Nephelometric assays- Urine 24 collection and kidney function test

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Urinary System

- Kidneys – remove excess water and waste products
- Ureters – drain urine into bladder
- Bladder – stores urine
- Urethra – drains urine to outside of the body
Urinary System

• Functions:
  • Removal of waste product from the body (mainly urea and uric acid)
  • Regulation of electrolyte balance (e.g. sodium, potassium and calcium)
  • Regulation of acid-base homeostasis
  • Controlling blood volume and maintaining blood pressure

• When wanting to evaluate the system
  • Urine
  • Blood

Why Measure Renal Function?

• Screening/Disease detection
• Disease monitoring
• Planning for renal replacement therapy
• Drug dose adjustment.
GFR

• Glomerular filtration rate (GFR) describes the flow rate of filtered fluid through the kidney.

• Specifically, it estimates how much blood passes through the glomeruli each minute. Glomeruli are the tiny filters in the kidneys that filter waste from the blood.

• Reference range:
  • GFR > 90 ml/min/1.73m²

(Note: GFR declines with age)

Glomerular Filtration Rate (GFR)

• Volume of blood filtered across glomerulus per unit time
• Best single measure of kidney function
Candidate markers for GFR

**Inulin**
- Filtered only
- Not made by body; must be injected

**Creatinine**
- An endogenous product of muscle metabolism; near-constant production
- Filtered, but a bit secreted

**Urea**
- An endogenous product of protein intake
- Filtered and absorbed; synthesis varies with diet

Creatinine Clearance to calculated GFR

- **Creatinine clearance** The amount of blood the kidneys can make creatinine-free each minute.

- **Reference ranges**
  - Serum Creatinine 60-120 umol/L
  - Creatinine Clearance 125 milliliters per minute
  - GFR > 90 ml/min/1.73m²
Creatinine Clearance

- Measure serum and urine creatinine levels and urine volume and calculate serum volume cleared of creatinine
- Requirements for 24 hour urine collection adds variability and inconvenience

\[
CrCl = \frac{\text{creatinine excreted/ unit time}}{[Cr]_{\text{serum}}} = \frac{[Cr]_{\text{urine}} \times V}{[Cr]_{\text{serum}}}
\]

- Therefore, it represents the volume of serum completely cleared of creatinine per unit time
- Since virtually all creatinine is cleared via glomerular filtration, it closely approximates the GFR
Creatinine Clearance

EXAMPLE:

UCr = 72 mg/dl
SCr = 2.0 mg/dl
V = 2 liters
time = 24 hours

\[
CrCl = \frac{72 \text{ mg/dl} \times 2000 \text{ ml/day}}{2.0 \text{ mg/dl} \times 24 \text{ hrs/day} \times 60 \text{ min/hour}} = 50 \text{ ml/min}
\]

Limitations of Creatinine Clearance

• Only valid at steady state—[Cr]_{serum} must be stable

• Trimethoprim, cimetidine lower tubular Cr secretion and lower CrCl without changing GFR:

• Becomes more inaccurate at low GFR
Another Problem with Creatinine Clearance

• Must be done on a properly collected, timed urine sample--patient error
• How can we check accuracy of any timed urine collection?

Estimated GFR (eGFR)

• Alternative to GFR.
• The serum level of an endogenous marker is related to the reciprocal of the level of GFR and can be used to estimate the GFR without a urine collection.
• More Practical.
How is Chronic Kidney disease (CKD) defined?

There are 2 ways that a person can be diagnosed with chronic kidney disease:

• Kidney damage for ≥3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased glomerular filtration rate (GFR), manifest by either:
  • Pathological abnormalities.
  • Markers of kidney damage, including abnormalities in the composition of blood or urine, or abnormalities in imaging tests.
• GFR<60 ml/min/1.73m² for ≥3 months with or without kidney damage.

Stages of Chronic Kidney Disease

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (ml/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or ↑GFR</td>
<td>≥ 90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mild ↓GFR</td>
<td>60 - 89</td>
</tr>
<tr>
<td>3</td>
<td>Moderate ↓GFR</td>
<td>30 - 59</td>
</tr>
<tr>
<td>4</td>
<td>Severe ↓GFR</td>
<td>15 – 29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure</td>
<td>&lt; 15 (or dialysis)</td>
</tr>
</tbody>
</table>

*National Kidney Foundation, Kidney Disease Outcome Quality Initiative (K/DOQI), Clinical practise guidelines for bone metabolism and disease in chronic kidney disease.
Am J Kid Dis. 2003; 42: S1-S201
Laboratory Assessment of Kidney Function: What can we measure?

• CKD Criterion 1 (Other than GFR)
  • Assessment of proteinuria/albuminuria
  • Markers of kidney disease other than proteinuria or decline GFR
    • Presence of formed elements in the urine.
    • Measure other renal functions (such as acidification or concentration)
    • Renal structural abnormalities (imaging)
    • (Novel biomarkers).

• CKD Criterion 2 (GFR based)
  • Measure GFR
    • 24 hours urine for Creatinine clearance
    • Inulin clearance
    • Iothalamate clearance
  • Estimated GFR (blood test)

Creatinine as a Marker of GFR

• Byproduct of muscle turnover, is constant from day to day in an individual in the absence of disease (where muscle is rapidly damaged like in rhabdomyolysis) or change in muscle mass.

• Small amount is secreted by kidney tubules. Therefore, creatinine clearance will always overestimate GFR to some extent.

• Muscle mass varies with age/sex/size

• Creatinine is a very small molecule that is freely filtered in the kidney. Hence, clearance of creatinine is proportionate to GFR.
Creatinine as a Marker of GFR: It works but ...

- Serum creatinine numbers are plotted against measured GFR in a large number of individuals with and without kidney disease.
- Serum creatinine increases as GFR falls
- However, creatinine does not increase very much until GFR is well below 60 mL per minute per 1.73 m²
- Such a person already would have stage 3 chronic kidney disease.

How Can We Turn the Serum Creatinine Into a Better Estimate of GFR?

- Equations that use demographics to estimate muscle mass
- Cockroft-Gault equation
  - This equation estimates creatinine clearance based upon serum creatinine together with age, gender, and weight.
- MDRD equation (Modification of Diet in Renal Disease study) to estimated GFR.
  - This study only included patients with chronic kidney disease, who were placed on various diets in order to determine if a low-protein diet helped preserve kidney function.
  - The investigators then used data in their study to develop an equation that best estimated GFR.
  - One problem with the MDRD equation is that it was developed using only persons with CKD, and therefore does not perform very well in normal individuals.
Revised eGFR equation

\[ \text{GFR (mL/min/1.73 m2)} = \]
\[ 175 \times (\text{Scr}) - 1.154 \times (\text{Age}) - 0.203 \times (0.742 \text{ if female}) \times (1.212 \text{ if African American}) \]

eGFR equation works, but it's not perfect

- The MDRD equation estimates GFR very well in patients with known chronic kidney disease.
- The MDRD equation underestimates GFR in persons with normal kidney function.
How Can We Turn the Serum Creatinine Into a Better Estimate of GFR?

- CKD-EPI equation
  - Recent refinement of MDRD study.
  - Included normal and CKD patients in study population.

What about Cystatin C?

- Low-molecular-weight basic protein (13 kDa)
- Freely filtered and metabolized in the kidney.
- Compared to creatinine, production of cystatin C is much less influenced by a person’s age, gender, and size.
- Therefore, blood levels of cystatin C better reflect GFR than serum creatinine, and over recent years much effort has been spent evaluating cystatin C as a GFR marker.
What about Cystatin C?

- The Mayo Clinic renal laboratory has recently evaluated a new cystatin C assay.
- It is a particle-enhanced turbidometric immunoassay and can be run on a chemistry autoanalyzer.
- This confers certain lab advantages including high-capacity, quick turnaround time, and lower-cost compared to other platforms.

Cystatin C Equations Categorize Patients Slightly Better Than MDRD eGFR

- Worth considering in Kuwait Health care system.
24 hours urine test (Albumin)

Understanding Urine Albumin
• Albumin is a protein found in the blood. A healthy kidney does not let albumin pass into the urine. A damaged kidney lets some albumin pass into the urine.

24 hours urine test (Albumin)

• Reference range:
Urine albumin:
• Normal: 0-29 mg/24 hours
• High: 30-300 mg/24 hours
• Very High and Nephrotic: > (greater than) 300 mg/24 hours

Increased urine albumin might indicate renal damage or diabetes
eGFR

- eGFR calculation has been recommended by National Kidney Foundation whenever a serum creatinine is performed in adults

Tests that predict kidney disease

- eGFR
- Urine Albumin/Creatinine Ratio
  - The urine albumin test or albumin/creatinine ratio (ACR) is used to screen people with chronic conditions, such as diabetes and hypertension, that put them at an increased risk of developing kidney disease.
To summarize:

1. Use the Creatinine Clearance as the best estimate of GFR
2. Use the Serum Creatinine/Cystatin C to follow renal function over time
3. Use the BUN to help assess GFR, volume status, and protein intake