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Come valutare la velocità di filtrazione glomerulare e quale metodo è considerato il più affidabile?

In depth review

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ABSTRACT

The prevalence of chronic kidney disease (CKD) continues to rise globally, paralleled by an increase in associated morbidity and mortality, as well as significant implications for patient quality of life and national economies. Chronic kidney disease often progresses unrecognized by patients and physicians, despite diagnosis relying on two simple laboratory measures: estimated glomerular filtration rate (eGFR) and urine analysis. GFR measurement has been grounded in renal physiology, specifically the concept of clearance, with creatinine identified as a suitable endogenous marker for estimating creatinine clearance (CrCl). On this foundation, various equations have been developed to calculate CrCl or estimated GFR (eGFR) using four variables that incorporate creatinine and certain demographic information, such as sex and age. However, creatinine measurement requires standardization to minimize assay variability across laboratories. Moreover, the accuracy of these equations remains contentious in certain patient subgroups. For these reasons, additional mathematical models have been devised to enhance CrCl estimation, for example, when urine collection is impractical, in elderly or debilitated patients, and in individuals with trauma, diabetes, or obesity. Presently, eGFR in adults can be immediately measured and reported using creatinine-based equations traceable through isotope dilution mass spectrometry. In conclusion, leveraging insights from renal physiology, eGFR can be employed clinically for early diagnosis and treatment of CKD, as well as a public health tool to estimate its prevalence.

KEYWORDS: renal function markers, creatinine, cystatin C, inulin, iohexol

Introduction

The prevalence of chronic kidney disease (CKD) continues to escalate globally, accompanied by an increase in morbidity, mortality, and significant implications for the quality of life of patients and the economies of nations. Any clinical condition resulting from a reduction in the number of functioning nephrons can progress into chronic renal failure, defined by the KDIGO guidelines as "abnormalities in kidney structure or function, present for 3 months, with health implications" [1]. In the real world, chronic kidney disease is a silent ailment often progressing unnoticed by patients and physicians, although the diagnosis relies on two simple laboratory measures: estimated GFR (eGFR) and urine analysis (screening for albuminuria/proteinuria). The glomerular filtration rate remains the premier comprehensive indicator of renal function as it assesses renal clearance and is directly related to the functioning renal mass, serving to classify CKD into stages, calculate medication dosages, and prepare for invasive studies with contrast medium. Early diagnosis of chronic kidney disease aids in delaying progression and reducing associated morbidity and mortality.

Identification of the Glomerular Filtration Process for GFR Measurement in Clinical Practice

Carl Ludwig (1816-1895), pioneered of glomerular filtration identified the glomerulus as a filter. This filtration is regulated by the hydrostatic pressure and modulated by the contraction and vasodilation of the afferent and efferent arterioles. He further hypothesized that the filtered volume decreased along the tubules due to reabsorption, thereby concentrating the end products in the urine [2]. However, to apply the concept of GFR in clinical settings, it was imperative to identify a solute removed solely by filtration, without reabsorption or secretion in the nephron. Later Paul Rehberg pinpointed creatinine as such a solute, given its endogenous production, filtration, and presumed lack of reabsorption or excretion.

Cockcroft-Gault equation

Creatinine Clearance = $\frac{140-\text{age (years)} \times \text{weight (kg)}}{72 \times \text{serum creatinine (mg/dl)}}$ x 0.85 (if female)

MDRD-4 (simplified)

Estimated Glomerular Filtration Rate (mL/min/1.73 m^2) =
= 175 (Serum Creatinine in mg/dl x 0.011312)$^{-1.154}$ x (age in years)$^{-0.203}$
x (0.742 if female) x (1.212 if African American/black)

CKD-EPI (2009)

Estimated GFR = 141 x min(S_{Cr}/κ, 1)$^{\alpha}$ x max(S_{Cr} /κ, 1)$^{-1.209}$ x 0.993^{Age} x 1.018 [if female] x 1.159 [if Black]
SCr (standardized serum creatinine) = mg/dL., K = 0.7 (females) or 0.9 (males), α = −1.329 (female) or−0.411 (male), Min = indicates the minimum of S_{Cr}/K or 1, max = indicates the maximum of S_{Cr}/K or 1, Age = Years

FAS (2016)

1) Estimated GFR = 107.3/(SCr/ *Q*) for age ≤ 2 to ≤ 40 years
2) Estimated GFR = 107.3/(Scr/ *Q*) x $0.988^{(age-40)}$ for age > 40 years
Q: the mean or median SCr value for age/sex-specific healthy populations

CKD-EPI cystatin C equation

Estimated Glomerular Filtration Rate (mL/min/1.73 m^2) =
= 133 × min(Scys/0.8, 1)$^{-0.499}$ × max (Scys/0.8, 1)$^{-1.328}$ × 0.996^{Age} [× 0.932 if female]
Scys = serum cystatin C, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scys/κ or 1

CKD-EPI creatinine-cystatin C

Estimated Glomerular Filtration Rate (mL/min/1.73 m^2) =
135 × min(Scr/κ, 1)$^{\alpha}$ × max(Scr/κ, 1)$^{-0.601}$ × min(Scys/0.8, 1)$^{-0.375}$ × max(Scys/0.8, 1)$^{-0.711}$ × 0.995^{Age} [× 0.969 if female] [× 1.08 if black]
Scr = serum creatinine, Scys = serum cystatin C, κ is 0.7 for females and 0.9 for males, α is −0.248 for females and −0.207 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

Figure 1. Comparative summary of GFR estimation equations, including Cockcroft-Gault, simplified MDRD-4, CKD-EPI creatinine and cystatin C, and the FAS method. These formulas incorporate variables such as age, weight, serum creatinine, and patient demographics to determine renal function.

Estimation of GFR with Endogenous Markers

Creatinine-Based Glomerular Filtration Estimation

Creatinine remains the most widely utilized endogenous marker for estimating renal function in clinical practice, research, and animal models. It is a waste product of regular muscle metabolism. Creatinine, not being protein-bound, is freely filtered by the glomeruli; however, its synthesis is not constant, as it is determined by daily protein intake and muscle trophism. It is also subject to both secretory and reabsorptive mechanisms [3]. These conditions restrict the utility of creatinine as a renal function marker. Gender differences in tubular secretion have also been documented: males may secrete more creatinine than females, which could result in discrepancies in GFR estimation between male and female animals [4].

The initial method to measure creatinine, developed in 1886, was the alkaline picric acid reaction of Jaffé (a colorimetric method). This method's interference with chromogens, such as bilirubin, glucose, or hemoglobin, led to inaccuracies in humans. In rodents, non-specific chromogens could overestimate creatinine by a factor of five. Different methods have been adapted to measure serum creatinine. The enzymatic determination, now considered the reference method in rodents, was validated in 2007 with various reactions with the aid of creatininase, creatinase, and sarcosine oxidase [5]. The measurement of creatinine in serum is prone to different types of error, interferences and imprecision. Serum creatinine certainly represents the most practical and least expensive measurement for stable glomerular filtration rate, however it presents some limitations in the interpretation of the results which may be secondary to both tubular secretion and the presence of muscle mass and protein intake. Even the absolute value of creatinine is subject to some variations such as the reference intervals of each analysis method of each laboratory with the risk of altering each glomerular filtration rate analysis equation. There are limitations in estimating creatinine secondary to muscle trophism because it is a product of muscle catabolism and results difficult in patients with extremely low or high muscular mass (e.g., anorexia, obesity or weight lifter). Creatinine is secreted by tubules and this explains why creatinine cleareance overestimates true GFR. Drugs, such as trimethoprim and cimetidine, also interfere with this tubular secretion and this explains why during their intake there is an increase in creatinine values without evident alterations in GFR. The absolute value of creatinine could be altered in some pathological conditions such as liver failure and rhabdomyolysis. The absolute value of creatinine has physiological limits for an accurate estimate of the glomerular filtration rate [20].

Creatinine Clearance Over 24 Hours and Estimation of GFR Using Endogenous and Exogenous Markers

24-hour creatinine clearance has been a prevalent method for assessing GFR in animal models. Yet, it is crucial to acknowledge that the limitations of serum creatinine as a renal function marker impact the precision and accuracy of the 24-hour collection [6]. Blood samples are necessary to measure serum creatinine.

GFR Estimation Using Cystatin-C

Cystatin-C (CysC) is a low molecular weight protein (13KDa) of the family of cysteine protease inhibitors. It is produced by all the nucleated cells of the body**,** filtered by the glomerulus, and then reabsorbed and metabolised by tubular epithelial cells, excluding its use for clearance on 24 hour urine. Like cratinine, the determination of cystatin C is influenced by factors such as sex, age and chronic inflammatory state [7], but it provides a more precise estimate of glomerular filtration as it is not affected by variables such as muscle mass and activity, or dietary protein intake.

GFR Estimation with Exogenous Markers: Inulin Clearance

The fructose polymer inulin has always represented a specific method for medical students for measuring glomerular filtration [8] due to the intrinsic characteristics of the molecule; in fact inulin is not metabolised, does not bind to plasma proteins and is freely filtered by the glomeruli without being reabsorbed or secreted by the tubular cells. However, considering inulin as the gold standard of the glomerular filtration method presents some limitations: the high cost and cumbersome methods for developing the process such as use with radioactive markers, poor solubility in water and demanding preparation for the solution to be injected (substance dissolve, filter and heat at high temperatures for many hours to remove inulin fragments). Once prepared, inulin is administered as a single intravenous bolus or continuous infusion and plasma and/or urine are collected at different times to calculate clearance. All these steps do not make this method universal.

Sinistrin: The New Inulin?

The measurement of GFR can also be obtained by evaluating the kinetics of Sinistrin FITC and in particular by estimating the half-life. Sinistrin has the advantage of having a lower molecular weight (3500 Da) compared to inulin, it is hightly soluble in aqueous solvents at room temperature, it can be used and labeled with FITC fluorescein [9]. Unlike inulin, it does not require any filtration and has the advantage of being able to be used using transcutaneous devices. An instrument composed of two LEDs is required for measuring fluorescence and transcutaneous GFR. The method consists in the intravenous infusion of Sinistrin with the FITC chromophore which emits the fluorescence captured by the instrument. Transcutaneous measurement has proven to be a good method for measuring renal function in murine models and has the advantage, especially in animals, of measuring glomerular filtration in the absence of particular traumas [21].

Transcutaneous Methods for GFR Measurement

To determine glomerular filtration, the intravenous injection of a sinister FITC molecule was studied and then the variation in fluorescence was studied using a device positioned on the skin. The change in fluorescence is used to calculate the elimination half-life of the marker and then convert the half-life data to GFR (ml/min). The main advantage of this method is its non-invasiveness, however it has limitations as it is an indirect method for measuring GFR and therefore requires conversion factors. The main advantage is its independence from blood/urine sampling and laboratory tests with real-time GFR examination, however a limitation to be evaluated is the high cost of the device ($1000) which makes it impractical for clinical practice [10].

Radiolabeled Tracers

The two most commonly used radiolabeled markers are ethylenediaminetetraacetic acid with radioactive chromium-51 (51Cr-EDTA) and diethylenetriamine pentaacetic acid with radioactive technetium-99 (99mTc-DTPA), both of which are low molecular weight and freely filtered by the glomerulus.

The method consists in measuring the plasma and urinary clearance of single intravenous injections of radiolabeled substances or alternatively intraperitoneal injection [11]. Blood and urine samples are taken and processed using a gamma counter that estimates GFR. 99mTc-DTPA has been used in healthy male Wistar rats and in animals with chronic kidney disease or doxorubicin-induced nephritic syndrome [12]. The main limitation of this technique derives from the use of radioisotopes,

which are not easy to find and which require special authorization and specific conservation; furthermore it presents toxicity for operators who must use specific precautions and careful waste management.

99mTc can dissociate from DTPA and up to 13% of 99mTc-DTPA can bind to plasma proteins, resulting in an underestimate of GFR [13]. These markers could be useful for verifying GFR but are not preferable in clinical practice.

Non-Radiolabeled Contrast Agents in GFR Assessment

Among the various possibilities for measuring GFR is iothalamate, an ionic contrast agent derived from tri-iodobenzoic acid with a molecular weight of 637 Kda. Bell proposed a rapid HPLC method to detect iothalamate and para-aminohippuric acid in rat serum and urine [14], giving an estimate of both GFR and renal blood flow. This method is not easy to apply as it involves both central venous catheterization, a method not without serious side effects, and the simultaneous collection of blood and urine.

Iohexol/Iohexol-DBS

Iohexol (Omnipaque™, GE Healthcare) is a molecule used as a contrast agent. It is excreted unmetabolised by glomerular filtration, without reabsorption or secretion by renal tubular cells without undergoing hepatic metabolism or interference with blood cells. Its use as a reference method for measuring GFR was established almost 30 years ago in humans [15]. In recent years, the filtration of iohexol in mice has been studied by intravenous injection and subsequent blood sampling for pharmacokinetic analysis. Iohexol is measured by HPLC chromatographic analysis. Schultz et al. described the plasma clearance of iohexol in rats in 2014 using liquid chromatography-electrospray-mass spectrometry (LC-ESI-MS). They administered different doses of iohexol via the tail vein to male HsdRCCHan:WIS rats, and the animals were sacrificed at different times after infection with iohexol (15, 30, 60, and 90 minutes) to obtain blood samples. Passos et al. validated the plasma clearance of iohexol in rats [16] against the “classical” gold standard, inulin clearance, using capillary electrophoresis, observing a correlation between iohexol and inulin clearance ($r = 0.792$). However, the procedure required large amounts of blood. Carrara proposed the measurement of GFR through experiments on mice using the following scheme: administration of iohexol (129.4 mg) intravenously and subsequent determination on four blood samples after the infusion at times (20, 40, 120, 140 minutes) [17]. While Luis-Lima proposed a further simplified scheme with fewer side effects, always in mice; intravenous administration of 6.47 mg of iohexol and subsequent blood sampling (approximately 10 µL each) after the infusion at times (15, 35, 55 and 75 minutes) with determination of iohexol by HPLC-UV on the blood and with factor correction equal to 0.89. The advantage of both methods was represented by the fact that they were comparable in their results not only in mice with normal renal function but also in mice with CKD and with a single kidney following nephrectomy [16].

This method has the advantage of using a small quantity of blood, approximately 10 µL, offering the advantage of carrying out serial samples over time to evaluate the progress of renal function.

Rodríguez-Rodríguez AE et al. have proposed the possibility of using dried blood samples (DBS) while maintaining adequate precision in sample processing [16]. The method consisted of sampling 5 µL of blood with heparin tubes at times 15, 30, 45, 60, 75 minutes after the infusion of Iohexol and subsequent drying of the blood sample on filter paper (Whatman 903, GE Healthcare) to 24 hours and subsequent extraction with 5% perchloric acid with centrifuge [18]. The measurement of Iohexol

was carried out with the HPLC method; this procedure showed high precision in the determination of GFR in mice.

Turner established a new method of determining GFR using Iohexol with two blood samples and compared it to better known methods such as inulin, creatinine and cystatin-C [19]. Intravenous infusion of 25 mg/kg of Iohexol was performed and blood samples were taken at times 2, 5, 10, 20, 30, 60, 90, 120, 180, 240, 300 minutes; the result shawed that the samples taken at the 30 and 90 minute periods represented the average of the values of all eleven blood samples. Thus, Iohexol was proposed as a method to determine GFR through a single intravenous infusion of 25 mg/kg of Iohexol, with subsequent measurements taken within 30 and 90 minutes.

Iohexol represents a precise method for measuring GFR however it may have measurement errors due to sample preparation**.**

Conclusions

The study of the various methods for calculating GFR is still a topic of study today so that we can achieve a simple, rapid and reproducible measurement in every peripheral structure. The ideal method should avoid 24-hour urine collection, reduce the amount of blood, avoid radiolabeled substances and speed in sample calculation. We have listed several types, each with potential disadvantages. Creatinine and cystatin-C, despite being widely used, sometimes have limitations in determining the real GFR. Radiolabeled markers (99mTc-DTPA and 51Cr-EDTA) are cheap but unsafe and should be replaced with an alternative method. Inulin represents the most precise method but is difficult to reproduce in a clinical environment due to the costs and complexity of the procedure. Iothalamate is less precise than inulin but more convenient and easier to use. Iohexol represents a precise and safe method but to date it has been studied in mouse models. An alternative may be represented by fluorescent markers such as FITC inulin or FITC sinistrin, also used in the transcutaneous method with the advantage of instantaneous measurement and no use of optimal methodical blood sampling in animals [6]. In conclusion, the method for measuring GFR should depend on the care setting, the resources available, the experience of the researcher and the safety and well-being of the animals.

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