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

- ~76,900 new cases in 2016 in the USA
- ~16,390 deaths due to bladder cancer
- 4th most common cancer in men and 9th in women (1 in 44 people)
- 9th most common cause of cancer death (F>M)
- ~75% non-muscle invasive bladder cancers (superficial bladder cancers), Ta, Tis, T1
- ~30% to 70% recurrence
- ~5% to 15% progression (<1% LG Ta)
- >535,000 people in the US are survivors of this cancer
- Highest per patient cost from dx to death of all cancers
- $4.1 billion/year spent to tx bladder cancer

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.

Urin cytologi
Cytologisk undersøkelse av eksfolierte cancerteller 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 suspekte 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 hydret for å sikre flest mulig bevarte celler i urinen (ungå cytolyse). En prøve tatt under pågående makroskopisk hematuri kan være vanskelig å tolke.

Urin cytologi er mest pålitelig for påvising av svulster av høy malignitetsgrad og CIS. Svuister med lav malignitetsgrad gir positiv urin cytologi i langt færre tilfelle. Tolknin av det cytologiske preparat kan være problematisk av flere grunner: Lavt cellettall, degenerative/irritative forandringer (for eksempel steinsykmom) og terapiinduserte forandringer (BCG, stråleterapi). Det er derfor viktig at man anvender et laboratorium med erfaring i cytologisk vurdering og gir adegvate kliniske opplysninger til patologen (70) (evidensgrad C).
The urinary tract – the basics

Upper urinary tract

Lower urinary tract
Histology of the Urinary Bladder

Lumen of bladder
Transitional epithelium
Lamina propria

Muscular layer (Detrusor muscle)

Adventitia (with fat cells)

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

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

Transitional epithelium
Basement membrane Lamina propria
Benign nuclei: oval, with nuclear groove, point towards top (normal polarity) and not hyperchromatic

- Umbrella cells
- Intermediate cells
- Basal cells
- Urothelium
- Lamina propria
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

<table>
<thead>
<tr>
<th>Relative composition of plasma and urine in normal men</th>
<th>plasma g/100 ml</th>
<th>urine g/100 ml</th>
<th>concentration in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>90–93</td>
<td>95</td>
<td>—</td>
</tr>
<tr>
<td>protein</td>
<td>7–8.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>potassium</td>
<td>0.02</td>
<td>0.15</td>
<td>×7</td>
</tr>
<tr>
<td>calcium</td>
<td>0.01</td>
<td>0.015</td>
<td>×1.5</td>
</tr>
<tr>
<td>magnesium</td>
<td>0.0025</td>
<td>0.01</td>
<td>×4</td>
</tr>
<tr>
<td>chloride</td>
<td>0.37</td>
<td>0.6</td>
<td>×2</td>
</tr>
<tr>
<td>phosphate</td>
<td>0.003</td>
<td>0.12</td>
<td>×40</td>
</tr>
<tr>
<td>sulfate</td>
<td>0.003</td>
<td>0.18</td>
<td>×60</td>
</tr>
<tr>
<td>ammonia</td>
<td>0.0001</td>
<td>0.05</td>
<td>×500</td>
</tr>
</tbody>
</table>

Not the perfect cell preservation medium!!
Clinical Indications of Urine Cytology

- Hematuria
- Follow-up of patients treated for Urothelial Carcinoma (UC)
- High-risk of bladder cancer

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

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

- RBC Cast
- Renal Tubular Cast
- Corpora Amylacea
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....
**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

- 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
Urethral Brushings
Urethral Brushings
Ileal Conduit

- Surveillance of ureters and renal pelves 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
Urine contemplation

Avicenna, physician and philosopher (980 – 1037), advocated systematic analysis of urine:

- Colour
- Density
- Sediment – calculus, abscess or tumor
- Odor – tumor

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 test whether dogs can distinguish between patients with bladder cancer and healthy controls and the percentage of correctly identified bladder cancer cases. Participants 61 patients (mean age 72.6 years) with histologically confirmed bladder cancer were recruited. Design Encoded urine samples were presented to each dog for formal test. Results All dogs were able to correctly identify the urine sample of 1 in 7 (14%) patients. Conclusions Although these anecdotal results remain unsupported by formal statistical analysis, they suggest that dogs may be able to detect bladder cancer.

Although these anecdotal results remain unsupported by formal statistical analysis, they suggest that dogs may be able to detect bladder cancer.
The age of uroscopy...

Historic medical practice of visually examining a patient's urine for pus, blood, or other symptoms of disease.
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).

<table>
<thead>
<tr>
<th>#</th>
<th>Answer</th>
<th>Bar</th>
<th>Response</th>
<th>%</th>
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<tbody>
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<td>I agree with this statement</td>
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<td>127</td>
<td>85%</td>
</tr>
<tr>
<td>2</td>
<td>I disagree with this statement</td>
<td></td>
<td>22</td>
<td>15%</td>
</tr>
<tr>
<td>3</td>
<td>Comments/Suggestions:</td>
<td></td>
<td>18</td>
<td>12%</td>
</tr>
</tbody>
</table>
The move to standardise...
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 example:

Irregulære, degenererte urotelceller af usikker betydning....
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

- **Stage Tis (in situ)**: Affects the lamina propria.
- **Stage Ta**: Affects the epithelium.
- **Stage T1**: Affects the lamina propria.
- **Stage T2a**: Affects the superficial muscle.
- **Stage T2b**: Affects deep muscle.
- **Stage T3a**: Affects surrounding fat/tissue (microscopically).
- **Stage T3b**: Affects surrounding fat/tissue or peritoneum.
- **Stage T4**: Affects nearby or distant organs.

Diagram showing the layers of the bladder and the locations of each stage.
Pathogenesis of Urothelial Carcinoma

Eva M. Wojcik and Stefan E. Pambuccian

Papillary Pathway

80%

Normal Urothelium

Hyperplasia

Genetically Stable FGFR3 (~85%)

Low Grade Carcinoma

RAS (?)

Recurrence

High Grade Carcinoma

Non-Papillary Pathway

20%

9p-, 9q-
p16

Dysplasia

Genetically Unstable p53 (~60%)

Carcinoma in situ

<10%

Invasive Carcinoma
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
“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”? 
Mr. Smith - You have a bladder cancer
What really matters?

High Grade Urothelial Carcinoma
Diagnostic Categories

HGUC

Everything

Hope

Positive

Negative

Atypical/Suspicious

Reality
Classifications

WHO 1973

Papilloma

Grade I

Grade II

Grade III

Papilloma

PUNLMP

Low Grade

High Grade

WHO/ISUP 2004

~ 10-20%

~ 50-60%

~ 80-90%

URINE CYTOLOGY SENSITIVITY

Very high probability that we are going to be wrong
### Evolution of the Classification

<table>
<thead>
<tr>
<th>Cytologic Classification</th>
<th>Histologic Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papanicolaou 1947&lt;sup&gt;5&lt;/sup&gt; (Papanicolaou Classification System)</td>
<td>Hopkins Template&lt;sup&gt;6&lt;/sup&gt;</td>
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<tr>
<td>Koss 1985&lt;sup&gt;10&lt;/sup&gt;</td>
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<tr>
<td>Murphy 1984&lt;sup&gt;11&lt;/sup&gt;</td>
<td>Layfield et al 2004&lt;sup&gt;13&lt;/sup&gt; (Papanicolaou Society of Cytopathology)</td>
</tr>
<tr>
<td>Ooms &amp; Veldhuizen 1993&lt;sup&gt;12&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Benign cells, ATY 1 cells, few clusters</td>
</tr>
<tr>
<td>II</td>
<td>Clusters, nuclear elongation, few ATY 2 cells</td>
</tr>
<tr>
<td>III</td>
<td>Malignant tumor cells, many ATY 2 cells</td>
</tr>
<tr>
<td>IV</td>
<td>Suspicious</td>
</tr>
<tr>
<td>V</td>
<td>TCC, grade 2</td>
</tr>
<tr>
<td></td>
<td>Papilloma</td>
</tr>
<tr>
<td></td>
<td>TCC, grade 3</td>
</tr>
<tr>
<td></td>
<td>Papilloma</td>
</tr>
<tr>
<td></td>
<td>PUNLMP</td>
</tr>
<tr>
<td></td>
<td>LGUC</td>
</tr>
<tr>
<td></td>
<td>HGUC</td>
</tr>
<tr>
<td></td>
<td>TCC, grade 3</td>
</tr>
</tbody>
</table>

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.

Owens et al. Cancer Cytopathology 2013
NEW paradigm

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

AUC \rightarrow SHGUC \rightarrow HGU

- LGUN – Low Grade Urothelial Neoplasm
Surprisingly little data...


Table 1. Prospective study.

<table>
<thead>
<tr>
<th>Cellularity</th>
<th>Sensitivities</th>
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<tr>
<td></td>
<td>AUC+</td>
</tr>
<tr>
<td>&lt;10 per 10 hpfs</td>
<td>60.5</td>
</tr>
<tr>
<td>≥10 per 10 hpfs</td>
<td>95.2</td>
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<tr>
<td>$P$ value</td>
<td>0.0001</td>
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<tr>
<td>&lt;20 per 10 hpfs</td>
<td>68.3</td>
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<tr>
<td>≥20 per 10 hpfs</td>
<td>100.0</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Volume is important...

Adequacy of Urine Specimens (Adequacy)
Let's 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

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
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 $\rightarrow$ Negative for High Grade Urothelial Carcinoma

How about these?
What is Atypia

- Positive Suspicious
- Atypical
- Negative
High-Grade Urothelial Carcinoma in Urine Cytology With Jet Black and Smooth or Glassy Chromatin

Andrew A. Renshaw, MD and Edwin W. Gould, MD

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 (250 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 (≥50 abnormal cells), and frequent necrosis. Cancer Cytology 2018;126:6-8. © 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, although this can vary widely. The Paris System for Reporting Urinary Cytology proposes a standardized terminology that only seeks to diagnose high-grade...
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

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 h-
Negative for High Grade Urothelial Carcinoma
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

Atypia → Suspicious → Positive
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???
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.”

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; Dorothy L. Rosenthal, MD; and Christopher J. VandenBussche, MD, PhD

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 “suspicous for high-grade urothelial carcinoma” (SHSUC) 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 SHSUC altered patient management among nonurologists. METHODS: Specimens signed out as AUC-H or SHSUC 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 (34% vs 53%; P < 0.001) when the specimens were ordered by nonurologists versus urologists, despite having similar rates of high-grade urothelial carcinoma on follow-up biopsy (67% vs 66%). When specimens ordered by nonurologists were diagnosed as SHSUC, these patients received more follow-up (100%) compared with those whose specimens were diagnosed as AUC-H (44%; P < 0.001). 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 < 0.001). CONCLUSIONS: Use of the word “suspicous” 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.
<table>
<thead>
<tr>
<th></th>
<th>Follow-Up, %&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Biopsy, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC-H, nonurologist</td>
<td>39.5% (17/43)</td>
<td>14% (6/43)</td>
</tr>
<tr>
<td>SHGUC, nonurologist</td>
<td>100% (15/15)</td>
<td>60% (9/15)</td>
</tr>
<tr>
<td></td>
<td>&lt;.0001</td>
<td>&lt;.0005</td>
</tr>
<tr>
<td>AUC-H, urologist</td>
<td>NA</td>
<td>73% (344/473)</td>
</tr>
<tr>
<td>SHGUC, urologist</td>
<td>NA</td>
<td>75% (197/261)</td>
</tr>
<tr>
<td>P (AUC-H vs SHGUC), urologist</td>
<td>NA</td>
<td>.43</td>
</tr>
</tbody>
</table>
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
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
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)
How about these???

Negative for HGUC

Suggestive of LGUN
Approach to Diagnosis in Urinary Tract

Are there fibrovascular cores?
- No
- Yes

Cytologic atypia present?
- No
- Yes

Mild

Degree of atypia?
- Severe

Reason for mild atypia? (treatment etc.)
- Yes
- No

Quantity of atypical cells?
- Rare, <5-10 cells
- Many

Negative

LGUN

Atypical

Suspicious HGUC

Positive HGUC

G. Barkan, MD
Nuclear: Cytoplasm Ratios

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

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

![Graph showing rate of atypia from 2008 to 2016]
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
Melanoma  

ADC  

Lymphoma  

Clear cell adc bladder  

Melanoma
Squamous Cell Carcinoma

- 5% of bladder cancers
- Pure squamous cell carcinoma rare – associated with caliculi, 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

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
Nuclear/cytologic atypia

- NFHG
- AUC/SHGUC: 8%-30%
- HGUC

Probability of high grade UC

Low
Moderate/High
Certain

Ancillary Testing
Ancillary Urine Based Techniques

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

Do you actually HAVE a question?

Are you trying to show off?

Are you sure it’s not a dumb question or that the speaker already answered it?

I don’t think so...

Do you really need to ask the question in public or could you follow up with him/her later?

Someone else might have the same question.

Are you the Seminar organizer asking a question because no one else is and the awkward silence is making everyone uncomfortable?

Yes

Thank God. Please ask the question and let’s get out of here!

No

Ok, you have a legitimate question. Do you actually care about the answer?

Yes!

FINE, ask your question.

No

Not really, I just want to show off.

Go for it.

Are you trying to show off?

Yes

Proceed with Caution.

No

Maybe.
References


