

Serum Creatinine: Not So Simple!

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Abstract

Measuring serum creatinine is cheap and commonly done in daily practice. However, interpretation of serum creatinine results is not always easy. In this review, we will briefly remind the physiological limitations of serum creatinine due notably to its tubular secretion and the influence of muscular mass or protein intake on its concentration. We mainly focus on the analytical limitations of serum creatinine, insisting on important concepts such as reference intervals, standardization (and IDMS traceability), analytical interferences, analytical coefficient of variation (CV), biological CV and critical difference. Because the relationship between serum creatinine and glomerular filtration rate is hyperbolic, all these CVs will impact not only the precision of serum creatinine but still more the precision of different creatinine-based equations, especially in low or normal-low creatinine levels (or high or normal-high glomerular filtration rate range).

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Introduction

Serum creatinine is one of the most commonly measured products in clinical chemistry laboratories worldwide. The analysis of this product is not expensive too.

For these reasons, this measurement is often considered easy and reliable. However, as we will describe in this review, measuring serum creatinine is not free of problems. Like every analyte, the measurement of creatinine in serum is prone to different types of error, interferences and imprecision [1–4]. From a clinical perspective, nephrologists know too well that one important limitation of this measurement is due to the fact that serum creatinine will vary not only with glomerular filtration rate (GFR) but also with muscle mass, because it is a product of muscle catabolism [2, 3, 5]. This dependency on muscular mass will make the renal interpretation of creatinine results difficult in patients with extremely low or high muscular mass (e.g., anorexia, obesity or weight lifter) [6, 7]. Probably still more important in daily clinical practice is that this association with muscular mass explains why similar serum creatinine values will correspond to different levels of GFR in patients (or subjects) of different age, gender or ethnicity [2, 8, 9]. Other limitations can be briefly discussed. First, creatinine is secreted by tubules, and this explains why creatinine clearance overestimates true GFR. Still more problematic is that this overestimation is quite unpredictable and might vary in the same patient with declining GFR [2, 10–12]. Drugs can also interfere with this tubular secretion, the most well-known being trimethoprim and cimetidine. This can lead to an increase in serum creatinine values when GFR remains constant [12, 13]. Second, serum creatinine can be influenced by diet. Meals rich in proteins

such as cooked red meat can increase the serum creatinine. The GFR itself also increases with such food intakes [2, 5, 14–16]. Third, some authors have described extra-renal clearance of serum creatinine, possibly by intestinal bacteria, which could be relevant in advanced chronic kidney disease (CKD) [17]. Finally, the production of creatinine, from muscular creatine, could be influenced negatively in severe hepatic disease and positively in rhabdomyolysis [2, 18]. We can name these types of interactions or sources of imprecision as “physiologic limitations” of serum creatinine. In the rest of this editorial, we focus on analytical errors or imprecision in the creatinine measurement.

Reference Intervals for Serum Creatinine

As there are age and gender differences in creatinine generation, the determination of population-based normal reference intervals has been the subject of detailed studies by Pottel et al. [8] and Ceriotti et al. [9], who determined age/gender-based intervals for children, adolescents, adults and older adults, particularly for Caucasians. Less detailed information is available for other ethnicities [19]. After birth, serum creatinine rapidly decreases to a value of approximately 0.25 mg/dL during the first month of life and it then starts to increase linearly with age. Serum creatinine remains constant for the average healthy subject between 20 and 70 years of age, with a mean of 0.90 mg/dL and normal reference interval (0.63–1.16 mg/dL) for (white) men and with a mean of 0.70 mg/dL and normal reference interval (0.48–0.93 mg/dL) for (white) women. Above the age of 70 years, serum creatinine starts to slowly increase again in both genders. These reference ranges may serve as a first tool to warn the presence of a possible kidney dysfunction. However, it has been argued that these population-based normal reference ranges are not very useful for the early detection of kidney impairment due to nephrotoxic drugs [20]. It was claimed that when an individual’s serum creatinine-level increases but remains within the population-based reference interval, it may still be indicative as an early warning signal for an upcoming kidney dysfunction. Sottas et al. [20, 21] proposes to use the percentage of change from baseline for each individual. The availability of longitudinal individual serum creatinine measurements (i.e., serial measurements) may allow the chance to move progressively from population-based to patient-based reference intervals, allowing faster and more accurate decision-making on

the individual’s kidney function and possible early detection of kidney dysfunction and early referral to the nephrologist.

Estimating GFR Equations: A Solution That Generates Other Issues

It could be considered controversial in 2017 to assert that the estimating GFR (eGFR) by creatinine-based equations does not contain more information than the biomarker concentration itself, even if some authors have already claimed this [22, 23]. It is true that the use of eGFR allows one better to take into account the variation of serum creatinine due to ethnicity, gender and age, these being the variables in the current eGFR equations [24–28]. The relationship between serum creatinine and GFR is hyperbolic. Converting serum creatinine to eGFR results in a scale that allows for easier interpretation of the decline in kidney function. As an example, in a 60-year-old Caucasian male subject, a serum creatinine value increasing from 0.6 to 1.2 mg/dL ($\Delta 0.6$ mg/dL) will have had a decrease in eGFR (using the CKD-Epidemiology Collaboration (CKD-EPI) equation) of 109–65 mL/min/1.73 m² ($\Delta 44$ mL/min/1.73 m²). For the same patient with a baseline serum creatinine of 1.2 mg/dL, the same increase of $\Delta 0.6$ mg/dL (to 1.8 mg/dL) will correspond to a decrease of eGFR of “only” $\Delta 25$ mL/min/1.73 m² (from 65 to 40 mL/min/1.73 m²) [29, 30]. In other words, a negative exponent (more or less close to “–1”, which corresponds to the “inverse”) is applied in the current recommended eGFR equations to better reflect the true mathematical association between GFR and serum creatinine. However, this mathematical construction is not flawless. Indeed, this inverse relationship also has consequences for the error or imprecision in eGFR calculated from serum creatinine measurements and so the variability of serum creatinine has a serious impact on the variability of eGFR results. Recent recommended equations include only variables such as age, gender and ethnicity [24–28], whereas previous studies also considered weight [31]. Therefore, the variability in serum creatinine explains close to 100% of current eGFR variability in longitudinal studies or in studies with repeated eGFR measurements (on a relatively short period) [32, 33]. Indeed, age will not change more than once a year, whereas change of gender is quite exceptional. If eGFR has some advantages, we must be careful and remind that eGFR can also amplify errors included in the serum creatinine values, as we illustrate in the next paragraphs.

How to Measure Serum Creatinine

Are There Differences between Methods? Are These Differences Relevant?

Serum creatinine can be measured either by enzymatic or the so-called Jaffe methods [1–4, 34]. Both are colorimetric methods. In Jaffe methods, serum creatinine reacts with picrate to give a yellow-orange colour that can be quantified. This reaction is, however, not fully specific, as picrate can also react with other components, known as pseudo-chromogens (acetoacetate, pyruvate, keto-acids, proteins, glucose, and ascorbic acid) [2–4, 35]. The Jaffe assays are also prone to other complex interactions with bilirubin or even specific drugs [36]. Different technical improvements in the last decades have increased the precision of the Jaffe assays (kinetic and/or rate-blanked assay, compensated Jaffe assay etc.). It is beyond the scope of the present work to describe these techniques into details, but whatever the improvement, a certain degree of imprecision still remains [4]. Enzymatic assays are based on different and successive enzymatic reactions [3, 4]. Enzymes used will vary according to the manufacturer. The analytical specificity and the sensitivity of enzymatic assays are better than for Jaffe assays. Comparing both methods, the analytical precision (CV_A for analytical coefficient of variation) is systematically better for the enzymatic assays [36, 37]. Importantly, for low creatinine concentrations as may occur in children [36, 38]), the results of the serum creatinine with the Jaffe reaction will be higher than with the enzymatic assay. In terms of precision, the added value of the enzymatic assay is important in samples with low or normal to low creatinine concentrations. Therefore, enzymatic assays should be favoured in specific populations like in paediatric patients or patients with hyperfiltration but also in specific situations where Jaffe assays are known to be subject of interferences (bilirubin, ketoacidosis etc.). The gain in precision (i.e., a smaller random error) with the enzymatic assays as compared to Jaffe assays is an intrinsic characteristic of the assay and is totally independent of the standardization procedure, which improves the systematic error (for both Jaffe and enzymatic assays). On the other hand, the added value of enzymatic assays compared to Jaffe assays is quite negligible in higher serum creatinine ranges.

Both for enzymatic and Jaffe methods, different assays are available on the market from different manufacturers. Before standardization, each assay had its own characteristics and each assay was calibrated with specific material provided by the manufacturer. For example, different

Jaffe assays would lead to different serum creatinine results [39–43]. Compared to non-calibrated assays, using calibrated creatinine (and creatinine-based equations specifically developed for such standardized assays) leads to a modest but significantly better performance for eGFR [44]. However, harmonization of creatinine measurement between laboratories is especially important in population studies. Indeed, the lack of standardization between assays (or inter-assay variability) has significant consequences on our knowledge of CKD prevalence [45–47]. But it also has an impact on the longitudinal monitoring of renal function in individuals. For a specific individual, the systematic difference could reach 0.2 mg/dL, which is not negligible. Thus, for a 60-year-old man, a Jaffe assay could give a result of 1.12 mg/dL, whereas the same sample assayed with another Jaffe could give 1.32 mg/dL. The corresponding eGFR results will be 71 and 58 mL/min/1.73 m² (with CKD-EPI equation). Because of the hyperbolic relationship between serum creatinine and GFR, the impact of such differences in the creatinine results will be higher for low (paediatrics), normal or close-to-normal serum creatinine values, whereas for high serum creatinine values (low GFR levels), the impact will be negligible. The same example with a serum creatinine of 3.0 mg/dL (and 3.2 mg/dL with the other assay) will give CKD-EPI results of 22 and 20 mL/min/1.73 m², respectively.

Standardization, Traceability, Bias and Precision

The concept of the standardization of creatinine measurement may look simple. The basic idea is that all laboratories calibrate their creatinine assays against calibration material provided by manufacturers for which the creatinine concentration has been determined with a higher order method, namely, tandem mass-spectrometry detectors coupled with liquid or gas chromatographs. Indeed, the measurement of serum creatinine by mass spectrometry is both accurate and very reproducible. Since the Creatinine Standardization Program has requested the manufacturers to standardize their creatinine assays to an isotope dilution mass spectrometry (“IDMS”) reference measurement procedure, we can theoretically expect that the same sample will give the same result in any laboratory in the world, whatever the method (Jaffe or enzymatic) and manufacturer, since the calibrators will all be “traceable” to the higher-order method [41, 48].

But several independent studies have shown that results obtained with so-called IDMS traceable methods (notably Jaffe assays and some dry enzymatic methods) still provide results that were quite far away from the

“true value,” as determined with a reference method [49, 50]. Importantly, this occurs most of the times when dealing with lower creatinine values, whereas, once again, this is the range of values with the largest impact on eGFR variability. To end this paragraph with a more optimistic view, we can assert that most enzymatic assays on the market in 2017 are well calibrated on IDMS [51]. Enzymatic assays have reached the goal to decrease the inter-assay variability and thus to decrease systematic differences (i.e., bias) between assays [52]. However, the systematic error due to the bias inherent to potential lack of calibration is only one part of the potential error linked to the serum creatinine measurement. The second type of error is random error, or imprecision, due to the intrinsic performance of the measurement. This error is expressed by the CV_A . As already mentioned, this error is also lower for enzymatic assays (around 2%) than for Jaffe ones (around 5.5%) [36, 42, 53]. The only way to reduce the CV_A of a given assay would be to perform tests in duplicate or triplicate and to consider the mean of the results. However, this is neither practical nor cost-effective.

Beyond Analytical Variation: The Biological Variation

Analytical variation is not the only source of variability in serum creatinine measurements. Indeed, for every analyte, there is also biological variation expressed in an intra-individual CV (CV_I ; within-subject variation). This variation is physiological, independent of the analytical CV and cannot be reduced [54]. Probably, part of the biological variation in serum creatinine is due to biological variation in “true” measured GFR. Briefly, CV_I is determined by calculating CV on repeated measurements in the same conditions (fasting, same moment of the day) in the same “stable” patients on a relatively short period of time. The CV_I^2 is then obtained by subtracting CV_A^2 from the global CV^2 . The CV_I of creatinine is presented in the literature and is 4.3% (Ricos-Fraser) [54, 55], updated to 5.95% on the Westgard blog (<https://www.westgard.com/biodatabase1.htm>), resulting from the analysis of 28 different studies. An important concept on the variability of serum creatinine is the critical difference or least significant change [56]. The critical difference is the smallest change of 2 results from the same individual that cannot be due to chance. The critical difference is calculated from both CV_I and CV_A ($[= 1.414 \times 1.96 \times (CV_A^2 + CV_I^2)^{0.5}$] [30, 56]. With the Ricos CV_I (4.3%) and CV_A for Jaffe (5.5%) and enzymatic (2%) methods, we calculated the critical difference for serum creatinine as 19 and 13%, respectively. Taking the same example of a 60-year old man,

this means that for a same actual GFR, the serum creatinine concentration of 1.12 mg/dL actually may vary between 0.91 and 1.33 mg/dL if the Jaffe assay is used or between 0.97 and 1.27 mg/dL if the enzymatic assay is used. Using the CKD-EPI equations, this range of non-different serum creatinine values is converted to eGFR values that may vary between 58 and 92 mL/min/1.73 m² for Jaffe serum creatinine and between 61 and 84 mL/min/1.73 m² for the enzymatic assay results. The intrinsic variability of creatinine is thus not so negligible when it is used in the eGFR equation. The relevance of this variation will be, once again, important in adults and especially in children with normal or close to normal serum creatinine values.

How Could We Still Decrease the Variability in eGFR?

To decrease the analytical component of creatinine variability, a relatively simple recommendation is to use enzymatic assays (to decrease the random error) and IDMS traceable assays (to decrease the systematic error). This recommendation is especially simple because most enzymatic methods have shown to be effectively calibrated to IDMS [37, 51]. However, even with the lowest possible CV_A (around 2% for usual assays), the error due to CV_I still remains. To overcome this problem, a possible solution could be to use other biomarkers than serum creatinine. Cystatin C and beta-trace protein are 2 possible alternatives [57–60]. However, these 2 markers have their own variability. For cystatin C, large efforts both from experts and manufacturers have led to a better standardization of the measurement (with the development of an international certified reference material ERM-DA471/IFCC provided by the International Federation for Clinical Chemistry and Laboratory Medicine to manufacturers [61] or the development of mass spectrometry method to measure cystatin C [62, 63]) [63, 64]. Such standardization between assays does not exist for beta-trace protein. Regarding cystatin C, CV_A but also CV_I are basically not different from serum creatinine measured by enzymatic assays (with a critical difference of 13%) [65] and the hyperbolic relationship with GFR is also true for cystatin C [57, 66, 67]. If we consider the same critical difference for creatinine and cystatin C, the effect of the variability of cystatin C on cystatin C-eGFR will be even slightly higher, as the exponent applied for cystatin C in an eGFR-equation is even slightly higher than that used for creatinine. The same 60-year old man with a plasma cystatin C at 1 mg/L will have an eGFR of 78 mL/min/1.73 m² (with the CKD-EPI equation-based on cystatin C only [57]), but could have lab result values of 0.87–1.13 mg/L, which

correspond to eGFR ranging from 66 to 94 mL/min/1.73 m². More interestingly is the use of eGFR including different biomarkers, the most known being the equations using both creatinine and cystatin C [27, 57, 66, 67]. Basically, in these equations, the exponent applied to each biomarker is logically lower than the exponent applied when a single biomarker is used, but because the biomarkers (with their exponent) are multiplied in the combined equation, the mathematical effect is more or less the same than for eGFR with a single biomarker. However, there is a lower probability that variability of both biomarkers are simultaneously and extremely affected by the same analytical variability (but also by the same non-GFR determinants [68]), forcing the eGFR-prediction to drift away from the true GFR. In fact, there is higher chance that the errors inherent to each biomarker compensate each other. This analytical lower error is a possible explanation, among others, for the better precision (i.e., lower random error) observed with combined equations. Taking the example of the 60-year-old man with a creatinine and cystatin C concentration of 1.12 mg/dL and 1.0 mg/L, respectively, and using a critical difference of 13% for both parameters, the eGFR value will be 75 mL/min/1.73 m², but the range will be between 64 and 90 mL/min/1.73 m², this range not being very different than the one observed with only one biomarker-based eGFR prediction. However, the risk that both biomarkers randomly change or vary to “extreme” values (to 1.33 mg/dL for creatinine and to 1.13 mg/dL cystatin C) at the same time, by pure chance, is extremely low.

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Conclusions

In this editorial, we have briefly reviewed the well-known physiological reasons that make the serum creatinine an imperfect GFR biomarker. Beyond these physiological reasons, there are also purely analytical reasons for the imprecision of serum creatinine and still more in the imprecision of eGFR. Enzymatic methods and the use of combined biomarkers are probably useful to improve the precision of the eGFR equations. Several data have yet confirmed this point [69–71]. But we do not know if the added value of both strategies (enzymatic and/or combined biomarkers) to estimate GFR at the individual level or in a population is sufficient enough to justify the higher cost of these methods or strategies compared to one basic Jaffe creatinine measurement. Such strategies could be useful in large clinical trials, especially in cohorts without measured GFR results, but their true added value still needs to be better characterized [72].

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Disclosure Statement

The authors declare they have no conflicts of interest.

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