Did Creatinine Standardization Give Benefits to the Evaluation of Glomerular Filtration Rate?

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ABSTRACT

During the last decade, a lot of efforts has been made to improve the evaluation of renal functions. Measured Glomerular Filtration Rate (GFR) remains the only valuable test to confirm or confute the status of chronic kidney disease (CKD) and is recommended by Kidney Disease Global Outcomes guidelines when estimation of GFR is not reliable. However, in routine clinical practice, serum creatinine remains the one of the most prescribed biological parameters and is an undeniable factor, alone or in association with other parameters, of the estimation of GFR. Since many years, a great improvement in the creatinine measurements was realized because of the standardization of the methods and fabrication of an international standard with concentration near to physiological ones (SRM967). Standardization according to Isotopic Dilution Mass Spectrometry dramatically improves the analytical performances of creatinine assays resulting in a more accurate estimation of GFR using creatinine based equations. Indeed, the standardization of creatinine improves the analytical performance by reducing the bias and removing the influence of the interfering substances.

However, biological variability of creatinine is not affected by analytical standardization and remains a limitation to the use of creatinine in some selected populations, having extreme ages or weights like children, elderly subjects, obese or malnourished populations. Standardization of creatinine assays result in a clear improvement of estimated GFR in general population but alternative methods should be used when creatinine production or metabolism is impaired.

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INTRODUCTION

Today, serum creatinine (SCr) is still one of the most prescribed analyses in medical laboratories to estimate the glomerular filtration rate (GFR) [1] and it is now recommended to integrate its value in a predictive equations. But creatinine is still used in some parts of the world to evaluate kidney function. Since methods for measuring SCr is potentially prone to several interferences, e.g. with bilirubin or pseudochromogens [2-4], the imprecision of the SCr measurement has been improved from the initial manual Jaffe method with important innovations. Earlier in the 1970s, the automatization of the methods began [5-7], followed by the development of kinetic measurements and by the emergence of enzymatic methods, almost free from interference by pseudochromogens like proteins [2-4, 8, 9]. Finally, the development of GC-IDMS or LC-IDMS as reference methods allowed the emergence of IDMS traceable assays [10].

However, limitations of creatinine as a potential GFR biomarker is not restricted to analytical considerations. First, creatinine levels are dependent of muscle mass since creatinine is a product of muscle catabolism of creatine phosphate [11, 12, 13]. Extremely low or extremely high muscular mass could result in a misinterpretation [14, 15]. Secondly, a tubular secretion of creatinine exists and this secretion could be responsible for an overestimation of GFR especially during the course of chronic kidney disease [11, 16-18]. Third, Serum creatinine can also 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 [11, 13, 19-21]. Fourth, some authors have described extrarenal clearance of serum creatinine, possibly by intestinal bacteria, which could be relevant in advanced chronic kidney disease (CKD) [22]. Finally, the production of creatinine, from muscular creatine, could be influenced negatively in severe hepatic disease and positively in rhabdomyolysis [11, 23].

These sources of imprecision are “physiologic limitations” of serum creatinine and one can only be conscious of them. But the standardization of methods is actually required for reducing analytical errors like bias in the creatinine measurement. We present here the actions made during the last decade resulting in standardization of creatinine measurements and their possible consequences on GFR estimation.

HOW CAN WE STANDARDIZE CREATININE MEASUREMENT METHODS?

The concept of the standardization of creatinine measurement was simple. The Creatinine Standardization Program was created by NKDEP’s Laboratory Working Group in collaboration with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the European Communities Confederation of Clinical Chemistry (now called the European Federation of Clinical Chemistry and Laboratory Medicine) to reduce interlaboratory variation in creatinine assay calibration. The National Institute for Standards and Technology (NIST) has released a standard reference material (SRM 967 Creatinine in Frozen Human Serum) for use in establishing calibrations

for routine creatinine measurement procedures, with demonstrated commutability with native clinical specimens in routine methods. These materials were value-assigned with the gas chromatography (GC) -isotope dilution mass spectrometry (IDMS) and liquid chromatography (LC)-IDMS reference measurement procedures [24]. A concentration of 88,4 μmol/L (1mg/dL) was chosen since this value is comprised in the critical range 1.0-1.5 mg/dL that allows clinical laboratories to verify that method performances follow recommendations (Total error in creatinine measurement should not increase the variability in eGFR more than 10% in eGFR at a serum creatinine concentration of 1.0 mg/dL) [3]. A new SRM 967a was prepared with two sub-pools, with one having normal levels of creatinine (Level 1, 0.8 mg/dL±0.1 mg/dL), and the other spiked with crystalline creatinine to achieve an elevated level of creatinine (Level 2, 4.0 mg/dL±0.2 mg/dL) to explore a wide range of creatinine values.

Since the Creatinine Standardization Program has requested the manufacturers to standardize their creatinine assays to an 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 [25, 26].

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 [27, 28]. 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. Finally, we can assert that most enzymatic assays on the market in 2017 are IDMS-calibrated [29]. Enzymatic assays have reached the goal to decrease the inter-assay variability and thus to decrease systematic differences (i.e., bias) between assays [30]. However, the systematic error due to the bias inherent to calibration is only one part of the potential error linked to the serum creatinine measurement.

WHY CREATININE STANDARDIZATION LED TO REDUCING INTERFERENCES IN CREATININE METHODS?

The first goal to reach when you try to standardize a method is to find a process which allows you to get a specific method. Two types of methods are used to determine creatinine concentrations: enzymatic and Jaffe’s methods. Both are colorimetric methods but since the first ones are using enzymatic reactions, they are more specific than the Jaffe’s ones [27].

In 1886, Jaffe [31] described complex formation between picric acid and creatinine in an alkaline environment. Since then, several colorimetric methods based on Jaffe’s observation were commercialized [32]. The total error budget of colorimetric methods was rather due to bias than to imprecision, in particular for low creatinine concentrations. This bias is due to the analytical interference by pseudo-chromogens for the Jaffe group [33] or to the calibration used in the dry chemistry method [34]. The earlier processes to reduce the interference of pseudo-chromogen effect of proteins [35] on the reactions based on alkaline picrate were deproteinization or dialysis. Today, however, analyzers use untreated serum or plasma, making creatinine assays using alkaline picrate reaction prone to the so-called ‘‘protein error’’ [33]. On average, this effect produces a positive difference of 27 μmol/L creatinine compared with enzymatic methods [33]. Moreover, before standardization, each assay was calibrated with specific material provided by the manufacturers

and particular processes. For example, different Jaffe assays would lead to different serum creatinine results [3, 25, 34, 36, 37]. Compared to non-calibrated assays, using IDMS traceable creatinine (and creatinine-based equations specifically developed for such standardized assays) leads to a modest but significantly better performance for eGFR [38].

However, harmonization of creatinine measurement between laboratories is especially important in population studies and on the longitudinal monitoring of renal function in individuals, with great influence on the establishment of reference intervals. Ceriotti et al., when trying to identify universally applicable reference intervals for creatinine via a systematic review of the literature, concluded that only data obtained with enzymatic assays had to be considered because of the higher specificity of this analytical approach [39]. They explained their choice because the subtraction of 18–25 µmol/L to eliminate protein-related unspecific interference on alkaline picrate assays significantly improves the correlation of these assays with enzymatic ones. In this situation, the obtained reference intervals are very similar to those of the enzymatic methods. However, on individual samples, especially at the low creatinine concentrations found in children, large differences can be seen.

Indeed, since the relationship between sCr and eGFR is actually exponential, it implies that small sCr differences will greatly impact the GFR values at low SCr values (corresponding to high GFR values) but the same difference will have minimal impact at high SCr values (corresponding to low GFR values). Therefore, if we consider an analytical error of 17.6 µmol/L in creatinine measurement for a 60 year-old man presenting a creatinine value of 98.6 µmol/L, this value is not different from 116.2 µmol/L. The corresponding GFR values with the CKD EPI study equation will be 71 or 58 ml/min/1.73 m2, respectively. The same example with a serum creatinine of 264 µmol/L and 281.6 µmol/L with the other assay will give CKD-EPI results of 22 and 20 mL/min/1.73 m2, respectively [3, 40-43]. A relative low analytical error of 17.6 µmol/L creatinine can therefore be responsible for a misclassification in the staging of CKD.

Is standardization responsible for the improvement of the imprecision of creatinine assays?

Comparing the analytical imprecision of both methods, the coefficient of variation (CV) is systematically better for the enzymatic assays [2, 44]. For low creatinine concentrations presented by children [2], the serum creatinine concentrations measured with the Jaffe reaction will be higher than with the enzymatic assay. Therefore, one may prefer enzymatic assays in specific populations like in children or in patients with hyperfiltration but also in specific situations where some Jaffe's methods are subject to interferences like bilirubin, ketoacidosis etc.

The gain in imprecision (due to 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 only improves the systematic error.

DID STANDARDIZATION GIVE BENEFIT TO EGFR?

Another source of variability of creatinine is biological variation expressed in an intra-individual CV. This variation is physiological, independent of the analytical CV and cannot be reduced by standardization [44].

Indeed, when combining the intra individual CV (5.95%) and analytical CV for Jaffe (5.5%) and enzymatic (2%) methods, in a 60-year old man, this means that for a given GFR, the serum creatinine concentration may vary for a creatinine

concentration of 88.4µmol/L between 80.1 and 117 µmol/L if the Jaffe assay is used or between 85.4 and 111.8 µmol/L if the enzymatic assay is used. Using the CKD-EPI equations, this range of non-different sCr values leads 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 with normal or close to normal serum creatinine values and especially in children.

CONCLUSION

Standardization of creatinine assays is effective in 2017. This improvement in creatinine measurements has decreased the analytical component of creatinine variability and for assessing the transferability of creatinine results, a relatively simple recommendation is to use enzymatic assays (to decrease the random error) and IDMS traceable assays (to decrease the systematic error). Today enzymatic methods have shown to be effectively calibrated to IDMS [29, 44]. However, with an analytical imprecision of 2% (for usual assays), the error due to intra-individual biological variation still remains. Thus, to overcome this limitation in selected populations (extreme age or body size, muscle diseases including severe denutrition, vegetarian diet…) recommendation is to measure GFR [1].

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