Available online at www.sciencedirect.com
ScienceDirect
Clinical Biochemistry 41 (2008) 498–503
CLINICAL BIOCHEMISTRY

Cystatin C could be a replacement to serum creatinine for diagnosing and monitoring kidney function in children

Raul Narvaez-Sanchez [a,*], Luz Gonzalez [b], Alba Salamanca [a], Myriam Silva [a], Dora Rios [a], Sinay Arevalo [b], Ricardo Gastelbondo [b], Javier Sanchez [c]

[a] Universidad del Rosario, Facultad de Medicina, Bogotá, Colombia
[b] Fundación Cardioinfantil — Instituto de Cardiología, Bogotá, Colombia
[c] Universidad Autónoma de Madrid, Facultad de Medicina, Madrid, Spain

Received 20 September 2007; received in revised form 15 January 2008; accepted 21 January 2008
Available online 7 February 2008

Abstract

Background: Chronic kidney disease (CKD) is a worldwide public health problem. Glomerular filtration rate (GFR) is accepted as the best way to diagnose and monitor kidney function. Plasma Cystatin C (CysC) has been proposed as a better marker of GFR than serum creatinine (SCr), but it is not widely used because of some drawbacks with CysC assays. Our purpose is to determine the diagnostic accuracy of CysC and SCr for GFR estimation in children, using 99Tc-DTPA clearance (Cl_{Tc}) as the reference standard. We also discuss some of the economic implications of these tests, in order to guide clinicians when to use CysC or SCr for the diagnosis or monitoring of CKD.

Methods: Data were collected from 109 Colombian outpatients aged less than 18 years referred for determination of GFR because of suspected or definite renal insufficiency. The cost of each test was determined in Bogotá, Colombia, and in Madrid, Spain.

Results: Using a GFR of 90 mL/min as a cut-off value, we found: CysC sensitivity 75%, specificity 84%, and area under ROC curve (AUC) 0.84. SCr sensitivity 46%, specificity 100%, and AUC 0.72. Using a GFR of 70 mL/min as a cut-off value, we found: CysC sensitivity 100%, specificity 48%, and AUC 0.94. SCr sensitivity 77%, specificity 91%, and AUC 0.81. In all calculations predictive values behave correspondingly and ranges were narrow at CI 95%. In AUC, $p=0.0001$. Cost per enzymatic test in Bogotá: CysC U$ 27; SCr U$ 2. Cost per enzymatic test in Madrid: CysC U$ 3; SCr U$ 0.08.

Conclusion: CysC is a very interesting option, and could be a replacement to serum creatinine for diagnosing and possibly for monitoring kidney function in children.

Keywords: Sensitivity and specificity; Glomerular filtration rate; Creatinine; Cystatins

Introduction

Chronic kidney disease (CKD) is a worldwide public health problem [1], but is often only clinically evident in its advanced stages. In Colombia there are nearly 15,000 patients with terminal CKD: the annual cost of treating these patients and associated complications exceeds the annual budget for rebuilding and upgrading hospital facilities [2]. The recorded prevalence of infantile CKD in Colombia is one of the lowest in America (just 2 cases per million per year) but this is probably due to enormous under-diagnosis [3], similar to that found in all non-industrialized countries. The incidence of CKD is growing around the world [4], in part because of detection through routine laboratory measurements [1]. In addition, adverse outcomes and costs of CKD in the Third World are growing [5].

Glomerular filtration rate (GFR) is the best overall measure of kidney function [6]. Estimation of GFR is used in the detection of CKD, and for monitoring renal function. Changes in GFR can predict the progression of disease and the risk of secondary complications. A decrease in GFR precedes kidney failure in all

Abbreviations: CKD, Chronic kidney disease; GFR, Glomerular filtration rate; CysC, plasma Cystatin C; SCr, serum creatinine; Cl_{Tc}, 99Tc-DTPA clearance; PPV, positive predictive value; NPV, negative predictive value; AUC, Area under ROC curve.

* Corresponding author.
E-mail address: raul.narvaez@uam.es (R. Narvaez-Sanchez).
URL: http://rincon.uam.es/dir?cw=877832031250000.

0009-9120/$ - see front matter © 2008 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.clinbiochem.2008.01.022

forms of progressive kidney disease and is associated with cardiovascular morbidity and mortality [7]. Early recognition of kidney disease and successful intervention may improve the prognosis in cardiovascular disease [8,9]. Therefore methods to estimate GFR must be accurate and cost effective.

SCr is the marker worldwide commonly used to estimate GFR, but it is a controversial test because serum creatinine production rate varies with muscle mass and the dietary intake, and thus on age, sex, race and the catabolic or anabolic state of the patient. CysC is a cationic non-glycosylated protein, formed from 120 aminoacids with a weight of 13.3 kDa. It is produced at a constant rate in lysosomes of all nucleated cells of the body, and is present in diverse biological fluids like serum, semen and cerebrospinal fluid [10]. It is cleared exclusively through the kidney and therefore its concentration depends solely on the GFR. In 1985 was demonstrated for the first time its powerful negative correlation with the GFR [11,12]. It has been proposed as a marker to estimate GFR since it can detect changes in GFR earlier than SCr [13–15] and recently identified in urine as a marker of tubular dysfunction [16]. But CysC has a wide interindividual variation and there are no standardized methods to measure it [17–20] nor validated equations to estimate GFR from CysC. CysC has been claimed to be independent of body mass [13] but it has been recently controverted [21]. CysC can be affected by several clinical conditions [22]. In addition, one CysC test is many times more expensive than one SCr test, which is an important consideration when frequent testing is necessary.

Methods

All procedures involving patients and data were in accordance with the Helsinki Declaration, the Health Ministry of Colombia policies concerning ethical principles for medical research, and with the consent of the Ethics Committees of Universidad del Rosario and Fundacion Cardioinfantil — Bogotá (FCI). Informed consent was signed by one patient's relative in addition to the assent by the patient if 7 years of age or older.

Patient population and samples

From April to September 2006 data were prospectively collected from 109 outpatients aged between 1 and 18 years. Patients were referred to the Pediatric Nephrology service from other services of FCI for the determination of GFR because of suspected CKD (patients with a risk factor as arterial hypertension, obesity, autoimmune diseases, repeated urinary tract infections, lower urinary tract obstruction or diabetes) or definite CKD. Patients satisfying inclusion criteria were referred to Nuclear Medicine of FCI for blood sampling and gamma imaging.

The inclusion criteria were: order of the nephrologist for measuring GFR; age > 1 year, < 18 years; acceptation of the child (if age > 7 years) and the child's relative to participate in the study. The exclusion criteria were: incapacity of the child's relative to understand the informed consent; acute infection, particularly nephritis or something that causes vomiting and/or diarrhea; neoplasia of any etiology in palliative treatment; hospitalized child; hepatic or thyroidal disease; Black race (because it is not clear if the SCr observed racial differences in adults are the same in children).

Once in the Nuclear Medicine service, a cannula was inserted into the patient's *vena basilica* and blood samples for CysC and SCr were taken (heparinized tube for CysC and dry tube for SCr). Immediately after blood sampling, 99Tc-DTPA was given through the same cannula and gamma images obtained using a gamma camera Hawkeye (General Electric; St Giles, UK) in optimal state of maintenance. GFR and renal plasmatic flux were measured by computerized analysis using Gates technique [23] by a specialist in nuclear medicine. We used Cl_{Tc} as a reference standard because of its excellent agreement with clearances of inulin and iothalamate, and it correctly classifies the target condition [24–26]. The supplier of the technetium used guarantees a quality standard higher than 95%.

The sample for SCr was processed immediately by biochemists in the FCI Clinical Laboratory using the VITROS250 analyzer (Johnson and Johnson; Ramsey, Minnesota, USA), by dry chemistry and two-point kinetics, with coefficient of variation less than 1.16%. The machine is calibrated daily with control sera from Randox Chemistry (Oceanside, CA, USA) and external quality control is ensured by the American College of Pathologists.

The samples for CysC were stored at −20 °C for not more than three months and analyzed, by biochemists, in batches by immunonephelometry in a BN 100 analyzer (Dade Behring, Germany), with coefficient of variation less than 2.0%, at the Clínica de la Policía in Bogotá, where technical maintenance and calibration of equipment are guaranteed by VelezLab enterprises (Bogotá, Colombia).

Table 1
Patient's characteristic

	N (%)
Age (mean±SD)	8.49±4.74
Age (years)	
1–5	30 (27.5)
5–9	28 (25.7)
9–13	33 (30.3)
>13	18 (16.5)
Gender	
Female	53 (48.6)
Male	56 (51.49)
Weight (kg) (mean±SD)	28.20±15.35
Height (cm) (mean±SD)	121.93±29.15
Stage (based on reference [1])	
GFR* ≥ 90	61 (56)
GFR from 60 to 89	24 (22)
GFR from 30 to 59	14 (12.8)
GFR from 15 to 29	3 (2.7)
GFR ≤ 15	7 (6.4)
Diagnostic groups	
Group 1: UTI, obstructive uropathy, VUR**	61 (56)
Group 2: Congenital kidney pathology***	15 (13.8)
Group 3: Glomerular disease****	22 (20.2)
Group 4: Others*****	11 (10.1)

* Absolute GFR determined by Cl_{Tc}, in mL/min.
** UTI: Antecedents of urinary tract infection; VUR: vesico-ureteral reflux.
*** Hypoplasia, dysplasia, agenesis.
**** Nephrosis, Haematuria, Proteinuria, Alport, etc.
***** Tubular pathologies, Arterial hypertension, Obesity.

Table 2
Biochemical parameters among diagnostic groups (mean±SD)

	All	Diagnostic group			
		1	2	3	4
N	109	61	15	22	11
SCr (mg/dL)*** [a]	1.24±2.13	1.12±1.92	0.56±0.25	2.40±3.26	0.5±0.32
CysC (mg/L)**	1.45±1.66	1.53±1.90	0.93±0.29	1.91±1.79	0.91±0.26
Cl_{Tc} (mL/min)*	87.55±36.26	86.64±34.96	88.13±29.12	79.91±45.40	107.18±28.21

Kruskal–Wallis test: * not significant; ** $p \leq 0.05$; *** $p \leq 0.001$.
[a] To convert mg/dL to μmol/L of creatinine, multiply by 88.

The readers of the index test and reference standard were blind (masked) to the results of the other tests.

The cost of SCr and CysC tests was provided in December 2006 by Instituto del Riñón, FCI Clinical Laboratory and VelezLab enterprises (all three from Bogota, Colombia), and from Hospital Universitario La Paz Biochemistry Unit (Madrid, Spain). Respective currencies were converted to U. S. dollars.

Statistical method

Sample size of 109 children was calculated for sensitivities of CysC (81%) and SCr (94%) informed by Dade Behring Marburg [27] for a two-side test with a significant level of 5% and power of 80% [28,29]. Sample size was stratified by kidney function in a relationship 1:3 (27 should be no CKD patients and 79 should be CKD patients). Continuous variables were summarized by mean, range and standard deviation. Distributions of qualitative variables were calculated. The distribution of biochemical parameters among diagnostic groups was co-pared using Kruskal–Wallis and Mann–Whitney *U* tests. Kidney dysfunction was defined as a GFR ≤ 90 mL/min or GFR ≤ 70 mL/min to calculate sensitivity, and specificity and to draw a ROC curve. These are widely used cut-off values in studies comparing CysC and SCr [24]. In reference to the gold standard, sensitivity and specificity were calculated by standard formulae [30]. The positive and negative predictive values of each test were calculated. The Lin correlation coefficient [31] was calculated to measure the concordance among quantitative variables CysC and SCr. 95% confidence intervals were calculated. Finally, the ROC curve was drawn and area under the curve was measured. Differences between ROC curves were estimated according to DeLong methods [32]. Confidence intervals for the AUC were calculated according to Hanley and McNeil [33] All statistical analyses and graphs were carried out at significant level of 5% and power of 80% using STATA software v9 and Statistical Package for the Social Sciences v14 (SPSS Inc, Chicago, USA).

Results

109 patients younger than 18 years were included, with mean age of 8.5 years (SD±4.7). 49% were female. Demographic characteristics, stages and diagnostic groups are shown in Table 1. As expected, SCr and CysC levels were inversely related to Cl_{Tc} ($\rho = -0.49$, $p = 0.0001$ for SCr; $\rho = -0.67$, $p = 0.0001$ for CysC) and the Lin coefficient between levels of CysC and SCr is positive ($\rho = 0.6$, $p = 0.0001$).

Levels of SCr vary significantly between diagnostic groups ($p \leq 0.001$) with the highest value in group 3 (mean 2.4 mg/dL–211 μmol/L-) and the lowest in diagnostic group 4 (mean 0.5 mg/dL–4 μmol/L-). CysC levels also vary significantly ($p \leq 0.05$) showing the highest value in group 3 (mean 1.9 mg/L) and the lowest in group 4 (mean 0.9 mg/L) (Tables 1 and 2).

Sensitivity and specificity

We used two clinically significant cut-off values based on the measured GFR, 90 and 70 mL/min. Using 90 mL/min, SCr was found to have a sensitivity of 46% (95% CI; 31.6–60.7), a specificity of 100% (95% CI; 92.6–100), a positive predictive value (PPV) of 70% (95% CI; 59.2–86.6) and a negative predictive value (NPV) of 100% (95% CI; 81.5–100). CysC was found to have a sensitivity of 75% (95% CI; 60.1–85.9), a specificity of 84% (95% CI; 71.4–91.4), a PPV of 78% (95% CI; 63.2–88.5) and a NPV of 81% (95% CI; 68.7–89.4) (see

Table 3
Sensitivity and specificity for SCr and CysC

	Sensitivity [a]	Specificity [a]	*p*	AUC	*p* [b]
70 mL/min					
SCr (mg/dL) [c]	77.4 (58.4–89.7)	91 (81.8–96)	<0.0001	0.81 (0.72–0.94)	0.004
CysC (mg/L)	100 (90.2–100)	48.4 (35.9–61.2)	<0.0001	0.94 (0.92–0.98)	
90 mL/min					
SCr (mg/dL) [c]	46 (31.6–60.7)	100 (92.6–100)	<0.0001	0.72 (0.60–0.82)	0.004
CysC (mg/L)	75 (60.1–85.9)	83.6 (71.4–91.4)	<0.0001	0.84 (0.76–0.92)	

AUC: area under the ROC curve.
[a] 95% CI.
[b] compared AUC by DeLong methods.
[c] To convert mg/dL to μmol/L of creatinine, multiply by 88.

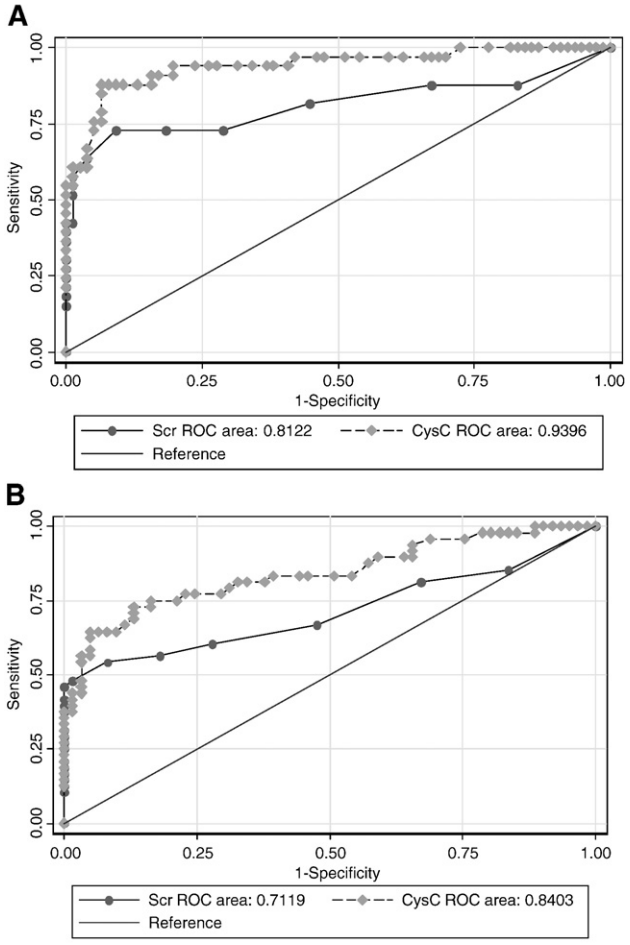

Fig. 1. ROC curves for SCr and CysC when kidney dysfunction is defined as GFR ≤ 70 mL/min (A) or GFR ≤ 90 mL/min (B).

Table 3). Optimum cut-offs on serum values were 1.05 mg/dL (92.4 μmol/L) for SCr and 1.01 mg/L for CysC. Using a GFR of 70 mL/min as a reference cut-off, SCr was found to have a sensitivity of 77% (95% CI; 58.4–89.7), a specificity of 91% (95% CI; 81.8–96), a PPV of 77% (95% CI; 58.4–89.7) and a NPV of 91% (95% CI; 81.8–96). CysC was found to have a sensitivity of 100% (95% CI; 90.2–100), a specificity of 48% (95% CI; 35.9–61.2), a PPV of 58% (95% CI; 45.9–68.6) and a NPV of 100% (95% CI; 86.2–100) (see Table 3). Optimum cut-offs on serum values were 0.85 mg/dL (74.8 μmol/L) for SCr and 0.87 mg/L for CysC.

ROC curves (Fig. 1) were drawn at the reference cut-off values. At 90 mL/min the AUC for SCr is 0.72 ($p=0.0001$; 95% CI 0.6–0.82) and for CysC is 0.84 ($p=0.0001$; 95% CI 0.76–0.92). Using 70 mL/min as a cut-off, the areas are 0.81 ($p=0.0001$; 95% CI 0.72–0.94) for SCr and 0.94 ($p=0.0001$; 95% CI 0.92–0.98) for CysC. The difference in AUC, which is based on paired data, is statistically significant ($p=0.004$).

Costs per test

Cost per enzymatic test in Bogotá, Colombia: CysC U$ 27; SCr U$ 2. Cost per enzymatic test in Madrid, Spain: CysC U$ 3; SCr U$ 0.08.

Discussion

CKD is a major worldwide problem, its prevalence is rapidly increasing and most of the patients remain undiagnosed. Accurate, cost-effective and widely available tests are required for diagnosis of CKD and to detect its progression to allow treatment at an early stage. Several methods exist to estimate GFR, and the accuracy (sensitivity plus specificity) of all of them tends to be highest for a normal GFR, and lowest with a reduced GFR [5]. Traditionally SCr has been used because it is readily available and cheap, but its limitations, especially in pediatric population, make it necessary to continue the search for better tests. Therefore our purpose was to determine the diagnostic accuracy of CysC and SCr for GFR estimation in children, using 99Tc-DTPA clearance as the reference standard, and to discuss some of the economic implications of these tests, in order to guide clinicians when to use CysC or SCr for the diagnosis or monitoring of CKD.

This is the first study of CysC in Colombian children. The population in our study comprised almost every child which, because of some urinary episode, was referred to nephrologists for measurement of GFR, and thus included a high number of healthy children. Data from all the patients were included in tests of sensitivity and specificity, and the variety of analyzed pathologic conditions presents a range of values for the analyte that includes those likely to be encountered in routine application. We had just two cases in treatment with corticosteroids during the study; none of them was out of ranges among their diagnostic groups or stages. We had a large number of patients presenting urinary tract infections and lower urinary tract obstruction, but CKD associated with autoimmune diseases, arterial hypertension and obesity are increasing in incidence in Colombia: Hispanics increasingly living the "American way of life" but in an environment lacking efficient primary health care services, leading to an increase in CKD-related incidence and mortality.

Radioisotopic measurements are the reference test of choice in patients of any age and any degree of renal impairment. A relative limitation of the radioisotopic study, at least for CKD of obstructive origin and short evolution (like most of our cases) is that this test will give an indication of the mass of nephrons functionally blocked by the endocapsular hypertension secondary to the obstruction but anatomically healthy and then recoverable if the obstruction were relieved, rather than GFR [26].

In our study, the correlation between SCr and CysC was similar to that found in children by other groups [34–36]. Those good correlation and concordance show that these tests report with similar accuracy. Our results suggest that they both are more useful in glomerular than in tubular pathologies. ROC curves (Fig. 1) were drawn at two clinically very important GFR cut-off values. At 90 mL/min the area under the curve for CysC is greater than that for SCr. In terms of sensitivity, this GFR region is what should be aimed at since early detection leads to early follow-up and possibly better management of a progressive disease. Even though, the clinician must think that there might be almost one in every five patients followed while not needing it.

With a GFR cut-off value of 70 mL/min, CysC and SCr areas increase[1] and the difference between them does not change. When one analyzes panel A of Fig. 1 (GFR 70 ml/min) and draws a diagonal line from 100% sensitivity to 0 specificity, the point of intersection for CysC gives a sensitivity of approximately 83% and a specificity of 86%. For SCr they are 74 and 73% respectively. A similar exercise for panel B of Fig. 1 (GFR 90 ml/min) gives a sensitivity of 76% and a specificity of 76% for CysC and 62 and 68% respectively for SCr. Accordingly, on both occasions, CysC performs better than SCr to evaluate kidney function in children. If a 100% specificity is the common denominator to all comparisons, then at a GFR of 70 ml/min, the sensitivity of CysC is approximately 43% and that of SCr is 48%, whereas at a GFR of 90 ml/min, their respective sensitivities are 55 and 46%. These data verified that CysC is more sensitive than SCr and is a better test in early stages of CKD, but are in conflict with several authors' findings [37,38] who suggest that CysC has no diagnostic advantage over SCr in more advanced disease.

Many studies that have found CysC measurement advantageous over SCr have used the Jaffe method for SCr determination. When enzymatic creatinine and cystatin methods are used as in our study, the advantage of CysC over SCr is less obvious. In addition, both tests are affected by several conditions, particularly by body mass, a key issue in pediatrics. Enzymatic and non enzymatic methods, and prediction equations, have been validated and standardized for SCr but not for CysC. We conclude that CysC is a very interesting option and could be a replacement to serum creatinine for the diagnosis and possibly for monitoring kidney function in children. We agree with Risch et al [19] that standardization of CysC assays will allow better comparison of study results and CysC-based GFR estimates, and should produce recommendations for the use of CysC and its related equations.

An important part of our findings is that one CysC test is more expensive than one SCr enzymatic test, 14 times in Bogotá and 37 times in Madrid (Let us remember that frequent testing is usually necessary in CKD). In cost/benefit terms, monitoring CysC in established CKD could not be justified. The cost of a test is not a major argument given the objective of reducing or preventing renal insufficiency in children, but the clinician must think, on one hand, that the cost of running a CysC test could be too great for it to outweigh the slight benefit in sensitivity, and on the other hand, that the reduced sensitivity of SCr could cause a missed case of renal insufficiency to go unnoticed for an extended period of time, resulting in an increased cost of care. Therefore, if the purpose of the testing strategy is to monitor for progression of kidney disease in someone already diagnosed, an estimated GFR from SCr, improved through validated equations as recommended by task groups [1] should be used. However, if the purpose of testing is to detect disease in a population of children at risk for kidney disease, a more complete cost analysis would be necessary to choose a test.

[1] The increase in AUC does not indicate that tests perform better when GFR reduces. When a lower GFR cut-off value is used, more positive cases are included above this value, increasing sensitivity and reducing specificity, and therefore increasing the probability of false positives.

It is foreseeable that if measurement of CysC becomes more widespread, the cost of the test will reduce, but it still may not be as cheap as SCr measurement, and SCr will keep its superb specificity and availability. Standardization of CysC assays and its related equations is necessary. In the meantime, clinicians must consider if CysC is the test they need, particularly in cost/benefit terms.

Acknowledgments

We are thankful to VelezLab Ltd. (distributor for Dade Behring in Colombia) for training our personnel to carry out CysC tests. We appreciate very much Dr. Vanessa Skelton for correcting the English in this article.

References

[1] Kidney Disease Outcome Quality Initiative (KDOQI). Clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002;39:S1–266.
[2] Gómez C. El peso de la insuficiencia renal crónica en el sistema de salud. El Pulso, No. 77. Medellín, Colombia; February 2005.
[3] Gastelbondo R, Mesa M. Etiología y estado actual de la insuficiencia renal crónica en pediatría. Pediatría 2000;vol 35(No 4).
[4] Barsoum R. Chronic kidney disease in the Developing World. N Engl J Med 2006;354:997–9.
[5] Narvaez-Sanchez R, Silva M, Salamanca A, Ríos D. Are the techniques for glomerular filtration rate estimation reliable? Rev UDCA, 2006;9:115–20.
[6] Stevens L, Coresh J, Greene T, Levey A. Assessing kidney function — measured and estimated glomerular filtration rate. N Engl J Med 2006;354:2473–83.
[7] Sarnak M, Katz R, Stehman-Breen C, Fried L, Swords N, Psaly B, et al. The Cardiovascular Health study. Cystatin C concentration as a risk factor for heart failure in older adults. Ann Intern Med 2005;142:497–505.
[8] Levin A. Editorial: Cystatin C, serum creatinine, and estimates of kidney function: searching for better measures of kidney function and cardiovascular risk. Ann Intern Med 2005;142:586–8.
[9] Shlipak M, Sarnak M, Katz R, Fried L, Sehger S, Newman A, et al. Cystatin C and the risk of death and cardiovascular events among elderly persons. N Engl J Med 2005;352:2049–60.
[10] T. Okay. Cistatina C: um novo marcador de funçao renal.
[11] Newman D. Cystatin C. Ann Clin Biochem 2002;39:89–104.
[12] Swan S. The search continues—an ideal marker of GFR. Clin Chem 1997;43:913–4.
[13] Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Cystatin C — A new marker of glomerular filtration rate in children independent of age and height. Pediatrics 1998;101:875–81.
[14] Herget-Rosenthal S, Marggraf G, Hüsing J, Göring F, Pictruck F, Janssen O, et al. Early detection of acute renal failure by serum cystatin C. Kidney Int 2004;66:1115–22.
[15] Paz J, Muñoz J, Arévalo S. Comparación entre cistatina C, creatinina plasmática, fórmula de Cockroft – Gault y gammagrafía renal con DTPA para estimación de la tasa de filtración glomerular. Trabajo de investigación presentado para optar al título de Especialista en Nefrología. Bogotá: Facultad de Medicina. Universidad del Rosario; 2004.
[16] Herget-Rosenthal S, van Wijk JA, Bröcker-Preuss M, Bökenkamp A. Increased urinary cystatin C reflects structural and functional renal tubular impairment independent of glomerular filtration rate. Clin Biochem 2007;40:946–51.
[17] Keevil B, Kilpatrick E, Nichols S, Maylor P. Biological variation of cystatin C: implications for the assessment of glomerular filtration rate. Clin Chem 1998;44:1535–9.
[18] Gabutti L, Ferrari L, Ferrari N, Mombelli G, Marone C. Does cystatin C improve the precision of Cockcroft and Gault's creatinine clearance estimation? J Nephrol 2004;17:673–8.

[19] Risch L, Drexel H, Huber A. Differences in glomerular filtration rate estimates by 2 cystatin C-based equations. Clin Chem 2005;51:2211.
[20] Madero M, Sarnak M, Stevens L. Serum cystatin C as a marker of glomerular filtration rate. Curr Opin Nephrol Hypertens 2006;15:610–6.
[21] MacDonald J, Marcora S, Jibani M, Roberts G, Kumwenda M, Glover R, et al. GFR estimation using cystatin C is not independent of body composition. Am J Kidney Dis 2006;48:712–9.
[22] Demirtas S, Akan O, Can M, Elmali E, Akan H. Cystatin C can be affected by nonrenal factors: a preliminary study on leukemia. Clin Biochem 2006;39:115–8.
[23] Gates G. Glomerular filtration rate: estimation from fractional renal accumulation of 99Tc-DTPA (stannous). AJR 1982;138:565–70.
[24] Roos J, Doust J, Tett S, Kirkpatrick C. Diagnostic accuracy of cystatin C compared to serum creatinine for the estimation of renal dysfunction in adults and children — A metaanalysis. Clin biochem 2007;40:383–91.
[25] Shore R, Koff S, Mentser M, Hayes J, Smith S, Smith J, et al. Glomerular filtration rate in children: determination from the Tc-99m-DTPA renogram. Radiology 1984;151:627–33.
[26] Salerno L, Curiale B, Pepe S, Corrao S, Mirabile D. GFR measuring with 99mTc-DTPA: limits in obstructive acute renal failure. Panminerva Med 1991;33:30–4.
[27] Dade Behring Marburg. Instructive brochure for cystatin C N latex technique, edition; November 2001.
[28] Obuchowski N. Sample size calculations in studies of test accuracy. Stat Methods Med Res 1998;7:371–92.
[29] Li J, Fine J. On sample size for sensitivity and specificity in prospective diagnostic accuracy studies. Statist Med 2004;23:2537–50.
[30] Fleiss JL. Statistical methods for rates and propotions. 2nd ed. New York: John Wiley & Sons, Inc.; 1981.
[31] Lin L. A concordance correlation coefficient to evaluate reproducibility. Biometrics 1989;45:255–68.
[32] DeLong E, DeLong D, Clarke-Pearson D. Comparing the areas under two or more correlated receiver operating curves: a nonparametric approach. Biometrics 1988;44:837–45.
[33] Hanley J, McNeil B. The meaning and use of the area under the receiver operating characteristic (ROC) curve. Radiology 1982;143:29–36.
[34] Zappitelli M, Parvex P, Joseph L, Paradis G, Grey V, Lav S, et al. Derivation and validation of cystatin C-based prediction equations for GFR in children. Am J Kidney Dis 2006;48:221–30.
[35] Dharnidharka V, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. AJKD 2002;40:221–6.
[36] Grubb A, Nyman U, Björk J, Lindström V, Rippe B, Sterner G, et al. 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.
[37] Christensson A, Grubb A, Nilsson J, Norrgren K, Sterner G, Sundkvist G. Serum cystatin C advantageous compared with serum creatinine in the detection of mild but not severe diabetic nephropathy. J Intern Med 2004;256:510–8.
[38] Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Cystatin C serum concentrations underestimate glomerular filtration rate in renal transplant recipients. Clin Chem 1999;45:1866–88.