Urine Cytology

Diagnostic Categories and Atypia

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Outline

• Indications and diagnostic accuracy of urine cytology
• Atypia in urine cytology
• Diagnostic categories
• The Paris system classification 2015
Introduction

• Majority of UT malignancies are UC
  – Urothelial carcinoma, 80-90%
  – Mixed Carcinoma- UC (5%)
  – Squamous cell carcinoma (5%)
  – Adenocarcinoma (2%)
  – Small Cell Carcinoma (1%)

• The main function of urine cytology is to diagnose urothelial carcinoma (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
4. 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)
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**
  • HGUC: > 90 %
  • LGUC: <50 %
Atypia in Urine Cytology
Diagnostic Categories

- JH created a template similar to Gyn TBS:
  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 TBS:
  1. Negative
  2. AUC-US (26%)
  3. AUC-H (5%)
  4. LG neoplasm
  5. HG neoplasm
  6. Non-diagnostic

- Cleveland Clinic
  1. Negative
  2. Atypical (14%)
  3. Suspicious for HG UC (2%)
  5. Positive

Rosenthal, cancer cytopath 2013
Diagnostic Categories Preferred by Urologists

1. Negative for HGUC
2. Suspicious for HGUC
3. Positive for HGUC
Should We Eliminate the “Atypical” Category?

- Approx 10-20% of urines classified as “atypical”
- Considerable inter-observer variability among pathologists as to what constitutes atypia
- Currently, 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
Variations in Atypical rate

• **Inter-institutional:**
  – Reported wide variation: 2-31%

• **Intra-departmental:**
  • ≈ 23,000 urine cases signed out by 12 cytopathologists at CC, over 3 yr period
    - All were cytopathology board certified
  • Variable experience ranging from 2-26 yrs

*Reynolds, Elsheikh, 2015*
• Wide variation in Atypical rate: 8-28% (avg 14%)
• Higher rates are not related to level of experience
• More dependent on individual threshold for atypia
Urine Dx’s Categorized by Pathologist
BMH, Indiana 2009

- % Negative
- % Atypical
- % Suspicious
- % Malignant
In Need of Standardization!

- Standard classification and terminology system
- Well defined and reproducible diagnostic criteria
- Uniform inter- and intra-departmental communications
- Consistent prognostic and management information leading to optimal patient care
The Paris System for Reporting Urinary Tract Cytology
Mission of the *Paris System* for Reporting Urinary Cytology:

Acknowledging that the target of urinary cytology is High Grade Urothelial Carcinoma (HGUC), we can potentially eliminate low grade urothelial carcinoma (LGUC) from our diagnostic menu unless the sample is a tissue equivalent, complete with fibrovascular stalk, a rare occurrence. The working group for the Paris System will define diagnostic categories based on pathologic evidence, testing cytomorphologic criteria for reproducibility as well as accuracy. *Our mission is to create a Cytologic System that is highly reliable as a non-invasive tool to determine those patients who need immediate cystoscopy vs. those who can be followed at an interval based upon risk stratification of their diagnostic category.*
TPS Diagnostic Categories

- Negative for HGUC
- Atypical Urothelial Cells
- Suspicious for HGUC
- High Grade Urothelial Carcinoma
- Low Grade Urothelial Neoplasm
- Other malignancies, both primary and secondary
Urinary Tract Histology

- **Superficial cell layer**
  one cell thick, superficial squames-size or larger, multinucleated

- **Intermediate cell layer**
  approximately 5 cell layers, parabasal-size cells

- **Basal cell layer**
  one cell thick, cuboidal-columnar
Ureter and Renal Pelvis

- Lining cells are larger and 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

- **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

- Voided urine is sparsely cellular, single cells-degeneration
Catheterization or washings specimens have many clusters.
TPS Diagnostic Categories

- Negative for HGUC
- Atypical Urothelial Cells
- Suspicious for HGUC
- **High Grade Urothelial Carcinoma**
- Low Grade Urothelial Neoplasm
- Other malignancies, both primary and secondary
High Grade Urothelial CA

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

Non-superficial and non-degenerated (viable) urothelial cells

- High N/C ratio > 0.5-0.7 (required)
- Hyperchromasia, moderate-severe (required)
  - and one of the following
- Irregular clumpy chromatin
- Irregular nuclear membranes

At least 5-10 abnormal cells
- Based on pathologist’s level of comfort
- Voided vs. upper tract instrumented specimen
• Single cells and/or disorganized clusters
• Irregular, hyperchromatic nuclei
- Pleomorphic bizarre cells, enlarged eccentric nuclei, coarse dark chromatin
TPS Diagnostic Categories

• Negative for HGUC
• Atypical Urothelial Cells
• **Suspicious for HGUC**
• High Grade Urothelial Carcinoma
• Low Grade Urothelial Neoplasm
• Other malignancies, both primary and secondary
Suspicious for HGUC - TPS Criteria

• Same criteria as HGUC:
  − Viable deep urothelial cells
  − High N/C ratio > 0.5-0.7
  − Marked Hyperchromasias
  − Irregular clumpy chromatin
  − Irregular nuclear membranes

• Less than 5-10 abnormal cells
  − Based on pathologist’s level of comfort
  − Voided vs. upper tract instrumented specimen
Coy Cells

- Suspicious for **HGUC**
- 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
Differential Diagnosis of HGUC

- Human polyoma viral infection
- Therapy effect
- Stones and reactive changes
- Other malignancies
Human Polyomavirus

- DNA virus (Papova)
- Immunocompromised and healthy individuals
- Important cause of allograph 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, clumped</td>
</tr>
</tbody>
</table>
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
**Therapy Effect**

**Thiotepa & Mitomycin C**
- Intravesical Rx of sup UC
- Repair-like changes

**BCG Vaccine**
- Treatment of CIS
- Granulomas, mild atypia

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

Photo from Murphy WM. Urinary Cytopathology. ASCP Press, 2000
Lithiasis

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

- Inflammation & debris in background may be misinterpreted as tumor diathesis
- May be impossible to distinguish from LGUC
- Occasionally marked cytologic atypia, including nuclear pleomorphism, coarsely granular chromatin, mitotic figures $\rightarrow$ false-positive diagnosis of HGUC
Lithiasis

- Important source of false positive Dx for LGUC and HGUC
- Clinical history not reliable: filling defect in upper UT → stone vs. neoplasm
- Persistent atypical features (weeks) → aggressively worked up for neoplasia
TPS Diagnostic Categories

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• Other malignancies, both primary and secondary
Mild Nuclear Atypia

- Single cells with enlarged and irregular nuclei (no significant hyperchromasia)
- Most common and most frustrating
Atypical Urothelial Cells (AUC)-TPS

Definition:

1. Atypia that falls short of “Suspicious” or “HGUC”
2. Degenerative changes where nature and degree of atypia cannot be explained
AUC - TPS Criteria

1. Non-degenerated, Non-superficial urothelial cells

- High N/C ratio > 0.5 (required) and one of the following:
  - Hyperchromasemia, mild-moderate
    - Compared to benign urothelial or squamous cell nuclei
  - Nuclear Irregularity, significant
  - Irregular clumpy chromatin, mild
• Nuclear irregularity, high NC ratio, but no significant hyperchromasia, compared to benign urothelial cells
High NC ratio, mild hyperchromasiasia

High NC ratio, nuclear irregularity
AUC- TPS Criteria

2. Degenerated non-superficial urothelial cells

- High N/C ratio and hyperchromasias
- Extensive degeneration of nuclei and/or incomplete cytoplasm
AUC- Exclusions

• **Mere presence of degeneration does not equate to AUC**

• **Excludes atypia secondary to known conditions:**
  • Polyoma virus, stones, reactive/repair, therapy, instrumentation, etc.
Negative

Lost nuclear membrane

AUC

Intact nuclear membrane, hyperchromatic
TPS Diagnostic Categories

- Negative for HGUC
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- Suspicious for HGUC
- High Grade Urothelial Carcinoma
- **Low Grade Urothelial Neoplasm**
- Other malignancies, both primary and secondary
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
  - No discriminating cytologic features between PUNLMP and LGUC
  - Wide range of sensitivities 0-73% (Avg 25-40%)

Whisnant, 2003
Mcroskey 2015

- Compared biopsy proven LGUC cytologies (98 cases) to negative cytologies (53 cases)
- Instrumented urine specimens
- Evaluated 17 published cytologic features
- All cases were examined blinded to histology
- No single cytologic feature was found to be helpful in DDX, except for papillary clusters with fibrovascular cores (2/98 cases)
Few cells with enlarged slightly irregular nuclei.
Clusters in voided urine

- Papillary clusters (without fibrovascular core are not associated with increased risk of neoplasia
- Should place less reliance on presence or shape of clusters
- More emphasis on nuclear features

(Deshpande & Mckee, Cancer Cytopathol, 2005)
Low Grade Urothelial Neoplasm - TPS Criteria

- 3D papillary clusters (extreme nuclear overlapping) with fibrovascular cores - very rare

- Diagnosis should also be qualified as Neg for HGUC
• Tight papillary clusters with atypia and extreme nuclear overlapping or ocean of cells- instrumented
May consider LGUN in presence of mass or correlated LGUN biopsy- Neg for HGUC
Differential Diagnosis of LG Urothelial CA

- Reactive/reparative changes
- Upper urinary tract sampling
- Instrumentation effect
- Lithiasis
• Reactive changes and repair
Upper Urinary Tract specimens

- Direct sampling of upper UT is effective in detecting HGUC, but poor for low grade lesions
- Sensitivity: LGUC 37% vs. HGUC 80% *Barkan 2015*
- Normal upper UT epithelium shows more atypia than lower UT and occasionally more than LGUC
  - N/C ratio, nuclear irregularities, papillary clusters
- Almost impossible to distinguish low grade UC from upper tract benign changes
• Negative cystoscopy, biopsy & followup
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)
ThinPrep

Instrumentation effect
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
TPS Diagnostic Categories

• **Negative for HGUC**
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• High Grade Urothelial Carcinoma
• Low Grade Urothelial Neoplasm
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Negative for HGUC-TPS Criteria

- If there is a known cause for “atypia” - it’s Negative
  - Reactive urothelial cells
  - Instrumentation effect
  - Upper urinary tract specimens
  - Changes associated with lithiasis
  - Polyoma viral cytopathic effect
  - Post-therapy effects
  - Clusters without fibrovascular cores or atypia
Negative for HGUC - TPS Criteria

• This category also includes “LGUN”

Sample Dx:
- Negative for HGUC
- Changes consistent/suggestive of LGUN
Nuclear / cytologic atypia

- Low
- Moderate/High
- Certain

AUC-Suspicious: 8%-30%

Probability of high grade UC

Slide courtesy of D. Rosenthal, MD
## 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>
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<tr>
<td>DNA ploidy</td>
<td>62 (45-86)</td>
<td>89 (76-100)</td>
<td>IA, FCM</td>
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<td>BTA</td>
<td>60 (32-100)</td>
<td>77 (40-96)</td>
<td>POC, Ref</td>
<td>False +</td>
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<tr>
<td>NMP22</td>
<td>67 (47-81)</td>
<td>72 (60-86)</td>
<td>Ref</td>
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<tr>
<td>ImmunoCyt</td>
<td>50-100</td>
<td>69-79</td>
<td></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>
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<tr>
<td>Microsatellite Analysis</td>
<td>83-95</td>
<td>83-100</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>FISH</td>
<td>69-87</td>
<td>85-97</td>
<td>Ref</td>
<td></td>
</tr>
</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

- 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
- ? FISH positive AUC treated as Susp/ HGUC
Impressive Sensitivity results:

• Surveillance UC patients:
  – FISH +/ cystoscopy-/ cytology- $\rightarrow$ 65% recurrent CA (within 29 months)
  – FISH- $\rightarrow$ 13% recurrent CA

• Hematuria surveillance:
  – FISH+/cytology- (30%) $\rightarrow$ 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
  – 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
Summary

- Urine cytology is 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
Dogs Sniff Out Cancer

- **Willis et al, British Medical Journal 2004:**
  - Dogs correctly identified urine from cancer patients: 41% success rate vs. 14% chance alone (Pathologist sensitivity for Dx of LGUC 25-40%)
  - Suggested that tumor-related volatile compounds are present in urine imparting a characteristic odor
Summary

- “Atypical” diagnoses should not be used for reactive/reparative changes → Negative for HGUC
- TPS provides strict cytologic criteria and aims to establish a standardized practical approach to urinary cytology classification
Is there a pathologist-or dog- in the house?
Thank You!