It's Time to Clear the Fog about Kidney Function Testing

John Toffaletti, PhD
Director of Blood Gas, Clinical Pediatric, and Outpatient Laboratories
Professor of Pathology
Duke University Medical Center
Chief of Clinical Chemistry; VA Med Center
Durham, NC

Main Points of My Talk

- Measured GFR (mGFR) is a poor kidney function test.
- Creatinine is a better renal function test than often believed.
- Cystatin C works synergistically with creatinine.
- Brief review of methods for measuring GFR and “clearance”.
- The eGFR is a normalized creatinine; it is NOT a mGFR!
- What does the CKD-EPI eGFR improve?
- The future of creatinine, cystatin C, and eGFR measurements.
- Briefn mention of markers for acute kidney injury such as NGAL.

Lab Tests Discussed Today

- **Creatinine**
- **eGFR**
- **measured GFR (mGFR)**
- **Cystatin C**
- **Brief mention of Acute Kidney Injury tests:**
  - **NGAL:** neutrophil gelatinase-associated lipocalin
  - **Cystatin C**

Methods for mGFR are Difficult:

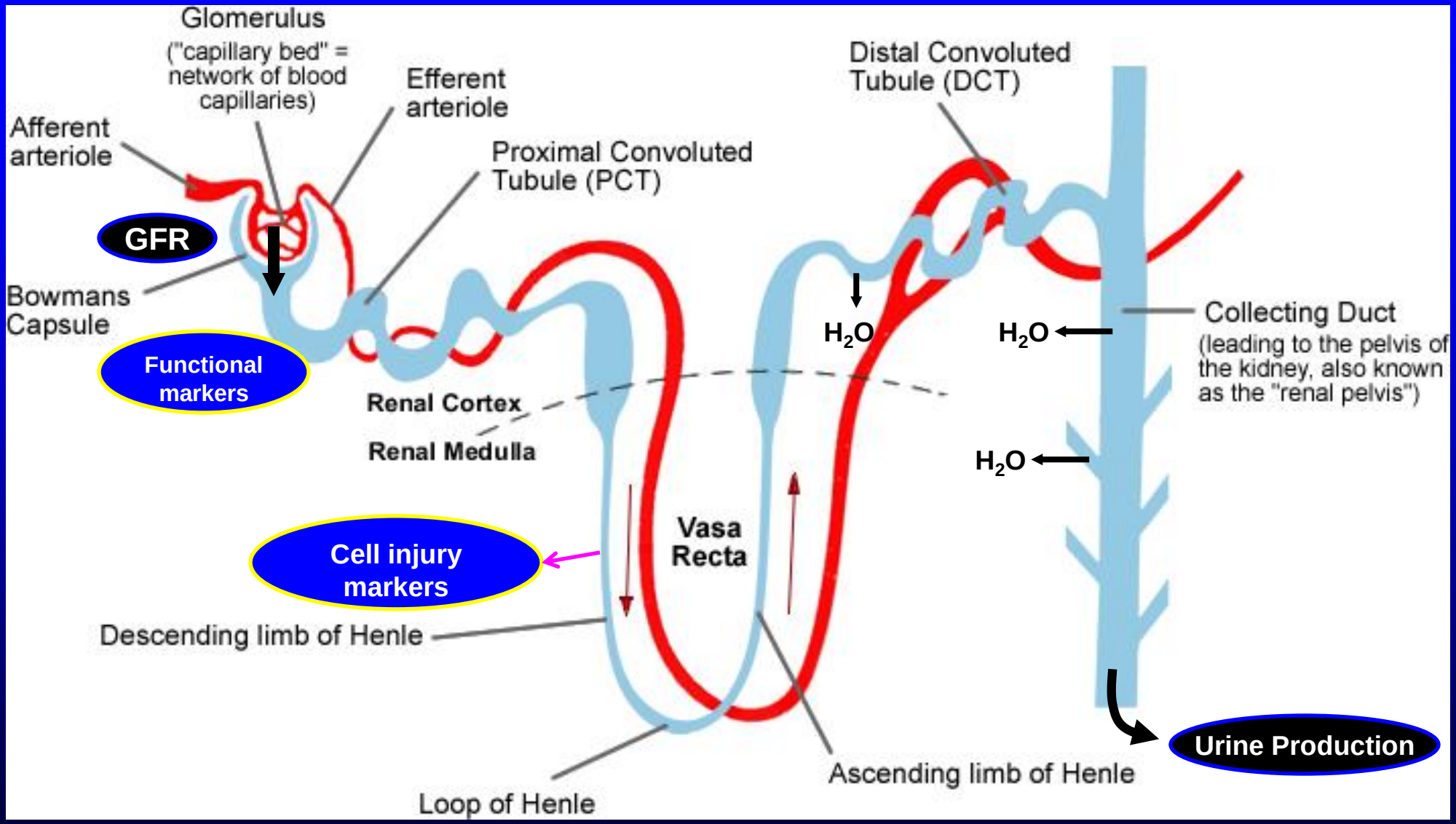
Require bolus injection or plasma infusion, urine collection, and measurements of marker

- **Inject or infuse a marker (inulin, iothalamate, iohexol), or measure an endogenous marker (creatinine).**
- **Collect one or more blood samples.**
- **Some require collection of an accurately-timed urine sample.**
- **mGFR calculated by various methods:**
  - **"Clearance": Urine vol (mL/min) X [urine] / [plasma]**
  - **Plasma disappearance: Rate of decay in plasma: GFR = V_D X (0.69 / $T_{1/2}$)**
- **Procedures are all tedious, slow, and expensive.**
- **Agreement between mGFR methods is variable.**

Acute and Chronic Kidney Diseases Share Common Causes

Conditions That May Cause or Increase Susceptibility to: Acute Kidney Injury / Chronic Kidney Disease
Hyper-inflammatory response (sepsis);
Diabetes; Autoimmune reaction; Advanced age;
Toxins/Nephrotoxic Drugs;
Chronic diseases; Critical illness;
Major surgery, especially with CP Bypass;
Trauma, Burns, Radiocontrast agents, Dehydration.

Diagram of a Human Nephron



Modified from: http://www.ivyroses.com/HumanBody/Urinary/Urinary_System_Nephron_Diagram.php

Chronic Kidney Disease

A slow, progressive loss of functional nephrons lasting over three months that leads to:

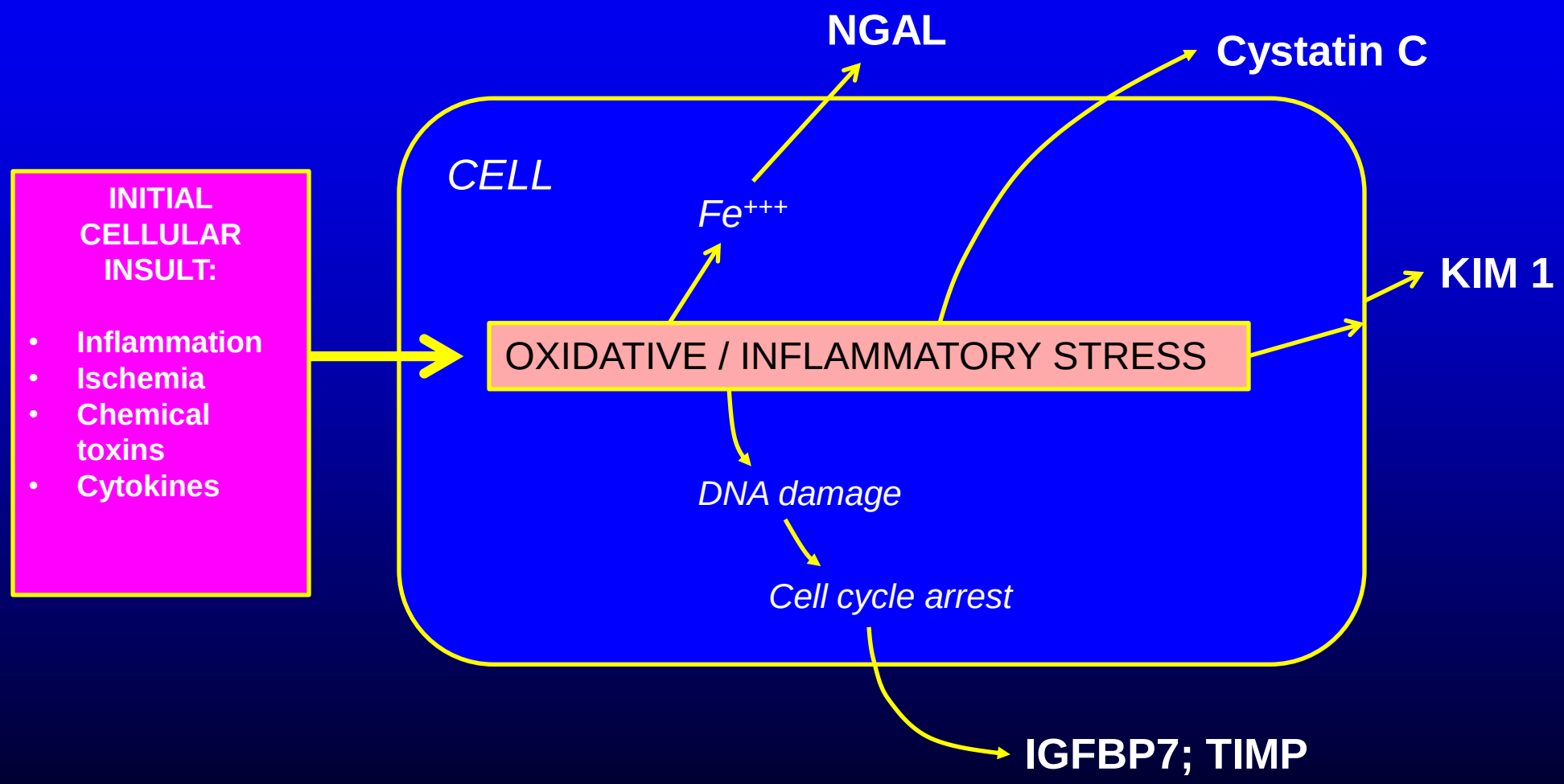
- **Proteinuria:**
  - **Progresses from occasional (early stages) to persistent.**
- **Gradual decrease in GFR:**
  - **rise of serum creatinine and cystatin C.**
- **Hypertension, bone disease due to poor ion regulation, 2^0 hyperparathyroidism.**

Early diagnosis and treatment can save kidney function!

What Would be the Ideal Marker for Chronic Declining Kidney Function?

- **GFR ?**
  - **Has large individual and population variations.**
  - **Very cumbersome test.**
- **Serum marker ?**
  - **Creatinine, cystatin C**
- **Number of lost functioning nephrons?**
  - **Yes, but we cannot measure this, so we are stuck with mGFR or a serum marker.**
    - » **This led to the development of the eGFR (estimated GFR) calculated from plasma creatinine and/or cystatin C.**

Cellular Production of Molecules Associated with Acute Kidney Injury



What Would be the Ideal Marker for Acute Kidney Injury?

- GFR ?
  - Neither sensitive nor practical: time consuming and too much variation.
- Serum marker of lost nephrons or GFR?
  - Creatinine, cystatin C, eGFR are useful but rise slowly.
- Marker for some insult to kidney cells:
  - NGAL, KIM, cystatin C, etc.
  - These might change earlier than creatinine, but are not highly specific for kidney disease.

Cystatin C in Renal Function

- Cystatin C is a small protein (MW ~13,000) that:
  - Is produced at a constant rate by all nucleated cells.
  - Is freely filtered by the glomerulus, then catabolized by the renal tubules = none appears in urine.
- It is also affected by some non-renal conditions and factors:
  - Inflammation, steroids, diabetes, weight, and other factors.
- Serum levels are less affected by muscle mass, diet, gender or race than is creatinine. However:
  - Reference ranges change with age:

  < 1y: 0.65-1.50 mg/L; 1-17y: 0.50-1.27 mg/L; > 17y: 0.53-0.95 mg/L

Cystatin C vs Creatinine

- Serum cystatin C and serum creatinine levels are about equally sensitive for decreased GFR.
- Cystatin C also has about the same population variation and within-individual variations as serum creatinine:
  - Scand J Clin Lab Invest 2009; 69: 831-836 (adults)
  - Scand J Clin Lab Invest 2010; 70: 54-59 (children)
  - Clin Chim Acta 2008; 395: 115-9
- Cost, availability, and familiarity are also issues.

Cystatin C Has Clinical Usefulness

- **Less affected by diet and muscle mass than creatinine.**
- **Recommended to confirm CKD when eGFR is 45-59 mL/min/1.7m^2** *(Stage 3A CKD).*
- **Is a better predictor of mortality in patients with CKD.**
- **Rises sooner that creatinine in AKI:**

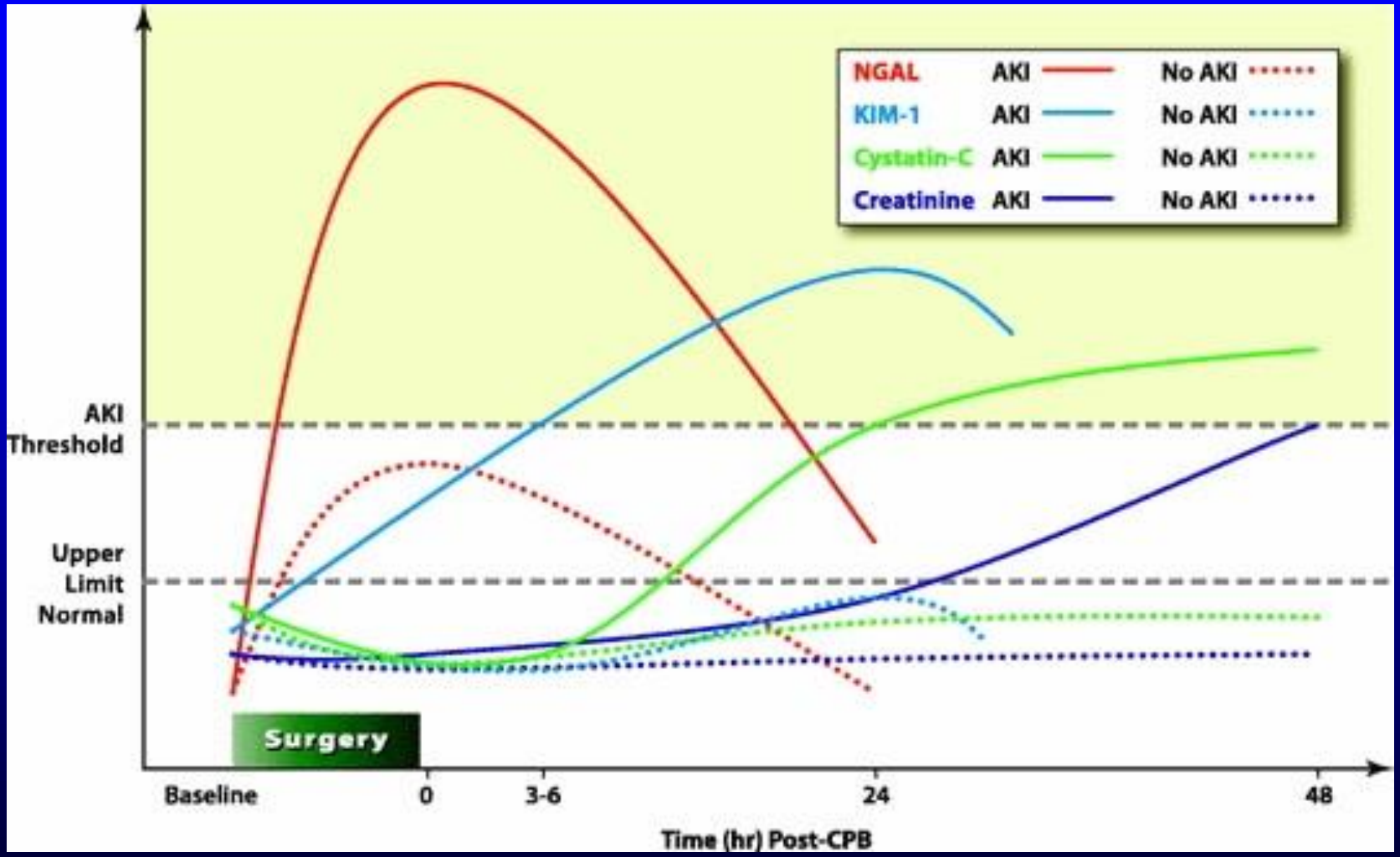
  Among 442 general ICU patients, cys C indicated acute kidney injury earlier than did serum creatinine ***(Nephrol Dial Transplant 2010; 25: 3283-3289)*** **:**

  - **In 342 pts, neither increased**
  - **In 17 pts, creat increased before cys C**
  - **In 66 pts, cys C increased before creat**
  - **In 17 pts, both increased at same time**

NGAL (Neutrophil Gelatinase-Associated Lipocalin)

- **NGAL is normally expressed at very low levels.**
- **NGAL expression is markedly inhanced by renal tubular cells in response to free Fe^{3+} ions that are liberated in response to ischemic or toxic injury.**
- **Plasma and urine NGAL are early markers for AKI, especially when the timing of the kidney insult is known, such as post cardiac surgery, sepsis, trauma, or radiocontrast exposure.**

AKI Biomarkers:Typical Time Courses after Cardiac Surgery

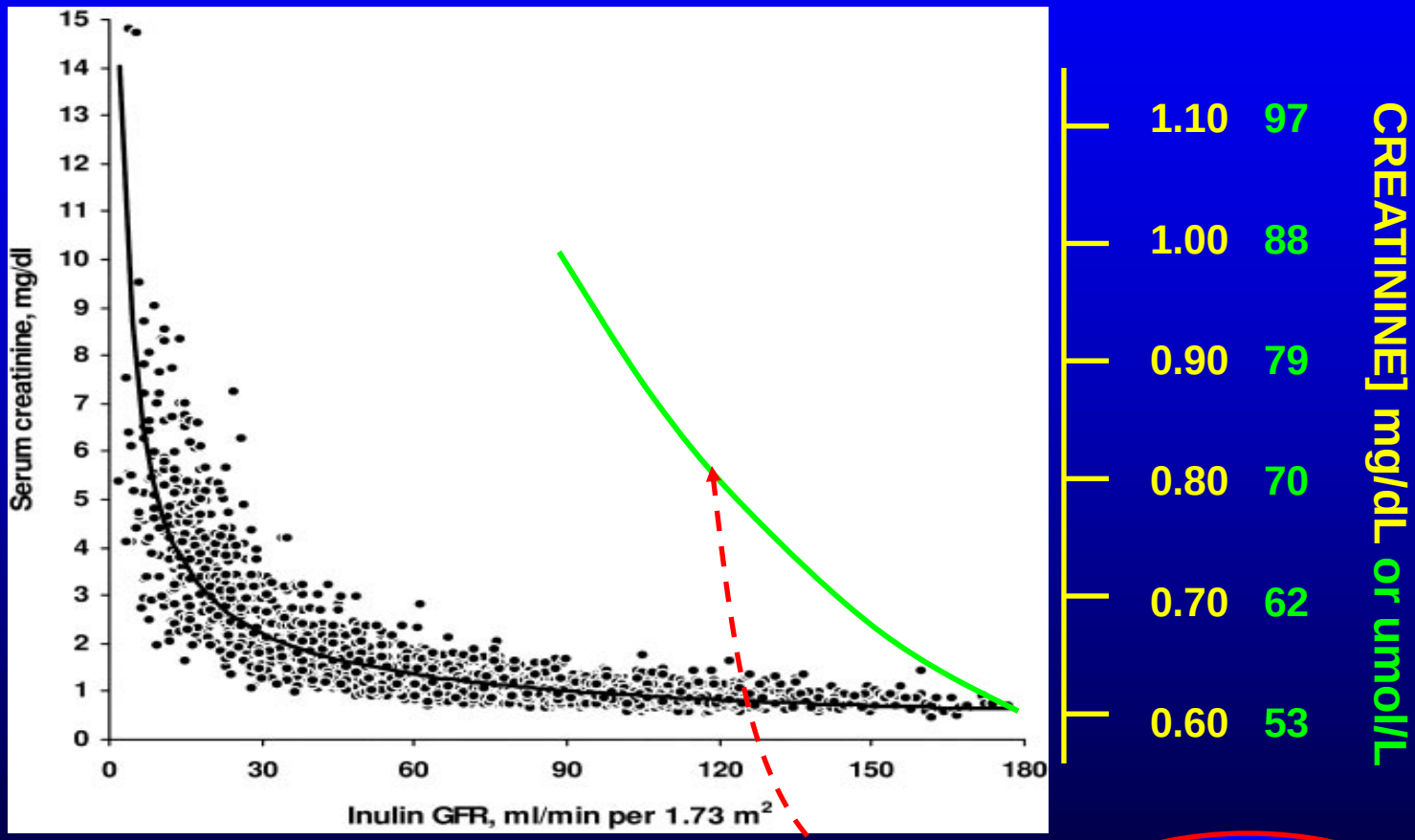


Anesthesiology 2010; 112: 998-1004

Some Big Lies Through History

- **The Earth is flat** *(Homer, Thames, many others)*
- **We got it all** *(many surgeons)*
- **I'll call you tomorrow** *(many men, some women)*
- **You'll go blind if you keep doing that** *(many mothers)*
- **email will never catch on** *(Toffaletti, circa 1990)*
- **It's simply plug and play** *(software experts)*
- **Serum creatinine does not increase until 50% of nephrons are lost** *(many kidney experts)*

Plot of Serum Creatinine vs GFR by Inulin Clearance



0.10 mg/dL scale makes Sensitivity appear much better!

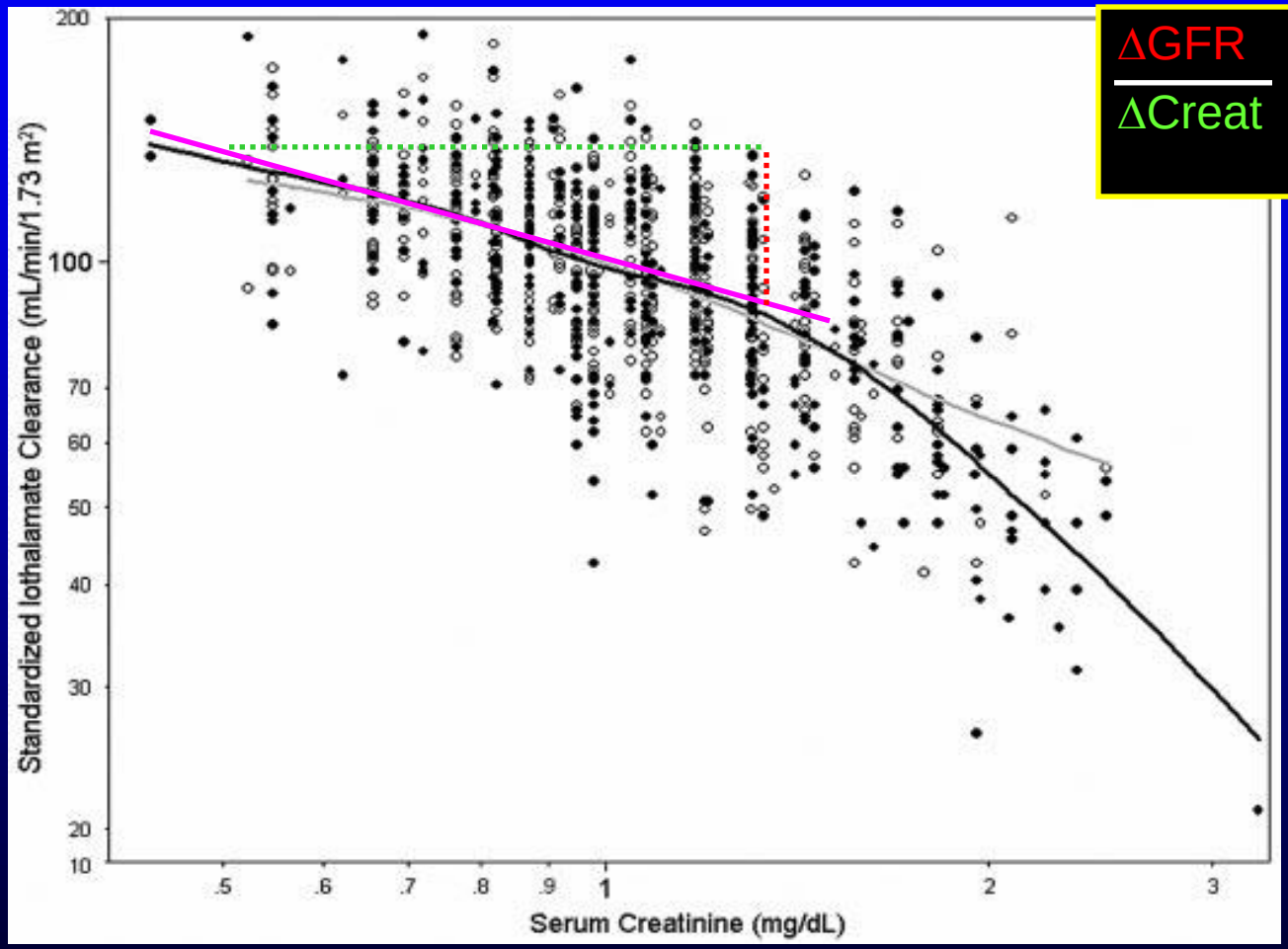
From Figure 1 in: Clin J Am Soc Nephrol 2009; 4: 899-906.

Plasma Creatinine as a Renal Function Test

- **Good: An increase is usually specific for diminishing renal function.**
- **Good: Within-individual variation is small.**
- **Bad: Population variation is large:**
  - **Creatinine varies by age, gender, and race (muscle mass)**
  - ***Protein intake*, drugs, and exercise may also affect blood levels of creatinine.**
- **Bad?: Lacks sensitivity for early detection of declining renal function *(Somewhat true, but is GFR any better?).***

Iothalamate GFR vs Creatinine (log scale) in Polycystic Ovary Disease

J Am Soc Nephrol 2006; 17: 854-62



$$\frac{\Delta GFR}{\Delta Creat} = \frac{140 - 85}{0.5 - 1.3} = \frac{-40\%}{+60\%}$$

What Was the Purpose of the eGFR Equations?

- **Based on belief that measured GFR was the "gold standard" for assessing kidney disease.**
- **Because of the wide reference range for serum creatinine, increased creatinine in an individual might go unnoticed by physicians.**
- **The "experts" then tried to develop an equation to calculate an eGFR from the creatinine result that would numerically mimic the measured GFR determined by iothalamate clearance.**
- **Using kidney disease stages based on mGFR, they used the eGFR to categorize individuals into various stages of kidney disease.**
- **While the older MDRD eGFR reported only when less than 60 mL/min/1.73m^2, the more recent CKD-EPI equation can allow all values of eGFR to be reported.**

So What is an "eGFR"?

- It is a creatinine / cystatin C that is normalized for age, gender, and race…then mathematically manipulated to look like a GFR.
- However, the eGFR is NOT a mGFR!
- And that is a good thing!

Old and New eGFR Equations for Predicting GFR In Adults

Cockcroft-Gault Equation (Nephron 1976;16:31):

$$\text{GFR} = \frac{(140 - \text{age}) \times \text{Weight}}{72 \times S_{Cr}} \times 0.85 \text{ (if female)}$$

Abbreviated MDRD Equation (Ann Intern Med 1999 and later):

$$\text{eGFR (IDMS)} = 175 \times (S_{Cr})^{-1.15} \times (\text{Age})^{-0.20} \times (0.742 \text{ if female}) \times (1.21 \text{ if black})$$

The new CKD-EPI equation (Ann Intern Med 2009;150:604):

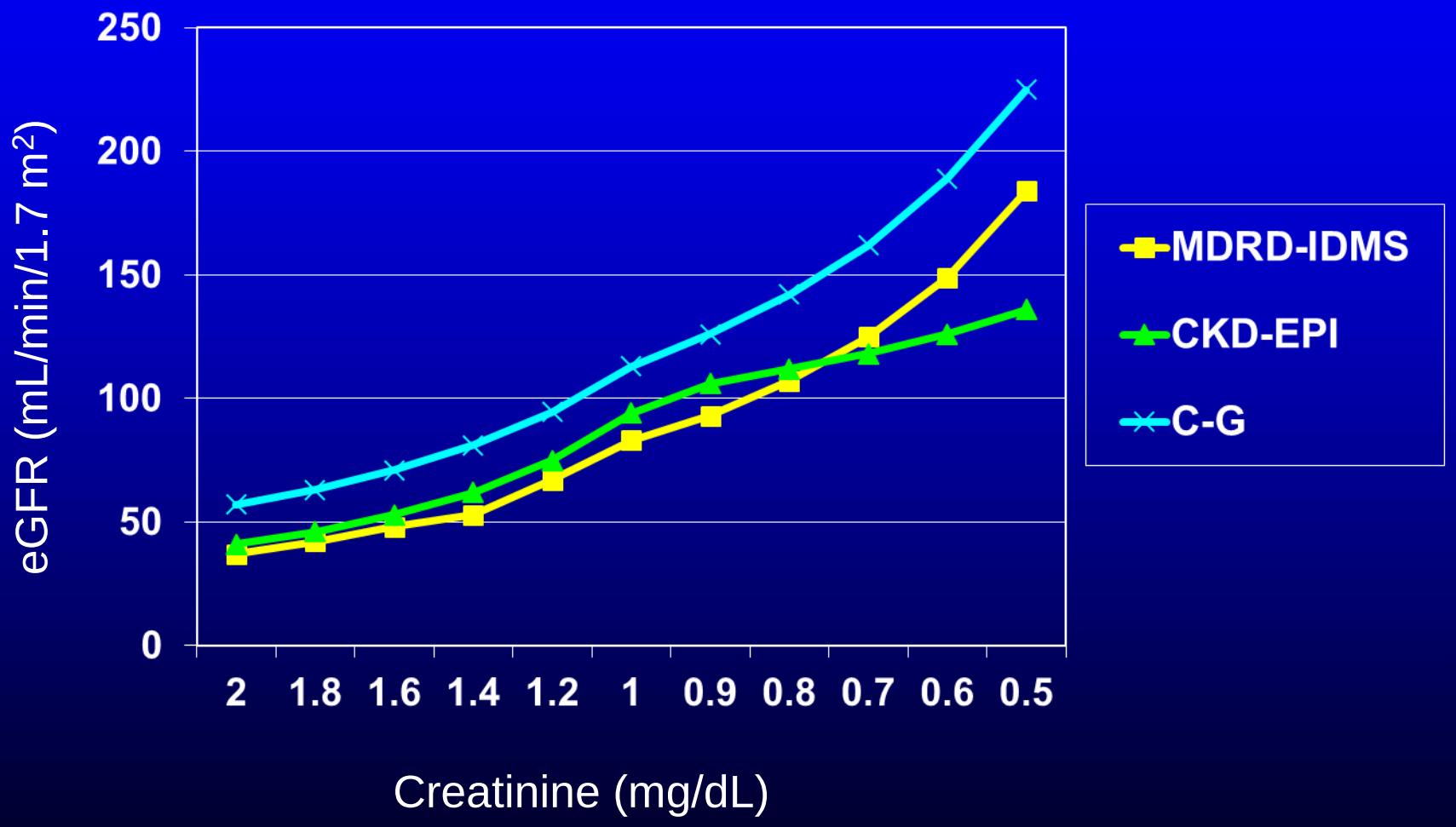
$$\text{eGFR} = 141 \times \mathit{min}(S_{Cr}/k,1)^{a} \times \mathit{max}(S_{Cr}/k,1)^{-1.21} \times (0.99)^{\text{Age}} \times (1.018 \text{ if female}) \times (1.16 \text{ if black})$$

And here is an eGFR for Cystatin C (Clin Chem 2014; 60(7): 974-86:

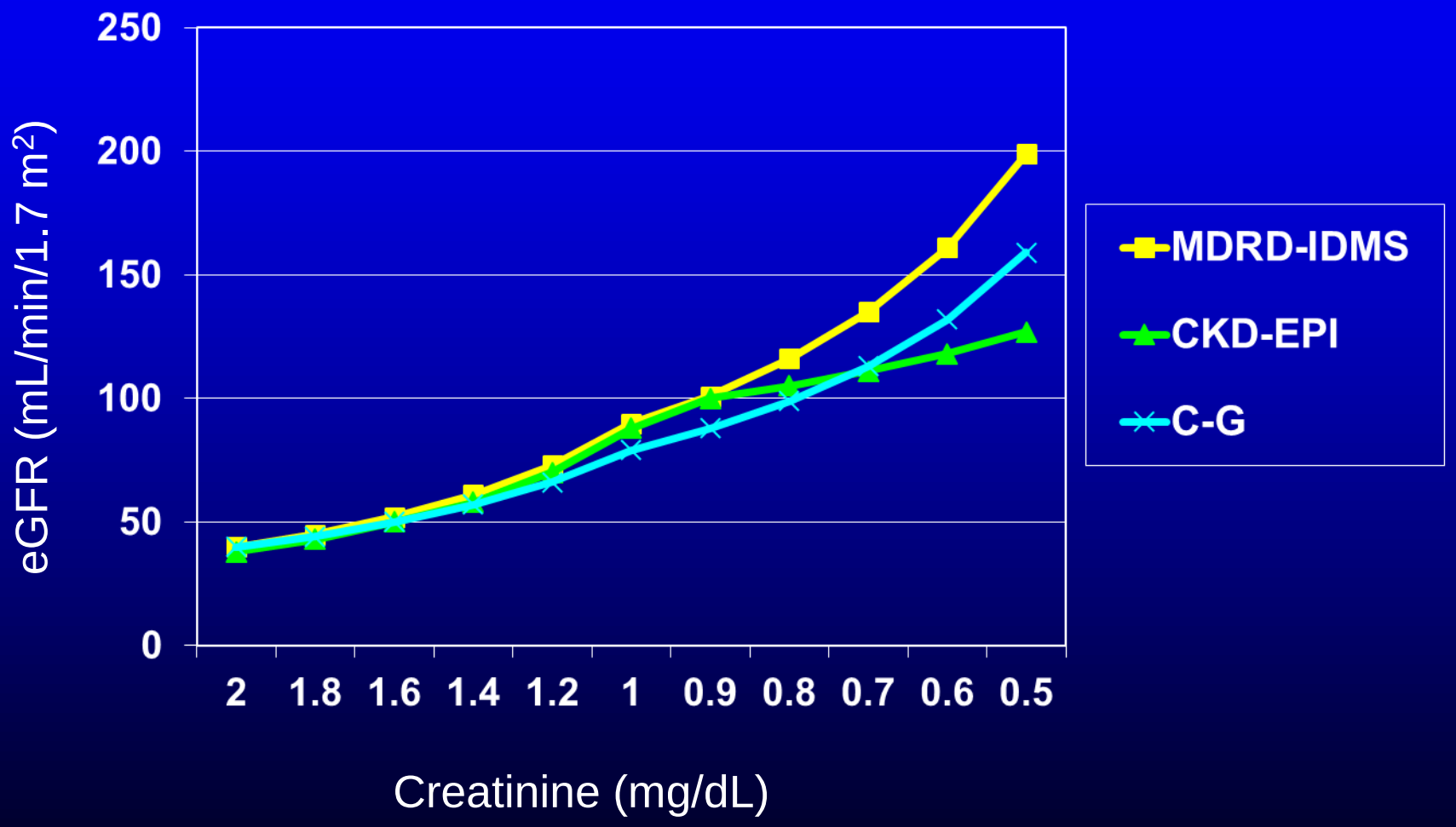
$$\text{eGFR}_{\text{cysC}} = 130 \times \text{cystatin C}^{-1.069} \times \text{Age}^{-0.117} - 7$$

How Will the New CKD-EPI eGFRs Differ From Other eGFRs?

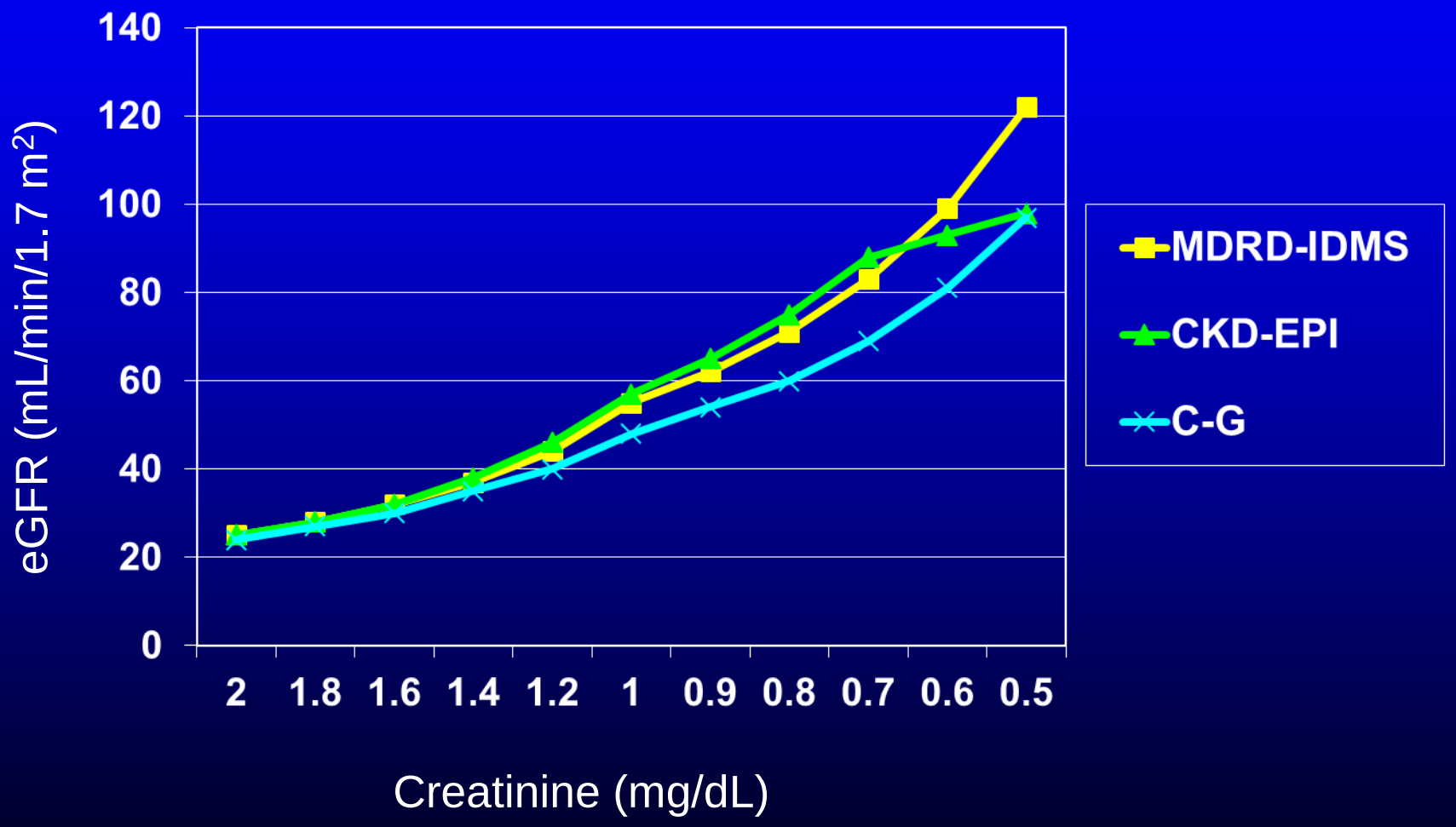
eGFR: MDRD-IDMS, CKD-EPI, C-G: 40 yr old white male; 180 lbs



eGFR: MDRD-IDMS, CKD-EPI, C-G: 70 yr old black male; 180 lbs



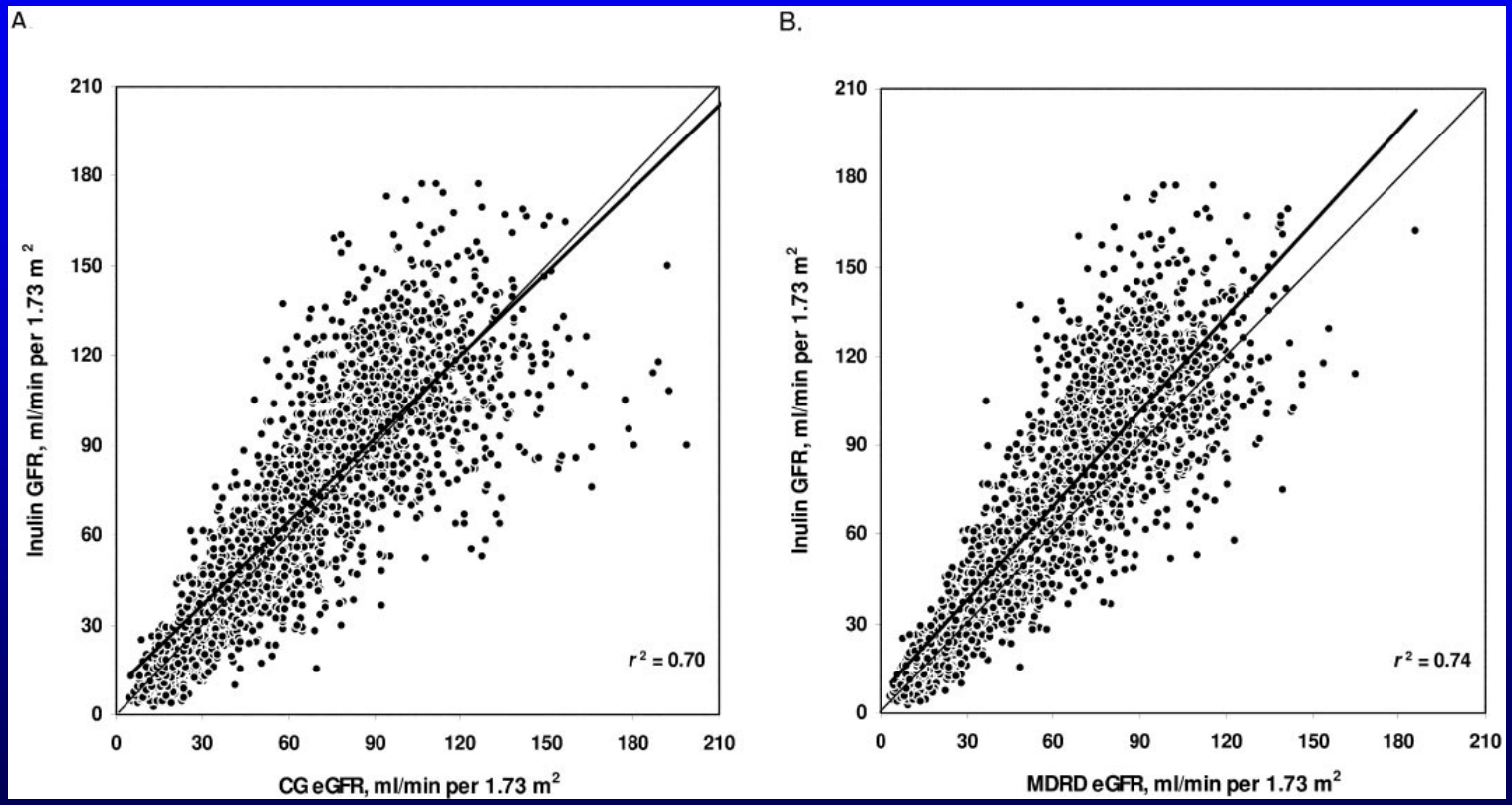
eGFR: MDRD-IDMS, CKD-EPI, C-G: 70 yr old white female; 130 lbs



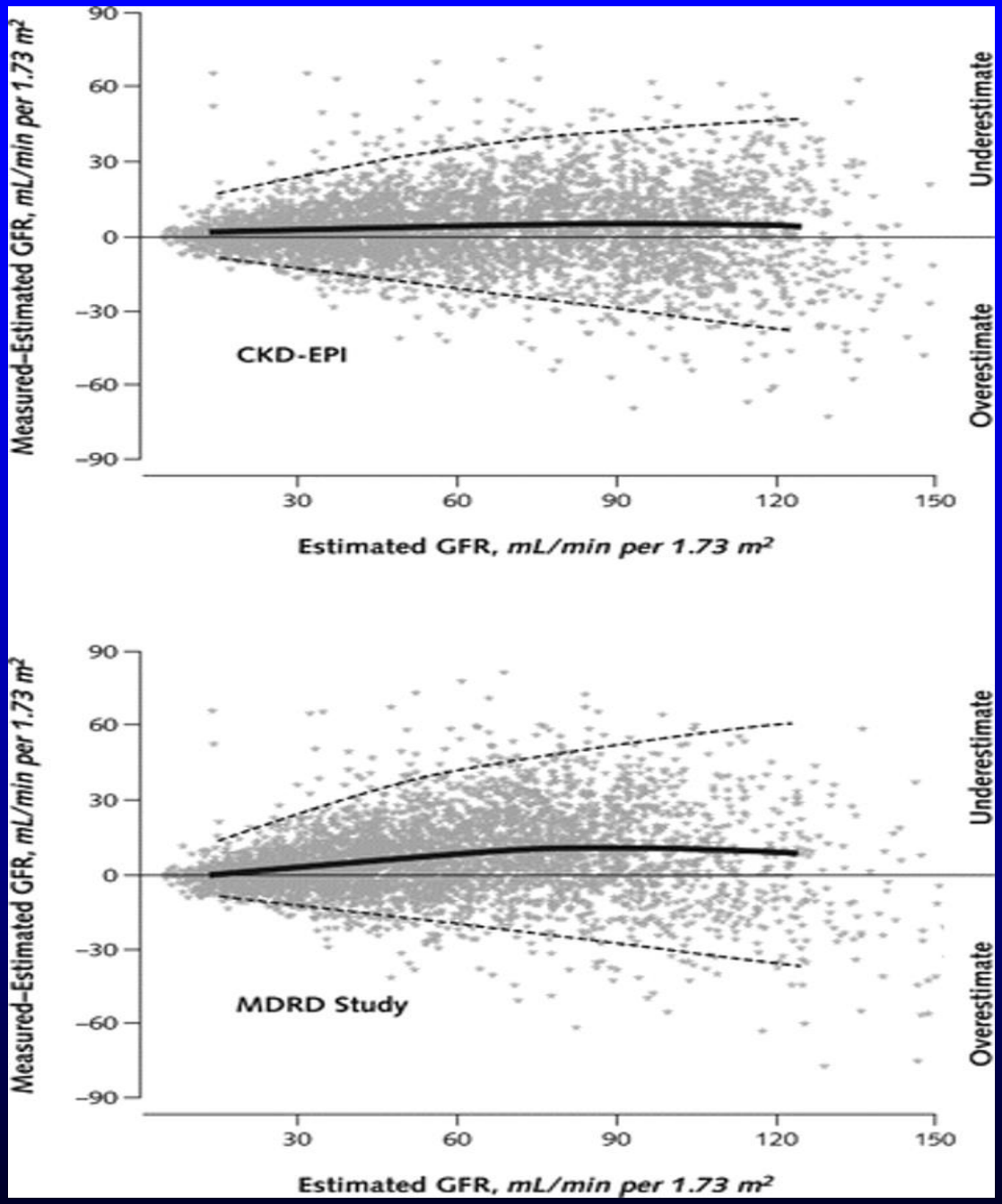
Do ANY Equations Provide an eGFR from Creatinine or Cystatin C that Reliably Predicts mGFR?

Simple Answer: NO
…and again, that is okay!

Plots of Inulin GFR vs C-G eGFR and MDRD eGFR



From Figure 2 in: Botev R, et al. Clin J Am Soc Nephrol 2009; 4: 899-906.



84.1% agree within 30%

Performance of the CKD-EPI and MDRD Study equations in estimating measured GFR in the external validation data set.

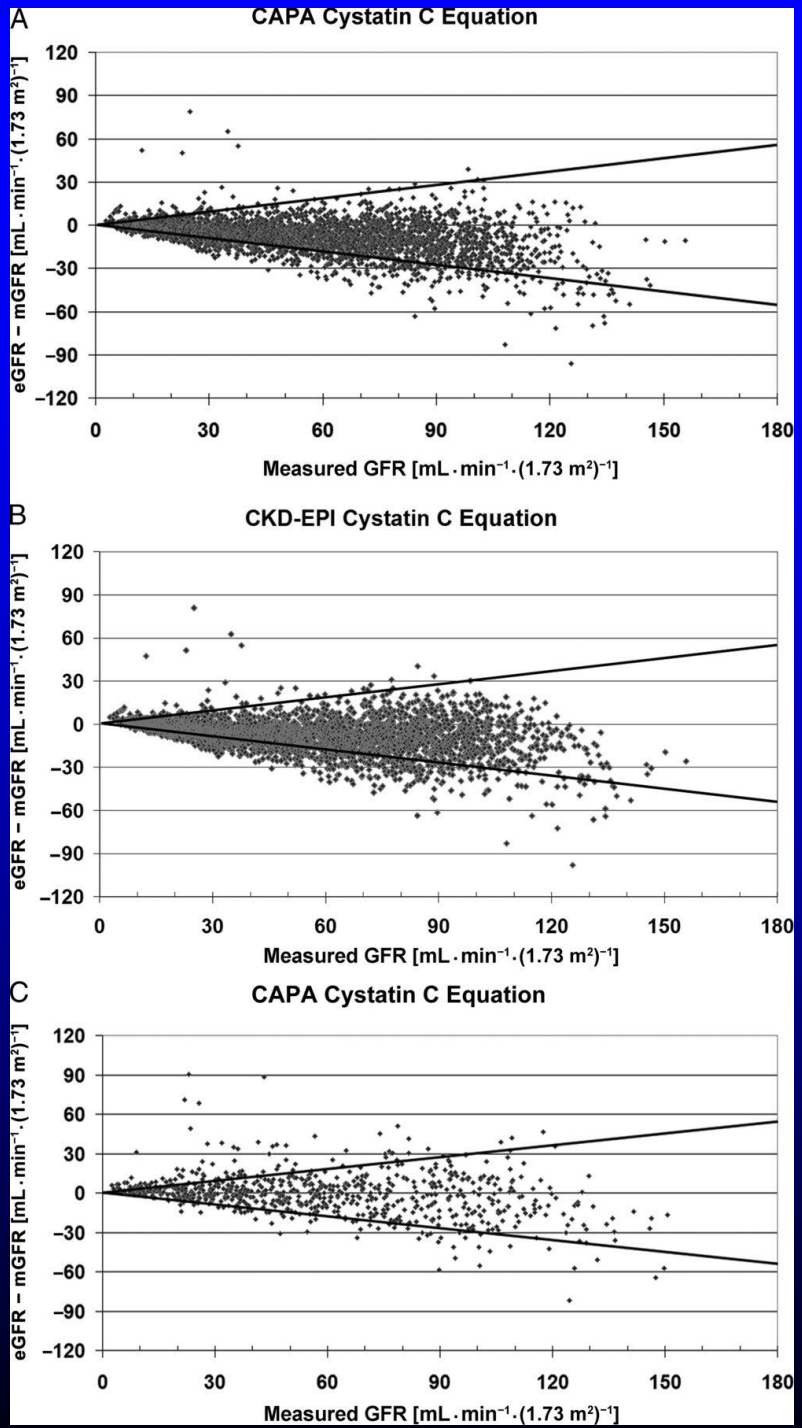
Both panels show the difference between measured and estimated versus estimated GFR

80.6% agree within 30%

Levey A S et al. Ann Intern Med 2009;150:604-612

©2009 by American College of Physicians

Plots of $eGFR_{Cys\ C}$ vs $mGFR_{iohexol\ or\ inulin}$



A and B:
3495 Swedish Adults (iohexol clearance)

C:
763 Japanese Adults (inulin clearance)

From Figure 1 in: Grubb A, et al. Clin Chem 2014; 60(7): 974-986..

What should the goal of the eGFR be?

To agree with the measured GFR? NO!

Unfortunately, some groups have made a career trying to do this.

To provide a parameter to alert non-kidney specialists of potential kidney disease? Yes!

To provide a convenient marker indicating deteriorating kidney function independent of gender, race and age? YES, we hope!

Here is a Report Validating a New eGFR Equation Based on 4 Metabolites

Validation of a Metabolite Panel for a More Accurate Estimation of mGFR Using Quantitative LC-MS/MS *(Clin Chem 2019; 65:3: 406-418)*

Tiffany A. Freed, Josef Coresh, Lesley A. Inker, Douglas R. Toal, Regis Perichon, Jingsha Chen, Kelli D. Goodman, Qibo Zhang, Jessie K. Conner, Deirdre M. Hauser, Kate E.T. Vroom, Maria L. Oyaski, Jacob E. Wulff, Gudny´ Eirı´ksdo´ ttir, Vilmundur Gudnason, Vicente E. Torres, Lisa A. Ford, and Andrew S. Levey

This study used LC-MS/MS methods to measure 4 serum metabolites that correlated with mGFR:
N-acetylthreonine, pseudouridine, phenylacetylglutamine, and tryptophan

Then developed an equation ($eGFR_{met}$) based on these 4 metabolites to predict the measured GFR. The % of agreement within 30% of the mGFR were:

$eGFR_{creat}$ to mGFR = 87%
$eGFR_{cysC}$ to mGFR = 88%
$eGFR_{cr\text{-}cysC}$ to mGFR = 93%
$eGFR_{met}$ to mGFR = 90%

Here is an Editorial That Has it Right!

Measured GFR as "Gold Standard": All that Glitters is Not Gold? *(Clin J Am Soc Nephrol 2011; 6: 1813-4)*

Chi-yuan Hsu and Nisha Bansal

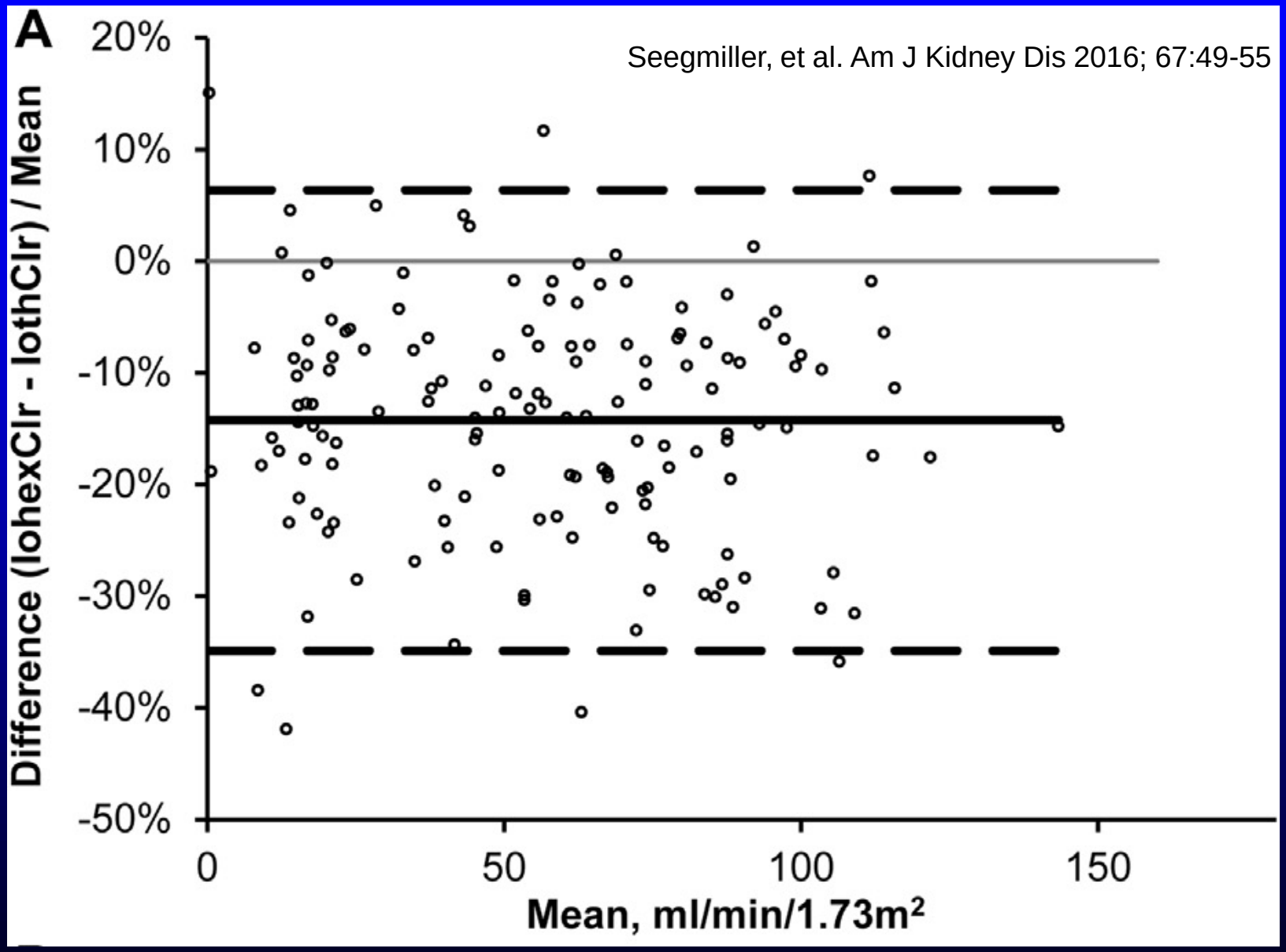
Their last paragraph says it all:

"Investigations regarding eGFR and mGFR have occupied much time and attention of many in academic nephrology. We believe it is time to reassess these efforts. The clinical purpose of assessing a patient's renal function is to anticipate complications (and) enable better screening and treatment decisions. Determining with great accuracy a certain physiological parameter --- actual GFR --- is a less important goal."

Measured GFR by Any Method (Creatinine, Iothalamate, Iohexol, etc) Has Many Faults

- **Has very large analytical variation**
- **Has large within-individual variation**
- **Variation or interference to urine (creatinine, iothalamate) measurement:**
  - **20 fold dilution for urine creatinine.**
- **mGFR is much more variable than serum creatinine and is not a sensitive diagnostic tool!**

Differences Between Iohexol GFR and Iothalamate GFR



Reference Ranges for mGFR as Wide as for Serum Creatinine

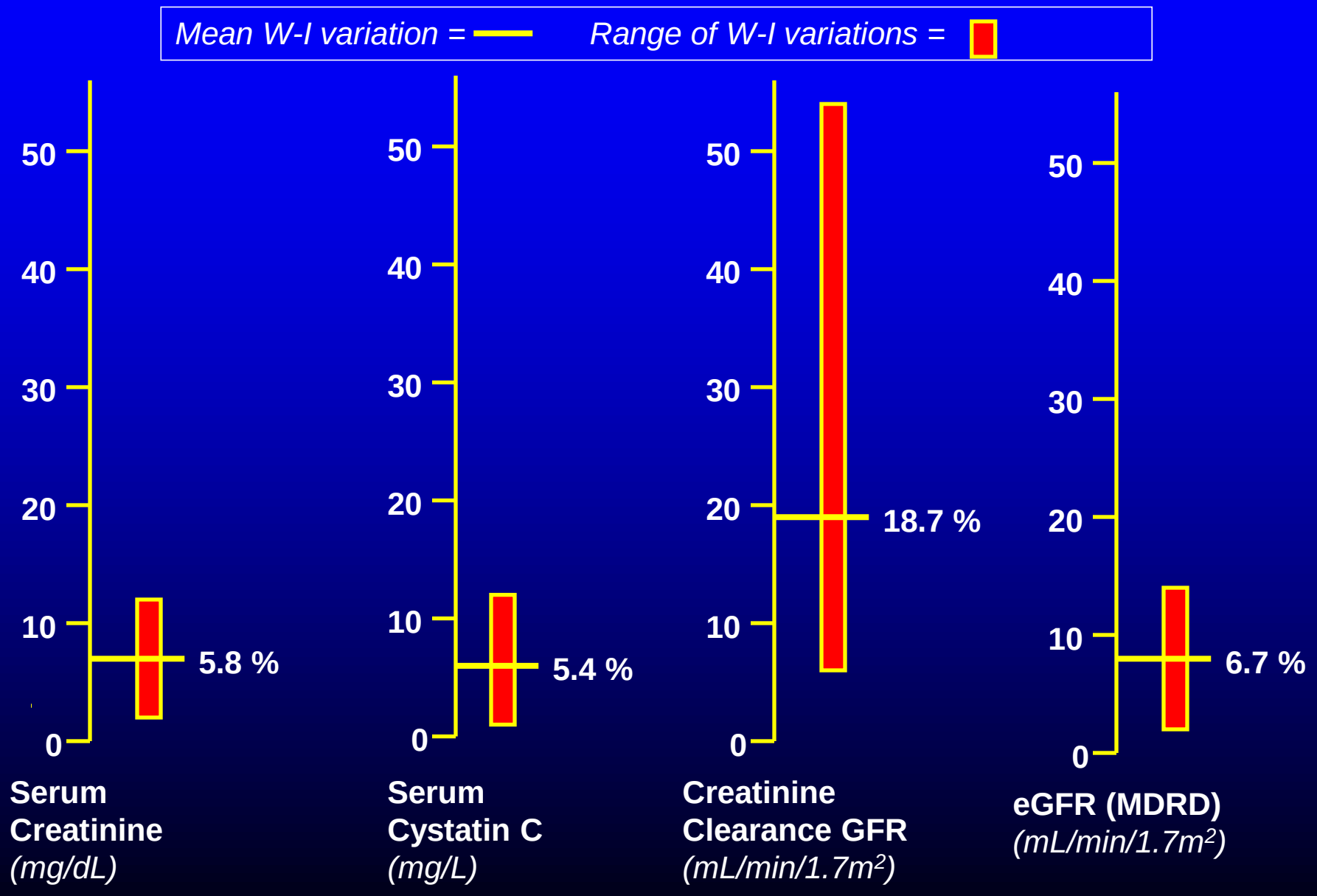
Parameter	Healthy Persons *(n = 501)*	
	Mean ± 2 SD	***Range***
Serum creatinine *(mg/dL)*	**0.73 – 1.37** ***(Ratio = 1.88)***	**0.6 – 1.6**
Iothalamate GFR *(mL/min/1.73 m^2)*	**67 – 135** ***(Ratio = 2.01)***	**63 – 177**

Rule AD, et al. Using serum creatinine to estimate GFR: accuracy in good health and in chronic kidney disease. Ann Intern Med 2004; 141: 929-937.

And:

mGFR Also Has a Large Within-Individual Variation

Creatinine and Cystatin C have Much Less Within-Individual Variation than Measured GFR *(31 persons over 6 months: Clin Chim Acta 2008; 395: 115-9)*



GFR vs Stage of Chronic Kidney Disease:

Some Recent Changes

CKD Stage	Description	GFR (mL/min/1.73m²)
1	Kidney damage with normal GFR	≥ 90
2	Kidney damage with mild ↓ GFR	60-89
3	Moderate ↓ GFR	30-59
4	Severe ↓ GFR	15-29
5	Kidney failure	< 15 (or dialysis)

New stages:
3a: 45 - 59
3b: 30 - 44

New Albuminuria Categories:
A1: <30 mg/g
A2: 30-300
A3: >300

Normal Range for GFR is 67-135

Reportable Range for MDRD eGFR

Clin Chem 2013; 59: 462-465.

Newer Chronic Kidney Disease Stages:

Assessed by GFR and Albuminuria

Table 2. Risk Assessment for CVD in CKD

		Urine Albumin/Creatinine Ratio (mg/g)		
CKD Stages	GFR	10-29	30-299	>300
1	90+			
2	89-60			
3A	59-45			
3B	44-30			
4	29-15			
5	< 15			

CVD, cardiovascular disease; CKD, chronic kidney disease; GFR, glomerular filtration rate.
Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group[9]

If eGFR is Here to Stay, What is It's Future?

- **Should all values be reported? I believe Yes.**
- **The use of both eGFR and urine albumin should improve diagnostic usefulness:**
  - **Minimize false positives based on GFR alone.**
- **Having creatinine and cystatin C together is beneficial, but expensive.**
- **Having serial measurements of creatinine or cystatin C or eGFR are beneficial.**

Summary of Conclusions

- Measured GFR is NOT a gold-standard kidney function test:
  - All are very cumbersome tests, and have large population and individual variations.
- Creatinine is still a very good renal function test:
  - More sensitive than often believed.
  - Far more precise than mGFR.
  - Serial measurements have added clinical value.
- Cystatin C:
  - Can be a useful when creatinine/eGFR are equivocal
  - Has clinical value in both AKI and CKD (but not going to replace creatinine)
- New CKD classification system with both eGFR and urine albumin may have improved clinical use.
- Optimal tests for AKI are based on cell injury marker, such as NGAL.
- Optimal tests for CKD = functional marker for glomerular loss: creatinine, cys C, eGFR.

My PhD advisor (Hill Gitelman, MD) once said that collecting an accurate 24 hour urine is one of the great challenges of modern medicine. But I came up with a possible solution…..

However, The IRB Did Not Approve My Method of Ensuring Accurate Collection of Urine

Thank You For Your Attendance and Attention!