Laboratory Evaluation of Kidney Function

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University of Utah CME Statements

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• Speakers are also expected to openly disclose intent to discuss any off-label, experimental, or investigational use of drugs, devices, or equipment in their presentations.

• This speaker has nothing to disclose.
Objectives

• At the end of this presentation, participants should be able to:

  ▫ Diagram the functional unit of the kidney.

  ▫ Compare and contrast the markers used to evaluate glomerular filtration rate.

  ▫ List criteria for staging chronic kidney disease.
Outline

• Review of the kidneys
  ▫ Anatomy
  ▫ Physiology
  ▫ Pathophysiology

• Assessment of kidney function in chronic disease
  ▫ Laboratory tests
  ▫ Clinical practice guidelines
Case Study

My doctor just told me I have stage 2 kidney disease.

My doctor just told me I have stage 3 kidney disease!
Anatomy and Physiology
Overview of Kidneys

• Functions:
  ▫ Regulation of homeostasis
    • Electrolytes
    • Water
    • Acid-base balance
  ▫ Remove waste and toxins from the body
    • Filtration and excretion
  ▫ Synthesize hormones
Anatomy

- **Size:** ~12 cm long and ~150 g
- **Receive 25% of cardiac output**
  - Cardiac output ~3 L/min
  - Kidneys ~0.75 L/min
- **Filters** 180 L per day
- **Produces urine**
  - 0.4-2 L per day
Nephron

- Functional unit of the kidney
  - 0.6 – 1.5 million per kidney
- Composed of:
  - Glomerulus
  - Proximal tubule
  - Loop of Henle
  - Distal tubule
  - Collecting duct

http://www.unckidneycenter.org/kidneyhealthlibrary/glomerulardisease.html
Glomerulus

• Function: to filter plasma to form an ultrafiltrate

• Size exclusion

• Charge exclusion

Ultrafiltrate: Reabsorption and Excretion

- **Ions/electrolytes**
  - Na\(^+\), K\(^+\), Cl\(^-\), Ca\(^{2+}\), PO\(_4\)^{3-}, Mg\(^{2+}\), SO\(_4\)^{2-}, HCO\(_3\)^{-}, H\(^+\)
- **Water**
- **Small molecules**
  - Glucose
- **Waste products**
  - Creatinine, urea
Proximal Convoluted Tubule

- The most metabolically active part of the nephron
  - 60-80% of reabsorption

- Driving force is active transport of Na$^+$
  - Water follows Na$^+$

- Filtrate volume decreases

http://www.uic.edu/classes/bios/bios100/lecturesfo4am/lect21.htm
Loop of Henle

- **Descending limb**
  - Permeable to water
  - Impermeable to solutes ($\text{Na}^+$, $\text{Cl}^-$)

- **Ascending limb**
  - Impermeable to water
  - Permeable to solutes ($\text{Na}^+$, $\text{Cl}^-$)
Distal Convoluted Tubule

- Reabsorption of Na$^+$
  - Active transport
  - Cl$^-$ follows Na$^+$
  - Water

- Reabsorption of Ca$^{2+}$

- Excretion of K$^+$

http://www.uky.edu/~mtp/Diuretic_Drugs.htm
Collecting Duct

- Determines final concentration of urine
- Normally impermeable to water reabsorption
  - Responds to external signals

http://www.uic.edu/classes/bios/bios100/lecturesf04am/lect21.htm
Final Product: Urine

- Healthy urine:
  - 0.4-2 L/day
  - Clear, amber colored
  - pH 5.0-6.0
  - Osmolality: 50-1400 mOsm/kg
  - Protein: 50-80 mg/day
    - Albumin: <30 mg/day
  - Glucose: <0.5 g/day
Pathophysiology
Chronic Kidney Disease (CKD)

- A progressive decline in kidney function
  - Decreased filtration
  - Progresses to end stage renal disease
    - Dialysis or kidney transplant

Definition: a decreased glomerular filtration rate or signs of kidney damage that persist >3 months

- Occurs over many years
  - Often asymptomatic
Chronic Kidney Disease (CKD)

• >26 million Americans have CKD

• Risk factors include:
  ▫ Diabetes
  ▫ Hypertension
  ▫ Family history of kidney disease
Overall Medicare Part D & non-Part D costs in patients with CKD, by year

Point prevalent Medicare CKD patients age 65 & older.

+$33 billion for ESRD
All-cause rehospitalization or death within 30 days after live hospital discharge, 2010

January 1, 2010 point prevalent Medicare patients, age 66 & older on December 31, 2009, unadjusted. Includes live hospital discharges from January 1 to December 1, 2010.

USRDS 2012 ADR
Geographic variations in adjusted prevalent rates (per million population), 1997

USRDS 2009
Geographic variations in adjusted prevalent rates (per million population), 2002

USRDS 2009
Geographic variations in adjusted prevalent rates (per million population), 2007

December 31 point prevalent ESRD patients. By HSA; rates adjusted for age, gender, & race. Excludes patients residing in Puerto Rico & the Territories.

USRDS 2009
The Role of the Laboratory

Tests to evaluate kidney function
Tests of Kidney Function

- Creatinine
- Glomerular filtration rate
- Urine albumin
- Cystatin C
- Urinalysis
  - Dipstick
  - Microscopic
- Blood urea nitrogen (BUN)
- Osmolality
- Urine protein
- Urine protein electrophoresis
- Kidney stone assessment
  - Calcium, citric acid, uric acid, oxalate
Creatinine
Case Study

My serum creatinine is 1.1 mg/dL.

My serum creatinine is also 1.1 mg/dL. Why do I have stage 3 kidney disease?

*Stage 2

*Stage 3
Creatinine

- Creatinine – a waste product of creatine
  - In muscles, phosphocreatine is used as an energy source

\[
\text{Creatine} \xrightarrow{\text{Creatine kinase}} \text{ATP} \xrightarrow{\text{ADP}} \text{H}_2\text{O} \xrightarrow{\text{Creatinine}} \text{Pi} \xrightarrow{\text{phosphocreatine}}
\]
Creatinine Assay

- **Jaffe Reaction/Alkaline Picrate**

  ![Chemical Structures]

  - creatinine
  - picrate

  **Product** formed under alkaline conditions.

- **Interferences**
  - Protein, glucose, bilirubin, hemoglobin
  - Acetoacetate – diabetic ketoacidosis
Enzymatic Reaction: Creatinininase and Creatinase

Creatininase

Creatinine + H₂O → Creatine

Creatine + ATP → Sarcosine + Urea

Sarcosine oxidase

Sarcosine + O₂ + H₂O → Formaldehyde + Glycine + H₂O₂

Peroxidase

Indicator (reduced) + H₂O₂ → Indicator (oxidized) + 2H₂O

- Fewer interferences
- More expensive, less widely available
Glomerular Filtration Rate
Glomerular Filtration Rate (GFR)

- Glomerular filtration rate (GFR)
  - $\text{GFR} = \text{rate (mL/min) at which substances in plasma are filtered through the glomerulus}$
  - Best indicator of overall kidney function
  - Can be measured or calculated using a variety of markers
GFR and Chronic Kidney Disease

- National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI)
  - 2002 Clinical Practice Guidelines for Chronic Kidney Disease

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (mL/min/1.73 m²)</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>
Markers for GFR

- Ideal characteristics:
  - Freely filtered at the glomerulus
  - No tubular secretion or reabsorption
  - No renal/tubular metabolism

- Exogenous or endogenous
  - Exogenous – not normally present in the body
    - Inulin
  - Endogenous – normally present in the body
    - Creatinine

- Radiolabeled or non-radiolabeled
Direct Measures of GFR: Clearance

- \[ C = \frac{U \times V}{P} \]
  - \( C \) = clearance
  - \( U \) = urinary concentration
  - \( V \) = urinary flow rate (volume/time)
  - \( P \) = plasma concentration

- Clearance = GFR
Inulin Clearance

• Gold standard for renal clearance
  ▫ Freely filtered at glomerulus
  ▫ No tubular metabolism
  ▫ No tubular reabsorption or secretion

• Protocol
  ▫ IV infusion
  ▫ Blood samples
  ▫ Urine catheter

• Limitations
  ▫ Expensive, hard to obtain
  ▫ Difficult to assay
  ▫ Invasive
Creatinine to Calculate GFR

• Advantages
  ▫ Endogenous
  ▫ Produced at ~constant rate per day
  ▫ Routinely measured
  ▫ Freely filtered at glomerulus
    • Inversely related to GFR
  ▫ Not reabsorbed or metabolized by renal tubules
  ▫ Assays are standardized

• Disadvantages
  ▫ Estimate of GFR
  ▫ Is secreted by renal tubules
    • ~10%
    • Secretion increases as kidney function decreases
Estimated Glomerular Filtration Rate (eGFR)

- **MDRD equation**
  - $\text{GFR (mL/min/1.73 m}^2) = 175 \times (S_{\text{Cr}})^{-1.154} \times (\text{age})^{-0.203} \times 0.742 \times 1.210$
  - If female
  - If African American

- **Study group:**
  - Primarily caucasian
  - Patients with kidney disease (mean GFR = 40 mL/min/1.73 m²)

- **Limitations**
  - Less accurate in patients with normal GFR
    - Often reported “>60 mL/min/1.73 m²”
  - May be less accurate in some other ethnicities
Estimated Glomerular Filtration Rate (eGFR)

- **CKD-EPI equation:**
  \[
  \text{GFR} = 141 \times \min(\text{SCr}/\kappa, 1)^\alpha \times \max(\text{SCr}/\kappa, 1)^{-1.209} \times 0.993^{\text{age}} \times 1.018 \times 1.159
  \]
  - SCr = serum creatinine (mg/dL)
  - \(\kappa = 0.7\) (female) or 0.9 (male)
  - \(\alpha = -0.329\) (female) or -0.411 (male)
  - Min = minimum of SCr/\(\kappa\) or 1
  - Max = maximum of SCr/\(\kappa\) or 1

- **Study population**
  - Patients with and without kidney disease

- **Performance**
  - Similar to MDRD equation at lower GFR
  - Improved performance at higher GFR

- **Limitations**
  - More recent equation
  - Not in widespread use

*Ann Intern Med 2009; 150:604*
MDRD vs CKD-EPI
Creatinine Considerations

• Creatinine is related to muscle mass
  ▫ eGFR calculations may be influenced by:
    • Age
    • Sex
    • Race
    • Body builders
    • Body habitus (amputees)
    • Vegetarian/recent ingestion of cooked meat
Serum Creatinine vs GFR

Case Study

- Calculate GFR (SCr = 1.1 mg/dL)
  - [http://www.nephron.com/MDRD_GFR.cgi](http://www.nephron.com/MDRD_GFR.cgi)

<table>
<thead>
<tr>
<th>GFR Calculations</th>
<th>His</th>
<th>Hers</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDRD (mL/min/1.73 m²)</td>
<td>71</td>
<td>51</td>
</tr>
<tr>
<td>CKD-EPI (mL/min/1.73 m²)</td>
<td>77</td>
<td>56</td>
</tr>
</tbody>
</table>

51 yo male

56 yo female
Urine Albumin and Protein
Case Study

My urine ACR is 120 mg/g.

Well my urine ACR is only 40 mg/g.

*Stage 2
*S\textsubscript{cr} 1.1 mg/dL
*\text{eGFR} = 77 mL/min/1.73 m\textsuperscript{2}

*Stage 3
*S\textsubscript{cr} 1.1 mg/dL
*\text{eGFR} = 56 mL/min/1.73 m\textsuperscript{2}
Albuminuria: Beyond Kidney Disease

- Albuminuria = the presence of albumin in urine
- Albuminuria is an independent marker for cardiovascular disease morbidity and mortality
  - Non-diabetic
  - Non-hypertensive

*Circulation* 2005; 112:969
Urine Albumin

- Immunoturbidimetric or nephelometric

- Reference interval:
  - <30 mg/d
  - Albuminuria = Albumin to creatinine ratio (ACR) >30 mg/g

- Limitations
  - No reference method
  - No reference material for urine albumin
    - Recommended standardization against serum albumin reference material (CRM 470)
Serum Cystatin C
Cystatin C

• ~13 kD protein
  ▫ Cysteine protease inhibitor
  ▫ Produced by all nucleated cells
    • Constant production rate

• Freely filtered by glomerulus
  ▫ No tubular secretion or reabsorption
• Is metabolized by the tubules
• Serum concentrations are unaffected by:
  ▫ Muscle mass, diet
Cystatin C Assay

- Nephelometry

- Limitations
  - Not standardized
  - Not widely used
  - Expensive – compared to creatinine
Estimated Glomerular Filtration Rate (eGFR)

- **CKD-EPI cystatin C equation**
  - \( GFR = 133 \times \min(\text{SCysC}/0.8, 1)^{-0.499} \times \max(\text{SCysC}/0.8, 1)^{-1.328} \times 0.996^{\text{age}} \times 0.932 \)
  - \( \text{SCysC} = \) serum cystatin C (mg/L)
  - Min = minimum of \( \text{SCysC}/0.8 \) or 1
  - Max = maximum of \( \text{SCysC}/0.8 \) or 1

- **Limitations**
  - Extensively metabolized by the renal tubules
  - New equation, not widely used

Clinical Practice Guidelines
Kidney Disease Improving Global Outcomes

- KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease
  - Sponsor: National Kidney Foundation
KDIGO 2012 Clinical Practice Guideline: Staging of CKD

<table>
<thead>
<tr>
<th>GFR Category</th>
<th>GFR (mL/min/1.73 m²)</th>
<th>Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>≥90</td>
<td>Normal or high</td>
</tr>
<tr>
<td>G2</td>
<td>60-89</td>
<td>Mildly decreased</td>
</tr>
<tr>
<td>G3a</td>
<td>45-59</td>
<td>Mildly to moderately decreased</td>
</tr>
<tr>
<td>G3b</td>
<td>30-44</td>
<td>Moderately to severely decreased</td>
</tr>
<tr>
<td>G4</td>
<td>15-29</td>
<td>Severely decreased</td>
</tr>
<tr>
<td>G5</td>
<td>&lt;15</td>
<td>Kidney failure</td>
</tr>
</tbody>
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KDIGO 2012 Clinical Practice Guideline: Albuminuria

• “The term microalbuminuria should no longer be used by laboratories.”
  ▫ ~30-300 mg/day of albumin

<table>
<thead>
<tr>
<th>Category</th>
<th>Albumin to creatinine ratio (mg/g)</th>
<th>Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>&lt;30</td>
<td>Normal to mildly increased</td>
</tr>
<tr>
<td>A2</td>
<td>30-300</td>
<td>Moderately increased</td>
</tr>
<tr>
<td>A3</td>
<td>&gt;300</td>
<td>Severely increased</td>
</tr>
</tbody>
</table>

Kidney Int Suppl 2013; 3(1).
KDIGO 2012 Clinical Practice Guideline: Assessing CKD

## Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012

<table>
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<tr>
<th>GFR categories (mL/min/1.73 m²)</th>
<th>Persistent albuminuria categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Normal or high</td>
<td>A1 Normal to mildly increased</td>
</tr>
<tr>
<td>G2 Mildly decreased</td>
<td>A2 Moderately increased</td>
</tr>
<tr>
<td>G3a Mildly to moderately decreased</td>
<td>A3 Severely increased</td>
</tr>
<tr>
<td>G3b Moderately to severely decreased</td>
<td></td>
</tr>
<tr>
<td>G4 Severely decreased</td>
<td></td>
</tr>
<tr>
<td>G5 Kidney failure</td>
<td></td>
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</table>

### Persistent albuminuria categories

<table>
<thead>
<tr>
<th>A1</th>
<th>A2</th>
<th>A3</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 mg/g &lt;3 mg/mmol</td>
<td>30-300 mg/g 3-30 mg/mmol</td>
<td>&gt;300 mg/g &gt;30 mg/mmol</td>
</tr>
</tbody>
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Kidney Int Suppl 2013; 3(1).
Case Study

<table>
<thead>
<tr>
<th></th>
<th>His</th>
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<tbody>
<tr>
<td>Age</td>
<td>51</td>
<td>56</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>CKD-EPI eGFR (mL/min/1.73 m²)</td>
<td>77</td>
<td>56</td>
</tr>
<tr>
<td>ACR (mg/g)</td>
<td>120</td>
<td>40</td>
</tr>
<tr>
<td>Kidney Disease Stage</td>
<td>2</td>
<td>3</td>
</tr>
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KDIGO 2012 Clinical Practice Guideline: Assessing CKD

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<table>
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<tr>
<th>GFR categories (mL/min/1.73 m²)</th>
<th>Description and range</th>
<th>Persistent albuminuria categories</th>
</tr>
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<tbody>
<tr>
<td>G1</td>
<td>Normal or high ≥ 90</td>
<td>A1 Normal to mildly increased</td>
</tr>
<tr>
<td>G2</td>
<td>Mildly decreased 60-89</td>
<td>A2 Moderately increased</td>
</tr>
<tr>
<td>G3a</td>
<td>Mildly to moderately decreased 45-59</td>
<td>A3 Severely increased</td>
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<td>G5</td>
<td>Kidney failure &lt;15</td>
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Kidney Int Suppl 2013; 3(1).
Therapeutic Interventions
Recommended Management: CKD

- **Blood pressure regulation**
  - ACE-inhibitors

- **Diet**
  - Low protein (<0.8 mg/kg/day)
  - Low salt (<2 g/day)

- **Blood glucose control**
  - HbA\textsubscript{1c} = ~7.0%

- **Supplements:**
  - Vitamin D
  - Bicarbonate

*Kidney Int Suppl 2013; 3(1).*
Summary

- The nephron is the functional unit of the kidney
  - The glomerulus is a key regulator of filtration rate and filtration selectivity
- Progression of chronic kidney disease is commonly monitored using glomerular filtration rate and albuminuria
- Numerous laboratory methods exist to evaluate glomerular filtration rate
  - Exogenous vs endogenous markers
  - Direct vs calculated
- KDIGO 2012 Clinical Practice Guidelines:
  - 6 stages of chronic kidney disease based on GFR
  - 3 stages of albuminuria
References

- ARUP SOP’s: Creatinine, Total Protein Urine, Microalbumin Urine, Cystatin C Serum
- CDC, [www.cdc.gov](http://www.cdc.gov)
- National Kidney Foundation, [www.kidney.org](http://www.kidney.org)
Questions?