

Urinalysis Notes:

II. Diagnosing kidney disease is based on abnormal findings

- A. Abnormal urinalysis
- B. Decreased GFR as determined by increased serum creatinine (NL = 1.0 to 1.4mg/dL)
- C. Abnormal imaging studies, such as with an ultrasound
- D. Abnormalities discovered on history and physical (H&P)

III. Abnormal renal function

A. We need to ask several questions to determine the cause of abnormal function:

- 1. Where is the location of the problem?
 - a. Is the problem pre-renal? Is the problem before the kidney?
 - b. Is this an intrinsic kidney disease? Is the problem within the kidney?
 - c. Is the problem post-renal? Is there a drainage problem after the kidney?
- 2. What is the chronicity of onset?
 - a. Is this an acute problem?
 - b. Is this a chronic problem?
- 3. If intrinsic kidney disease is present, where in the kidney is the defect?
 - a. Is there a problem with the glomerulus?
 - b. Is there a problem with the tubule?
- 4. What is the mechanism of abnormal renal function?
 - a. Is there an inflammatory process occurring?
 - b. Is this a non-inflammatory problem?
 - i. Is the problem associated with a metabolic disorder?
 - ii. Is the problem associated with ischemia?
- 5. What is the nature of the cause of renal dysfunction?
 - a. Is there a systemic disease causing renal dysfunction?
 - b. Is the problem limited to the kidney?

B. To help us answer these questions, the following provide useful information:

- 1. A urinalysis
- 2. History and records of old lab data showing previous urinalysis records and serum creatinine levels.
- 3. A physical exam
- 4. Kidney imaging studies, such as an ultrasound

C. The information we gather determines the management of the patient.

IV. A urinalysis is easy to do, it is inexpensive, it can be done in the office of your practice by any personnel, and it gives a lot of useful information.

A. Urinalysis Technique

- 1. Obtain a fresh urine sample ("pee in a jar") and perform urinalysis within 30-60 minutes of collection.
- 2. The sample should be a clean catch midstream and if possible obtain the sample in the morning.
- 3. Once collected, the urine is centrifuged at 3000 rpm for 3-5 minutes to separate the urine and obtain a supernatant and a sediment or pellet.
 - a. The sediment is evaluated with light microscopy
 - b. The supernatant is used to measure the chemicals present

4. A dipstick is used to evaluate the supernatant
 - a. The dipstick has several pads on it to allow us to evaluate the several chemicals in the supernatant of the urine.
 - b. Each pad undergoes a color change due to a reaction caused by the presence of various chemicals, such as glucose, blood, bilirubin, and others (discussed below)
 - c. The reactions are allowed to proceed for 1-2 minutes
 - d. The color change is then compared to standard calibrated colors
 - e. The results are qualitative or semiquantitative, meaning their measurements are classified as a small amount, a moderate amount, or a large amount.
- V. Parameters measured by evaluation of the supernatant: specific gravity, pH, glucose, ketones, bilirubin, nitrites, leukocyte esterase, and protein
- A. Specific gravity
1. Used as an index of urine concentration
 2. Specific gravity is a comparison between the mass of a certain volume of supernatant and the mass of an equal volume of distilled water
 3. Specific gravity is used as an indicator of urine osmolality to evaluate how concentrated the urine is, i.e., it tells us what the kidney is doing with water and solutes.
 4. There is a direct correlation between specific gravity and urine osmolality
 - a. Maximally dilute urine has a specific gravity = 1.002, which correlates with an osmolality = 50-100 mosmol/kg
 - b. Maximally concentrated urine has a specific gravity = 1.030-1.040, which correlates with an osmolality = 1000-1200 mosmol/kg
 - c. Urine which is isotonic to plasma (isosthenuric) has specific gravity = 1.010 which correlates to an osmolality = 300 mosmol/kg
 - i. Isosthenia indicates renal tubular damage, *so this is a good number to remember: specific gravity = 1.010 when tubular damage has occurred.*
 - ii. If the renal tubules are damaged, they cannot concentrate nor dilute the urine so the urine remains isotonic to the plasma.
 5. There are limitations when using specific gravity to measure urine concentration
 - a. Specific gravity is affected by the number and weight of solutes
 - b. Urine osmolality is only influenced by the number of solutes
 - c. Thus, an unusually high solute weight will make specific gravity an inaccurate measure of urine concentration
 - i. Glucose is a high molecular weight molecule and has a high mass in comparison with other solutes therefore if glucose is in the urine (glucosuria) then the specific gravity will indicate a higher osmolality than is actually present
 - ii. IV contrast media also causes an increased specific gravity that misrepresents osmolality, indicating a higher osmolality than is present.
- B. Urine pH
1. Is considered the least useful parameter
 2. Normal urine pH with a normal protein diet = 4.5-6.5 (the urine is normally acidic)
 - a. The kidney removes the acid produced from dietary protein metabolism
 - b. Situations where pH can be diagnostically helpful:
 - i. To assess metabolic acidosis
 - (a) During metabolic acidosis, the urine pH should be very acidic, <5.
 - (b) If metabolic acidosis is present but the urine pH is not lowered then this suggests distal renal tubular acidosis in which the kidney is not excreting enough NH_4^+ to get rid of the H^+ .
 - ii. To assess or diagnose urinary tract infections (UTIs)

- (a) The kidney is not physiologically capable of making markedly alkaline urine
- (b) If the urine pH = 8, then suspect a non-physiologic cause, such as a UTI caused by a bacteria, such as a *Proteus* species, which splits urea into ammonia (NH₃) and cause the urine to be alkaline.

C. Glucose

1. Normal urine has no glucose, because the kidney does not waste essential nutrients
2. Used to screen for certain systemic diseases such as hyperglycemia and diabetes mellitus
 - a. If blood glucose concentration exceeds the reabsorptive capacity of the Na/glc cotransporter in the proximal tubule, then glucosuria develops
 - b. The cotransporter obeys saturation kinetics and when overwhelmed glucose spills out into the urine.
 - c. When blood glucose exceeds about 180 (overt diabetic levels), then glucosuria and osmotic diuresis result
3. Glucosuria with a normal blood glucose level implies proximal tubule damage

D. Ketones

1. The body is normally metabolizing carbohydrates and does not make a lot of ketones
2. Thus normal urine has no ketones because the kidney will conserve the small amount of ketones that are present
3. Situations in which an increased amount of ketones are produced
 - a. Severe fasting or if patient has been sick with vomiting and diarrhea
 - b. Diabetic ketoacidosis
 - c. Alcoholic ketoacidosis
4. If blood ketone concentration exceeds the reabsorptive capacity of the Na-ketone cotransporter in the proximal tubule, then ketonuria develops
5. The dipstick is sensitive to acetoacetate and acetone
 - a. Acetone results from spontaneous decarboxylation of acetoacetate
 - b. The other ketoanion, β -hydroxybutyrate, is not detected, but ketones are usually made in equal molar amounts, so the dipstick is a good screen

E. Bilirubin

1. Urine dipstick for bilirubin is a screen for abnormal hepatobiliary function
2. Bilirubin is a metabolic byproduct of the breakdown of the heme moiety of hemoglobin
3. Bilirubin is carried to the liver, conjugated, and excreted with bile into the duodenum
4. Conjugated bilirubin is water soluble and is freely filtered by the glomerulus
 - a. Bilirubin is a waste material, so the kidney does not reabsorb it
 - b. Increased plasma levels of conjugated bilirubin result from impairment of transfer from the liver to the bile or from the biliary tract to the intestine
 - c. Elevated plasma levels result in positive bile in the urine
5. Unconjugated bilirubin is not water-soluble
 - a. When first broken down by the reticuloendothelial system, bilirubin is unconjugated
 - b. Unconjugated bilirubin cannot be excreted in the urine

F. Nitrites in the urine are indicative of a UTI

1. Nitrites are not present in normal urine, but nitrates are normally present
2. Nitrite occurs in the urine when there is bacteria that have nitrate reductase enzymes, specific enzymes that reduce nitrate to nitrite
 - a. Almost always gram-negative organisms
 - b. These are the most common organisms that infect the urinary tract:
 - i. *Proteus* sp.
 - ii. certain *E. coli*

- iii. *Kiebsiella pneumoniae*
- iv. *Pseudomonas aeruginosa*

G. Leukocyte esterase

1. A neutrophil-specific esterase
2. Its presence in the urine implies that an inflammatory process is occurring in the kidney or urinary tract
3. Indicates pyuria, the presence of white blood cells (WBCs) in the urine
4. WBCs are not present in normal urine

H. Protein in the urine

1. Suggests that something might be wrong at the glomerular level
2. Normal urine has < 150 mg/day of protein
 - a. Most of the normal protein in the urine is Tamm-Horsfall mucoprotein, which is produced by the thick ascending limb of the loop of Henle; its function is unknown and it is often found as a component of casts.
 - b. Low molecular weight proteins (LMWPs) are normally found in the urine
 - c. Only micro amounts (<20mg/dL) of albumin are normally found in the urine
3. To determine protein concentration in the urine:
 - a. Measure the amount of protein excreted over a 24 hour period
 - i. =3-3.5 g/day is the nephrotic range
 - ii. The nephrotic range is the amount of protein loss that is usually required to give the nephrotic symptoms of edema and hypoalbuminemia
 - b. Determine the ratio of urine protein concentration to urine creatine concentration
 - i. This is a simpler way to estimate total protein excretion per day
 - ii. The lab measures both protein and creatinine in mg/dL
 - iii. The advantage is that you don't have to do a timed urine collection
 - iv. Closely parallels the g/day measurement of protein in a 24-hour urine sample
 - v. A ratio of 5 means that the urine protein concentration is about 5 g/day
4. The following table shows the correlation of a 24-hour urine protein with the dipstick:

<u>24 hr urine (mg/day)</u>	<u>Dipstick</u>
<150	0
150	trace
300	1+
1000	2+
3000-4000	3+
>5000	4+

5. How protein can get in the urine
 - a. The most common way is via an alteration in the permeability of the glomerular capillary
 - i. Normally, the filter restricts most all protein from entering the urine
 - ii. When there is impaired permeability, albumin is found in the urine and will be the most abundant protein found in the urine because albumin is the most abundant protein found in the serum
 - iii. The dipstick is sensitive to albumin only
 - iv. Non-albumin proteins can be measured with sulfosalicylic acid
 - (a) A volume of urine is added to an equal volume of sulfosalicylic acid and all proteins are precipitated out
 - (b) A visual assessment of the precipitate size is used
 - b. A proximal tubule defect can allow proteins to get into the urine

- i. LMWPs are filtered at the glomerulus
- ii. Under normal conditions, the proximal tubule will reabsorb these LMWPs by pinocytosis and degrade them to amino acids
- iii. A defect in the proximal tubule will allow LMWPs into the urine
- c. Overflow of excess amounts LMWPs, most commonly due to multiple myeloma (MM) can result in urinary protein
 - i. Excessive amounts of immunoglobulin light chains are generated in MM
 - ii. The reabsorptive capacity of the proximal tubule is exceeded
 - iii. These light chains are not detected by the dipstick but would result in a positive sulfosalicylic acid test
 - (a) If all of the protein in the urine is albumin, then the sulfosalicylic acid test will correlate with the dipstick measurement
 - (b) In a case such as multiple myeloma, the dipstick would be negative for protein (albumin), but the sulfosalicylic acid test would detect a lot of protein (light chains)

V. Other parameters measured in the supernatant without the dipstick

- A. Measure urine sodium to determine what the kidney is doing with sodium
- B. Measure urine chloride when assessing metabolic alkalosis
- C. Measure urine potassium in hyper- or hypokalemia to determine if the kidney is contributing to the problem
- D. Measure creatinine to determine the fractional excretion of sodium and evaluate renal function.

VI. Evaluation of the urine sediment

- A. When the dipstick is normal, we do not usually look at urine sediment
 - 1. If negative for urine blood, then no red blood cells (RBCs) would be present
 - 2. If negative for urine leukocyte esterase, then no WBCs would be present
 - 3. An exception is if there are signs of renal tubule abnormalities such as hyperkalemia or renal insufficiency (abnormal creatinine); in these situations, it would be useful to look at urine sediment
- B. Preparation to obtain the sediment
 - 1. Centrifuge the urine and separate it into supernatant and sediment
 - 2. Pour off the supernatant
 - 3. Bang the test tube to re-suspend the small amount of liquid still present
 - 4. Pipette a small drop onto a glass slide and cover with a cover slip
 - 5. Look at the slide under low power and then high power
 - 6. No stains are required and no oil is used
- C. The sediment is used to identify any casts and cells that may be present in the urine
 - 1. Red blood cells (RBCs) and hematuria
 - a. There are situations in which there are no RBCs in the urine sediment but the dipstick is positive for blood, for example:
 - i. With massive hemolysis free hemoglobin is freely filtered and the dipstick pad reacts with the hemoglobin but no RBCs are seen in the sediment.
 - ii. With severe muscle injury or rhabdomyolysis free myoglobin is freely filtered and the dipstick pad also reacts with myoglobin but no RBCs are seen in the sediment
 - b. The following are situations in which RBCs are in the urine sediment:
 - i. Kidney stones
 - ii. Trauma
 - iii. Prostatic disease
 - iv. Cancer of the prostate, bladder, or kidney

- c. Determining if RBCs are coming from the kidney or from a post-renal origin
 - i. In both situations, the dipstick would be positive for blood and the sediment would contain RBCs
 - ii. The differences between renal origin and extra- or post-renal origin:
 - (a) RBCs from a postrenal origin (e.g., coming from the bladder) will be round and uniform in size and shape, and the presence of blood clots further confirms an extra-renal or post-renal origin.
 - (b) RBCs that are associated with glomerular bleeding will often be fragmented and dysmorphic due to mechanical and osmotic trauma acquired as the RBC traversed the glomerular capillary and traveled through the renal tubules; and, the presence of red cell casts and proteinuria are further indicators that there is a problem of glomerular bleeding
 - d. A few slides of RBCs in the urine were shown
 - i. The RBCs in the urine are more pale and yellow than RBCs in the blood
 - ii. RBCs are smaller than white blood cells (WBCs) and have no nucleus
 - iii. Dysmorphic RBCs in the urine are shrunken and pinched in appearance and are suggestive of glomerular bleeding.
2. WBCs and pyuria
- a. Normal urine has very few WBCs (NL = 0-2 WBCs/hpf)
 - b. Urinary WBCs are usually indicative of infection or inflammation at some site along the urinary tract.
 - i. White cell casts indicate that the source of inflammation is in the kidney, such as with acute pyelonephritis or acute interstitial nephritis.
 - ii. Pyuria (WBCs in the urine) can also be seen with glomerular inflammation, but usually hematuria and proteinuria will also be present
 - c. Some slides of WBCs in the urine showed a granular, "glittering" appearance in the urine and are often referred to as "glitter cells"; the WBCs have an irregular nucleus and are larger than RBCs but smaller than epithelial cells.
 - d. Pyuria usually indicates a bacterial infection, a UTI.
3. Squamous epithelial cells found in the urine come from the bladder, urethra, or vagina (not from the kidney).
- a. It is common to see a few in the urine
 - b. The better the urine catch (midstream urine from a cleaned urethra), the fewer epithelial cells will be present
 - c. These squamous epithelial cells are pancake-shaped with a central, pyknotic nucleus
4. Renal epithelial cell come from the tubules of the kidney and are not normally present in large amounts in the urine.
- a. Increased numbers suggest tubular injury
 - b. These cells are larger than WBCs, have a round nucleus, and the cytoplasm is not glittery like WBCs
5. The sediment is also used to identify casts in the urine; casts consist of precipitated proteinaceous material that is congealed in the tubular lumen, usually the collecting ducts, giving the casts a cylindrical shape with regular margins.
- a. The major matrix protein of casts is the Tamm-Horsfall protein
 - b. There are 4 types of casts: hyaline, granular, cellular, and waxy
 - i. Hyaline casts are mostly composed of the Tamm- Horsfall matrix and have

no diagnostic significance.

ii. Granular casts indicate pathology but are non-specific and imply either glomerular disease or tubular disease and often form in proteinuric states

iii. Cellular casts identify the kidney as the source of the RBCs, WBCs, or epithelial cells and these casts indicate that pathology of the kidney is present

(a) Red cell casts are specific but not sensitive for inflammatory glomerular disease.

(b) White cell casts indicate pyelonephritis, infected medullary interstitium, or intense glomerular nephritis; extra-renal infections, such as a bladder infection, will not have casts but will have WBCs

iv. Waxy casts are markers for chronic disease and locate the injury to the kidney; waxy casts are thought to represent successive stages of cellular casts.

6. If lipids are found in the sediment, fatty casts may be present and identified with a polarizer of a light microscope and have a "Maltese cross" appearance.

a. Lipiduria is often associated with heavy proteinuria and come from small molecular weight cholesterol-containing lipoproteins which have been filtered

b. Lipiduria is essentially diagnostic of glomerular disease and when proteinuria is also present, indicates nephrotic syndrome.

VII. Summary of the main renal syndromes

A. Glomerular diseases include the nephrotic and nephritic syndromes

Nephrotic syndrome	vs.	Nephritic syndrome
Proteinuria, >3g/day (3+/4+) Urine Protein:Creatinine ratio >3 Hypoalbuminemia Low plasma oncotic pressure Edema May have lipiduria No blood or RBCs GFR and BP are usually normal Examples: in children, minimal change disease membranous nephropathy focal glomerulosclerosis diabetic glomerulopathy		mild to moderate proteinuria (~1g/day or 1+/2+) urine Protein:Creatinine ratio ~1 Normal serum albumin Normal plasma oncotic pressure Retention of salt and water Hypertension with edema ?Signs of volume overload, e.g. shortness of breath Blood or RBCs are present (hematuria) GFR is usually decreased and BP is increased Example: post-streptococcal glomerulonephritis

B. Mixed nephrotic-nephritic syndromes often occur and the following characteristics are generally present:

1. Heavy proteinuria
2. Intense inflammation and pyuria with hematuria
3. An example: Seen in lupus (SLE) as a diffuse proliferative glomerulonephritis

C. Mesangial disease of the kidney is characterized by inflammation of the mesangium.

1. Recall that the glomerular capillary loops are surrounded by the mesangium but with inflammation of the mesangium, the capillary loops remain normal
2. GFR is normal with no proteinuria and normal serum creatinine
3. Gross hematuria is present with RBCs or Red cell casts in the urine
4. An example: IgA nephropathy

D. Tubular Diseases of the kidney most often result from ischemic injury that causes acute tubular necrosis.

1. Recall that 90% of the renal blood flow goes to the cortex leaving the medulla most susceptible to ischemic injury.
2. The vasa recta system supplies blood to the medulla and the deeper areas of the medulla have less oxygen tension

E. Nephrotoxins can cause acute tubular injury; examples of nephrotoxins:

1. Overdose or abuse of analgesics, e.g. ibuprofen
2. Aminoglycosides
3. Amphotericin B
4. Heavy metals, often used with chemotherapeutic agents like cisplatin
5. Iron moieties of free hemoglobin and myoglobin
6. IV contrast media

F. Obstruction of the drainage system from the kidney will injure the medullary tubules causing the tubules to decrease reabsorption and causing the glomerulus to decrease filtering--Basically, obstructive uropathy "backs things up like a clogged sink"

- G. Allergic interstitial nephritis is NOT an infection but is an allergic inflammatory response characterized by sterile pyuria (WBCs in the urine but no bacteria) with eosinophils present in the urine

VIII. Comments on urine volume

- A. Urine volume or urine output is NOT a marker for kidney function; GFR is.
 - 1. Patients with advanced renal failure can have adequate urine output of 800-1500mL/day, but this tells us nothing about GFR and kidney function.
 - 2. Urine output is primarily determined by water intake
- B. Although normal urine volume does not exclude renal dysfunction, an extremely low urine output or no urine output does indicate abnormal kidney function
 - 1. Oliguria is defined as a low urine output of <500mL/day and because it takes at least 500-600mL of urine output per day to rid the body of the daily solute load, this can cause problems
 - 2. Anuria is defined as a urine output of <100mL/day and indicates a post-renal obstruction until proven otherwise.
 - a. Pre-renal failure almost never causes anuria
 - b. Intrinsic renal disease almost never causes anuria