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

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Chronic kidney disease (CKD) is an increasing clinical problem. Although clinical risk factors and biomarkers for the 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. Because 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 using new minimally invasive or noninvasive 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 to review fluorescent technologies to deliver patient-specific GFR.
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The Clinical Problem

Chronic kidney disease (CKD) is 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 stages 3 (glomerular filtration rate [GFR], 30-59 mL/min/1.73 m^2) and 4 (GFR, 15-29 mL/min/1.73 m^2). Using estimated GFR (eGFR) definitions in population studies, patients classified as having CKD have an increased risk for cardiovascular events, progression, dialysis therapy, and death.[2] This important clinical information, including the overall rate of CKD, has been challenged on the individual patient level because estimating equations have wide dispersion ($\pm30\%$ 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 lack of human tissue to interrogate and compare with animal models at structural and molecular levels, as is being successfully accomplished in glomerular diseases[7] and

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transplantation, are also barriers to success. Therefore, individualization of therapy in CKD is not presently possible and likely will not 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. Because 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 GFR therapeutic registration end points in clinical trials.

Understanding and Quantifying GFR as a Measure of Kidney Function

Serum Creatinine as a Surrogate Marker of GFR

GFR, which measures the quantity of water 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 level as a marker in CKD have been recently reviewed by Levey et al.[15] Although it is well known that reductions in GFR secondary to chronic kidney injury are accompanied by increases in Scr levels, the insensitivity of this surrogate marker as an estimate of GFR, especially in GFRs > 60 mL/min/1.73 m^2, is often underappreciated.[16-18] One of the reasons for this is illustrated in Fig 1A, a plot of measured GFR (mGFR) versus Scr level. The graph is divided into insensitive, sensitive, and highly sensitive regions for Scr level 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 level. To illustrate this point, consider what a change of 0.3 mg/dL in Scr level 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 shown in Fig 1B. I use 0.3 mg/dL because most would consider this a significant change in Scr

level. One can see the amount of GFR loss at several initial Scr levels for this change. In the insensitive region of the GFR-Scr curve (Scr, 0.8 mg/dL), a change of 0.3 mg/dL represents a loss of >33 mL/min/1.73 m^2 of baseline GFR for a woman and 24 mL/min/1.73 m^2 for a man. In the sensitive portion of the curve (Scr, 2.0 mg/dL), an increase of 0.3 mg/dL represents a loss of 4 and 5 mL/min/1.73 m^2, respectively, whereas in the highly sensitive portion (Scr, 4.0 mg/dL), it represents a $1.0-$mL/min/1.73 m^2 change in GFR. Therefore, one can see why Scr level 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 patients with CKD, 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 patients with CKD are more prone to acute kidney injury? I think so.

The discrepancy between what a linear increase in Scr level means at different starting Scr levels 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 level 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 for the loss in kidney function to be detectable due to the insensitivity of Scr level. Can we trust Scr level or eGFR to deliver on this end point in patients with CKD with mild reductions in GFR or adequate renal reserve? Can we trust that the therapeutic agent will be effective in late-stage CKD given the fibrosis and endothelial and tubular epithelial changes that have already occurred?

Other factors limit the utility of Scr level 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 in which Scr level 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,
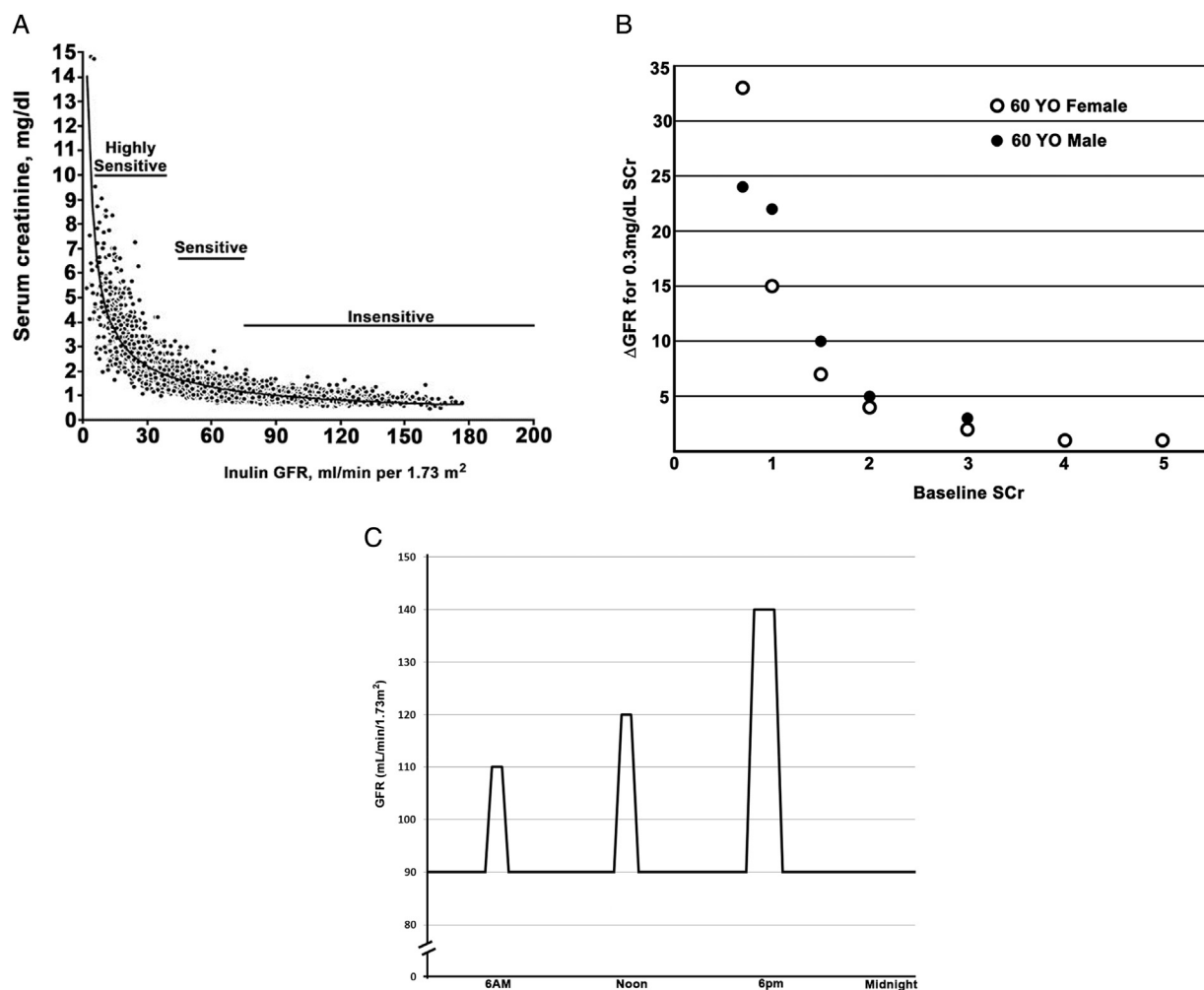
Figure 1. Properties of glomerular filtration rate (GFR). (A) Serum creatinine (SCr)- versus inulin-measured GFR. Adapted from Botev[20] with permission of the American Society of Nephrology. (B) Baseline SCr level of an idealized 60-year-old (YO) white man and woman versus the change in GFR that it would take to result in a 0.3-mg/dL change in SCr level (the definition of acute kidney injury). (C) Idealized change in GFR with meals throughout the day.

or glucagon infusion,[27,28] as shown in Fig 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 also been 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 patients with CKD or aging patients. Several studies have shown no predictable effect on

renal reserve in patients with CKD 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 a 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 (Fig 1C). Therefore, a fasting Scr level and 24-hour urine sample 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 patient 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, an mGFR done while the patient is fasting underestimates the mean 24-hour GFR, whereas an mGFR obtained following a protein meal over-estimates the 24-hour average daily GFR.

Chronic hyperfiltration occurs early in some diabetic patients and may portend 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 (Fig 2).

Renal reserve has also been shown by many investigators to be chronically activated in critical care situations in which 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 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 with postdonation baseline values. Following kidney donation, Scr level 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 m^2. The predonation stimulated GFR was 143 mL/min/1.73 m^2. Therefore, loss of 1 kidney, or 50% of total GFR,

resulted in an increase of only 0.33 mg/dL in Scr level, a loss of 41 mL/min/1.73 m^2 of basal GFR, but a loss of 71 mL/min/1.73 m^2 total GFR. This was exactly half 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 more than 50 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 or reabsorbed from urine, so that mGFR would be equal to 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 timed urine collections enable 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 because they distribute into the extracellular fluid (ECF) volume to occupy the total ECF volume. Therefore, disappearance of the exogenous filtration marker from plasma volume 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 plasma volume and ECF compartments, which typically requires 1 to 2 hours. When equilibrium is reached between plasma and ECF, removal from plasma occurs only via the kidney. This is why 1-compartment models require several hours of recurrent blood draws for an accurate determination, and determination can either be by plasma sampling or using a noninvasive fluorescent detector to quantify ECF fluorescence.

A 2-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 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
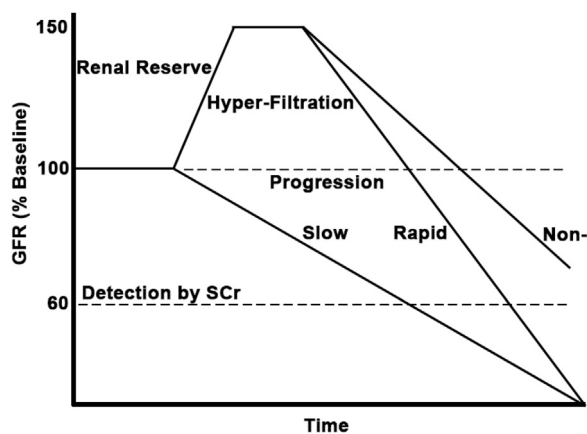

Figure 2. An idealized schematic shows progressive loss of glomerular filtration rate (GFR) in 3 different patients. Two patients had early but long-term renal reserve activation, as some diabetic patients have, resulting in hyperfiltration. The third patient lost total kidney function without activating 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 (SCr) level, detection is late or not at all.

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

Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate.

more time points and the closer they are obtained to the completed injection, the better the estimate. Disappearance of the GFR marker is then broken down into movement into the ECF and clearance by the kidney. The rate for kidney clearance is determined by the dose divided by the area under the curve.[39-43]

With the development of suitable contrast agents, medical imaging, for instance, magnetic resonance imaging techniques, will be beneficial for providing kidney regional and total kidney blood flow and GFR.[44-47] Disadvantages of these technologies are the high expense, low degree of accessibility, 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 nonionizing radiation are either minimally invasive or noninvasive. 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 on this idea, as is shown by recent publications (Table 1).[60,63-70] Initial studies in mice showed that a single injection of fluorescein isothiocyanate (FITC)–tagged inulin could be used to quantify GFR with normal, reduced, and increased GFRs.[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 have 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] Nonfluorescent 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 that 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 a factor of 200.

However, all 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 2-Compartment GFR Measurement Technique With 2 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 2-component fluorescent marker mixture, a software analysis program for the 2-compartment model, and a detector.[79]

The first component is a 2-marker injectate composed of a plasma volume indicator and GFR indicator. The plasma volume marker is a high-molecular-weight 150-kDa inert marker with a prolonged stable phase in plasma, used to quantify plasma volume based on dilutional principles.

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,[60] 2010; Wang et al,[69] 2012
FITC-sinistrin	ECF; single-compartment model	Skin	Decay rate	Rats	Schock-Kusch et al,[68] 2011; Schock-Kusch et al,[67] 2009
^{99m}Tc-DTPA	ECF; single-compartment model	Arm	Decay rate	Human	Rabito et al,[64] 2010
Carbustyril ^{124}Eu-DTPA	ECF; single-compartment model	Skin	Decay rate	Rats	Rabito et al,[65] 2005
Pyrazine derivatives	ECF; single-compartment model	Skin	Decay rate	Rats	Poreddy et al,[63] 2012; Rajagopalan et al,[66] 2011
FITC-inulin	Vasculature; 2-compartment model	Venous	GFR	Rats, mice	Qi et al,[40] 2004
FITC-inulin	Vasculature and urine	Venous	PV, GFR	Mice	Bivona et al,[71] 2011
FITC-sinistrin	ECF; continuous infusion	Skin	Decay rate	Rats	Schock-Kusch et al,[72] 2012
FITC-sinistrin	ECF; single-compartment model	Skin	Decay rate	Rats	Shmarlouski et al,[78] 2015
FITC-sinistrin	ECF; single-compartment model	Skin	Decay rate	Dogs, cats	Steinbach et al,[73] 2014

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

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 plasma volume 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 area under the curve. In summary, this 2-marker approach gives the t_0 concentration, not an estimated one. This improves the accuracy of the area under the curve and dramatically reduces the time required and 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 nontoxic 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. Use of a red fluorescent large dextran plasma volume marker and freely filterable green fluorescent small dextran GFR reporter molecules allows for GFR determinations.[41,79] Direct plasma volume determinations, using the large 150-kDa dextran plasma volume marker, also minimize potential estimation errors in t_0 due to variations in plasma volume with disease states. It also avoids movement of the molecule into the extracellular space, even in disease states like sepsis, thus allowing for stable measurement over time. Therefore, one can determine plasma volume by determining the amount of dilution of the plasma volume indicator when equilibrium within the plasma has been reached in 10 to 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 1-marker approach is used in a 2-compartment model.

The second component necessary for a rapid bedside determination is a software analysis program using the 2-compartment model.[41] The GFR rate constant and apparent volume of distribution of the plasma volume 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 plasma volume indicator molecule.[41] Since its introduction,[80] the 2-compartment model has been applied in a number of renal studies in animal models[40,42,81] and 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 plasma volume 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 because it gives an exact plasma volume and also minimizes the number of early plasma samples necessary because no extrapolation to t_0 is necessary. Presently, 3 plasma samples taken at 15, 60, and 120 minutes postinjection are required for

determination of both plasma volume 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 plasma volume 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 patients with CKD.

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 2-marker 2-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 at 1 hour poststimulation. Measuring renal reserve would also give early insight into hyperfiltration in diabetic patients, 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.

ACKNOWLEDGEMENTS

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