Non-protein nitrogenous substances (NPN)

A simple, inexpensive screening test a routine urinalysis is often the first test conducted if kidney problems are suspected. A small, randomly collected urine sample is examined physically for things like color, odor, appearance, and concentration (specific gravity); chemically, for substances such as protein, glucose, and pH (acidity/alkalinity); and microscopically for the presence of cellular elements (red blood cells [RBCs], white blood cells [WBCs], and epithelial cells), bacteria, crystals, and casts (structures formed by the deposit of protein, cells, and other substances in the kidneys’ tubules). If results indicate a possibility of disease or impaired kidney function, one or more of the following additional tests is usually performed to pinpoint the cause and the level of decline in kidney function. **1-Glomerular function tests** depend on examination of substances which depend on glomerular function for their elimination (creatinine, urea, and eGFR). The determination of non-protein nitrogenous substances (NPN) in the blood has traditionally been used to monitor renal function. The majority of these compounds arise from the catabolism of proteins and nucleic acids

**Non-protein nitrogenous substances (NPN):** The most important NPN compounds used in evaluation of kidney functions are urea, creatinine, creatine uric acid, and ammonia. As clearance levels decrease, blood levels of creatinine, urea, and uric acid increase.

**1-Blood Urea Nitrogen (BUN): Glomerular function test:**

It is formed in the liver from protein catabolism by releasing free amino groups (\(-\text{NH2}\)). Then it is transported to blood and then excreted in urine by kidneys. It represents about more than 75% of excreted NPN compounds. Urea is the major excretory product of protein metabolism. Its levels are affected by high protein intake, catabolic states, post-surgery and trauma, and gastro-intestinal hemorrhage, all of which cause increased urea production from protein.

**Physiology of Urea:**

Most of the urea in the glomerular filtrate is excreted in the urine, although some urea is reabsorbed by passive diffusion during passage of the filtrate through the renal tubules. Under conditions of normal flow and normal renal function, about 40% of the filtered urea is reabsorbed; when the flow rate is decreased, the amount passively reabsorbed increases. The amount reabsorbed depends on urine flow rate and extent
of hydration. Small quantities of urea (≤10% of the total) are excreted through the gastrointestinal tract and skin. Small of urea portion is diffused to intestine to be cleaved by bacterial urease enzyme to CO2 and NH3. NH3 is partially lost in stool and partially reabsorbed to blood. The concentration of urea in the plasma is determined by renal function and perfusion, the protein content of the diet, and the rate of protein catabolism. The BUN test measures the amount of nitrogen contained in the urea. High BUN levels can indicate kidney dysfunction, but because BUN is also affected by protein intake and liver function, the test is usually done together with a blood creatinine, a more specific indicator of kidney function.

The injection or ingestion of steroids produces a rise in BUN as do stressful situations that cause the adrenal gland to secrete additional cortisol. For these reasons, the measurement of serum creatinine is a better indicator of kidney status than is that of BUN although in many cases, they go up and down simultaneously.

**Urea nitrogen** : serum 6-20 mg/dl, urine 24-h 12-20 g/day

**Pathophysiology of Urea:**

increased BUN is seen in the prerenal, renal, and post-renal factors. An elevated concentration of urea in the blood is called azotemia not uriceimia. Very high plasma urea concentration accompanied by renal failure is called uremia.

This condition is eventually fatal if not treated by dialysis or transplantation.

**Prerenal azotemia** is caused by reduced renal blood flow.

It is increased the plasma urea levels without increased plasma creatinine.

Less blood is delivered to the kidney; consequently, less urea is filtered.

Causative factors include; • Decreased renal blood flow (renal ischemia) as in congestive heart failure (↓COP). • Shock and Significant decrease in blood volume.

**Renal azotemia**: Decreased renal function causes an increase in plasma urea concentration as a result of compromised urea excretion. Intra- Renal causes of elevated urea include acute and chronic renal failure.

**Post-renal azotemia** can be due to obstruction of urine flow anywhere in the urinary tract by renal calculi, tumors of the bladder or prostate, or severe infection. It is
increased plasma urea levels with increased plasma creatinine as in: as in renal stones, prostatism or urinary tract tumours

**The major causes of decreased plasma urea concentration include** low protein intake and severe liver disease. Plasma urea concentration is decreased during late pregnancy (when the fetus is growing rapidly and utilizing maternal amino acids) and infancy as a result of increased protein synthesis. And in starvation,

Differentiation of the cause of abnormal urea concentration is aided by calculation of the **urea nitrogen/creatinine ratio**, which is Normally 10-20.

Prerenal conditions tend to elevate plasma urea, whereas plasma creatinine remains normal, causing a high urea N/creatinine ratio.

A high urea N with an elevated creatinine is usually seen in post-renal conditions.

A low urea N/creatinine ratio is observed in conditions associated with decreased urea production, such as low protein intake, acute tubular necrosis, and severe liver disease.

**NB:** Serum urea Nitrogen mg/dL = Serum urea mg/dL X 0.467.

**Creatine and Creatinine: Glomerular function test**

Creatinine is formed from creatine and creatine phosphate in muscle and is excreted into the plasma at a constant rate related to muscle mass. Plasma creatinine is inversely related to glomerular filtration rate (GFR) and, although an imperfect measure, it is commonly used to assess glomerular filtration function.

**Physiology of Creatine and Creatinine:** Creatine is synthesized primarily in the liver from arginine, glycine, and methionine. It is then transported to other tissues, such as muscle, where it is converted to creatine phosphate, which serves as a high-energy source. About 1-2% of the total muscle creatine pool is converted daily to creatinine.

Creatinine is released into the circulation at a relatively constant rate that has been shown to be proportional to an individual’s muscle mass. With normal kidney function, then, the amount of creatinine in the blood remains relatively constant and normal.
Clinical Application of Creatine and Creatinine: Plasma creatinine concentration is a function of relative muscle mass, the rate of creatine turnover, and renal function.

Creatinine is filtered freely unchanged at the glomerulus and eliminated without significant reabsorption or secretion in the tubules.

The serum creatinine is a better indicator of renal function than either that of BUN or uric acid because it is not affected by diet, exercise, or hormones. Therefore the glomerulus filtration rate (GFR) was most often assessed by determining the urinary creatinine clearance.

Creatinine clearance: It is a measure of the amount of creatinine eliminated from the blood by the kidneys. Owing to creatinine not being significantly reabsorbed or secreted by the renal tubules, CrCl provides a measure of the glomerular filtration rate (GFR). It is calculated as follows;

\[
\text{Creatinine clearance} = \frac{U \times V}{P} \times 1.73/A
\]

- **U** = Urine creatinine (mg/dL).
- **P** = Plasma or serum creatinine (mg/dL).
- **V** = Urine flow rate (mL/minute).
- **A** = Body surface area (m²).
- **1.73/A** = factor normalize clearance for average body surface area

However, CrCl changes more linearly in proportion to renal mass loss than does plasma creatinine or urea, so is the best measure of progress in chronic renal failure.

It is of no value in acute renal failure since it needs a steady state situation to obtain a meaningful result. Normal CrCl is about 120 ml/min. Creatinine clearance may be used as indicator for GFR because:

- Creatinine is endogenously produced.
- Creatinine is released into body fluid at constant rate.
- Its plasma level maintained within narrow limits.
- Its plasma level not affected by dietary factors.
But Creatinine clearance overestimates GFR because a small amount of creatinine is reabsorbed by the renal tubules and up to 10% of urine creatinine is secreted by the tubules. However, CrCl provides a reasonable approximation of GFR. 

Creatinine concentration decreases with age beginning in the fifth decade of life.

Pathophysiology. Creatinine

Renal causes of increased plasma creatinine include: Diseases with loss of nephrotic functions e.g. acute and chronic glomerulo-nephritis. Diseases with increased pressure on the tubular side of nephrones e.g. urinary tract obstruction due to prostatic enlargement. Plasma creatinine is a relatively insensitive marker and may not be measurably increased until renal function has deteriorated more than 50%.

Low plasma creatinine: Creatinine production is determined by the size of creatine pool hence a smaller muscle mass leads to daily lower creatinine production.

Physiologically pregnancy is accompanied with decreased plasma creatinine level. Also, females and children show low plasma creatinine levels when compared with adult men. Pathologically low plasma level of creatinine is found in wasting diseases, starvation, and in patients treated with corticosteroids due to their protein catabolic effect.

Laboratories are making increasing use of estimated GFR derived from creatinine estimations, more especially because of the problems with measuring creatinine clearance((24 h) urine collection).

Note: Creatine In muscle disease such as muscular dystrophy, poliomyelitis, hyperthyroidism, and trauma, both plasma creatine and urinary creatinine are often elevated. Plasma creatinine concentrations usually are normal in these patients. Measurement of creatine kinase is used typically for the diagnosis of muscle disease
3- Uric Acid:

Uric acid is a metabolite of nucleic acids, purines, and nucleoproteins catabolism and is the end product of protein (purine) metabolism in man. Although it is filtered by the glomerulus and secreted by the distal tubules into the urine, most uric acid is reabsorbed in the proximal tubules and reused. Uric acid is relatively insoluble in plasma and, at high concentrations, can be deposited in the joints and tissue, causing painful inflammation.

**Physiology of Uric Acid:** Purines, such as adenosine and guanine from the breakdown of ingested nucleic acids or from tissue destruction, are converted into uric acid, primarily in the liver. Uric acid is transported in the plasma from the liver to the kidney, where it is filtered by the glomerulus. Reabsorption of 98% to 100% of the uric acid from the glomerular filtrate occurs in the proximal tubules. Small amounts of uric acid are secreted by the distal tubules into the urine. Renal excretion accounts for about 70% of uric acid elimination; the remainder passes into the gastrointestinal tract and is degraded by bacterial enzymes. Nearly all of the uric acid in plasma is present as monosodium urate. At the pH of plasma (pH < 7) urate is relatively insoluble; at concentrations greater than 6.8 mg/dL, the plasma is saturated. As a result, urate crystals may form and precipitate in the tissues. In acidic urine (pH < 5) uric acid is the predominant species and uric acid crystals may form.

**Clinical Application of Uric Acid:** Uric acid is measured to assess inherited disorders of purine metabolism, to confirm diagnosis and monitor treatment of gout, to assist in the diagnosis of renal calculi, to prevent uric acid nephropathy during chemotherapeutic treatment, and to detect kidney dysfunction. Increased levels of uric acid have been observed in renal failure, chronic lead poisoning, polycythemia, some leukemias, and toxemia of pregnancy.

Male 3.5-7.2 mg/dl

Female 2.6-6 mg/dl

Chronic renal disease causes increased uric acid concentration because filtration and secretion are impaired. However, uric acid is not useful as an indicator of renal function because many other factors affect its plasma concentration.

Hypouricemia is less common than hyperuricemia and is usually secondary to severe liver disease.
**Ammonia:**

Ammonia is formed in the deamination of amino acids during protein metabolism. It is removed from the circulation and converted to urea in the liver. Free ammonia is toxic; however, ammonia is present in the plasma in low concentrations. Ammonia (NH₃) is produced in the catabolism of amino acids and by bacterial metabolism in the lumen of the intestine. Some ammonia results from anaerobic metabolic reactions that occur in skeletal muscle during exercise. Ammonia is consumed by the parenchymal cells of the liver in the production of urea. At normal physiologic pH, most ammonia in the blood exists as ammonium ion (NH₄⁺).

Ammonia is excreted as ammonium ion by the kidney and acts to buffer urine.

Clinical conditions in which blood ammonia concentration provides useful information are hepatic failure, Reye’s syndrome, and inherited deficiencies of urea cycle enzymes.

Measurement of urine ammonia can be used to confirm the ability of the kidneys to produce ammonia

**Note 1:** Reye’s syndrome (the disease is preceded by a viral infection and the administration of aspirin).

**Note 2:** Ammonia is of use in the diagnosis of inherited deficiency of urea cycle enzymes.