Pathology of bladder cancer and biomarker

Dr Fam Xeng Inn
UKMMC
Type of bladder cancer

- Urothelial carcinoma (transitional cell carcinoma)- 95%
- Squamous cell carcinoma (SCC)- 5%
- Adenocarcinoma- 1%
- Small cell carcinoma, rhabdomyosarcoma- children
- Bladder pheochromocytoma
- Bladder lymphoma
Histology of the Urinary Bladder

(a) Micrograph of the bladder wall (17X)  
(b) Epithelium lining the lumen of the bladder (360X)
Urothelial cell carcinoma

- arises from transitional epithelial cells that are adjacent to the basement membrane.
- Development of papillary tumor that projects into the bladder lumen
- if untreated, eventually penetrates the basement membrane, invades the lamina propria, and then continues into the bladder muscle.
1973 WHO grading

*Urothelial papilloma*

Grade 1: well differentiated
Grade 2: moderately differentiated
Grade 3: poorly differentiated

2004 WHO grading system [papillary lesions]

*Urothelial papilloma (completely benign lesion)*

Papillary urothelial neoplasm of low malignant potential (PUNLMP)

Low-grade (LG) papillary urothelial carcinoma

High-grade (HG) papillary urothelial carcinoma
Histologic Spectrum of transitional cell carcinoma (urothelial carcinoma [UC])

2004 WHO

1973 WHO

PUNLMP Low grade High grade
Histological classification and stage of newly diagnosed bladder cancer in a population-based study from the Northeastern United States

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<table>
<thead>
<tr>
<th>WHO/ISUP classification</th>
<th>Carcinoma in situ</th>
<th>Papilloma</th>
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<th>Grade 2</th>
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<td>144</td>
<td>59</td>
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</table>
- Papillary urothelial neoplasm of low malignant potential
- orderly arrangement of cells within papillae
- minimal architectural abnormalities
- minimal nuclear atypia irrespective of the number of cell layers.
Low grade

- overall orderly appearance
- easily recognizable variation of architectural and or cytologic features even at scanning magnification.
- Minimal variation of polarity and nuclear size, shape, and chromatin texture
- definitive cytologic atypia.
- Mitotic figures are infrequent
- Note the highest grade of abnormality
High grade

- predominantly or totally disorderly appearance at low magnification.
- both architectural and cytologic abnormalities.
- Architecturally, cells appear irregularly clustered and the epithelium is disorganized.
- Cytologically, there is a spectrum of pleomorphism ranging from moderate to marked.
- The nuclear chromatin tends to be clumped and nucleoli may be prominent.
- Mitotic figures, including atypical forms, are frequently seen at all levels of the urothelium.
- to comment on is marked nuclear anaplasia.
Carcinoma in situ (CIS)
- flat, HIGH GRADE, non-invasive urothelial carcinoma.
- It can be missed at cystoscopy
- considered as an inflammatory lesion if it is not biopsied.
- multifocal
- bladder, upper urinary tract, prostatic ducts, prostatic urethra
**CIS**

- presence of cells with large, irregular, hyperchromatic nuclei
- may be either present in the entire thickness of the epithelium or only part of it
- Usually designated as severe dysplasia or marked atypia
Classification of CIS

- Primary: isolated CIS with no previous or concurrent papillary tumours and no previous CIS;
- Secondary: CIS detected during follow-up of patients with a previous tumour that was not CIS;
- Concurrent: CIS in the presence of any other urothelial tumour in the bladder
WHO 2004 grading system (flat lesions):
- Hyperplasia (flat lesion without atypia or papillary aspects)
- Reactive atypia (flat lesion with atypia)
- Atypia of unknown significance
- Urothelial dysplasia
- Urothelial CIS is always high-grade
Squamous Cell Carcinoma

- derived from bladder urothelium with pure squamous phenotype
- constitutes around 5% of all urinary bladder carcinomas
- Associated with chronic cystitis:
  - stone
  - urinary retention
  - indwelling catheter
- Smoking
- Bladder diverticular
Squamous Cell Carcinoma

- Schistosomiasis is the major cause of squamous cell carcinoma of the bladder in African countries.
- El-Bolkainy reported, in Egypt, 82% of patients with bladder carcinoma were found to harbor Schistosoma haematobium eggs in the bladder wall.
- S. haematobium, S. mansoni, and S. japonicum.
- The eggs are found embedded in the lamina propria and muscularis propria of the bladder wall.
- The deposition of Schistosoma eggs commonly provokes a severe inflammatory response and fibrosis.
Squamous Cell Carcinoma

- Tumor appears nodular and has a plaquelike, irregular surface.
- There is deep invasion into the muscularis and often involvement of the extravesical organs.
- Most of the tumors are large, exophytic, and necrotic and bulge into the bladder cavity.
- Less responsive to radiation and chemotherapy.
- Localized bladder SCC: radical cystectomy, pelvic lymphadenectomy.
Adenocarcinoma

- 2% of primary bladder tumors
- Primary - arises from the bladder
- Urachus - arise from the urachus
- Metastatic - adenocarcinoma that has metastasized to bladder
- Patient must be evaluated for site of origin other than bladder
- CT thorax-abdomen - pelvic
- Endoscopic examination
- Tumour maker
- Mamagram, chest X-ray
Adenocarcinoma

- Exstrophic bladder
- CEA elevated
- Worse prognosis than TCC
- Not responding to radiation, chemotherapy and intravesical therapy
- Radical cystectomy + pelvic lymphadenectomy
Small Cell Carcinoma

- poorly differentiated, malignant neoplasm that originates from urothelial stem cells
- has variable expression of neuroendocrine markers
- Morphologically, it shares features of small cell carcinoma of other organs, including the lung
- Usually present with invasion of the muscularis propria
- Stain positive for chromogranin A and Synaptophysin
Small Cell Carcinoma

- 50% present with metastasis
- Should undergo metastatic evaluation
- M0: systemic etoposide and cisplatin
- Respondent: radical cystectomy +/- pelvic radiation
- M1: systemic chemotherapy
Biomaker
Biomaker

- UroVysion
- Microsatellite Analysis
- ImmunoCyt/uCyt+
- Nuclear matrix protein 22
- Cytokeratin
- BTA-stat
- BTA trak
Bladder cancer urinary cytology revealed a sensitivity of 38.0% and a specificity of 98.3% respectively.

Sensitivity increased significantly with malignancy grade.

In high grade tumours sensitivity improved from initial 52.2% up to 78.3%.
UroVysion

- technique that uses fluorescently labeled DNA probes to assess cells for genetic alterations.
- chromosome enumeration probes (CEPs): hybridize (stick to) the pericentromeric regions of chromosomes and are used to enumerate the number of chromosomes in a cell.
- locus-specific indicator probes (LSI): hybridize to genes of interest, such as the HER2, P53, or other genes.
- Fluorescence microscope to assess copies fluorophores in the nucleus of a cell
UroVysion

- most bladder cancers possess chromosomal abnormalities and that the degree of aneuploidy and structural chromosomal abnormalities (deletions and gains) increase with increasing tumor grade.
- FISH allows assessment of interphase cells for chromosomal abnormalities.
- chromosome 3 (CEP3): red
- chromosome 7 (CEP7): green
- chromosome 17 (CEP17) aqua
- 9p21 locus (LSI 9p21) location of the P16 tumor suppressor gene: gold
- 4 probes provide a higher sensitivity than just 1 or 2 probes
- 4 is the maximum number that one can easily have in a single probe set owing to spectral overlap of the different fluorophores light emissions.
Hybridization is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters.

Four types of genetic abnormalities: polysomy, tetrasomy, trisomy, and homozygous 9p21 deletion.

Cells with gains (ie, 3 or more copies) for 2 or more of the 4 probes in the same cell are referred to as “polysomic” cells.

Polysomy generally correlates with presence of a high-grade tumor.

meta-analysis, the pooled sensitivity and specificity of UroVysion was reported to be 72% (69% to 75%) and 83% (82% to 85%), respectively.
Microsatellite Analysis

- Microsatellites are highly polymorphic DNA-repeat regions and common to all of the eucaryotic genomes
- Alterations of microsatellite DNA are an integral part of neoplastic progression
- Valuable as clonal markers for the detection of human cancers
- Loss of heterozygosity has been reported for bladder carcinoma in regions of chromosomes 4, 5, 8, 9, 11, and 17 and is considered a major event in the carcinogenesis of bladder cancer
Microsatellite Analysis

- Urine was collected, treated, centrifuged, then DNA extraction
- Extracted DNA from each sample was amplified by PCR for targeted polymorphic microsatellite markers
- localized on chromosomes 4, 6, 8, 9, 11, 13, 14, 16, 17, and 20.
- Anne et al reported the overall sensitivity of the test was 84% for detecting bladder cancer.
ImmunoCyt/uCyt+

- Immunocytochemical test developed by Fradet and Lockhard in 1997
- fluorescent-labeled antibodies to 3 markers
- antibody is directed against a high molecular weight form of glycosylated carcinoembryonic antigen 19A211 and is labeled red
- two antibodies, LDQ10 and M344, are directed against mucins, which are cytoplasmic antigens specific for bladder cancer and are labeled with fluorescein green
- Allar verified tumor specificity of these antigens.
- M344 expression being present in 71% of Ta-T1 tumors.
- 19A211 high-molecular-weight carcinoembryonic antigen expression found in 90% of Ta-T1 tumors
ImmunoCyt/uCyt+

- Mucins are high-molecular-weight glycoproteins found on epithelial cell surfaces.
- In urothelial malignancy, these glycoproteins are not as heavily glycosylated, thereby exposing a portion of the protein backbone.
- Red and green fluorescence is evaluated and quantified using a fluorescence microscope with a dual filter for fluorescein (the green marker) and Texas Red (the red marker).
- Sample result is considered positive if at least 1 cell is seen to fluoresce green or red.
- A negative test result shows no fluorescence.
ImmunoCyt/uCyt+

- at least 500 cells without fluorescent signal must be observed on the slide before the sample can be called negative
- the need for proper training in performing the test and a learning curve with the assay.
- Vriesema found high interobserver variability on ImmunoCyt/uCyt test.
- Sensitivity for new bladder cancer is ranging from 63.3 to 86.3%
Nuclear matrix protein 22

- NMP-22 is a specific nuclear matrix protein found in the cell nucleus.
- Involve in DNA synthesis, RNA transcription, and the regulation of gene expression, including proper distribution of genetic materials to daughter cells during mitosis.
- Malignant neoplasia elevates intracellular NMP-22 at least 25 fold
- Resulting in shedding of the protein into the urine.
Nuclear matrix protein 22

- An NMP-22 test kit is manufactured by Matritech (Newtown, MA)
- detect the concentration of NMP in voided urine
- values greater than 7 NMP units/ml are usually considered suggestive of a tumor
Cytokeratin

- Cytokeratins are the major structural proteins in the cytoplasm of epithelial cells and their derivatives.
- Each type of epithelial cells synthesizes at least one type I and one type II keratin, which co-polymerize into filaments.
- Keratin filaments are characterized by tissue-specific expression patterns from early embryogenesis onwards.
- Important in defining tissue structure and potential function.
Cytokeratin

- found in bladder urothelium.
- potential value has been suggested as tumor markers for bladder malignancy.
- Different CK markers have been evaluated for bladder cancer diagnosis in urine samples
  - urinary bladder antigen (measuring urinary fragments of CK8 and CK18)
  - CYFRA 21-1 (quantifying CK19)
  - tissue polypeptide-specific antigen (determining CK18)
  - reverse transcriptase-polymerase chain reaction detecting CK-20 in voided urine specimen.
BTA-stat

- BTA stat used monoclonal antibodies generated against human complement factor H (hCFH) in urine.
- Bladder tumor associated antigen was identified as human complement factor H related protein (hCFHrp)
- similar in composition, structure and function to human complement factor H (hCFH).
- bladder tumor antigen stat test, performed in a disposable kit.
- detects bladder tumor-associated antigen within 5 minutes of placing fresh untreated urine.
BTA TRAK

Principle of the BTA TRAK Assay

- Add assay diluent
- Add patient urine sample
- Incubate 1 hr. @ 37°C
- Add conjugate
- Incubate 1 hr. @ 37°C
- Add substrate
- Incubate 30 min @ 37°C
- Add stop reagent
- Mix
- Wash
- Wash
- Measure absorbance @ 405 nm

- No sample preparation or pretreatment
- Standard 96-well format
- Delivers quantitative results in 2.5 hours
- Adaptable to most automated systems
Ellis established the decision threshold for BTA assay at 14 kilounits/L, corresponding to 97% specificity.
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<th>Markers (or test specifications)</th>
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<th>Sensitivity for high-grade tumours (%)</th>
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None of these markers have been accepted for diagnosis or follow-up in routine urology or in guidelines