Review

The usefulness of cystatin C and related formulae in pediatrics

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Abstract

Serum creatinine does not share the properties of an ideal marker of glomerular filtration rate (GFR) like inulin, but continues to be the most widely used endogenous marker of GFR. In the search of a better biomarker of GFR, the small molecular weight protein cystatin C has been introduced with features more similar to that of inulin, such as constant production and no non-renal elimination. However, it has not enjoyed widespread use despite its significantly improved diagnostic performance in the detection of impaired GFR and its independence of body composition. A variety of formulae based on either cystatin C or creatinine or both have been developed to estimate GFR. We summarize the currently used methods of GFR measurement, their limitations and analytical errors. The review also summarizes the history, features and the feasibility of cystatin C measurements as well as the most widely used formulae for the estimation of GFR in children. The diagnostic performance of the cystatin C derived eGFR formulae at various levels of GFR is also discussed. An eGFR formula derived from pooled studies analyzing both creatinine and cystatin C, and using a biology-based mathematical approach may be advantageous.

Keywords: chronic kidney disease; creatinine; cystatin C; estimated glomerular filtration rate (eGFR).

Introduction

It is widely accepted that kidney function is best measured as glomerular filtration rate (GFR) (1). GFR cannot be measured directly. However, if a substance in the plasma is in a stable concentration, physiologically inert, and freely filtered at the glomerulus, but is not secreted, reabsorbed, synthesized, or metabolized by the kidney, the amount of that substance filtered at the glomerulus is equal to the amount excreted in the urine (2). The gold standard for the GFR measurement is inulin clearance (3). In 1934, Richards found that the polysaccharide inulin (a fructose polymer made from the Jerusalem artichoke) is freely filtered through colloidion membranes but not absorbed, while studying water reabsorption in the renal tubule of amphibians (4). Inulin shares many features of an ideal marker of glomerular filtration, namely exclusive elimination by glomerular clearance with no tubular secretion and no non-renal excretion (5).

Inulin clearance

Inulin clearance is widely regarded as the gold standard for measuring GFR. The classic method of measuring inulin clearance requires an intravenous infusion with timed urine collections over a period of several hours. This is costly and cumbersome. In the traditional way developed by Homer Smith (6), an inulin bolus is given intravenously to increase the concentration in the extra-cellular water that needs to be equilibrated. This is then followed by an intravenous infusion for a period of several hours until a steady concentration in the plasma is obtained. In clinical practice, this is rarely achieved because the amount of inulin infused has to be estimated using surrogate markers of the renal function. This process can over- or under-estimate the amount of inulin that is required. Accurate urine collections are necessary over a period of a few hours. Ideally, the bladder should be catheterized and emptied, and saline washout and air displacement should be used at the end of each collection period. To minimize collection errors, several collection periods are required, typically three times per hour. In addition, there are considerable difficulties with the measurement of inulin. Reliable methods using reversed-phase high-performance liquid chromatography have only been published after the millennium (7). Because measuring inulin clearance is cumbersome and there are methodological problems with its accurate measurements and high inter- and intra-assay variability if methods other than mass spectrometry are used (5), alternate methods of measuring GFR have been developed.
Nuclear medicine GFR methods

Nuclear medicine techniques of GFR estimation have been in use as a replacement for inulin clearance since the 1970s (2). The main advantage of using a radiolabeled compound with characteristics that are similar to inulin is its immediate determination by counting the radioactivity. Today’s nuclear medicine methods form a new standard for GFR measurements because of its easily assayed, radiolabeled and stable compounds that meet the criteria of a good GFR marker, namely clearance only by glomerular filtration without tubular secretion and extra-renal elimination. The most widely used single bolus-injection technique utilizes the intravenous injection of a suitable compound at a precisely known quantity. It is important that no material is injected interstitially and all matter enters the intravascular space, which may be particularly challenging in the case of young children. Extravasion will cause significant overestimation of GFR (8). After the injection, the plasma is sampled from the opposite arm. The measured concentrations are plotted against time (Figure 1).

The different substances used for nuclear medicine GFR measurement are listed in Table 1.

The accuracy of the subsequent calculations are affected by a number of factors, in particular the exact time and the frequency of the sampling. It is generally recognized that at least three sampling points are required (22). Unfortunately, there is poor standardization amongst most centers, and only two-point sampling time points are chosen to reduce the number of venipunctures in children.

Furthermore, most centers utilize only one-compartmental models. A single compartmental model results in some overestimation of GFR (23). Late sampling (4 and/or 5 hour concentrations after injection) could overcome this problem (24), but is rarely implemented. For a more accurate assessment of the extracellular water (especially important in children with altered fluid status, for instance if they are on long-term diuretics), an early sampling point is required (25). Also, if one-compartmental model is utilized, appropriate corrections for the overestimation should be employed (26).

What are the particular problems with GFR measurement in children?

The body of a child is in constant change. To accommodate for this change, GFR is normalized to body surface area, which is considered to be the best denominator, although the extracellular volume is also considered (27). While all nephrons are terminally differentiated at birth, only the juxtaglomerular glomeruli are used at birth and there is continuous recruitment of additional glomeruli until 18–24 months of age. Ideally, GFR should be reported as age-independent z-scores, rather than as absolute GFR or GFR/body surface area (28). In the first year of life, and during puberty, there are growth spurts and rapid increase of muscle mass, requiring special considerations for the calculation of GFR (29), especially in adolescent males (30). The most important determinant of GFR is height, which when taken into account led to the famous Schwartz formula (31), a GFR estimation model based on the height creatinine ratio. Gold-standard measurements of GFR are cumbersome and invasive, and those children at risk requiring frequent GFR monitoring, such as transplant recipients (32) are already subject to numerous other tests. As such, accurate non-invasive measurements of GFR are essential, and endogenous markers are required.

Endogenous surrogate markers of GFR

The earlier stated methods of inulin clearance and nuclear medicine methods are invasive and involve undesirable effects, such as radiation exposure or the need for multiple blood samples and urinary catheterization. Therefore, it has become a preferred practice to use an endogenous marker that is produced at a constant rate, shares the features of inulin, and thus eliminates the need for a compound injection. Popper and Mandel proposed the use of serum creatinine in 1937 (33), which remains to be the most widely used marker for GFR estimation in spite of its shortcomings. This marker remains the most widely used marker for estimation of GFR. Where serum endogenous markers are considered as suitable markers of GFR, the National Kidney Foundation guidelines recommend the use of serum marker based prediction equations for GFR estimation and rules against the use of serum markers alone in the assessment of renal function, as discussed in ref. (34).

Limitations of serum creatinine as endogenous marker of GFR

Serum creatinine is the most widely used endogenous marker to predict GFR. Creatinine is a metabolic product of creatine and phosphocreatine found in muscle and as such reflects
Table 1  Commonly used nuclear medicine radioisotopes or cold exogenous markers for GFR measurement.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Tracer</th>
<th>Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>51Cr-ethylenediamine tetra-acetic acid (EDTA) clearance (9)</td>
<td>51Chrome</td>
<td>Europe, widely studied in children (10, 11)</td>
</tr>
<tr>
<td>99mTc-diethylenetriamine penta-acetic acid (DTPA) (12)</td>
<td>99Technetium</td>
<td>North America, widely studied in children (13, 14)</td>
</tr>
<tr>
<td>125I-iothalamate (15)</td>
<td>125Iodine or cold</td>
<td>North America and Europe, widely studied in children (16, 17), used as subcutaneous infusion in Montreal (18)</td>
</tr>
<tr>
<td>Iohexal (19, 20)</td>
<td>Cold</td>
<td>Scandinavia, North America (21)</td>
</tr>
</tbody>
</table>

muscle mass and varies little from day to day (35). Serum creatinine measurement is widely available at low cost. However, while creatinine may be a feasible marker of GFR in populations with near normal GFR, it has multiple limitations for individuals. There is substantial inter- and intra-patient variability due to differences in muscle mass (36). In childhood, there is age and muscle mass dependency of serum creatinine and accurate assessment of normal GFR even with the use of body length/creatinine ratios (see below) remains difficult. In certain pediatric patient clientele, such as patients with spina bifida (37), neuromuscular disease, anorexia nervosa, or liver cirrhosis, serum creatinine is completely unusable because of the abnormal muscle mass in these children who are often wheelchair bound (38, 39). In addition, the production of creatinine is not constant; the rate of turnover is also variable (40). It is also known that creatinine is also undergoing tubular secretion (41). Creatinine is secreted in the proximal convoluted tubule by active transport similar to that of organic cations. Tubular secretion of creatinine divided by insulin clearance increases progressively from 0.16 in adult patients with normal GFR to 0.92 in patients with GFR <40 mL/min (12). Furthermore, there used to be considerable variability in the reference range for serum creatinine based on the method used for its determination (42, 43). Proficiency testing surveys published periodically by the College of American Pathologists, evaluated the variability in creatinine measurement among various methods used by different institutions across the US and demonstrated substantial variability (44). This will make the validation of older methods to estimate GFR based on serum creatinine, measured by different techniques, subject to debate. Only more recently, the traceability of the creatinine measurements to higher-order reference methods [isotope dilution-mass spectrometry (IDMS) reference method] improved accuracy of creatinine measurements (45). This paper established improved pediatric reference intervals that may be adopted by any laboratory serving a similar population (predominantly Caucasian). Clinical implications of creatinine standardization have recently been reviewed (46).

Overall, the method used for serum creatinine is of high importance. Calculation of GFR based on serum creatinine measured by the alkaline picrate method is limited because of method non-specificity, low values in children, particularly in infants, and lack of appropriate GFR formulae. Therefore, enzymatic methods are preferred, as widely suggested in the literature (47).

However, the problem of tubular secretion of serum creatinine cannot be addressed with the methodology of measurement. Approximately a decade ago, blockade with H2 antagonists revealed promising results that may overcome the significant problem of tubular secretion of serum creatinine (48, 49). The use of cimetidine protocols in children remains scarce (50). Creatinine clearance determinations involving timed urine collections may provide greater accuracy but are difficult for pediatric patients to perform, time-consuming, and impractical for routine use. Only recently small molecular proteins have emerged as potentially superior endogenous markers of GFR (51).

Small molecular weight proteins as markers of GFR

Small molecular mass proteins have long been proposed as markers of GFR as they are normally almost freely filtered through the normal glomerular membrane (52, 53). In a normally functioning kidney these small molecular weight proteins should then be almost completely reabsorbed and degraded by the proximal tubular cells. Several proteins have been tested, such as β-2 microglobulin and β-trace protein [analyzed in children in ref. (13)], however, of all these markers, cystatin C appears to be the most promising.

What is cystatin C?

Cystatin C is a small molecular weight protein that was initially known as γ-trace protein. The amino acid sequence of the single polypeptide chain of human cystatin C was determined in 1981 (54). Two years later, cystatin C was identified as an inhibitor of cysteine proteases after discovering significant homology with the sequence of chicken cystatin – both proteins had a sequence identity of 44% (55).

Cystatin C is produced by all human nucleated cells, since immunochemical and Northern blot studies of human tissues and cell lines have shown that cystatin C and/or its mRNA is present in all investigated cell types (56). Likewise, investigations of the production of cystatin C by human cell lines in culture have displayed that all cell lines that were investigated all secrete cystatin C (57). Determination of the structure of the human cystatin C gene and its promoter has demonstrated that the gene is of the house-keeping type, which indicates
a stable production rate of cystatin C by most nucleated cell types (56). Earlier studies of the serum level of cystatin C in large patient cohorts have failed to correlate the serum level to any pathophysiological state besides those affecting the glomerular filtration rate, which also is compatible with a stable secretion of cystatin C from most human tissues (58). However, very large doses of glucocorticoids have recently been described to increase the production of cystatin C (59, 60) whereas low and medium doses of glucocorticoids do not seem to alter the production of cystatin C (61). Also, thyroid dysfunction can affect cystatin C levels (62), but our group found no association with thyroid function markers in recent studies (63, 64).

The reference values for cystatin C obtained in a carefully selected population are 0.75±0.09 mg/L for children aged 4–19 years, 0.74±0.10 mg/L for males and 0.65±0.09 mg/L for females (aged 20–59 years), and 0.83±0.10 mg/L for older individuals (> or =60 years) (65). In the first year of life, renal function matures physiologically. Accordingly, much higher cystatin C values up to 2.8 mg/L were found at birth. These are subject to a rapid decline after birth reflecting maturation of kidney function (66). Age dependency also has to be considered in adults. New reference intervals with more detailed age distribution from central Europe have recently been published on 985 healthy subjects older than 25 years (67).

Studies of the handling of human cystatin C in rats have shown that the plasma renal clearance of cystatin C is 94% of that of the generally used GFR-markert 51Cr-EDTA and that cystatin C thus is practically freely filtered in the glomeruli (68). At least 99% of the filtered cystatin C is degraded in the tubular cells. When the GFR of a set of rats was variably lowered by constricting their aortas above the renal arteries, the renal plasma clearance of cystatin C correlated strongly with that of 51Cr-EDTA with a linear regression coefficient of 0.99 and with the y-intercept not being statistically different from 0 (69).

After these encouraging studies, additional studies suggested that the reciprocal of cystatin C correlates better with a gold standard GFR measurement than the reciprocal of serum creatinine (70–72). Cystatin C as a marker of GFR was found to be independent of body composition (36, 73, 74). Development of automated and rapid particle-enhanced immunoturbidimetric and immunonephelometric methods, also more precise than the original radioimmuno- or enzyme-linked immunosorbant assays (75, 76), has allowed large-scale use of serum cystatin C as a clinically useful GFR-marker (76).

The diagnostic performance of cystatin C in comparison with serum creatinine was first analyzed in 2002 with a meta-analysis of 46 studies, in both adults and children (77). The pooled data analysis compared correlation coefficients between GFR and the reciprocals of serum creatinine and cystatin C in 3703 individuals and found significantly better correlations (mean r=0.816 [95% confidence interval (CI) 0.804–0.826] vs. mean r=0.742 [95% CI 0.726–0.758]). ROC plots were available for a pooled sample size of 997 individuals, again showing a significantly better area under the [ROC] curve (mean=0.926 [95% CI 0.892–0.960] vs. mean r=0.837 [95% CI 0.796–0.878]). This meta-analysis suggests that cystatin C is superior to serum creatinine for the detection of impaired GFR in cross-sectional studies. More recently, another meta-analysis from 24 studies (n=2007) also confirmed that the diagnostic accuracy favored cystatin C (78), although in this more recent study, the diagnostic odds ratios started to overlap, most probably due to the IDMS testing of serum creatinine.

The need for standardization of cystatin C, similar to the IDMS traceability of serum creatinine

It should be highlighted that problems due to lack of standardization of cystatin C lead to similar problems as was highlighted above with regards to serum creatinine and the need for IDMS traceability (45). Results obtained with the two main assays that are commercially available, namely the DAKO kit (turbidimetric, PETIA) and the Siemens Healthcare assay (nephelometric, PENIA), can be quite different, as recently demonstrated with the publication of reference intervals for healthy term and preterm infants. The results with the PENIA method in our study (79) were significantly lower than those reported by Harmoinen et al. (80). Similar problems occurred when the results of the CKiD study were re-analyzed using PENIA, which lead to substantially better results (81). Recently, Anders Grubb reported the first certified standardized reference material for cystatin C (82). World-wide standardization, as just shown in the previous paragraph, is a powerful tool towards improving the diagnostic accuracy of GFR biomarkers, and the authors of this review are convinced that standardization of cystatin C similar to that of creatinine will further enhance the diagnostic performance of cystatin C.

Endogenous markers as basis for GFR estimation models

Studies have continuously reported the accuracy of serum endogenous marker based GFR estimation models in measuring GFR over the serum levels of such markers alone (34). As such, numerous GFR estimation models have been developed to estimate GFR using creatinine and cystatin C as base markers. Some of these formulae where either creatinine based, cystatin C based or formed by a combination of both markers.

Estimating GFR using creatinine

First and foremost, creatinine standardization is of paramount importance to account for the analytical variability of different creatinine methods. The various formulae cannot be compared if the creatinine measurements are not standardized (46). Fortunately, the National Kidney Disease Education Program (NKDEP), the College of American Pathologists (CAP), and
the National Institute for Standard and Technology (NIST) have collaborated to prepare a serum-creatinine reference material (NIST 967) with demonstrated commutability with native clinical specimens in routine methods. These materials are value-assigned with the gas chromatography-isotope dilution mass spectrometry (GC-IDMS) and liquid chromatography (LC)-IDMS reference measurement procedures (83). Nearly all clinical laboratory methods are now expected to have calibration traceable to an IDMS reference measurement procedure, which may lead to better formulae for the estimation of GFR.

A wide range of creatinine based formulae were developed over the years to estimate GFR. The most commonly used creatinine based formulae are the Cockcroft-Gault (84), the Modification of Diet in Renal Disease (MDRD) formula (85), which later was replaced with a four-variable version and adjusted for standardized creatinine (86) and the chronic kidney disease-epidemiology collaboration CKD-EPI formula (87). The (CKD-EPI) equation was reported to perform with higher level of accuracy than the MDRD equation (87).

In the case of children, the most widely-used formula is the Schwartz-formula (31) together with modifications for adolescent boys (30) and infants (29). The Counahan-Barratt formula may be slightly more suitable for GFR estimation based on serum creatinine in children (88, 89).

It should be emphasized that adult formulae cannot be used in children. This was shown clearly for the Cockcroft-Gault formula (90) and the MDRD formula (91). The bias worsens with younger age. The literature on validation of the CKD-EPI formula in children remains scarce. One study of 850 children and adults aged 5–95 years comes to the conclusion that the formula should not be used for children and youth (92).

Recently, the Chronic Kidney Disease in Children (CKid) study recruited a cohort of approximately 600 children with chronic kidney disease in the USA and Canada that involved creatinine being referenced to IDMS (93). This has led to an improved Schwartz formula (21).

The commonly used creatinine based GFR estimation formulae are summarized in Table 2, some of which were also derived for children.

### Estimating GFR using cystatin C

Recent research has reported cystatin C to be less dependent on muscle mass than creatinine and as a result simpler GFR prediction equations were derived from cystatin C compared to creatinine (13, 70, 96). These models proved to give a more accurate and precise measurement of GFR than creatinine based equations with less bias, irrespective of anthropometric data, especially in identifying patients with GFR below 60 mL/min/1.73 m² (97). Cystatin C based estimation formulae were also shown to correctly classify CKD patients in 61.5%–72.0% compared to 62.1%–64% of the creatinine based formula (97). Some authors have also suggested replacing the classic Schwartz formula with GFR calculated from serum cystatin C in children since serum cystatin C concentrations are only slightly overestimated while creatinine levels show considerable bias (98).

One important aspect is that pediatric formulae based on cystatin C work well in adults, as could be shown for both the Filler formula (98), which was prospectively validated in a cohort of adult renal transplant recipients (99), and Anders Grubb’s formula $GFR = 84.69 \times \text{cystatin C (mg/L)}^{-1.609} \times 1.384$ (if child <14 years) (91).

In contrast, a large number of different cystatin C based prediction models were produced using a plethora of different mathematical models, with the inclusion of different clinical variables, variable methods for the measurement of cystatin C, variations in cystatin C calibrators, and the different ethnic backgrounds (100). Perhaps the most striking differences of the accuracy of different formulae occurred when the same patients were compared using turbidimetric (21) vs. nephelometric methods (81). Based on that study it appears that the Siemens Healthcare cystatin C nephelometry assay

### Table 2

Equations to predict glomerular filtration rate using creatinine.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>GFR = (170) × (Scr)^−0.999 × (age)^−0.109 × (urea)^−0.170 × (albumin)^−0.101 × (0.762 if female) × (1.18 if black)</td>
</tr>
<tr>
<td>b</td>
<td>GFR = (186) × (Scr)^−1.159 × (age)^−0.203 × (0.742 if female) × (1.21 if black)</td>
</tr>
<tr>
<td>c</td>
<td>GFR = [(140–age) × (weight)]/[(Scr^×72) × (0.85 if female)]</td>
</tr>
<tr>
<td>d</td>
<td>GFR = [6700/(Scr × 88.4)] × (weight/4) − (urea/2) − [100/(height)^2] + 35 (if male) or 25 (if female)</td>
</tr>
<tr>
<td>e</td>
<td>GFR = 141 × min(Scr/κ, 1) × max(Scr/κ, 1) − 1.209 × 0.993 × Age × 1.018 [if female] × 1.159 [if black], where κ = 0.7 for females and 0.9 for males. α = −0.329 for females and −0.411 for males, min indicates the minimum of Scr/kor 1, and max indicates the maximum of Scr/k or 1</td>
</tr>
<tr>
<td>f</td>
<td>GFR = (42.3) × (height/Scr)^0.76</td>
</tr>
</tbody>
</table>

As most pediatric formula are a variation of the formula $GFR = a \times [\text{height}/\text{creatinine}]^b$, the CKD formula in its latest iteration is given for children, which provides for 84.3% of eGFR within 30% and 40.4% within 10% of measured iohexol GFR. Age in years, height in meters, Scr (serum creatinine) in mg/dL (convert to SI unit μmol/L multiply by 88.4), weight in kg. a Glomerular filtration rate (GFR) in mL/min/1.73 m². b MDRD, Modification of Diet in Renal Disease, urea in mg/dL (convert to SI unit 0.357 mmol/L multiply by 0.357), albumin in g/dL (convert to SI unit g/L multiply by 10). c GFR in mL/min, weight in kg. d Urea in mmol/L, height in meters. e Height in meters.
may be preferable. There is a need for the development of a standardized cystatin C based GFR prediction model (97). Certainly, the recently developed standardized reference material for cystatin C forms a most important step towards this goal (82).

The commonly used cystatin C based GFR estimation formulae are summarized in Table 3 some of which were also derived for children.

### Combined creatinine and cystatin C based GFR estimation equations

Several other GFR estimation equations were developed using the combination of both serum markers, creatinine and cystatin C. Schwartz and co-workers were able to demonstrate that a cystatin C and creatinine-based eGFR formula (GFR = 41.6 \cdot \text{[height/creatinine]}^{0.443} \cdot \text{[1.8/cystatin C]}^{0.477} ) was able to result in a correlation coefficient of R^2 = 0.843 between the measured and estimated GFR with 90.1% of eGFR within 30% of measured GFR (81). Similarly, Zappitelli et al. also demonstrated the highest accuracy with a formula combining cystatin C and serum creatinine (107). In adults, Tidman et al. also reported a greater accuracy for GFR estimation using combined models than the cystatin C or the Creatinine based estimation equations (108). Also, the combined models have shown to correctly classify CKD more frequently and to have the best performance in those with near-normal renal function (97). Of these well-known formulae are the Bouvet et al. (101) and the Zappitelli et al. (107) in children.

The list of formulae for the estimation of GFR based on cystatin C±serum creatinine is constantly expanding. Several important themes emerge when comparing the various formulae:

- Some formulae are based on cystatin C alone. These formulae are convenient and do not require any additional data in most cases. Some of them have been prospectively validated in adults and in children (99).
- Some formulae use both creatinine and cystatin C (21, 107). These formulae require significant additional information, which may include height, gender and urea. Formulae that include creatinine require more data, such as gender, ethnicity etc. While formulae with cystatin C alone can be quite useful for both children and adults, there is not much point in using a formula based on both creatinine and cystatin C for adults and children, similar to creatinine-based eGFR formula (90).
- A wide variety of “gold-standard methods” have been employed to derive the eGFR formula. This in itself provides considerable bias, especially if only two sampling points are used (see Table 3). The proper use of two-compartmental model has been rather limited as well.

A very detailed analysis of these formulae was recently performed by our group (97). The diagnostic accuracy of various cystatin C equations varies with different levels of GFR. We compared various cystatin C equations across GFR strata <60, <90, ≥135, and ≥150 mL/min per 1.73 m^2 for an accurate prediction and appropriate classification of the measured GFR. The CKiD (21), Zappitelli-CysEq (107),

### Table 3 Equations to estimate glomerular filtration rate (mL/min/1.73 m^2) using serum creatinine and cystatin C.

<table>
<thead>
<tr>
<th>Cystatin C-based, mg/L</th>
<th>Formulae</th>
<th>Glomerular filtration rate measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boıkenkamp (70)</td>
<td>eGFR = 137/CysC–20/4</td>
<td>Filtration markers</td>
</tr>
<tr>
<td>Bouvet (101)</td>
<td>eGFR = [(SCr (mg/l)/96)–(0.35×(20.20))], [(CysC (mg/l)/12)–(0.56×(0.19))], [([Body weight/45] (0.30×(0.17))), [Age/(40×(0.16))].</td>
<td>Inulin SS: 60, 120, 180</td>
</tr>
<tr>
<td>CKiD (21)</td>
<td>eGFR = 70.1/5/cysC × 0.635</td>
<td>[1.8/CysC (mg/L)]^{0.474} \times[30/urea]^{0.169} \times[141/1.4]^{0.188}</td>
</tr>
<tr>
<td>Filler (98)</td>
<td>Log eGFR = 1.962 + [1.123 × log(1/CysC)]</td>
<td>Ioixol Details in (102)</td>
</tr>
<tr>
<td>Grubb (91)</td>
<td>eGFR = 84.69 × CysC^{–1.69×}(×(1.38 if age &lt;14 years))</td>
<td>51Cr-EDTA SS: 60, 120, 180, 240</td>
</tr>
<tr>
<td>Hoek (103)</td>
<td>eGFR = –4.32 + (80.35 × CysC)</td>
<td>99Tc-DTPA Cold ioixol SS: &gt;240, details not specified</td>
</tr>
<tr>
<td>Larsson (104)</td>
<td>eGFR = 77.239 × (CysC^{–2.623})</td>
<td>123I-Iothalate Cold ioixol SS: 240</td>
</tr>
<tr>
<td>Le Bricon (105)</td>
<td>eGFR = 78/CysC+4</td>
<td>123I-Iothalate Cold ioixol SS: 60, 90, 120, 150, 180</td>
</tr>
<tr>
<td>Rule (106)</td>
<td>eGFR = 66.8 × CysC^{–4.36}</td>
<td>51Cr-EDTA Cold ioixol SS: 60, 105–120</td>
</tr>
<tr>
<td>Zappitelli (CS-CrEq)</td>
<td>eGFR = (507.76 × CysC^{0.535} × SCr^{0.547})/40.45</td>
<td>Cold ioixol SS: 60, 120</td>
</tr>
</tbody>
</table>

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CysC, cystatin C; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet and Renal Disease; SCr, serum creatinine; SS, serum samples; US, urine samples. Creatinine in SI unit umol/L, except the CKD formula\(^2\), which used a different unit (mg/dL) convet to umol/L multiply by 88.4. Age in years, body weight (kg), height (m), urea [urea in mg/dL (0.357 mmol/L)]. eGFR units: in mL/min.
and Zappitelli-CysCrEq (107) equations had a higher accuracy, estimated by eGFR values within 10% and 30% of the respective (99m)Tc DTPA, in the GFR categories <60 and <90 mL/min per 1.73 m², whereas the Bökenkamp (70), Bouvet (101), and Filler (98) equations had a greater accuracy in the GFR categories ≥135 and ≥150 mL/min per 1.73 m². The Bouvet, CKiD, Filler, Zappitelli-CysEq, and Zappitelli-CysCrEq equations had a greater sensitivity to classify GFR <60 and <90 mL/min per 1.73 m², whereas the Bökenkamp equation had a higher sensitivity for GFR ≥135 and ≥150 mL/min per 1.73 m². In short, formulae always perform best in the range where most of the patients were recruited. As such, the CKiD formula (21) may be particularly good for patients with a GFR between 25 and 75 mL/min. The method of determination of cystatin C also plays an important role. As outlined above, the re-analysis of the CKiD study using the Siemens Healthcare assay rather than the DAKO assay gave convincingly better results (81). Given the variability in the performance of various equations with GFR, an ideal equation that can be applied to all remains a challenge. Short of an individualized approach based on GFR levels by center, further research on refining the equations should focus on data pooling, ensuring the quality of the gold-standard method, and choosing a mathematical model that best resembles the naturally occurring decline of isotope measurements in the time concentration curve. Ideally, non-linear mixed pharmacokinetic models that adjust for extracorporeal volume, gender, ethnicity and age as well as selecting an appropriate model with the number of compartments should be utilized. Perhaps the Bayesian approach for the WinNonLin derived GFR calculations employed by Bouvet et al. can maximize the quality of the gold-standard method of measuring GFR (101). As outlined above, standardized calibration and uniformity in using cystatin C assays can improve the prediction by cystatin C equations (109).

**Cystatin C as a potential marker for volume assessment and cardiovascular outcomes**

In patients with chronic kidney diseases with or without dialysis, their volume status is an important but difficult clinical assessment. In our recent publication, we have found that in patients who are on dialysis with no residual kidney function, the cystatin C reduction ratio per single hemodialysis treatment is negatively influenced by ultrafiltration rate (64). As a result, cystatin C level, its volume of distribution and its kinetics relating to volume status, needs to be further explored.

Furthermore, the current evidence explaining the biological plausibility of the association between cystatin C levels and cardiovascular outcomes are scant, despite a significant number of studies that have found strong associations in adult population. Likely, the mechanisms are partly due to the fact that cystatin C is a marker of reduced renal function, which is associated with cardiovascular outcome. However, in patients with cardiovascular diseases, studies have shown similar associations independent of residual renal function (110). A systematic review of 15 studies on “cardiovascular disease and cystatin C” showed that cystatin C level >1.3 mg/L is a risk factor for the occurrence of fatal and non-fatal cardiovascular events (111). Furthermore, recently published studies have provided some possible explanations to the mechanisms of cystatin C leading to an increase in cardiovascular diseases. Cystatin C is a base proteinase inhibitor and is produced by all nucleated cells. Xie et al. demonstrated that oxidants induce cystatin C elevation. Cystatin C regulates Cathepsin B activity and its elevation can affect cardiac extracellular remodelling (112). In addition, there is a positive association between cystatin C and monocyte levels which can lead to atherosclerosis (113). One might argue that atherosclerosis is not prevalent among children with chronic kidney disease, however, there clearly is evidence for vascular disease with media calcifications in these patients, leading to the major cause of death (32). Finally, Funayama et al. have shown that cystatin C level is an independent predictor for vasospastic angina (114). Clearly, these studies have demonstrated some of the possible mechanisms by which this important marker can lead to increased cardiovascular diseases and alter left ventricular mass. More studies are therefore needed. Therefore, cystatin C can be an important marker for both assessing kidney function and predicting cardiovascular outcomes. The influence of these cardiovascular morbidities on the variability of both creatinine and cystatin C levels as well as the accuracy of eGFR estimations has been understudied.

**Limitations of prediction equations**

Although prediction equations have shown to be more accurate estimators of GFR than the serum levels of the markers alone, these prediction equations tend to have limitations to their use. Prediction equations have shown to represent only the relationship between the marker and its non-GFR determinants (74). They also vary across populations and over time which would yield inaccurate estimations when applied to different populations from the one used in developing these formulae (81). The use of prediction equations may also be limited by other laboratory factors, such as the different calibrators used in determining the cystatin C blood levels, non-accurate methods for cystatin C determination, and the higher cost associated with cystatin C in the case of the cystatin C based equations (115). If one is to control for these varying parameters, a universal model can be developed using the appropriate statistical approach.

**Conclusions**

In summary, gold standard GFR measurements are cumbersome and invasive. It is without doubt that cystatin C offers significant advantages over serum creatinine as endogenous markers of GFR. The Schwartz formula is a good estimate of GFR in children with normal body composition, but for more accurate assessment across the lower levels of GFR, the CKiD or Filler or Zappitelli formula offers significant advantages. In certain conditions, such as spina bifida patients, only cystatin C-derived eGFR correlates with measured GFR. Significant
variability exists with regards to high quality of the “gold-standard GFR measurement” across the various studies. Very few centers use appropriate sampling frequency and appropriate two-compartmental models to determine the GFR. The pre-analytical error related to the body composition, anthropometry variability, body surface area calculation vs. extra-cellular volume and intra- and inter-patient variability of the GFR measurements has been understudied. This also applies to the effect of the patient cohort’s GFR range on the diagnostic performance of the various formulae. The best approach towards a better formula for worldwide use would be the pooling of data to generate more robust formulae with appropriate validation cohorts. The importance of standardization and calibration of the cystatin C assays also cannot be underestimated. Nonetheless, of all endogenous markers, cystatin C appears to be the best surrogate for GFR and it is hoped that serum creatinine be combined or replaced for the estimation of GFR in children.

Conflict of interest statement

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