The Role of Cytology in Detecting Urological Neoplasia

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Role of urine cytology

First line investigation of patients with haematuria, LUTS, unexplained recurrent UTIs.

Monitoring of patients following treatment of known urothelial carcinoma.

Advantages: Cheap, quick, non-invasive

Disadvantage: Low sensitivity for diagnosis of low grade UC
## Alternatives to urine cytology

<table>
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<th>Median sensitivity</th>
<th>Range (min–max)</th>
<th>No. pts.</th>
<th>Median specificity</th>
<th>Range (min–max)</th>
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<td>13–75</td>
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<td>94</td>
<td>85–100</td>
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</table>

The data applies to patients under surveillance for UCC, na not analyzed. See appendix for references.

Accuracy of urine cytology – the Oxford experience

Consecutive series of 778 patients referred to a one-stop haematuria diagnosis clinic.

Criteria for referral: at least one episode of macroscopic haematuria and age >40 years.

On the same day patients underwent examination by a nurse specialist followed by urine cytology, CTU and flexible cystoscopy.

After a follow-up of 21-66 months, 156/778 (20%) patients were diagnosed with urothelial carcinoma (UC) of bladder on rigid cystoscopy and biopsy.
Accuracy of urine cytology – the Oxford experience

Retrospective review of urine cytology reports.
Reports scored as:
0 = inadequate or no specimen
1 = benign
2 = atypical probably benign
3 = atypia of uncertain significance
4 = atypia suspicious of malignancy
5 = malignant.
Frequency of bladder cancer on follow-up

![Bar chart showing frequency of bladder cancer on follow-up based on urine cytology scores.](chart.png)
### Accuracy of urine cytology – the Oxford experience

<table>
<thead>
<tr>
<th>Cytology Score</th>
<th>Bladder UC</th>
<th>Upper tract UC</th>
<th>No UC</th>
<th>Total</th>
<th>% malignant</th>
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<tr>
<td>0</td>
<td>12</td>
<td>1</td>
<td>32</td>
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<td>467</td>
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<td>39</td>
<td>4</td>
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<td>86</td>
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<tr>
<td>Total</td>
<td>156</td>
<td>20</td>
<td>602</td>
<td>778</td>
<td>23</td>
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</table>
Is there a role for urine cytology?

There were no cases of histologically proven urothelial carcinoma for which voided urine cytology was positive and CTU and flexible cystoscopy were negative.

52 patients had abnormal cytology, despite normal cystoscopy and CTU and underwent:
- Repeat urine cytology
- Further imaging
- Cystoscopy under general anaesthetic with retrograde studies

Total cost £50,535
Causes of errors in urine cytology

361 urine cytology specimens with histological follow-up

Diagnostic assignments:
1. Benign
2. Atypical
3. Suspicious
4. Malignant

Cytology-histology discrepancies (≥2 step) in 208 (41%)

Retrospective review of cytology/histology slides.

Interpretation discrepancy - error in disease categorisation

Sampling discrepancy - the diagnostic lesion not present on slide

Causes of errors in urine cytology

49% of discrepancies resulted in harm (unnecessary further investigations, delayed diagnosis >1 month)

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Adjudicated Root Cause</th>
<th>Interpretation</th>
<th>Sampling</th>
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<tr>
<td></td>
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<td>Cytologic</td>
<td>Histologic</td>
</tr>
<tr>
<td>Voided (n = 164)</td>
<td></td>
<td>44</td>
<td>11</td>
</tr>
<tr>
<td>Instrumented lower tract (n = 35)</td>
<td></td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Upper tract (n = 9)</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (n = 208)†</td>
<td></td>
<td>57 (27.4)</td>
<td>16 (7.7)</td>
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</table>

Methods for reducing errors in urine cytology

Diagnostic standardisation

Use of “experts” – cytologists differ in frequency of errors

Reducing proportion of “atypical” reports, shifting to definitive diagnostic categories

Double slide reading with two or more blinded cytopathologists

Improve specimen handling and processing to reduce sampling errors – majority of interpretation errors occur in suboptimal specimens (degenerate/few tumour cells/obscuring debris)
Normal urine

Few urothelial cells
N:C ratio < 1:2
finely granular chromatin
Superficial / umbrella cells
  contain abundant cytoplasm,
  vacuolation +/-
  one or more nuclei, which may vary in size
  –fine chromatin
  –small nucleoli +/-
Normal urine

Squamous cells
more common in females
(vulvovaginal/trigone origin)
in males probably from terminal
urethra
generally superficial and
intermediate

Seminal vesicle cells
maybe accompanied by
spermatazoa
oval in shape, hyperchromatic
nuclei, brown cytoplasmic
pigment
Normal urine

Squamous cells
more common in females
(vulvovaginal/trigone origin)
in males probably from terminal
urethra
generally superficial and
intermediate

Seminal vesicle cells
maybe accompanied by
spermatozoa
oval in shape, hyperchromatic
nuclei, brown cytoplasmic
pigment
Normal specimen - instrumented

Catheterisation/ureteric washings & brushings

Cellular specimen, representation from deeper layers
rounded cell clusters with smooth cytoplasmic border
small sheets
in ureteric w&b : very large superficial cells,
Fine chromatin, regular nuclear outlines
Normal specimen – ileal conduit

poorly preserved intestinal cells, mucus, inflammatory cells
no. of intestinal cells variable, show marked degeneration with pyknotic nuclei, cytoplasmic eosinophilic degeneration
in late stages poor cellularity due to mucosal atrophy
General pitfalls

Overinterpreting cells with low N:C ratio as cancer cells
Mistaking papillary aggregates as a reliable sign of low grade neoplasia (remember - instrumentation, calculi, infection, crypts in trabeculated bladder)
Mistaking regenerative/reactive/reparative cells for cancer
Treatment-associated changes
radiotherapy, chemotherapy

Inflammation associated with calculi
Low grade urothelial carcinoma

Features suggesting low grade neoplasia:

- true papillary clusters with fibrovascular cores
- increased cellularity
- increased N:C ratio, irregular nuclear outlines, cytoplasmic homogeneity
Pitfalls in diagnosis of low grade UC

Degenerate urothelial cells
  pyknotic nuclei, evidence of karyorrhexis
cytoplasmic eosinophilic inclusions
Instrumentation effects
Calculi
  cellular sample with papillary groups, inflammatory cells, RBCs
history helpful!
High grade UC & CIS

Increased cellularity
Poorly cohesive sheets, dispersed cells
Enlarged nuclei, densely hyperchromatic
Invasive lesions - ‘dirty’ necrotic background, inflammatory cells
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Pitfalls in diagnosis of high grade UC

Polyoma virus infection (BK virus)
Pitfalls in diagnosis of high grade UC

Polyoma virus infection (BK virus)
Infected 90% of population (usually asymptomatic)
Latent infection in urothelium and renal tubules with reactivation with immunosuppression
Urinary excretion of BKV-infected urothelial cells (decoy cells) in 20-30% of patients following renal transplantation

Polyoma virus large T-antigen is intranuclear and difficult to demonstrate in cytology specimens.
High grade UC is more frequent post-renal transplantation

3-4 fold increased risk of urothelial carcinoma post-renal transplantation relative to general population.

Different demographics and pathology in transplant patients

<table>
<thead>
<tr>
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<th>Non-tx urothelial tumours</th>
<th>Post-transplant</th>
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<tbody>
<tr>
<td>Mean age</td>
<td>60</td>
<td>44</td>
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<tr>
<td>Sex ratio</td>
<td>M:F 4:1</td>
<td>M=F</td>
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<tr>
<td>Pathology</td>
<td>low grade non-invasive</td>
<td>high grade invasive</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>75%</td>
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Polyoma virus T-Ag is oncogenic

T-Ag inactivates the pocket protein family, including pRb.

BKV T-Ag activates the DNA methyltransferase 1 gene DNMT1 is associated with tumourigenesis through tumour suppressor gene hypermethylation.

Expression of T-Ag induces high-grade bladder tumours in transgenic mice.

No apparent association of PV T-Ag and urothelial tumours in immunocompetent humans.
Nuclear staining for T-Ag in post-transplant urothelial carcinoma but not adjacent non-neoplastic urothelium.

Roberts et al. *Brit J Cancer* 2008;99:1383
65 year old Caucasian female
BKV reactivation 5 months post-transplant, diagnosed on urine cytology, no biopsy-proven BKVN
2.5 years post-transplant: biopsy for graft dysfunction – IFTA and active infiltrate, T-Ag negative.

Urine cytology:
Urine cytology 3 months later:

Bladder biopsy:
Non-urothelial tumours

Small cell carcinoma
Loose aggregates of tumour cells.
High N:C ratio, nuclear molding, salt and pepper chromatin.
Non-urothelial tumours

Prostatic carcinoma
Urine cytology low sensitivity for the diagnosis of prostatic carcinoma.
Well differentiated tumours show glandular differentiation with mucin.
Poorly differentiated tumours resemble high grade urothelial carcinoma.
Non-urothelial tumours

Prostatic carcinoma
Urine cytology low sensitivity for the diagnosis of prostatic carcinoma. Well differentiated tumours show glandular differentiation with mucin. Poorly differentiated tumours resemble high grade urothelial carcinoma.
Non-urothelial tumours

Lymphoma
Dispersed single cells, no cohesive aggregates.
May be difficult to differentiate from chronic inflammation.
Monomorphic lymphoid cells ± blasts.
Clinicopathological correlation essential.
Non-urothelial tumours

**Lymphoma**
Dispersed single cells, no cohesive aggregates.
May be difficult to differentiate from chronic inflammation.
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Clinicopathological correlation essential.
General advice

Consider specimen type
If excessive degenerative changes, ask for repeat specimen

If ‘atypia’, ask for clinical history:
Calculi
Polyomavirus infection
Previous tumour diagnosis
BCG, radiotherapy, cytotoxic drugs