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9. GFR - WHERE ARE WE NOW?

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9.1 Abstract

The availability of a worldwide standard for creatinine is an important milestone for the improvement of GFR estimations for adults. However, an unacceptable interlaboratory variation is still observed which is mainly due to differences in calibration. In adults, the MDRD formula allows to obtain a reliable GFR estimation. Systematic reporting of eGFR by clinical laboratories helps to identify patients at risk for developing end stage renal failure. Care has to be taken when using estimated GFR values for drug dose adjustment. The use of enzymatic creatinine assays is recommended. Updating the currently used estimation formulas for calculating GFR in children is far from easy.

Low molecular mass marker proteins like Cystatin C and beta trace protein can be regarded as an attractive practical alternative for assessing GFR since they only require a determination in serum or plasma and are better suited in the blind range of creatinine.

9.2 Introduction

Determination of serum or plasma creatinine concentrations are of importance because of its central role in the assessment of renal function and the use of creatinine values for estimation of glomerular filtration rate (GFR) (1). For adults, estimating equations have been developed from the Modification of Diet in Renal Disease (MDRD) Study (2). The recent availability of the international NIST SRM 967 creatinine standard means an important milestone in the further improvement of GFR estimation (3). For adults, an improved GFR-estimating equation based on serum creatinine values traceable to IDMS reference measurement procedures has been recently presented (4). Clinically validated adaptations of creatinine-based formulas for estimating GFR in children are about to be published.

On the other hand, evidence is growing that serum concentrations of low molecular mass marker proteins can be considered as an interesting alternative for estimating renal function (5). In the present review, the various possibilities for assessing GFR are discussed.

9.3 Exogenous markers

Reference values for GFR are often expressed as a value adjusted to adult ideal body surface area. These values work well for many clinical situations, but in subjects with an atypical body mass, they may not accurately reflect renal function.

The reference method to determine GFR is the urinary clearance of inulin during a continuous intravenous infusion. Alternatively, the plasma clearance of inulin can be determined, which does not require urine collection (6). Similarly, iothexol and iothalamate are radiographic contrast agents that can be used as exogenous GFR markers comparable to inulin and Cr⁵¹-EDTA (7). They can be measured by HPLC. Exogenous markers are very accurate but are expensive and rather impractical and therefore mainly restricted to research use.

9.3.1 Creatinine assays

Creatinine is by far the most commonly used biochemical marker of renal function. The commonest principle for assaying creatinine is the so-called Jaffe reaction (11). Since Jaffe only observed a complex formation between picric acid and creatinine in alkaline environment in 1886 and never described an analytical method, variation amongst "Jaffe method" recipes is broad (8). The analytical bias of current creatinine methods is still disappointing: the liquid enzymatic based and the compensated Jaffe method showed a small positive bias, whereas a major positive bias was observed for the creatinine iminohydrolase (9) and the uncompensated Jaffe method (9). This bias is due to the analytical interference by pseudochromogens for the Jaffe group and the calibration used in the dry chemistry method (9). Interlaboratory variation for creatinine is still unacceptably high; which leads to an unacceptable variation in the estimation of kidney function.

9.3.2 Global creatinine restandardization

The NKDEP, CAP, and NIST have collaborated to prepare a human serum-creatinine reference material with acceptable commutability with native clinical specimens. These materials are value-assigned with the GC-IDMS and LC-IDMS reference measurement procedures (3). The materials are designated NIST SRM 967. Implementing traceability of serum creatinine assays to GC- or LC-IDMS will lead to changes in the clinical decision-making criteria currently used for serum creatinine concentrations and creatinine clearance. In 2008 - 2009, the process of implementation of the new ID-MS standardization by the IVD industry is ongoing. Use of serum creatinine concentrations or equations to estimate GFR requires knowledge of the calibration of the serum creatinine assay (10).

9.3.3 Correcting for non-specificity based on average values for adults

In the earliest manual methods, serum creatinine was assayed by the Jaffe reaction after deproteinisation, eliminating the pseudo-chromogen effect of proteins (11). Early automated methods used dialysis membranes to prevent interference from plasma proteins. Today, analyzers use undiluted serum and plasma, making them prone to the "protein error" in the alkaline picrate reaction (11). In the serum of adults, this effect produces a positive difference of about 27 $\mu\text{mol/L}$ creatinine compared with HPLC or enzymatic methods (11). Because urine contains relatively little or no protein, the protein error affects only creatinine determinations in serum or plasma.

Therefore, creatinine clearance is underestimated when creatinine methods affected by protein error are used. For calculating GFR, this positive bias is greatly compensated by the overestimation attributable to tubular secretion of creatinine, which is relatively more important in children (11).

In order to comply with new regulations, manufacturers of Jaffe based methods can restandardize their creatinine assays using a compensation, a mathematical correction which compensates for analytical non-specificity due to the protein error. Since children have lower reference ranges for total protein, this protein error is considerably smaller in children (11). In consequence, use of restandardized Jaffe-type assays results in overcompensation when used in children or infants.

The enzymatic methods manage to measure the serum creatinine more correctly (9). Due to the elimination of analytical non-specificity in these methods, the lower enzymatic creatinine result (when the result has not been adjusted to Jaffe-like results) leads to a marked increase of creatinine clearance estimations because of the increased effect of tubular secretion on test results. Paradoxically the analytical improvement makes creatinine less suited as a GFR marker in pediatric medicine (12).

When creatinine clearance is measured following administration of cimetidine (a blocker of tubular secretion of creatinine), the effect of tubular secretion can be corrected. The cimetidine protocol allows estimating of GFR in a clinical setting. However it cannot be used on a wide scale.

9.3.4 Calculated creatinine clearance in adults

For adults, the currently recommended GFR estimating equation has been developed from the Modification of Diet in Renal Disease (MDRD) study (2, 4). The coefficients of this GFR estimating equation have recently been adapted for the new ID MS creatinine standardization. Clinicians and laboratorians should therefore be very careful: when using these formulas the coefficients used in the MDRD formula should always match with the creatinine calibration used. It should be noted that the MDRD formula measures GFR which is not exactly the same as the earlier Cockcroft & Gault formula for creatinine clearance estimation. In contrast to the Cockcroft & Gault formula, the MDRD equation does not require the body mass so that it can be preported more easily by clinical laboratories. For estimated GFR values exceeding 60 mL/min, no exact eGFR values should be reported by the laboratories as the uncertainty of the serum creatinine determination is too important in that range. Also in subjects younger 18 or older than 70, the MDRD formula has not been validated. The MDRD formula is excellently suited for detecting patients at risk for developing end - stage renal disease. However, for adjusting drug dose in patients with a reduced renal clearance, the MDRD formula is to be handled with care since the vast majority of available pharmacokinetical data collected during the last three decades have been based upon the Cockcroft & Gault formula (dating from 1976). Relative differences between Cockcroft & Gault and MDRD results are most pronounced in the elderly.

9.3.5 Calculated creatinine clearance in children

The bias in serum creatinine concentration in the lower range is a major concern in pediatrics due to the lower reference ranges for serum or plasma creatinine in infants and children (12). For estimating GFR in children and infants, the Schwartz and the Counahan-Barratt equations are recommended (12, 13). Both provide GFR estimates based on a constant multiplied by the child's height divided by the serum creatinine concentration. The values for the constant used in both equations differ considerably (12). Since these formulas have been validated 30 years ago, reassessment of formulas for estimating GFR using enzymatic creatinine assays is ongoing. Enzymatic creatinine methods are recommended (14). However, it is clear that it will be difficult to develop reliable formulas for compensated Jaffe results.

9.3.6 Cystatin C, a promising alternative

Serum concentrations of low-molecular mass marker proteins are primarily determined by GFR. An ideal marker has to have a constant production rate and should not vary in its concentration in situations with an acute-phase reaction. Cystatin C (Cys C) shares these properties. It is a 13 kDa cysteine protease and is produced by all nucleated cells. In normal conditions, serum Cys C is almost completely filtered by the glomerulus and largely catabolized by the tubules. Since serum Cys C concentration is closely correlated with the GFR, serum Cys C has been introduced as a GFR marker (5). Studies comparing Cys C and creatinine as marker of GFR generally showed diagnostic superiority of serum Cys C vs. serum creatinine concentrations. In the blind range of creatinine, Cys C proves to be a superior marker. Formulas have been developed allowing reliable estimation of GFR based on Cys C (12). Unlike creatinine, serum Cys C reflects GFR independent of age, gender, height, and body composition. Because of its low individuality, Cys C has fewer inherent limitations as a screening test for detecting deteriorating GFR than serum creatinine. However, clinicians should be cognizant of extrarenal conditions (upregulation in certain tumours) and pharmacological factors (e.g. glucocorticoid treatment) that can influence the results of serum Cys C assays (12). Also thyroid dysfunction affects serum Cys C concentration by influencing the production rate of the protein (12). Serum creatinine concentrations are lower in malnutrition and lead to overestimation of GFR, while Cys C levels are unaffected (12).

Cys C-based GFR estimates show significantly less bias and serves as a better estimate for GFR (12). Cys C can be measured using immunochemical methods in a highly reproducible manner. Validation of a candidate primary recombinant reference material by an IFCC working group is ongoing.

9.4 Other protein markers

Beta trace protein (BTP) or prostaglandin D synthase is a glycoprotein with a molecular mass of 23 000–29 000, depending on the degree of glycosylation (5). BTP has been introduced for the measurement of kidney function in the creatinine-blind range. International standardization of BTP is still lacking.

Beta 2 microglobulin (11.3 kDa) has been advocated as a GFR marker (12), but its serum concentration can increase as an acute-phase reactant (5). Beta 2 microglobulin has the disadvantage of being increased in patients with several malignancies, particularly lymphoproliferative disorders (5, 12). Like Cys C and BTP, beta 2 microglobulin has the advantages of age and muscle mass independence (5).

9.5 Conclusions

Despite the stricter regulations and the technical progress in laboratory automation, between-laboratory variation of Jaffe based methods has not decreased over the last decade. Analytical bias in creatinine assays needs to be reduced and non-specificity bias should be improved (9). The creatinine standardization issue has major clinical consequences which are far beyond the significance of the parameter itself. Apart from the conventional calculation of the creatinine clearance, also the calculation of the clearance using derived formulas is a key element in the assessment of renal function and the calculation of the correct dose of many drugs which are characterized by a narrow therapeutic index and a renal elimination mechanism. The MDRD formula is recommended for identifying individuals at risk for developing renal insufficiency. However, care should be taken when estimated GFR values are used for dose calculation of drugs since literature data are still mostly based on the older Cockcroft & Gault formula. Data obtained by Cockcroft&Gault and MDRD equations are not per se interchangeable.

When introducing revised serum creatinine calibration to be traceable to IDMS, laboratories will need to communicate the following to clinicians: the serum creatinine reference interval will change to lower values, calculations of estimated GFR used to adjust drug dosages will be affected by the decreased creatinine values, measured and calculated creatinine clearance values will increase, and the corresponding reference interval will be different.

In view of the difficulties in adapting creatinine assays to the new calibrators in the pediatric concentration range in a uniform way, the low molecular mass proteins Cys C and BTP offer promising alternatives for calculating GFR in children. In comparison with serum creatinine, these proteins have a better diagnostic sensitivity for detection of impaired GFR (12). Although some caveats have to be taken into account when interpreting test results, protein-based GFR calculations only require serum values. The progress in the standardization of these protein assays will enable the wide-scale use of these methods.

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