Scandinavian Journal of Clinical & Laboratory Investigation, 2012; 72: 73–77

informa
healthcare

ORIGINAL ARTICLE

Improved estimation of glomerular filtration rate (GFR) by comparison of $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$

ANDERS GRUBB[1], ULF NYMAN[2] & JONAS BJÖRK[3]

[1]*Department of Clinical Chemistry, Lund University Hospital, Lund, Sweden,* [2]*Department of Radiology, University of Lund, Lasarettet Trelleborg, Trelleborg, Sweden, and* [3]*Competence Centre for Clinical Research, Lund University Hospital, Lund, Sweden*

Abstract

Objective. GFR-prediction equations based upon cystatin C and creatinine have better diagnostic performance in estimating GFR than equations based upon only one of the two markers. The present work concerns in what way a comparison between separate estimations of GFR based upon cystatin C ($eGFR_{cystatin\ C}$) or creatinine ($eGFR_{creatinine}$) can be used to evaluate the diagnostic performance of a combined cystatin C- and creatinine-based estimation of GFR. ***Methods.*** The difference between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ was compared with measured GFR (iohexol clearance) and a combined cystatin C- and creatinine-based estimation of GFR in a Swedish-Caucasian cohort of 857 adult patients. ***Results.*** A difference between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ of $\geq 40\%$ indicated a markedly reduced diagnostic performance of the combined cystatin C- and creatinine-based estimation of GFR. ***Conclusion.*** Comparison of the agreement between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ can be used to evaluate the diagnostic performance of combined cystatin C- and creatinine-based estimations of GFR. If 'threshold values' for discordance are exceeded, it must be considered whether the clinical context requires the use of an invasive gold standard method to measure GFR. In some clinical contexts either creatinine or cystatin C are known to be invalidated as markers of GFR and in these situations the use of only the cystatin C- or the creatinine-based GFR estimate should be considered when the 'threshold values' are exceeded.

Key Words: *Kidney function, immunoassays, kidney diseases, cystatin C, creatinine*

Introduction

GFR-prediction equations based upon cystatin C ($eGFR_{cystatin\ C}$) or creatinine ($eGFR_{creatinine}$) may produce estimated GFR-values, of which 80–85% are within $\pm 30\%$ of GFR measured by invasive gold standard methods. However, the highest percentages of estimated GFR-values within $\pm 30\%$ of measured GFR are obtained using GFR-prediction equations based upon both cystatin C and creatinine ($eGFR_{cystatin\ C + creatinine}$) [1–9]. The performance of $eGFR_{creatinine}$ is reduced *inter alia* if a patient has an abnormally low or high muscle mass, recently ingested boiled meat or is treated with a drug that influences the tubular secretion of creatinine. The performance of $eGFR_{cystatin\ C}$ is reduced *inter alia* if a patient is treated with large doses of glucocorticoids. In such clinical situations the diagnostic performance of a GFR-prediction equation based upon both cystatin C and creatinine may be inferior to those equations based upon only one of the GFR-markers [1]. See also www.egfr.se. However, such situations may not always be recognized by those ordering a GFR-estimate or by the laboratory performing the tests. It has therefore been suggested that a comparison between separate estimations of GFR based upon cystatin C or creatinine can be used to evaluate the diagnostic performance of a combined cystatin C- and creatinine-based estimation of GFR [1]. The present work concerns the relation between the agreement between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ and the diagnostic performance of $eGFR_{cystatin\ C + creatinine}$.

Correspondence: Anders Grubb, MD, PhD, Department of Clinical Chemistry, Lund University Hospital, SE 221 85 Lund, Sweden. Tel: + 46 46 173964. E-mail: anders.grubb@med.lu.se

(Received 30 June 2011; accepted 18 October 2011)

ISSN 0036-5513 print/ISSN 1502-7686 online © 2012 Informa Healthcare
DOI: 10.3109/00365513.2011.634023

Material and methods

The patient population studied was identical to the one previously used to analyse various equations to combine creatinine and cystatin C to predict GFR [8]. It consisted of adult patients (Swedish-Caucasians ≥ 18 years) consecutively referred to the Departments of Clinical Chemistry, University Hospitals of Lund and Malmö for determination of GFR by iohexol clearance. Simultaneous measurements of plasma creatinine, plasma cystatin C, weight and height were performed and age and gender recorded.

The Lund population consisted of 451 patients (225 females) and the Malmö population of 425 patients of whom 19 patients were excluded because of missing plasma creatinine values ($n = 6$), missing plasma cystatin C values ($n = 8$) or technical assay errors ($n = 5$) leaving 406 subjects in the Malmö cohort. All procedures involving subjects and data were in accordance with the Helsinki Declaration of 1975 concerning ethical principles for medical research involving human subjects.

The characteristics of the two cohorts and the combined set ($n = 857$) are shown in Table I and included 12 patients with neurological diseases and secondary muscular atrophy. Common indications for referral were diagnosis and follow-up of chronic kidney disease, evaluation of renal function prior to dosage of drugs cleared by the kidneys, evaluation of potential renal donors, follow-up of unilaterally nephrectomized patients, pre-operative evaluation of patients with hyperparathyroidism and control of renal transplants ($n = 44$).

Determination of iohexol clearance

Five mL of iohexol (Omnipaque 300 mg iodine/mL, GE Healthcare, Oslo, Norway) were administered intravenously in an antecubital vein. Iohexol clearance (referred to as 'measured GFR') was calculated from plasma clearance of a single plasma sample of iohexol [10] drawn at varying times, normally 4 hours after injection, according to expected GFR as determined by plasma creatinine concentration and anthropometric data. The exact time of administration and blood sampling were documented by a specialist nurse. Plasma iohexol concentrations were determined by high-pressure liquid chromatography with a total analytical variation of 2–4% (coefficient of variation, CV%) at the range of iohexol concentrations normally encountered during the study [11]. The Dubois formula was used to adjust the measured GFR values to 1.73 m^2 body surface area [12].

Determination of plasma creatinine

Plasma concentrations of creatinine were determined at Lund University Hospital by an enzymatic colorimetric assay on a Hitachi Modular P analyzer (Roche Diagnostics, Mannheim, Germany) and with a calibrator traceable to primary reference material with values assigned by isotope dilution mass spectrometry (IDMS) [13]. At Malmö University Hospital a modified Jaffe colorimetric method was used on a Beckman LX20 analyzer (Beckman Coulter, Inc., Fullerton, CA, USA) employing zero-point calibration and a calibrator traceable to primary reference material with values assigned by IDMS [14,15]. Total analytical variation (CV%) of the enzymatic method in Lund was 1.4–3.0% at concentrations of creatinine between 60 and 578 μmol/L and 2.2–2.8% at concentrations between 53 and 631 μmol/L for the Jaffe method in Malmö.

Determination of plasma cystatin C

Plasma cystatin C levels were determined by an automated particle-enhanced immunoturbidimetric method [16] using a Hitachi Modular P analysis system, reagents (code Nos LX002, S2361, X0973, X0974) obtained from DakoCytomation (Glostrup, Denmark) and following the procedure recommended by the reagent producer. The procedure had a total coefficient of variation of 2.1% at a cystatin C level of 1.0 mg/L and of 1.7% at a level of 4.0 mg/L. All samples were analysed within one day after collection or frozen at –20°C until analysed.

Table I. Demographic and anthropometric patient characteristics, plasma creatinine, plasma cystatin C, and iohexol clearance given as median values (2.5 and 97.5 percentiles) in the Lund and Malmö cohorts as well as in the combined set.

Parameters	Lund ($n = 451$)	Malmö ($n = 406$)	Combined ($n = 857$)
Age (years)	58 (24–83)	61 (26–85)	59 (26–85)
Females	225 (50%)	152 (37%)	377 (44%)
Total body weight (kg)	73 (46–115)	78 (49–111)	75 (48–112)
Height (cm)	170 (151–189)	173 (152–190)	171 (152–189)
Body surface area (m^2)	1.83 (1.45–2.31)	1.92 (1.49–2.33)	1.88 (1.46–2.31)
Body mass index (kg/m^2)	25 (18–39)	26 (18–38)	25 (18–38)
Plasma creatinine (μmol/L)	92 (39–400)	136 (54–623)	106 (44–545)
Cystatin C (mg/L)	1.18 (0.79–3.07)	1.53 (0.85–4.06)	1.30 (0.81–3.82)
Iohexol clearance (mL/min per 1.73 m^2)	63 (11–124)	42 (8–115)	55 (9–121)

Prediction equations

The Lund-Malmö creatinine-based equation ($eGFR_{creatinine}$) with age and gender [17] and the Grubb cystatin C-based equation ($eGFR_{cystatin\ C}$) based on adults and including gender [2] were selected for the present analysis. Plasma creatinine (pCr) is expressed in μmol/L, plasma cystatin C (pCy) in mg/L, age in years and ln denotes the natural logarithm. Both equations express relative GFR in mL/min per 1.73 m^2 body surface area.

Lund-Malmö creatinine equation ($eGFR_{creatinine}$)

$$GFR = e^{X - 0.0124 \times age + 0.339 \times \ln(age) - 0.226\ (\text{if female})}$$

$$X = 4.62 - 0.0112 \times pCr\ (\text{if } pCr < 150\ \mu mol/L)$$

$$X = 8.17 + 0.0005 \times pCr - 1.07 \times \ln(pCr)\ (\text{if } pCr \geq 150\ \mu mol/L)$$

Grubb cystatin C equation ($eGFR_{cystatin\ C}$)

$$GFR = 86.49 \times pCy^{-1.686} \times 0.948\ (\text{if female}),$$

equivalent with

$$GFR = e^{4.46 - 1.686 \times \ln(pCy) - 0.053\ (\text{if female})}$$

GFR estimates from the combined use of the two analytes ($eGFR_{cystatin\ C + creatinine}$) were based on the arithmetic mean of $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$, which has proved as accurate as more complex equations [8].

Statistical evaluation

All statistical analyses were conducted using SPSS release 18.0.1. (SPSS Inc, Chicago, USA). In the statistical testing we regarded *p*-values in the order of 0.05 as moderate evidence against the null hypothesis, whereas *p*-values in the order of 0.001 or below were regarded as strong evidence against the null hypothesis [18]. The present study focused on the *accuracy* of the arithmetic mean of $eGFR_{cystatin\ C}$ and $eGFR_{creatinine,}$ denoted $eGFR_{cystatin\ C + creatinine}$, in relation to the *agreement* between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$. The *accuracy* of $eGFR_{cystatin\ C + creatinine}$ was reflected by the absolute percentage error:

$|eGFR_{cystatin\ C + creatinine} - \text{measured GFR}|\ /\ \text{measured GFR}$, and summarized as the percentage of estimates within 30% (P_{30}) and 10% (P_{10}) of measured GFR [19]. The *agreement* was reflected by the difference%, i.e. the absolute difference $|eGFR_{cystatin\ C} - eGFR_{creatinine}|$ expressed in percent relative to the arithmetic mean $eGFR_{cystatin\ C + creatinine}$.

The following analyses were made:

(1) Pearson's and Spearman's correlation coefficients (denoted r and r_s) were used to evaluate the overall association between accuracy (absolute percentage error) and agreement (difference%).
(2) The accuracy categorized as P_{30} and P_{10} was evaluated in relation to agreement (difference%) rounded to nearest integer and then categorized as $< 10\%$, 10–19%, 20–29%, 30–39% and $\geq 40\%$ difference. Fisher's exact test was used to evaluate differences in P_{30} and P_{10} across categories of agreement.
(3) Measured GFR is related to both accuracy and agreement and may thus confound the association between agreement and accuracy. To account for such confounding we modelled accuracy, i.e. P_{30} and P_{10}, respectively, using logistic regression with measured GFR and difference% as continuous covariates.
(4) We calculated an 'improvement index', defined as the proportion of all GFR estimates for which $eGFR_{cystatin\ C + creatinine}$, but not both $eGFR_{cystatin\ C}$ and $eGFR_{creatinin}$, were inaccurate according to P_{30} and P_{10}, respectively. This index represents the upper limit of the improvement in accuracy that could be obtained if the most accurate eGFR (i.e. $eGFR_{cystatin\ C + creatinine}$, $eGFR_{cystatin\ C}$ or $eGFR_{creatinin}$) was consistently applied for each patient. Note that the sum of, e.g. P_{30} and the corresponding improvement index for P_{30} can never exceed 100%.
(5) The improvement index depends on the accuracy, i.e. the potential for improvement is higher when accuracy is low. To account for such confounding in the association between agreement and the improvement index, we modelled the improvement index using logistic regression with inaccuracy (absolute percentage error) and difference% as continuous covariates.

Results

The overall association between difference% and absolute percentage error was not consistent ($r = 0.13$, $p < 0.001$ but $r_s = 0.05$, $p = 0.12$), however, P_{30} was clearly decreased for differences between $eGFR_{cystatin\ C}$ and $eGFR_{creatinin}$ exceeding a 'threshold value' of 40% (Table II; $p = 0.02$ when comparing P_{30} for 30–39 and $\geq 40\%$ difference). The dip in accuracy when expressed as P_{10} seemed to occur already at 30–39% difference, but the statistical evidence for this dip was weak ($p = 0.09$ when comparing P_{10} for 20–29 and 30–39% difference). Measured GFR was noticeably lower for differences exceeding 40%, but the suggested inverse association between difference% and P_{30} remained clear when measured GFR was adjusted for using logistic regression ($p = 0.001$), whereas the association between difference% and P_{10} remained weaker ($p = 0.05$).

Table II. Accuracy of $eGFR_{cystatin\ C + creatinine}$, the arithmetic mean of $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$, calculated as the percentage of estimates within 30% (P_{30}) and 10% (P_{10}) of measured GFR in relation to the difference% between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$, defined as $|eGFR_{cystatin\ C} - eGFR_{creatinine}| / eGFR_{cystatin\ C + creatinine}$. The improvement index, defined as the proportion of all GFR estimates where $eGFR_{cystatin\ C + creatinine}$, but not both $eGFR_{cystatin\ C}$ and $eGFR_{creatinin}$, were inaccurate within 30% and 10% of measured GFR, is also presented.

Difference %	Median measured GFR (mL/min per 1.73 m^2)	Accuracy (%)		Improvement index (%)	
		30%	10%	30%	10%
<10 ($n=220$)	59	90.0	43.6	1.8	8.2
10–19 ($n=200$)	59	91.5	48.5	2.5	25.0
20–29 ($n=175$)	61	94.3	45.1	5.7	39.4
30–39 ($n=120$)	54	90.0	35.0	8.3	52.5
≥ 40 ($n=142$)	39	79.6	40.8	18.3	30.3
Total ($n=857$)	55	89.5	43.4	6.4	28.4

The improvement index generally suggested higher potential for improvement in accuracy when the difference between $eGFR_{cystatin\ C}$ and $eGFR_{creatinin}$ was considerable (Table II). This association between the agreement (difference%) and the potential for improvement in accuracy remained evident when differences in accuracy across levels of agreement were adjusted for using logistic regression ($p < 0.001$ both for improvement in P_{30} and in P_{10}).

Discussion

The diagnostic performance of $eGFR_{creatinine}$ is reduced *inter alia* if a patient has an abnormally low or high muscle mass, recently ingested boiled meat or is treated with a drug that influences the tubular secretion of creatinine. In these clinical contexts the diagnostic performance of $eGFR_{cystatin\ C}$ is generally unaltered. However, the performance of $eGFR_{cystatin\ C}$ is impaired if a patient is treated with large doses of glucocorticoids and in this situation the performance of $eGFR_{creatinine}$ is still acceptable. Although GFR-prediction equations based upon both cystatin C and creatinine ($eGFR_{cystatin\ C + creatinine}$) generally are superior to GFR-prediction equations based upon either cystatin C ($eGFR_{cystatin\ C}$) or creatinine ($eGFR_{creatinine}$) this may not be the case in these specific clinical contexts. Although these contexts may be easily recognized in some cases, they will not invariably be recognized. There may also be additional, not yet identified, clinical contexts invalidating either cystatin C or creatinine as useful markers for GFR. Comparing $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ might be helpful to identify both known and unknown causes when neither cystatin C nor creatinine are suitable as a marker for GFR [1]. To be able to efficiently use such a comparison, it must be known when the discordance between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ is large enough to indicate such a condition. The present study based upon measured GFR and $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ in a patient cohort of 857 Swedish-Caucasian adult patients indicates that if the discordance is 40% or more, the diagnostic performance of $eGFR_{cystatin\ C + creatinine}$ is markedly reduced. Such discordance should initiate a more careful evaluation of the clinical context to disclose conditions invalidating either creatinine or cystatin C as a GFR marker. If such conditions are identified, GFR might be best estimated using a prediction equation based upon only the non-invalidated marker. If such conditions are not identified, it should be realized that the estimation of GFR is unreliable and that an invasive, gold standard, measurement of GFR might be required.

It should be realized, that the discordance value, the 'threshold value', indicating requirement of a further evaluation of the clinical context to improve estimation of GFR, as presented in this work, is influenced by the actual patient cohort and by the equations used for estimating GFR, i.e. $eGFR_{cystatin\ C}$, $eGFR_{creatinine}$ and $eGFR_{cystatin\ C + creatinine}$. For other patient cohorts might contain proportionally more, or fewer patients, with conditions invalidating either cystatin C or creatinine as a GFR marker. The more patients with such conditions in the cohort, the greater the potential for improvement of eGFR by comparison of $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$. The equations for $eGFR_{cystatin\ C}$, $eGFR_{creatinine}$ and $eGFR_{cystatin\ C + creatinine}$ will also influence the 'threshold value' by being more or less sensitive for patient characteristics reducing the value of creatinine and cystatin C as markers for GFR.

It is possible to calculate an 'improvement index', defined as the proportion of all GFR estimates for which $eGFR_{cystatin\ C + creatinine}$, but not both $eGFR_{cystatin\ C}$ and $eGFR_{creatinin}$, are inaccurate according to P_{30} or P_{10}. This index will represent the upper limit of improvement in accuracy that could be obtained, if the most accurate eGFR (i.e. $eGFR_{cystatin\ C + creatinine}$, $eGFR_{cystatin\ C}$ or $eGFR_{creatinin}$) was consistently applied. In the present study the improvement index was 18.3 % for a discordance threshold of $\geq 40\%$, which means that if an accurate evaluation of the relevant clinical conditions could be performed for each patient the P_{30}-value of 79.6% for $eGFR_{cystatin\ C + creatinine}$, would

theoretically increase to $79.6 + 18.3 = 97.9\%$. This 'improvement index' will, exactly like the 'threshold value' of discordance, also be influenced by the actual patient cohort and by the equations used for eGFR, i.e. $eGFR_{cystatin\ C}$, $eGFR_{creatinine}$ and $eGFR_{cystatin\ C + creatinine}$ and for the same reasons.

Acknowledgements

The investigation was supported by grants from the Swedish Science Research Council (Project No. 5196) from the Alfred Österlund Foundation, Greta and Johan Kock Foundation, Medical Faculty of the University of Lund and from Region Skåne.

Declaration of interest: The authors have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Grubb A. Non-invasive estimation of glomerular filtration rate (GFR). The Lund model: simultaneous use of cystatin C- and creatinine-based GFR-prediction equations, clinical data and an internal quality check. Scand J Clin Lab Invest 2010;70:65–70.

[2] Grubb A, Nyman U, Björk J, Lindström V, Rippe B, Sterner G, Christensson A. Simple cystatin C-based prediction equations for glomerular filtration rate compared with the Modification of Diet in Renal Disease prediction equation for adults and the Schwartz and the Counahan-Barratt prediction equations for children. Clin Chem 2005;51: 1420–31.

[3] Rule AD, Bergstralh EJ, Slezak JM, Bergert J, Larson TS. Glomerular filtration rate estimated by cystatin C among different clinical presentations. Kidney Int 2006;69: 399–405.

[4] Bouvet Y, Bouissou F, Coulais Y, Séronie-Vivien S, Tafani M, Decramer S, Chatelut E. GFR is better estimated by considering both serum cystatin C and creatinine levels. Pediatr Nephrol 2006;21:1299–306.

[5] Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, Xu JS, Huang SM, Wang LN, Huang W, Wang M, Xu GB, Wang HY. Improved GFR estimation by combined creatinine and cystatin C measurements. Kidney Int 2007;72:1535–42.

[6] Tidman M, Sjöström P, Jones I. A comparison of GFR estimating formulae based upon s-cystatin C and s-creatinine and a combination of the two. Nephrol Dial Transplant 2008;23:154–60.

[7] Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, Rossert J, Van Lente F, Bruce RD, Zhang YL, Greene T, Levey AS. Estimating GFR using cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. Am J Kidney Dis 2008; 51: 395–406.

[8] Nyman U, Grubb A, Sterner G, Björk J. Different equations to combine creatinine and cystatin C to predict GFR. Arithmetic mean of existing equations performs as well as complex equations. Scand J Clin Lab Invest 2009;69:619–27.

[9] Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009;20:629–37.

[10] Jacobsson L. A method for calculation of renal clearance based on a single plasma sample. Clin Physiol 1983;3:297–305.

[11] Krutzen E, Bäck SE, Nilsson-Ehle I, Nilsson-Ehle P. Plasma clearance of a new contrast agent, iohexol: a method for the assessment of glomerular filtration rate. J Lab Clin Med 1984;104:955–61.

[12] DuBois D, DuBois E. A formula to estimate the approximate surface area if height and weight be known. Arch Intern Med 1916;17:1275–83.

[13] Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, vanLente F. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med 2006;145:247–54.

[14] Mårtensson A, Rustad P, Lund H, Ossowicki H. Creatininium reference intervals for corrected methods. Scand J Clin Lab Invest 2004;64:439–41.

[15] Rustad P, Felding P, Franzson L, Kairisto V, Lahti A, Mårtensson A, Hyltoft Petersen P, Simonsson P, Steensland H, Uldall A. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. Scand J Clin Lab Invest 2004;64:271–84.

[16] Kyhse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindström V, Grubb A. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. Clin Chem 1994; 40:1921–6.

[17] Björk J, Bäck SE, Sterner G, Carlson J, Lindström V, Bakoush O, Simonsson P, Grubb A, Nyman U. Prediction of relative GFR in adults: new improved equations based on Swedish Caucasians and standardized plasma-creatinine assays. Scand J Clin Lab Invest 2007;67:678–95.

[18] Sterne JA, Davey Smith G. Sifting the evidence – what's wrong with significance tests? BMJ 2001;322:226–31.

[19] Stevens LA, Zhang Y, Schmid CH. Evaluating the performance of equations for estimating glomerular filtration rate. J Nephrol 2008;21:797–807.