191 Understanding Genitourinary System Cytology: From Morphology to Molecular Pathology

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This session will provide a comprehensive review of the morphologic criteria and guidelines for the cytologic diagnosis of genitourinary neoplasms and non-neoplastic lesions using real cases to segue into discussion. The subject matter is comprised of 2 sections: Urinary tract cytology and renal FNA cytology. The salient morphological features of genitourinary tract cytology, the current state-of-the-art ancillary tests, and the pitfalls associated with them will be addressed. The practical integration of ancillary tests (DNA ploidy, biomarkers, FISH, and other novel tests designed to detect malignancy) in urine cytology and renal FNA's will be discussed. Approach to differential diagnoses and diagnostic role of ancillary studies in renal cytology will be covered in light of the newer sensitive imaging techniques and personalized treatment modalities of the 21st century.

- Learn the indications, cytomorphologic features, and potential pitfalls of urine cytology.
- Recognize normal renal cytology, common, and uncommon lesions in renal aspirations, and understand limitations in diagnosing renal FNA's.
- Understand the current role and potential pitfalls of emerging new technologies and ancillary techniques in urine and renal cytology; and review integrated approach (using biochemical, molecular tests, morphological and clinical findings) the in the diagnosis of genitourinary tract cytopathology.

FACULTY:

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 Entire Pathology Team
 Cytopathology
 Cytopathology (Non-Gynecologic)
 2.0 CME/CMLE Credits

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Understanding Genitourinary System Cytology: From Morphology to Molecular Pathology

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I. URINE

Introduction to Urinary Tract Cytopathology

Urothelial carcinoma (UC) of the bladder is a challenging condition for both urologists and pathologists. For the urologist, the challenge is to predict at an early stage which patient will have further tumor occurrences or will develop invasive properties. For the pathologist, the challenge is mainly to detect low grade lesions. By definition, the nuclear differences between low grade urothelial carcinoma and normal urothelium are subtle and very subjective. Additionally, reactive processes due to treatment, lithiasis of the urinary tract or viral infections may be difficult to distinguish from neoplastic processes.

A sensitive non-invasive test to detect bladder cancer remains an elusive but highly desirable goal. Although urine cytology is highly sensitive for detection of high grade UC, its sensitivity for low grade cancer remains unacceptably low. Consequently, invasive cystoscopy remains the mainstays for the diagnosis of bladder urothelial carcinoma and it is used to monitor patients with high risk for recurrence and progression.

Therefore there is an obvious need for a development of additional more sensitive tests that could detect urothelial neoplasia. In this context, a number of techniques, including DNA ploidy by image analysis, flow cytometry, or laser scanning cytometry, image analysis-based morphometry, immunohistochemistry, cytogenetics or urine chemical assays such as NMP-22 and BTA have been proposed to be used in conjunction with cytologic examination.

New technologies, however, should not diminish the need for a proper cytologic evaluation. Successful urine cytology depends upon numerous factors:

- Basic knowledge of anatomy, histology and function of the GU tract
- Availability of clinical information: sex, age, type of specimen, symptoms, cystoscopic and radiographic findings, previous history
- “Common language” with surgical pathologist and clinician
- Application of adjunct tests
- Understanding and acceptance of limitations

Cellular and non-cellular components of normal urine specimens

A specialized type of epithelium (transitional epithelium or, currently recommended name, urothelium) is lining a lower collecting system which includes bladder and urethra,
and upper collecting system which includes renal pelvis, calyceal system and ureters. Urothelium is a multilayer epithelium, composed of 6 to 7 layers of cells. The main role of the urothelium is to form a blood/urine barrier.

Cells normally found in urine:

- Urothelial cells – basal cells, intermediate cells, superficial (umbrella) cells
- Squamous cells – contaminant, trigone, squamous metaplasia

Other cells found in urine:

- Glandular cells – prostatic, endometrial, cystitis glandularis, paraurethral glands
- Renal tubular cells
- Leukocytes, lymphocytes and RBC’s
- Seminal vesicle cells
  Sporadically, degenerated seminal vesicle cells can be seen in urine specimens, particularly from older patients. Seminal vesicle cells in urine specimens often have a bizarre appearance, with greatly enlarged nuclei and foamy, fragmented cytoplasm. The chromatin is hyperchromatic, degenerated and smudgy. In contrast, the chromatin of malignant cells is coarse. As in prostatic specimens, seminal vesicle cells may be distinguished from cancer cells by the presence of a golden-brown lipofuscin pigment. Often, spermatozoa accompany seminal vesicle cells. These cells also have an abnormal DNA content.

Non cellular elements:

- Crystals
- Casts
- Sperm
- Corpora Amylacea
- Lubricant
- Mucus
- Fibrin
- Pollen
- Alternaria
- Microconidia

Types of Urinary Tract Specimens

Voided Urine:  - the most convenient and easily obtained
               - contamination from external genitalia and vagina
               - degenerated epithelial cells (eosinophilic inclusions)
Catheterized urine:  
- lack of contamination from external genitalia  
- more cellular – pseudopapillary fragments

Bladder washing/barbotage:  
- more cellular  
- better preservation  
- monolayered sheets, pseudopapillary fragments and single cells  
- multinucleated cells  
- columnar intermediate and deep cells

Ileal conduit:  
- hypercellular  
- dirty background of mucus and bacteria  
- columnar cells of ileal epithelium  
- degenerated urothelial cells with cytoplasmic inclusions  
- karyorrhexis

Renal pelvis washing/brushing:  
- high cellularity  
- well-preserved  
- sampling of a specific area

Inflammatory Conditions

A. Noninfectious

- Interstitial cystitis: Non-specific cytologic findings
- Eosinophilic cystitis: Eosinophils and reactive urothelial cells
- Hemorrhagic cystitis: Non-specific cytologic findings, abundant erythrocytes
  Could be infectious or non-infectious
  Etiology: Escherichia coli; adenoviruses, papovavirus; influenza A; cyclophosphamide, and radiation induced.

B. Infectious

- Viral
  Human Polyoma Virus: Human polyoma viruses are small, non-enveloped, double-stranded DNA viruses that are classified into two main strains, BK and JC. The JC strain of the virus is associated with progressive multifocal leukoencephalopathy. The BK strain of the virus affects the kidney and can be detected in the urine. A primary BK virus infection occurs during childhood and is usually subclinical. Over 90% of adults are seropositive for BK viral antibodies. The BK virus generally remains latent in the kidney, but intermittent viruria is demonstrable in 0.3% of
healthy adults. The infection is reactivated in individuals with various degrees of immunological deficits. BK virus-infected cells are characterized by the presence of single, large, homogenous, basophilic inclusions occupying most of an enlarged nuclear area. Because of the nuclear abnormalities, the infected cells can easily be misclassified as malignant cells, and have been previously described as “decoy cells” The other type of cells commonly seen in PV infection are the “empty cells”, described by Koss. In addition, urothelial cells affected by BK virus have an abnormal DNA content.

**Cytomegalovirus:** Larger cell with perinuclear halo and both cytoplasmic and nuclear inclusions

**Adenovirus:** Homogenous basophilic intranuclear inclusion, with multiple small irregular inclusions, and nuclear clearing

**Herpes:** Multinucleation, margination of the chromatin, and molding of the nuclei

- **Fungal:**

  **Candida:** is the most common fungal organism affecting the bladder. More common in immunosuppressive patients and diabetes. If seen accompanied by numerous squamous cells, bacterial organisms in women possibility of vaginal contamination should be raised.

- **Parasitic:**

  **Trichomonas:** Rare sexually transmitted infection, frequently associated with genital coinfection. The organisms is a light gray, pear shaped protozoan ranging in size between 15- 50μm, with cytoplasmic eosinophilic granules.

  **Schistosoma:** Endemic in Africa and some parts of Asia. The organisms are transmitted via fresh water snails. The eggs are oval, ranging between 100-150 μm, with a terminal (S. hematobium) or lateral (S. mansoni) spine.

- **Bacterial**
  
  Fecal flora and malakoplakia (rarely - blue targetoid calciosherules (Michaelis-Gutmann bodies)

**Urolithiasis:**

This one of the most common pitfalls in urinary cytology. Patients may present with hematuria and/or filling defect. Cytology specimens may be cellular and three dimensional fragments composed of cells exhibiting significant pleomorphism may be
seen. Often clinical history is crucial to avoid a false positive diagnosis. However, stones can co-exist with a neoplasm.

**Treatment Related Changes**

- **Cyclophosphamide:**
  - high N/C ratio
  - large nuclei
  - hyperchromasia and degeneration
  - granular and wispy cytoplasm

- **Mitomycin and Thiotepa:**
  - umbrella cells mostly affected
  - marked nuclear enlargement
  - multinucleation
  - hyperchromatic, granular chromatin

- **Radiation:**
  - cytomegaly
  - nucleomegaly
  - preserved N/C ratio
  - multinucleation
  - nuclear and cytoplasmic vacuoles

- **BCG:**
  - granulomas
  - inflammation
  - multinucleated giant cells, histiocytes

**Urothelial Carcinoma**

The American Cancer Society predicts that approximately 131,000 new cases of bladder cancer will be diagnosed in the year 2009 in the United States and approximately 28,100 people will die of the disease. Only about 50% of these new cases will be detected by routine cytologic examination. At presentation, the majority of bladder cancers (75%) will be superficial. Of these, 50% - 70% will recur and 10% - 20% will progress. This natural history of bladder cancer results in a very high overall disease prevalence. As a matter of fact, the urinary carcinoma is described as the most prevalent form of cancer. This is mainly due to two factors typical of superficial disease. The recommended frequent follow up and the duration of this because of low risk of mortality due to disease.

**Epidemiology**

- Highest rate in North America, Western Europe
- Lowest rate in Japan
- Male:Female – 3:1
• Median age at Dx - > 65 years

**WHO/ISUP consensus classification (1998) of urothelial neoplasms**

- **Papillary Noninvasive**
  - Papilloma
  - Inverted papilloma
  - Papillary urothelial neoplasm of low malignant potential - PUNLMP
  - Papillary urothelial carcinoma, low grade
  - Papillary urothelial carcinoma, high grade

- **Invasive Neoplasms**
  - Lamina propria invasion (superficial urothelial carcinoma)
  - Muscularis propria (detrusor muscle) invasion

<table>
<thead>
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<tr>
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<td>Low malignant potential</td>
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<tr>
<td>TCC II</td>
<td>Low grade UC</td>
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<tr>
<td>TCC III</td>
<td>High grade UC</td>
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**Urothelial Carcinoma – Low Grade**

Low grade tumors are characterized by:

- Increased cellularity
- Presence of papillary, cohesive clusters
- Mild to moderate pleomorphism
- Eccentric, mildly enlarged nuclei
- Mild irregularity in nuclear membrane
- Granular, even chromatin
- Homogenous cytoplasm
- Inconspicuous nucleoli
- Low – moderate sensitivity and specificity

The cytologic and architectural criteria are unreliable in diagnosing low grade lesions. Also low grade lesions having very little chance of progression, and if there is a papillary low grade lesion the chance of the urologist seeing it in cystoscopy is very high. Therefore, in practice, unless papillary fragments are seen with intact fibrovascular cores, low grade urothelial carcinoma should not be diagnosed on cytology.
Urothelial Carcinoma – High Grade

High grade tumors are characterized by:

- Increased cellularity
- Presence of loose clusters and single cells
- Moderate to marked pleomorphism
- Eccentric, enlarged, pleomorphic nuclei
- Irregular nuclear membrane
- Coarse chromatin
- +/- prominent nucleoli
- Squamous or glandular differentiation
- High sensitivity and specificity

Other bladder neoplasms

Primary

- Squamous cell carcinoma
- Adenocarcinoma
- Small cell carcinoma

Squamous cell carcinoma

This is a rare tumor, accounting for less than 5% of all bladder carcinomas. It is most frequently associated with schistosomiasis, chronic inflammation, and urolithiasis. Well differentiated squamous cell carcinoma shows cells with dense cytoplasm, and orangophilia (if keratinizing), hyperchromatic small nuclei. In contrast, poorly differentiated carcinoma has pleomorphic, hyperchromatic cells with high N:C ratio, prominent nucleoli and a necrotic background. The co-presence of urothelial carcinoma and dysplastic cells arising in the genital tract can not be reliably distinguished cytological from squamous cell carcinoma arising in the bladder. In the presence of dysplastic squamous cells in the urine a differential diagnosis of urothelial carcinoma with squamous differentiation, squamous cell carcinoma, and dysplastic cells arising in the genital tract should be raised.

Adenocarcinoma

Adenocarcinoma is also a rare tumor, accounting for less than 2% of all bladder carcinomas. May arise in the bladder or urachus. Well differentiated adenocarcinoma; forms glandular structures, or sheds as isolated columnar cells with hyperchromatic nuclei and amphiphilic, finely vacuolated cytoplasm. Poorly differentiated carcinoma could have signet ring cells or cells with high N:C ratio, and prominent nucleoli. Tumors cells are usually positive for Cytokeratin 20 and Cytokeratin 7 (CK7 has a varying range of positivity 0-82%). In general CDX-2 and villin are negative in
adenocarcinoma arising in the bladder, however, there are reports stating otherwise as well.

**Small cell carcinoma**

As in other areas of the body this is a highly aggressive malignancy, accounting for less than 1% of the bladder carcinomas. Majority of small cell carcinomas are seen in combination with urothelial carcinoma, and the most common symptoms are hematuria, dysuria, and paraneoplastic syndromes. The cytology is identical to small cell carcinomas seen elsewhere; small round to oval cells, with high N:C ratio, scant cytoplasm, hyperchromatic nucleus with coarse- ‘salt and pepper’ chromatin, nuclear molding, and occasional mitotic figures. The tumor cells are usually positive for at least one of the following chromogranin, synaptophysin, and CD 56.

**Secondary**

- ~ 10% of bladder tumors
- Majority (~ 70%) – direct invasion: prostate, cervix, uterus, GI tract
- Distant metastases – malignant melanoma, carcinomas of stomach, breast, kidney and lung

**Ancillary Techniques in Urine Cytology**

- DNA ploidy
  - Flow cytometry
  - Static image analysis
  - Laser Scanning Cytometry

- Morphometry

- Cytogenetic alterations and urothelial tumor markers
  - Microsatellite Instability Assays
  - FISH
  - Blood (ABO) group antigens and Lewis X
  - Urothelial tumor-associated monoclonal antibodies
  - CK 20
  - E-cadherin
  - P53
  - The BARD Bladder Tumor Antigen - BTA™
  - The Nuclear Matrix Protein- NMP22™
  - Telomerase
  - Multiprobe FISH Assay – UroVision™
  - Cyfra 21-1
  - ImmunoCyte
  - Survivin
DNA Ploidy

The evaluation of DNA ploidy in urinary specimens is becoming accepted adjunct test used for both diagnostic and prognostic purposes. In general, low grade urothelial carcinomas are diploid and high grade tumors are aneuploid. As a result, aneuploidy is a strong indicator of high grade malignancy as well as carcinoma in situ. Also aneuploidy in conjunction with suspicious cytology is highly predictive of tumor recurrence. In addition, it has been shown that DNA ploidy analysis provides independent prognostic information.

Definitions:

**DIPLOID:** Normal (2c or 2N) amount of DNA corresponding to 46 chromosomes; cells in G0/G1 stage of the cell cycle; DNA index – DI = 1

**ANEUPLOID:** Abnormal (increased or decreased) amount of DNA

**TETRAPLOID:** Double amount of DNA (4c or 4N DNA)

**HYPERPLOID:** DNA content > 5c

Diploid histogram

![Diploid Histogram](image)

Aneuploid histogram

![Aneuploid Histogram](image)
DNA Ploidy – Flowcytometry

Flow cytometry (FCM) was first applied to study bladder washings from patients with urinary carcinomas. Numerous early studies reported the usefulness of this technique in the management of bladder cancer. It has been documented that flow cytometric DNA ploidy results correlate well with cystoscopic and cytologic findings. When FCM and urine cytology were used together an improved diagnostic ability was observed. However, recent reports did not confirm these early findings. In addition, FCM requires numerous cells, therefore only bladder washes are suitable specimens.

DNA Ploidy – Static Image Analysis

The alternative and most commonly used technology to evaluate a DNA ploidy in urine specimens is image analysis (IA). Image analysis is a broad term, encompassing morphometry, densitometry and even neural networks. Typically, the term is used to describe an integrated, interactive computer-based system in which measurements of specific cellular features, including the amount of DNA, are analyzed. In this method, cells (100 – 200) are visually selected by operator. All urine specimens are suitable for analysis. Nuclear DNA ploidy is evaluated on Feulgen-stained slides.

In the DNA staining method, developed by Feulgen and Rossenbeck, hydrochloric acid is used to hydrolyze the ribose-purine bonds in the DNA to give sugar aldehyde residues, and a dye is then coupled stoichiometrically to the sugar aldehyde. The staining intensity becomes a measure of the DNA content in the nucleus. The absorption spectrum maximum for the Azure A stain is 620 nm. The IOD is assumed to be equivalent to the amount of DNA present in the nuclei.

DNA Ploidy – Laser Scanning Cytometry (LSC)

The Laser Scanning Cytometer (LSC) is a newly developed instrument highly suited for DNA ploidy analysis. The LSC combines features of both flow and image
cytometry and is capable of measuring multicolor fluorescence, light scatter and location of cells fixed to a microscopic slide. It is capable of rapid and automatic measurements of a large number of cells. In addition, cell location is recorded so the cells of interest can be relocated for a morphologic examination and classification.

For DNA analysis the cells are stained with propidium iodide (PI) and cytokeratin conjugated with fluorescein isothiocyanate (FITC). PI stoichiometrically intercalates with the nucleic acids on the DNA strand. The fluorescence signal from a cell is proportional to its DNA content. PI-stained nuclei emit fluorescence light at wavelengths between 580 and 650 nm. The emission color is red. FITC emits light at wavelengths between 488 and 525 nm. The emission color is green.

**Pitfalls in DNA Ploidy**

- Superficial cells
- Polyoma virus infected cells
- Seminal vesicle cells

**Comparison between FCM, IA and LSC**

<table>
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<th>FEATURES</th>
<th>FCM</th>
<th>IA</th>
<th>LSC</th>
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<tr>
<td>Applicability to routine cytology specimens</td>
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<tr>
<td>Correlation with morphology</td>
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<td>+++</td>
<td>+++</td>
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<tr>
<td>Specimen preparation</td>
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<td>Speed</td>
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<td>Labor intensity</td>
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<tr>
<td>Operator dependency</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>Measurement of multiple parameters</td>
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**DNA Ploidy – Summary**

- Low grade carcinoma – diploid
- High grade carcinoma – aneuploid
- Rare benign conditions – aneuploid
Help distinguish urothelial atypia from carcinoma
Not a screening test
Follow up of patients with history of urothelial carcinoma
Complement routine cytology

Morphometry

Morphometry has been defined as the “quantitative description of a structure”. In practice, this term is usually applied to quantitative techniques that measure features of size, shape, and texture in two dimensions and/or spatial relationships from cells or other tissue structures. The need for measurement comes from the recognition that interobserver and intraobserver diagnostic decisions are poorly reproducible. Morphometry has several advantages over conventional visual assessment: objectivity, reproducibility and the ability to detect changes too subtle to be visually appreciated in individual cells. Therefore morphologic diagnostic accuracy and precision can be improved by applying this technique.

Morphometry – summary
- Valuable diagnostic tool
- Important prognostic information
- Potential for automation
- Mostly under investigation

Molecular Alterations in Bladder Carcinoma

A number of studies aimed at defining loss of heterozygosity have shown a general chromosomal instability in UC with loss of parts of chromosome 9 at early stages and of chromosomes 11, 13, 3, 4, 8, 17 and 18 during further development of the tumor. It has been postulated that two different tumor suppressor gene loci on chromosome 9 are involved as tumorigenic events in bladder cancer. It was also postulated that loss of heterozygosity of 9p might be associated with the development of tumor with more aggressive behavior.

Fluorescence in situ hybridization (FISH) has been demonstrated as a viable method for determination of chromosome specific anomalies in cells obtained from urine specimens for early tumor detection or recurrence. Recently, a multi-color FISH Probe Mixture designed for interphase cell analysis for detection and quantification of chromosome 3, 7, 17 and the 9p21 region has been made commercially available (UroVysion™ Multi-color FISH Probe Mixture, Vysis).

Tumor Markers in Urothelial Carcinoma
Lewis X - the only blood group antigen with potential prognostic application.

Immunocyt™ -M344, 19A211, LDQ10; sensitivity – 86%, specificity – 79%

CK 20 - RP-PCR; sensitivity – 91%

E-cadherin - up-regulated in papillary tumors, expression gradually lost in high grade, invasive carcinomas

p53 - sensitivity in voided urine – 23.5%, specificity – 75%

Telomerase - telomerase activity is measured using a telomeric repeat amplification protocol (TRAP). This method requires a minimum 30 ml of urine. The reported sensitivity ranges from 56 to 89% and specificity ranges from 70 to 96.4%.

BTA™ - The original BTA test is a latex agglutination assay which detects the presence of basement membrane antigens that have been isolated and characterized in the urine of bladder cancer patients. Reported sensitivity of the BTA test for detection of recurrent bladder tumors ranged between 32% and 74%, and the specificity ranged between 40% and 96%. Modification in the original BTA test resulted in the development of a single step, five-minute, immunochromatographic assay, BTA Stat and BTA TRAK assay. These assays detect a human complement factor H related protein that is produced in vitro by several human bladder cancer cell lines but not other epithelial cell lines. The BTA Stat shows an improved sensitivity and specificity as compared to the original BTA test. At this moment, despite numerous controversial reports, most authors would agree that this test could be utilized only as an adjunct to cytology, particularly for the detection of recurrent tumors.

NMP™ - NMP22 is an enzyme immunoassay for the quantification of nuclear matrix proteins that comprise the internal structural framework of the nucleus. The antibodies in this assay recognize two domains of the nuclear mitotic apparatus protein. This is a quantitative assay with a recommended cut off point of 10 IU/ml.

CYFRA 21-1 enzyme-linked immunoabsorbent assay kit (ELISA-CYFRA 21-1; CIS Bio International, Gif-Sur Yvette, France. The assay uses two monoclonal antibodies, BM 19-21 and KS19-1, to recognize cytokeratin 19 fragments.

Overall median sensitivity and specificity
(Adapted from Lotan and Roehrbon, Urology 61: 109-118, 2003)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Patients (n)</th>
<th>Sensitivity (%)</th>
<th>Patients (n)</th>
<th>Specificity (%)</th>
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<td>Immunocyt</td>
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II. FNA OF RENAL LESIONS

Fine needle aspiration (FNA) of kidney masses have been performed for the diagnosis of mass lesions, confirmation of advanced neoplasia and metastases, staging of tumors, and rarely as therapeutic aspiration of cystic lesions.¹ In the past the decision of whether to perform a nephrectomy used to be based on radiographic features and size, precluding the use of FNA.² Today where treatment is not limited to surgery the indications for renal FNA have expanded.

The indications of renal FNA in solid masses include:

1. Patients with presumed malignant lesions who aren’t candidates for resection. These include patients with unresectable primary tumors, patients with metastatic disease and patients with another primary tumor with other comorbidities to preclude surgery.
2. Cases where partial nephrectomy or laparoscopic morcellation is preferred over radical nephrectomy
3. Cases where non surgical treatment methods such as minimally invasive methods are preferred.
4. Cases where preoperative/neoadjuvant chemotherapy or biological response modifiers are preferred.
5. Cases where pretreatment molecular/ cytogenetic typing of the tumor is recommended to individualize the treatment of choice.

The indications of renal FNA in cystic masses include:

1. Simple cysts for therapeutic aspiration (this is more of an indication of the past, today most simple cysts are just followed up).
2. Radiologically indeterminate cystic lesions.

Prerequisites and technique for Renal FNA: A platelet count of > 70,000/ml is required; and the patients on anticoagulants should stop taking them 2 days prior to the biopsy. The FNA is performed under conscious sedation in addition to local anesthesia, with 20-23 gauge needle (spinal or Chiba) following an 18 gauge guide needle under US (and rarely CT) image guidance.

Complications of Renal FNA are very rare and include: perirenal hemorrhage, pneumothorax, infection, A-V fistula, urinoma. There are very few reports of needle tract seeding.³⁴

Renal FNA Statistics: The accuracy of FNA of kidney in diagnosing tumors range from 73% to 94 %, the sensitivity 50-90%, the specificity 50-93%. The diagnostic yield quoted
in earlier papers are as low as 40%, however with the newer imaging techniques it has risen to up to 95%.

Nevertheless, there are several challenges in diagnoses: differentiation of the normal renal elements, identifying well differentiated renal cell carcinomas (RCC), differentiating between oncocytoma and chromophobe RCCs, and differentiating between high grade papillary RCC, urothelial carcinoma, collecting duct carcinoma and metastatic carcinomas. Another potential problem is necrotic, hemorrhagic, or cystic tumors, which may lead to a false negative diagnosis. Currently there is no consensus on the adequacy criteria however it has been suggested to deem soft tissue and/or normal kidney elements only, and blood and/or necrotic tissue, and scant cellularity (few well-preserved cells) as unsatisfactory on solid renal mass FNA. In practice a sample is deemed satisfactory if it is sufficiently cellular to conjure a differential diagnosis and to render a specific diagnosis. Cystic lesions, on the other hand are tricky, even though abundant fluid is aspirated the smear could be acellular; or it could be composed of macrophages only therefore it is difficult to define adequacy in cystic lesions.

Normal Kidney Cytology:

**Glomeruli:** Cellular globular/papillary structures composed of spindled and round cells. Prominent capillary loops could be identified. Differential diagnosis (DDX): Papillary RCC (shows atypia and true fibrovascular cores)

**Proximal Convoluted Tubules:** Rare cells with indistinct cytoplasmic borders, abundant granular cytoplasm, and low N:C ratio. DDX: Oncocytoma, Chromophone renal cell carcinoma (RCC) (both have sharply defined cell borders)

**Distal Convoluted Tubules:** Rare groups of small cells with clear or granular cytoplasm with well-defined cell borders and inconspicuous nucleoli. These are smaller, flatter epithelial cells compared to the proximal tubular cells. DDX: Clear cell RCC (shows cytoplasmic vacuolization and nuclear atypia in higher grades), papillary RCC (shows atypia and papillae)

**Collecting Ducts:** Small, tight clusters of small cells with scanty cytoplasm, high N:C ratio, indistinct cellular borders and inconspicuous nucleoli. DDX: High grade papillary carcinoma, collecting duct carcinoma, metastatic adenocarcinoma (all these entities show more cellular atypia and have more conspicuous nucleoli)

**Cystic Lesions:** The majority of renal mass lesions (70-85%) are cysts; and they are mostly benign, acquired, and solitary. Renal cysts are classified radiologically according to the likelihood of the cyst being benign/malignant (Bosniak System) Category I being benign, IV being most likely malignant, II and III indeterminate. It is controversial whether or not to perform FNA on simple cysts (Bosniak I), however it is becoming a common practice to opt for FNA when there is suspicion of malignancy (Bosniak II-IV). The incidence of a cyst harboring RCC ranges between 1-25%, and the negative predictive value of a renal cyst FNA is low. In case of multiple cysts, the differential
diagnosis includes cysts due to long term dialysis or transplantation (these have a 9% chance of developing renal cell carcinoma)\textsuperscript{18}, and autosomal dominant (adult type) polycystic disease of the kidney. FNA of most simple cysts including the cases with multiple cysts yield clear, pale straw-colored fluid which shows a few foamy macrophages and no epithelial elements.

**Benign Lesions:**

**Xanthogranulomatous Pyelonephritis (XP):** Inflammatory sequela of chronic suppurative renal infection (Proteus or E.coli), often associated with an obstruction. The peak age incidence is between 4th and 6th decades. Cytology shows a cellular aspirate with foamy histiocytes singly and in clusters, multinucleated giant cells, and neutrophils.\textsuperscript{19} DDX: Clinically, radiographically and pathologically can be confused with renal cell carcinoma (RCC has a round nucleus compared to the kidney-bean shape in XP, the nucleoli are more prominent in RCC). A panel of immunohistochemical stains (ipox) and histochemical stains could differentiate reliably between the two (XP: PAS, LMWtCK, EMA (-) and CD 68 (+)).

**Renal Abscess:** The aspirate is cloudy, white to yellow. Cytology shows acute inflammatory cells. Gram negative organisms are the most common agent.

**Renal infarct:** Radiographically, they present as a wedge-shaped lesion. Cytology shows necrotic glomeruli and tubules. Rarely atypia, cytoplasmic vacuolization, prominent nucleoli could be seen.\textsuperscript{20} DDX: RCC (atypia is more pronounced)

**Metanephric adenoma:** Rare tumors that may arise from renal tubule epithelium. It is most commonly seen in women in 5\textsuperscript{th} decade. Cytology shows aggregates of small tubules and glomeruloid, tight short papillae composed of bland cells with scant cytoplasm, fine chromatin and rare nucleoli. Rare foci of necrosis may be present. DDX: Wilms tumor (WT1 (+)), papillary RCC (usually has more cytoplasm, longer papillae; and are pancytokeratin and EMA (+) unlike MA), metastatic papillary carcinoma of the lung or thyroid (clinical history plus pancytokeratin, EMA TTF-1 or thyroglobulin (+))

**Renal cortical (papillary) adenoma:** Small papillary renal cell tumors <0.5 cm in diameter occur commonly and rarely become malignant. In the recent WHO classification they are classified as renal cortical adenoma.\textsuperscript{21} Cytologically these tumors are same as papillary RCC.

**Angiomyolipoma:** is composed of smooth muscle, adipose tissue and vessels. About 20\% of cases are associated with tuberous sclerosis complex. Owing to high vascularity it may be confused with renal cell carcinoma on angiogram. Cytology shows a highly cellular aspirate with spindled to epithelioid cells singly and in clusters. Stromal cells are round to oval nuclei with fine chromatin, and inconspicuous nucleoli. Sporadic intranuclear inclusions could be seen. Pleomorphism, mitotic figures and necrosis are very rare. Ipox: HMB45 (+) and CD10 (-).
**Oncocytoma:** Account for 3-5% of renal masses. Cytology shows loosely cohesive small groups and isolated cells with abundant eosinophilic, granular cytoplasm with distinct cell borders, round nuclei with fine chromatin and occasional binucleation. However sometimes nuclear atypia, pleomorphism and prominent nucleoli could be seen making it difficult to differentiate from RCC. DDX: Proximal tubular cells (the granules of the cytoplasm spill out in benign tubular cells and the cytoplasmic membranes are indistinct), hepatocytes (hepatocytes have a polygonal cytoplasm and are hepar1 (+)), chromophobe RCC (see below for detailed discussion), clear cell RCC (unlike clear cell RCC, oncocytoma is pancytokeratin (+), vimentin (-), CD10 (-)). In case of scant cellular specimen where further studies (ipox, electron microscopy) can not be done the lesion is better classified as an ‘oncocytic neoplasm’ on cytology.

**Malignant Lesions:**

**Renal Cell Carcinoma (RCC):** This is the most common tumor of the kidney. It is most prevalent in males in the 5th –7th decades. It is important to distinguish between types of RCC since they have different prognostic and treatment implications.

**Clear Cell RCC:** Approximately 75% of RCC are clear cell type, and they are associated with chromosome 3p deletions. The cytologic features are of a cellular aspirate composed of large clusters and sheets of cells with abundant and vacuolated cytoplasm, low N:C ratio and eccentric nucleus. DDX: Tubular cells, macrophages, adrenal, hepatocytes (because of the bland nature of low grade RCC it could be mistaken for benign elements, however benign tubular cells and macrophages are not found in large clusters, hepatocytes have a more granular and polygonal cytoplasm and several ipox could be used to differentiate RCC from adrenal neoplasms: EMA, vimentin, CD 10 (+) in RCC, inhibin, melan A, synaptophysin, calretinin (+) in Adrenal cortical neoplasms)

**Papillary RCC:** Approximately 15% of RCC are papillary, and they are associated with trisomy chromosome 7, 16, and 17. These tumors are usually small and peripheral; they could be multifocal and associated with cortical adenomas. The prognosis is better than of the clear cell type. Cytology shows a cellular aspirate with the malignant cells arranged in a papillary configuration around fibrovascular cores. The cells could have a granular or vacuolated cytoplasm with rare intracytoplasmic hemosiderin deposits. N:C ratio is high, the nuclei are uniform; and rarely intranuclear grooves could be identified. Occasional foamy macrophages and rare Psammoma bodies could also be seen. DDX: Benign distal tubular cells, clear cell RCC, urothelial carcinoma, collecting duct carcinoma, metastatic carcinomas (Ipox aids in the diagnosis i.e. Like other RCC: EMA, Low molecular weight CK (+), Mucin, CEA (-); unlike other RCC CK7 (+); unlike Collecting Duct Ca: High molecular weight CK (K903) (-))

**Chromophobe RCC:** Only 3-5% of RCC are of chromophobe type and are associated with multiple chromosomal deletion. These tumors also have a better prognosis than clear cell RCC. Cytology shows a cellular aspirate composed of broad ribbons and loosely
cohesive groups of cells with fluffy/flocculent to granular cytoplasm with focal vacuolization and distinct cell borders. There is frequent binucleation and nuclear size variation. Diff-Quik stain shows a perivascular reticulated zone. DDX: Oncocytoma, clear cell RCC (Oncocytomas could have a membraneous staining of Hale’s colloidal iron whereas Chromophobe RCC are usually diffusely positive. Due to morphologic, molecular, and antigenic similarities (such as c-kit (+)22 in both tumors) it is thought that these tumors could be of similar descent i.e. different expressions of the same morphologic spectrum, but detailed studies are still needed to verify this theory. A variety of ipox have been reported to have a differentiating value between chromophobe RCC and oncocytoa ks-cadherin (+/-)23, caveolin 1 (+/-)24, CD 3 (-/+), CD63 (diffuse+/apical or polar+)26 although to this date the gold standard still electron microscopy where oncocytoas show abundant cytoplasmic mitochondria and chromophobe RCC show cytoplasmic microvesicles).

Sarcomatoid RCC: Accounts for 3-5% of renal cell carcinomas. It is defined by the presence of a high grade spindle cell component, with or without epithelioid differentiation. If the epithelial component is not seen CK (+) cells are necessary for diagnosis. It is not a subtype for RCC, it rather represents dedifferentiation of any type of RCC. The prognosis bad with a median survival of 6 months. Cytology shows malignant high grade spindle cell component in a background of RCC. Undersampling could fail to detect the sarcomatoid component thus if there is a radiologic suspicion for sarcomatoid differentiation (different echogenic areas) it is important to ask for additional samples for an accurate diagnosis. DDX: Metastatic high grade sarcoma (sarcomatoid cells are EMA, CK (+), sma (+/-), vimentin (+))

Collecting Duct Carcinoma: Very rare adenocarcinoma arising from the collecting ducts epithelium. Cytology shows cells arranged in small clusters, papillary configuration or single cells with scant dense to vacuolated cytoplasm, high N:C ratio, hyperchromatic nuclei, and prominent nucleoli. DDX: High grade RCC, High grade UC, metastatic carcinomas (cytology alone can not distinguish these entities, ipox is of help; i.e. CDC ulex and mucin (+)).

Renal Medullary Carcinoma: Clinical entity seen in young black men with sickle cell trait. Cytology reveals cohesive cellular groups with vacuolated cytoplasm, indented nuclei, irregular membranes, coarse or vesicular chromatin.27 DDX: High grade urothelial carcinoma, metastatic carcinoma.

Urothelial Carcinoma (UC): This is the most common tumor of the renal pelvis, accounting for 10% of all malignant renal tumors. It is most commonly seen in men in 7th decade1,28. There is a significant association with synchronous or metachronous urothelial tumors in other sites so the surgical approach is different than of RCC therefore differentiation on cytology is important. The low grade (LG) tumors cytologically reveal sheets and papillae composed of cells with dense, nonvacuolated cytoplasm with large hyperchromatic nuclei. High grade (HG) tumors reveal single or small clusters of cells with scanty dense to wispy cytoplasm, high N:C ratio, with large hyperchromatic nuclei.
and cercariform cells. DDX of LG UC: Papillary RCC (less layers of epithelial elements compared to UC). DDX HG UC: Collecting duct carcinomas, metastatic papillary neoplasms (e.g. lung, thyroid) and papillary RCC (history and ipox helps in accurate diagnosis. UC are CK 7, CK 20, uroplakin III and thrombomodulin (+))

**Metastatic Carcinomas:** The most common primary sources are breast, lung, intestine, opposite kidney, and stomach. Metastatic tumors of the kidney are often bilateral and multifocal. The history of a previous primary lesion and an ipox aid in the diagnosis.

**Other rare lesions:** Lymphoma (secondary involvement of diffuse large B-cell lymphoma is the most common), sarcoma (leiomyosarcoma is the most common type).

### Summary of immunohistochemical profiles of common renal neoplasms

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UC= Urothelial carcinoma, PRCC=Papillary renal cell carcinoma, CDC=collecting duct carcinoma, RCC=Renal cell carcinoma, CRCC=Chromophobe renal cell carcinoma, CK 7=cytokeratin 7, LMW= low molecular weight, HMW= high molecular weight, CEA= carcinoembryonic antigen
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Superficial (umbrella) cells

Lithiasis

Human Polyoma Virus effect

Seminal vesicle cell
Treatment effect – BCG related granuloma

Urothelial carcinoma

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DNA Ploidy - Image Analysis

DNA ploidy – laser scanning cytometry

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