Atypical/malignant urothelial and decoy cells – a cytologist‘s perspective

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UREX2015, Ljubljana, Sept 25 – 26, 2015
OUTLINE

• Aim of urinary cytology
• Cytopreparatory techniques
• Malignant cells
• Decoy cells
• Atypical cells
• Ancillary techniques
AIM OF URINARY CYTOLOGY
Urine cytology - and cytology of other fluid samples from urothelial tract

- Non-invasive test*
- Tumor detection and diagnosis
- Follow-up of patients after treatment of bladder tumors – detect recurrence or new primary (↑risk)
- Exceptionally for screening of high risk population

*compared to cystoscopy = gold standard
Hematuria

- Infection of urinary tract
- Bladder tumor (malignancy*)
- Urinary tract stones
- Benign prostate hyperplasia
- Prostate cancer
- .....and more

*painless hematuria*
Cytology samples

- Spontaneous/voided urine
- Bladder washing

Less common:
- Catheterised urine
- Retrograde catheterisation of ureters (renal pelvis)
- Urine from nephrostoma
- Neobladder (Bricker)

Illustr. From Koss’ Diagnostic Cytology

Urine ? Origo of cells: bladder, + renal pelvis, ureter, urethra
Spontaneous/voided urine

• Simple and cheap dg method
• Morning urine: most cells, least preserved (acid pH of urine!)
• Second morning urine
• 3 samples, 3 consequent days
• Prefixation? (2% polyethylene glycol (Carbowax)+50% to 70% ethanol)
Bladder washing

• First bladder emptied by catheter
• During or prior to cystoscopy
• Instilling / recovering 3-4x of 50 – 100 ml normal saline
• Cellular specimens of well preserved urothelium

• Prefixation? (2% polyethylene glycol (Carbowax)+50% to 70% ethanol)
CYTOPREPARATORY TECHNIQUES
Processing of fluid sample

- Centrifuge for 10 min → direct smear of sediment
- Membrane filtration, imprint
- Cytospin
- Liquid based cytology techniques

SAME PREPARATION METHOD FOR ANY FLUID SAMPLE FROM THE URINARY TRACT
Method – fixation and staining

Fixation:
• Alcohol based fixatives
• Spray (Carbowax/ethanol)

Staining:
• Papanicolaou
Exfoliated urothelial cells

- Cell morphology depends on sampling method and technique of processing
Cytology of urothelium

exfoliation

a – cells of superficial layer – umbrella cells

b- cells of intermediate layers

(c – cells of basal layer)
Cells in urine

- Urothelium
- Renal tubules
- Surrounding organs
- Macrophages, blood cells
Cells in urine

- Clean background
- Few Eri, Lci
- Scanty urothelial cell preservation?
- Squamous cells (F)
Bladder washing

- Numerous cells, single or in groups, (3D - dg mistakes)
- Umbrella cells
- Intermediate layer cells
MALIGNANT CELLS
Cytologic signs of malignancy

- Single cells, small groups, poor cohesion
- Variable size and shape - polymorphous
- \[ \uparrow \text{N/C ratio} \]
- Cytoplasm – ?perservation of cell
- Irregular nuclear shape
- Hyperchromasia, coarsely granular, unevenly distributed chromatin
- Mitoses
- Nucleoli, multiple, large

HIGH GRADE malignant urothelial cells

EASY CYTOLOGICAL DIAGNOSIS
HIGH GRADE
Invasive/infiltrating (non-papillary) urothelial carcinomas

*Cells with evident malignant morphology*

- Background: inflammatory cells, blood, necrosis (abundant)
- Single keratinized cells

- Urinary cytology: sensitivity $\sim 80-90\%$, specificity $\sim 100\%$
HIGH GRADE
Papillary (non-invasive) Urothelial Carcinomas, High Grade

*Cells with evident malignant morphology*

- Urinary cytology: sensitivity ~80-90%, specificity ~100%

Majority of former WHO grade III exfoliate evidently malignant cells
20-30% of former WHO grade II – atypia less pronounced
HIGH GRADE
Non-invasive: urothelial carcinoma - in situ

*Cells with evident malignant morphology*

**DD - invasive urothelial carcinoma**
- No necrosis, scanty erythrocytes, polymorphs (not reliable)

- Sensitivity of cytology for detection of CIS high (70-100%), specificity ~100%
LOW GRADE
Non-invasive Papillary Urothelial Neoplasms (papilloma, PUNLMP, former WHO grade I carcinoma)

- **Cytol signs of malignancy not obvious**
- Uniform cells
- Slightly ↑ nuclei (similar to benign changes)
- Chromatin bland, small nucleoli
- Clean background, Ery
- ?shape of groups (DD: instrumentation, inflammation, stones)
- **Fibrovascular cores**
Cytology of urothelial tumors

Urinary cytology not reliable for dg of LG papillary tu

Sensitivity for detection of LG papillary tumors low:
0-73% (or 16% and 39%)
Other types of bladder carcinoma

Squamous cell carcinoma

DD females: ca of genital tract
(urine? direct extension)
Other tumors of bladder

**Adenocarcinoma** –
primary rare

- Primary?
- Direct invasion - Prostate? Colorectal?
- Mestastasis?

PSA+
Tumors of upper urinary tract – renal pelvis and ureters

Basic investigation:
cells from spontaneous/voided urine

Cytology similar to bladder neoplasms
DECOY CELLS
Differential diagnosis of atypical/malignant cells:

- Malignant urothelial cells - urine
- Polyoma effect on urothelial cells - urine
...a person or thing used to mislead or lure someone into a trap
Decoy cells – by L. Koss&Ricci, 1950

- Infection with polyoma virus in childhood - almost universal
- (Re)activation
- Immuno compromised patients: after chemo, Tx, AIDS, diabetics
- No obvious reason! – 0,5-0,6% urines, can last weeks, months, no consequences for health
- .. in urothelial cells (BK strain)
Decoy cells - urine

Decoy cells - urine

• Single cells – immuno competent
• Numerous cells: immuno compromised – after Tx
Immunocytochemical staining

- Antibody detecting large T antigen of SV40
- “pan“ anti-polyoma antibody (BK, JC, SV40)
- Not all decoy cells +
ATYPICAL (UROTHELIAL) CELLS
Classification of urinary cytology

- Atypia
Goal of The Paris System, first proposed in 2013:

Cytology can **Find The High Grade Cancers**
Diagnostic Categories of TPS: 2014

• Adequacy Statement
• Negative for HGUC

- Benign urothelial and squamous cells
- Reactive urothelial cells
- Polyoma Viral Cytopathic Effect: **Decoy cells**
- Benign Urothelial Tissue Fragments (BUTF)
- Post-therapy effect
- Changes associated with lithiasis

To be published soon (Nov 2015!)
Post-therapy effect

- *Post irradiation*
- *After intravesical chemoth – mitomycin*
- *After immunotherapy with Bacillus Calmette-Guérin (BCG) = weakened Mycobacterium bovis*
Changes associated with lithiasis

- Numerous urothelial cells – abrasion/stones
- 3D bally or “papillary” groups
- Cell and nuclear atypia

- DD: ca + stones!!!
Atypical urothelial cells – TPS criteria

• N/C ratio of >0.5
• Nuclear hyperchromasia
• Irregular clumped chromatin
• Irregular nuclear membranes: shapes and thickness
Suspicious for HGUC – TPS criteria

- N/C ratio of at least 0.7
- Marked hyperchromasia
- Markedly clumped chromatin
- Deformed nuclear membranes
- Cells of HGUC but..
- Too few (<10), not well perserved!
ANCILLARY TECHNIQUES
Reliability of cytological diagnosis

- LG papillary tumors
- Other urothelial carcinomas
- Atypia/suspicious

Ancillary non-invasive techniques

CYTOLOGY BASED

- to avoid repeating (painful) cystoscopy for follow-up
UroVysion FISH Test

UroVysion™

to detect aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus via fluorescence in situ hybridization (FISH) in urine specimens from persons with hematuria suspected of having bladder cancer

(First approved by FDA only for surveillance of patients with bladder cancer)

http://www.urovysion.com/ ™ Abbott Molecul Inc., Des Plaines, IL, USA
UroVysion FISH Test

• High-grade UC
  • no additional benefit compared to cytology!

• Low-grade UC
  • increased sensitivity from 25% to 75%
  • usually visible by cystoscopy

• Main indications:
  • Atypical/suspicious urothelial cells
  • Control after intavesical BCG treatment
  • Upper urinary tract malignancy
  • Surveillance after transurethral resection
  • Hematuria in patients with increased risk of urothelial carcinoma
  • Note: large reactive cells can be aneuploid/tetraploid!

Bubendorf L, Acta Cytol 2011; Dimashkieh H et al. CCP. 2013
CONCLUSIONS

ADVANTAGE in diagnosis of:

• High grade (urothelial) tumors
• ... including urothelial carcinoma in situ
• Highest risk of morbidity and mortality

LIMITATION in diagnosis of:

• Low-grade papillary tumors
• Differentiation of LG papillary tumor from “space occupying lesions” of renal pelvis and ureter
• Low risk of morbidity and mortality
the Paris System for Reporting Urinary Cytology

www.cytopathology.org
www.cytology-iac.org

Courtesy of Prof. Dorothy Rosenthal et al.