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Rethinking CKD Evaluation: Should We Be Quantifying Basal or Stimulated GFR to Maximize Precision and Sensitivity?

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Abstract

Chronic kidney disease (CKD) remains an increasing clinical problem. Although clinical risk factors and biomarkers for development and progression of CKD have been identified, there is no commercial surveillance technology to definitively diagnose and quantify the severity and progressive loss of glomerular filtration rate (GFR) in CKD. This has limited the study of potential therapies to late stages of CKD when FDA-registerable events are more likely. Since patient outcomes, including the rate of CKD progression, correlate with disease severity, and effective therapy may require early intervention, being able to diagnose and stratify patients by their level of decreased kidney function early on is key for translational progress. In addition, renal reserve, defined as the increase in GFR following stimulation, may improve the quantification of GFR based solely on basal levels. Various groups are developing and characterizing optical measurement techniques utilizing new minimally invasive or non-invasive approaches for quantifying basal and stimulated kidney function. This development has the potential to allow widespread individualization of therapy at an earlier disease stage. Therefore, the purposes of this review are to suggest why quantifying stimulated GFR, by activating renal reserve, may be advantageous in patients and review fluorescent technologies to deliver patient-specific GFR.

Keywords

Renal Reserve; glomerular filtration rate (GFR); estimated GFR; measured GFR; Hyperfiltration; Fluorescent GFR Determinations; Diabetic Nephropathy; Plasma Volume; One-compartment GFR model; 2-Compartment GFR Model; Chronic Kidney Disease (CKD); therapeutic success; kidney disease progression; renal function; filtration marker; surrogate marker; serum creatinine; early detection; FDA registration; review

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The Clinical Problem

Chronic kidney disease (CKD) remains an untreatable and progressive disease process affecting up to 10% of the US population and its prevalence is increasing.[1] The growth has primarily been in patients with CKD stage 3 (glomerular filtration rate [GFR], 30–59 ml/min/1.73m^2) and stage 4 (GFR, 15–29 ml/min/1.73m^2). Using estimated GFR (eGFR) definitions in population studies, patients classified as having CKD have an increased risk of cardiovascular events, progression, dialysis, and death.[2] This important clinical information, including the overall rate of CKD, has been challenged on the individual patient level because the estimating equations have wide dispersion (±30% around the mean with 10% outliers), and thus may under- or overestimate kidney function in a large number of patients.[3]

Of major clinical importance is the advancement of therapeutic options to prevent and or minimize progression and the long-term consequences of CKD. Agents effective against many different plausible preclinical therapeutic targets have been developed and advanced into clinical trials for CKD. Unfortunately, these clinical trials have not been successful and what was once seen as an attractive large unmet need by pharmaceutical and biotech companies is now being viewed with skepticism. Why the failure to translate highly effective preclinical therapies into effective clinical trials? This has often been attributed to inappropriate preclinical models[4–6], and, in response, new more appropriate models are being developed. The multifocal pathophysiologic nature of CKD and the lack of human tissue to interrogate and compare to animal models at structural and molecular levels, as is being successfully accomplished in glomerular diseases[7] and transplantation, are also barriers to success. Therefore, individualization of therapy in CKD is not presently possible and likely won't be in the near term.

However, we must ask if we have developed the clinical techniques and approaches to quantify progression and the response to therapy. Since a reduction in GFR is the most appropriate parameter of progression and therapeutic success, only when we are able to achieve this will we be able to classify biomarkers of early and progressive disease and quantify US Food and Drug Administration (FDA) GFR therapeutic registration end points in clinical trials.

Understanding and Quantifiying GFR as a Measure of Kidney Function

Serum Creatinine as a Surrogate Marker of GFR

Glomerular filtration rate, which measures the quantity of plasma filtered through glomeruli within a particular period, is a physiologic process and as such a direct indicator of global kidney function. It became the cornerstone of nephrology beginning with Homer Smith and in the distant past was actively measured in clinical studies.[8] Recent reviews have identified chromium 51 (^{51}Cr)–EDTA, iothalamate, or iohexol as reasonably accurate methods for measuring GFR, if inulin clearance is not possible, and have delineated some of their limitations.[9–11] Unfortunately, these techniques and the adaptations that followed remain cumbersome and expensive and require a prolonged time for sample processing, and thus are not clinically practical. Therefore, physicians base diagnostic and therapeutic decisions in

millions of patients daily primarily using a surrogate marker of GFR, the patient's serum creatinine (Scr) concentration.[12–14]

The advantages and limitations of Scr as a marker in CKD have been recently reviewed by Levey et.al.[15] While it is well known that reductions in the GFR secondary to chronic kidney injury are accompanied by increases in Scr, the insensitivity of this surrogate marker as an estimate of GFR, especially in GFRs above 60 ml/min/1.73 m2, is often underappreciated.[16–18] One of the reasons for this is illustrated in Figure 1A, a plot of measured GFR (mGFR) versus Scr. The graph is divided into insensitive, sensitive, and highly sensitive regions for Scr as an estimate of GFR, based on the slope of the curve. In higher GFR regions it takes a very large change in GFR to result in even a small quantifiable change in Scr. To illustrate this point, consider what a change of 0.3 mg/dl of Scr means for an individual patient, calculated using the CKD-EPI (CKD Epidemiology Collaboration) creatinine equation for eGFR for the ideal 60-year-old white man and woman as is shown in Figure 1B. I use 0.3 mg/dl as most would consider this a significant change in Scr. One can see the amount of GFR loss at several levels of initial Scr for this change. In the insensitive region of the GFR-Scr curve (Scr, 0.8 mg/dL) a change of 0.3 represents a loss of over 33 ml/min/1.73 m2 of baseline GFR for the woman and 24 mL/min/1.73 m2 for the man. In the sensitive portion of the curve (Scr, 2.0 mg/dl) an increase of 0.3 Scr represents a loss of 4 and 5 ml/min/1.73 m2, respectively, whereas in the highly sensitive portion (Scr, 4.0 mg/dL) it represents a 1.0–ml/min/1.73 m2 change in GFR. Therefore, one can see why Scr or eGFR provides variable sensitivity as a measure of GFR change over the GFR spectrum. Functionally this may explain the increased incidence of acute kidney injury in CKD patients, because the amount of GFR lost can be more readily quantified when the slope of the Scr-GFR relationship is steeper. Is this in part why CKD patients are more prone to acute kidney injury? I think so.

The discrepancy between what a linear increase in Scr means at different starting levels of Scr brings up another important point, especially when one considers that therapeutic trials in CKD are undertaken in patients with moderate to advanced CKD. Clinical trialists have shifted the study population to those having a large change in Scr for a small change in GFR to enhance their ability to detect the signal. Thus, patients with CKD stage 3b or 4 are studied rather than patients starting with a more normal GFR, who would have to have a far greater loss of GFR the loss in kidney function to be detectable due to the insensitivity of Scr. Can we trust Scr or eGFR to deliver on this end point in CKD patients with mild reductions in GFR or adequate renal reserve? Can we trust the therapeutic agent will be effective in late-stage CKD given the fibrosis, endothelial and tubular epithelial changes that have already occurred?

Other factors limit the utility of Scr in patients with CKD, and these limitations have been reviewed.[15,19] Therefore, formulas derived from large population studies have been created to account for patient weight, age, sex and race. However, these formulas face challenges when applied to the individual patient[20–25] even in situations where Scr is stable. In a recent review it was stated that "In usual practice, an eGFR equation is defined as having sufficient accuracy when at least 75% of the estimates fall within ±30% of the measured GFR."[26,p2066].

Renal Reserve

Basal GFR may not be an adequate marker of kidney function because it does not take into account renal reserve. Renal reserve is defined as the increase in GFR above basal fasting values that is activated by stress, an oral protein load, or amino acid, dopamine or glucagon infusion[27,28] as shown in Figure 1C. The increase in GFR is accompanied by a reduction in renal vascular resistance and a congruent increase in renal plasma flow such that the filtration fraction remains constant. Renal reserve can be stimulated using several approaches. Initial studies were accomplished using animal and vegetable proteins, with animal proteins being more stimulating. Response was found to be proportional to the amount of meat given, with maximal stimulation occurring approximately 150 minutes after ingestion.[27,29] Other approaches used amino acid infusion, and a mixture of amino acids was marketed for this purpose (Freamine III, Baxter).[30] Infusions of dopamine or glucagon have been also used.[27,31,32]

Renal reserve is thought to be proportionally lost as basal GFR declines, but very little is known about renal reserve in CKD or in aging patients. Several studies have shown no predictable effect on renal reserve in CKD patients over a wide range of GFRs.[29,33,34] That is, renal reserve, as a percentage of basal GFR, could be high in patients with a low basal GFR or low in patients with a high baseline GFR. Therefore, with a basal measurement of GFR, we do not really know the true potential or maximal stimulated GFR, and we do not know if renal reserve is being used up to offset measurable reductions in basal GFR as maximal GFR diminishes. Thus, the difference between a patient with progressive decline in GFR and a patient with a stable GFR may be that the stable patient has renal reserve that is being depleted and thus basal GFR does not change.

Glomerular filtration varies throughout the day primarily due to meals that result in GFR increases (figure 1C). Therefore, a fasting serum creatinine and a 24-hour urine for creatinine clearance are integrated results of these variations. The percentage change in GFR after a meal depends on the amount of protein ingested and the patient's available renal reserve. For instance, in a hyperfiltering diabetic pateint or a patient with advanced CKD lacking renal reserve, the change in GFR after a protein-rich meal may be very small as a percentage of baseline GFR.[32,35] In addition, a mGFR done while the patient is fasting underestimates the mean 24-hour GFR, while an mGFR obtained following a protein meal overestimates the 24-hour average daily GFR.

Chronic hyperfiltration occurs early in some diabetic patients and may portend a higher risk for progression of CKD.[34,36] This results in high filtration rates and can last for a prolonged period prior to a quantifiable decline in basal GFR, figure 2.

Renal reserve has also been shown by many investigators to be chronically activated in critical care situations where 24-hour creatinine clearances, as a measure of GFR, have been increased to mean levels of 170 ml/min for many days in patients on ventilators, and in patients who have received vasopressors for hemodynamic support.[37] This has been termed augmented renal clearance and leads to underdosing medications cleared by the kidneys with important clinical consequences. Activation of renal reserve also occurs in transplant donors. For example, in kidney donors studied prior to and after donation,[38] basal and stimulated

GFRs have been measured predonation and compared to postdonation baseline values. Following kidney donation, Scr was found to increase from 0.96 ± 0.15 (standard deviation) to 1.29 ± 0.24 mg/dL while basal GFR decreased from 113 to 72 ml/min/1.73 m2. The predonation stimulated GFR was 143 ml/min/1.73 m2. Therefore, loss of one kidney, or 50% of total GFR, resulted in only an increase of 0.33 mg/dl in Scr, a loss of 41 ml/min/1.73 m2 of basal GFR, but a loss of 71 ml/min/1.73 m2 total GFR. This was exactly half of the total predonation stimulated or total GFR, implying complete engagement of the renal reserve of the remaining kidney.[28]

Determining GFR

The rapid and accurate clinical determination of GFR has been a goal for over fifty years. The clinical utility for an mGFR is listed in Box 1. To measure GFR accurately, the ideal GFR marker should be small, retained within the vasculature, not protein bound, and freely filtered across the glomerulus. It should not be secreted into the urine or reabsorbed from the urine, so that the mGFR would be equal to the urinary clearance of the marker after its intravenous infusion. Inulin, a small fructose polymer that is neither secreted, reabsorbed, nor metabolized, and is cleared only by glomerular filtration, is the reference standard GFR marker. A constant intravenous infusion of an exogenous filtration marker allows a steady state plasma concentration, and collection of timed urine collections enables assessment of urinary clearance to approximate the GFR. However, this approach is costly, unwieldy, error prone, and time consuming.[12] Plasma clearance of inulin and other small molecular weight compounds used to quantify GFR can be assessed without the requirement for a timed urine collection, however, neither inulin nor other markers are retained exclusively within the plasma volume (PV) because they distribute into the extracellular fluid (ECF) volume to occupy the total ECF volume. Therefore, the disappearance of the exogenous filtration marker from the PV is governed by both ECF space distribution and kidney clearance. To remove the ECF distribution component from the equation, the single compartment model uses the terminal elimination phase constant k because it relates directly to kidney removal of the compound after equilibration of the marker between the PV and ECF compartments, which typically requires 1–2 hours. Once equilibrium is reached between the plasma and ECF, removal from the plasma only occurs via the kidney. This is why one compartment models require several hours of recurrent blood draws for an accurate determination, and determination can either be by plasma sampling or using a non-invasive fluorescent detector to quantify ECF fluorescence.

A two compartment model can also be used with a bolus injection of a single marker compound. In this case the initial concentration of the marker at time zero (t_0) and the plasma volume of the injected compound are estimated by obtaining many samples in the first few minutes after injection and extrapolating the concentrations obtained back to t_0 on the x axis. The more time points, and the closer they are obtained to the completed injection, the better the estimate. The disappearance of the GFR marker is then broken down into movement into the ECF and the clearance by the kidney. The rate for kidney clearance is determined by the dose divided by the area under the curve (AUC).[39–43]

With the development of a suitable contrast agents, medical imaging, for instance magnetic resonance imaging (MRI) techniques, will be beneficial for providing kidney regional as well as total kidney blood flow and GFR.[44–47] The disadvantages of these technologies are the high expense, the low degree of accessibility, the challenges of repeating the study, and the patient needing to be transported for the study.

Translating Fluorescence Measurements Into Clinical Observations

Progress in the Area of Fluorescence Tissue Imaging

Optical techniques that use non-ionizing radiation are either minimally invasive or non-invasive. They can empower the diagnosis of diseases with high sensitivity, speed, and accuracy. In particular, quantitative fluorescent approaches have been developed for determining numerous kidney functions including GFR.[48–62]

Several groups of investigators and commercial entities are working in this idea as is shown by recent publications (Table 1).[60,63–70] Initial studies in mice showed a single injection of FITC (fluorescein isothiocyanate)-tagged inulin could be used to quantify GFR with normal, reduced, and increased GFR.[40] Continuous infusions of FITC-inulin have also been used with success in mice[71] to quantify hyperfiltration in diabetic mice. Sinistrin, a derivative of inulin with increased solubility, and FITC-sinistrin has been used in bolus and continuous infusion studies in rats.[67,68,72] These later studies were accomplished using a transcutaneous fluorescence excitation emitter and an emission monitor that detects ECF fluorescence through the skin. Transcutaneous detection of fluorescent sinistrin has also been used in cats and dogs[73], and a detector that allows continuous monitoring has been developed for use in awake mice.[74] Non-fluorescent sinistrin has been successfully used safely in humans with various levels of kidney function, including patients with augmented renal clearance.[75,76] Pyrazine dyes have also been studied in rodent models to quantify a rate of clearance constant, and recent data indicate they have an adequate toxicity profile.[77] Finally, lifetime-decomposition measurements of FITC-sinistrin have been used to quantify GFR[66,78] and have allowed a reduction in FITC-sinistrin dose by factor of 200.

However, all of these studies had to use a single compartment model because the fluorescent signal comes from the ECF. This approach requires equilibration of the injected fluorescent marker with the ECF, and this large volume of distribution and quenching of fluorescence by skin increases the amount of marker needed for adequate signals. It also only yields a rate constant for marker removal from the ECF and not a true GFR. To convert the rate constant to GFR, one has to have an accurate determination of ECF volume or use estimating equations for ECF.[43] The use of estimating equations works reasonably well in “normal” individuals but in patients with an altered ECF, such as with edema or ascites, adequate equations to estimate ECF volume have not been developed. This minimizes the utility of this approach in CKD patient populations.

Developing a Bedside Two-Compartment GFR Measurement Technique With Two Markers

Translation to the clinic requires refining an approach initially developed in rats that would allow rapid direct quantification of vascular fluorescence for quantitative analysis of GFR and plasma volume.[60] Three components are necessary to translate from the microscope to the bedside: a two component fluorescent marker mixture, a software analysis program for the two-compartment model, and a detector.[79]

The first component is a two-marker injectate composed of a PV indicator and GFR indicator. The PV marker is a high molecular weight 150 kDa inert marker with a prolonged stable phase in the plasma, used to quantify PV based on dilutional principles. This marker eliminates the need for many early blood samples because extrapolation of the GFR marker back to zero for determination of the t_0 concentration of the marker is not necessary. In this case the t_0 concentration of the GFR marker can be determined directly, knowing the PV and dose of small marker injected. The freely filtered small molecular weight glomerular filtration indicator is again used to determine the rate of movement into the ECF and clearance by the kidneys based on dose and AUC. In summary, this two-marker approach gives the t_0 concentration, not an estimated one. This improves the accuracy of the AUC and dramatically reduces the time required and the number of plasma samples that must be drawn.[79]

Design of the large and small molecular weight markers requires inert uniform-sized molecules that are highly water soluble, such as dextrans, that can be covalently labeled with non-toxic readily differentiated molecules with different detection properties, such as fluorescent dyes. Dextrans are inert and highly water soluble molecules that can be prepared at various sizes, with low dispersion about the mean, and have been used clinically for many decades. They are also easy to covalently label with fluorescent molecules, for which they have a high conjugation ratio. These characteristics allow for very low milligram doses and volumes to be given. Thus small quantities of fluorescent dextrans are needed, which has important clinical safety and commercial implications. The use of a red fluorescent large dextran PV marker and freely filterable green fluorescent small dextran GFR reporter molecules allows for GFR determinations.[41,79] Direct PV determinations, using the large 150-kDa dextran PV marker, also minimizes potential estimation errors in t_0 due to variations in PV with disease states. It also avoids movement of the molecule into the extracellular space, even in disease states like sepsis, thus allowing for a stable measurement over time. Therefore, one can determine PV by determining the amount of dilution of the PV indicator once equilibrium within the plasma has been reached in 10–15 minutes. Knowing this value the t_0 concentration of the small GFR marker can be calculated directly. This is a critical number that is only estimated when a one marker is approach is used in a two compartment model.

The second component necessary for a rapid bedside determination is a software analysis program using the two-compartment model.[41] The GFR rate constant, and apparent volume of distribution (V_d) of the PV indicator molecule, can be measured by monitoring the plasma concentration of the fluorescently labeled GFR filtration molecule over time and dilution

equilibrium value of the PV indicator molecule.[41] Since its introduction,[80] the two-compartment model has been applied in a number of renal studies in animal models[40,42,81] as well as in humans.[82–84] It has been shown to be an effective approach in plasma clearance analysis and GFR determination.

The third component is detection, and this requires plasma sampling and a fluorescence detector. The number of plasma samples includes an early sample for PV determination. This sample can also be used to determine the initial starting concentration of the GFR clearance marker at t_0. This is a major advantage as it gives an exact PV and also minimizes the number of early plasma samples necessary since no extrapolation to t_0 is necessary. Presently, three plasma samples taken at 15, 60 and 120 minutes postinjection are required for determination of both PV and mGFR. This shortens the time necessary for the study, and the fluorescent indicators allow for an immediate readout, thus avoiding time-consuming biochemical- or radioactivity-based determinations.

Another advantage of determining the PV with a compound that has a long half life is the ability to resample the plasma at different time points and determine the effect of volume addition or removal maneuvers such as fluid boluses or diuretics in CKD patients.

A rapid determination of GFR has multiple advantages (Box 1). It also allows for measurement of stimulated GFR and therefore renal reserve. To accomplish this either an intravenous infusion of an amino acid mixture or a protein meal can be used.[30,34] Using a two-marker, two compartment model this can be done either following a basal measurement of GFR by repeating the study after stimulation or without a baseline measurement by a GFR study one hour poststimulation. Measuring renal reserve would also give early insight into hyperfiltration in diabetics allowing for earlier initiation of therapies to reduce intraglomerular pressure. It will also allow for individualization of patient care and rational development of other biomarkers of disease and progression.

In summary, early identification and determination of the extent of GFR loss in CKD will allow for early treatment, as well as enrollment and appropriate stratification in clinical studies. Determination of basal GFR and stimulated GFR would allow for more precise and reproducible GFR measurements and the ability to follow progressive loss of GFR in all patients, even when renal reserve is limiting changes in basal GFR. Fluorescence technologies will provide the ability to quantify basal and stimulated GFR, thus allowing for individual care.

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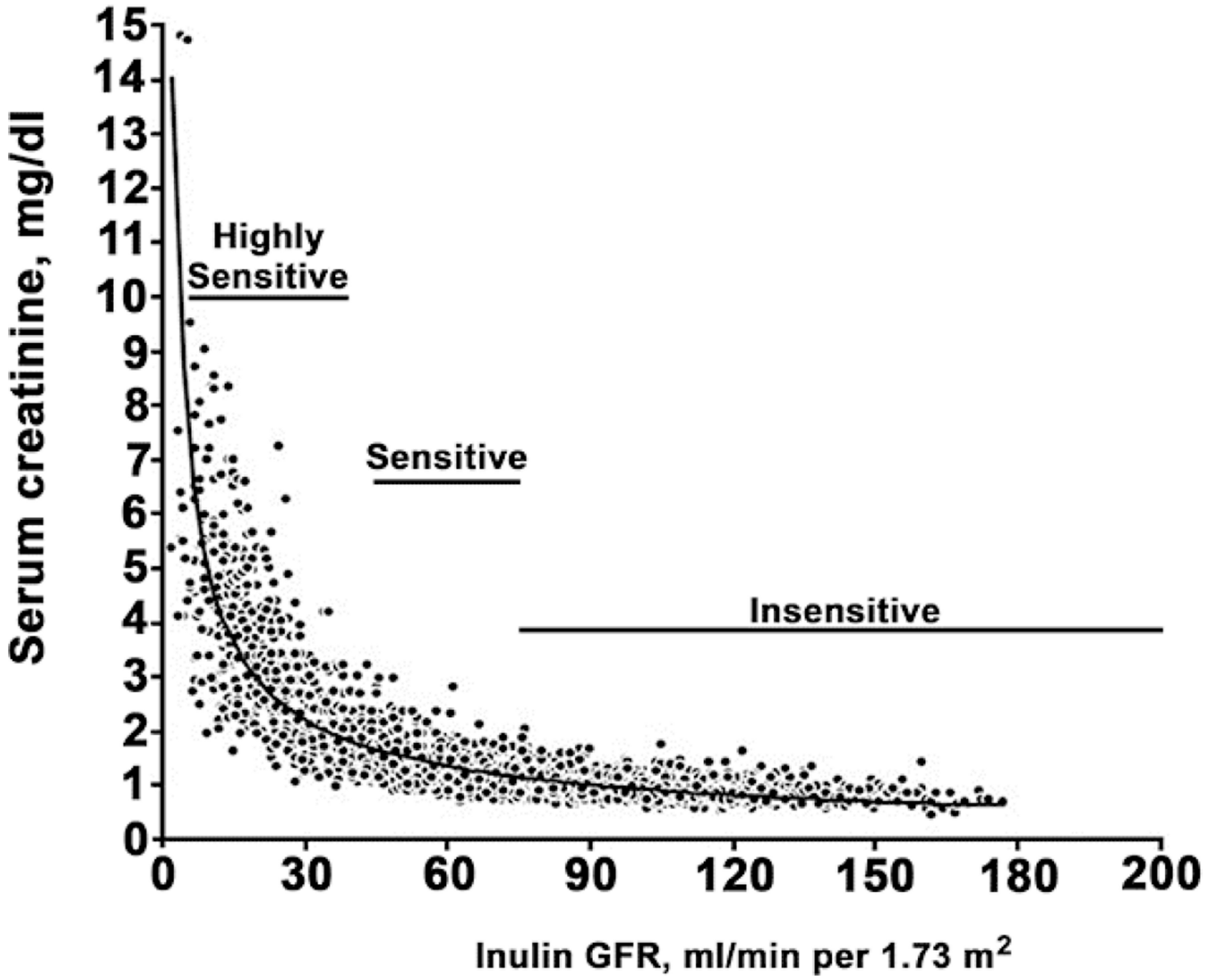
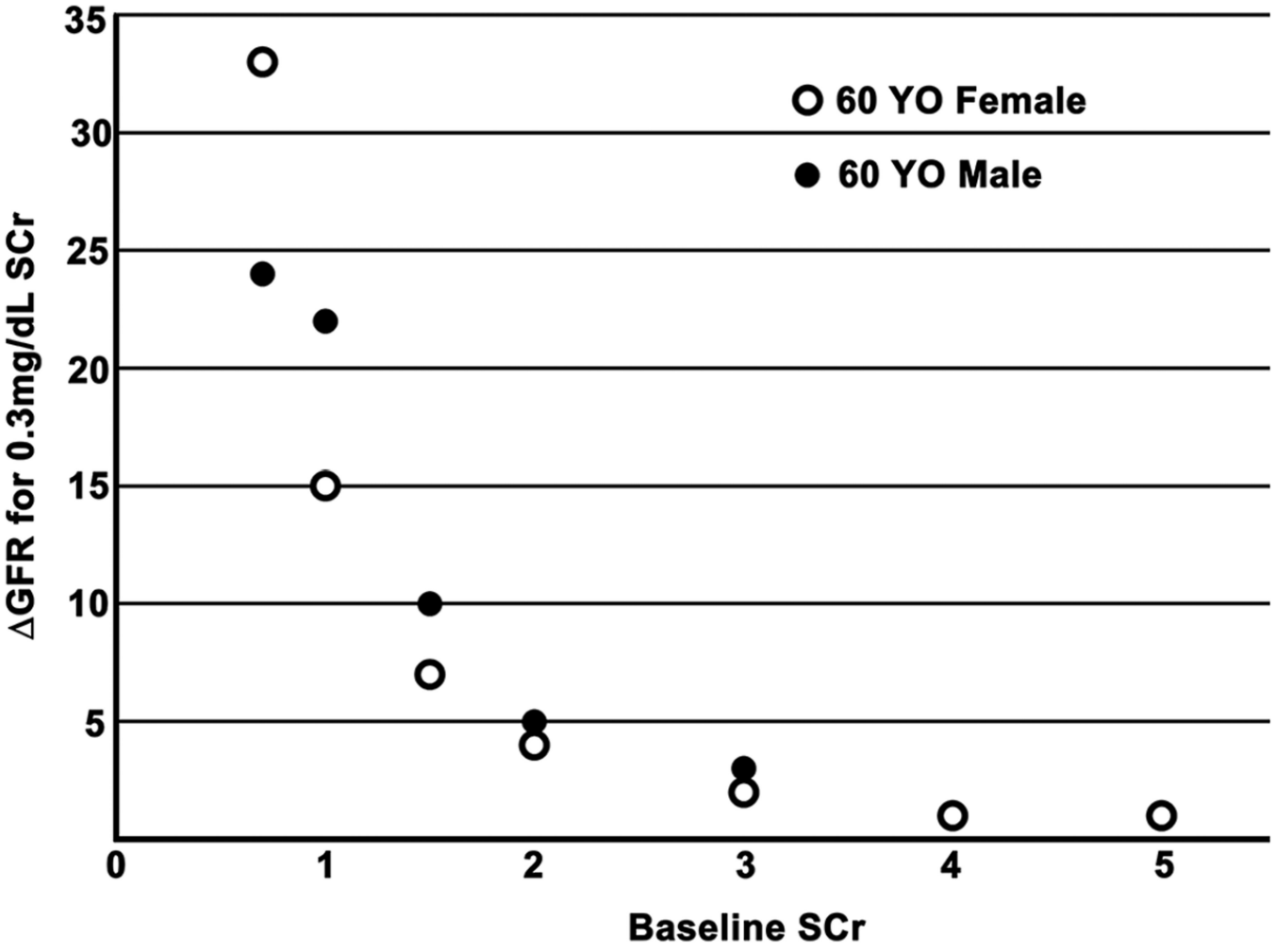
Box 1

Clinical Applications for Basal and Stimulated measured GFR

- Screening test for CKD in high-risk patients with or without preexisting kidney damage
  - Diagnosis
  - Quantifying severity of injury
  - Quantify rate of decline in GFR
  - Quantifying renal reserve
- Screening for hyperfiltration in diabetic patients to identify an early signal of pending CKD development
- Drug-dosage adjustments in patients with acute or chronic decreased kidney function for nephrotoxins and renally cleared medications
- Risk assessment of acute kidney injury and recovery of function after injury
- Monitoring patients who require repeated administration of nephrotoxic drugs such as cisplatin
- Indirect assessment of visceral organ perfusion, such as during prolonged anesthesia
- Following therapeutic responses in either baseline or stimulated GFR
- Screening test for kidney donation

CKD, chronic kidney disease; GFR, glomerular filtration rate





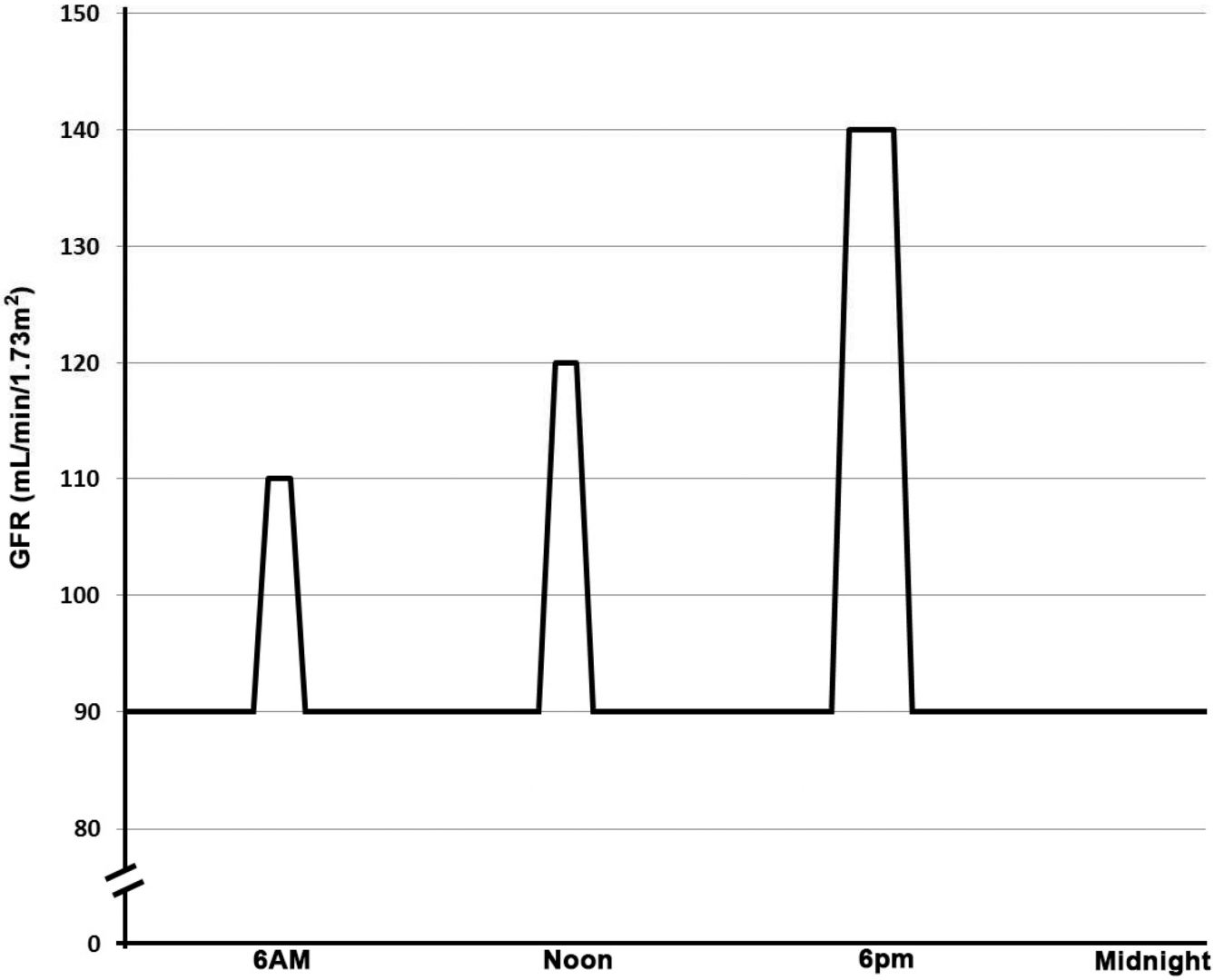


Figure 1.

Properties of GFR. A. Serum creatinine versus inulin measured GFR. Adapted from Botev[20] with permission of the American Society of Nephrology.

B. Baseline serum creatinine of an idealized 60-year-old Caucasian male and female versus the change in GFR that it would take to result in a 0.3mg/dl change in serum creatinine (the definition of acute kidney injury).

C. Idealized change in GFR with meals throughout the day.

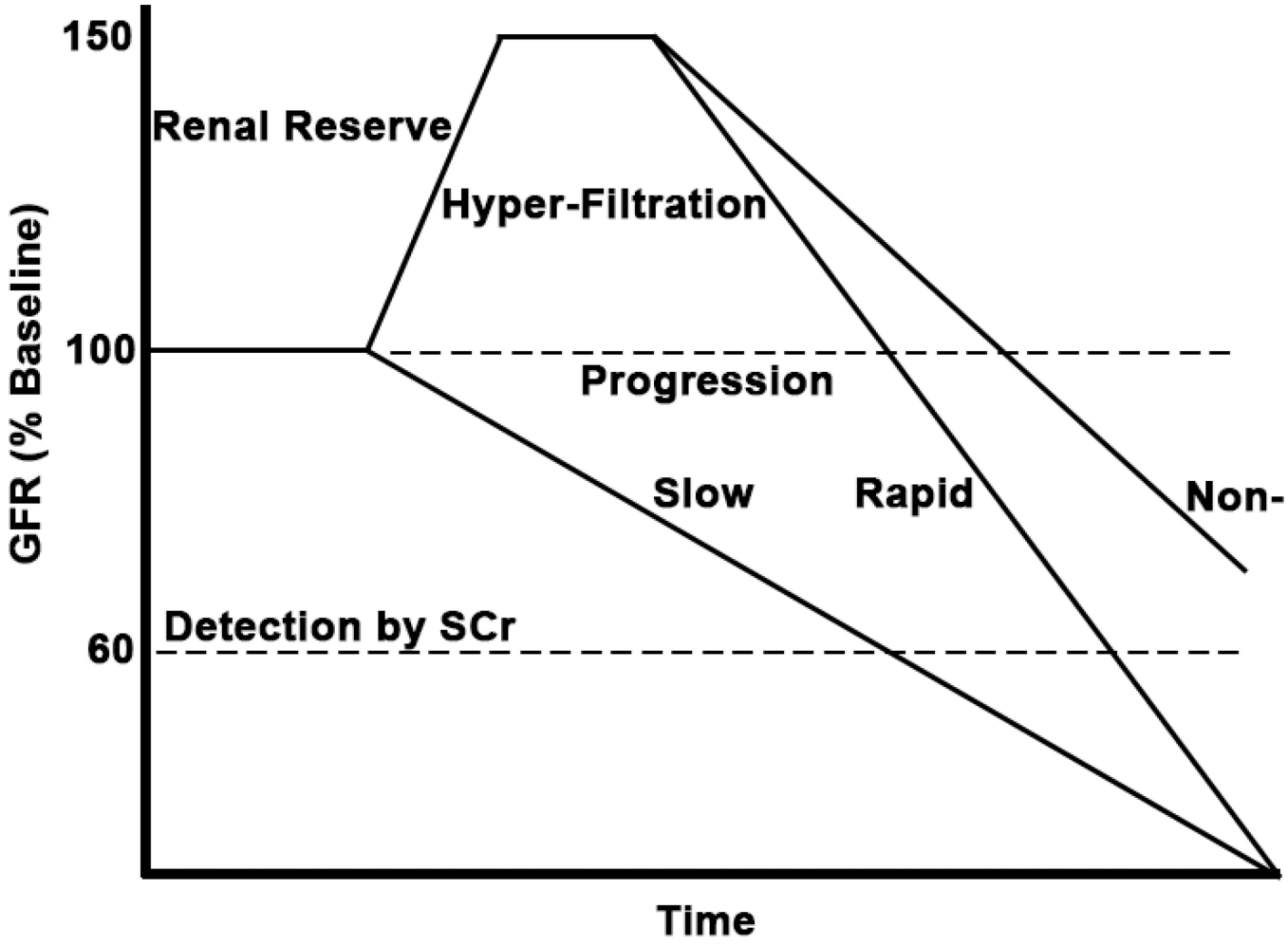


Figure 2.

An idealized schematic showing progressive loss of GFR in three different patients. Two patients had early but chronic activation of renal reserve, as some diabetic patients have, resulting in hyperfiltration. The third patient lost total kidney function without activating renal reserve. This reduces the slope of GFR loss but has the same clinical end point. In all cases if detection depends on serum creatinine, detection is late or not at all.

Table 1

Fluorescent Probe Determination of GFR

Molecular Marker	Sample Space	Detector Site	Resulting Determination(s)	Species	References
Dextrans: 150-kDa Rh & 5-kDa FITC	Vasculature; 2-Compartment Model	Venous	PV, GFR	Dogs	Wang et al 2010[60]; Wang et al 2012[69]
FITC-Sinistrin	ECF; Single-Compartment Model	Skin	Decay Rate	Rats	Schock-Kusch et al 2011[68]; Schock-Kusch et al 2009[67]
^{99m}Tc-DTPA	ECF; Single-Compartment Model	Arm	Decay Rate	Human	Rabito et al: 2010[64]
Carbustyril ^{124}Eu-DTPA	ECF; Single-Compartment Model	Skin	Decay Rate	Rats	Rabito et al 2005[65]
Pyrazine derivatives	ECF; Single-Compartment Model	Skin	Decay Rate	Rats	Poreddy et al 2012;[63] Rajagopalan et al 2011[66]
FITC-Inulin	Vasculature; 2-Compartment Model	Venous	GFR	Rats, Mice	Qi et al 2004[40]
FITC-Inulin	Vasculature and Urine	Venous	PV, GFR	Mice	Bivona et al 2011[71]
FITC-Sinistrin	ECF; Continuous Infusion	Skin	Decay Rate	Rats	Schok-Kusch et al 2012[72]
FITC-Sinistrin	ECF; Single-Compartment Model	Skin	Decay Rate	Rats	Shmarlouski et al 2015[78]
FITC-Sinistrin	ECF; Single-Compartment Model	Skin	Decay Rate	Dogs, Cats	Steinbach et al 2014[73]

ECF, extracellular fluid; FITC, fluorescein isothiocyanate (a green fluorescent dye); GFR, glomerular filtration rate; PV, plasma volume; ^{99m}Tc, technetium 99m; DTPA, diethylenetriamine pentaacetic acid; Rh, rhodamine (a red fluorescent dye)