Clinical Utility of Creatinine- and Cystatin C–Based Definition of Renal Function for Risk Prediction of Primary Cardiovascular Events in Patients With Diabetes

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OBJECTIVE—To assess the cardiovascular risk of diabetic subjects with chronic kidney disease (CKD) based on different estimated glomerular filtration rate (eGFR) equations and to evaluate which definition of CKD best improves cardiovascular risk prediction of the Framingham Cardiovascular Risk Score (Framingham-CV-RS).

RESEARCH DESIGN AND METHODS—CKD was defined as eGFR <60 mL/min/1.73 m^2, estimated by the creatinine-based Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations and a cystatin C–based equation (CKD-CysC). Cox regression was used to estimate hazard ratios (HRs) of subjects with CKD for incident cardiovascular events in a cohort of 1,153 individuals with diabetes (baseline age 50–74 years). Furthermore, the CKD definitions were added individually to a reference model comprising the Framingham-CV-RS variables and HbA_{1c}, and measures of model discrimination and reclassification were assessed.

RESULTS—During 5 years of follow-up, 95 individuals had a primary cardiovascular event. Crude HRs were increased for all CKD definitions. However, after adjusting for established cardiovascular risk factors, HRs for both creatinine-based CKD definitions were attenuated to point estimates of 1.03, whereas the HRs for the cystatin C–based CKD definition remained significantly increased (HR 1.75 [95% CI 1.07–2.87]). Extension of the reference model by the different CKD definitions resulted in an increase in the *c* statistic only when adding CKD-CysC (from 0.638 to 0.644) along with a net reclassification improvement of 8.9%.

CONCLUSIONS—Only the cystatin C–based CKD definition was an independent risk predictor for cardiovascular events in our diabetic study cohort and indicated a potentially better clinical utility for cardiovascular risk prediction than creatinine-based equations.

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Chronic kidney disease (CKD) is a frequent disease in the elderly, especially among older adults with diabetes (1,2). However, epidemiologic data about the prevalence of CKD in patients with diabetes remain sparse and the accuracy of the different estimating equations to assess renal function in clinical routine is still debated (1,3,4).

CKD can be classified with an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2 (CKD stages 3–5) (5). The most commonly used equation to estimate glomerular filtration rate (GFR) is the serum creatinine–based abbreviated Modification of Diet in Renal Disease (MDRD) equation (6), although it is well known that it underestimates GFR in the normal and high-normal range (7). Recently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation has been introduced as a better means of estimate eGFR in observational research (8). However, data from patients with diabetes comparing the CKD-EPI and MDRD equations are still limited (8). Performance of creatinine-based eGFR in patients with diabetes and nephropathy lacks accuracy to monitor kidney function (9), especially in the early phases of renal impairment, and it can take years until other signs of a glomerulopathy such as albuminuria appear (10). Therefore, cystatin C–based estimating equations are suggested to show better clinical utility compared with creatinine-based equations (11,12).

Which formula is best to be used to classify CKD in subjects with diabetes is an important question, especially because effective interventions exist to reduce the risk for cardiovascular disease and progression to end-stage renal disease (13). However, no study thus far has compared the MDRD and CKD-EPI formulas with a cystatin C–based equation in patients with diabetes (14). An eligible end point to shed further light on this question is an estimated predictive value of each equation for cardiovascular disease because CKD is clearly associated with cardiovascular end points, independent of established cardiovascular risk factors (15,16).

Therefore, the objective of this analysis is to estimate the prognostic utility of serum creatinine– and cystatin C–based CKD definitions for incident cardiovascular events in subjects with diabetes.

RESEARCH DESIGN AND METHODS

—This investigation is based on the ESTHER Study (Epidemiologische

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Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung [German]), an ongoing cohort study with details previously described (17–19). Briefly, 9,953 subjects, aged 50–74 years at baseline, were recruited by their general practitioners during a routine health check-up between 2000 and 2002 in the German federal state of Saarland. The ESTHER Study has been approved by the ethics committees of the Medical Faculty of the University of Heidelberg and the Medical Association of Saarland and is being conducted in accordance with the Declaration of Helsinki.

Data collection

Information on sociodemographic characteristics, smoking behavior, alcohol consumption, physical activity, and prevalent diseases (e.g., diabetes and hypertension) was obtained by a standardized questionnaire. Medication at baseline, height, weight, systolic blood pressure, and HDL and LDL cholesterol were assessed and documented on a standardized form by the general practitioners during the health check-up along with the information on whether the study participant had fasted overnight as requested. Furthermore, blood and urine samples were taken during the health check-up, centrifuged, shipped to the study center, and stored at −80°C.

Laboratory measurements

Serum creatinine measurements were performed by the kinetic Jaffe method (interassay coefficient of variation [CV] 6%). Serum cystatin C concentrations were measured by immunonephelometry on a Behring Nephelometer II (Dade-Behring Diagnostic, Marburg, Germany) (interassay CV 3.8%). Urinary albumin concentration was measured with an immunonephelometry assay (interassay CV 5.2%) in spot urine sample. Interleukin (IL)-6 concentrations were determined with the Human IL-6 Quantikine HS ELISA kit (R&D Systems, Wiesbaden, Germany). C-reactive protein (CRP) was measured by immunoturbidimetry with the wrCRP antibody (Bayer, Leverkusen, Germany) on the ADVIA 2400. HbA_{1c} was measured from whole blood samples with high-performance liquid chromatography on the Variant II (Bio-Rad, Munich, Germany). Total cholesterol and triglycerides measurements were determined from serum samples by a high-performance liquid chromatography method calibrated with the Synchron LX multicalibrator system (Beckman Coulter, Galway, Ireland). All measurements were performed in a blinded fashion.

Study population

Participants of the ESTHER baseline examination were excluded from this analysis if they did not have physician-diagnosed diabetes (n = 8,526). Subjects with diabetes were defined if this was documented on the health check-up form by the treating physicians or if antidiabetes medication was prescribed. Furthermore, subjects with a primary myocardial infarction or stroke before baseline (n = 236), without determined creatinine or cystatin C levels (n = 9), with a non-European country of origin (n = 7) and subjects lost to follow-up right after baseline (n = 22) were excluded, resulting in a final study population of n = 1,153 subjects with diabetes.

End point definition

We defined a composite end point of cardiovascular events of myocardial infarction, stroke, or cardiovascular death. Cardiovascular deaths were identified via death certificates obtained from public health departments of Saarland, and all deaths coded with ICD-10 code I were counted. Vital status could be ascertained by information from a mortality register of the public health departments of Saarland or by received questionnaires from the participants for 99.1% of the cohort's baseline participants, and an ICD code for the leading cause of death from death certificates was available for 92.4% of the deceased. Incidences of nonfatal myocardial infarction and nonfatal stroke were ascertained at 2- and 5-year follow-up by standardized questionnaires sent to the study participants with subsequent validation of self-reported cases by medical records obtained from the study participants' general practitioners. The latter could be obtained for 82.3 and 80.5% of myocardial infarctions and strokes, respectively. Lack of validation was due to nonresponse of the general practitioners. Self-reports were accepted for definition of incident cases as they were confirmed by general practitioners in 83.5 and 70.1% of validated cases, respectively.

Estimation of GFR and definition of CKD

GFR was estimated using the equations of the MDRD (6) and CKD-EPI (8) and by a cystatin C–based estimating equation (eGFR-CysC) according to Arnal-Dade (11):

$$\text{eGFR (MDRD)} = 186.3 \times (\text{creatinine})^{-1.154} \times \text{age}^{-0.203} \times 0.742 \text{ (if female)} \quad (1)$$

$$\text{eGFR (CKD-EPI)} = 141 \times \min(\text{creatinine}/k, 1)^{\alpha} \times \max(\text{creatinine}/k, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018 \text{ (if female)} \quad (2)$$

$$\text{eGFR-CysC} = 74.835/(\text{cystatinC}^{1.333}) \quad (3)$$

where k is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates the minimum of creatinine/k or 1, and max indicates the maximum of creatinine/k or 1.

In equations, eGFR was expressed in milliliters per min per 1.73 meters squared, weight in kilograms, cystatin C in milligrams per liter, serum creatinine in milligrams per deciliter, and age in years. Special calculations for blacks were not considered because all final study participants with diabetes were of nonblack origin. CKD was defined by an eGFR <60 mL/min/1.73 m^2 (5). For illustration of the agreement or disagreement of the three CKD definitions, Fleiss-Cohen κ coefficients, which use quadratic weights for deviations, were calculated (20).

Statistical analyses

Baseline characteristics of the study population were described in subjects who had a cardiovascular event during follow-up and compared with subjects without a cardiovascular event (Wilcoxon rank-sum test for continuous measures, χ^2 test for proportions). Furthermore, all baseline characteristics were dichotomized and their associations with eGFRs by creatinine- and cystatin C–based equations were assessed by Wilcoxon rank-sum tests. In addition, Spearman correlation coefficients were determined for the three eGFRs among each other and of the eGFRs with continuously quantifiable cardiovascular risk factors.

Cox proportional hazards models were used to assess hazard ratios (HRs) and Kaplan-Meier survival curves for the three CKD definitions compared with subjects without CKD at baseline with respect to incident cardiovascular events during follow-up (log-rank test for comparison of Kaplan-Meier curves). Models were adjusted for age, sex, total cholesterol–to–HDL ratio,

systolic blood pressure, usage of ACE inhibitors or angiotensin receptor blockers (ARBs), and current smoking, as well as for BMI, HbA_{1c}, diabetes type, and IL-6 to further adjust for the severity and length of diabetes and inflammatory processes—all strongly modulating the cardiovascular risk in subjects with diabetes (18,21). Multiple imputation was used to adequately deal with missing covariate values (Supplementary Data).

To assess the predictive value of the three CKD definitions for cardiovascular risk prediction, they were added individually to the Framingham Cardiovascular Risk Score (Framingham-CV-RS). Measures of model fit, discrimination, reclassification, and calibration were assessed with Cox proportional hazards regression. Model fit was assessed by the likelihood ratio test and Akaike Information Criterion (AIC). Whereas the likelihood ratio usually increases with the adding of variables to a risk score, the AIC punishes adding variables that do not substantially increase model fit. Ultimately, the model with the lowest AIC is considered as best fitting the data. Discrimination of the models was compared on the basis of the areas under the receiver operating characteristic curves (AUCs) (synonymous to *c* statistic). It was tested for different AUCs according to the method of Hanley and McNeil, taking into account that the AUCs were derived from the same cases (22). The AUC has well-known limitations to detect an improvement of a risk score by an additional biomarker, even if it is strongly associated with the disease (23). Therefore, the net reclassification improvements (NRIs) by adding each CKD definition were calculated according to the risk strata 0–2.5, >2.5 to 5, >5 to 10, and >10% of predicted probability for a cardiovascular event (24). Lower cut points for the risk strata than the usually used cutoffs of 5, 10, and 20% for 10-year risk prediction were chosen because of the 5-year follow-up duration of the cohort (23). For an improved net reclassification, adding a variable to a risk score should lead to more case subjects who move up a risk category than down and, if possible, also to more control subjects who move down a risk category than up. Furthermore, the integrated discrimination improvements (IDIs) were assessed that estimate the extended model's improvement in the difference in predicted probabilities for case subjects (which should increase) and control subjects (which should decrease) across all possible cut points (24,25). Calibration of all assessed risk scores was verified by May and Hosmer's simplification of the Gronnesby-Borgan test (26). The study sample was divided into quintiles according to the study participants' ranks in an estimated risk score. *P* values >0.05 for the comparison of observed and expected cases indicate good model calibration.

Besides eGFR, other measures of renal damage, such as micro- or macroalbuminuria, are used to assess kidney function. Therefore, the aforementioned statistical analyses were also carried out for micro- or macroalbuminuria, defined by urinary albumin ≥20 mg/L, and compared with the results for the different CKD definitions.

All statistical tests were two sided, with $P < 0.05$, and all analyses were conducted with the software package SAS, version 9.2 (SAS Institute, Cary, NC). In a sensitivity analysis, all analyses were repeated excluding subjects with imputed covariate data.

RESULTS—At baseline, the median age of the 1,153 included study participants with diabetes was 64 years (interquartile range 60–69), 577 (50%) were males, and the median diabetes duration was 5 years (1–11). Thirty-nine subjects (4%) reported onset of diabetes before the age of 40 years and were considered to have type 1 diabetes. During follow-up, 91 subjects died, and the response rate of surviving participants in the 2- and 5-year follow-up was 97.2 and 87.6%, respectively.

Associations of baseline characteristics with cardiovascular events during follow-up and eGFR estimated by different equations

Table 1 shows the baseline characteristics of the study population stratified by cardiovascular event status during follow-up. IL-6, CKD based on eGFR-CysC (CKD-CysC), urinary albumin, micro- or macroalbuminuria, and HbA_{1c} were significantly associated with the outcome ($P < 0.05$), and sex, age, hypertension, systolic blood pressure, physical inactivity, total cholesterol, and low-dose aspirin were associated with the outcome with $P < 0.20$. CKD as defined by the creatinine-based GFR-estimating equations was not associated with incident cardiovascular events ($P = 0.51$ for both equations).

A lower eGFR was associated with the presence of most cardiovascular risk factors (Table 2). GFRs estimated by the MDRD and CKD-EPI equations show a similar pattern of associations with the assessed risk factors. Compared with the creatinine-based estimating equations, eGFR-CysC was on the one hand not associated with sex, smoking, or use of statins but was on the other hand associated with low education, alcohol abstinence, BMI, micro- or macroalbuminuria, and insulin dependency in a statistically significant manner. Correlation coefficients of continuous cardiovascular risk factors and eGFRs by the three equations were all between −0.34 and 0.08 (Supplementary Table 1). Relatively high inverse correlations ($r = -0.21$ to -0.34) were observed for age with all three eGFR equations, for total cholesterol with the creatinine-based equations, and for IL-6 with the cystatin C–based equation. In a comparison of the different eGFRs, a higher correlation coefficient (difference of at least 0.10) was observed for the creatinine-based eGFRs with regard to total cholesterol and triglycerides and for eGFR-CysC with regard to BMI, IL-6, and urinary albumin. Whereas the GFRs estimated by the MDRD and CKD-EPI equations were very highly correlated ($r = 0.98$), they showed a less pronounced correlation with eGFR-CysC ($r = 0.44$ and $r = 0.46$, respectively; all *P* values <0.001).

Risk for major cardiovascular events of subjects with CKD, based on different GFR-estimating equations

The prevalence of CKD at baseline by means of the different estimating equations was 14.6% (95% CI 12.6–16.8) by MDRD, 15.5% (13.5–17.8) by CKD-EPI, and 14.8% (12.9–17.0) by cystatin C. The creatinine-based formulae identified nearly the same study participants with CKD (165 identical/17 not identical), and Fleiss-Cohen κ indicated very good agreement (κ = 0.94 [95% CI 0.92–0.97]). However, despite a comparable prevalence, the cystatin C–based equation identified a quite different population with CKD compared with the creatinine-based MDRD (79 identical/181 not identical; κ = 0.37 [0.30–0.45]) and CKD-EPI (86 identical/178 not identical; κ = 0.40 [0.33–0.48]) equations.

During a median follow-up time of 5.0 years (25th–75th percentile 4.8–5.2), 44 participants had a primary stroke (38 physician confirmed), 40 had a primary myocardial infarction (37 physician confirmed), and 39 died of a cardiovascular cause. Overall, 95 individuals met the criteria of the composite end point "major cardiovascular event" (88 physician confirmed). The

Table 1—*Description of the study population of patients with diabetes, stratified by cardiovascular event status during follow-up*

Characteristic	*n* (with/without CVE)[a]	Subjects without CVE during FUP	Subjects with CVE during FUP	*P*
Sex (% male)	95/1,058	49.3	57.9	0.11
Age (years)	95/1,058	64 (60–68)	66 (61–70)	0.07
Education ≤9 years (%)	91/1,012	81.3	75.8	0.20
Current smoking (%)	94/1,020	16.4	21.3	0.22
Alcohol consumption (g/day)	82/939	3 (0–12)	3 (0–13)	0.84
Diagnosed hypertension (%)[b]	95/1,058	73.7	80.0	0.18
Use of ACE inhibitor or ARB (%)	94/1,058	54.7	59.3	0.71
Systolic blood pressure (mmHg)	93/1,035	140 (130–156)	150 (135–160)	0.05
Physical inactivity (%)[c]	95/1,050	26.0	32.6	0.16
BMI (kg/m^2)	95/1,055	29.2 (26.7–32.2)	28.4 (26.0–31.8)	0.23
Total cholesterol (mg/dL)	95/1,058	215 (179–246)	221 (186–254)	0.16
Fasting LDL cholesterol (mg/dL)	50/560	140 (116–168)	148 (122–174)	0.25
HDL cholesterol (mg/dL)	64/662	46 (37–55)	43 (37–53)	0.33
Fasting triglycerides (mg/dL)	75/881	143 (97–213)	141 (95–207)	0.88
Prescribed statins (%)	94/1,058	8.2	8.5	0.92
Prescribed low-dose aspirin (%)	94/1,058	13.3	19.2	0.12
CRP (mg/L)	95/1,051	2.6 (1.3–5.7)	3.4 (1.5–7.7)	0.11
IL-6 (pg/mL)	84/975	2.5 (1.4–5.2)	**3.3 (1.9–6.9)**	**0.024**
CKD (MDRD) (%)	95/1,058	14.4	16.8	0.51
CKD (CKD-EPI) (%)	95/1,058	15.3	17.9	0.51
CKD (cystatin C) (%)	95/1,058	14.0	**24.2**	**0.007**
Urinary albumin (mg/L)	95/1,052	11.3 (11.3–12.5)	11.3 (11.3–46.0)	**0.004**
Micro- or macroalbuminuria (mg/L)				**0.005**
<20	62/833	79.2	65.3	
20–199 (microalbuminuria)	21/152	14.4	22.1	
≥200 (macroalbuminuria)	12/67	6.4	12.6	
Type 1 diabetes (%)[d]	39/833	4.1	8.3	0.10
HbA_{1c} (%)	94/1,057	6.8 (6.1–7.6)	**7.0 (6.1–8.5)**	**0.048**
Use of antidiabetes drugs (%)	94/1,058	49.9	53.2	0.54
Insulin dependency (%)	94/1,058	15.8	18.1	0.56

Data are percent or median (1st–3rd quartile) unless otherwise indicated. CVE, cardiovascular event; FUP, follow-up. Boldface numbers indicate a level of significance of $P < 0.05$. [a]Numbers do not always add up to the total of $n = 1,153$ as a result of missing values. [b]Documented in medical records or prescribed antihypertensive drugs. [c]Performs <1 h of moderate or vigorous physical activity per week. [d]Assumed for those with self-reported age at diagnosis of diabetes before age of 40 years.

incidence rate for major cardiovascular events was 18.5 per 1,000 person-years.

Differences in proportions of event-free survivors during 5 years of follow-up of subjects with CKD based on the different equations are illustrated in Kaplan-Meier survival curves in Supplementary Fig. 1. The curves of subjects with CKD are below the curves of subjects without CKD for all equations. However, the difference was not significant for the MDRD ($P = 0.45$) and CKD-EPI ($P = 0.41$) equations, whereas a significant difference was observed when the CKD-CysC definition was used ($P = 0.003$).

Incidence rates of cardiovascular events and HRs estimated in crude models were increased in subjects with prevalent CKD compared with subjects without CKD for all three eGFR-estimating equations (Table 3). However, the highest HR that was also the only statistically significant one after full adjustment (model 2) was found for the cystatin C–based estimating equation (HR 1.75 [95% CI 1.07–2.87]), which was comparable in magnitude with the HR of micro- or macroalbuminuria (1.55 [1.00–2.45]) (Supplementary Table 2). Including both CysC-CKD and micro- or macroalbuminuria in the fully adjusted model resulted in only small attenuations of their respective HRs. In contrast, the creatinine-based equations had HRs close to the no-effect value of 1.

Predictive value of CKD definitions for incident cardiovascular events

Table 4 shows the results of the evaluation of a model comprising the Framingham-CV-RS variables [age, sex, log(total cholesterol), log(HDL), log(systolic blood pressure), use of antihypertensive treatment, and current smoking] and HbA_{1c} (reference model) with respect to the prediction of cardiovascular events during follow-up in subjects with diabetes and compares it with models comprising the same variables extended by CKD defined by three different definitions. The reference model showed a good overall model fit and calibration. It was not further improved by adding any of the creatinine-based CKD definitions, but numbers improved when CKD-CysC was added. The AUC of 0.638 of the reference model decreased when the MDRD and CKD-EPI CKD definitions were added and increased to 0.644 when CKD-CysC was added. This was in line with a higher AIC of the models containing creatinine-based CKD definitions compared with the AIC of the reference model, an indication of worse model fit. However, none of the differences in AUCs between models were statistically significant (all $P > 0.05$).

Net reclassification improvement by CKD was higher when the CKD definition

was based on the cystatin C–based equation (8.9%) than when it was based on the MDRD (2.2%) or CKD-EPI (3.2%) equations. However, all P values were >0.05. A similar pattern was seen for IDIs with 1.15% ($P = 0.010$) for the cystatin C–based CKD definition and 0.48% for both creatinine-based CKD definitions ($P = 0.028$ for both equations). Measures of model fit, discrimination, calibration, and reclassification of micro- or macroalbuminuria were comparable in magnitude or slightly higher compared with those reported for CysC-CKD (Supplementary Table 3). The result patterns did not change when a complete case analysis was carried out excluding subjects with imputed covariate values or when eGFR was included as a continuous measure (data not shown).

CONCLUSIONS—In this prospective cohort study comprising 1,153 subjects with diabetes from a primary care setting, we found a strong and statistically significant association of prevalent CKD with incident cardiovascular events during follow-up when CKD was defined by a cystatin C–based estimating equation but not when CKD was defined by the commonly used creatinine-based ones. In addition, typical measures of model fit and clinical accuracy showed a trend toward higher values when the cystatin C–based CKD definition was added to the Framingham-CV-RS compared with CKD definitions by creatinine-based equations. Therefore, compared with creatinine-based equations, cystatin C–based eGFR equations may have better clinical utility for identifying subjects at high risk for cardiovascular events in patients with diabetes and may be helpful in improving risk assessment in this high-risk group.

Cystatin C is a low–molecular-weight protein that is produced at a constant rate by all nucleated cells, is freely filtered in the glomeruli, and is almost completely reabsorbed and catabolized by the proximal renal tubular cells. Its serum concentrations are mainly determined by GFR (27). It has been suggested that cystatin C levels are generally not influenced by extrarenal factors such as age, muscle mass, or sex, and evidence accumulates for the value to estimate GFR by means of cystatin C in both patients with and patients without diabetes. Although different cystatin C–based formulae have been developed, irrespective of the formula used, the accuracy and precision by cystatin C methods in general seem to be better

Table 2—*Differences in GFR, estimated by different estimating equations, according to presence or absence of cardiovascular risk factors in subjects with diabetes*

		eGFR (mL/min/1.73 m^2)								
		MDRD			CKD-EPI			Cystatin C		
Risk factor	*n* (without/with risk factor)[a]	Without risk factor	With risk factor	*P*	Without risk factor	With risk factor	*P*	Without risk factor	With risk factor	*P*
Male sex	576/577	82 (65–100)	89 (73–107)	**<0.001**	83 (65–95)	88 (72–97)	**0.005**	81 (68–98)	82 (68–96)	0.97
Age ≥65 years	602/551	90 (74–108)	79 (63–100)	**<0.001**	92 (76–100)	79 (61–92)	**<0.001**	87 (75–103)	76 (64–90)	**<0.001**
Education ≤9 years	211/892	86 (72–106)	85 (69–104)	0.66	87 (70–96)	86 (69–96)	0.91	86 (73–99)	81 (68–97)	**0.031**
Current smoking	927/187	84 (69–102)	95 (74–114)	**<0.001**	84 (69–95)	94 (74–101)	**<0.001**	81 (68–98)	84 (69–99)	0.32
No alcohol	582/439	86 (72–103)	86 (72–108)	0.74	87 (71–95)	87 (69–98)	0.28	82 (70–99)	81 (65–94)	**0.016**
Hypertension[b]	297/856	88 (73–107)	84 (68–103)	**0.014**	89 (74–98)	85 (67–95)	**0.003**	87 (77–103)	79 (66–94)	**<0.001**
Use of ACE inhibitor or ARB	409/743	86 (71–107)	85 (67–99)	**0.048**	87 (71–98)	85 (66–94)	**0.035**	84 (70–99)	78 (64–92)	**<0.001**
Physical inactivity[c]	841/304	87 (71–105)	80 (63–100)	**0.001**	87 (71–97)	80 (62–95)	**0.001**	82 (70–99)	77 (63–92)	**<0.001**
BMI ≥30 kg/m^2	655/495	86 (71–105)	85 (68–105)	0.54	86 (70–96)	86 (68–96)	0.89	82 (70–99)	80 (66–93)	**0.002**
Total cholesterol ≥240 mg/dL	807/346	88 (72–109)	80 (63–94)	**<0.001**	88 (71–98)	80 (63–93)	**<0.001**	80 (67–94)	84 (71–101)	**0.002**
LDL cholesterol ≥160 mg/dL	417/193	86 (70–107)	84 (69–101)	0.42	87 (70–96)	85 (70–95)	0.54	81 (69–98)	82 (67–96)	0.98
HDL cholesterol <40 mg/dL	510/216	84 (69–102)	86 (69–105)	0.61	84 (69–95)	86 (68–97)	0.42	81 (68–98)	79 (65–95)	0.10
Triglycerides ≥200 mg/dL	680/276	88 (72–109)	81 (63–98)	**<0.001**	88 (71–97)	82 (63–94)	**<0.001**	81 (70–96)	80 (65–96)	**0.022**
Statins	1,057/95	86 (70–106)	78 (61–93)	**<0.001**	87 (70–97)	78 (61–92)	**<0.001**	81 (68–98)	82 (61–98)	0.35
Low-dose aspirin	993/159	86 (70–105)	81 (65–100)	0.06	87 (70–96)	81 (64–94)	**0.022**	82 (69–98)	76 (62–90)	**<0.001**
CRP ≥3 mg/L	618/528	87 (71–108)	83 (67–100)	**0.002**	87 (71–97)	84 (68–95)	**0.025**	82 (71–99)	80 (66–94)	**<0.001**
IL-6 ≥6 pg/mL	832/227	87 (72–105)	81(60–109)	**0.004**	87 (72–96)	81 (60–96)	**0.002**	82 (70–98)	76 (61–92)	**<0.001**
Albuminuria ≥20 mg/L	895/252	86 (70–104)	86 (67–108)	0.79	87 (70–96)	86 (65–96)	0.56	82 (70–98)	77 (58–93)	**<0.001**
Type 1 diabetes[d]	833/39	87 (71–106)	75 (53–94)	**0.005**	87 (70–96)	76 (54–95)	**0.049**	82 (69–98)	74 (51–92)	**0.007**
HbA_{1c} ≥7.0%	643/508	84 (69–103)	87 (71–106)	0.13	85 (69–96)	88 (71–97)	0.11	81 (69–94)	82 (67–99)	0.30
Insulin dependency	968/184	86 (70–105)	82 (65–99)	0.10	87 (70–96)	82 (63–95)	0.20	82 (69–98)	78 (60–92)	**0.002**

Data are median (25th–75th percentile) unless otherwise indicated. Boldface numbers indicate a level of significance of $P < 0.05$. [a]Numbers do not always add up to the total of $n = 1,153$ as a result of missing values. [b]Documented in medical records or prescribed antihypertensive drugs. [c]Performs <1 h of moderate or vigorous physical activity per week. [d]Assumed for those with self-reported age at diagnosis of diabetes before the age of 40 years.

Table 3—*Incident primary cardiovascular events during follow-up according to CKD at baseline, defined by different eGFR equations*

	Subjects with diabetes	
	Without CKD	With CKD
CKD (MDRD), *n*	985	168
Incident CVE, *n*; PY	79; 4,399	16; 731
Incidence rate per 1,000 PY	18.0	21.9
Crude model[a]	1 (ref.)	1.23 (0.69–2.05)
Adjusted model 1[b,c]	1 (ref.)	1.13 (0.64–1.97)
Adjusted model 2[b,d]	1 (ref.)	1.03 (0.58–1.83)
CKD (CKD-EPI), *n*	974	179
Incident CVE, *n*; PY	78; 4,357	17; 772
Incidence rate per 1,000 PY	17.9	22.0
Crude model[a]	1 (ref.)	1.25 (0.71–2.05)
Adjusted model 1[b,c]	1 (ref.)	1.12 (0.65–1.95)
Adjusted model 2[b,d]	1 (ref.)	1.03 (0.59–1.81)
CKD (cystatin C), *n*	982	171
Incident CVE, *n*; PY	72; 4,437	23; 692
Incidence rate per 1,000 PY	16.2	33.2
Crude model[a]	1 (ref.)	**2.02 (1.24–3.18)**
Adjusted model 1[b,c]	1 (ref.)	**1.79 (1.10–2.91)**
Adjusted model 2[b,d]	1 (ref.)	**1.75 (1.07–2.87)**

Data are HR (95% CI) unless otherwise indicated. CVE, cardiovascular event; PY, person-years. Boldface numbers indicate a level of significance of $P < 0.05$. [a]Unadjusted (eGFR only). [b]Estimated in joint analyses in 20 imputed complete datasets. [c]Adjusted for age, sex, total cholesterol–to–HDL cholesterol ratio, systolic blood pressure, use of ACE inhibitors or ARBs, and smoking status. [d]Adjusted for variables of adjusted model 1 plus BMI, HbA_{1c}, diabetes type, and IL-6.

compared with creatinine-based formulae (27,28). For diabetes patients, Pucci et al. (29) observed a higher predictive value in the early detection of reduced renal function (determined by the iohexal plasma clearance method) when the GFR was estimated by cystatin C compared with GFR estimation by the creatinine-based MDRD and Cockroft-Gault formulae. Particularly poor precision and accuracy of creatinine-based eGFR equations with respect to the range of 60–89 mL/min/1.73 m^2 (determined by the isotopic ^{51}creatinine EDTA method) have also been described by others (9,30). However, it should be noted that in contrast, the validity of creatinine-based equations is higher in diabetic patients with very poor renal function (31). Nevertheless, evidence accumulates for better accuracy and precision of eGFR-CysC compared with eGFR based on creatinine levels in subjects with diabetes, especially in early changes of kidney function.

The prevalence of CKD in our cohort as assessed with all of the different equations was 14.6% (MDRD), 14.8% (cystatin C), and 15.5% (CKD-EPI). A large Italian cohort study of Caucasian diabetic patients with a mean age of 67 years observed a prevalence of CKD of 18.7 and 17.2% according to the MDRD and the CKD-EPI equations, respectively (32). Considering the slightly lower mean age of our cohort, these prevalences compare favorably with those estimated in our cohort. Although the prevalence of CKD-CysC was comparable with the creatinine-based ones, it has to be kept in mind that the agreement in identified CKD patients assessed by Fleiss-Cohen κ coefficients was fair. This is consistent with the low correlation coefficients of eGFR-CysC with GFRs estimated by the creatinine-based equations. Furthermore, eGFR-CysC was more strongly correlated with BMI and IL-6, and CKD-CysC was more strongly associated with low education, physical inactivity, and insulin-dependent diabetes compared with creatinine-based GFR or CKD definitions, respectively. Another cross-sectional analysis showed that diabetes, higher CRP, higher white cell blood count, and lower serum albumin were associated with higher serum cystatin C but lower serum creatinine (33). These differences in associations with important risk factors for atherosclerosis development may partly explain the higher incidence rate of cardiovascular events observed in subjects with CKD-CysC compared with subjects with a creatinine-based CKD definition (16,34) and, furthermore, the fair agreement of the CKD definitions.

The overall incidence rate of 18.5 cardiovascular events per 1,000 person-years in our cohort (50% men; mean age 64 years and free of previous myocardial infarctions or strokes) was comparable with the incidence rate of 21.0 cardiovascular events per 1,000 person-years in a British cohort of diabetic patients (100% men, mean age 63 years and free of coronary heart disease) (35). This is the first cohort study with diabetic patients that compares the associations of creatinine- and cystatin C–based CKD definitions with incident cardiovascular events. The measures of model fit, discrimination, and reclassification of a cystatin C–based estimating equation raised the potential for a better prognostic utility compared with creatinine-based ones, which is in line with previous prospective studies conducted in the general population (36) or patients with coronary heart disease (12,37). Several groups are currently investigating new approaches to predict cardiovascular risk by combining CKD-CysC and albuminuria results for staging of CKD (38). We did not see substantially different patterns when we added albuminuria to the fully adjusted models (data not shown), and the difference between the creatinine- and cystatin C–based equations basically remained unchanged. Nevertheless, we observed in our cohort a significant association of micro- or macroalbuminuria and cardiovascular events, independent of presence of CKD in the model, and moreover, we showed a clinical utility of micro- or macroalbuminuria in cardiovascular risk prediction at least as high as those of CysC-CKD. This supports the view that a combination of these measures to define CKD may have additional benefits for cardiovascular risk prediction over the current definitions.

When looking at the results, the following limitations and strengths should be considered. We observed statistically significant IDIs but not NRIs for adding the different CKD definitions to the Framingham-CV-RS. This may be due to the substantially lower power of the NRI compared with the IDI. Nevertheless, the NRI results should be interpreted with caution (39).

The most important limitation with respect to the study design is the lack of a gold standard measurement for GFR (i.e., insulin clearance). In addition, we only had single measurements of creatinine and cystatin C. These limitations however, are present in most of the cited epidemiological studies and may indeed lead to an overestimation of CKD prevalence.

A strength is that the ESTHER study is a population-based cohort, a fact supporting

the external validity of our study. However, it is important to note that our results can only be generalized for older Caucasian adults with diabetes (age range 50–74 years). The assumption that subjects who reported onset of the disease before the age of 40 have type 1 diabetes is imprecise and was only used to give a crude estimate of the proportion of subjects with type 1 diabetes in the study cohort (40).

Currently, available data on eGFR-CysC equations still have to be considered to be very limited, and further studies in younger subjects, subjects of different ethnicities, and subjects with type 1 diabetes have to be carried out to corroborate the findings.

In conclusion, our study in patients with diabetes shows a strong association of prevalent CKD with incident cardiovascular events when CKD was defined by a cystatin C–based equation but not when CKD was defined by creatinine-based ones. Furthermore, we showed that the cystatin C–based CKD definition may have better clinical utility for identifying subjects at high risk for cardiovascular events in patients with diabetes than the commonly used creatinine based equations. If corroborated in other studies, our results have important implications for clinical practice because they point out the potential benefits of cystatin C measurements for the monitoring of kidney function in patients with diabetes.

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Table 4—*Measures of model fit, discrimination, calibration, and reclassification of risk models without and with a CKD definition in the prediction of the composite cardiovascular end point*

	Framingham model plus HbA_{1c}[a]	Framingham model plus HbA_{1c} plus CKD (MDRD)[a]	Framingham model plus HbA_{1c} plus CKD (CKD-EPI)[a]	Framingham model plus HbA_{1c} plus CKD (CysC)[a]
Model fit				
Likelihood ratio	22.7 (df = 8, **P = 0.004**)	24.9 (df = 9, **P = 0.006**)	24.9 (df = 9, **P = 0.006**)	27.7 (df = 9, **P = 0.001**)
AIC	1,285	1,287	1,287	1,282
Discrimination: *c* statistic (AUC)	0.638	0.637	0.636	0.644
Calibration: observed events/expected events (*P*)[b]				
Quintile 1	13/9.2 (0.20)	13/9.2 (0.22)	13/9.3 (0.21)	11/8.8 (0.45)
Quintile 2	14/13.7 (0.93)	15/13.7 (0.73)	15/13.7 (0.73)	14/13.3 (0.84)
Quintile 3	12/18.0 (0.16)	11/17.7 (0.11)	12/17.7 (0.17)	18/17.2 (0.85)
Quintile 4	20/22.3 (0.62)	19/22.2 (0.49)	18/22.4 (0.36)	16/21.5 (0.24)
Quintile 5	36/31.9 (0.46)	37/32.1 (0.38)	37/31.9 (0.37)	36/34.2 (0.76)
Reclassification (95 case subjects/1,058 control subjects)				
Subjects with CKD, n_{up}/n_{down}[c]	1 (ref.)	15/14	15/13	18/15
Subjects without CKD, n_{up}/n_{down}[c]	1 (ref.)	99/111	98/110	134/195
NRI (*P*)	1 (ref.)	2.2 (0.71)	3.2 (0.57)	8.9 (0.16)
IDI (95% CI), *P*	1 (ref.)	0.48 (0.14–0.83), **0.028**	0.48 (0.14–0.82), **0.028**	1.15 (0.57–1.74), **0.010**

Data are proportions (%) unless otherwise indicated. Boldface numbers indicate a level of significance of $P < 0.05$. [a]Variables of the Framingham model: age, sex, log(total cholesterol), log(HDL cholesterol), log(systolic blood pressure), use of antihypertensive treatment, and current smoking (yes/no). Measures of model fit, discrimination, calibration, and reclassification shown were estimated in imputed complete dataset no. 1 because it had the median *P* value of the likelihood ratio test for the Framingham model of the 20 imputed datasets. [b]The study sample was divided into quintiles according to the study participants ranks in an estimated risk score. $P > 0.05$ for the comparison of observed and expected cases indicates good model calibration. [c]According to four categories: 0–5, >5 to 10, >10 to 15, and >15% of predicted probability for a cardiovascular event in the following 5 years.

D.R. participated in the development of the study concept and design, the acquisition of data, and critical revision of the manuscript for important intellectual content and obtained funding. B.S. and D.R. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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