Insights Into the Clinical Lab's Most Misunderstood Tests: Creatinine, eGFR, Cystatin C and mGFR

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Disclosures

- Receive consultation fees from Roche Diagnostics, and Werfen (formerly IL)
  - (No products will be mentioned in this talk)

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Learning Objectives

- Describe the advantages and disadvantages of various kidney function tests: Creatinine, eGFR, cystatin C, and measured GFR.
- Identify the classification system for CKD based on GFR and urine albumin.
- Discuss the early change of creatinine in blood in declining kidney function.
- Explain the basic methodology of mGFR by both urine clearance and plasma disappearance methods.
- Examine the large variability of measured GFR methods and that they are not “gold standards” at all.
- Inspect the clinical utility of following within-individual longitudinal (serial) changes in creatinine/eGFR and/or cystatin C, and how this eliminates the need to adjust for race, sex, or age differences.

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Some Big Lies Through History

- The Earth is flat (Homer, Thames, many others)
- I'll call you tomorrow (many men, some women)
- Email will never catch on (Toffaletti, circa 1990)
- You'll go blind if you keep doing that (many mothers)
- It's simply plug and play (software experts)
- We got it all (many surgeons)
- Serum creatinine does not increase until 50% of nephrons are lost (many kidney experts)
- The gold standard for detecting kidney disease is the **measured** GFR (too many)
- We need more equations for eGFR (self-appointed experts)

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Journey to an Alternate Universe:

Historical Development of the Estimated Cardiac Output Equation based on hs-Troponin

- In 1960: Professor Sheistkopf (world expert) declares that cardiac output (CO) is the "gold standard" for detecting cardiac disease.
  - From 1970 to 1995, CK-MB, then later, Troponin, comes along as surrogates to detect myocardial damage.
  - In 2000, self appointed experts develop equation using Troponin to estimate Cardiac Output (eCO).
  - In 2010, these experts develop the CHD eCO equation based on hs-Tn, claiming it is more accurate at estimating CO.
  - eCO = (1/ Tn) x 0.97 x Age x 1.08 *(if female)* x 1.14 *(if Black)*
    - All clinical labs have to modify their reports to use this new eCO equation.
    - Few, if any, studies show *m*CO is clinically superior to serum markers.
  - These experts becomes very famous and own several yachts, homes in Malibu, Swiss Alps, French Riviera, and a high-rise condo in NYC, driving Ferraris, Porsches, and Rolls.

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Image of Nephron: Functional vs tubular damage markers

GFR = glomerular filtration rate

Cys C = cystatin C

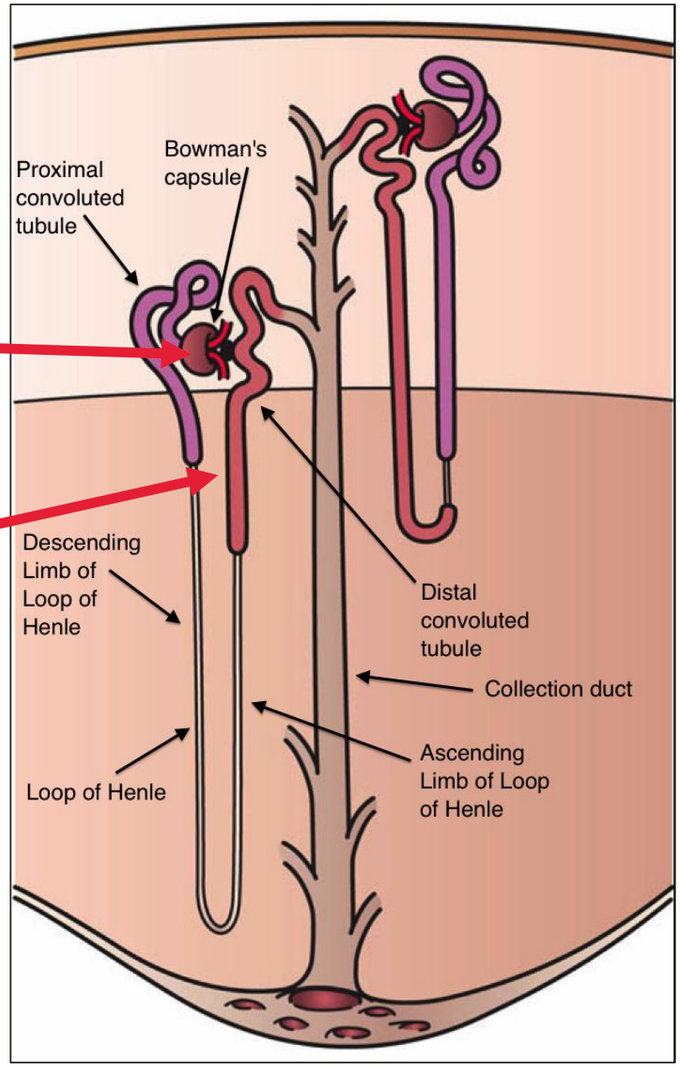
mGFR = measured GFR

NGAL = neutrophil gel lipocalin

Nephrocheck = device for acute kidney injury

Functional Markers of GFR: Creatinine, Cys C, mGFR

Markers of tubular damage: NGAL, KIM-1, α-1-microglob, Nephrocheck



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

- Measured GFR (mGFR) ?
  - Has large physiologic variation and very cumbersome test.
  - However, measured GFR is a well-recognized parameter by physicians, and it has history.
- Serum marker ?
  - Creatinine, cystatin C: these are good.
- Number of lost functioning nephrons ?
  - This is the ultimate test, but we cannot measure this.
- This has led to the eGFR (estimated GFR) calculated from the plasma creatinine and/or cystatin C.

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Why Do We Even Need to Calculate an estimated GFR?

Why not simply measure the GFR?

Answer: It’s because the measured GFR is a &*X$bz@ process!

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Brief History of Developing eGFR Equations for Adults

- 1976: Cockroft and Gault equation predicts GFR from serum creatinine, age, sex, and weight (race not included).
- 1999: The MDRD eGFR equation predicts mGFR from serum creatinine.
- 2009: The CKD-EPI equation is touted as “more accurate” than the MDRD equation for predicting measured GFR.
- Other eGFR equations are developed for various countries: *“It is likely impossible to develop a single accurate ethnicity-specific equation or correction factor that would allow precise eGFRcr reporting in all populations in different countries.” Clin J Am Soc Nephrol. 2021;16(6): 963–965.*
- 2017: Some call attention to bias inherent in eGFR equations that include a factor based on race.
- 2020-21: An NKF and ASN task force examine how the inclusion of race in eGFR equations affects persons = the CKD-EPI (2021) eGFR.

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GFR vs Stage of Chronic Kidney Disease:

Original and Some Recent Changes

CKD Stage	Description	GFR (mL/min/1.73m^2)
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

Clin Chem 2013; 59: 462-465.

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KDIGO 2012 Classification for CKD based on GFR and Albuminuria (mg Alb/g Creat)

Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012

GFR categories (ml/min/ 1.73 m²) Description and range				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Use eGFR as an initial benchmark for further workup

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eGFR Creatinine Equations for Adults

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

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

MDRD Equation (Ann Intern Med 1999;130:461):

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

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

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

The new CKD-EPI 2021 equation (JASN 2021):

$$\text{eGFR} = 142 \times \mathit{min}(S_{Cr}/k,1)^{a} \times \mathit{max}(S_{Cr}/k,1)^{-1.20} \times (0.994)^{\text{Age}} \times (1.012 \text{ if female}) \sout{\times (1.16 \text{ if black})}$$

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What Were Recommendations for JASN 2021 Report?

- Immediately implement the CKD-EPI 2021 creatinine equation without race variable.
- Increase national efforts to facilitate routine and timely use of cystatin C, especially to confirm eGFR in clinical decision making.
- Encourage and fund research to develop new, more accurate eGFR equations based on new endogenous filtration markers.
- Utilize these to eliminate racial and ethnic disparities.

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What has been the continual goal of these eGFR equations from 1999 to 2021?

It has been to improve the agreement of eGFR results to measured GFR results.

What did that “improved accuracy” of the CKD-EPI 2009 equation look like?

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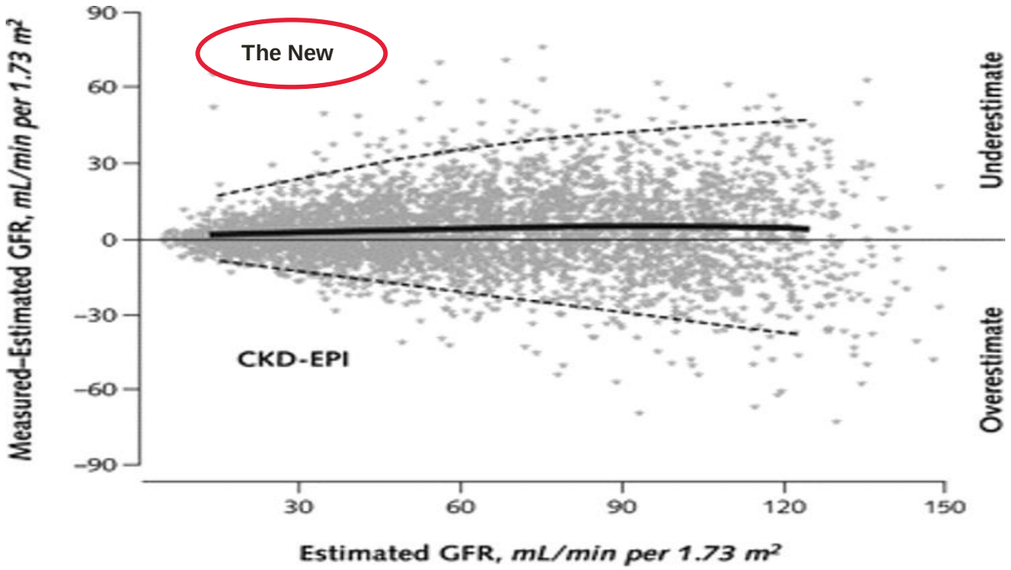
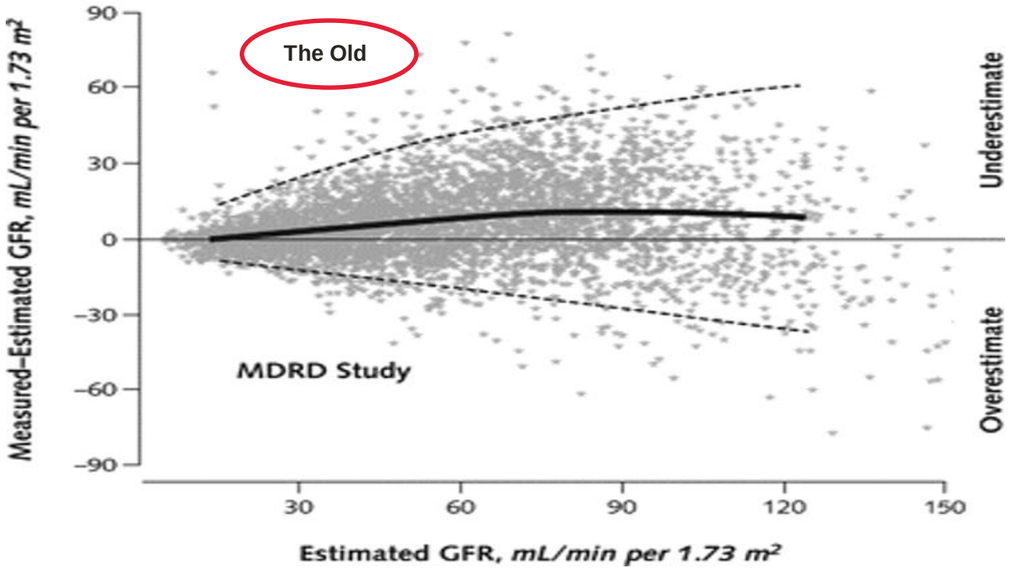


84.1% agree within 30%

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

A ±30% error had to be accepted



80.6% agree within 30%

Levey AS, Stevens LA, Coresh J, et al.
Ann Intern Med 2009;150:604-612

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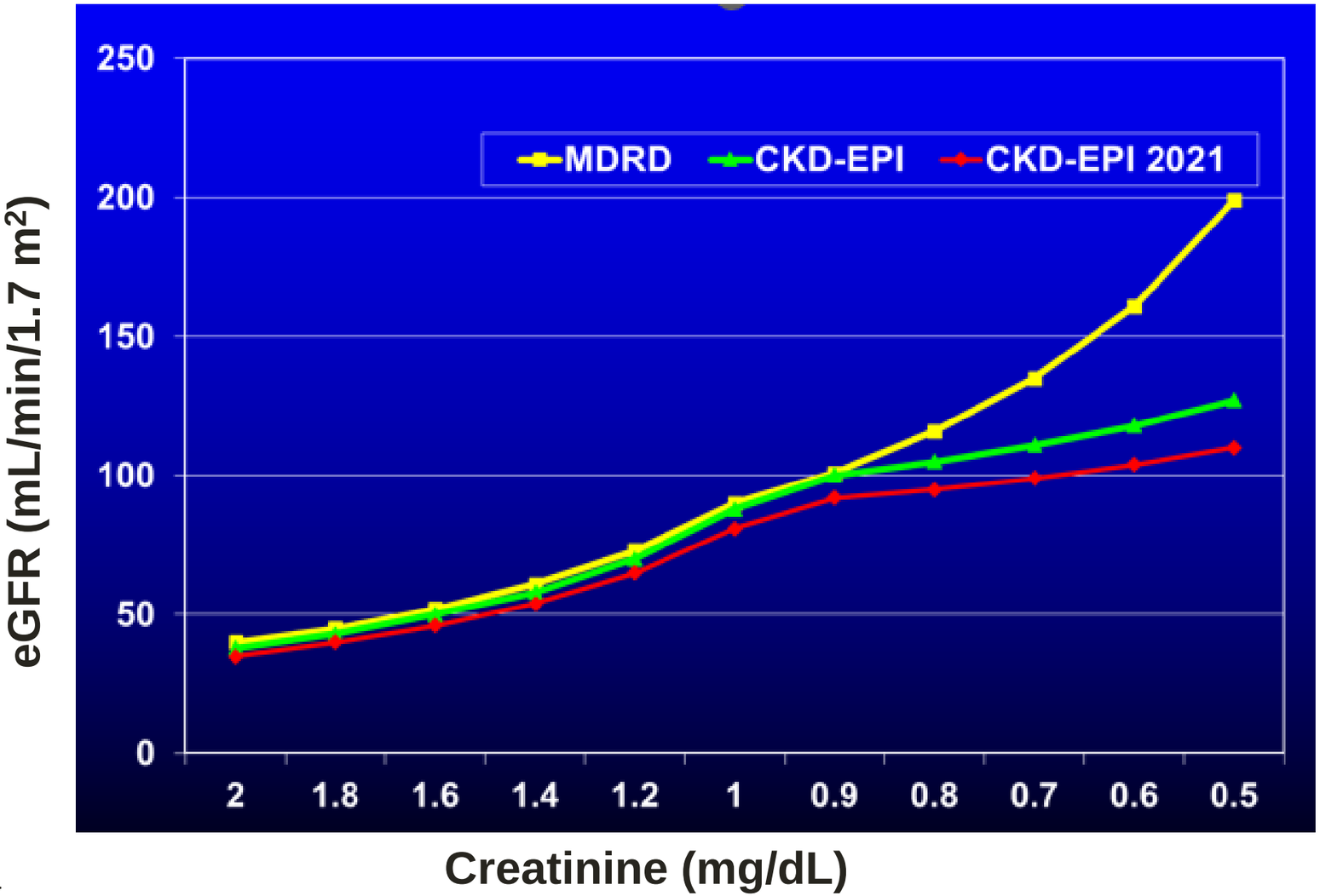
Plots of MDRD, CKD-EPI, and 2021 eGFRs vs Creatinine: 70 yr old black male



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Do ANY Equations Provide an eGFR (from Creatinine) that Accurately Predicts mGFR?

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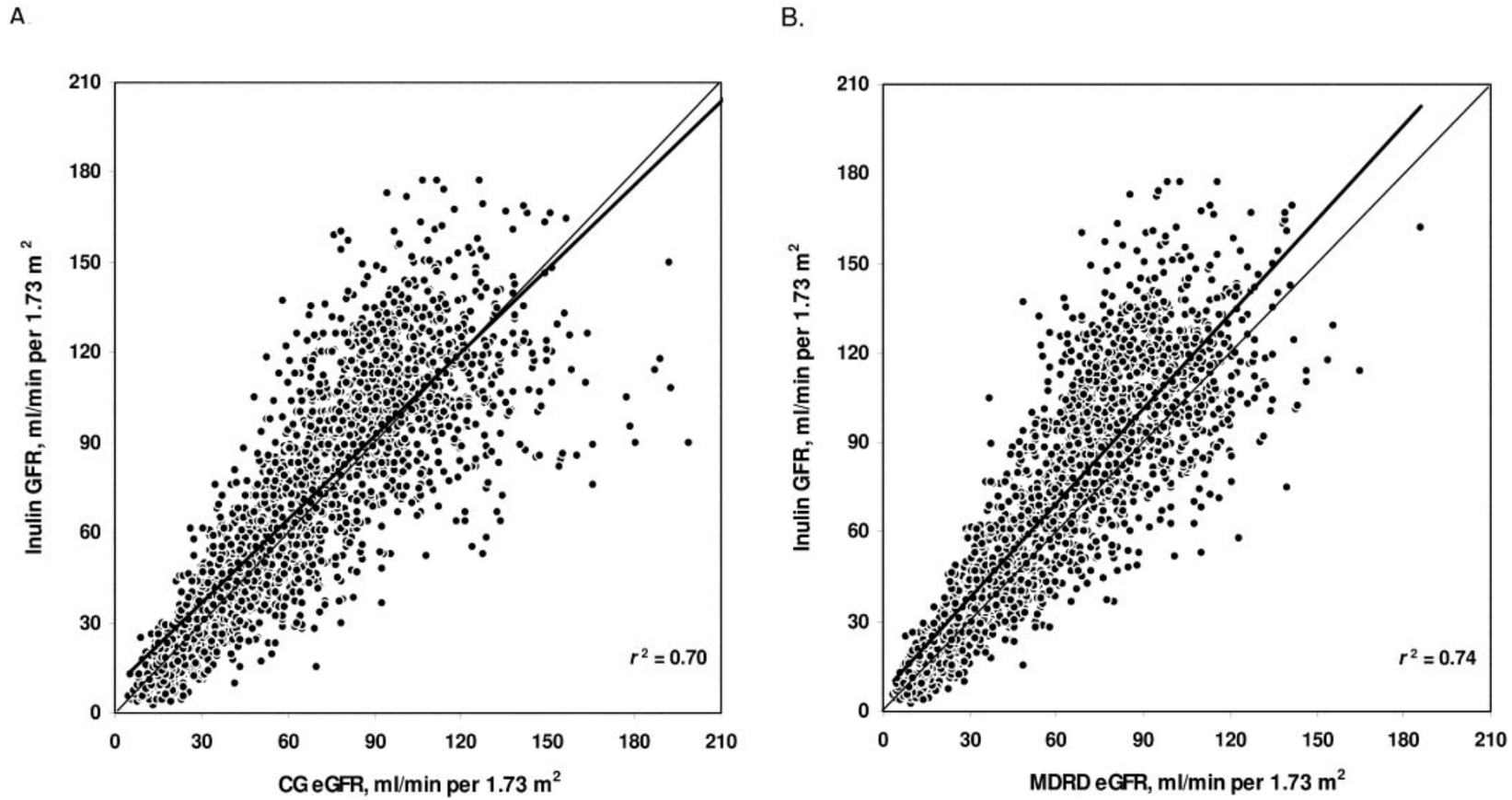
Plots of Inulin mGFR vs C-G eGFR and MDRD eGFR



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

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Questions:

So, is the creatinine, cysC, or eGFR the culprit…

or is the measured GFR the culprit?

Is Measured GFR* Really a “Gold-Standard”?

*By Inulin, Iothalamate, Iohexol, Creatinine, Cr-EDTA, etc.

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Is the eGFR Useful? YES!

- Physicians are tuned in to understanding what a GFR number means.
- It is useful as a general guide for evaluating kidney function in a patient.
  - *Especially for initial evaluations*
- Compared to mGFR, it is much more convenient, less invasive, and less expensive.
  - Remember, the eGFR is really a creatinine or cystatin C that has been numerically changed to look like a mGFR.

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Methods for Measuring GFR:

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

- Inject or infuse inulin, iothalamate, iohexol, ^{51}Cr-EDTA, etc) or use endogenous marker (creatinine).
- Collect multiple blood samples and sometimes also collect accurately-timed urine samples.
- GFR calculated by various methods:
  - Urine “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, invasive, expensive, imprecise, and accurate ??

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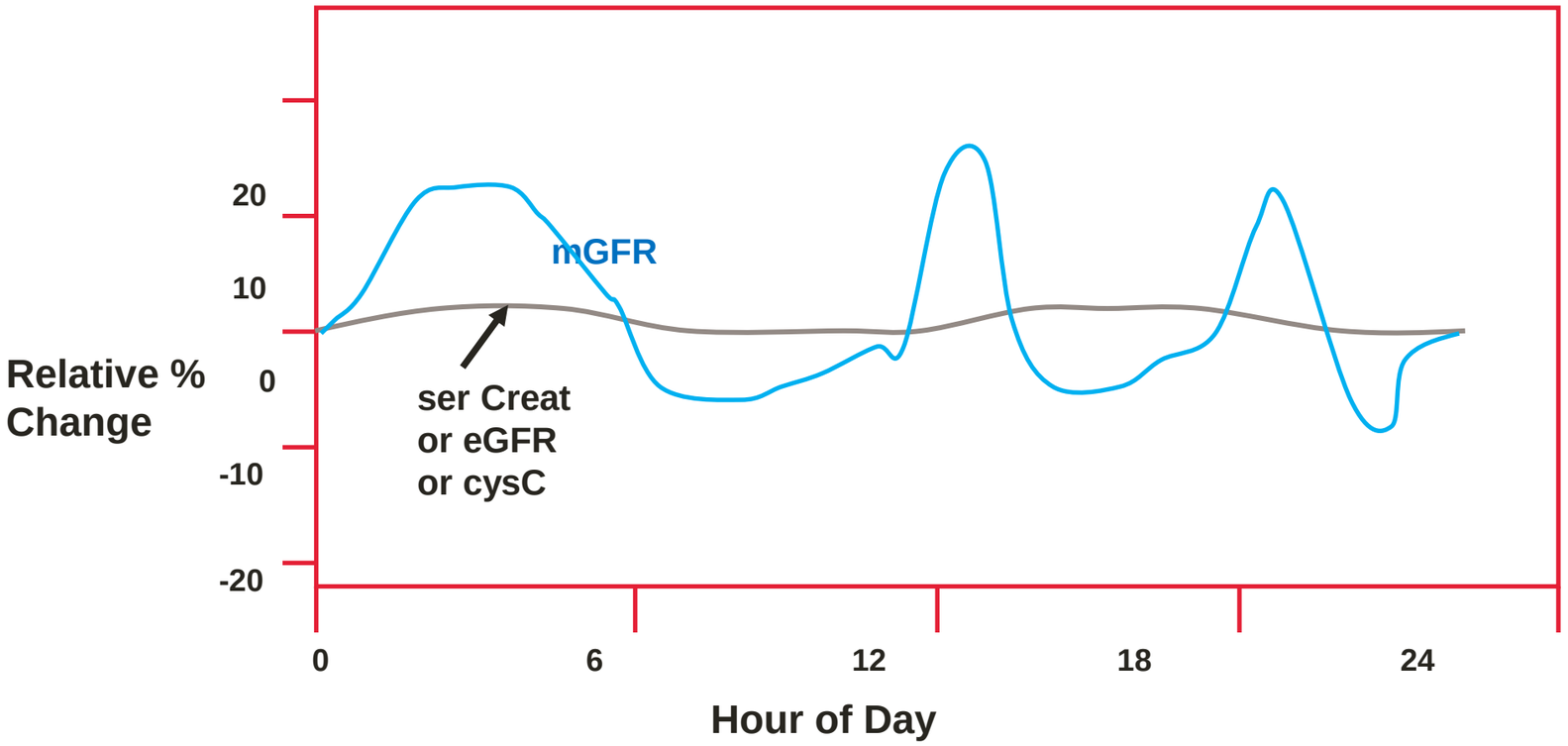
My Conclusion: Measured GFR and Serum Creatinine/eGFR Have Inherently Different Regulation Patterns



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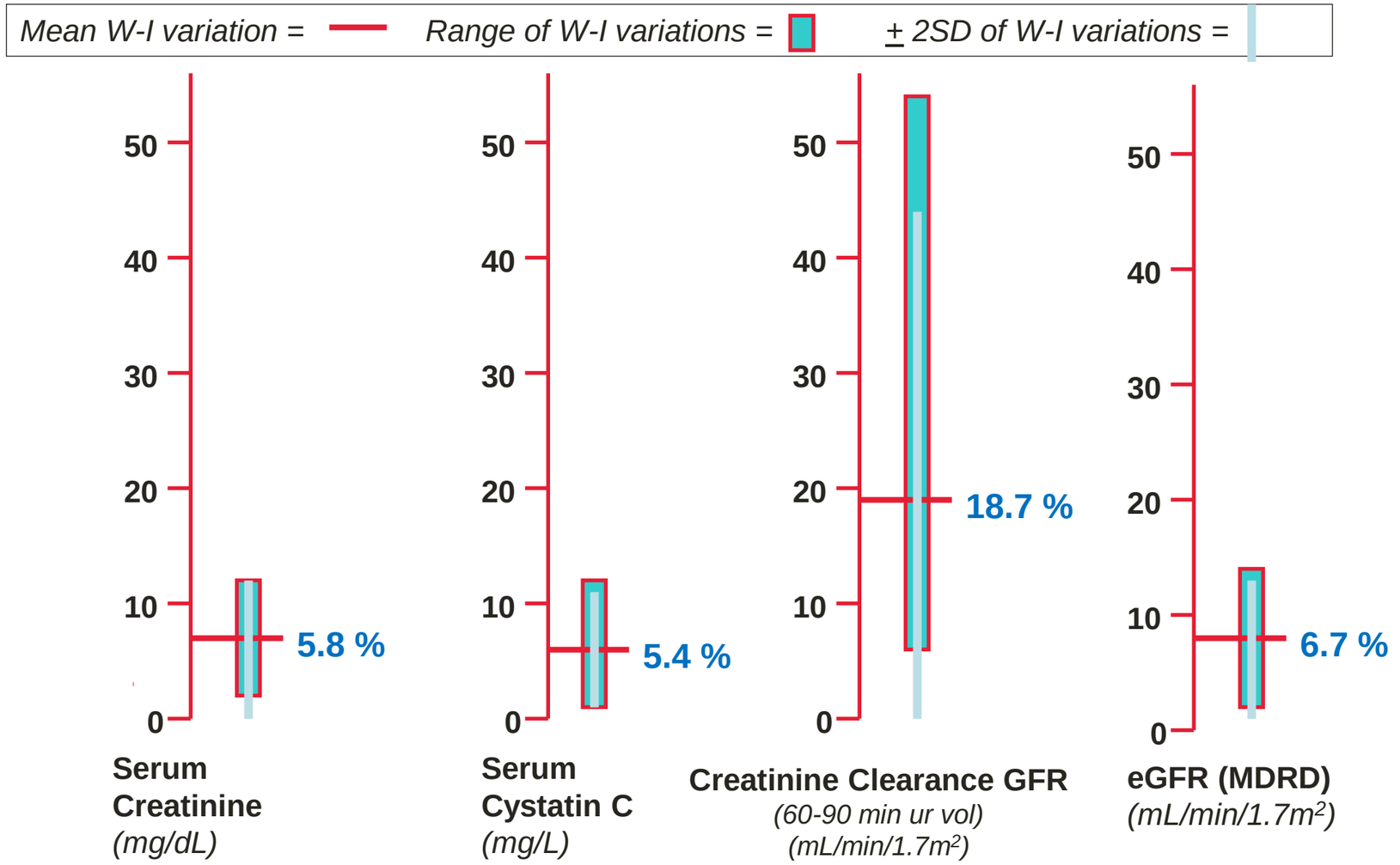
Within-Individual Variation (%CV) of Renal Function Tests on 31 Healthy Persons *(Clin Chim Acta 2008; 395: 115-9)*



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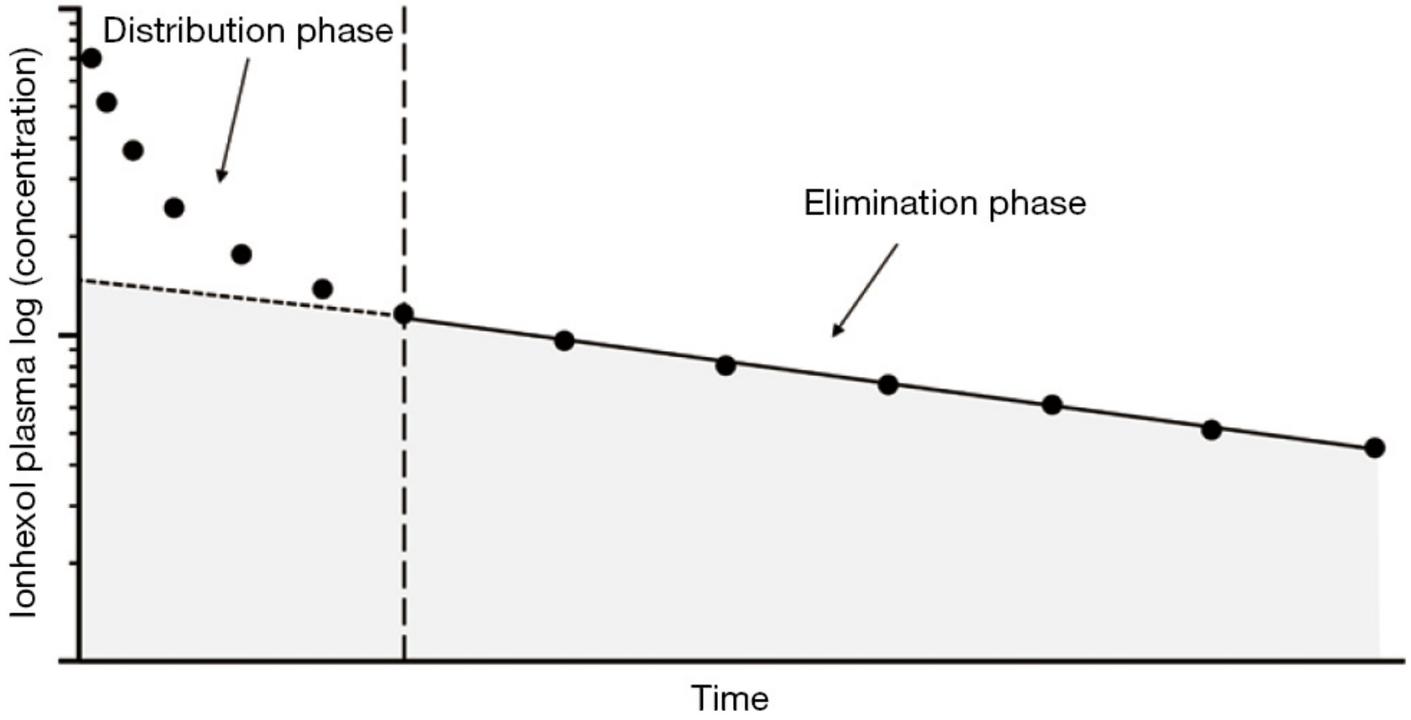
Interpreting the Iohexol Clearance Test for mGFR



- Inject 5–10 mL of iohexol solution.
- Collect multiple blood samples to measure plasma iohexol disappearance.
- The early phase reflects the distribution between the intra- and extra-vascular volumes.
- The later phase corresponds to elimination by the kidney.

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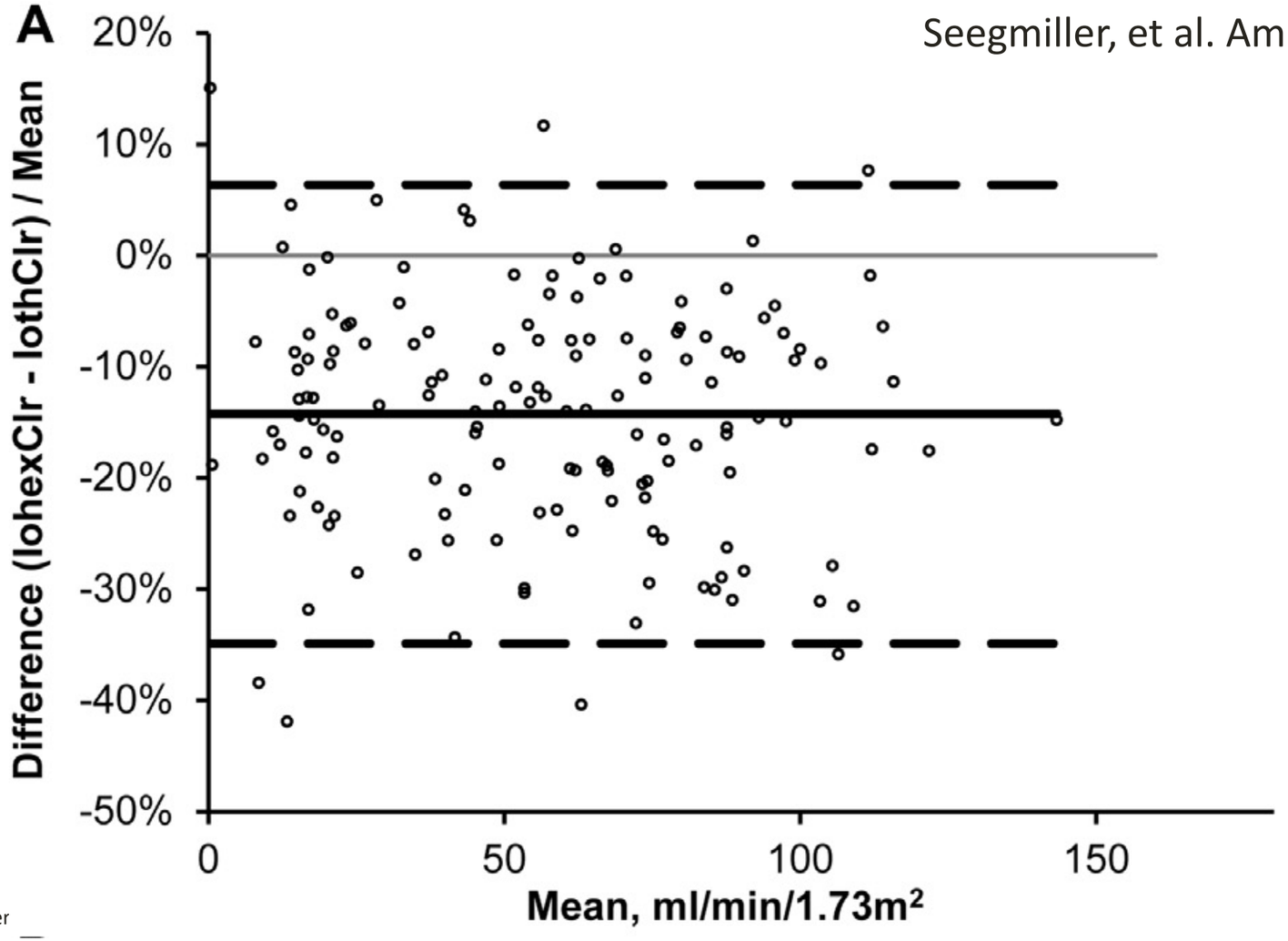
Differences Between Iohexol mGFR and Iothalamate mGFR



Seegmiller, et al. Am J Kidney Dis 2016; 67: 49-55

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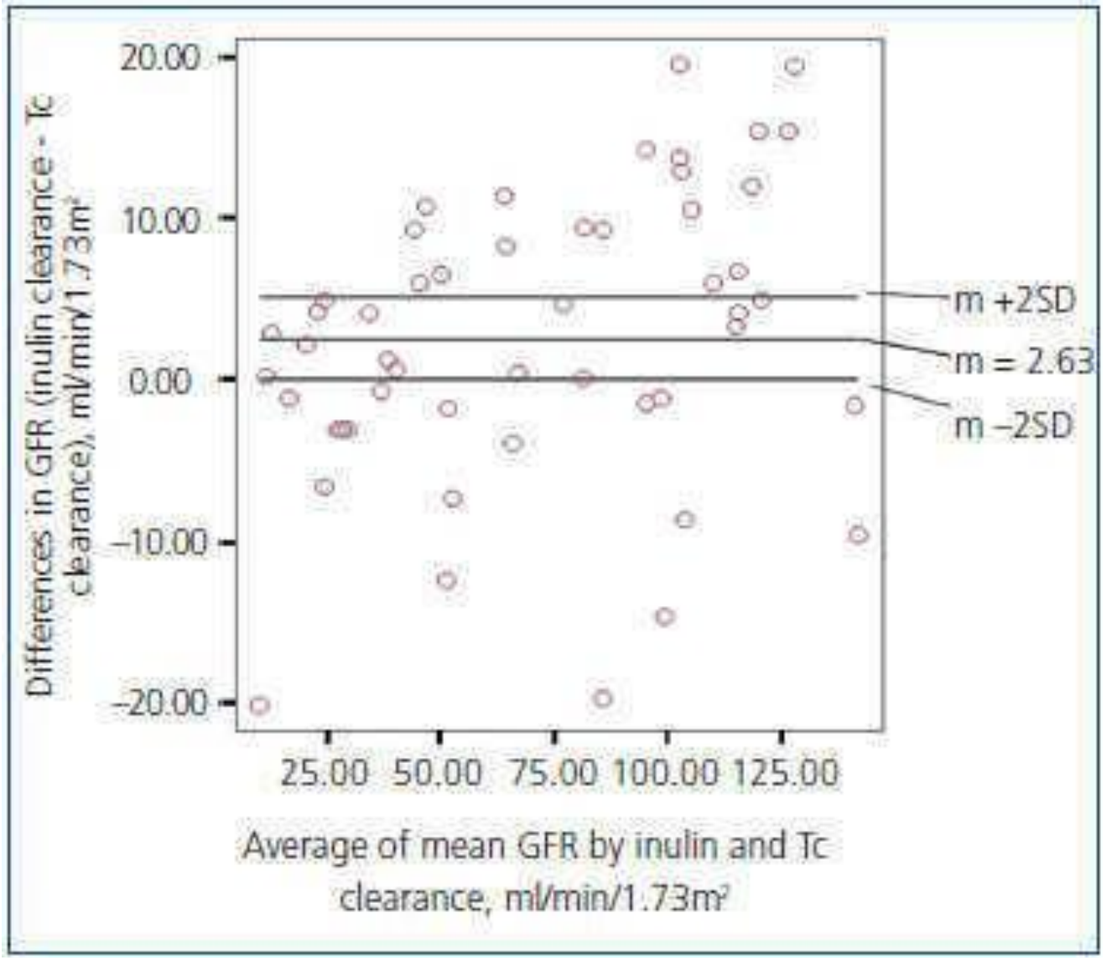
Discordance Between GFR Measured by Inulin Clearance and Technetium Clearance



Nephrologia 2010; 30(3): 324-30.

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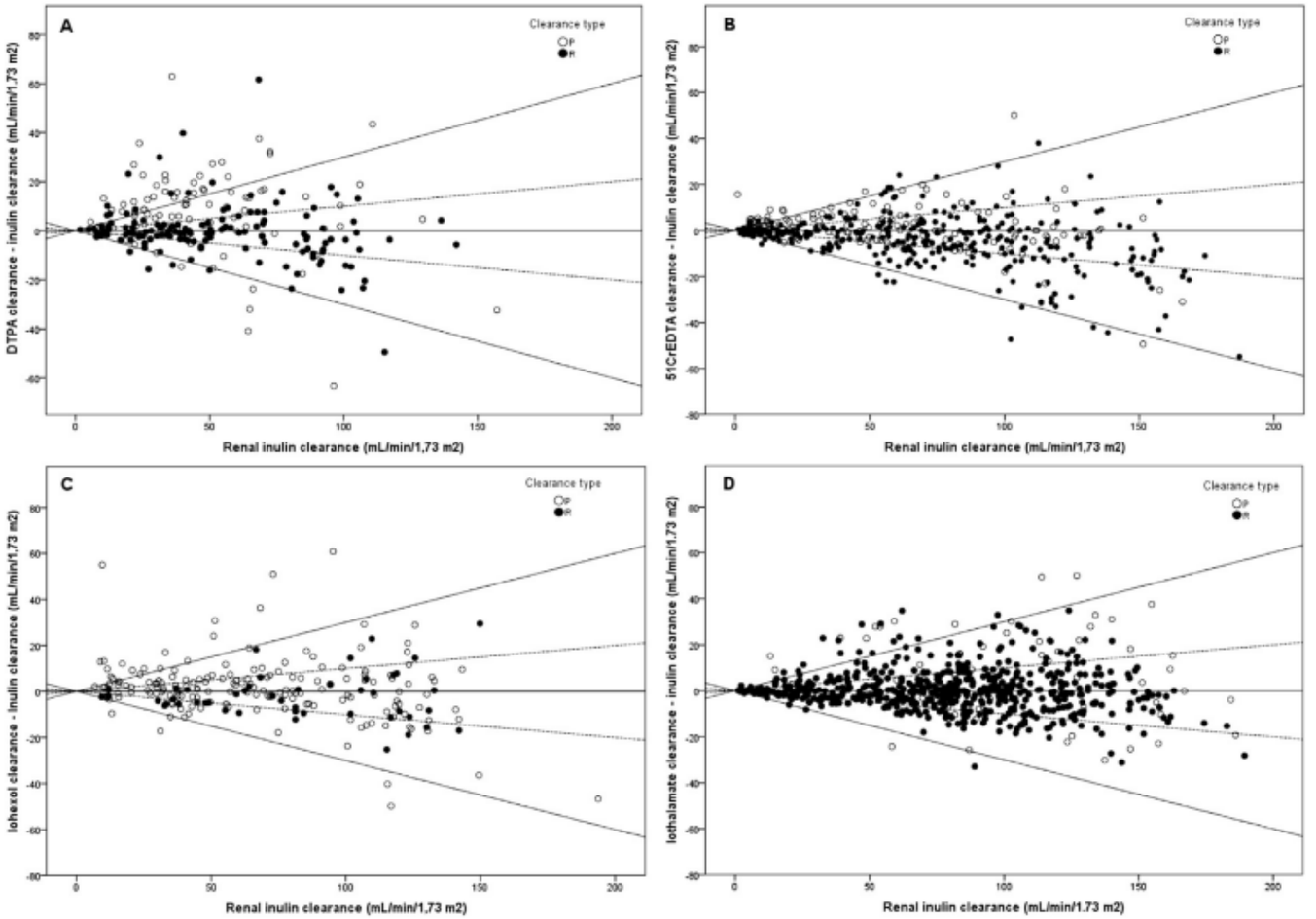
Soveri I, et al. Measuring GFR: A Systematic Review.



Am J Kidney Dis. 2014;64(3):411-424

Figure 2. Differences reported in various studies between **measured glomerular filtration rates** (mGFR) by (A) DTPA, (B) ^{51}Cr-EDTA), (C) Iohexol, or (D) Iothalamate in relation to renal inulin clearance (P, plasma clearance, R, renal clearance). The proportion of errors that did not exceed 30% (P_{30}) limits (solid lines) and P_{10} limits (dashed lines) are shown.

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We Must Correct the Perception that Serum Creatinine Does not Increase Until 50% of Nephrons are Lost

- This originates from an often-referenced 1985 study by Shemesh et al. concluding that:
  - Large decreases in mGFR are associated with insignificant changes in sCr in early kidney impairment.
  - Unfortunately, this has been widely cited as fact, even in the CKD-EPI 2021 study.
- While the Shemesh study was an excellent work on creatinine handling by kidney tubules, their clinical conclusion was based more on physiology than clinical study.
  - Their Table 3 shows serum creatinine increased from 1.4 to 2.3 mg/dL as inulin clearance mGFR decreased from 61 to 32 mL/min/1.73 m^2 in glomerulopathic patients.

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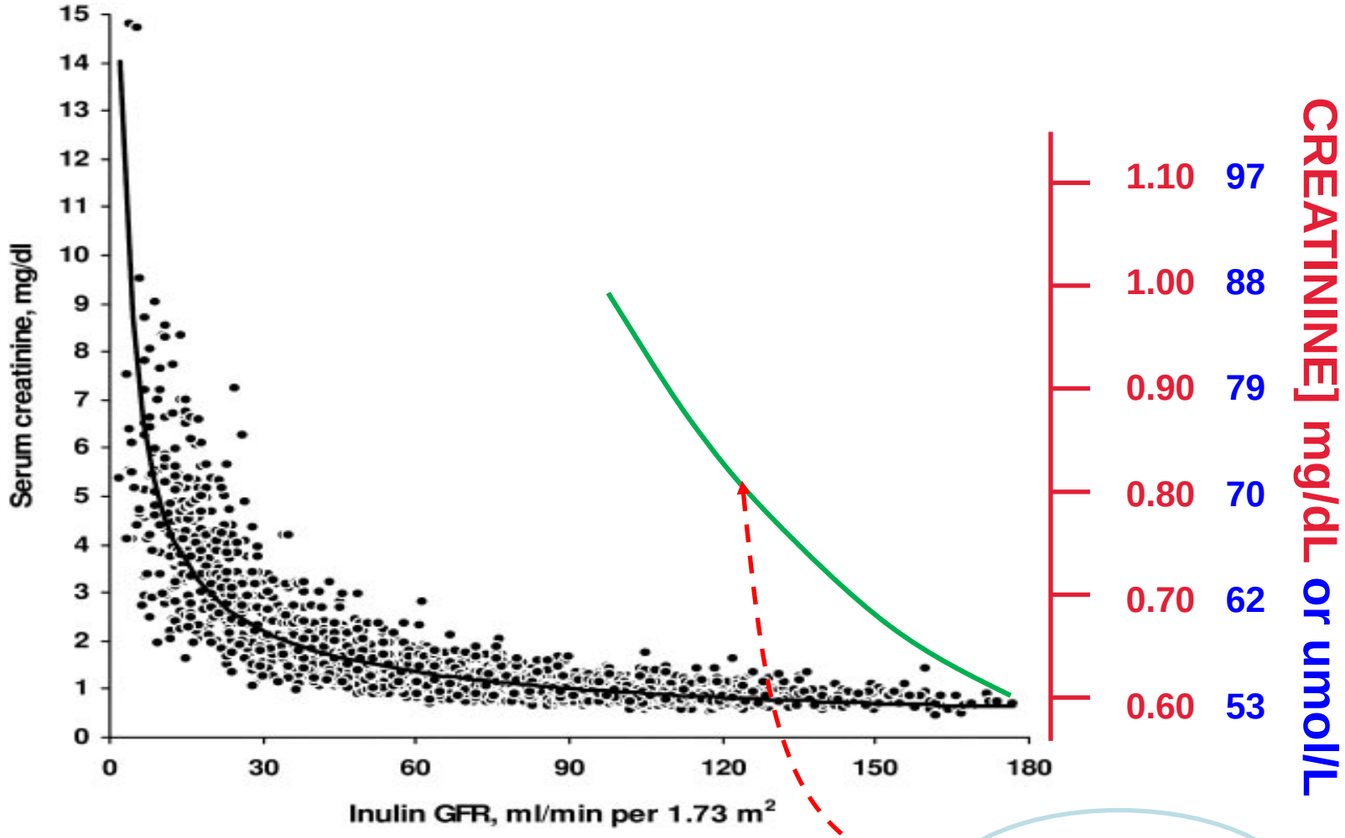
A Misleading Plot of Serum Creatinine vs GFR by Inulin Clearance



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

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When Is Cystatin C Most Useful?

- Useful in evaluating CKD when $eGFR_{Creat}$ is 45-59 mL/min/1.73m^2 w/o albuminuria (CKD Stage 3a).
- When serum creatinine is less reliable:
  - Extremes of muscle mass
  - Pediatrics
  - Racial differences
  - Elderly
  - Diet

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When Might Measured GFR (mGFR) be Most Useful?

- When serum creatinine and cystatin C disagree substantially.
- When $eGFR_{Creat}$ is 45-59 mL/min/1.73m^2 w/o albuminuria (CKD Stage 3a).
- As a confirmatory test before significant medical decisions are made related to kidney dysfunction.

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Here are several studies that demonstrate how Creatinine, eGFR, CysC, etc. should be evaluated.

These studies evaluate their clinical utility, and …

they clearly show that serum creatinine does change in the early stages of nephron loss.

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Onuigbo and Agbasi concluded that within-individual trajectories of patients' serum creatinine are a most useful diagnostic tool for managing patients

- They illustrated this with cases from a variety of patients with acute kidney injury and/or CKD.
- Seemingly small changes in serum creatinine allowed earlier identification of patients with milder kidney impairment.
- Following within-individual changes are independent of variables such as age, sex, race, or nationality.

Diabetic nephropathy and CKD – Analysis of individual patient serum creatinine trajectories: A forgotten diagnostic methodology. J Clin Med 2015;4:1348–68.

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Bhavsar et al. followed over 800 African Americans with hypertensive CKD for a mean of 103 months

- They compared mGFR, sCr, eGFR, sCysC, and β-trace protein (BTP) concentrations to predict end-stage renal disease (ESRD).
- For 246 participants who developed ESRD during follow-up, higher concentrations of each marker were strongly and significantly associated with higher risk of ESRD.

Bhavsar NA, et al. Am J Kidney Dis 2011;58:886–93

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Kim et al. evaluated over 1300 patients in stages 3 to 5 CKD (eGFR <60 mL/min/1.73 m^2)

- 134 of the 1300 patients progressed to ESRD, requiring either dialysis or kidney transplant.
- They concluded that slope of eGFR calculated from either sCr or sCysC were equivalent in predicting which patients in CKD stages 3 to 5 progressed to ESRD.

Creatinine-and cystatin C-based estimated glomerular filtration rate slopes for the prediction of kidney outcome: a comparative retrospective study. BMC Nephrol 2019;20:214.

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Spanaus et al. followed changes of sCr, cysC, BTP, and mGFR by iohexol in 177 patients during progression of CKD

- Patients were studied for periods of 3 to 84 months.
- All 3 markers increased progressively with decreasing mGFR:
  - Their diagnostic performance for detecting even minor decreases in kidney function were similar, with BTP slightly better.
- Creatinine, even within the reference interval, increased in the early stages of declining kidney function as detected by iohexol mGFR.
- Changes of each serum biomarker, including sCr, strongly correlated with mGFR, and that each was useful for diagnosing early changes in kidney function as mGFR decreased from > 120 to < 60 mL/min/1.73 m^2.
  - *Clin Chem 2010;56:740–9.*

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Figure from Spanaus study showing creatinine, CysC, and b-trace protein vs Iohexol mGFR

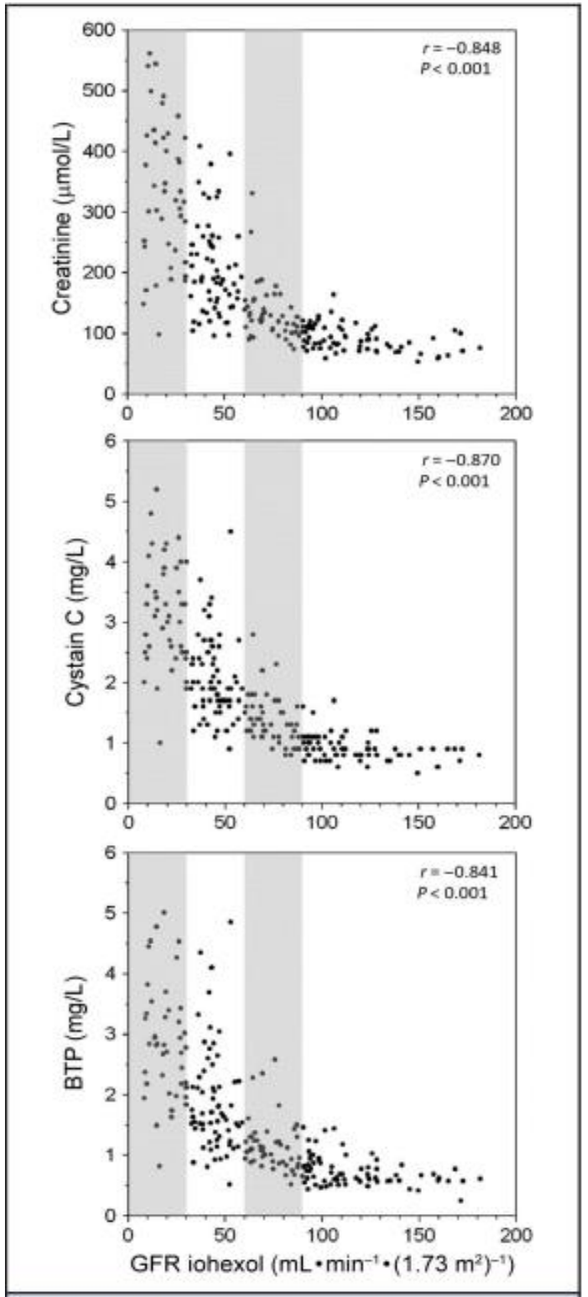

Fig. 1. Relationship between serum concentrations of cystatin C, BTP, creatinine, and GFR measured by iohexol clearance in 227 patients with primary CKD at baseline.

Clin Chem 2010; 56(5): 740-9

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Study of Imprecision of Iothalamate mGFR

- Evaluated data from 1995 participants in the MDRD and AASK studies with at least 2 baseline iothalamate mGFRs.
  - mGFRs averaged 62 days apart.
- Found that mGFRs had substantial variability across visits:
  - 8% varied by > 30%; 4% varied by 25 – 30%; 87% varied by $\leq$ 25 %.
  - They defined “consistent results” between visits as $\leq$ 25% difference.
- While they did show the variation of mGFRs between these 2 visits …

Kwong D, Stevens LA, … Levey AS, Coresh J. Imprecision of urinary iothalamate clearance as a gold-standard mGFR decreases the diagnostic accuracy of kidney function estimating equations. Am J Kid Dis 2010; 56: 39-49.

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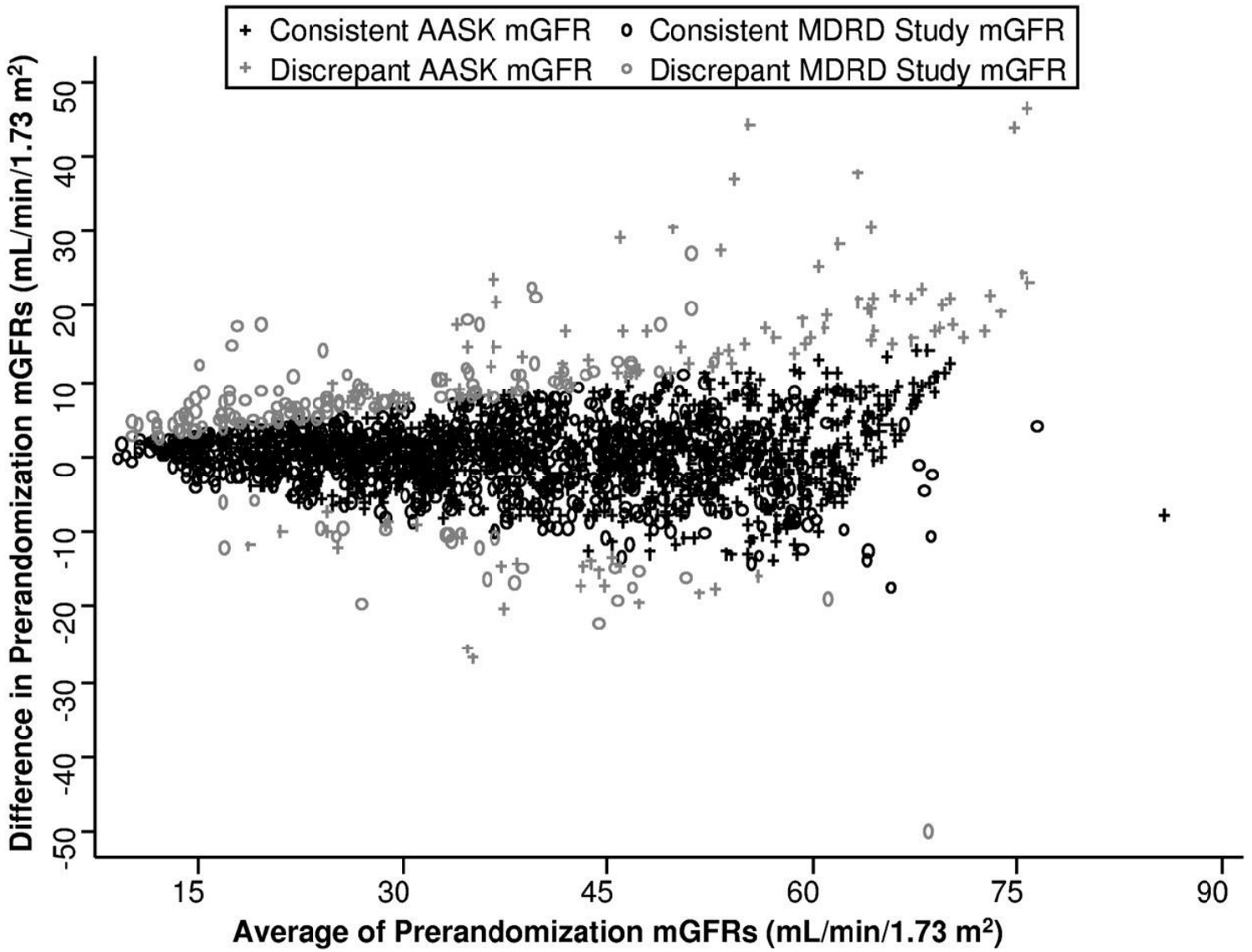
Differences Between the 2 Baseline mGFRs



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Study of Imprecision of Iothalamate mGFR

- … they concluded that:
  - "… the variation in iothalalamate mGFR… substantially impacts the accuracy of these eGFRs."
- Unfortunately, they also concluded that:
  - "Nevertheless, this gold-standard mGFR performed better than eGFRs" (MDRD $_{Cr}$, CKD-EPI $_{CysC}$, and CKD-EPI $_{Cr\text{-}CysC}$ equations) *in predicting itself!*

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I've complained a lot, so let's look at… Future Needs for sCr and eGFR

- A national effort to encourage developing baseline creatinine, eGFR and/or cystatin C on all appropriate persons.
- Improve precision of serum creatinine methods:
  - Requires improved methods; this is challenging!
    - Enzymatic creatinine methods are typically better.
  - Note: IDMS standardization improves long-term method stability but has little impact on method precision.

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More Future Needs for $_{ser}$Creatinine and eGFR

- Emphasize value of serial measurements:
  - Within-individual changes of creatinine and/or eGFR eliminate variables of race, sex, nationality, and age.
- Develop specific guidelines for interpreting within-individual changes in serum creatinine, eGFR, and/or serum CysC that warrant referral to nephrology:
  - An increase of 0.20 mg/dL (18 umol/L)?
  - An increase of 0.30 mg/dL (27 umol/L)?
  - An increase of 20% or 30%?
    - Shavit, et al noted each 0.20 mg/dL increase was associated with an increased mortality (Kidney Blood Pressure Res 2012)
  - An appropriate time interval for changes: 3, 6, or 12 months?
    - These are similar to recommendations in AKI.

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Summary of Points

- Creatinine is a good renal function test:
  - More precise than mGFR and increases early in disease.
- The eGFR is useful in calling attention to possible kidney disease.
- Measured GFR is not a “gold-standard” kidney function test:
  - Cumbersome, lengthy, invasive, and expensive, and has large population and individual variations.
- Cystatin C can be useful clinically:
  - When creatinine/eGFR are equivocal, but not going to replace creatinine measurements anytime soon.
- For both eGFR and creatinine measurements, the use of serial or longitudinal within-individual measurements needs to be encouraged.
  - This has been done for years to confirm AKI.
- **No more eGFR equations, *please!***

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Thank you

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2019 report showing why we do not need more eGFR equations: “Validation of a metabolite panel for a more accurate estimation of GFR using LC-MS/MS”

- Concluded that an equation based on 4 metabolites more accurately estimated mGFR.
- Metabolites were:
  - N-acetylthreonine, pseudouridine, phenylacetyglutamine, tryptophan
- Relative accuracy within $\pm$ 30% $(1 - P_{30})$ compared to mGFR:
  - $eGFR_{Creat}$: 87%
  - $eGFR_{cysC}$: 88%
  - 4 metabolite panel : 90%
  - $eGFR_{Cr\text{-}cysC}$: 91%

Freed TA, Coresh J, Inker LA, …Levey AS. Clin Chem 2019; 65(3): 406-418.

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Reference Intervals (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.

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