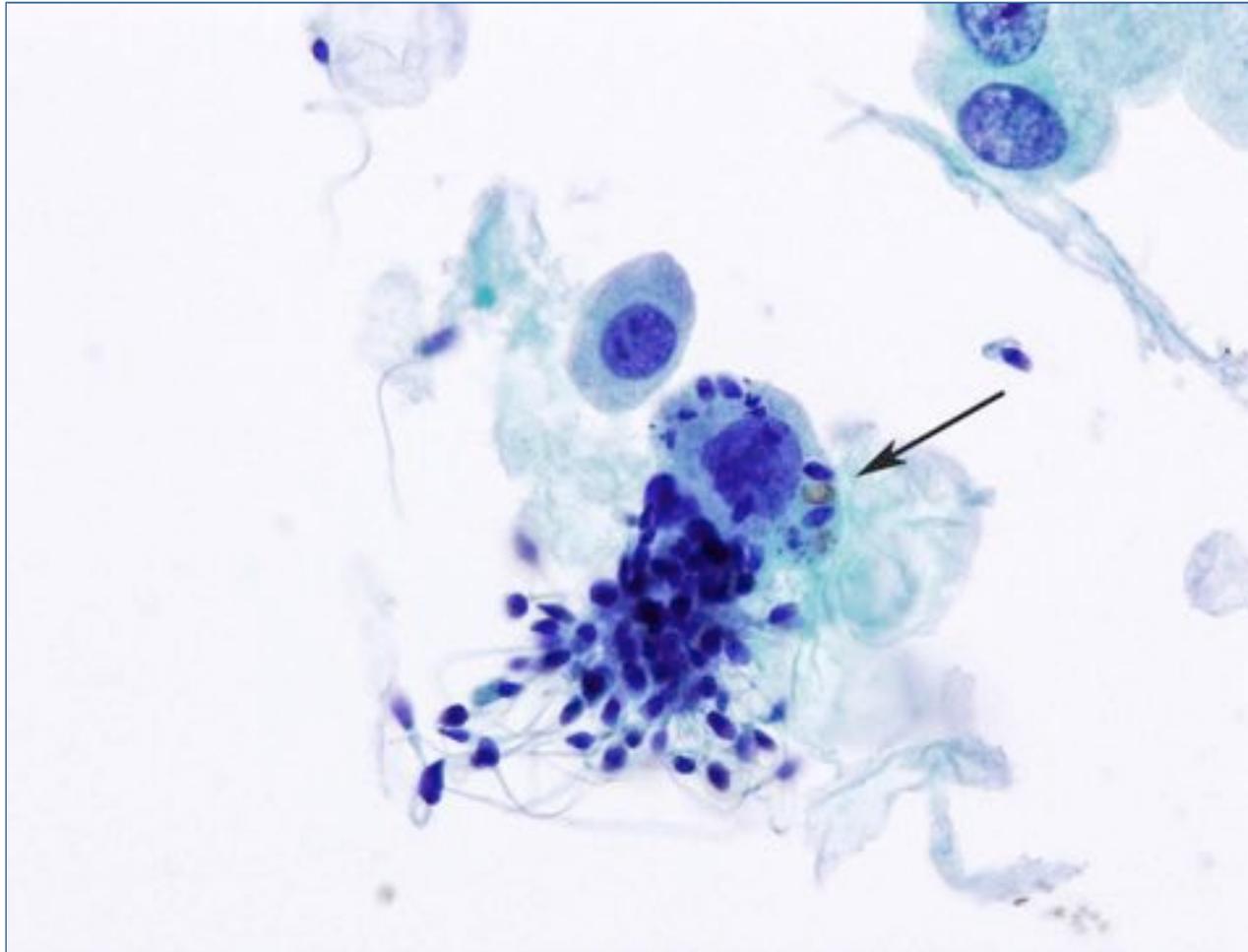
# Malakoplakia



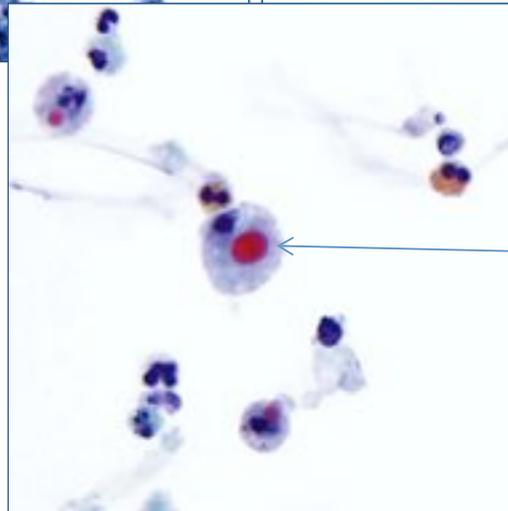
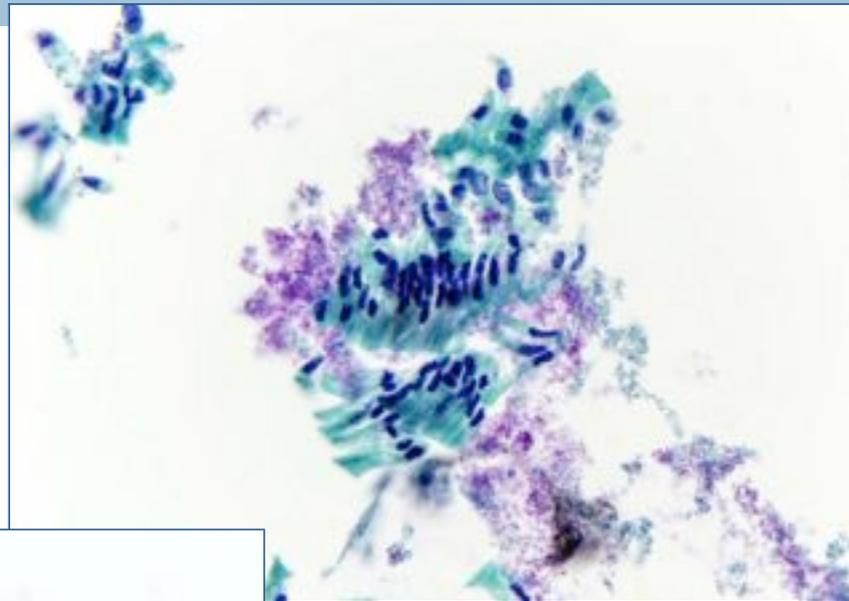
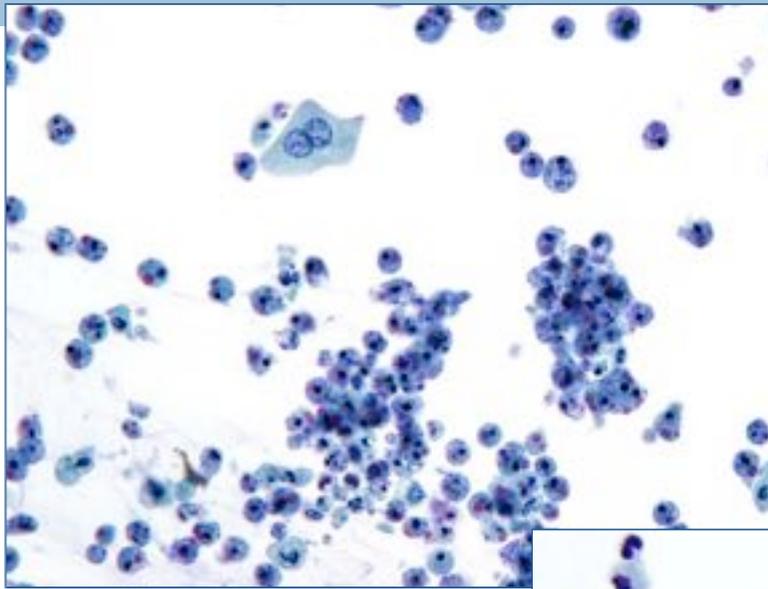
## Malakoplakia with Michealis Gutmann Bodies

Malakoplakia: Histiocytes with abundant granular cytoplasm filled with bacteria and bacterial fragments

# Seminal Vesicle Cells



# Bladder Diversion Urine



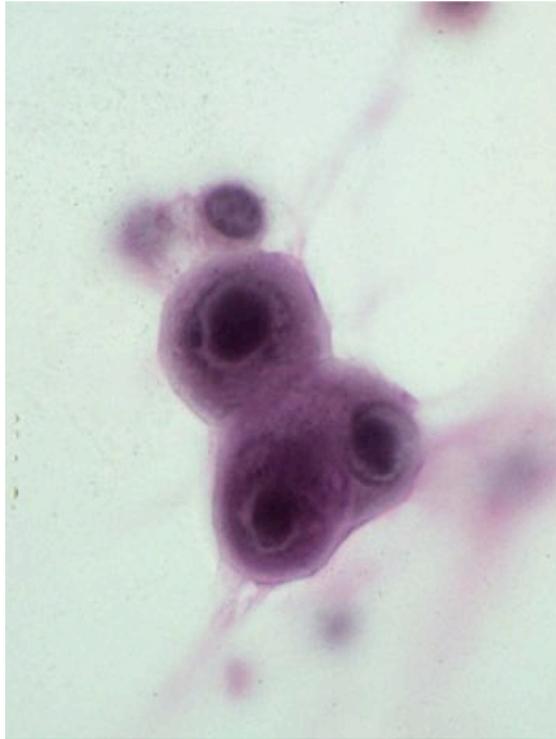
Melamed – Wolinska body

# Infections -Viral

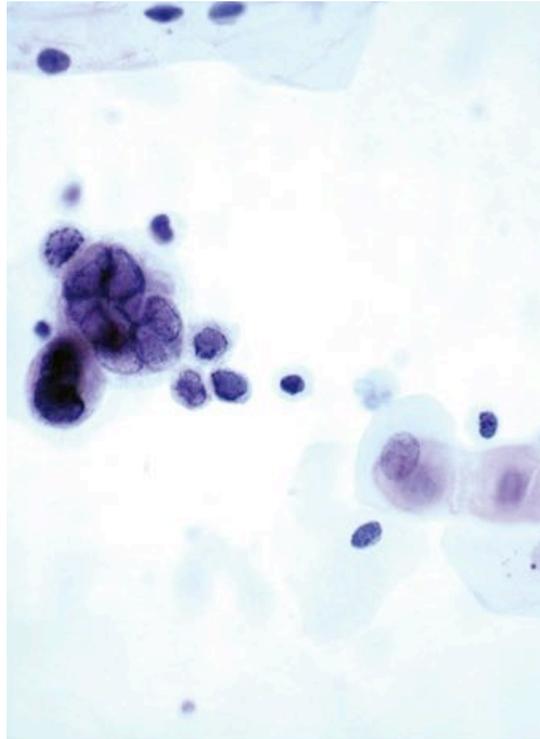


- ❑ Polyomavirus
  - ❑ Infects both healthy and immunocompromised individuals
  - ❑ 4% of urine specimens
  - ❑ No clinical significance in immunocompetent
- ❑ Herpes: Uncommon, immunocompromised patients
- ❑ CMV: Most commonly effects renal tubular cells
- ❑ HPV: Vaginal contamination

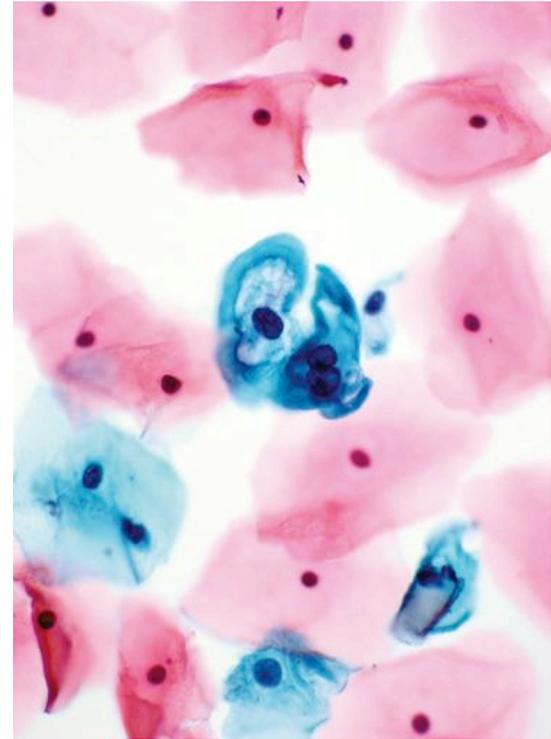
# Infections - Viral



CMV

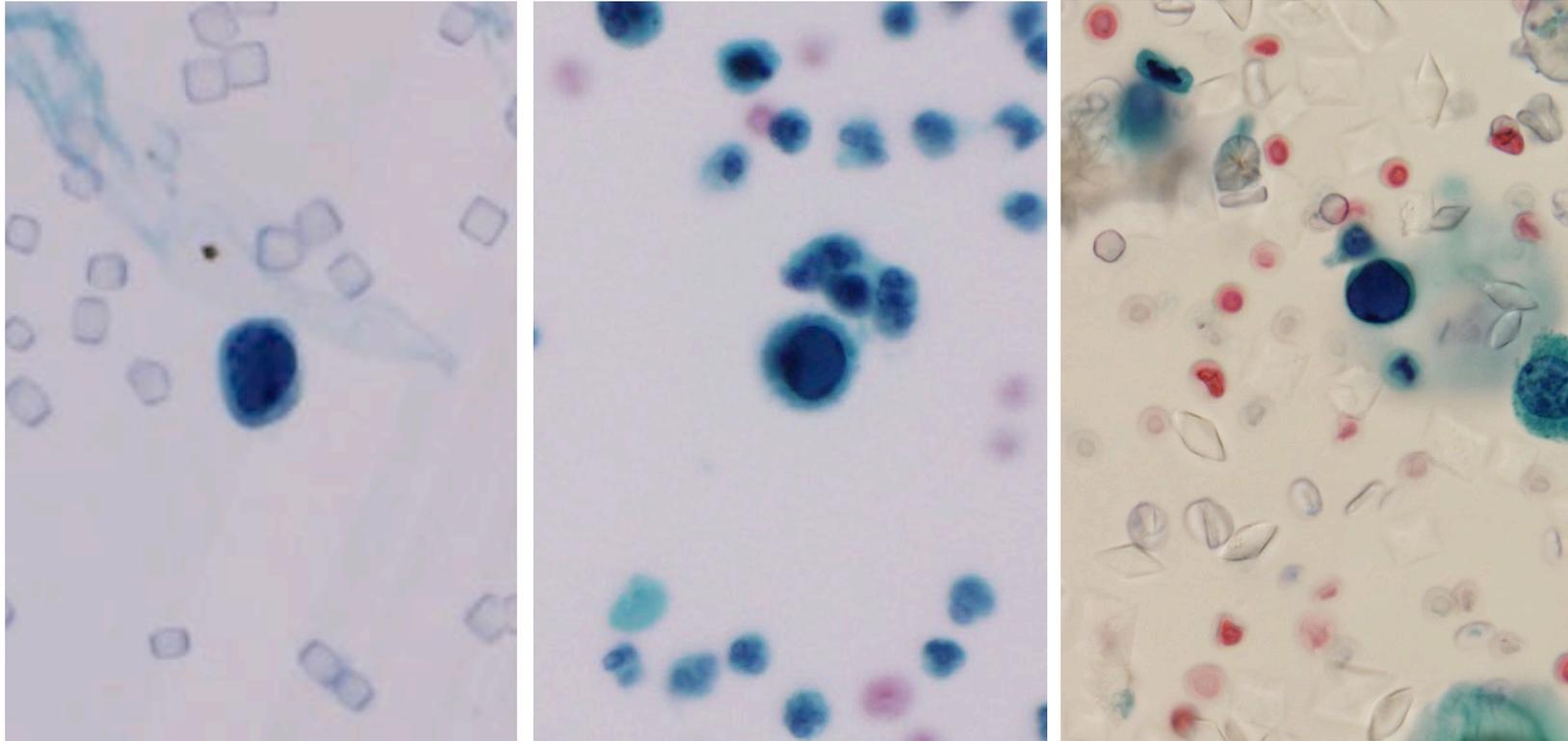


HSV



HPV

# Infections - Viral

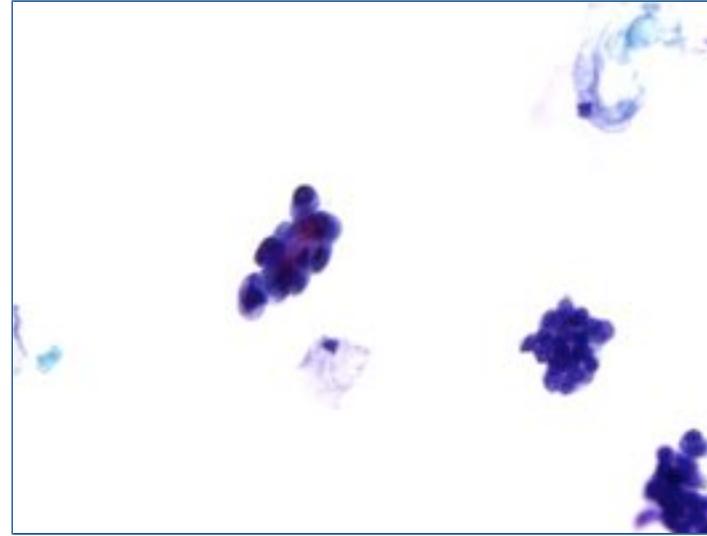
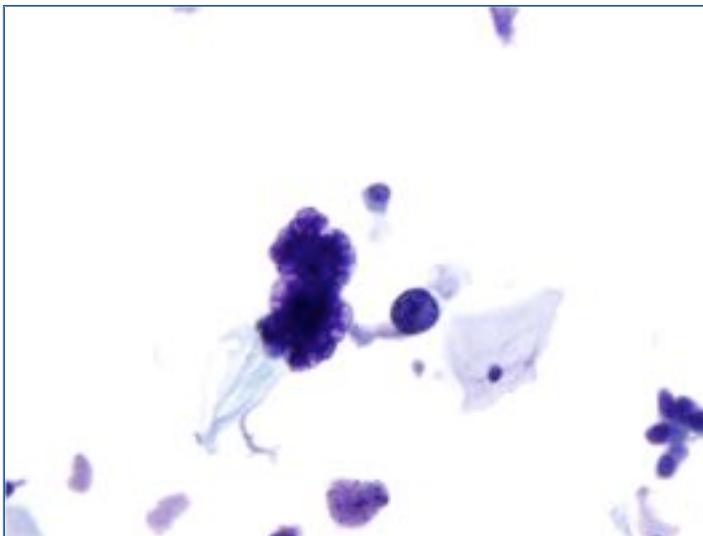
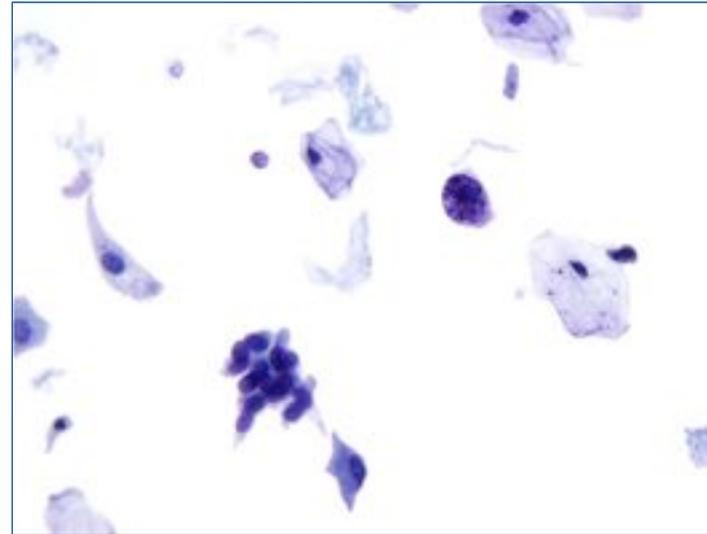
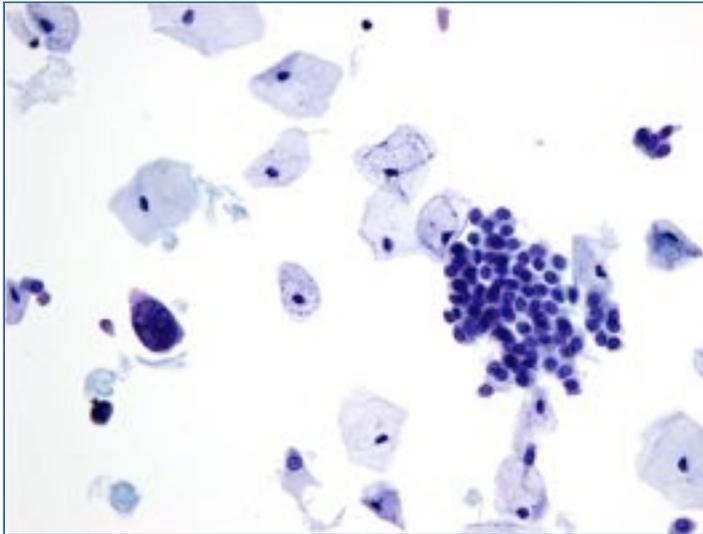


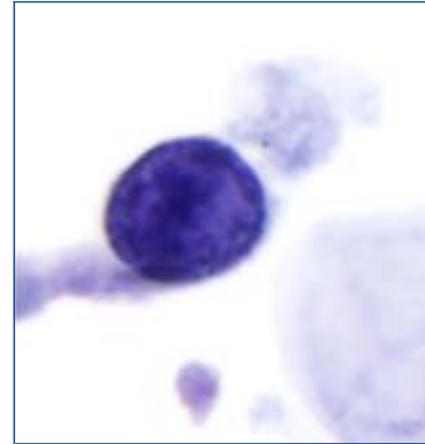
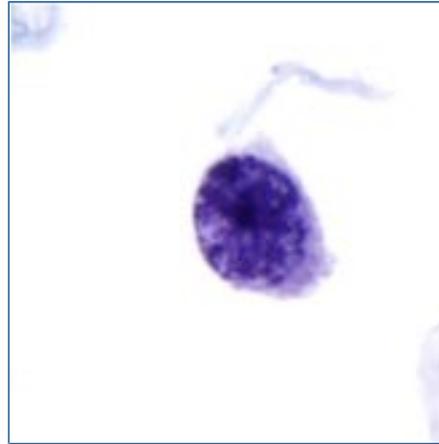
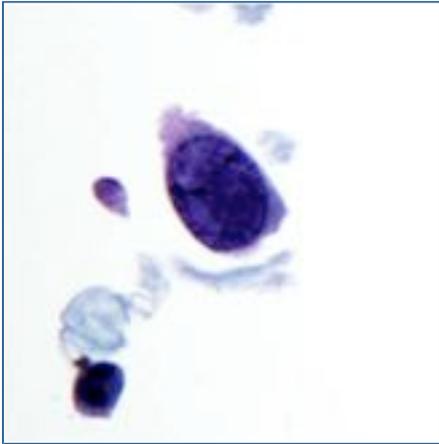
Polyomavirus Cytopathic Changes



Things are never that easy.....

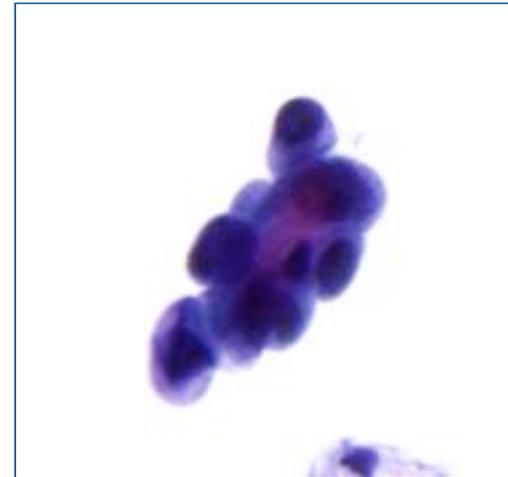
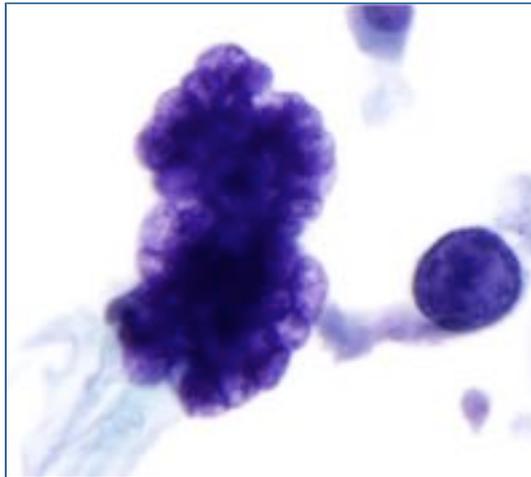
**88-year-old man with a history of T1 HGUC previously treated by local excision. F/U bx negative. Cystoscopy - negative.**



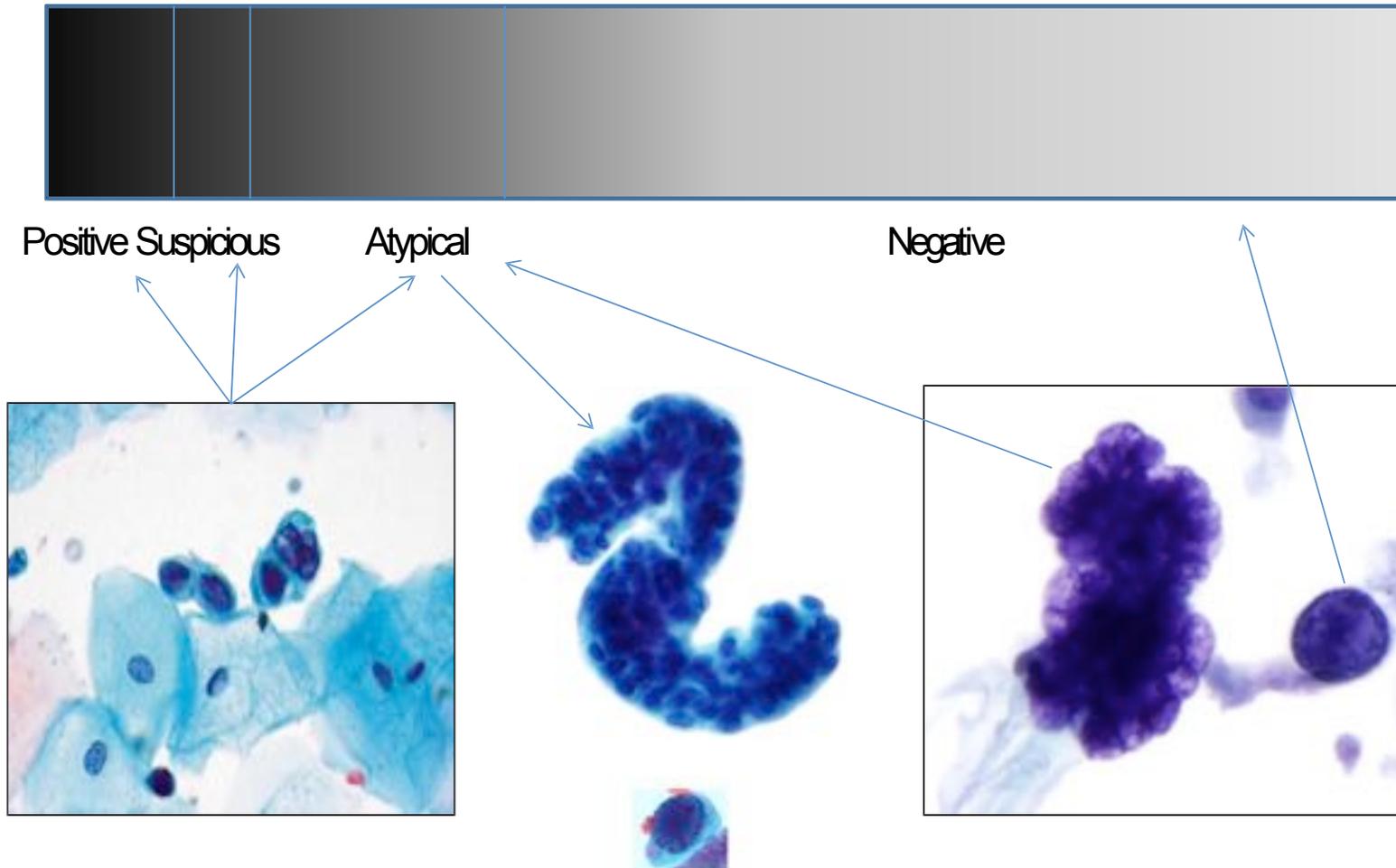


- Polyoma → Negative for High Grade Urothelial Carcinoma

**How about these?**



# What is Atypia



## High-Grade Urothelial Carcinoma in Urine Cytology With Jet Black and Smooth or Glassy Chromatin

Andrew A. Renshaw, MD <sup>1,2</sup> and Edwin W. Gould, MD<sup>1,2</sup>

**BACKGROUND:** Some high-grade urothelial carcinomas (UCs) in urine cytology can have jet black, smooth, or glassy chromatin, but to the authors' knowledge, the incidence and criteria for diagnosis are not well described. The current study was performed to define the incidence and appearance of high-grade UC in urine cytology in cytospin preparations with jet black and smooth or glassy chromatin. **METHODS:** Cytospin preparations from 331 cases with biopsy follow-up (230 benign/low-grade UCs and 101 malignant UCs) were reviewed. **RESULTS:** Cases with malignant cells with jet black and smooth or glassy chromatin were identified in a total of 60 cases (59.4% of all malignancies). These comprised 18 carcinoma in situ cases, 28 high-grade papillary UCs, 8 invasive UCs, 3 squamous cell carcinomas, 2 adenocarcinomas, and 1 melanoma. Of the 93 high-grade UCs, 51 (54.8%) had cells with either jet black and smooth or glassy chromatin. These cells were the only type of malignant cell in 6 of 101 cases (5.9%). All cases had at least 50 cells with jet black nuclei. Nuclei with jet black and smooth chromatin often were smaller than normal urothelial cells, often but not always elongate, had irregular nuclear outlines including pointed areas, and usually were accompanied by necrosis. Cells with glassy chromatin often were larger than normal urothelial cells, had rounder but still irregular nuclei, and also had frequent necrosis. **CONCLUSIONS:** Malignant urothelial cells in urine cytology with jet black chromatin are common and can be diagnosed as "positive for malignancy" based on their irregular nuclear outline, increased cellularity ( $\geq 50$  abnormal cells), and frequent necrosis. *Cancer Cytopathol* 2018;126:64-8. © 2017 American Cancer Society.

**KEY WORDS:** black; cytospins; Decoy; diagnosis; glassy; high grade; urine; urothelial carcinoma.

### INTRODUCTION

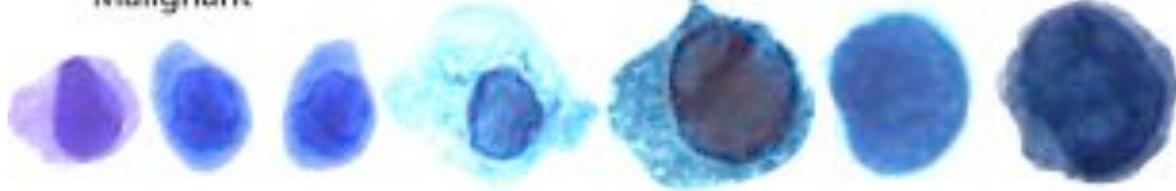
Urine cytology is a highly accurate test for high-grade urothelial carcinoma (UC), with a sensitivity and specificity as high as 79% and 95%, respectively,<sup>1-4</sup> although this can vary widely.<sup>5-17</sup> The Paris System for Reporting Urinary Cytology proposes a standardized terminology<sup>18-23</sup> that only seeks to diagnose high-grade

Benign (Decoy cells, Polyoma virus)

RBC 



Malignant



## High-Grade Urothelial Carcinoma on Urine Cytology Resembling Umbrella Cells

Andrew A. Renshaw · Edwin W. Gould

Department of Pathology, Baptist Hospital of Miami and Miami Cancer Institute, Miami, FL, USA

### Keywords

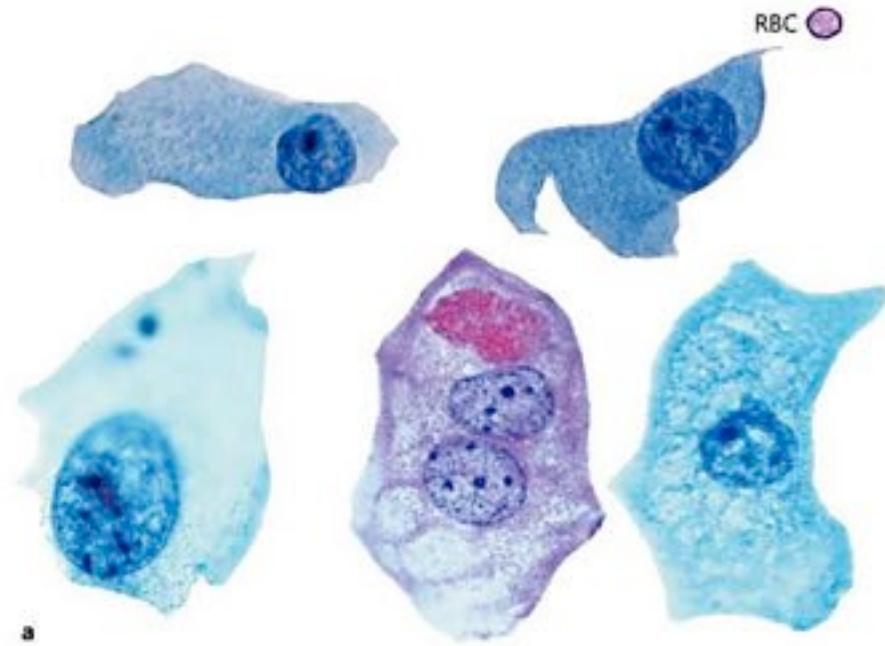
Urine · High-grade urothelial carcinoma · Diagnosis · Cytospin · Umbrella cells

### Abstract

**Context:** High-grade urothelial carcinoma (UC) cells have many appearances on urine cytology, but according to The Paris System, they can be easily distinguished from umbrella cells. **Objective:** We aimed to define the incidence and appearance of high-grade UC cells that resemble umbrella cells

### Introduction

Urine cytology is a highly accurate test for detecting high-grade urothelial carcinoma (UC), with a sensitivity and specificity as high as 79 and >95%, respectively [1-4], though these can vary widely [5-17]. The Paris System for Reporting Urinary Cytology has been proposed for providing the standardized terminology for UC [18-23], and includes a single set of strict diagnostic criteria derived from the literature: at least 5 abnormal cells [24], a nuclear-to-cytoplasmic (N/C) ratio >0.7 [12, 25], moderate hy-



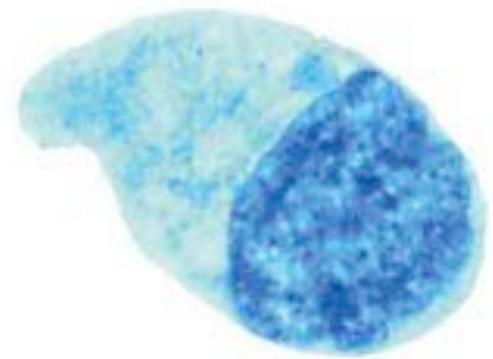
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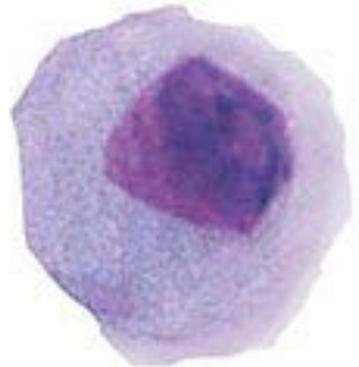
b

RBC 

Benign



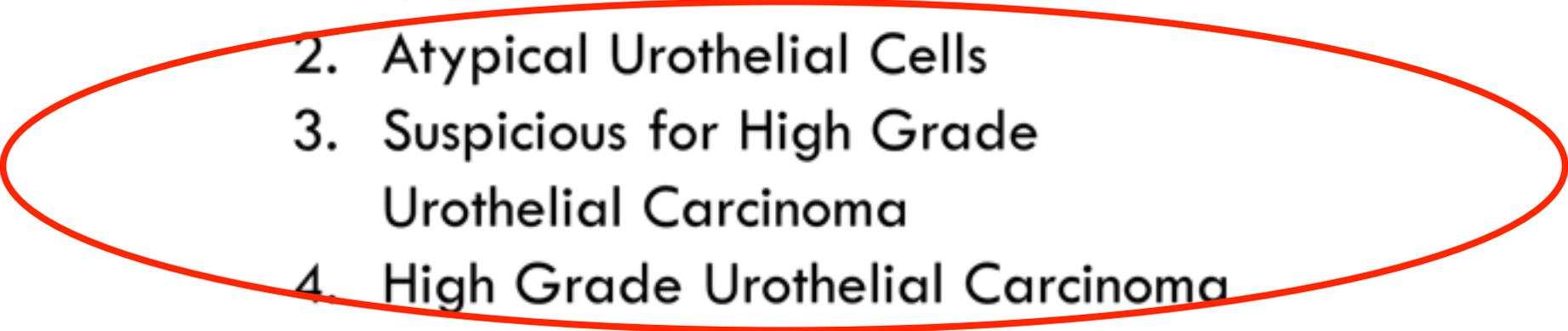
Malignant



**Negative for High Grade Urothelial Carcinoma**

# Diagnostic categories



1. Negative for High Grade Urothelial Carcinoma
  2. Atypical Urothelial Cells
  3. Suspicious for High Grade Urothelial Carcinoma
  4. High Grade Urothelial Carcinoma
  5. Low Grade Urothelial Neoplasm
  6. Other malignancies, both primary and secondary
- 

# What is atypia? Findings in literature

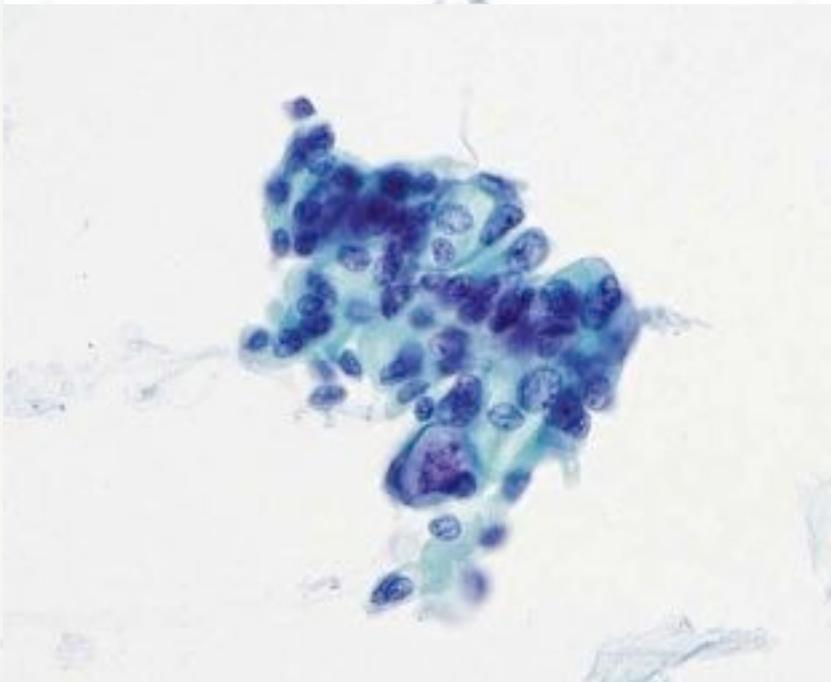
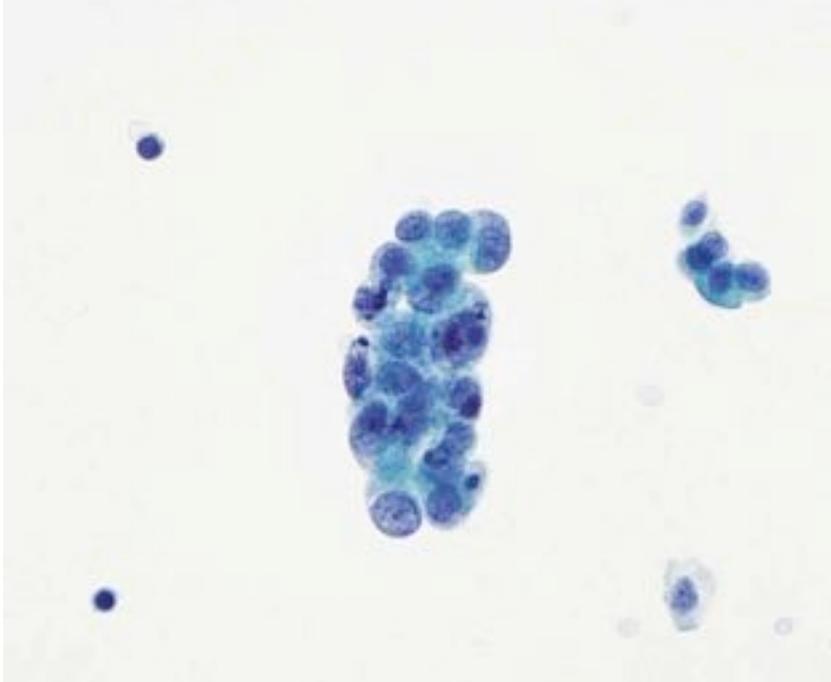
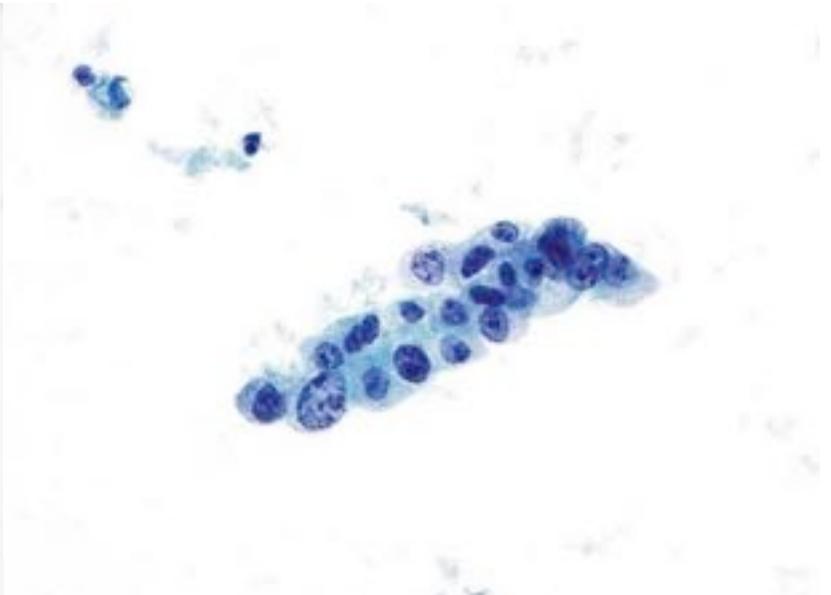
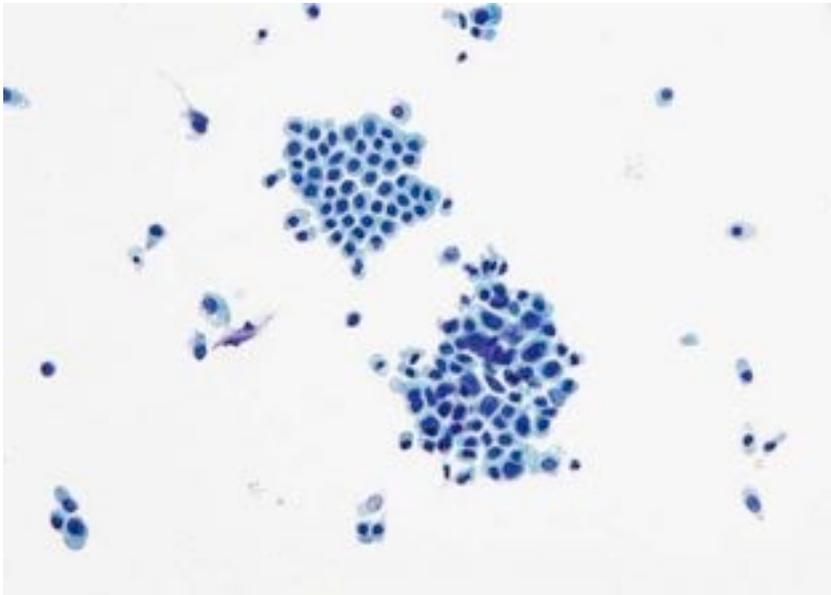
1. High nuclear cytoplasmic ratio ( $>0.7$ )
2. Nuclear hyperchromasia
3. Coarse, clumped chromatin
4. Irregular nuclear membranes



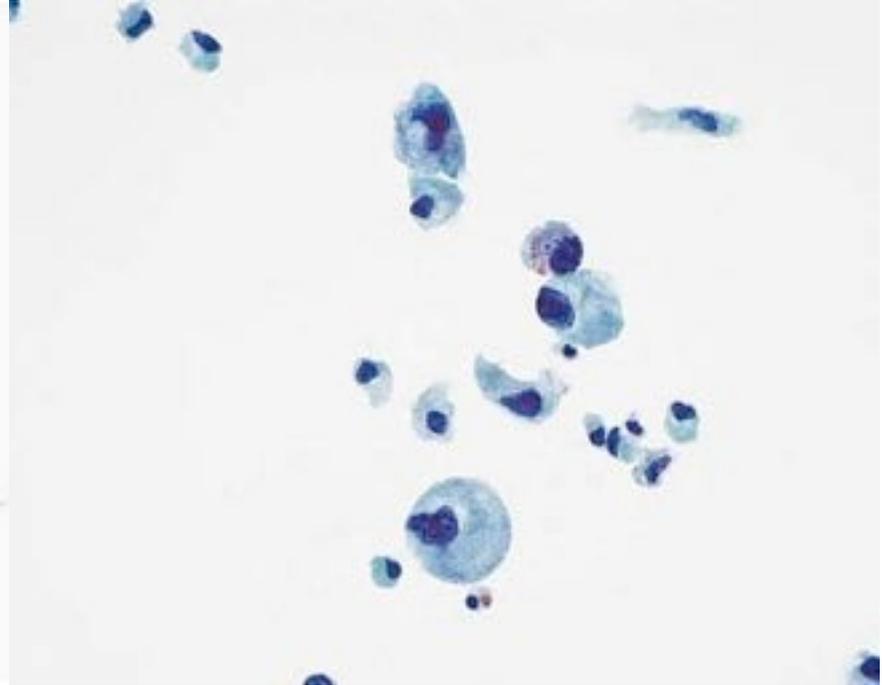
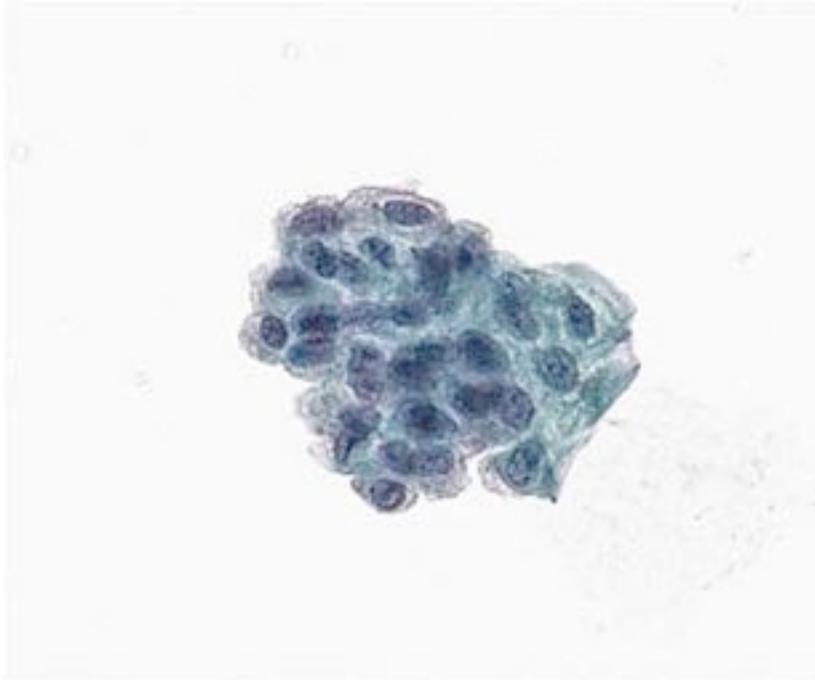
# Atypical Urothelial Cells (AUC)

## Criteria for AUC

- Non-superficial and non-degenerated urothelial cells with an **high N/C ratio > 0.5 (required)**  
*and one of the following:*
  - **Hyperchromasia** (compared to the umbrella cells or the intermediate squamous cell nucleus)
  - **Irregular clumpy chromatin**
  - **Irregular nuclear contours**



# Degeneration



# N:C ratio of 0.5???

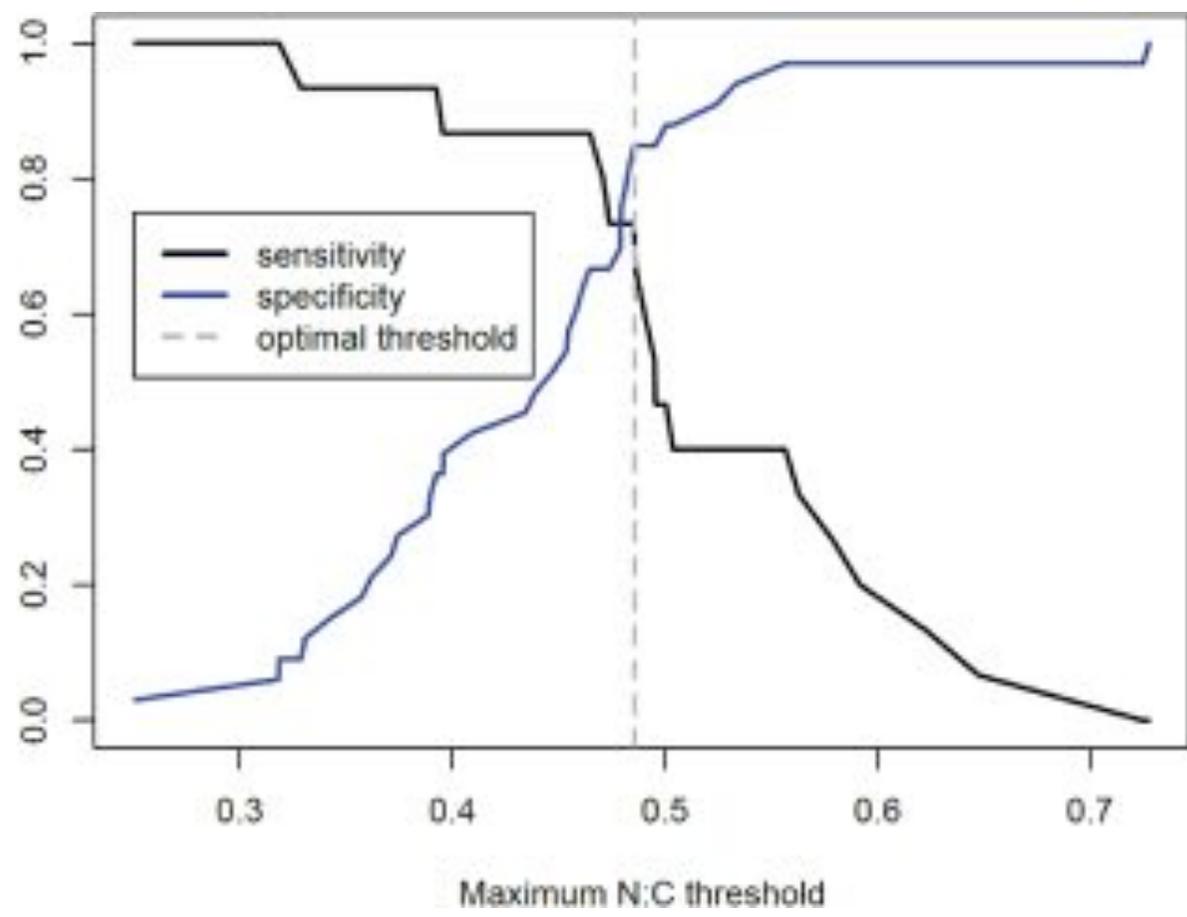
Original Article

## Digital Image Analysis Supports a Nuclear-To-Cytoplasmic Ratio Cutoff Value of 0.5 for Atypical Urothelial Cells

Jen-Fan Heng, MD<sup>1,2,3</sup>, Vivek Charu, PhD<sup>1,4</sup>, M. Lisa Zhang, MD<sup>5</sup>,  
and Christopher J. VandenBussche, MD, PhD<sup>6</sup>

**BACKGROUND:** An elevated nuclear-to-cytoplasmic (N:C) ratio of  $>0.5$  is a required criterion for the diagnosis of atypical urothelial cells (AUC) in The Paris System for Reporting Urinary Cytology. **METHODS:** To validate the N:C ratio cutoff value and its predictive power for high-grade urothelial carcinoma (HGUC), the authors retrospectively reviewed the urinary tract cytology specimens of 15 cases of AUC with HGUC on follow-up (AUC-HGUC) and 33 cases of AUC without HGUC on follow-up (AUC-N-HGUC). The number of atypical cells in each case was recorded, and each atypical cell was photographed and digitally examined to calculate the nuclear size and N:C ratio. **RESULTS:** On average, the maximum N:C ratios of atypical cells were significantly different between the AUC-HGUC and AUC-N-HGUC cohorts (0.53 vs 0.43,  $P = .00009$ ), whereas the maximum nuclear sizes of atypical cells ( $153.43 \mu\text{m}^2$  vs  $201.47 \mu\text{m}^2$ ,  $P = .69$ ) and the number of atypical cells per case (10.13 vs 7.88,  $P = .12$ ) were not found to be significantly different. Receiver operating characteristic analysis demonstrated that the maximum N:C ratio alone had high discriminatory capacity (area under the curve, 79.9%; 95% confidence interval, 64.19%-94.19%). The optimal maximum N:C ratio threshold was 0.486, giving a sensitivity of 75.3% and a specificity of 84.8% for predicting HGUC on follow-up. **CONCLUSIONS:** The identification of AUC with an N:C ratio  $>0.486$  has a high predictive power for HGUC on follow-up in AUC specimens. This justifies using the N:C ratio as a required criterion for the AUC category. Individual laboratories using different cytopreparation methods may require independent validation of the N:C ratio cutoff value. *Cancer Cytopathol* 2017;125:710-6. © 2017 American Cancer Society.

**KEY WORDS:** atypical urothelial cells (AUC); digital imaging; nuclear-to-cytoplasmic (N:C) ratio; The Paris System for Reporting Urinary Cytology; urinary tract cytology



# Suspicious for High-Grade Urothelial Carcinoma (Suspicious)

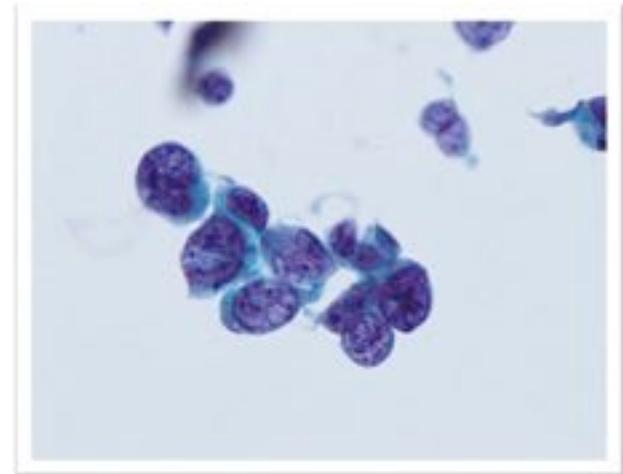
## Criteria for SHGUC

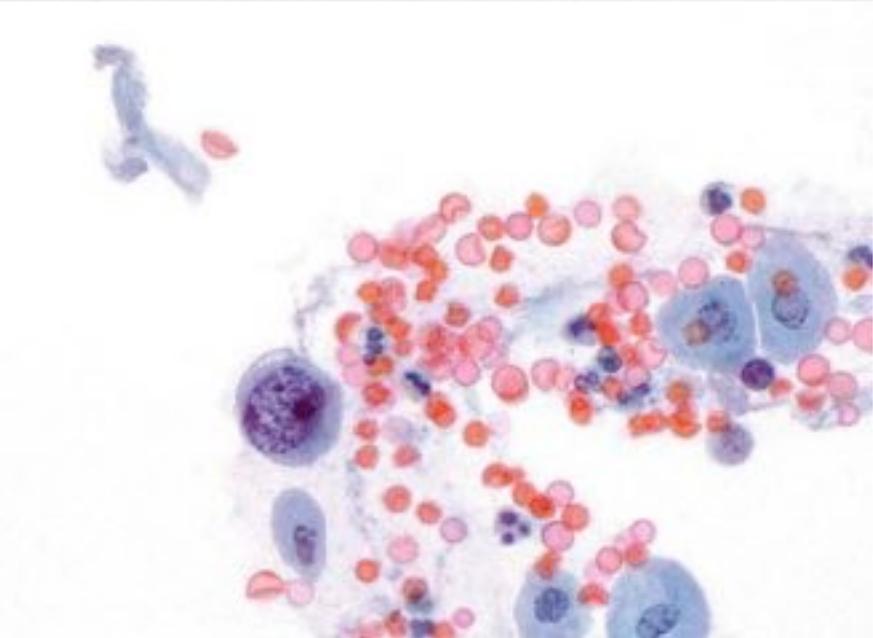
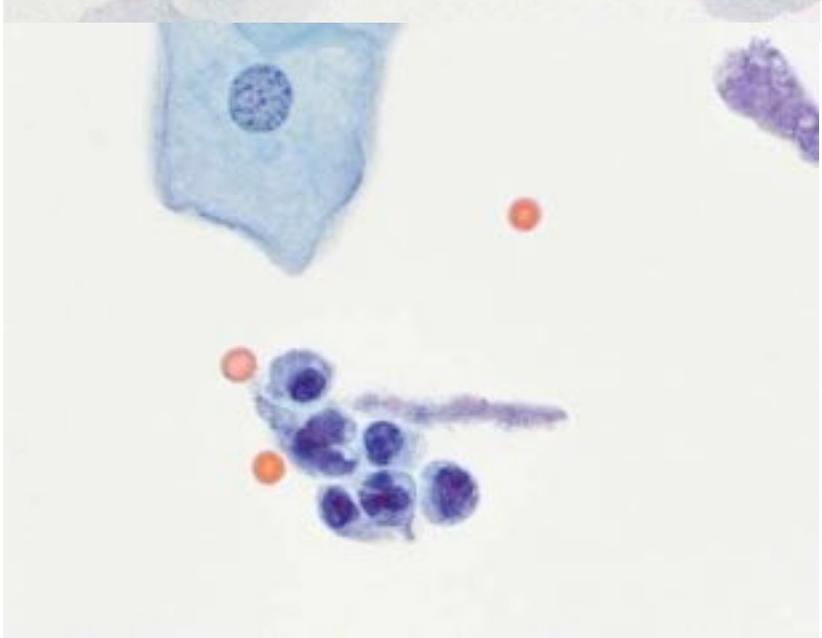
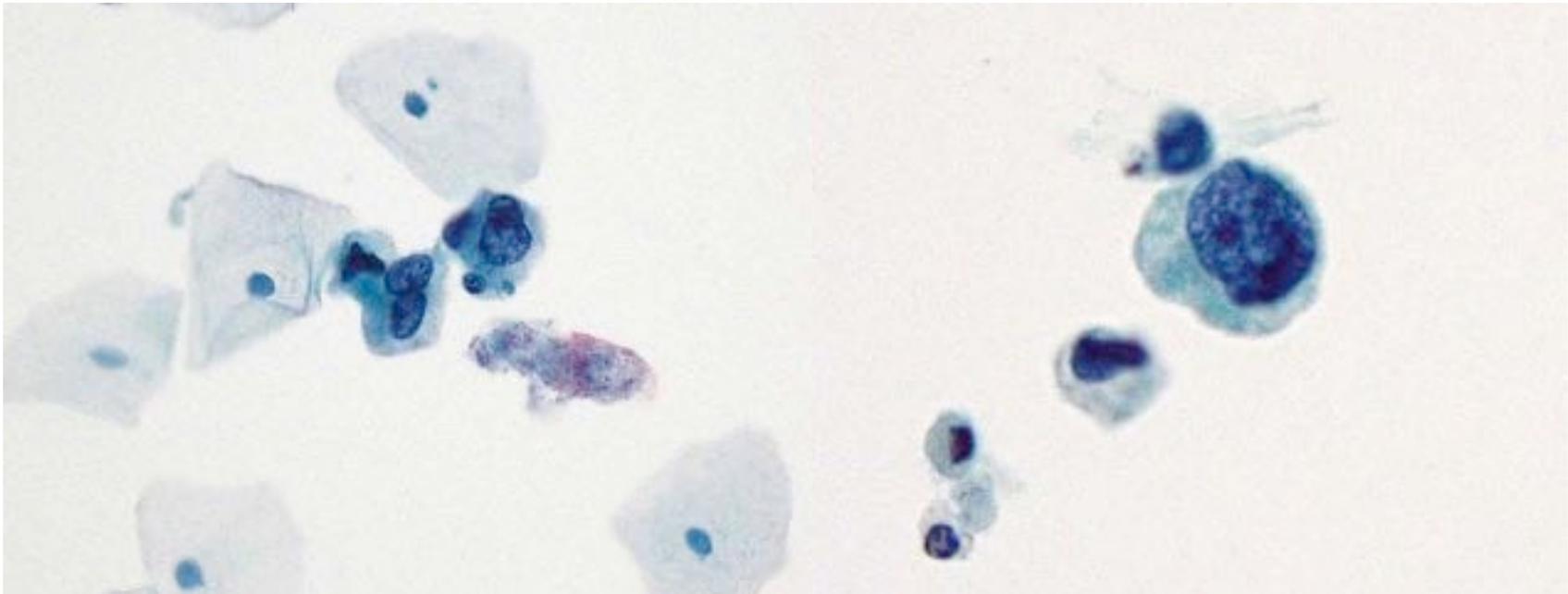
- Non-superficial and non-degenerated urothelial cells with an high **N/C ratio > 0.7** (required)
- **Hyperchromasia** (compared to the umbrella cells or the intermediate squamous cell nucleus) (required)

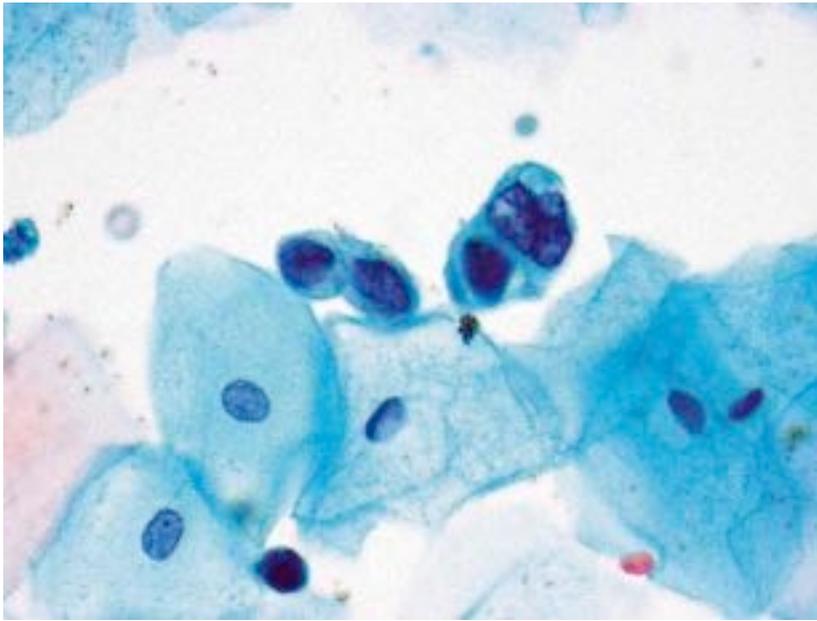
*and one of the following:*

- **Irregular clumpy chromatin**
- **Irregular nuclear membranes**

**<10 cells**







# Suspicious for HGUC vs. Positive HGUC

## Quantity matters..

“The number of atypical urothelial cells is an important criterion to classify urine cytology specimens into the ‘positive’ or the ‘suspicious’ categories. A cut-off number of **>10** cells to render a definitive diagnosis of HGUCA seems valid from the clinical standpoint .”

ORIGINAL ARTICLE

**Urine cytology: does the number of atypical urothelial cells matter? A qualitative and quantitative study of 112 cases**

Fadi Brimo, MD<sup>a,\*</sup>, Bin Xu, MD<sup>a</sup>, Wassim Kassouf, MD<sup>b</sup>,  
Babak Ahmadi-Kaliji, MD<sup>a</sup>, Michele Charbonneau, CT<sup>a</sup>,  
Ayoub Nahal, MD<sup>a</sup>, Yonca Kanber, MD<sup>a</sup>, Derin Caglar, MD<sup>a</sup>,  
Manon Auger, MD<sup>a</sup>

JASC 2015;4(4)232-238

5 – 10 cells – gray zone, based on experience, history,  
individual threshold, etc

# Not only quantity and quality matter..

Original Article

## When Words Matter: A “Suspicious” Urinary Tract Cytology Diagnosis Improves Patient Follow-Up Among Nonurologists

J. Judd Fite, MD, MBA<sup>1</sup>; Dorothy L. Rosenthal, MD<sup>1,2</sup>; and Christopher J. VandenBussche, MD, PhD <sup>1,3</sup>

**BACKGROUND:** Urinary tract cytology (UTC) specimens diagnosed using high-risk indeterminate categories such as “atypical urothelial cells, cannot exclude high-grade urothelial carcinoma” (AUC-H) or “suspicious for high-grade urothelial carcinoma” (SHGUC) have a high rate of detection of high-grade urothelial carcinoma on subsequent biopsy. Although urologists are familiar with such terminology, it is unclear whether patients receive appropriate follow-up when UTC is ordered by nonurologists. In the current study, the authors investigated whether the use of AUC-H versus SHGUC altered patient management among nonurologists. **METHODS:** Specimens signed out as AUC-H or SHGUC were identified from the archives of the study institution, which included periods of time before the use of the standardized Johns Hopkins Hospital template, during use of the Johns Hopkins Hospital template, and after institution of The Paris System for Reporting Urinary Cytology. **RESULTS:** Approximately one-half of the specimens diagnosed as AUC-H were not investigated further when ordered by nonurologists. Patients with specimens diagnosed as AUC-H received fewer subsequent biopsies (14% vs 53%;  $P < .001$ ) when the specimens were ordered by nonurologists versus urologists, despite having similar rates of high-grade urothelial carcinoma on follow-up biopsy (67% vs 68%). When specimens ordered by nonurologists were diagnosed as SHGUC, these patients received more follow-up (100%) compared with those whose specimens were diagnosed as AUC-H (44%;  $P < .001$ ). Patients with specimens ordered by nonurologists also received more follow-up biopsies when these were diagnosed as suspicious (60%) compared with patients whose specimens were diagnosed as AUC-H (14%;  $P < .001$ ). **CONCLUSIONS:** Use of the word “suspicious” for the high-risk indeterminate category results in greater follow-up among nonurologists ordering UTC specimens. *Cancer Cytopathol* 2018;000:000-000. © 2018 American Cancer Society.

**KEY WORDS:** indeterminate; suspicious; The Paris System for Reporting Urinary Cytology; urinary tract cytology; urine

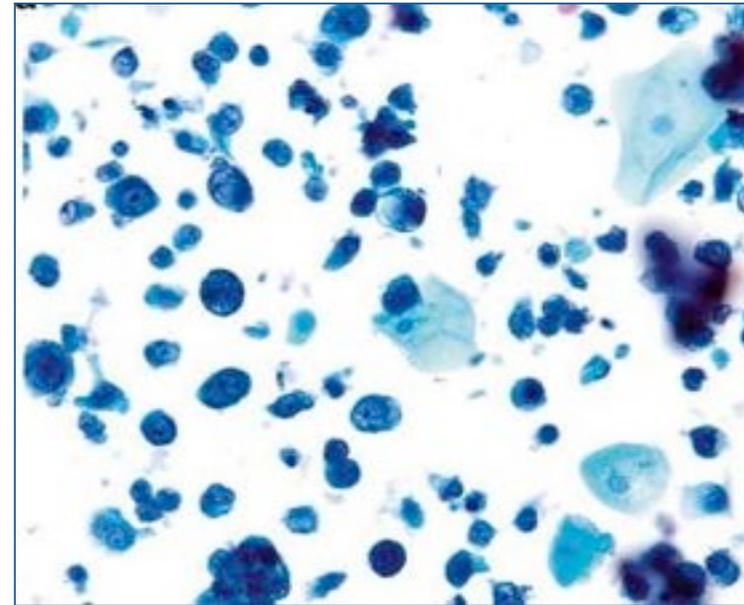
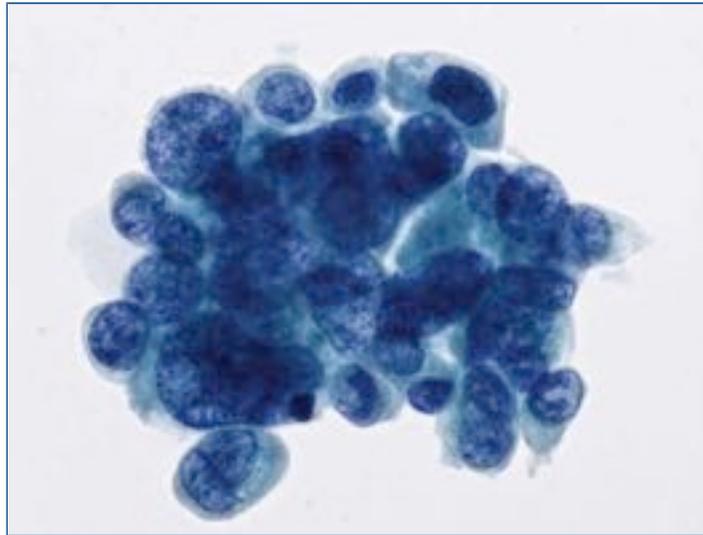
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	Follow-Up, % <sup>a</sup>	Biopsy, %
AUC-H, nonurologist	39.5% (17/43)	14% (6/43)
SHGUC, nonurologist	100% (15/15)	60% (9/15)
<i>P</i> (AUC-H vs SHGUC), nonurologist	<.0001	<.0005
AUC-H, urologist	NA	73% (344/473)
SHGUC, urologist	NA	75% (197/261)
<i>P</i> (AUC-H vs SHGUC), urologist		.43

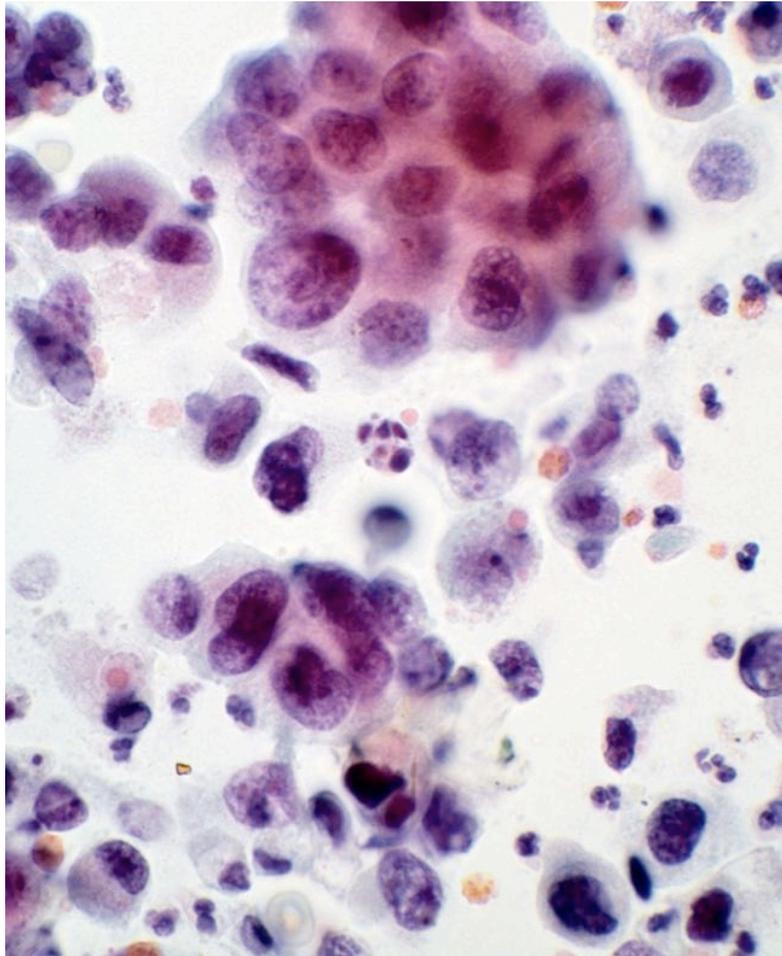
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# High-Grade Urothelial Carcinoma (HGUC)

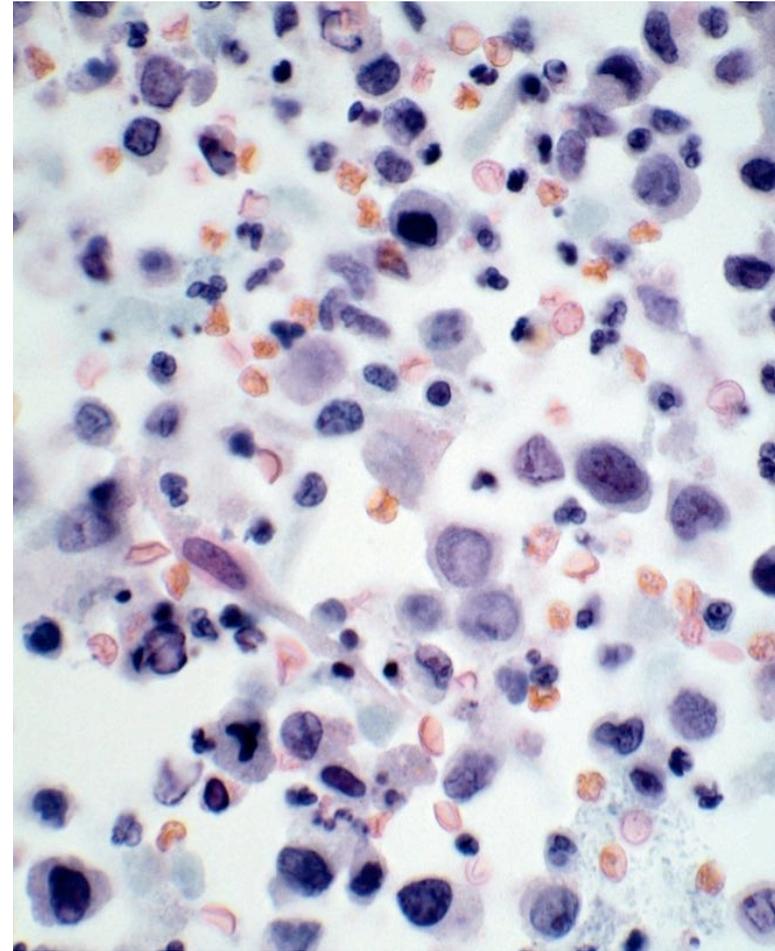
- Cellularity: At least 5–10 abnormal cells
- N/C ratio: 0.7 or greater
- Nucleus: Moderate to severe hyperchromasia
- Nuclear membrane: Markedly irregular
- Chromatin: Coarse/clumped



# High-grade UC



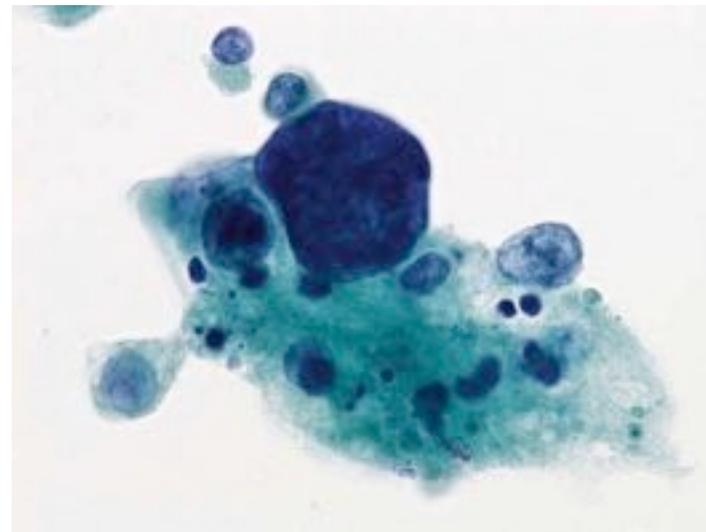
Bladder Washing



Squamous differentiation

## Other Notable Cytomorphologic Features

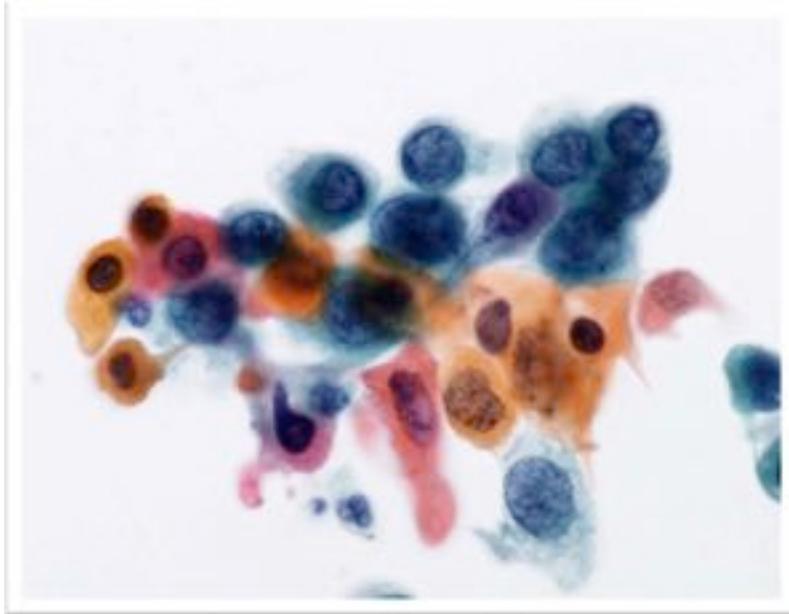
- Cellular pleomorphism
- Marked variation in cellular size and shapes, i.e., oval, rounded, elongated, or plasmacytoid (Comet cells)
- Scant, pale, or dense cytoplasm
- Prominent nucleoli
- Mitoses
- Necrotic debris
- Inflammation



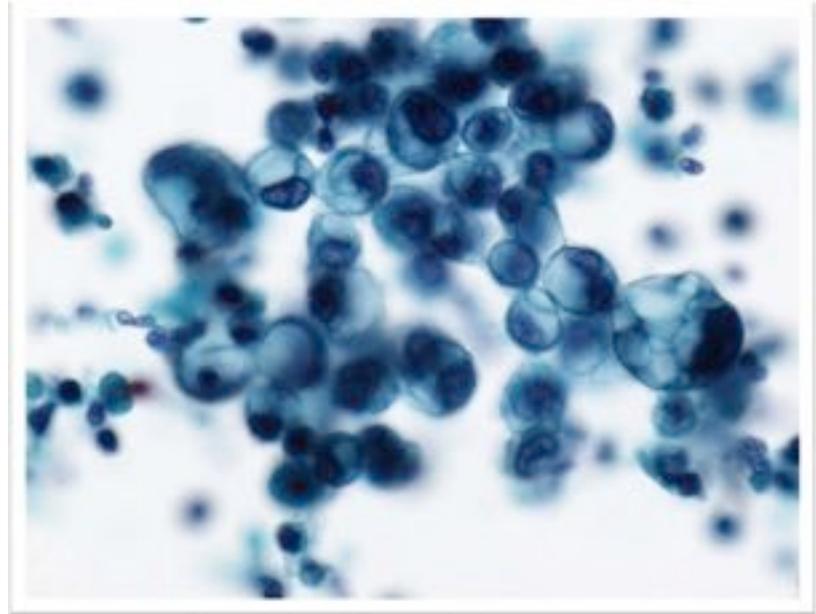
# High-grade UC - Differential Diagnosis



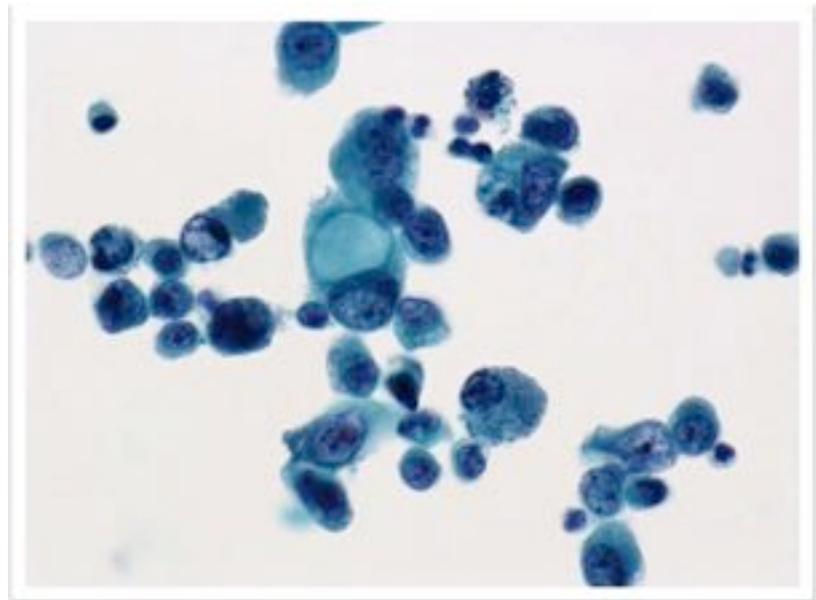
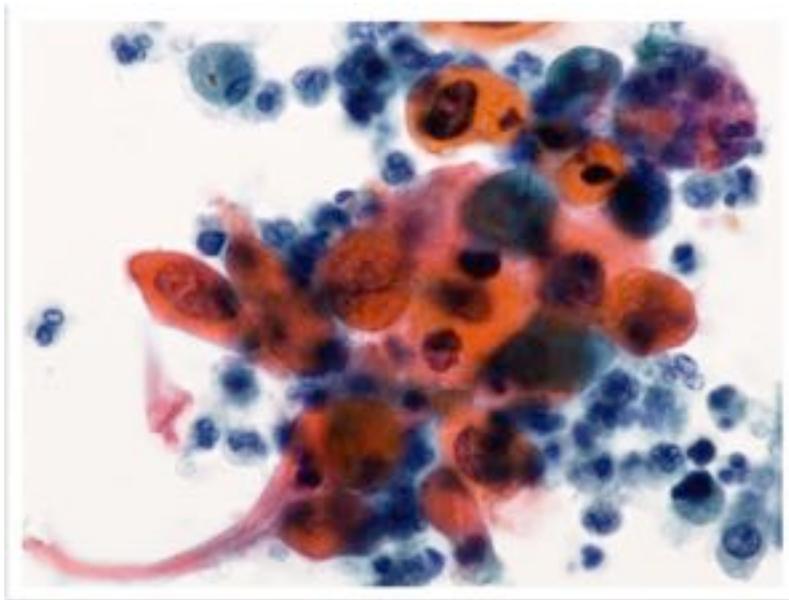
- ❑ Polyomavirus
- ❑ Stone atypia
- ❑ Normal upper tract washing or brushings
- ❑ Treatment effect
- ❑ Non specific reactive changes



Squamous differentiation



Glandular differentiation

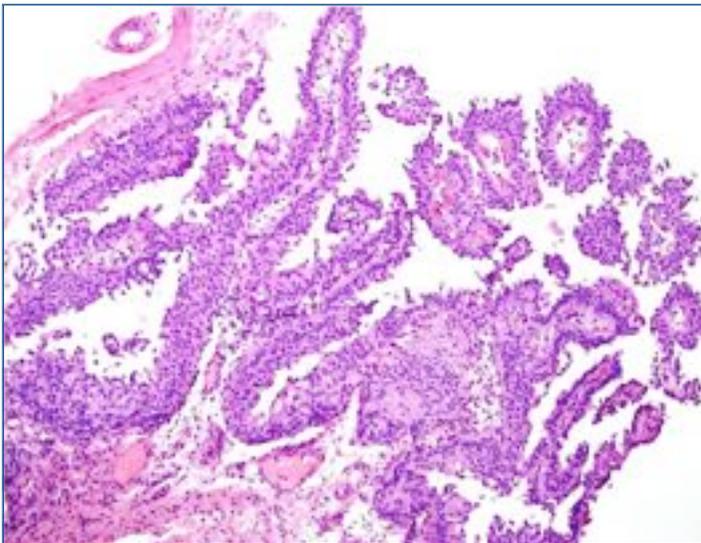


## What happened to Low grade urothelial neoplasia (LGUN)??

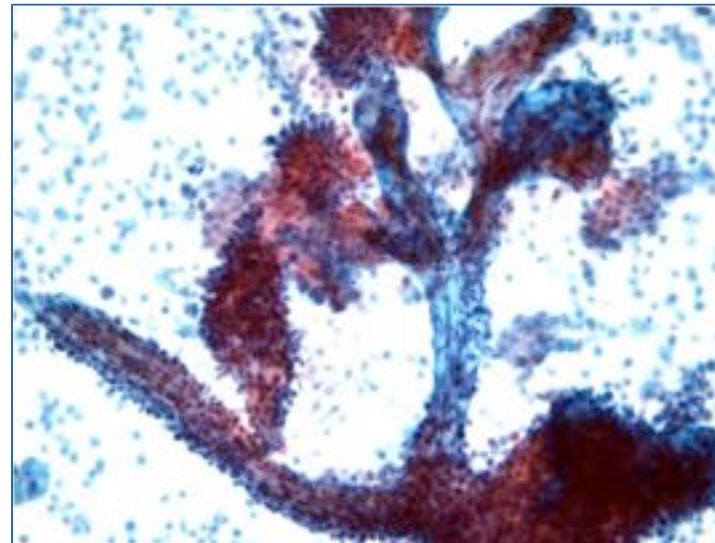
- Almost impossible to diagnose without a mini-biopsy with fibrovascular core
- Cytologically normal nuclei
- Is it truly a carcinoma?
- More common than HGUC
- BUT, not life threatening

## Low-Grade Urothelial Neoplasia (LGUN)

- LGUN - combined cytologic term for low grade papillary urothelial neoplasms (LGPUN) (which include urothelial papilloma, PUNLMP and LGPUC) and flat, low grade intraurothelial neoplasia



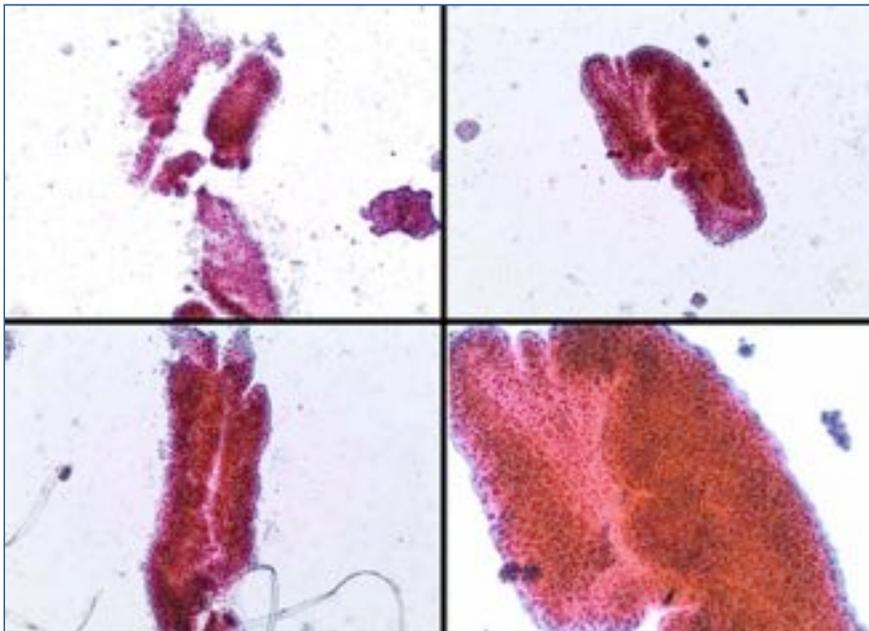
LGU  
C



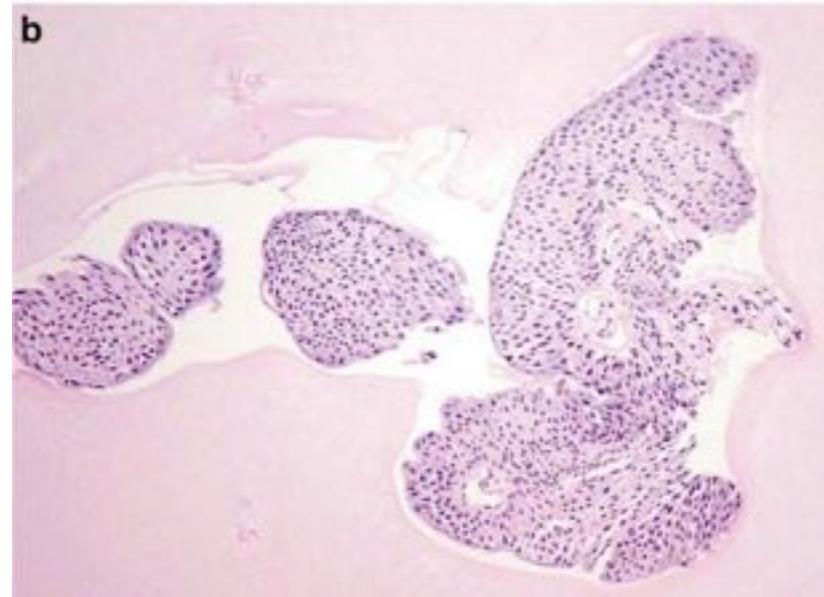
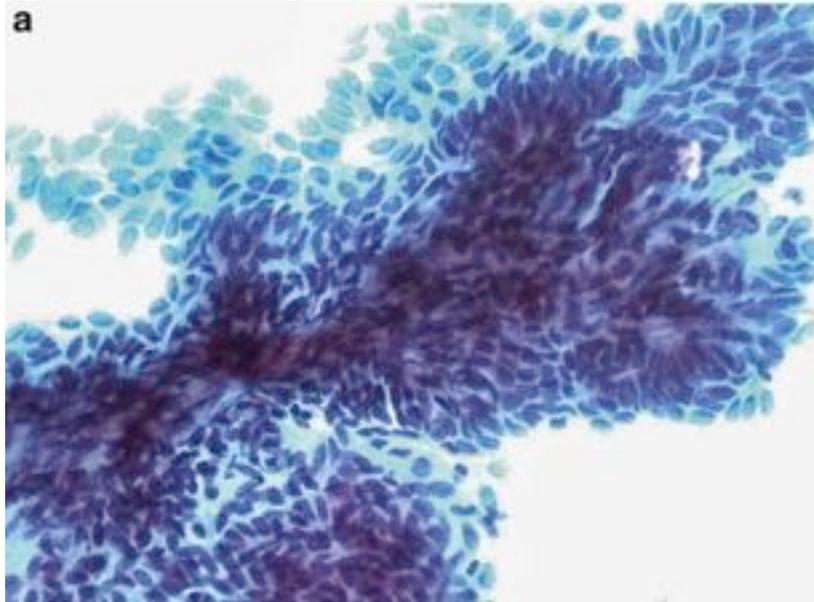
LGUN

## Cytologic Criteria of Low Grade Urothelial Neoplasia (LGUN) (regardless of the specimen type: voided or instrumented):

- Three-dimensional cellular papillary clusters (defined as clusters of cells with nuclear overlapping, forming "papillae") with fibrovascular cores with capillaries

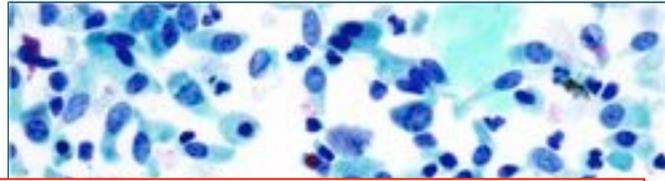
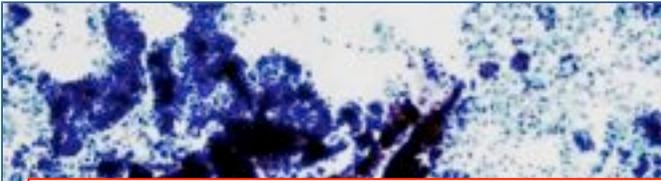


## Cytologic Criteria of Low Grade Urothelial Neoplasia (LGUN) (regardless of the specimen type: voided or instrumented)

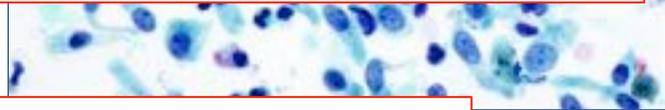
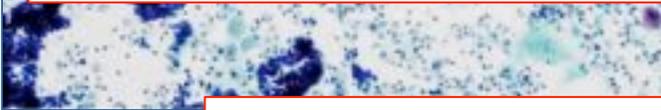


Cell Block

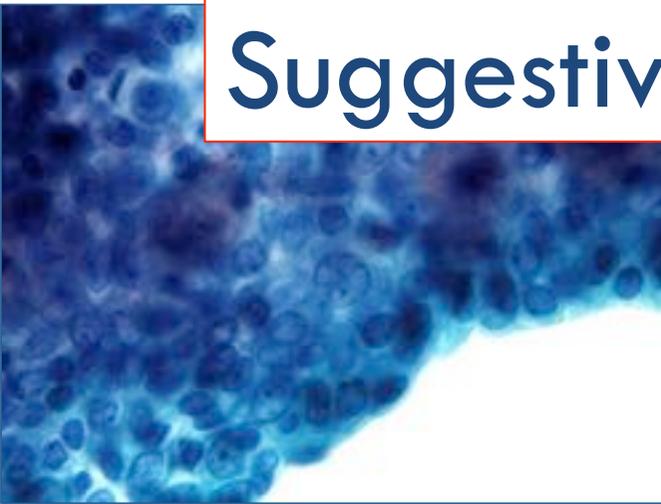
How about these????



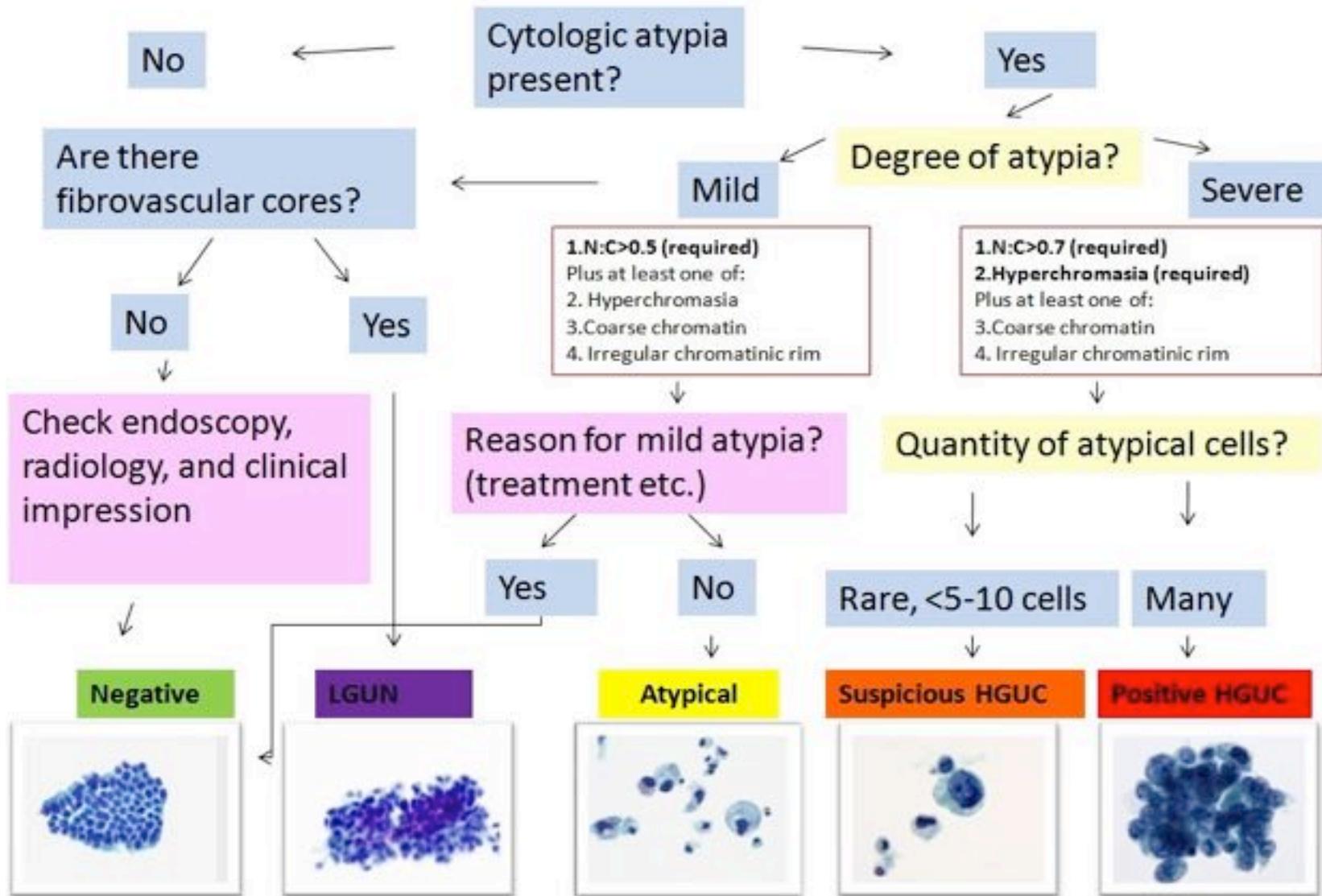
Negative for HGUC



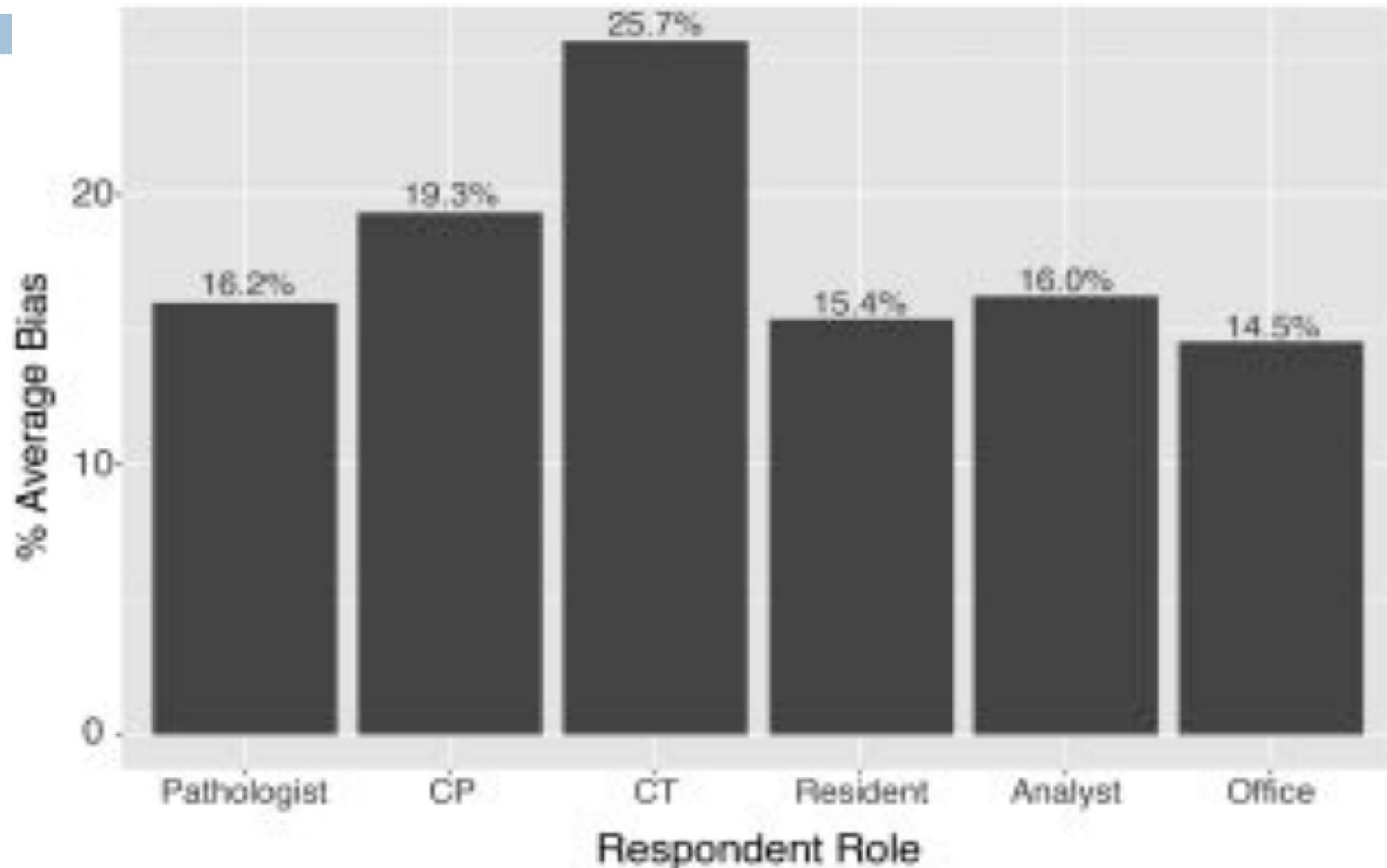
Suggestive of LGUN



# Approach to Diagnosis in Urinary Tract



# Nuclear: Cytoplasm Ratios



Zhang ML, Guo AX, VandenBussche CJ. Morphologists overestimate the nuclear-to-cytoplasmic ratio. *Cancer Cytopathol* 2016;124:669–677.

# What does the urologist do the cytology report?????



# Clinical Management

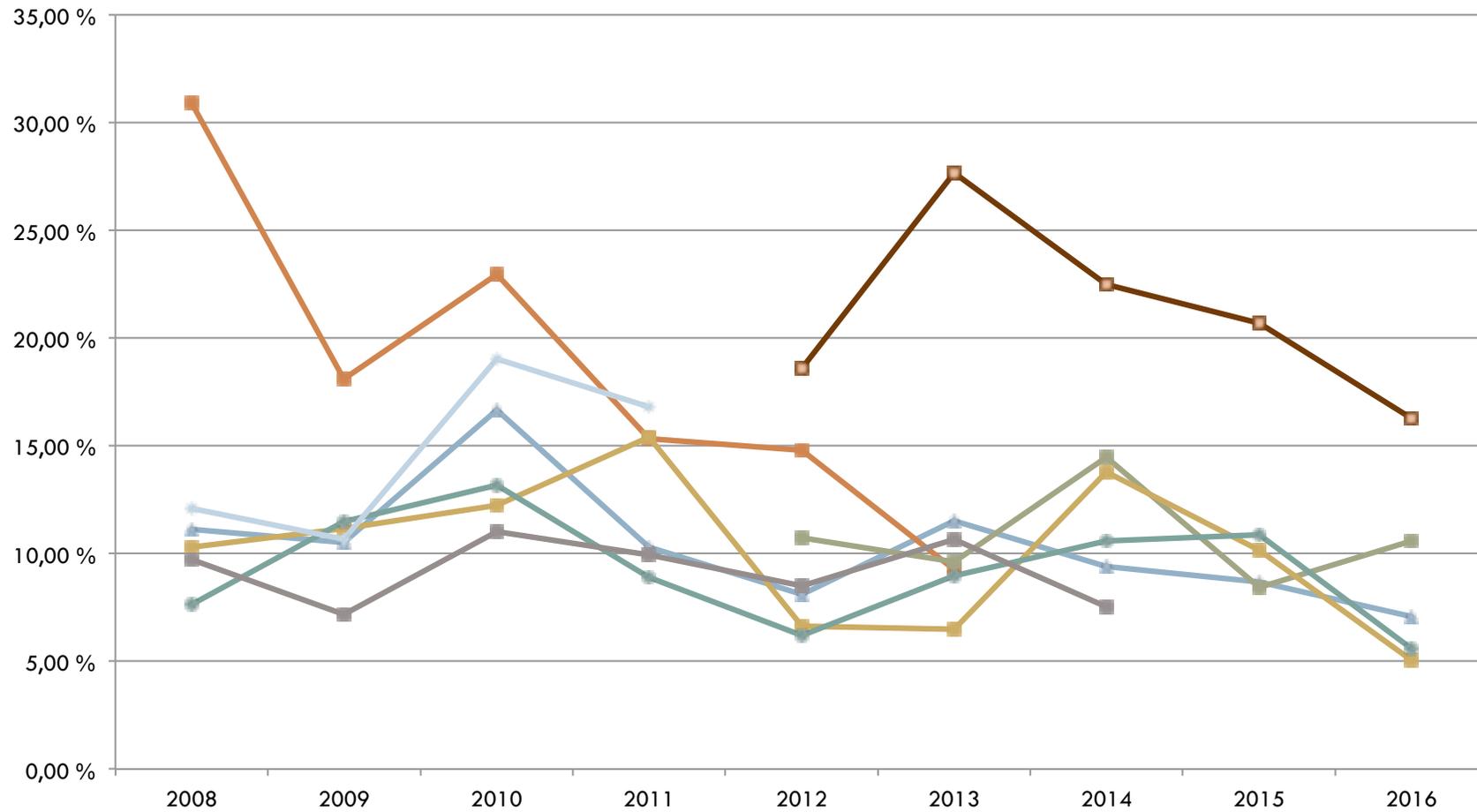


- From the standpoint of the urologist, the workup for AUC should be individualized based on the risk assessment of the patient
- From a practical standpoint, the clinical management of “suspicious for HGUC” is similar to a “positive for HGUC” diagnosis
- Transurethral resection establishes the histologic diagnosis and is therapeutic for most solitary low grade tumors

# Clinical Management

Category	Risk of Malignancy	Management
Unsatisfactory/Nondiagnostic	? (<5%)	Repeat cytology, cystoscopy in 3 months if increased clinical suspicion
Negative for HGUC	0-2%	Clinical follow up as needed
Atypical Urothelial Cells (AUC)	8-35%	Clinical follow up as needed. Use of ancillary testing.
Suspicious for HGUC	50-90%	More aggressive follow up, cystoscopy, biopsy
LGUN	~10%	Need biopsy to further evaluate grade and stage
High Grade UC	>90%	More aggressive follow up, cystoscopy, biopsy, staging
Other malignancy	>90%	More aggressive follow up, cystoscopy, biopsy, staging

# Rate of Atypia at Loyola per pathologist

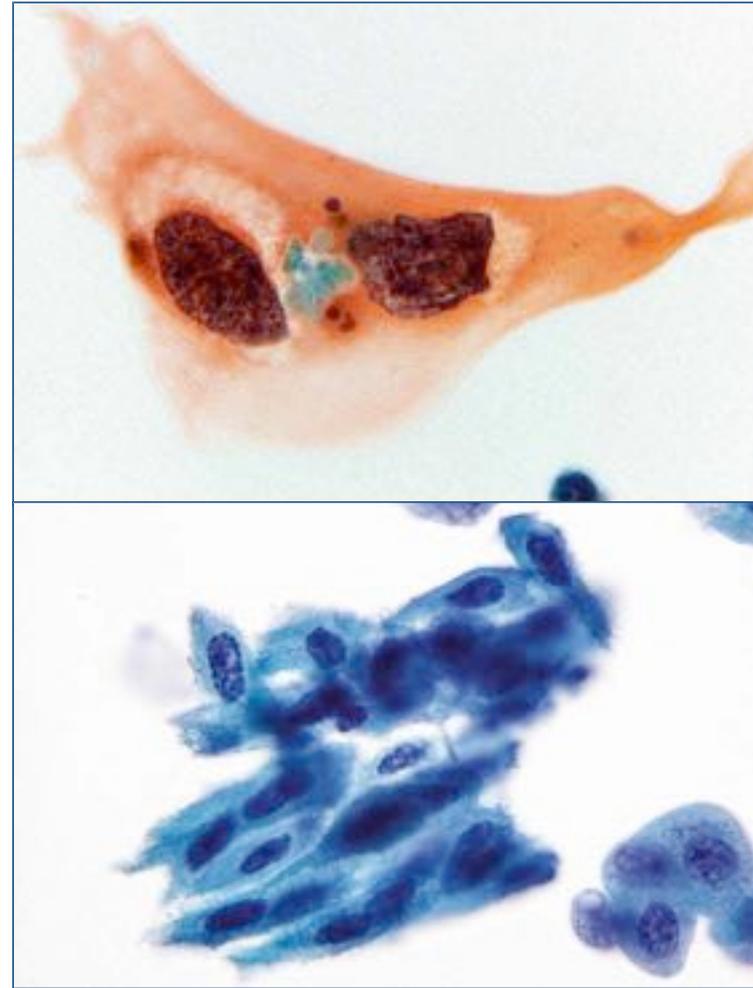
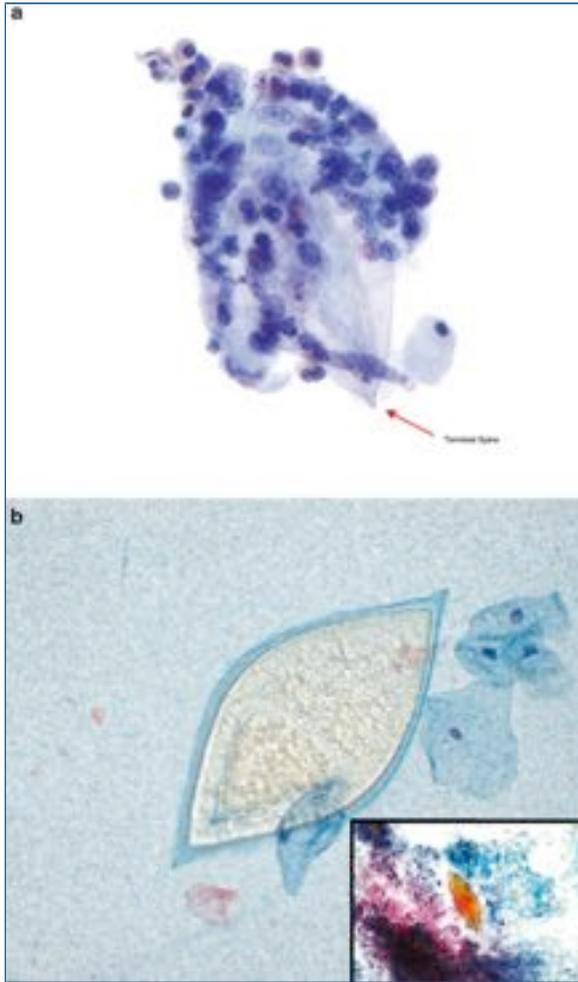


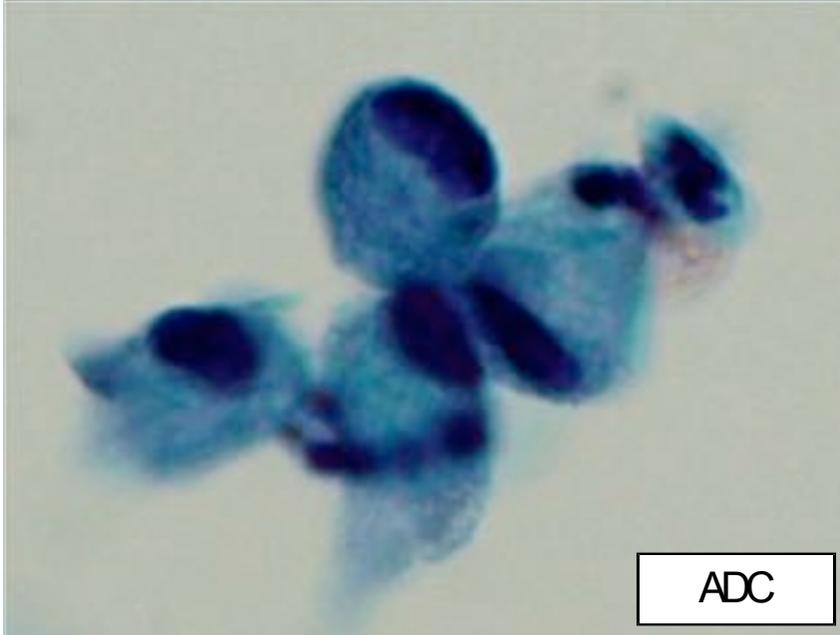
# Diagnostic categories



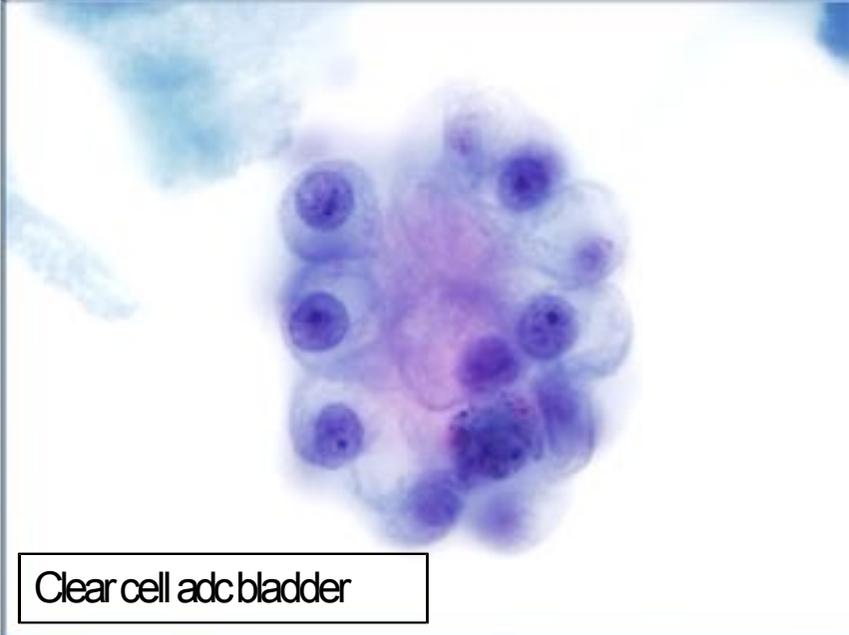
1. Negative for High Grade Urothelial Carcinoma
  2. Atypical Urothelial Cells
  3. Suspicious for High Grade Urothelial Carcinoma
  4. High Grade Urothelial Carcinoma
  5. Low Grade Urothelial Neoplasm
  6. Other malignancies, both primary and secondary
- 

## Other Malignancies Primary and Metastatic and Miscellaneous Lesions

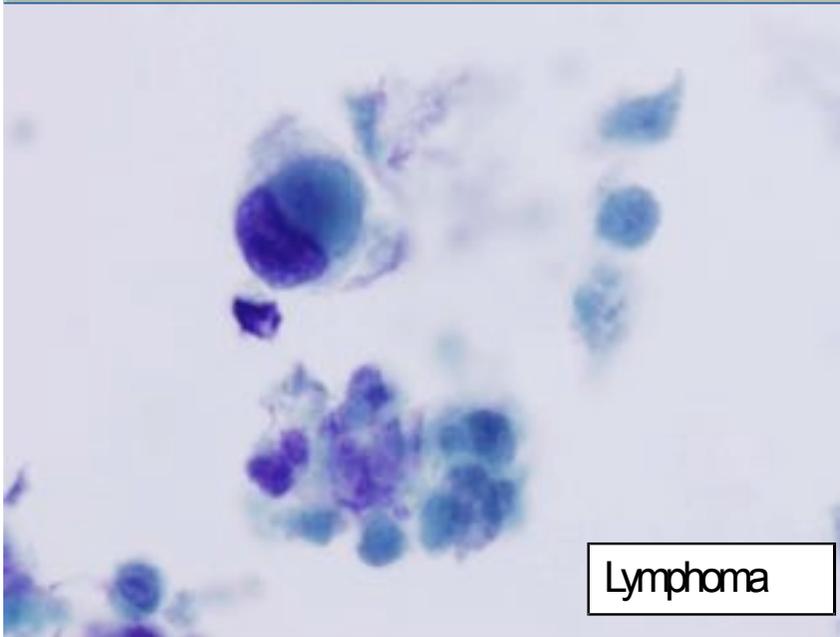




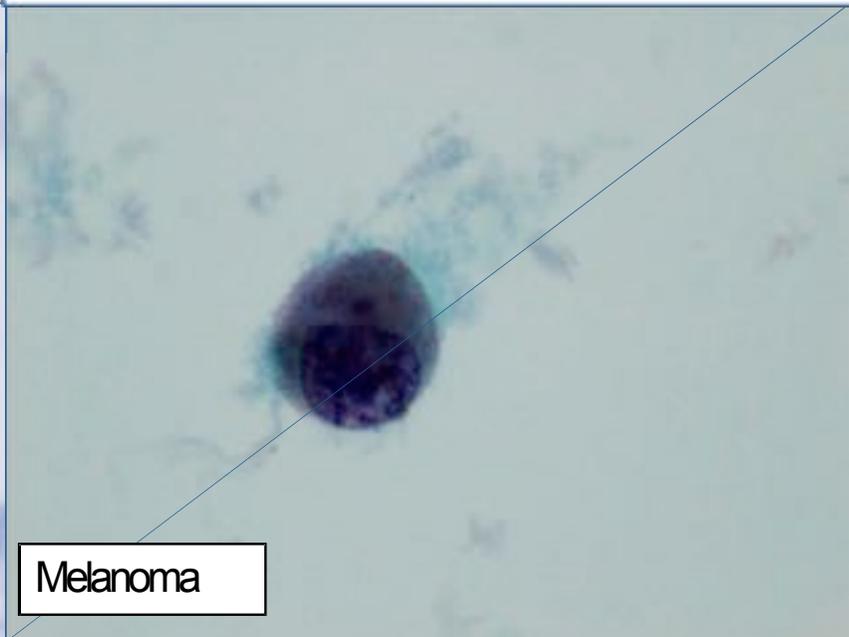
ADC



Clear cell adc bladder



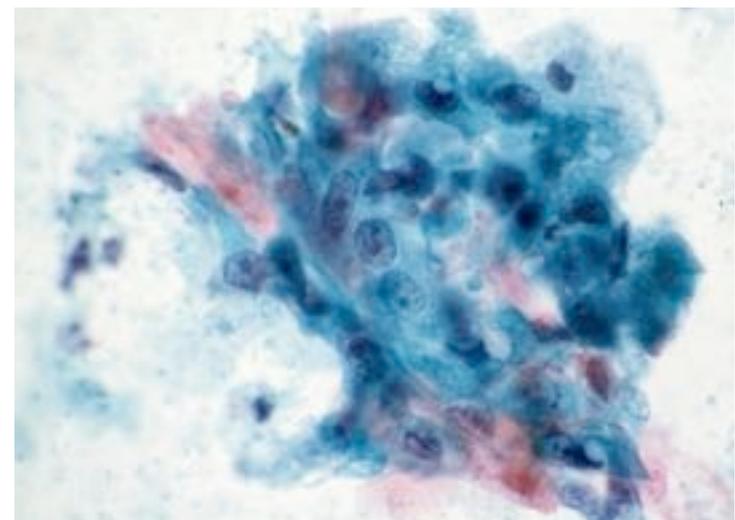
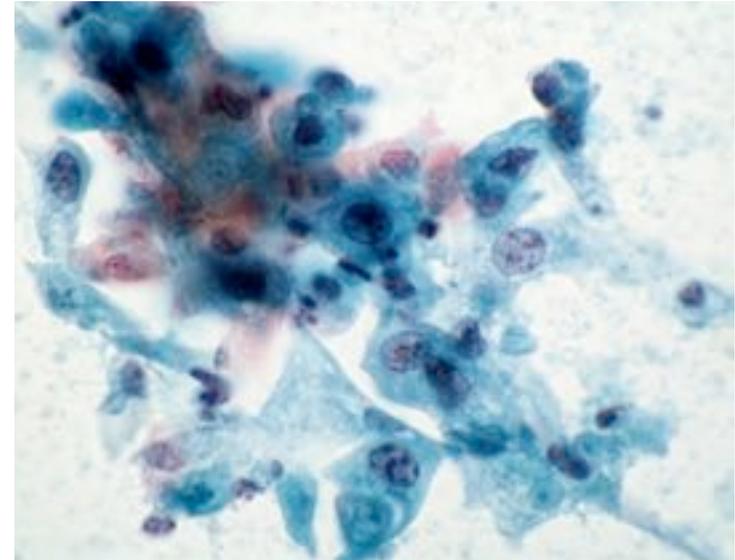
Lymphoma



Melanoma

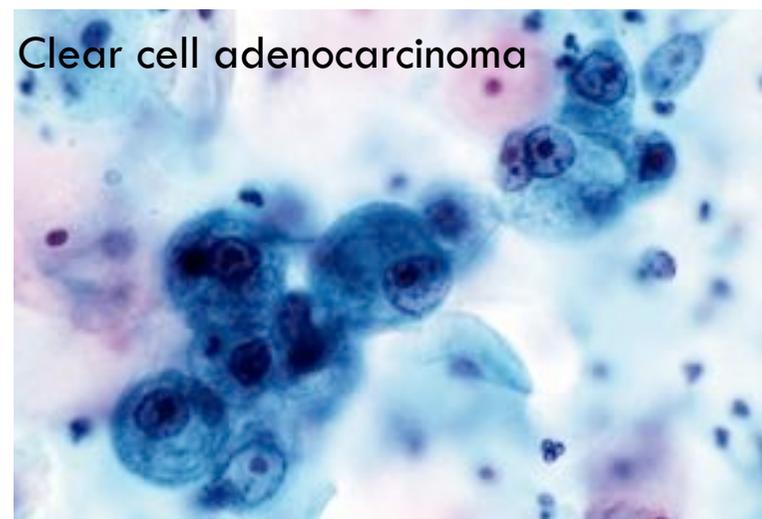
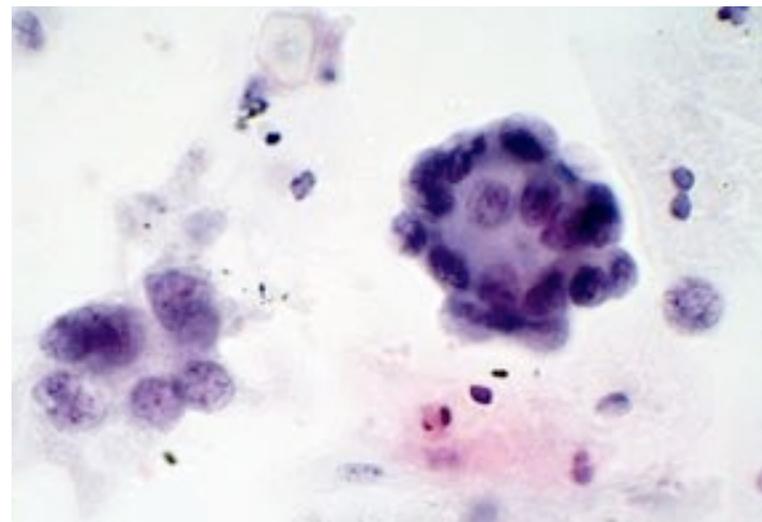
# Squamous Cell Carcinoma

- ❑ 5% of bladder cancers
- ❑ Pure squamous cell carcinoma rare – associated with calculi, diverticuli, schistosomiasis
- ❑ Squamous differentiation in UC
- ❑ Cytoplasmic keratinization
- ❑ Hyperchromatic angulated nuclei



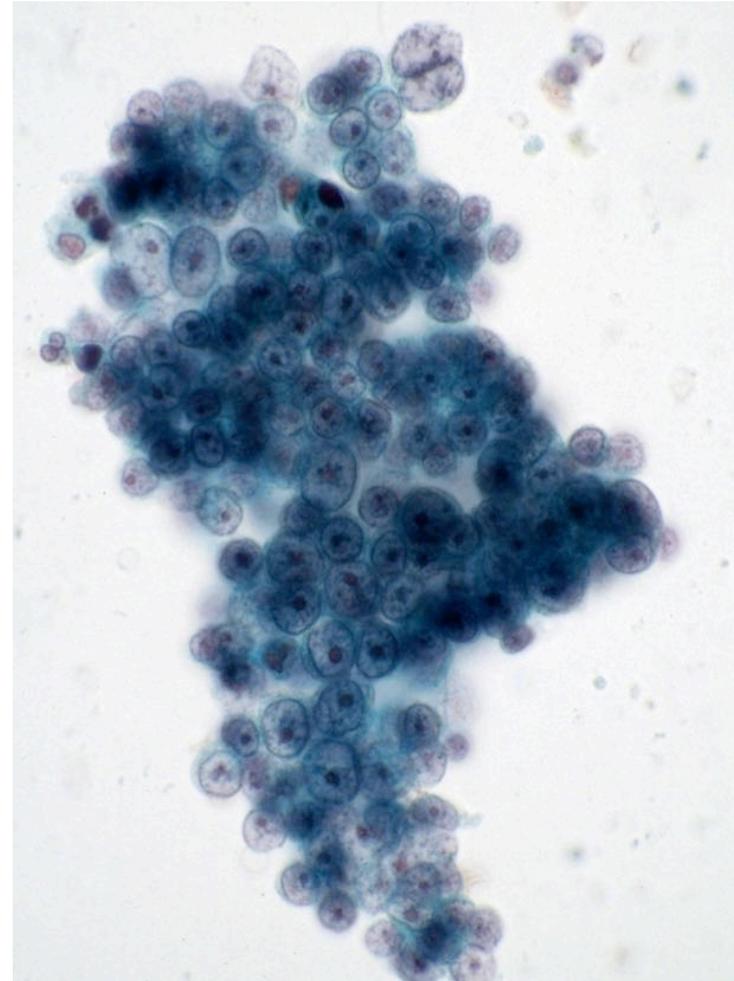
# Primary Adenocarcinoma

- ❑ Rare, <2% of bladder cancer
- ❑ Colonic type, most common
- ❑ Signet ring type
- ❑ Clear cell adenocarcinoma

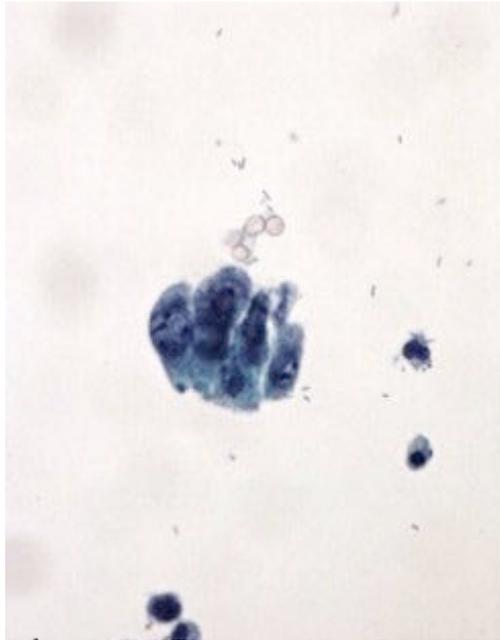


# Secondary Tumors

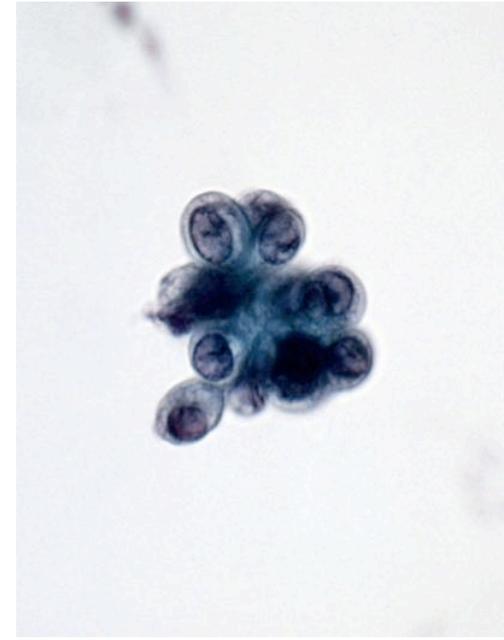
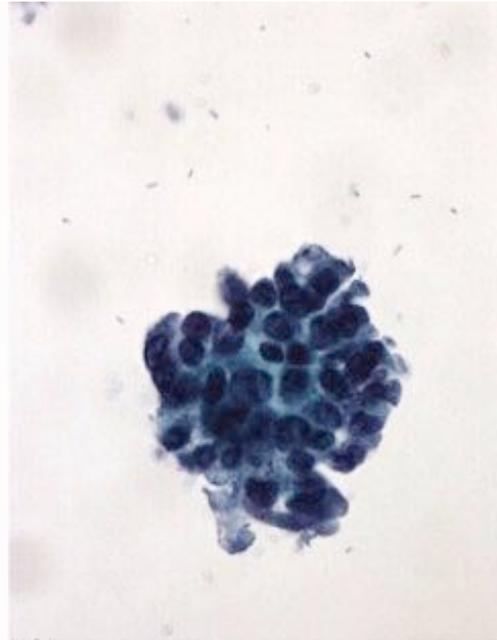
- ❑ Prostatic Adenocarcinoma
  - ❑ Seen in high-grade prostatic carcinoma
  - ❑ Large cohesive three dimensional clusters with ill-defined cell borders
  - ❑ Prominent nucleoli with relatively abundant cytoplasm
  - ❑ Dark nuclei resembling UC
  - ❑ History helpful !



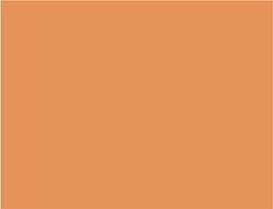
# Secondary Tumors



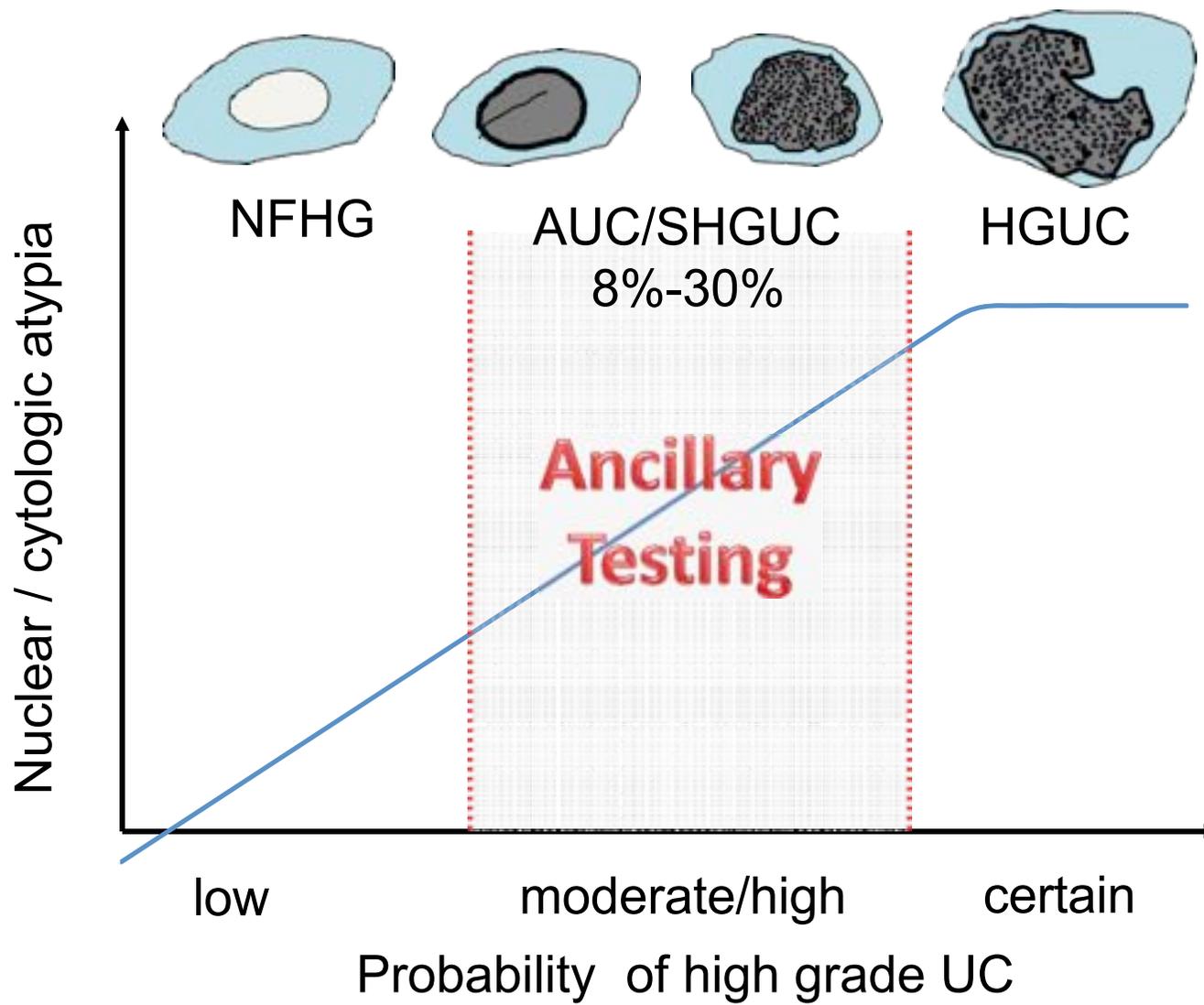
Colonic Adenocarcinoma



Endometrial  
Adenocarcinoma



# ANCILLARY TECHNIQUES



# Ancillary Urine Based Techniques

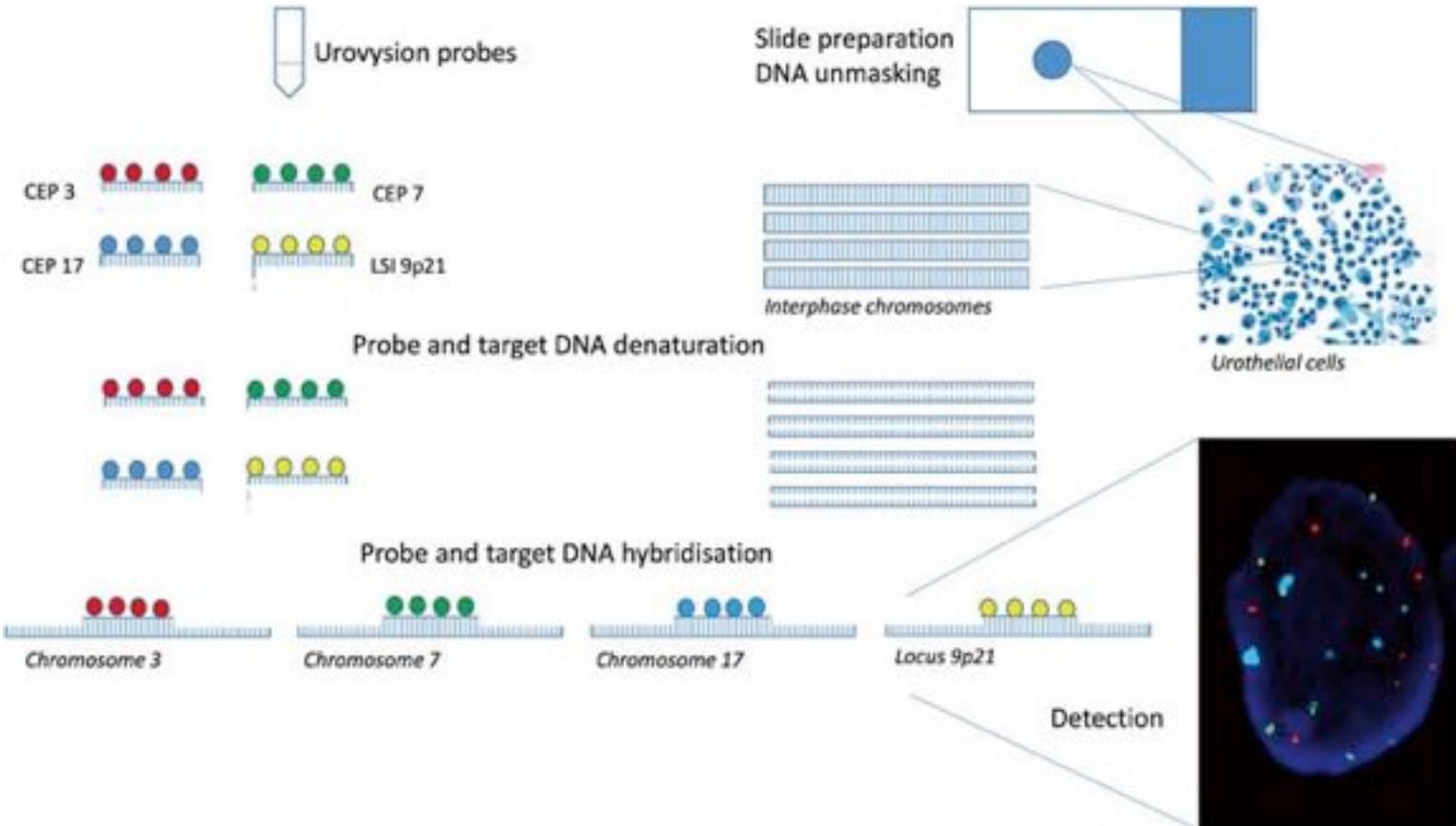


- ❑ DNA ploidy
- ❑ Bladder Tumor Antigen (Bard BTA stat<sup>®</sup>)
- ❑ Nuclear Matrix Proteins (NMP22<sup>™</sup>)
- ❑ UroVysion<sup>™</sup>
- ❑ ImmunoCyt/uCyt<sup>™</sup>
- ❑ Telomerase
- ❑ Hyaluronic Acid Hyaluronidase
- ❑ Fibrin-Fibrinogen Degradation Product

# UroVysion

- ❑ Chromosomal abnormalities in UC first described in 1990s
- ❑ Initial studies tested single chromosome probes
- ❑ Suklova *et al* published first study with multiple probes (10 probes tested)
- ❑ Highest sensitivity achieved with combination of 4 probes
  - ❑ Chromosome 3 (CEP)
  - ❑ Chromosome 7 (CEP)
  - ❑ Chromosome 17 (CEP)
  - ❑ Chromosome 9p21 (LSI probe)
- ❑ Sensitivity: 84%    Specificity: 92%
  - ❑ Cutoff: 5 abnormal cells

# Ancillary Studies in Urinary Cytology



# UroVysion



- ❑ Multicolor multitarget FISH UroVysion test approved by FDA in 2001
- ❑ Approved Indications:
  - ❑ Surveillance of patients with bladder cancer
  - ❑ Detection of bladder cancer in persons with hematuria suspected of having bladder cancer
- ❑ Meta-analysis of several studies by Hajdinijak
  - ❑ Sensitivity (72%) ; Specificity (83%)
- ❑ Targeted-UroVysion (CK7 immunophenotyping followed by UroVysion) improves diagnostic efficiency

# Summary



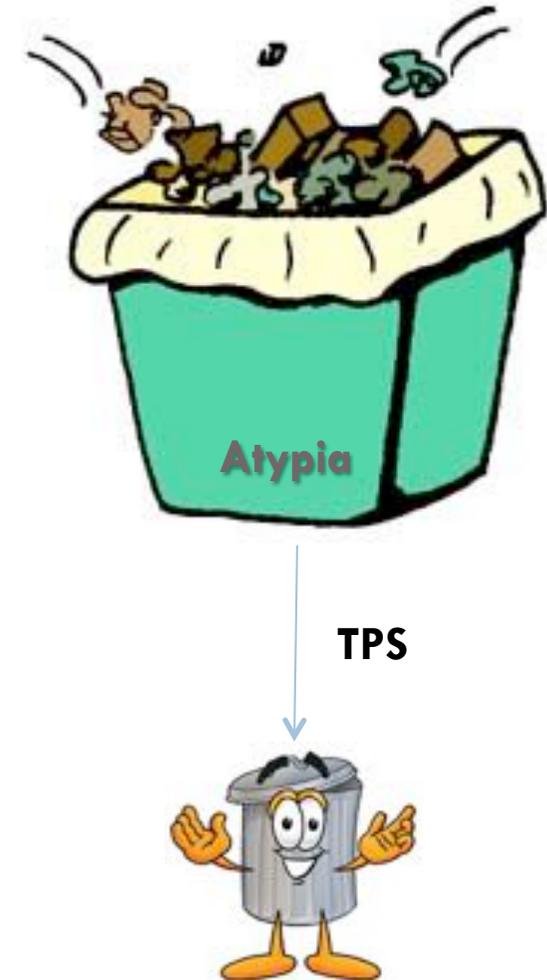
- ❑ Most urine specimens are negative
- ❑ Diagnosis of low-grade UC remains challenging due to overlapping features with reactive atypia
- ❑ Urine cytology has high accuracy for high-grade lesions
- ❑ FISH (UroVysion) more sensitive than cytology in detection of UC but produces more false positive results. Data suggest its use as a reflex test following equivocal cytologic diagnosis
- ❑ Upper tract urinary samples including FISH should be interpreted with reserve due to higher false positive rate

# FISH vs. Cytology

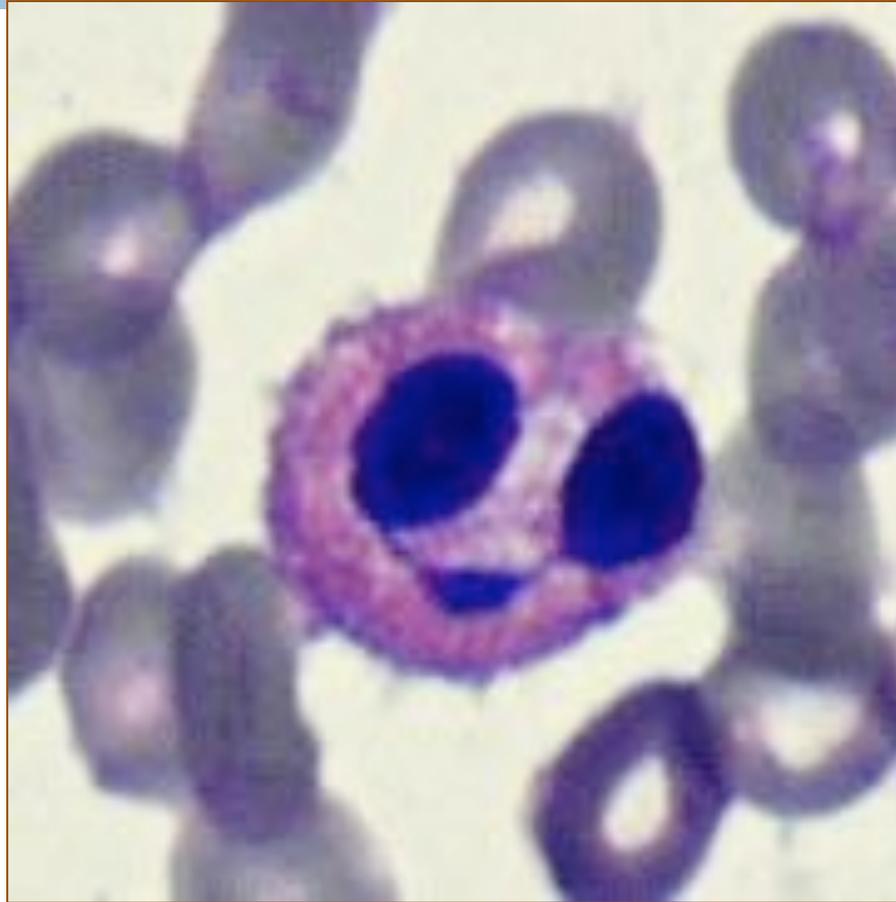
- FISH more sensitive but less specific than urine cytology
- PPV of urine cytology in HGUC > 90%
  - ▣ PPV of FISH: as low as 50%
  - ▣ Cytology = 7-10 times cheaper (Murphy 2009)
  - ▣ Combined FISH & Cytology
    - 98% sensitivity and > 95% specificity
- FISH-neg patients (low risk) may be allowed extended time intervals between cystoscopies

# Final take home message

- ❑ HGUC – this is the one that matters –  
Negative for HGUC
- ❑ The diagnosis “atypia” should not be used as  
a waste basket and dx should be based on  
criteria
- ❑ LGUN – new diagnostic category, based on  
presence of fibrovascular cores
- ❑ Not all malignant cells in urines are urothelial  
carcinoma
- ❑ Future studies are needed for validation of  
TPS

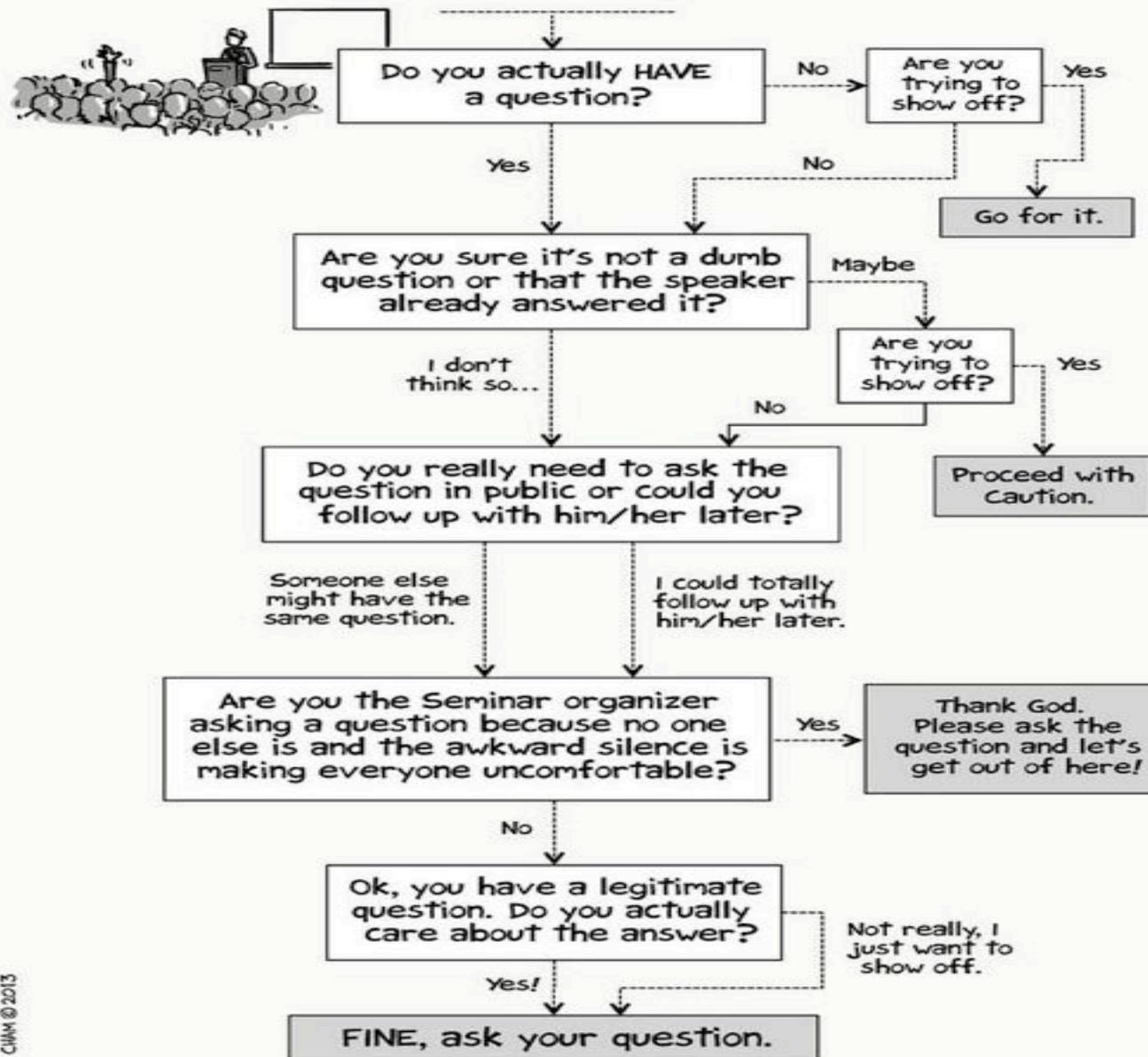


Thank you for listening!



Any questions?

# Should you ask a Question during Seminar?



# References

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