Comparison of estimated GFR equations based on serum cystatin C alone and in combination with serum creatinine in patients with coronary artery disease

Yusuf Çetin Doğaner, Ümit Aydoğan*, James Edwin Rohrer, Aydoğan Aydoğdu**, Tuncer Çaycı***, Cem Barçın****, Kenan Sağlam*****

Department of Family Medicine, Mayo Clinic; Rochester, MN-USA
Departments of *Family Medicine, **Endocrinology, ***Biochemistry, ****Cardiology, *****Internal Medicine, Gülhane Military Medical Faculty; Ankara-Turkey

ABSTRACT

Objective: Several new equations (GFRCKD-EPI-cr, GFRCKD-EPI-CysC, GFRCKD-EPI Cr+CysC) are used for the calculation of estimated GFR (eGFR) to evaluate renal function. These equations explicitly demonstrate the association of coronary artery disease (CAD) and severe renal impairment cases. However, these equations are considered insufficient to explain the relation with normal or mildly impaired eGFR and CAD. Our hypothesis was to indicate the inversely proportional relationship of eGFR values, calculated by the different equations, with the presence of CAD in patients with normal or mildly impaired renal function.

Methods: Eighty-eight patients who underwent elective coronary angiographic intervention were enrolled into the study. The study population was divided into two groups based on angiographic documents: patients with normal coronary arteries (CAD-) and patients with CAD (CAD +). These patients were stable and decided to implement angiography for the purpose of suspicion about CAD and control. Since it is thought that eGFR equations based on creatinine are inadequate to determine chronic kidney disease (CKD) and overestimate CKD diagnosis, cystatin C-based equations are considered an alternative. Due to the potential effects of inflammatory events of the markers used in equations, patients with diabetes mellitus, severe CKD, and inflammatory bowel disease were excluded from the study.

Results: The average age of all participants was 51.93±9.31 (32-65 years); 80.7% (n=71) was male. A statistical difference was found between the CAD (-) group and the CAD (+) group in terms of the variables of age (45.46±8.48 vs. 54.95±8.11, p<0.001), gender (67.9% vs. 86.7%, male, p=0.037), cystatin C values (1.37±0.34 vs. 0.85±0.39, p<0.001), and GFR equations defined by the Chronic Kidney Disease Epidemiology: GFRCKD-EPI-cr (85.86±14.20 vs. 79.45±10.25, p=0.018), GFRCKD-EPI-CysC (58.61±21.87 vs. 100.82±32.00, p<0.001), and GFRCKD-EPI Cr+CysC (68.29±13.49 vs. 90.75±18.34, p<0.001). After adjustment of the variables in multiple regression analyses, only age (OR, 1.199; 95% CI, 1.077 to 1.335, p=0.001), gender (OR, 8.252; 95% CI, 0.223 to 55.659, p=0.030), and the GFRCKD-EPI-CysC equation (OR, 1.059; 95% CI, 1.028 to 1.090, p<0.001) were detected as predictors for presence of CAD.

Conclusion: GFR equations based on cystatin C or combined with creatinine may have superiority to GFR equations based on creatinine alone in CAD patients. However, the impact of different variables on the GFRCKD-EPI-CysC equation should not be ignored in specific groups, such as CAD. (Anatol J Cardiol 2015; 15: 571-6)

Keywords: coronary artery disease, glomerular filtration rate, cystatin C

Introduction

Chronic kidney disease (CKD) is one of the major causes in the evaluation of etiologic risk factors of coronary artery disease (CAD) (1). The correct estimation of renal function may be decisive in appraising the prognosis in patients with CAD. Glomerular filtration rate (GFR) is a substantial indicator for kidney function in the diagnosis and treatment stages. More than 80% of laboratories state the estimated glomerular filtration (eGFR) if they have the capacity to measure serum creatinine values (2). Nevertheless, all creatinine-based equations have limitations due to variables affecting the serum creatinine level, including features, such as age, sex, and race (3). Recently, cystatin C, a non-glycosylated 13-kDa protein, has emerged as a novel marker of kidney function that is less influenced by muscle mass or diet (4, 5).

Several equations based on measured GFR or creatinine clearance have been improved for clinical usage. The renowned
equations are the Cockcroft-Gault (C&G) equation (6), the simplified Modification of Diet in Renal Disease (MDRD) equation, and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (1). Recent equations to estimate GFR based on cystatin C have now been developed, including a CKD-EPI cystatin C equation (7). The utility of estimating GFR by cystatin C versus creatinine-based equations for predicting presence of CAD has not been well studied (8). The most important reasons concerning the knowledge not being precise about cystatin C are the rare use of cystatin C in clinical practice, differences between standardized methods for analyzing cystatin C, lack of determination of which reference ranges should be used, and high costs for the assays of cystatin C compared with assays for creatinine (9). Analyses of those studies indicated that no patient sampling excluded glucose metabolism disorders completely.

We designed this study to compare the difference of GFR equations based on creatinine, cystatin C alone, and combinations of both in CAD patients in either normal and mildly decreased GFR levels (GFR>60 mL/min/1.73 m²). Our hypothesis was that the decline of all GFR equation values in the range of normal or milder GFR values was more likely to be associated with the presence of CAD. To confirm this hypothesis, eGFR equations using serum cystatin C alone and in combination with serum creatinine were compared with the equation using serum creatinine alone in patients who had undergone coronary angiography to detect the existence of CAD.

Methods

Study groups

Eighty-eight patients were enrolled into the study with suspicion of CAD who underwent coronary angiography under elective conditions between June 2010 and June 2011. All of these patients had applied to the cardiology outpatient clinic. The sample of the study was chosen from patients with GFR>60 mL/min/1.73 m². The aim of this sampling was to exclude the impact of severe CKD on the GFR equation based on cystatin C due to presence of CAD. CAD was accepted as any level of involvement in the coronary arteries according to angiographic results.

Patients with glucose metabolism disorders, severe chronic renal failure (eGFR ≤60 mL/min/1.73 m²), previous acute coronary syndrome (within 3 months) or cerebrovascular disease (stroke, transient ischemic attack, etc.), inflammatory bowel disease, and high sedimentation rate (>20 mm/h) were excluded from the study to document particularly the relationship of those markers (cystatin C and creatinine) with GFR equations. The initial eGFR values were measured by MDRD formula. Hypertension was defined as blood pressure >140/90 mm Hg or under current antihypertensive medication. The patients were informed about the purpose of the study. The patients agreed to participate in the study and signed the patient consent form. The Local Ethic Committee approved study.

Laboratory measurements

Laboratory test results had been analyzed within the last 72 hours, and all serum parameters were recorded prior to the angiographic intervention. All of these serum parameters tests were performed on a Hitachi 912 Chemistry Analyzer using Roche Diagnostic kits (Roche Diagnostics, Indianapolis, USA). The laboratory was a center in which internal and external validity tests are routinely performed. The patients were tested by oral glucose tolerance test (OGTT) to exclude the effects of glucose metabolism disorders.

Coronary angiographic interventions were performed through the right femoral artery or the left radial artery of patients by a cardiologist in the angiography laboratory using a Philips Integris 3000 (Philips Medical Systems, Best, and Netherlands). Coronary angiographies were performed upon stable patients group vary from the suspicious treadmill test results or for the purpose of controlling after PTCA or acute coronary syndrome at least 3 months ago. The patients were evaluated according to the angiographic results in terms of presence of CAD.

Patients who had any glucose metabolism disorder (DM, IGT, IFG) according to the OGTT test results or any previous history of glucose metabolism disorders; patients with overt kidney failure (eGFR <60 mL/min/1.73 m²); patients with acute coronary syndrome before angiography (within 3 months); patients with sedimentation value higher than 20 mm/hour; patients with thyroid functions disorders; and patients under 20 and over 65 years were excluded from the study. GFR values were calculated using the MDRD equation initially.

Assessment of cystatin C

Serum cystatin C measurements were analyzed by the enzyme-linked immunosorbent assay (ELISA) method using the Human Cystatin C ELISA kit A02 (Bio Vendor, Laboratorium´ medicína a.s., Czech Republic) in a laboratory on a BioTek reader (BioTek Instruments, Winooski, Vermont, USA). It has a strong correlation with immunoturbidimetric and latex nephelometric methods used in the measurement of cystatin C.

Statistical analysis

Analyses were performed with the use of the SPSS, version 15.0 (Chicago, IL, USA) package program. Definitive statistical methods were presented in numbers and percentages for categorical variables and as mean±standard deviation for numerical variables. The distribution of data was analyzed through the Kolmogorov-Smirnov test. Since the data were in a normal distribution, student t-test was performed for numerical variables. Categorical data were analyzed by Chi-Square analysis. The correlations between variables were analyzed through Pearson or Spearman test. Multiple logistic regression analysis was used to identify independent predictors of presence of CAD in terms of GFR measurements. This study should be accepted as preliminary, indicating the use of newer eGFR equations based on cystatin C and creatinine to predict the existence of CAD.
Arising from this fact, we did not calculate the study power. Statistical significance was accepted as $p<0.05$. The GFR-estimating equations used in the study are listed below (Table 1).

### Results

Of the 92 patients who underwent coronary angiography intervention, 4 were excluded due to the probability of impact in terms of pre-diabetic state (impaired fasting glucose). The short characteristic features and different GFR measurements are shown in Table 2. The average age of all participants was 51.93±9.31 (32-65 years), and 80.7% (n=71) was male; 68.2% (n=60) was diagnosed as CAD patients after the intervention for coronary angiography.

When the coronary arteries were examined due to the involvement of CAD, a negative correlation was detected between GFRCKD-EPI-cr and GFRCKD-EPI-CysC (r=-0.246, $p=0.021$; r=-0.497, $p<0.001$, respectively). A negative correlation was appointed between GFRCKD-EPI-cr and GFRCKD-EPI-CysC (r=-0.313, $p=0.003$). There was no sig-
significant relationship between the state of hypertension and eGFR equations.

The multiple logistic regression analysis revealed a significant portion of the variance in terms of independent predictors, including age, gender, and GFRCKD-EPI-CysC for presence of CAD. GFRCKD-EPI-cr was not significantly associated with the dependent variable. After the adjustment of independent variables in the multiple regression analyses, only age (OR, 1.199; 95% CI, 1.077 to 1.335, p=0.001), gender (OR, 8.252; 95% CI, 2.223 to 55.659, p=0.030), and the GFRCKD-EPI-CysC equation (OR, 1.059; 95% CI, 0.972 to 1.101, p=0.290) were detected as statistically related with the presence of CAD (Table 3).

### Discussion

In our study, a significant association was found between the state of presence of CAD and serum cystatin C values, age, gender, GFRCKD-EPI-Cr, GFRCKD-EPI-CysC, and GFRCKD-EPI-Cr+CysC. After the adjustment of covariates, patients with male gender, older age, and higher GFRCKD-EPI-cysC measurements were more likely to be associated with the diagnosis of CAD. In addition, a negative correlation was detected between GFRCKD-EPI-cr and the LAD, Cx coronary arteries, and the total number of involved coronary arteries. A positive correlation was detected for GFRCKD-EPI-cysC with the LAD, Cx, RCA, and total number of involved coronary arteries.

The usage of cystatin C-related studies in clinical practice and research projects has been growing within the last few years. The more likely of the epidemiological studies concluded a positive relationship between high cystatin C values and cardiovascular risk and developing cardiovascular events in a selected patient sample (10, 11), which was certainly contrary to previous immunohistochemical, experimental, and clinical studies (12-14). Although numerous studies imply that cystatin C is more sensitive than creatinine in the measurement of GFR (15, 16), the clinical role of cystatin C measurement has not been clarified yet. The hypothesis supported in prior studies was the better predictive value of cystatin C compared with creatinine to predict mortality and adverse cardiovascular events in the general population and the elderly (17, 18). In contrast with most of the studies, we detected that participants with a normal angiogram had higher serum levels of Cys-C compared to patients with CAD. Results of the present study confirmed the findings of few aforementioned studies reported a significant association between lower levels of cystatin C and progression of atherosclerosis. Moreover, in accordance with previous studies, the present study demonstrated an inhibitory role of cystatin C on the proteolytic activity of the atherosclerotic plaque.

Some authors have indicated that the equations combining creatinine and cystatin C might be more accurate for the diagnosis and monitoring of CKD (19-21). In another study, contrary to these findings, GFRCKD-EPI-CysC was also associated with CAD; however, the addition of serum creatinine values to the equation did not contribute a beneficial effect in estimating cardiovascular events. The same study group emphasized that when they measured the GFR values with the MDRD study equation and GFRCKD-EPI-cr, both equations had a similar relation with CAD, heart failure, and stroke (22). When the numbers of vessels involved by atherosclerosis level were examined in the present study, a negative correlation with GFRCKD-EPI-cr and a positive correlation with GFRCKD-EPI-CysC were reported. This different finding can be accepted as a reactive response of endogenous markers to the atherosclerotic process in coronary arteries. Contrary to most of the studies, since cystatin C values were lower in patients with CAD than in non-CAD patients, GFR equations based on cystatin C were higher in CAD patients compared to non-CAD patients in the present study. The discrepancy of these results might originate from other unknown predictors affecting the interaction between atherosclerosis and kidney functions and other complicated physiological pathways.

The CKD-EPI equations of GFR measurements (GFRCKD-EPI-cr, GFRCKD-EPI-CysC, GFRCKD-EPI Cr+CysC) were found significantly compared to the GFRMDRD and GFRC&G equations in our study. GFR equations of CKD-EPI with cystatin C alone or combined with creatinine were more significant compared to GFR equations of CKD-EPI with creatinine. Multivariate logistic regression analysis pointed out that only the GFRCKD-EPI-cysC equations among the equations of GFR measurements designated the risk of CAD significantly after adjustments of the covariates. GFRCKD-EPI-cysC measurement values were increasingly associated with the risk of presence of CAD. It was unexpected and the opposite result compared with most studies. We assumed that the most decisive reason for this result was the selection of the patient sample with GFR values of >60 mL/min/1.73m². The atherosclerotic burden of coronary arteries might make a contribution to the results in unknown pathophysiological pathways. Furthermore, the diagnostic value of cystatin C based on equations to predict GFR might disagree among different studies. The measurement techniques of GFR used in studies are not homogeneous and may have caused these different results (23).

Even patients with mildly decreased kidney function (eGFR of 60-89 mL/min/1.73m²) have CAD risk compared to those who

| Table 3. Descriptive and GFR evaluation predictors in CAD (+) patients compared to CAD (-) patients (n=88) (estimated GFR levels are given in mL/min/1.73 m²) |
|---------------------------------|------------------|------------------|------------------|
|                                | Odds ratio of association with presence of CAD | 95% CI | P       |
| Gender, male/female            | 8.252            | 0.223 to 55.659  | 0.030            |
| Age, years                     | 1.199            | 1.077 to 1.335   | 0.001            |
| GFRCKD-EPI-cr                  | 1.034            | 0.972 to 1.101   | 0.290            |
| GFRCKD-EPI-CysC                | 1.059            | 1.028 to 1.090   | <0.001           |

*Multivariate logistic regression analysis
CAD (-) - patients with normal coronary arteries, CAD (+) - patients with coronary artery diseases, GFRCKD-EPI-cr: glomerular filtration rate chronic kidney disease epidemiology collaboration- creatinine, GFRCKD-EPI-CysC: glomerular filtration rate chronic kidney disease epidemiology collaboration- cystatin C
have normal GFR levels (≥90 mL/min/1.73 m²) (24). CKD evaluated by the GFRCKD-EPI-CysC equations indicated better clinical benefits in diagnosing patients at a high risk of CVD compared with the GFRCKD-EPI-cr equations (25). Astor et al. (18) determined that a lower eGFR based on a cystatin C-related equation was strongly associated with a higher risk for cardiovascular mortality. Since this study was designed as a cross-sectional study, further analyses could not be made.

One of the strengths of our study departed to the others was the presence of exclusion criteria, including diabetes mellitus, thyroid disorders, all cancer types, and inflammatory processes, that might impress the cystatin C state. Thus, we tried to observe the net association between cystatin C and the various types of eGFR equations reflecting kidney function in CAD patients. In addition, only patients with a GFR measurement greater than 60 mL/min/1.73 m² were enrolled into the study to possess a homogeneous study sample.

Study limitations

The present study has several limitations. The number of the patient sample was low due to the exclusion criteria to avoid the effects on cystatin C values. The latter important limitation of our study is not to have GFR measurements. Therefore, we can not decide the best alternative test which either cystatin C or creatinine reflects the GFR as assigned by a Gold standard (i.e., inulin clearance). Since this study was designed as preliminary descriptive research, we could not follow up on the relation between the eGFR values and future cardiac events. Another limitation might be the generalizability of our findings. This study sample more likely included patients who were male (80.7%), aged 32-65 years, and caucasian. Therefore, these results may not be generalized to females, older adults, or other ethnic groups.

Conclusion

In conclusion, we believe that the GFRCKD-EPI-CysC equation or the combined equation with creatinine has superiority to GFR equations based on creatinine alone. However, as we obtained in our study, the influence of different kinds of unknown variables on the GFRCKD-EPI-CysC equation should not be ignored in specific groups, such as CAD disease.

Conflict of interest: None declared.

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