LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. Ask for blood tests that indicate nitrogen retention and interpret them in acute kidney injury (AKI) vs chronic kidney disease (CKD)
2. Ask for blood tests that evaluate regulatory kidney function and interpret them in AKI vs. CKD
3. Ask for and interpret the blood tests that evaluate the endocrine function in CKD
4. Ask for and interpret the main immunological investigations in diagnosis of glomerulonephritis
5. Use the on-line platform for GFR calculator and to interpret the results according to CRD stadialization
6. Ask for and interpret tests indicating the acute and chronic tubular dysfunction

Exploration of renal function has two major indications:

a) Diagnosis of renal disorders – in patients with: painful urination, miction disorders, quantitative changes and/or the presence of pathological compounds in urine

b) Monitoring of patients with high risk for developing renal disorders (patients with diabetes mellitus, hypertension, personal/familial history of kidney disease, chronic tm with potential nephrotoxic drugs). This monitoring is useful mainly for:

- Early detection of glomerular/tubular dysfunction
- Adjusting drug dosage according to the renal excretion capacity

Tests that explore the renal disorders can be classified in:

I. The biochemical blood assessment
II. Tests that explore glomerular dysfunction
III. Tests that explore tubular dysfunction
IV. Urine analysis

I. BIOCHEMICAL BLOOD ASSESSMENT

Blood tests that explore the alteration of the renal function, offering information regarding the subjacent etiological mechanisms.

A. Tests that explore the alteration of the renal function

The renal functions that can be explored through biochemical blood tests are:
- the uremic toxins excretory function
- the regulatory function
- the endocrine function

a) Tests that explore the excretory function. Indicators of nitrogen retention (azotemia)

Nitrogen retention or azotemia represents the increased concentration of serum catabolites (urea, creatinine, uric acid) which are indicators of decreased glomerular filtration rate (GFR). The excretion function is altered both in acute kidney injury, formerly known as acute renal failure, as well as in the chronic kidney disease, formerly known as chronic renal failure:

- **Acute kidney injury (AKI)** – represents the sudden decrease (but potentially reversible) of GFR, that usually occurs in healthy kidneys
- **Chronic kidney disease (CKD)** – represents the slow, progressive, and irreversible decrease of GFR < 60 ml/min/1,73 m², for at least 3 months, in kidneys with preexistent damage.

1. Serum urea

Source: Represents the final product of nitrogen catabolism, synthesized by the liver from ammonia during ureogenesis. Serum concentration of urea depends on:
- protein catabolism
- protein exogenous intake
- renal function (urea is filtered, is reabsorbed and is secreted tubulary)
- liver detoxification function
- extrarenal excretion (skin and GI tract during marked serum increased levels)
- volemia

Normal values: 15-45 mg/dL

Pathologic changes:
- Increased values:
  - Renal increase:
    o in AKI: urea is rapidly increasing (with 10-20 mg/dl/24 hours) and it can get to values of 200 - 400 mg/dl in less than 1 week
    o in CKD: plasma urea is increasing slowly, reaching at values > 300 mg/dl in the end stage of the disease (chronic uremia)
  - Extrarenal increase:
    o increased protein catabolism (e.g., digestive hemorrhages, massive tissue destructions, neoplasms)
    o massive protein ingestion
    o hypovolemia
- Decreased values:
  - end-stage cirrhosis (hepatic dysfunction)
  - malnutrition
  - hypervolemia

! Of note: serum urea is a good indicator of azotemia, which cannot yet be correlated with the severity of GFR decrease.

2. Blood urea nitrogen (BUN)

For establishing the etiology of azotemia in AKI, blood urea nitrogen can be determined by dividing the value of urea by 2.2.

Normal values for BUN: 7-18 mg/dL

3. Serum creatinine

- Source: It is a breakdown product of creatinphosphate in the muscle. Values are dependent on:
  - muscular mass (sex and age)
  - renal function (90% of the creatinine is filtered and 10% is secreted in the proximal tubule)

Normal values: 0.6 - 1 mg/dL (women) and 0.8-1.3 mg/dL (men).

Pathologic changes:
- Increased values:
  - Renal:
  - in AKI: urea is increasing (with 0.5-1 mg/dl at 24-48 hours and can rich at values ≥ 4 mg/dl in less than 1 week)
  - in CKD is increasing with 0.5-1 mg/dl at 1-2 years, reaching values > 10 mg/dl in the end stage of the disease
- Extrarenal increase:
  o tissue necrosis: rhabdomyolysis, IIIrd degree burns
  o hypovolemia
- Decreased values:
  o severe hepatic dysfunction
  o decreased muscle mass (muscle dystrophy, myasthenia gravis)
  o malnutrition
  o hypervolemia.

! Of note: serum creatinine is not a good indicator for the early onset of GFR decrease detection, because its serum level increases only when the number of functional nephrons decreases at 50-75%. Doubling of serum creatinine level signifies the reduction with 50% of the functional nephron mass.

4. BUN:Creatinine Ratio

Normal values: 10 - 20

Clinical value:
BUN: creatinine ratio is used for differential diagnosis of azotemia in acute kidney injury, which can be induced by a cause:
- prerenal (functional)
- renal (intrinsic)
- postrenal (obstructive)

- In PRERENAL azotemia – GFR decreases due to renal hypoperfusion induced by hypovolemia (hemorrhage, gastro-intestinal liquid loss, burns, congestive heart failure, acute pancreatitis):
  - at glomerular level: GFR decrease induces the decrease of filtered urea and creatinine
  - at tubular level: an important part of urea is reabsorbed in PCT, while the whole quantity of filtered creatinine is eliminated through urine
  - BUN: creatinine ratio > 20

- In RENAL azotemia – the most frequent cause of AKI is the acute tubular necrosis (ischemic or nephrotoxic) which induces the primary urine retrodiffusion at PCT and the
decrease of GFR due to blood renal flux disorders:
- at glomerular level: the GFR decrease induces the decrease of filtered urea and creatinine
- at tubular level: filtered urea and creatinine are reabsorbed in plasma, but the urea excess in plasma can be eliminated via extrarenal pathways (skin, gastrointestinal tract), while the excess of creatinine accumulates in plasma
- BUN: creatinine ratio < 10

- In POSTRENAL azotemia – GFR decreases due to urinary tract obstruction, induced by bilateral obstructive uropathy or the obstruction of a single kidney (anatomical/functional):
  - at glomerular level: the decrease of GFR induces the decrease of both filtered urea and creatinine
  - at tubular level: filtered urea and creatinine cannot be properly eliminated, due to renal obstruction, thus they both accumulate in blood
  - BUN: creatinine ratio is normal = 10-20

5. Uric acid

- Source: final catabolism product of purine nucleotides (within the liver). Serum concentration depends on:
  - nucleoproteins catabolism
  - renal function (uric acid is glomerulary filtered, and is reabsorbed/secreted in the PCT)

- Normal values: 2 – 7 mg/dl: M
  2 – 5,7 mg/dl: F

- Increased values (hyperuricemia):
  - Renal – uric acid is a good indicator of the end stage of CRD (of renal failure) when its plasma level reach values > 10 mg/dl (Table 1)
  - Extrarenal:
    o gout
    o increased nucleoproteins catabolism in leukemias, chemotherapy, radiotherapy

- Decreased values: hepatic failure

B. Tests exploring the kidney regulatory function

1. Serum electrolytes - plasma ionogram

Explores the function of conservation and elimination of plasma electrolytes being useful in appreciating the hydro-electrolyte imbalances induced by:
- AKI: the oligoanuric phase and then the diuretic phase (polyuric)
- CKD: the initial phase (compensatory phase) and then the terminal phase (renal failure)
- alteration of aldosterone secretion
- diuretics

Table 2. Normal values of plasma electrolytes

<table>
<thead>
<tr>
<th>Cations (mEq/l)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na⁺)</td>
<td>136-146</td>
<td></td>
</tr>
<tr>
<td>Potassium (K⁺)</td>
<td>3,5-5</td>
<td></td>
</tr>
<tr>
<td>Calcium (Ca²⁺)</td>
<td>1,25-2,72</td>
<td></td>
</tr>
<tr>
<td>Magnesium (Mg²⁺)</td>
<td>0,8-1,2</td>
<td></td>
</tr>
<tr>
<td>Anions (mEq/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride (Cl⁻)</td>
<td>98-106</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate (HCO₃⁻)</td>
<td>23-27</td>
<td></td>
</tr>
<tr>
<td>Phosphate (HPO₄²⁻)</td>
<td>1,3-2</td>
<td></td>
</tr>
<tr>
<td>Sulphate (SO₄²⁻)</td>
<td>0,1-0,65</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Changes of plasma ionogram in AKI and CRD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AKI (oligo-anuric phase)/ CKD (initial phase)</th>
<th>AKI (polyuric phase)/ CKD (terminal phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>K⁺</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>HPO₄²⁻</td>
<td>↑</td>
<td>N</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>↑</td>
<td>N</td>
</tr>
</tbody>
</table>

2. Acid-base balance parameters

Mark out the metabolic acidosis induced by altered acid-base balance in AKI and the final stage of CKD (reabsorption & generation of HCO₃⁻ simultaneously with H⁺ excretion).

Table 4. Normal values and pathological variations of acid-base parameters in renal failure.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NV</th>
<th>AKI/CRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7,35-7,45</td>
<td>↓</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/l)</td>
<td>23-27</td>
<td>↓</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>38-42</td>
<td>↓</td>
</tr>
</tbody>
</table>
3. Proteinemia and Electrophoresis (Table 5)

Mark out the hypoproteinemia and dysproteinemia induced by protein loss at glomerular membrane level, in nephrotic syndrome (Table 5).

Table 5. Normal values and pathological variations of proteinemia and electrophoresis in nephrotic syndrome.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NV</th>
<th>Nephrotic syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinemia (g/dl)</td>
<td>6.7 – 8.4</td>
<td>↓</td>
</tr>
<tr>
<td>ELFO (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumins</td>
<td>50-70</td>
<td>↓</td>
</tr>
<tr>
<td>$\alpha_1$ – globulins</td>
<td>3-6</td>
<td>N</td>
</tr>
<tr>
<td>$\alpha_2$ – globulins</td>
<td>7-10</td>
<td>↑</td>
</tr>
<tr>
<td>$\beta$ – globulins</td>
<td>11-14</td>
<td>↑</td>
</tr>
<tr>
<td>$\gamma$ – globulins</td>
<td>15-20</td>
<td>↓</td>
</tr>
</tbody>
</table>

4. Serum lipidogram

Normal values: Lipids = 400-800 mg/dl  
Total Cholesterol = 140-200 mg/dl

Serum lipids and total cholesterol increase in nephrotic syndrome.

C. Tests that explore the endocrine function

Are required in patients with CRD and include:

1. Serum level of erythropoietin

In patients with CKD, the secretion of erythropoietin is altered, so its serum concentration will be decreased, leading to severe normochromic anemia. Exogenous substitution therapy with human modified erythropoietin is effective in treating secondary anemia.  
Normal values: 4,3 – 29 UI/L

2. Serum level of parathyroid hormone (PTH)

Is useful for the diagnosis of secondary hyperparathyroidism that appears in CKD associated with mineral bone disease. PTH serum level increases due to secondary hyperPTH induced by hypocalcaemia and hyperphosphatemia ⇒ renal osteodystrophy.  
Normal value: 15-65 pg/ml

3. Serum level of 1,25-dihidroxi-colecaldiferol (1,25 -(OH)$_2$ – D$_3$):

The serum level of 1,25 -(OH)$_2$ – D$_3$ decreases due renal deficit in synthesis of the active form of vitamin D$_3$ (decrease of the activity of 1 $\alpha$-hydroxylase, responsible for the second hydroxylation)  
⇒ hypocalcaemia and osteomalacia  
Normal values: 30-80 pg/ml

B. IMMUNOLOGIC investigations

1. Serum autoantibodies:

- Antinuclear antibodies (ANA profile)  
  - represent a group of antibodies against the self cells nuclei.  
  - Their presence is specific for different autoimmune diseases (anti double stranded DNA antibody – systemic lupus; anti SSb/La antibody – Sjögren syndrome)

- Anti basal membrane antibodies  
  - Antibodies against type IV collagen at the level of the basal glomerular membrane and the alveoli  
  - rapidly progressive glomerulonephritis, associated or not to the pulmonary manifestations of Goodpature disease.

- ANCA (anti neutrophil cytoplasmic antibody)  
  - Antibodies against antigens from the plasma of neutrophils, seen typically in patients with vasculitis, that can associate different forms of glomerulonephritis.

2. Serum Complement - C3, C4

- The C3 component refers to the activation of the classical and alternant complement pathway while the C4 fraction refers to the activation of only the classical complement pathway (it’s concentration will be normal if the complement was activated only through the alternant pathway).  
- C3 levels will be decreased, while C4 component levels will be normal during poststreptococcal glomerulo-nephritis  
- Both components are decreased when there is a local renal high consumption (acute glomerulo-nephritis, lupus nephritis, cryoglobulinemic nephropathy)

3. Serum immunoglobulins and immuno-ELFO

- useful in determining the level of different immunoglobulines (i.e., IgA in mesangial IgA deposits nephropathy), or light $k$ and $\lambda$ chains (multiple myeloma).
II. TESTS THAT EXPLORE GLOMERULAR DYSFUNCTION

Glomerular dysfunction manifests by GFR decrease < 90 ml/min/1,73 m² WITH OR WITHOUT proteinuria.

GFR can be measured directly (the clearance renal method) or can be estimated indirectly (several formula).

1. Endogenous - creatinine clearance (CrCl)

Definition: The clearance of a substance refers to the removal of that substance from the blood, expressed as the volume of blood or plasma cleared of the substance per unit time.

Clearance can be calculated using the following formula:

\[ C = \frac{U \times V}{P} \]

Where:
- \( C \) = clearance (ml/min)
- \( V \) = urinary volume per minute (ml/min)
- \( U \) = urinary concentration of the substance (mg%)
- \( P \) = plasmatic concentration of the substance (mg%)

For the evaluation of GFR substances that are eliminated through glomerular filtration must be used, but that are NOT reabsorbed and are NOT secreted within the tubules. These requirements are fulfilled by exogenous substances (inulin, radioactive marked EDTA – that are not used in clinical practice) and only partially fulfilled by endogenous creatinine, that is eliminated through glomerular filtration up to 90% and 10% is secreted in the TCP. Therefore, GFR is overestimated by 10-20 ml/min/1,73m², but this is found to be within reasonable limits. However, in kidney failure the secreted fraction increases, therefore the clinical value of creatinine clearance being lost.

Normal values: 125±25 ml/min (M); 95±20ml/min (F) – for a body surface of 1,73 m².

*Of note:* CrCl CANOT identify a moderate decrease of GFR between 40 and 70 ml/min/1,73 m² (the „blind“ diagnosis zone of the creatinine clearance).

2. Assessment of the glomerular filtration rate (eGFR)

Due to the difficulty of the precise collecting of diuresis, especially in patients with low compliance, estimated GFR is based on only a single determination of serum concentration of creatinine and/or, more recently of cystatin C.

Estimated GFR is based on only one single determination of serum concentration of creatinine and/or cystatin C.

- **Cystatin C** is a low molecular weight protein, part of the lysosomal enzyme inhibitor family (mainly inhibits the cistein-proteinase) preventing the extracellular catabolism of peptides and proteins. This substance is produced by all nucleated cells, production rate being constant throughout life. **Cistatin C production is not influenced by muscle mass (sex, age), food intake or drugs.** Due to the low molecular weight and the positive electronic charge, cystatin C is freely filtered in the glomeruli and the is completely reabsorbed in the TCP, where it is also metabolized. Thus, in the absence of tubular lesions, cystatin C is NOT seen in the urine (is determined only in blood).

GFR can be measured using different formulas:

- **CKD-EPI** (Chronic Kidney Disease Epidemiology Collaboration) - more precise if GFR > 60ml/minute.
- **MDRD** (Modification of Diet in Renal Disease) - more precise if GFR < 60ml/minute

These formula can be accessed on line (mdrd.com) allowing the stadialization of CRD (Table 6) according to the plasma level of creatinine and/or cystatin C.

Table 6. CKD stadialization according to the GFR decrease rate and the severity of renal injury.

<table>
<thead>
<tr>
<th>Stage</th>
<th>GFR (ml/min/1,73 m²)</th>
<th>Severity of renal damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>&gt; 90</td>
<td>Chronic renal injury with normal GFR</td>
</tr>
<tr>
<td>G2</td>
<td>60 – 89</td>
<td>Chronic renal injury with GFR slightly decreased</td>
</tr>
<tr>
<td>G3a</td>
<td>45 – 59</td>
<td>CRD with slight to moderate decrease of GFR</td>
</tr>
<tr>
<td>G3b</td>
<td>30 – 44</td>
<td>CRD with moderate to severe decrease of GFR</td>
</tr>
<tr>
<td>G4</td>
<td>15 – 29</td>
<td>CRD with severe decrease of GFR</td>
</tr>
<tr>
<td>G5</td>
<td>&lt; 15</td>
<td>Renal failure</td>
</tr>
</tbody>
</table>
III. TESTS THAT EXPLORE TUBULAR DYSFUNCTION

**Tubular dysfunction** represents the decrease of the kidney ability to concentrate and dilute urine, rapidly installed in the acute tubular necrosis (ATN) or slowly, progressively in chronic kidney disease (CKD):

- **in ATN:**
  - Retrodiffusion of the primary urine at the PCT (proximal convoluted tubule) and the important decrease of GFR induce oliguria
  - The decrease of the kidney ability to concentrate and dilute urine, rapidly installed, induces the elimination of urine with the same density as the primary urine (isosthenuria)
  - Decreased tubular reabsorption capacity induces the increase of FENa⁺ (fractional excretion of sodium)

- **in CKD:**
  - The slow-progressive decrease of GFR is compensated by glomerular hyperfiltration at the level of remnant nephrons, which induces polyuria
  - The decrease of the kidney ability to concentrate and dilute urine, slowly installed, induces the elimination of urine with the same density as the primary urine (isosthenuria), which won’t be modified regardless the volume of diuresis (fixed isosthenuria).

I. Indicators of ACUTE TUBULAR DYSFUNCTION

1. **Urinary indices** explores the decrease of the kidney ability to concentrate urine in ATN:
   - **Urine Osmolarity** (UOsm, mOsmol/l) – measured in urine/24 hours
   - **urinary Na⁺** (UNa⁺, mmol/l) – measured in a spontaneous urine sample
   - **Fractional Excretion of sodium, FENa⁺** – calculated according to the following formula:
     \[
     FENa⁺ = \left(\frac{\text{Urinary Na⁺}}{\text{serum Na⁺}}\right) \times 100 / \left(\frac{\text{Urinary Creatinine}}{\text{serum Creatinine}}\right)
     \]
   - **Clinical value:** differential diagnosis of functional oliguria from prerenal azotemia (due to renal hypoperfusion, with normal tubular function) from organic oliguria in renal azotemia (due to ATN, with decreased tubular function) (Table 1)

<table>
<thead>
<tr>
<th>Table 1. Differential diagnosis of oliguria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of azotemia</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Prerenal</td>
</tr>
<tr>
<td>Renal</td>
</tr>
</tbody>
</table>

2. **Early markers of ATN**

NGAL/LCN2 (neutrophil gelatinase associated lipocalin or lipocain 2): - is a gelatinase binding protein, member of the lipocaine family, initially found in the granules of human neutrophils. NGAL is markedly expressed in injured tubular epithelial cells (NGAL has a role in the conservation of tubular function through apoptosis inhibition and proliferative response).
   - **is a „troponin-like” biomarker** which:
     - identifies ATN in an early stage (few hours after the onset), *before* the increase of serum creatinine (24-48 hours)
     - allows the differential diagnosis of oliguria from *renal azotemia* (increased NGAL level) and prerenal azotemia (normal NGAL level)
     - is used for the early prediction of ATN after cardio-pulmonary bypass and renal transplant
   - **is an independent risk marker for the progression of CKD,** even in patients with normal creatinine (correlates with GFR and proteinuria).
**CHECKPOINT**

*1. Which of the following factors influence the serum concentration of cystatin C?*
A. Dietary protein intake  
B. Detoxification liver function  
C. Sex and age  
D. Muscle weight  
E. None of the above

*2. Which of the following changes represent a consequence of endocrine function alteration in CRD?*
A. Increased EPO synthesis  
B. Normochromic normocytic anemia  
C. Hyperphosphatemia  
D. Hypercalcemia  
E. Secondary hyperparathyroidism

3. Which of the following aspects characterize the prerenal azotemia?
A. Slow and progressive decrease of GFR  
B. Decreased BUN: creatinine ratio < 10  
C. Increased BUN: creatinine ratio > 20  
D. Is induced by acute tubular necrosis  
E. Is induced by hypovolemia

4. Which of the following changes of plasma ionogram are present in the oligoanuric stage of the acute tubular damage?
A. Hyponatremia  
B. Hypokalemia  
C. Hypercalcemia  
D. Hypomagnesaemia  
E. Hyperphosphatemia

5. Which of the following changes define dysproteinemia in nephrotic syndrome?
A. Decreased albumins  
B. Increased $\alpha_1$ – globulins  
C. Decreased $\alpha_2$ - globulins  
D. Increased $\beta$-globulins  
E. Increased $\gamma$-globulins

6. What is the severity of renal dysfunction if the estimated GFR through MDRD formula is 50 ml/min/1.73 m$^2$?
A. Chronic renal lesion with mildly decreased GFR  
B. CRD with mild to moderate GFR decrease  
C. CRD with moderate to severe GFR decrease  
D. CRD with severe GFR decrease  
E. Renal failure

7. Which of the following explore the tubulary dysfunction from acute tubulary necrosis?
A. Plasma osmolarity  
B. Na$^+$ measured in a spontaneous urine sample  
C. Fractional Excretion of sodium  
D. Creatinine clearance  
E. Serum complement
CLINICAL CASES

1. 62 years of age patient, with type 2 DM, presents with HT and oliguria.

**Blood tests:**
- Urea = 160 mg/dl
- Creatinine = 2.3 mg/dl
- Uric acid = 8.5 mg/dl
- eGFR calculated with MDRD (mdrd.com) is 45 ml/min/1.73 m²
- Glycemia = 340 mg/dl

**What diagnostic(s) would you consider?**
**What further blood tests are justified? Argue!**

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2. 40 years of age patient, presents to the ER with malaise, fever, oliguria, with a suspicion of acute pancreatitis.
- Urea = 220 mg/dl
- Creatinine = 4.5 mg/dl
- Glicemia = 500 mg/dl
- K⁺ = 5.9 mEq/l
- Urinary osmolarity = 600mOsm/l
- FENa⁺ = 0.8%

**What diagnostic(s) would you consider?**
**What further blood tests are justified? Argue!**

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