Urological Tests in Clinical Practice

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Springer

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British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Control Number: 2006922752

ISBN-10: 1-84628-390-6 e-ISBN 1-84628-422-8 Printed on acid-free paper
ISBN-13: 978-1-84628-390-1

9 8 7 6 5 4 3 2 1

Springer Science+Business Media, LLC
springer.com

Preface

As this book was going to press, the Editor-in-Chief of the British Journal of Urology International introduced an interesting and timely phrase "finger tip urology". He has rightly pointed out that medical knowledge base is expanding so rapidly that no urologist can keep pace. The implication is that there is a desperate need for an easy reference source in many areas of urology. We think that this book is "spot on"!

Urological diagnosis is rarely possible without tests. And there are several tests to request. What investigation to order in which patient is always in the mind of those who care for urological patients. We hope that this book helps to resolve some of that dilemma.

The book is in two parts. Part one details the tests urologists today need to do, ranging from simple dipstix testing of urine to MR spectroscopy. No test is of value if one does not know when to apply it. Part two therefore outlines common urological conditions and the tests that need to be performed to reach a diagnosis. There is also a wealth of other information too; to give an example, TNM classification of tumors.

We hope that you as a busy resident in urology, newly appointed urologist, specialist urological nurse or other health professional allied to urology, will find this "finger tip information" of value in the management of common urological problems.

This book obviously contains a lot of information, and though quite substantial, is succinct and may therefore suffer from the usual disadvantage of over simplicity. But that is the whole purpose—providing a quick reference. We hope that you find this handy and useful in your practice.

Best wishes

N.Rao
S.Srirangam
G.Preminger

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Part II: Urological Conditions Requiring Investigations

Part I
Urological Investigations

Chapter 1
Urine Tests

(a) URINALYSIS

Urinalysis is a requisite examination in the assessment of all urological patients and comprises—

A. Physical examination of urine
B. Dipstick analysis
C. Microscopic analysis of urine

Appropriate specimen collection and transport is vital for accurate and clinically valid results.

Specimen collection and transport

Midstream specimen of urine (MSU)

The typically acidic and concentrated early morning urine (EMU) samples are more likely to detect a urinary tract infection (UTI), since red blood cells (RBC), white blood cells (WBC), and casts are best preserved in such a medium. In addition, overnight bacterial proliferation will also increase the yield from EMU samples.

- Males should retract the foreskin, clean the glans penis, and void with the foreskin retracted and collect a mid-stream sample without stopping urine flow
- Females are required to clean the vulva and separate the labia prior to specimen collection

Suprapubic aspiration of urine

- Gold standard technique for the diagnosis of a UTI
- May be required in patients with an equivocal result from an MSU
- Commonly employed in the pediatric population
- Best achieved by a sterile suprapubic puncture (with a spinal needle) under ultrasound guidance

Urine via intermittent catheterization

- Requires passage of a lo-fric® catheter to obtain a urine sample
- Discard initial urine and collect subsequent urine
- Even a sterile intermittent catheterization carries up to a 2% risk of introducing a new UTI

Fractionated urine samples

In males, although MSUs are adequate, in certain clinical conditions it might be appropriate to take different aliquots of urine to aid localization of the UTI (e.g., diagnosis of prostatitis).

- Voided bladder 1 (VB1)—first 5–10 mL of voided urine and represents urethral flora
- Voided bladder 2 (VB2)—mid-stream urine and best correlates with bladder urine
- Voided bladder 3 (VB3)—initial 2–3 mL of urine, containing expressed prostatic secretions (EPS), collected following prostate massage

In patients with a suspected sexually transmitted infection (STI), urethritis, or urethral discharge, a VB1 and VB3 specimen are sent separately for culture.

- A bacterial count greater than 10 times in VB3 compared to VB1 or 2 is diagnostic of prostatitis
- A leucocyte count >10 per high-power field in VB3 compared to VB1 or 2 is diagnostic of prostatitis
- An EPS or VB3 pH of >8 is suggestive of prostatitis
- A higher bacterial count in VB1 compared to VB3 is diagnostic of urethritis

Urine from indwelling catheters

Interpretation of urinalysis from patients on long-term indwelling catheterization is problematic for a variety of reasons, and unnecessary treatment may result in the emergence of antibiotic –resistant organisms.

- Bladder colonization is inevitable and can occur within 4 days of catheter placement
- A positive bacterial growth does not necessarily suggest a significant UTI
- Antibiotic therapy is unlikely to eradicate the targeted pathogen while the patient remains catheterized
- Colonizing flora may change over time

In such patients, a urine sample should only be sent if a UTI is suspected in a systemically unwell patient. Urine must be taken from the collection port and not from the catheter bag.

Urine from ileal conduits
Skin organism contamination is inevitable in patients with urinary diversions and therefore urostomy bag urine is not suitable. If clinically indicated, urine collection should be via a catheter introduced as far into the conduit as it will go.

Urine collection in children
- UTIs are common in children
- Urine collection can be difficult
- Toilet-trained children can provide an MSU
- Urine from children not toilet trained can be obtained by a "clean voided" bag sample, suprapubic aspiration, or transurethral catheterization

The relative merits and drawbacks are discussed in Table 1.1.

Urine transport
- Specimen must reach laboratory within 2 hours
- Delay can result in either over-proliferation or death of organisms
- Alternatively, store at a temperature of 4°C if a delay is encountered and analyze as soon as possible
- Beware that refrigeration can result in a decreased number of urinary leucocytes

A. Physical examination of urine
Color: The endogenously produced pigment, urochrome, gives urine its characteristic yellow-brown color. Since urochrome is excreted at a uniform rate (i.e., the same amount per hour), the color of urine varies primarily with urine output, which in turn is predominantly affected by the patient's hydration status. In addition, a variety of other compounds related to food, medication, and infection can alter the color of urine. Patients commonly complain of altered urine color and it is important to be aware of common urine color-altering factors, as listed in Table 1.2.

Turbidity: Cloudy urine is commonly caused by—
- Phosphaturia—will typically occur after consumption of a large meal or quantity of milk in susceptible patients.

TABLE 1.1. Comparision of urine collection techniques in children

	Clean voided bag samples	Suprapubic aspiration	Urethral catheterization
Technique	Clean perineum and external genitalia. Apply bag. Remove promptly and perform urinalysis after micturition	Insert a 1.5-inch, 22-gauge needle, 1–2 cm above symphysis pubis under ultrasound guidance; aspirate 5 mL of urine	Insert a 5 or 6 F urethral catheter into bladder using lidocaine lubricant jelly; discard first few drops of urine
Advantages	Non-invasive	Safe High sensitivity (>95%) Quick	Safe Successful in virtually 100% of cases
Disadvantages	Contamination—high false positive (63%) Positive culture may not be sufficient to commence antibiotic therapy	Hematuria, intestinal or viscus perforation (risk very small) Success rate variable—46–96% Can only be used in children <2 years age	Invasive Urethral trauma/hematuria False positives (80%) Takes longer than suprapubic aspiration Cannot be used in older children
Clinical comments	Positive results usually require validation by using an invasive technique	Gold standard	Correlates reasonably well with suprapubic aspiration

TABLE 1.2. Factors affecting urine color

Color	Causes
Red	Hematuria Hemoglobinuria Myoglobinuria Beet-root Blackberries Rifampicin Heavy metal poisoning (mercury, lead)
Yellow	Riboflavin Phenacetin
Orange	Concentrated urine (dehydration) Phenazopyridine Sulfasalazine
Blue or green	Biliverdin Dyes (methylene blue, indigo carmine) Cimetidine Promethazine
Brown	Hemorrhage Laxatives (e.g., senna) Urobilinogen Porphyria Rhubarb Aloe Anti-malarials (e.g., chloroquine) Antibiotics (e.g., nitrofurantoin, metronidazole) Methyldopa

Diagnosis is completed by either acidifying the alkaline urine to dissolve the excess phosphate crystals (urine turns clear) or by visualizing the precipitated phosphate crystals under microscopy

- UTI—pungent-smelling, cloudy urine is likely to be secondary to pyuria associated with an infective process
- Rare causes of turbid urine include chyluria (lymph fluid in urine), hyperoxaluria, and lipiduria

B. Urine dipstick analysis

Dipstick testing is useful in assessing patients with—

- Renal disease
- Urological disorders
- Metabolic disease not related to the kidneys

TABLE 1.3. Reference range for urine dipstick parameters

	Adult	Child
Color	Light straw to dark amber	Light straw to dark yellow
Turbidity	Clear	Clear
Odour	Aromatic	Aromatic
Specific gravity	1.005–1.040	1.005–1.030 (1.005–1.020 in newborns)
pH	4.5–8.0	4.5–8.0
Blood	Negative	Negative
Protein	Negative	Negative
Glucose	Negative	Negative
Ketones	Negative	Negative

Urine dipsticks use reagent strips, where a chemical reaction with an active compound results in color changes that provide important information on (1) urinary specific gravity, (2) pH, (3) blood, (4) protein, (5) glucose, (6) ketones, (7) WBC, (8) nitrites, and (9) bilirubin and urobilinogen. Normal findings are highlighted in Table 1.3.

Specific gravity (SG)
- Measure of total solute concentration
- Usually varies between 1.001 and 1.040
- SG of >1.020 is concentrated
- SG of <1.008 is considered dilute

The state of hydration and renal insufficiency are the two main determinants of urine SG.

- SG decreases with age as the renal concentrating ability diminishes
- Over-hydration, diuretic therapy, diabetes insipidus, and renal failure will all result in a decreased urine SG
- In renal failure (acute or chronic) the kidneys lose the ability to concentrate urine and the SG remains fixed at 1.010

pH
- Urinary pH ranges from 4.5 to 8
- pH of <5.5 is considered acidic
- pH of >6.5 regarded alkaline
- Urinary pH changes rapidly when in contact with air; therefore, prompt testing is essential

As a general rule, urinary pH reflects serum pH, but there is an important exception.

- In renal tubular acidosis (RTA), the urine will often remain alkaline in spite of the presence of acidic urine, due to urinary bicarbonate loss
- In severe type II RTA (proximal) the urine may occasionally become acidic, but will always remain alkaline even with severe type I RTA (distal)

Additionally, alkaline urine (pH over 7.5) suggests infection by a urea-splitting organism such as *Proteus*. Vegetarians commonly have alkaline urine due to low acid ingestion. Uric acid and cystine stones form in acidic urine (pH less than 5.5).

Blood

- Detection of blood is due to peroxidase-like activity of hemoglobin, which catalyzes the oxidation of a chromogen indicator, causing a color change in direct proportion to the amount of blood in urine
- Hematuria, hemoglobinuria, and myoglobinuria will all result in positive dipstick for blood
- Dipstick detection of hematuria (defined at >3 RBC/high-power field) has a sensitivity of over 90%
- There is also a higher false-positive rate

Common causes of false-positive results include—

(i) Urine contamination with menstrual blood
(ii) Dehydration
(iii) Exercise
(iv) Oxidizing agents
(v) Bacterial peroxidase

False-negative results may occur with poorly mixed urine or with patients on high doses of vit C. Microscopic evaluation is mandatory in any patients with positive dipstick test for hematuria.

Protein

- Proteinuria in adults is defined as the excretion of >150 mg of protein per day (normal protein excretion rate is 80–150 mg)
- Dipstick analysis will detect concentrations as low as 10 mg/dL

The protein concentration of urine alters the urine pH and this results in a change in the color of the pH-sensitive dye on the dipstick. Albumin, the primary urinary protein, causes the dipstick to turn green; the darker the green, the greater the urinary protein concentration.

Causes of false negatives include—

- Alkaline urine
- Dilute urine
- Urine in which albumin is not the primary protein

The detection of proteinuria on a dipstick is a measure of protein concentration and not protein excretion; therefore, measurement of 24-hour urinary protein excretion and protein electrophoresis is mandatory to rule out underlying renal disease.

Proteinuria can be classified by timing into—

1. Transient: common; occurs in children; also in patients with sepsis, cardiac failure and following exercise; spontaneous resolution
2. Intermittent: occurs in young men in the upright position due to increased renal vein pressure; not pathological
3. Persistent: pathological and requires further evaluation with 24-hour urine collection for protein excretion

(Proteinuria is further discussed in the section on 24-hour urine collection. Section 1f–page 28)

Glucose

Detection occurring due to a double oxidative reaction of the glucose with glucose oxidase on the dipstick is specific for glucose and does not occur with any other sugars. Patients with a positive glucose dipstick should be investigated for diabetes mellitus.

- Glucosuria should not be present in the normal healthy adult
- A positive dipstick suggests that the renal tubular reabsorption threshold has been exceeded. This corresponds to a serum glucose level of about 18 mg/L
- Nevertheless, the degree of glucosuria correlates poorly with hyperglycemia

Ketones

- Ketones (acetoacetic acid and acetone) are detected by the nitroprusside test

- Not usually present in urine from healthy adults
- Frequently associated with catabolic situations (with increased breakdown of body fat) such as diabetic ketoacidosis, starvation, and pregnancy

WBC

Pyuria is detected either by dipstick or microscopy. Leucocyte esterase, a leucocyte-specific isoenzyme, is the basis for dipstick testing. Common causes include—

- UTI
- Tumors
- Stones
- Glomerulonephritis
- Foreign bodies
- Fungal infection
- Tuberculosis
- Certain drugs (e.g., cyclophosphamide)

False-positive tests occur due to contamination. False-negative results are usually due to—

- Inadequate contact of dipstick with urine
- Increased urinary specific gravity
- Glycosuria
- Presence of urobilinogen
- Ascorbic acid in urine

Therefore leucocyte esterase in isolation is insufficient to diagnose a UTI, but in conjunction with positive urinary nitrites is strongly suggestive of infection. Its use should be confined to screening urine in the asymptomatic patient in the primary care setting.

Nitrites

- Common urinary gram-negative bacteria can convert nitrates to nitrites
- A positive nitrite dipstick has a high specificity for detecting bacteruria (>90%) but its sensitivity is variable (40–85%). Accuracy is also diminished in subclinical bacteruria ($<10^5$ organism/mL)
- Contamination can result in a false-positive result
- In the hospital setting, urine dipstick for leucocyte esterase and nitrites is not an adequate replacement for urine microscopy and culture in the detection of urinary tract infection

Bilirubin and urobilinogen

- Normal adult urine should contain very little urobilinogen and no bilirubin
- Patients with liver disease and biliary obstruction will have elevated levels of conjugated bilirubin which is water-soluble and will be detectable on dipstick
- Urinary bilirubin is pathological and warrants hepatobiliary investigations
- Urobilinogen is the end product of conjugated bilirubin metabolism, but is also increasingly detectable in urine in patients with hemolysis, hepatocellular disease, and gastrointestinal hemorrhage

C. Urine microscopy

Urine microscopy is useful, reliable, and cheap. Indications include—

- Suspected UTI
- Suspected acute glomerulonephritis
- Unexplained acute or chronic renal failure
- Hematuria
- Suspected urinary tract malignancy

Technique

- Obtain a clean catch, first morning (preferably) urine sample MSU
- About 10–15 mL is centrifuged at 3,000 rpm for 5 minutes, with the supernatant subsequently discarded
- 0.01–0.02 mL of the residual sediment is placed directly on the microscope slide and covered with a coverslip
- Microscopy should include examination at both low power (×100 magnification) and high power (×400 magnification). Low-power magnification is adequate for the identification of most cells, macrophages, and parasites, but high-power is required to discriminate between circular and dysmorphic RBC, and to identify crystals, bacteria, and yeast
- Careful inspection is performed for (1) cells, (2) casts, (3) crystals, (4) bacteria, (5) yeast, and (6) parasites

It is prudent to remember that the volume of urine visualized in one HPF represents 1/30,000 mL and false negatives, due to this volume constraint, are therefore inevitable.

1. Cells

RBC

Urine containing less than 3 RBC per HPF is considered normal. Microscopy is superior to urine dipstick in detection of hematuria (greater than 3 erythrocytes/HPF). Although both tests have a similar sensitivity, the specificity of dipstick analysis is lower (about 70%). Microscopy can also distinguish between hemoglobinuria (large number of erythrocytes seen) and myoglobinuria (erythrocytes absent).

Additional features can assist in localizing the origin of hematuria in glomerular and non-glomerular causes (see Table 1.4).

WBC

Pyuria is best diagnosed by microscopic examination of centrifuged urine sediment. Normal urine may contain up to 2 WBC per HPF in men, and up to 5 WBC per HPF in women. Significant pyuria requires >10 WBC per HPF.

- Fresh leucocytes (larger and rounder) are more suggestive of pathology, while old leucocytes (small and wrinkled) are usually seen in urine contaminated with vaginal secretions
- Large numbers of WBCs per HPF is highly specific for UTIs (especially if associated with hematuria), but various factors can affect the numbers of WBCs detected, including the intensity of the inflammatory response; hydration status of the patient; urine collection technique; and centrifugation and sampling technique
- Other causes of significant pyuria include almost any non-infective urinary tract pathology and results must be interpreted appropriately. Persistent abacterial pyuria should instigate investigations to exclude—
  - Urinary stones
  - Tumors
  - Urinary tuberculosis
  - Glomerulonephritis

The absence of pyuria, in conjunction with either a negative bacterial culture or with a growth of mixed organisms, is likely to be secondary to contamination. Moreover, even with isolation of a single urinary pathogen, the absence of pyuria would suggest a contamination/sampling error in over 85% of patients.

TABLE 1.4. Origin of hematuria in glomerular and non-glomerular causes

		Non-glomerular	
	Glomerular	Renal	Urological
Erythrocyte morphology	Typically dysmorphic (rarely round) Minimal hemoglobin Irregular cytoplasm distribution	Circular/normal morphology	Circular/normal morphology
Associated proteinuria	Significant proteinuria (2+ to 3+ on dipstick, 100–200 mg/dL)	Usually significant proteinuria	Usually absent
Casts	Usually contains red blood cell casts	Casts absent	Casts absent
Causes	Glomerulonephritis (GN) IgA nephropathy (Berger's disease) Mesangioproliferative GN Focal segmental proliferate GN Familial GN (Alport's syndrome) Membranous GN	Polycystic kidney disease Medullary sponge kidney Papillary necrosis Coagulopathy Renal tumors Exercise-induced Renal artery embolism/thrombosis Renal vein thrombosis	Urological tumors Stones UTI
Action	Serum creatinine 24-hour urine for protein excretion Referral to nephrologist Renal biopsy?	Rule out urological cause Treat underlying condition?	Urine cytology Cystoscopy Renal tract imaging

Epithelial cells

Microscopy may also reveal squamous, transitional, and renal tubular cells.

- Squamous cells originate from the vagina, urethra, or trigone and typically appear large with irregular cytoplasm and a small, central nucleus
- Transitional cells are smaller with prominent cytoplasm staining, and have a large nucleus. The features of malignant transitional cells are discussed later in the chapter on urine cytology
- Renal tubular cells are larger and uncommon but, if present, suggest a glomerular pathology

2. Casts

The Tamm–Horsfall mucoprotein, excreted by the renal tubular epithelial cells, forms the basic medium for all renal casts by entrapping any cells (e.g., RBC, WBC, and sloughed renal tubular cells) within the tubular lumen. Hyaline casts, containing only mucoproteins, are excreted normally and therefore are not considered pathological. Conditions that increase hyaline cast excretion include pyelonephritis, chronic renal failure, and physical exercise.

Careful microscopic inspection of cast contents will disclose a number of underlying conditions (see Table 1.5).

3. Crystals

A number of distinct crystals can be identified in the urine of normal patients, but are more frequently seen in the urine of patients with stone disease. Crystal precipitation is dependant on urine pH.

TABLE 1.5. Urinary casts

Casts	Clinical Significance
Hyaline	Present in normal urine (↑ in dehydration and proteinuria)
Red blood cells	Glomerular bleeding due to glomerulonephritis
White blood cells	Seen in acute pyelonephritis and acute glomerulonephritis
Cellular	Nonspecific renal damage resulting in sloughing of renal tubular cells
Fatty	Commonly seen in nephritic syndrome, lipiduria and hypothyroidism

- Alkaline urine → calcium phosphate, calcium triphosphate (struvite) crystals
- Acidic urine → calcium oxalate, uric acid and cystine stones

Crystals are discussed in more detail in the chapter on urine analysis for stones.

4. Bacteria

Although the results of urine culture are far more important than the results of microscopy, in the diagnosis of a UTI, an effort should be made to detect bacteria on urine microscopy, as normal urine is sterile and should contain no bacteria.

Nevertheless, contamination, either from poor sampling technique or vaginal/peri-urethral flora in a female, can often result in positive identification in urine. In males, detection of any bacteria should prompt further evaluation with a urine specimen. In females, a count of 5 bacteria/HPF reflects a colony count of about 100,000 organisms/mL and is diagnostic of a UTI.

The morphology of any observed bacteria can provide useful clues. The most common uropathogens are aerobic gram-negative rods and these have a characteristic bacillary shape. *Staphylococci* are seen in clumps, whereas *streptococci* typically form beaded chains. The presence of filamentous *lactobacilli* is suggestive of vaginal floral contamination.

5. Yeast

Candida albicans is the most common yeast cell found in urine, especially in patients with diabetes mellitus or long-term indwelling urinary catheters. Contamination can occur from a vaginal source. The characteristic appearance of *Candida* with budding and hyphae formation distinguishes it from other microorganisms.

6. Parasites

Patients with schistosomiasis (*S. hematobium*) usually originate from, or have travelled to North Africa or the Middle East, and inspection of their urine during the early/middle stage may reveal the characteristic parasitic ovum with its terminal spike. Examination of a terminal specimen of urine yields best results.

Trichomonas vaginalis are large flagellated organisms which can be easily seen in patients with urethritis.

Urine culture

- Diagnosis of a UTI requires bacterial growth in urine culture
- Urine should be cultured immediately or refrigerated at 4°C and analyzed within 24 hours

Although various techniques of urine culture and bacterial count estimation have been described, the standard surface plating and dip-slide methods are the most widely used.

Technique

1. **Standard surface plating**: a standard loop is dipped in urine (0.1 mL) and inoculated onto an agar plate. One half of the agar plate is blood agar (grows any bacteria), while the other is a more selective medium, such as MacConkey or eosin-ethylene blue, which grows gram-negative bacteria. Each bacterial rod or cocci cluster will form a colony after overnight incubation, which can be counted, identified, and multiplied by 10 to report the number of colony-forming units (cfu) per mL of urine. This technique is reasonably accurate and is widely recommended.

2. **Dip-slide method**: is simpler, less expensive, and less accurate than standard surface plating. A commercially available, double-sided, agar coated slide is dipped in urine, incubated overnight in its sterile container and the approximate bacterial count estimated by reading off a supplied picture chart. This semiquantitative method is useful for general practices or remote medical centers.

Interpretation

Though it is well recognized that significant bacteriuria represents a count of greater than 10^5 cfu/mL, this is perhaps an oversimplification

- Urine is a good culture medium (with a bacterial doubling time of 30–45 minutes) and, if allowed to incubate long enough, most bacteria will reach a count level of 10^5 cfu/mL
- Therefore "significant bacteriuria" only applies if the sample has been collected, transported, and cultured appropriately
- Nevertheless, 70–80% of women with a UTI will have 10^5 cfu/mL
- The majority of women with counts of $<10^2$ cfu/mL do not have a UTI and bacterial growth is due to contamination or delay in specimen transport

- A third of women with acute cystitis symptoms have a count of between 10^2 and 10^4 cfu/mL. In these patients, the commonly isolated pathogens include *E. coli*, *Proteus*, and *S. saprophyticus*. Therefore, in the symptomatic female, the appropriate threshold for defining "significant bacteriuria" is 10^2 cfu/mL of a known pathogen. A likely explanation for this phenomenon may be that frequent voids in symptomatic patients result in a decreased colony count
- Counts of $>10^5$ cfu/mL are due to contamination in up to 20% of patients (either from the vagina/perineum in infection-susceptible women or the foreskin in uncircumcised men)
- Contamination in men is uncommon; therefore, counts of 10^2 cfu/mL in a well-collected urine sample should be considered significant
- Identification of any bacteria in urine obtained by sterile suprapubic aspiration is clinically significant

Significant bacteriuria in adults in clinical practice can therefore be summarized as follows:

Females
- MSU showing $>10^3$ cfu/mL with acute uncomplicated cystitis
- MSU showing $>10^4$ cfu/mL with acute uncomplicated pyelonephritis
- MSU showing $>10^5$ cfu/mL with a complicated UTI

Males
- MSU showing $>10^4$ cfu/mL with a complicated UTI

Features that are indicative of a true UTI include—

1. Symptomatic patient
2. Single organism isolated
3. Repeat culture identifies same uropathogen
4. Pyuria (>10 WBC/HPF)
5. Significant bacteriuria (≥10 cfu/mL suprapubic aspiration or $>10^4$ cfu/mL from MSU)

Be aware that patients with a high fluid intake, urinary frequency, and/or on antibiotics may have a decreased bacterial count.

Features which decrease the likelihood of a UTI include—

1. No urinary symptoms
2. Mixed bacterial growth
3. Count $<10^4$ cfu/mL
4. No pyuria
5. Epithelial cells or lactobacilli on microscopy suggests vaginal contamination

(b) FLOW CYTOMETRY (DNA PLOIDY)

Introduction

Tumors are characterized by a higher percentage of proliferating cells, and therefore the presence of increased mitotic figures correlates with tumor aggressiveness in transitional cell carcinoma (TCC) of the urinary tract. Flow cytometry (FCM) can measure the DNA content of cells, and therefore objectively quantitate the aneuploid cell population and the proliferative activity (proportion of cells in S phase of the cell cycle) within a tumor.

Indications

Due to the fact that FCM has not demonstrated clinical superiority over urine cytology, its use remains sporadic. Potential indications include—

- As a screening tool, in combination with urine cytology, for the high-risk patient group
- As a prognostic indicator of muscle invasiveness
- As a predictor of recurrence, in combination with morphonuclear scoring
- To assess efficacy of intravesical therapy for TCC of the bladder

Specimen collection and analysis

- A large number of cells are required for pertinent analysis, and less than 50% of voided specimens have adequate cellularity for FCM
- Up to one-third have uninterpretable histograms due to cell degeneration in unfixed specimen
- Although FCM is best performed on bladder irrigation specimens, fresh, frozen or paraffin-embedded tumor tissues can also be used

To prevent cell degeneration, bladder irrigation samples must either be analyzed immediately, refrigerated and tested within 24 hours, or frozen for subsequent use.

Interpretation

- The majority of normal urothelial cells as well as grade I TCC are DNA diploid
- Most grade III are DNA aneuploid
- Suspect CIS if bladder irrigation specimen is DNA aneuploid and bladder biopsy DNA diploid

The majority of patients (97%) with cytological diagnosis of cancer have abnormal DNA ploidy, but only 5% of patients with normal cytology have abnormal DNA ploidy (see Table 1.6)

Prognosis: Grade II TCC are a heterogeneous group with equal numbers of DNA diploid and aneuploid cases. DNA ploidy adds more prognostic information in this group than the grade itself. In the superficial grade II TCC, the 5-year recurrence-free rates are 75% for DNA diploid compared to 25% for DNA aneuploid. In general, the trend with regards to prognosis is diploid > tetraploid > triploid to tetraploid.

Progression: In superficial TCC (grade I/II), progression to muscle invasion, in a tumor containing DNA diploid, DNA tetraploid, and DNA aneuploid cell populations, occurred in 2%, 10% and 50% of patients respectively. Nearly all grade III patients are aneuploid and FCM is not of value in their prognosis.

Survival: In CIS, the 5-year progression-free survival in patients with one and multiple DNA aneuploid populations is 67% and 20%, respectively. The mean survival time for DNA diploid T3a or less is 91 months versus 26 months for DNA aneuploid T3b or T4. Patients with low S-phase fractions (<11%) also have a longer recurrence-free survival than those with higher values.

Recurrence: The presence of a clear non-tetraploid DNA histogram is diagnostic of recurrent TCC. In patients with atypical cytological findings, 20% with abnormal DNA ploidy have a recurrence compared to only 5% with normal DNA ploidy. Combining FCM with cytology has a 95% sensitivity in detecting recurrent TCC in the bladder, with no loss of specificity. In

TABLE 1.6. DNA ploidy

DNA Status	Cytology Negative (%)
DNA diploid	98
DNA tetraploid	69
DNA aneuploid	8

addition, combining FCM with the morphonuclear score (an image analysis-based nuclear grading system) recurrence versus non-recurrence is predicted in 91% of low-grade disease.

Treatment monitoring: Patients treated with radiotherapy for muscle-invasive TCC have a clinical response in 100%, 54%, and 30% of cases for tumor that are DNA diploid, aneuploid, and multiploid, respectively. About 70% of patients with an S-phase fraction <11% survive 10 years in contrast to only 30% of patients with a fraction value of >11%. After radiotherapy, a DNA tetraploid population can be expected up to 2 years post-therapy and should not be interpreted as treatment failure.

The presence of DNA diploid cells in patients undergoing intravesical chemotherapy indicates a good response, whereas an aneuploid DNA histogram indicates treatment failure.

Drawbacks

- Invasive procedure (catheterization for specimen collection)
- Large number of cells required
- Only significant DNA chromosomal abnormalities lead to changes in the DNA ploidy detectable by FCM and minor chromosomal abnormalities are missed
- Not all TCC is associated with an abnormal DNA content
- FCM equipment not available in all units

- FCM measures cell DNA content (the degree of aneuploidy)
- Most grade III are DNA aneuploid
- 97% of patients with TCC and abnormal cytology have abnormal DNA ploidy
- Abnormal DNA ploidy is associated with poor prognosis (higher recurrence and progression, poorer response to treatment)
- General prognostic trend is diploid > tetraploid > triploid to tetraploid

(c) CYTOLOGY

Introduction

Urothelial cells are constantly being exfoliated into urine in both normal patients and those with transitional cell carcinoma (TCC)

of the urinary tract. These cells can be identified, fixed, and analyzed in order to determine cell morphology, thereby providing a convenient, non-invasive technique for the observation of high-risk patients. As a gold standard, voided urine cytology (VUC) is unquestionably inadequate and is plagued with problems of poor sensitivity. Nevertheless, while emerging novel urinary markers for the detection of TCC still remain largely unproven, VUC will have widespread use.

Indications

- Surveillance or screening for TCC or CIS in the urinary tract
- Frank hematuria in any patient >40 years of age
- Irritative lower urinary tract symptom in any patient >40 years of age (to detect CIS)

Specimen collection and analysis

One variable affecting the sensitivity of urine cytology is the type of specimen. Voided urine is easy to obtain, but generally hypocellular and degenerated. Contamination by skin and vaginal contents may also occur in females. The sensitivity is augmented when three specimens are obtained on separate days. For one, two, and three voided specimens, sensitivities of 41%, 41%, and 60% have been reported respectively.

- *Voided urine*: an early-morning sample is not suitable and the second morning provides the best specimen. Collect 50 mL in a universal container and send to the lab immediately
- *Catheterized urine samples and bladder washouts*: these have a higher cellularity and less contamination, but require an invasive procedure that may introduce instrument artefact. In addition, urine from patients with an indwelling catheter is unsuitable for analysis, as denuded normal epithelial cells may be interpreted as low-grade TCC. Saline bladder washouts are three times as accurate (80% for CIS) as voided urine since the mechanical action of barbotage enhances tumor cell shedding and better preserves for examination
- *Ureteric urine sampling*: the ureter is catheterized up to a point just below the level of suspected lesion. Urine from the other ureter is sent for control. False-negative rates are high (22% to 35%), but saline washes will improve overall sensitivity. Brush biopsy performed via a retrograde catheter improves yield, with a reported sensitivity of 91%, a specificity of 88%, and an

accuracy of 89%. Complications include ureteric bleeding and perforation

Analysis

- Urine must be sent for analysis as soon as possible after collection
- Following centrifuge, the cell pellet obtained is divided between two slides
- One is stained with Papanicolaou stain and the other with hematoxylin and eosin
- Microscopic analysis is performed

Interpretation

Primary goals of VUC are to—

- Recognize early flat lesions such as CIS before they invade
- Detect the 10% of superficial lesions destined to invade

Characteristically, TCC cells may appear singly or in small clusters, have large hyperchromatic nuclei with irregular, coarsely textured chromatin. Malignant cells identified in cytological specimens may be classified as—

- Low grade—correlate with histological grade I lesions and some grade II lesions
- High grade—correlate with some grade II lesions, all grade III lesions, and also with CIS

In patients who are observed for bladder TCC, the overall sensitivity of positive urine cytology is approximately 40–60%, but the sensitivity increases to about 90% for high grade disease and CIS.

A positive cytological diagnosis is highly predictive of TCC, even in the presence of normal cystoscopy. Malignant cells may appear in the urine long before cystoscopically detectable lesions emerge, leading to a seemingly inflated rate of false-positive results. The overall reported false-negative rate is 65%, but may be as high as 96% in low-grade tumors.

High-grade TCC and CIS

- VUC has excellent performance statistics in patients with high-grade lesion, and as such justifies its use as a screening and surveillance device
- Sensitivity is at least 90%