Urine Cytology
Challenges and Pitfalls

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Introduction
• Majority of UT malignancies are urothelial CA
• The main function of urine cytology is DX of UC
• Focus on a practical approach to DX of UC
  – Low grade urothelial neoplasms
  – High grade Urothelial CA
  – Other neoplastic, infectious and reactive processes addressed as they arise in the Diff DX
• “Atypical/Suspicious” urine cytology & potential pitfalls
  • Diagnostic categories and specimen adequacy
  • Role of Ancillary techniques in detection of UC

Indications
1. Establish Dx in symptomatic patients-hematuria
   – Most common, low yield (5-10% malignancy)
2. Screen high risk patients (exposure to industrial chemicals, metals, etc.)
3. Follow-up patients with Hx of UC
   • Complementary to cystoscopy and biopsy: detect small and hidden lesions (diverticuli, ureters, renal pelvis)
   • Urine cytology is the most reliable method for detecting urothelial CIS (> biopsies)
Types of Specimens

- **Voided urine**
  - Clean catch (avoid 1st morning specimens)
  - Poor preservation
- **Catheterized urine**
  - Not contaminated by genital epithelium
  - Poor preservation
- **Washings/Brushings**
  - Superior to voided urine - good preservation and cellularity
  - Localized - may not sample upper urinary tract and urethra
- **Ileal-conduit urine**

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**Urinary Tract Histology**

- **Superficial cell layer**
  One cell thick, size of superficial squames or larger, multinucleated
- **Intermediate cell layer**
  Approximately 5 cell layers, size of parabasal cells
- **Basal cell layer**
  One cell thick, cuboidal-columnar

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**Urinary Tract Histology**

- **Ureter and renal pelvis**
  Lining cells are larger, more pleomorphic than bladder (decreased cell turnover & exfoliation)
Normal Urinary Tract Cytology

• *Superficial urothelial cells*
  – Marked variation in size and shape (10-150 μ), low N/C
  – Often polygonal
  – Abundant pale cytoplasm, well defined borders
  – Occasional vacuolization
  – Round-oval nuclei, often multinucleated

Normal Urinary Tract Cytology 2

• *Deep urothelial cells*
  – Uniform in shape and size (10-20 μ)
  – Scant to moderate dense cytoplasm, distinct borders, fine vacuolization
  – Central nuclei, finely granular chromatin, small nucleoli

Normal Urinary Tract Cytology 3

*Squamous cells*
  – Metaplasia in urethra and bladder trigone
  – Contaminant from female genital tract (most common)

*Columnar Cells*
  – Rare in voided urine (Instrumentation)
  – Cystitis glandularis
  – Intestinal metaplasia
  – Prostate origin, renal tubules
  – GIT, female GT
  – Neoplasia

*Red Blood Cells*
Normal Urinary Tract Cytology

- Voided urine is variably cellular, single cells
- Catheterization or washings have many clusters

Specimen Adequacy in Urine Cytology

- Meaningful criteria not established - Arbitrary
  - Renshaw (in Cytology, Cibas and Ductman 2009)
    - Must have at least one urothelial cell
    - Absence of urothelial cells (except ileal conduit) = ND,
      - vaginal cells only, blood only, obscuring inflam.
    - Marked degenerative changes

Specimen Adequacy in Urine Cytology²

- Murphy (Bastacky et al, Cancer Cytopath 1999)
  - At least 15 deep urothelial cells (washings)
  - 5 deep cells (voided urine)
  - Only for patients followed for UC
Urinary Tract Malignancy

- Urothelial carcinoma, 80-90%
  - Non-invasive (papillary), 80%
  - Invasive
- Squamous cell carcinoma (5%)
- Adenocarcinoma (2%)
- Mixed Carcinoma- UC (5%)
- Small Cell Carcinoma (1%)

Diagnostic Accuracy of Urine Cytology

- **Number of Specimens**
  - Voided urine on 3 consecutive days
  - 50% accuracy (1 specimen)
  - 75-90% accuracy (3 specimens)

- **Patient Population**
  - High risk and history of CA

- **Tumor Grade**
  - HG UC: > 90 %
  - LG UC: <50 %

Grading Systems for Papillary UC

<table>
<thead>
<tr>
<th></th>
<th>1940 Ash</th>
<th>1973 WHO</th>
<th>2004 WHO/ISUP Urinary cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Papilloma</td>
<td>Papilloma</td>
<td></td>
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</tbody>
</table>
Criteria for Diagnosis of Neoplasms in Urine Cytology

<table>
<thead>
<tr>
<th></th>
<th>Low grade</th>
<th>High grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact with urine flow</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Shed cells</td>
<td>Few</td>
<td>Many</td>
</tr>
<tr>
<td>Cells sufficiently abnormal or</td>
<td>No</td>
<td>Marked</td>
</tr>
<tr>
<td>different form normal urothelial cells</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PUNLMP and Papilloma

- **PUNLMP**: Papillary structures lined by benign appearing urothelium with increased thickness
- **Papilloma**: normal thickness

PUNLMP and Papilloma 2

- **PUNLMP**: Recurrence = 25-45%
- Lacks capacity to invade or metastasize
- < 8% progression to HG UC
- **PUNLMP** and **Papilloma** are very difficult to recognize by cytology and would frequently escape detection (WHO/ISUP 2004)
Low Grade Urothelial CA

- Predominately papillary
- Capacity to invade (<20%)
- Rarely metastasizes
- Progression < 15%

Low Grade Urothelial CA

- Cytologic diagnosis of LGUC is problematic
  - Minimal shedding of neoplastic cells
  - Subtle cytologic alterations, difficult to distinguish from reactive changes, i.e. stones, instrumentation
  - Cytologic overlap between PUNLMP and LGUC (some cases indistinguishable)
  - Whisnant, 2003: No discriminating cytologic features between PUNLMP and LGUC, in 86 specimens

Cytology of LG Urothelial CA

- Generalized disagreement about accuracy of cytologic Dx and which criteria are most useful
  - Wide range of Sensitivity 0-73%
  - Overall sensitivity for LG UC 25-40%
Cytology of LG Urothelial CA
Murphy, AFIP Fascicle 1, 2004

- Readily recognized by experienced observers
  - Loose clusters
  - High N/C ratio
  - Extreme eccentricity of nuclei
  - Irregular nuclear borders
  - Finely granular chromatin with even distribution
  - Homogeneous cytoplasm
- All features rarely present in all cases and all cells

Cytology of LG Urothelial CA
Renshaw in Cytology. Diag principles and clinical correlates 2009

- Lumps PUNLMP and LG UC into one category
- Architectural
  - Papillary fragments
  - Irregular cell clusters
- Cytologic
  - ↑ N/C ratio
  - homogeneous cytoplasm
  - irregular nuclear membranes
- Above criteria are not specific
- Diagnostic sensitivity 30%, specificity 80%

LG Urothelial CA vs. Reactive changes
Raab et al. Cancer 1994

- Examined 82 bladder wash specimens (33 LG UC + 49 benign)
- Stepwise logistic regression analysis
- Architectural features not statistically significant
LG Urothelial CA vs. Reactive changes

Raab et al. Cancer 1994

• 3 key cytologic criteria useful for discriminating LG UC from reactive changes
  1. N/C ratio
  2. Irregular nuclear membrane
  3. Non-vacuolated (homogeneous) cytoplasm

• All 3 features present in only ½ of cases
• At least 2 key criteria = Sensitivity 85%, Specificity 96%
• ?? Value in voided urine
• Bladder washing- LGUC: ocean of cells

• voided urine- LG UC

Differential Diagnosis of LG Urothelial CA

• Reactive/reparative changes
• Instrumentation effect
• Lithiasis
• Upper urinary tract sampling
### Reactive/reparative Changes vs. Low Grade Urothelial CA

<table>
<thead>
<tr>
<th></th>
<th>Reactive</th>
<th>Low Grade UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architecture</td>
<td>tightly cohesive clusters</td>
<td>papillary clusters, crowded, loose</td>
</tr>
<tr>
<td>N/C</td>
<td>slight increase</td>
<td>moderate increase</td>
</tr>
<tr>
<td>Nucleus</td>
<td>central, smooth membrane</td>
<td>eccentric, slight irregular</td>
</tr>
<tr>
<td>Chromatin</td>
<td>finely granular, lg nucleoli nucleoli</td>
<td>finely granular, small</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>vacuolated</td>
<td>homogeneous</td>
</tr>
<tr>
<td>Background</td>
<td>inflamed or clean</td>
<td>clean</td>
</tr>
</tbody>
</table>

- Reactive changes and repair

### Instrumentation Effect

- Catheterized urine and bladder wash specimens
- Large pseudopapillary groups and 3D clusters
- Nuclear overlap and crowding
- Low N/C ratio
- Finely granular chromatin with even distribution
- Well defined cytoplasmic borders
- Nuclear palisading at periphery of clusters with abundant cytoplasm (cytoplasmic collar)
LG Urothelial CA vs. Instrumentation

- Cytologic criteria that showed statistical significance in descending order: bladder wash

<table>
<thead>
<tr>
<th>LGUC</th>
<th>Non-neoplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous cytoplasm</td>
<td>Vacuolated cytoplasm</td>
</tr>
<tr>
<td>↑ N/C ratio</td>
<td>Smooth border of cluster</td>
</tr>
<tr>
<td>Eccentric nuclei</td>
<td>Cytoplasmic collar</td>
</tr>
<tr>
<td>Irregular nuclear membrane</td>
<td>Abundant acute and chronic inflammation</td>
</tr>
<tr>
<td>Haphazard overlap of nuclei</td>
<td>Mixture of small and large urothelial cells</td>
</tr>
<tr>
<td>Ragged border of clusters</td>
<td></td>
</tr>
<tr>
<td>Nuclear hyperchromasia and enlargement</td>
<td></td>
</tr>
<tr>
<td>Nuclear pleomorphism</td>
<td></td>
</tr>
</tbody>
</table>

Chu et al, 2002
How Long is Cytology Abnormal after Cystoscopy?

- Evaluated 48 patients
- Examined urine before, immediately after, 1, 2, 7, 14 and 28 days
- Instrumentation effect was transient, mostly disappearing within 1 day after cystoscopy

McVey et al. BJU INT, 2004

Lithiasis

- Papillary clusters common
- Smooth bordered clusters
- Centrally placed nuclei, smooth nuclear membranes, finely granular chromatin
- Hyperchromatic smudgy nuclei (degenerative changes)
- Multinucleated giant cells

Lithiasis 2

- Inflammation & debris in background may be misinterpreted as tumor diathesis
- May be impossible to distinguish from PUNLMP or LGUC
- Occasionally there is cytologic atypia including nuclear pleomorphism, coarsely granular chromatin, mitotic figures → false-positive diagnosis of HG UC
**Lithiasis**

- Important source of false positive Dx for LG or HG UC
- Clinical history not reliable: filling defect in upper UT → stone vs. neoplasm
- Persistent atypical features (weeks) → aggressively worked up for neoplasia

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**Upper Urinary Tract specimens**

- Direct sampling of upper UT is effective in detecting HG UC, but poor for low grade lesions
- Normal upper UT epithelium shows more atypia than lower UT and occasionally more than LGUC
- High N/C ratio, enlarged nuclei, nuclear membrane irregularities
- Often present in papillary clusters
- Almost impossible to distinguish low grade UC from upper tract benign changes

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**Renal pelvis/ureter brushing**

- Negative cystoscopy, biopsy & followup
High Grade Urothelial CA

- Often invasive, 70% mortality
- 90% of pts dying of disease present initially with HGUC
- Can not reliably separate CIS from invasive HGUC
- High diagnostic accuracy of cytology
  - Sensitivity 80-90 %
  - Specificity > 95%

HGUC

- Disorganized clusters and single cells

Pleomorphic bizarre cells, enlarged eccentric nuclei, coarse chromatin and large nucleoli
**Differential Diagnosis of HG UC**

- Human polyoma viral infection
- Therapy effect
- Degenerative and reactive changes
- Upper urinary tract specimens
- Stones

**Human Polyomavirus**

- DNA virus (Papova)
- Immunocompromised and healthy individuals
- Important cause of allograft failure in renal transplant recipients
- Decoy Cells- infected nuclei:
  - Smudgy
  - Washed out
  - Reticulated

**Polyoma Virus**

- Diff DX is degenerated HG UC

<table>
<thead>
<tr>
<th></th>
<th>Polyoma virus</th>
<th>HG UC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Architecture</strong></td>
<td>Single cells</td>
<td>Single cells &amp; clusters</td>
</tr>
<tr>
<td><strong>Nuclear membrane</strong></td>
<td>Smooth, round</td>
<td>Marked irregularity</td>
</tr>
<tr>
<td><strong>Chromatin</strong></td>
<td>Uniform, smudgy, reticulated</td>
<td>Coarsely granular</td>
</tr>
</tbody>
</table>
Polyoma

HGUC

Therapy Effect

- Cytoxan & Busulfan
  - Systemic treatment of non urothelial malignancies
  - Hemorrhagic cystitis
  - Severe cytologic atypia may be indistinguishable from CA
  - Atypia more bizarre than usual HGUC
  - Atypia often has degenerative features

Photo from Modern Cytopathology.
Elsevier Science, 2004

Therapy Effect 2

Thiotepa & Mitomycin C
  - Intravesical treatment of superficial UC
  - Repair-like changes

BCG Vaccine
  - Treatment of CIS
  - Granulomatous inflam. and mild atypia

Radiation Change
  - Extreme cytomegaly, multinucleation, but low N/C ratio

Photo from Murphy WM. Urinary Cytopathology. ASCP Press, 2000
Potential Pitfalls

- **False Positive Diagnosis (1.3-15%)**
  - Instrumentation, stones, palpation
  - Chemo-Radiation therapy changes
  - Viral changes
  - Upper urinary tract specimens
  - Reactive/degenerative changes

Possible Additional Explanations of “False Positives”

- CIS sloughs easily in urine but maybe non-diagnostic on histology = False-FP
- Neoplasia may involve hidden inaccessible sites = False-FP
- Upper UT specimens (ureter, renal pelvis) are most difficult to interpret → overcall = True FP

Management of “False Positive” Cytology

- Positive cytology should be confirmed histologically before definitive treatment
- Positive cytology requires close follow-up
- Malignancy is found on follow up of most patients with positive cytology and negative initial clinical evaluation
Potential Pitfalls ²

• False Negative Diagnosis
  – Low grade UC
  – Renal cell and prostatic CA
  – Inadequate specimen
  – Degenerated cells

Renal Cell CA
• Urine Cytology plays no significant role in diagnosis
• Malignant cells are either shed late in course of disease or ND
• Cells often degenerated & indistinguishable from b9 renal tubular or urothelial cells
• Single cells, clusters, large eccentric nuclei, vacuolated granular cytoplasm

Prostate Carcinoma
• Almost always in pts with known Hx of advanced disease
• Easier to recognize WD carcinoma: abundant cytoplasm, large prominent nucleoli
• PD carcinoma difficult to distinguish from HGUC
Atypia in Urine Cytology
and
Diagnostic Categories

Diagnostic Categories
• JH created a template similar to Gyn BS:
  1. Negative
  2. AUC-US
  3. AUC-H
  4. LG neoplasm
  5. HG neoplasm
  6. Non-diagnostic

Rosenthal, cancer cytopath 2013

Diagnostic Categories
• JH created a template similar to Gyn BS:
  1. Negative for HGUC
  2. Suspicious for HGUC
  3. Positive for HGUC

  1. Negative
  2. AUC-US (26%)
  3. AUC-H (5%)
  4. LG neoplasm
  5. HG neoplasm
  6. Non-diagnostic

Rosenthal, cancer cytopath 2013
Should We Eliminate the “Atypical” Category?

- 10-20% of urines classified as “atypical”
- Considerable inter-observer variability among pathologists as what constitutes atypia
- Most urologists interpret “atypia” as negative or unhelpful

Arguments for Not Eliminating “Atypia”

- Significant proportion of malignant cases would be missed if “atypia” was eliminated
  - Malignant rate on FU: 23-68%
- Ancillary studies such as FISH can be helpful in those cases
- Dr. Wojcik

“Atypical” Urine Cytology

- Don't make “atypia” a waste basket
- May qualify “atypical” to R/O LG neoplasm
- “Atypical” diagnoses should not be used for reactive/reparative changes → Negative
- Don’t use “suspicious” terminology to R/O LG neoplasms
Diagnostic Categories- CC

- Negative
- Atypical
  - R/O LGUC /PUNLMP
- Suspicious
  - for HG UC/ malignancy
- Positive
  - HG UC or other malignancies

Total Urines at Cleveland Clinic
2008-2010 (17,800 cases)

Urine Dx’s Categorized by Pathologist
BMH, Indiana 2009
Common Sources of “Atypical” Urine Cytology

1. Papillary clusters in voided urine
2. Mild nuclear atypia- most common
3. Degenerated large atypical nuclei
4. Coy cells

1. Clusters in voided urine

- Papillary clusters are not associated with increased risk of neoplasia
  - >3 clusters in ThinPrep → high sensitivity, poor specificity 
  (Deshpande & Mckee, Cancer Cytopathol, 2005)
- Should place less reliance on presence or shape of clusters
  - Exception - fibrovascular core
- More emphasis on nuclear features: single cells, irregular enlarged nuclei, homogeneous cytoplasm

Not Atypical

- Deep urothelial cells and instrumentation effect
2. Mild Nuclear Atypia

Mild Nuclear Atypia

LGUC

Benign

LGUC
3. Degenerative Changes

Atypical Benign

Radiation

Degenerated large atypical nuclei
4. Coy Cells

- Suspicious finding
- Opposite of “decoy cells”
  - Often sparse in number
  - Been compared to “litigation cells” on Paps
  - Small cells with hyperchromatic irregular nuclei
  - India ink/coal black nuclei

Suspicious for HGUC/Malignancy

- Studied 58 pts for features predictive of HGUC
- 4 features were recognized in AUH:
  - Hyperchromasia, marked
    - obscuring nuclear detail
  - Irregular nuclear borders
  - Increased N/C ratio
  - Anisonucleosis, striking: often > 3:1 ratio

(VandenBussche, 2013)

Suspicious for HGUC²

- Hyperchromasia by far the strongest predictor for HGUC, even in absence of other features
- These features were predictive of HGUC in surveillance pts but not hematuria pts- stone atypia

(VandenBussche, 2013)
Management of Suspicious Urine Cytology

- Differs from “atypical” specimens (regarded as neg)
- Patients with persistent suspicious cytology or recurrent hematuria need further evaluation and follow up
- Patients with suspicious cytology and negative initial evaluation should have repeat urine cytology at 6-8 weeks

Ancillary Tests for Detecting & Monitoring UC

Surveillance of Patients with Hx of UC

- Sensitivity of cytology for HG cancer is > 90%
- Overall sensitivity for LGUC is unacceptably low: 25-40%
- Current standard of care:
  - Cystoscopy + cytology every 3-6 months for first year
  - reduced intervals subsequently
Detecting & Monitoring UC

- 75% of bladder cancers are superficial UC
- 50-70% of superficial UC recur after resection, and approx. 15% progress to invasive disease
- Clinical behavior of UC is difficult to predict, so frequent surveillance is necessary

Detecting & Monitoring UC 2

- Currently, urine cytology & cystoscopy are Gold Standards for detection of recurrent UC
- Limitations of cytology in detecting LGUC & invasive nature of cystoscopy → search for highly sensitive and specific non-invasive UC tumor markers

Ancillary Tests for Detecting & Monitoring UC

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity % (range)</th>
<th>Specificity % (range)</th>
<th>Lab</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine cytology</td>
<td>54 (35-68)</td>
<td>95 (83-100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA ploidy</td>
<td>62 (45-86)</td>
<td>89 (76-100)</td>
<td>IA, FCM</td>
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<tr>
<td>BTA</td>
<td>60 (32-100)</td>
<td>77 (40-96)</td>
<td>POC, Ref</td>
<td>False +</td>
</tr>
<tr>
<td>NMP22</td>
<td>67 (47-81)</td>
<td>72 (60-86)</td>
<td>Ref</td>
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</tr>
<tr>
<td>ImmunoCyt</td>
<td>50-100</td>
<td>69-79</td>
<td>Ref</td>
<td>False +</td>
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<tr>
<td>Telomerase</td>
<td>74 (62-93)</td>
<td>79 (60-99)</td>
<td>Ref</td>
<td>False +</td>
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<tr>
<td>Microsatellite Analysis</td>
<td>83-95</td>
<td>83-100</td>
<td>Ref</td>
<td></td>
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<tr>
<td><strong>FISH</strong></td>
<td>69-87</td>
<td>85-97</td>
<td>Ref</td>
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</tbody>
</table>
**FISH (UroVysion)**

- A 4-probe set that targets the common chromosomal abnormalities in UC
- **FISH+ results:**
  - Polysomy 3, 7, 17
    - Gain of 2 or more chromosomes
    - Seen mostly in HGUC, but not LGUC
  - Deletion 9p21
    - Seen in LGUC

**FISH 2**

- FDA approved for surveillance of patients with hematuria and history of UC
- Recommended in hematuria pts with other risk factors such as smoking hx and age > 45
- High sensitivity 69-93%, esp. for HGUC, lower for LG UC
- Lower specificity than urine: 50-85% vs. > 93%

**FISH 3**

**Impressive Sensitivity results:**

- Surveillance UC patients:
  - FISH +/- cystoscopy/- cytology- \(\rightarrow\) 65% recurrent CA (within 29 months)
  - FISH- \(\rightarrow\) 13% recurrent CA
- Hematuria surveillance:
  - 30% of pts are FISH+/cytology- \(\rightarrow\) 60% UC
- Post BCG therapy
  - FISH+ approx 10 times more likely to develop invasive cancer
**False-Positive FISH**

- Be careful about significance of FISH+ in upper tract cytology (not FDA approved for that purpose)
  - Limited value for upper tract tumor surveillance
  - High false + (Johannes, J Urol. 2010)
- Polyoma virus can cause false + FISH (approx 15%)
  - Usually in pts with high viral titers (renal transplant)

**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

**Dogs Sniff Out Cancer**

  - Dogs correctly identified urine from cancer patients: 41% success rate vs. 14% chance alone
  - Suggested that tumor-related volatile compounds are present in urine imparting a characteristic odor
Summary

• Urine cytology best applied to HGUC
• Cytology less helpful for detecting and monitoring LG neoplasms
  – Not major limitation
  – LG neoplasms rarely aggressive and can be readily detected by cystoscopy

Summary

• Ancillary studies are currently not recommended for routine applications
  – Can’t distinguish between aggressive and non-aggressive cancers
  – May act as adjunct to cytology in certain patients
  – Markers may detect lesions before they are clinically relevant
• Cystoscopy and histology remain as the gold standard for detecting primary and recurrent UC

Subclassifying Atypical Urinary Cytology

• Cell clusters in voided urine ➔ Negative
• Increased NC ratio without nuclear membrane irregularity ➔ Negative
• Increased NC ratio with nuclear membrane irregularity ➔ Atypical, R/O LGUC
• Poorly preserved cells/smudgy nuclei
  – Nuclear membrane not intact ➔ Negative
  – Intact and irregular nuclear membrane ➔ Atypical or Susp. for HGUC

Modified from Renshaw 2009
Is there a doctor -or dog- in the house?