Prediction and monitoring of chronic kidney disease

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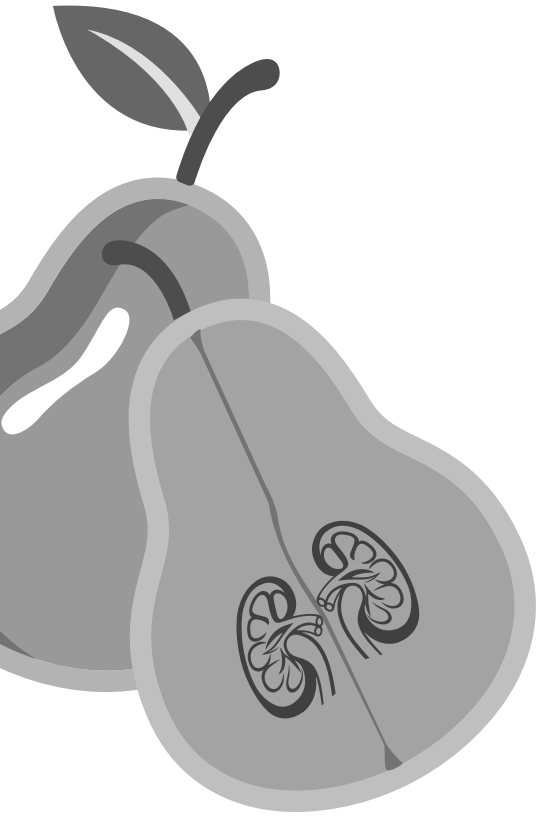
Chapter 6

Creatinine and Cystatin C- based eGFR slopes for monitoring change in kidney function over time



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Abstract

Background and objectives: Creatinine and Cystatin C are validated markers to estimate GFR. It is however unknown which marker performs best for monitoring eGFR change over time.

Design, setting, participants, and measurements: For this study, we used data from the PREVEND study, a large, community based prospective cohort study. We included participants with a least 2 creatinine and cystatin C measurements. eGFR slopes were calculated per individual using linear mixed models with eGFRs based on creatinine, cystatin C or creatinine plus cystatin C. Three methods were used to assess which marker is best for monitoring change in eGFR: comparison of within-individual variability (sum of squares of the repeated eGFR measures per individual (within-individual variability), of between-individual variability (standard deviations of the mean eGFR slopes), and of biological plausibility in two ways (R-squares of the regression models investigating associations of CKD progression risk markers with eGFR slopes, as well as associations with cardiovascular events (CVE) and all-cause mortality (ACM) with Cox regression).

Results: Mean $eGFR_{creat}$ slope was -0.89 ml/min/1.73m^2/year for 6,552 participants during follow-up for 11.3 (IQR 8.5-12.2) years. $eGFR_{creat}$ slopes had the lowest within- and between-individual variability. However, $eGFR_{cysc}$ slopes had the strongest associations with CKD progression risk markers (R^2 0.24 for $eGFR_{cysc}$ versus 0.06 for $eGFR_{creat}$, $P<0.05$). Both slopes had similar associations with ACM, whereas only $eGFR_{creat}$ slopes were associated with CVE.

Conclusion: In this study we found that cystatin C did not consistently outperform creatinine to monitor change in eGFR over time.

Introduction

Creatinine is the standard filtration marker for the estimation of GFR, yet it is known that the use of creatinine has limitations. For instance, in subjects with an abnormal muscle mass for age and/or gender, creatinine based GFR equations may yield unreliable results.[1] For this reason other filtration markers have been developed, such as cystatin C.[2] However, cystatin C also has non-GFR determinants, such as inflammation.[3] The CKD-EPI collaboration has recently developed equations to estimate GFR from age, gender, race and creatinine or cystatin C.[4,5] Earlier research has shown that $eGFR_{creat}$ and $eGFR_{cysc}$ have similar accuracy for estimating measured GFR at a single time point, and that accuracy is best when GFR was estimated with an equation which uses both creatinine and cystatin C ($eGFR_{creat/cysc}$).[5] Others have shown that single measurements of creatinine and cystatin C, and their respective eGFR values, are associated with cardiovascular endpoints and mortality.[6-9] In general, cystatin C and $eGFR_{cysc}$ had a stronger association with these end points than creatinine and $eGFR_{creat}$.

In clinical practice and epidemiological studies, however, kidney function is monitored using repeated measurements of eGFR over time rather than at a single time point. Serum levels of filtration markers can vary within an individual over time, because of change in kidney function, but also because of changes in non-GFR determinants, for example through by changes in muscle mass or meat intake for creatinine, and in case of infections and malignancies for cystatin C.[10,11] Therefore the filtration marker that performs best for single estimations of eGFR may not be the best marker for monitoring change of eGFR over time in an individual. It is not yet known what the best marker performs best in this respect. We aimed to address this question by hypothesizing that the best marker or combination of markers would result in eGFR slopes with the lowest within- and between-individual variability and the strongest associations with established chronic kidney disease (CKD) risk markers, and incidence of cardiovascular events and all-cause mortality.

Materials and methods

This study was performed using data of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study. The PREVEND study is designed to prospectively investigate the natural course of albuminuria and its relation to kidney and cardiovascular disease in a large cohort drawn from the general population. Details of this study have been described elsewhere.[22,23] In summary, 8,592 participants were included in 1997-1998 and were followed over time during a series of follow-up visits. The PREVEND study has been approved by the medical ethics committee of the University Medical Center Groningen. Written informed consent was obtained from all participants. We excluded subjects with less than 2 creatinine and cystatin C measurements (n=2,040), leaving 6,552 individuals for the analyses. Of these, 6463 participants completed the second examination between 2001 and 2003, 5531 the third examination between 2003 and 2006, 4785 the fourth examination between 2006 and 2008, and 3442 the fifth examination between 2008 and 2012.

Measurements

During the study, serum was collected from participants at baseline, and during the follow-up examinations, yielding a total of 4 follow-up visits. All serum samples were stored at -80°Celsius until creatinine and cystatin C were measured. Per participant, all samples were analyzed in the same run, thereby excluding inter-assay variability when assessing eGFR slope over time within a given individual patient. Creatinine was measured with an isotope diluted mass spectrometry traceable enzymatic method, and cystatin C was measured by a PETIA assay (Gentian, Moss, Norway) and calibrated against the international standard for cystatin C,[24] both on a Roche Modular auto-analyzer (Roche diagnostics, Mannheim, Germany). The intra- and inter-assay coefficients of variation were 0.9% and 2.9% for creatinine, and 4.1% and 3.3% for cystatin C, respectively.

Statistical analysis

Baseline characteristics are presented for all participants in the present analysis stratified according to quartiles of change in eGFR. Continuous data is presented as mean with standard deviation (SD) or as median and interquartile range (IQR) in case of non-normal distribution. Categorical data are presented as count and percentage. We used linear regression to calculate the p for trend across quartiles. The outcome measures for the present analyses were slope of estimated GFR (eGFR) expressed as change in eGFR in ml/min/1.73m^2 per year and incidence of cardiovascular events, all-cause mortality and a composite of cardiovascular events and all-cause mortality. eGFR was calculated using the CKD-EPI equations for creatinine, cystatin C and creatinine plus cystatin C.[5]

To investigate which marker was best for monitoring of eGFR slopes, we used 4 methods. First, for comparison of within-individual variability in eGFR slopes we calculated the total sum of squares for each individual using within-participant linear regression. For this analysis only data are used of the 5639 participants who had at least 3 measurements available, because linear regression using 2 available measurements would result in a sum of squares of zero. The medians of the total sums of squares were then compared using the related samples Wilcoxon signed rank test.

Second, we compared the between-individual variability in eGFR slopes per marker by assessing which method yielded the smallest SD of the average eGFR slope. We used linear mixed models to calculate individual eGFR slopes. We compared the SDs of the average slopes using Levene's test.

Third, we compared the biological plausibility of the eGFR slopes in two ways. To this end, we first performed univariable and multivariable linear regression analyses of the eGFR slopes to find the marker with the strongest associations with established CKD progression risk markers. We bootstrapped the analyses with 1,000 repetitions to obtain p-values for the difference in R^2 between the models. Established CKD progression risk markers were age, gender, current smoking, body mass index (BMI), systolic blood pressure (SBP), serum total cholesterol, glucose and urinary albumin creatinine ratio (UACR). The biological plausibility of eGFR slopes was also tested by comparing the associations of eGFR slopes with incident cardiovascular events and all-cause mortality. The eGFR slopes were recalculated using only baseline and the first follow-up eGFR measurement and time was set to zero at follow-up visit 1, to simulate a prediction model using past eGFR slope to predict future events. Five models were built: model 1: crude model with eGFR slope; model 2: as model 1 plus age and gender; model 3: as model 2 plus BMI, smoking and SBP; model 4: as model 3 plus glucose and cholesterol; model 5: as model 4 plus log transformed urinary albumin creatinine ratio.

As a sensitivity analysis, the linear regression models and the Cox regression models were repeated with z-scores of the marker(s) instead of eGFR, to rule out the effect of the variables age, gender and race that are incorporated in the GFR equations. To this end, the values for each marker were inversed (1/marker) and then expressed as a z-score for each individual (value in the individual minus the mean value in the study population, divided by the standard deviation). Composite z-scores were calculated for creatinine plus cystatin C (($z\text{-score}_{creat}$+$z\text{-score}_{cysc}$)/2).

All statistical analyses were performed using STATA (Stata Corp, TX, USA) and SPSS version 22 (IBM, www.ibm.com), and a P-value of <0.05 was adopted to indicate statistical significance.

Results

Baseline data

Baseline characteristics for all 6,552 participants with at least 2 study visits are listed in Table 1. The overall mean age was 49.5±12.1 years, 50% were female and 95% were Caucasian. Mean baseline $eGFR_{creat}$ was 96±15 ml/min/1.73m^2. Participants with the most rapid eGFR decline (Q1, with eGFR decline of more than 1.05 ml/min/1.73m^2 per year) were older, less often smokers, and had a slightly higher BMI and systolic blood pressure, more likely to have diabetes and had higher baseline creatinine and UACR levels (p for trend all <0.05).

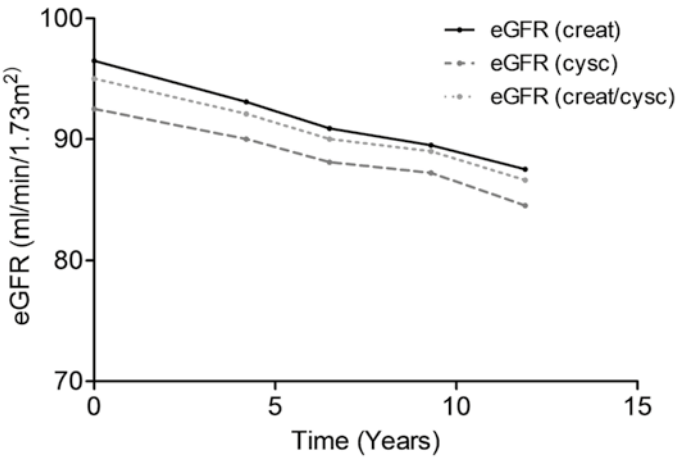

Figure 1 eGFR trajectories for creatinine (solid, black), cystatin C (dashed, dark grey) and creatinine/cystatin C (dotted, light grey).

Table 1 Baseline characteristics of participants in the PREVEND study stratified according to quartile of change in $eGFR_{creat}$ during follow-up.

		$eGFR_{creat}$ change per year (ml/min/1.73m²)				
	All	Q1	Q2	Q3	Q4	P(trend)
		< -1.05	-1.06 to -0.85	-0.86 to -0.69	> -0.70	
N	6552	1638	1638	1638	1638	
Age (years)	49.5±12.1	52.2±12.8	50.0±12.2	48.8±11.8	47.2±11.0	**<0.001**
Female (n(%))	3294 (50)	844 (51)	856 (52)	797 (49)	797 (49)	0.07
Caucasian race (n(%))	6249 (95)	1560 (95)	1566 (95)	1565 (95)	1558 (95)	0.9
Smoking (current) (n(%))	1812 (28)	399 (25)	441 (27)	481 (30)	491 (30)	**0.001**
BMI (kg/m²)	26.0±4.1	26.2±4.2	26.0±4.1	26.0±4.1	26.0±4.1	**0.05**
SBP (mmHg)	128±20	133±22	128±19	127±18	125±17	**<0.001**
DBP (mmHg)	74±10	75±10	73 ±10	73±9.1	73±9	<0.001
Total Cholesterol	5.6±1.1	5.6±1.1	5.6±1.1	5.6±1.1	5.6±1.1	0.5
Diabetes (n(%))	239 (4)	92 (6)	54 (3)	59 (4)	34 (2)	**<0.001**
Glucose (mmol/L)	4.8±1.2	5.0±1.5	4.8±1.0	4.9±1.1	4.7±0.8	**<0.001**
Creatinine (mg/dL)	0.81±0.18	0.81±0.19	0.78±0.17	0.79±0.15	0.87±0.17	**<0.001**
Cystatin C (umol/L)	0.89±0.17	0.92±0.19	0.88±0.18	0.86±0.15	0.89±0.15	**<0.001**
Single run baseline eGFR (ml/min/1.73m²)						
$eGFR_{creat}$	96±15	95±17	99±15	100±14	93±15	0.1
$eGFR_{cysc}$	82±19	89±20	94±18	97±18	94±17	**<0.001**
$eGFR_{creat/cysc}$	95±17	92±18	97±17	99±16	94±15	**<0.001**
UACR (mg/mmol)	0.77 (0.53-1.37)	0.85 (0.47-1.74)	0.78 (0.54-1.33)	0.75 (0.53-1.28)	0.72 (0.51-1.25)	**<0.001**[a]

Continuous variables are presented as mean±standard deviation or median (interquartile range). Categorical variables are presented as count (percentage).

Abbreviations: eGFR, estimated glomerular filtration rate; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; UACR, urinary albumin-to-creatinine ratio.

*: eGFR calculated with the CKD-EPI equation for Creatinine; a: P for natural log of UACR

Within-individual variability of eGFR slopes

The mean follow-up time was 11.3 (IQR 8.5-12.2) years. The eGFR trajectories for each marker or combination of markers are shown in Figure 1. This figure shows that the eGFR slopes derived from creatinine and cystatin C run parallel to each other and that the use of cystatin C resulted in lower eGFR values. The exact eGFR and number of participants per time point are listed in Supplemental Table 1. The results of the within-individual variability analyses are listed in Table 2. As to be expected, the sum of squares increased with the number of available measurements. For each subgroup of available eGFR measurements per individual (3, 4 or 5 available measurements), the sum of squares was consistently lowest for $eGFR_{creat}$ slopes. Overall, $eGFR_{creat}$ slopes also had the lowest sum of squares (95.2, IQR 44.6-195.0) and thus the lowest within-individual variability ($p<0.001$ compared to $eGFR_{cysc}$). Of note, the sum of squares of $eGFR_{creat/cysc}$ slopes was not significantly different from lower than the sum of squares of $eGFR_{creat}$ slopes.

Table 2 Within-individual variability: median and interquartile range of total sum of squares of eGFR slope per filtration marker

No of measurements (N)	$eGFR_{creat}$	$eGFR_{cysc}$	$eGFR_{creat/cysc}$
Overall (5639)	95.2 (44.6-195.0)[a]	125.0 (56.1-249.2)[a,b]	101.1 (47.9-194.3)[b]
3 (898)	44.5 (17.8-119.8)	59.7 (22.8-132.2)	48.1 (19.9-111.2)
4 (1476)	84.7 (38.8-180.7)	110.3 (47.9-229.3)	91.1 (42.1-178.6)
5 (3265)	116.1 (59.9-217.6)	154.8 (76.4-286.1)	124.6 (64.2-220.8)

Median (IQR) sum of squares is presented for the overall slopes for all subjects with at least 3 Creatinine measurements, and broken down by number of available measurements

a= $P<0.001$ for $eGFR_{creat\ SR}$ versus $eGFR_{cysc\ SR}$

b= $P<0.001$ for $eGFR_{cysc\ SR}$ versus $eGFR_{creat/cysc\ SR}$

Between-individual variability of eGFR slopes

The mean eGFR slope and standard deviation per marker is shown in Table 3. For creatinine this was -0.89±0.94 ml/min/1.73m^2/year, and for cystatin C -0.96±1.12 ml/min/1.73m^2/year (difference $P<0.001$). Pairwise comparison of the SDs of each slope showed that the SD for $eGFR_{creat}$ slopes was the smallest ($P<0.01$), meaning that $eGFR_{creat}$ slopes had the lowest between-individual variability when compared to $eGFR_{cysc}$ and $eGFR_{creat/cysc}$ slopes.

Table 3 Between-individual variability: slopes of eGFR Creatinine, Cystatin C, Creatinine + Cystatin C, and pairwise comparisons of SD

	Mean slope±SD	P for SD comparison		
		$eGFR_{creat}$	$eGFR_{cysc}$	$eGFR_{creat/cysc}$
$eGFR_{creat}$	-0.89±0.94			
$eGFR_{cysc}$	-0.96±1.12	**<0.001**		
$eGFR_{creat/cysc}$	-0.93±0.97	**<0.01**	**<0.001**	

Slopes are expressed as change in eGFR in ml/min/1.73m^2 per year. P-values from Levene's test comparing SDs.
Abbreviations are: eGFR, estimated GFR; creat, Creatinine; cysc, Cystatin C; CI, confidence interval.

Associations of established CKD progression risk markers with eGFR slopes

The associations of baseline CKD progression risk markers with eGFR slopes during follow-up are shown in Table 4. In the univariable models we found similar associations of risk markers with all slopes, although for each risk marker the standardized beta's of the association with $eGFR_{cysc}$ slopes were consistently higher than with $eGFR_{creat}$ and $eGFR_{creat/cysc}$ slopes. The multivariable linear regression analyses showed that variance of $eGFR_{cysc}$ slopes was better explained by CKD progression risk markers (R^2 0.24 for $eGFR_{cysc}$, as compared to 0.06 for $eGFR_{creat}$, P<0.05). The variance of the $eGFR_{cysc}$ slopes was predominantly explained by age and albuminuria (standardized beta's -0.36 and -0.12, respectively).

Associations of eGFR slopes with all-cause mortality and cardiovascular events

Table 5 shows the results of the Cox regression analyses. For this analysis we included 6,182 subjects who had eGFR slopes available for creatinine as well as cystatin C for the period between baseline and the first follow-up visit. During follow-up after the second study visit 250 subjects died (4%) and 266 subjects had a fatal or non-fatal cardiovascular event (4%). In the unadjusted as well as the adjusted analyses we found that the three eGFR slopes were similarly associated with all-cause mortality, but that only $eGFR_{creat}$ slopes were associated with cardiovascular events. After adjustment for covariates all these associations were lost, with the exception of the association of $eGFR_{creat/cysc}$ slope with all-cause mortality (HR 0.94, 95% CI 0.88-0.99).

Sensitivity analyses

As sensitivity analyses, the linear regression and Cox regression were repeated with z-scores of each marker instead of their eGFR values (Supplemental Tables 2 and 3). Both analyses yielded essentially similar results when compared to the main analyses. Again, CKD progression risk markers had the strongest associations with z-score slopes of cystatin C. The Cox regression analyses using z-score slopes also showed that the associations with all-cause mortality were similar between the markers and that only z-score slopes of creatinine were associated with cardiovascular events.

Table 4 Associations of eGFR slopes with established CKD progression risk markers

Slopes of:		$eGFR_{creat}$		$eGFR_{cysc}$		$eGFR_{creat/cysc}$	
		Univar	Multivar	Univar	Multivar	Univar	Multivar
Adjusted R^2			0.06		0.24*		0.19*
Age	Stand β	-0.18	-0.12	-0.45	-0.36	-0.38	-0.29
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Female	β	-0.01	-0.04	-0.00	-0.07	-0.02	-0.07
	p-value	0.4	<0.001	0.8	<0.001	0.2	<0.001
Smoking	β	0.03	0.02	0.05	-0.02	0.06	0.01
	p-value	0.003	0.1	0.001	0.1	<0.001	0.5
BMI	Stand β	-0.05	0.04	-0.23	-0.09	-0.18	-0.04
	p-value	<0.001	0.01	<0.001	<0.001	<0.001	0.004
SBP	Stand β	-0.16	-0.09	-0.31	-0.09	-0.28	-0.10
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Glucose	Stand β	-0.11	-0.06	-0.20	-0.04	-0.19	-0.06
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total Cholesterol	Stand β	-0.02	0.05	-0.14	0.03	-0.10	0.05
	p-value	0.1	<0.001	<0.001	0.004	<0.001	<0.001
Ln(ACR)	Stand β	-0.17	-0.11	-0.26	-0.12	-0.25	-0.13
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations are: eGFR, estimated glomerular filtration rate; cysc; Cystatin C; Stand, standardized; BMI, body mass index; SBP, systolic blood pressure; ACR, albumin to creatinine ratio

*=P<0.05 vs Creatinine

Table 5 Cox regression analysis of eGFR slopes between baseline and follow-up visit 1 with death and cardiovascular disease events

	Change in $eGFR_{creat}$		Change in $eGFR_{cysc}$		Change in $eGFR_{creat/cysc}$	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Death						
Model 1	0.90 (0.84-0.96)	0.002	0.85 (0.80-0.90)	**<0.001**	0.84 (0.79-0.89)	**<0.001**
Model 2	0.91 (0.86-0.97)	**0.003**	0.92 (0.87-0.97)	**0.003**	0.90 (0.85-0.96)	**0.001**
Model 3	0.93 (0.88-0.99)	0.03	0.94 (0.89-0.99)	**0.03**	0.93 (0.87-0.98)	**0.01**
Model 4	0.94 (0.88-0.99)	**0.03**	0.94 (0.89-0.99)	**0.04**	0.93 (0.88-0.99)	**0.02**
Model 5	0.95 (0.89-1.01)	0.07	0.95 (0.90-1.01)	0.09	0.94 (0.88-0.99)	**0.05**
Fatal and non-fatal CVE						
Model 1	0.94 (0.88-0.99)	**0.04**	0.96 (0.91-1.02)	0.1	0.94 (0.88-1.00)	0.06
Model 2	0.92 (0.87-0.98)	**0.01**	1.00 (0.94-1.06)	0.9	0.96 (0.89-1.02)	0.2
Model 3	0.94 (0.89-0.99)	**0.04**	1.02 (0.97-1.08)	0.5	0.98 (0.92-1.05)	0.6
Model 4	0.94 (0.89-1.00)	0.06	1.03 (0.97-1.09)	0.3	0.99 (0.93-1.05)	0.7
Model 5	0.95 (0.89-1.01)	0.07	1.03 (0.98-1.09)	0.3	0.99 (0.93-1.06)	0.8

Abbreviations are: eGFR, estimated glomerular filtration rate; cysc, Cystatin C; CVE, cardiovascular events. Hazard ratio is given per unit change in eGFR in ml/min/1.73m^2/year. Model 1: crude model; model 2: as model 1 + age and gender; model 3: as model 2 + bmi, smoking and systolic blood pressure; model 4: as model 3 + glucose and cholesterol; model 5: as model 4 + ln(UACR)

Discussion

In this analysis of the PREVEND study data we aimed to find the best marker for monitoring change in eGFR over time using four methods. In the first two analyses, we found that the within- and between individual variability was lowest for $eGFR_{creat}$ slopes. In addition, slopes of $eGFR_{cysc}$ slopes had the strongest association with established CKD progression risk markers at baseline. Lastly, the association of the three eGFR slopes with all-cause mortality was similar. Interestingly, only $eGFR_{creat}$ slopes were associated with cardiovascular events. Collectively, we found that no marker consistently outperformed the other.

The three statistical methods we used aimed to address the different aspects of eGFR slopes. First, we investigated the within-individual variability of changes of eGFR slopes using the sum of squares. Within-individual variability of eGFR slopes can have four sources: change in kidney function, biological variability of the marker, assay variability, and the eGFR equation used. Literature indicates that the biological variability of creatinine and cystatin C is similar.[12] Furthermore, in the PREVEND study, all markers were measured per individual in a single run thereby excluding day-to-day variability as a source of assay variability. Additionally, the other factors used in the GFR equation do not change over time and are equal for all markers. Furthermore, analyses of z-score slopes of the markers instead of eGFR slopes yielded essentially similar results, thus excluding a significant effect of differences in relative weight of the non-filtration variables in the GFR equations. Therefore the eGFR metric with the lowest sum of squares is likely the marker with the strongest association with true GFR decline, in this case $eGFR_{creat}$ slopes.

Second, we investigated the between-individual variability by comparing the standard deviations of the population mean eGFR slopes. As the eGFR slope for each marker was measured in the same participants, the variation of eGFR decline should be the same for each marker in case it were perfect filtration markers. As a result, the slope with the smallest SD has the highest precision, and theoretically may be expected to be closest to the true eGFR slope. However, this only holds true if all tested markers only reflect kidney function, and are not affected by other body processes, such as inflammation or aging. If these non-GFR factors have more impact on the serum concentration of the marker than GFR itself, this can lead to a falsely low SD. Therefore, the results of this analysis should be interpreted in combination with the results of the other analyses. In our study, we found the lowest between-individual variability for $eGFR_{creat}$ slopes.

Third, we investigated the biological plausibility of the eGFR slopes, by investigating their biological plausibility association of the eGFR slopes with established CKD progression risk markers. We found that $eGFR_{cysc}$ slopes had the strongest association with CKD progression kidney risk markers, both in models with the $eGFR_{cysc}$ slopes as well as with z-score slopes the marker. Others have also found that cystatin C based eGFR slopes have stronger associations with CKD progression risk markers than those based on creatinine.[13]

Fourth, we investigated the association of eGFR slopes with incident cardiovascular events and mortality. We found that the eGFR slopes for both markers had similar associations with all-cause mortality, and surprisingly only $eGFR_{creat}$ slopes were associated with cardiovascular events. It has been shown previously that creatinine-based eGFR decline is associated with an increased risk of

developing end stage renal disease (ESRD), cardiovascular events and mortality.[14-18] Two studies have previously examined the association of eGFR slopes or percentage change in eGFR for creatinine and cystatin C with cardiovascular disease and mortality.[18,19] The study by Ku et al investigated 942 participants of the CRIC-study, which compared the change in eGFR over 2 years for creatinine and cystatin C to mGFR and creatinine clearance with regards to the association with end-stage kidney disease, cardiovascular events, congestive heart failure and all-cause mortality.[19] The second study was performed by Rebholz et al. using data of in the ARIC study, a large community based cohort study conducted in the United States.[18] As in our study, $eGFR_{creat}$ slopes had a stronger association with cardiovascular events than cystatin C based eGFR slopes in both the CRIC and ARIC study. Moreover, in ARIC $eGFR_{creat}$ slopes even had a stronger association with cardiovascular events than slopes of mGFR. In the ARIC study eGFR slopes of all markers were associated with cardiovascular disease, whereas in our cohort only slopes of $eGFR_{creat}$ showed a significant association. Furthermore, as in our study, $eGFR_{cysc}$ based slopes had the strongest association with all-cause mortality in the ARIC study, whereas the CRIC study found that none of the slopes had an association with all-cause mortality.

The differences between our findings and those of the CRIC and ARIC study may have been caused by differences in study population and statistical analyses. For example, our cohort was a community-based cohort and CRIC is a CKD-cohort, and it is possible that the presence of CKD weakens the association of (e)GFR slopes with mortality. Furthermore, only crude Cox regression models were analyzed in CRIC, so there is no information as to whether these (e)GFR slopes had an independent association with end points. For the comparison of our results to the findings of the ARIC study, the discrepant findings may have been caused by a combination of a different statistical approach (ARIC did not have data on albuminuria, and the rate of eGFR decline was expressed as percentage decline over 6 years), and a higher event rate in the ARIC study.

A limitation of this study that needs to be addressed is that mGFR was not available in the PREVEND study. Therefore we could not use change in GFR measured by an exogenous filtration marker (mGFR) as an independent referee to find the best endogenous marker for the assessment of eGFR slopes. However, mGFR assessment is laborious, cumbersome for participants and expensive. It is therefore not feasible to perform repeated a gold standard measurements of GFR in a large epidemiological studies such as PREVEND. In addition, others have shown that mGFR also suffers inaccuracy and bias, and may be less of a gold standard than often assumed.[20,21] The strength of this study is that the PREVEND study is a large, community-based study with a long follow-up duration with several follow-up examinations, allowing analyses of eGFR slopes. Furthermore, creatinine and cystatin C were measured in a single run, thus ruling out day-to-day variation as a possible source of measurement error.

To conclude, in this study we found that eGFR slopes based on creatinine are not consistently outperformed by cystatin C nor by the combination of creatinine and cystatin C with regards to within- and between individual variability and biological plausibility. Therefore, at this time it seems inappropriate to replace or combine creatinine with cystatin C for monitoring kidney function over time.

References

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Supplemental Tables

Supplemental Table 1 Mean eGFR per time point (ml/min/1.73m^2)

	Time (years)				
eGFR equation	0	4.2	6.5	9.3	11.9
N	6414	6463	5531	4785	3442
Creatinine	96.6±15.6	93.1±15.6	90.9±15.6	89.5±15.5	87.5±15.4
Cystatin C	93.4±18.6	90.0±19.7	88.1±20.0	87.2±20.3	84.5±19.6
Creatinine/Cystatin C	95.6±16.6	92.1±17.3	90.0±17.5	89.0±17.7	86.6±17.2

Supplemental Table 2 Associations of z-score biomarker slopes with established CKD risk factors

Multivariable		Creatinine		Cystatin C		Creat/CysC	
		Univar	Multivar	Univar	Multivar	Univar	Multivar
Adjusted R^2			0.04		0.17*		0.08
Age	Stand β	-0.12	-0.06	-0.37	-0.28	-0.22	-0.014
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Female	β	-0.002	-0.004	-0.00	-0.003	-0.00	-0.004
	P-value	**<0.001**	**<0.001**	0.5	**<0.001**	0.4	**<0.001**
Smoking	β	0.001	0.001	-0.003	-0.00	-0.005	0.002
	P-value	**0.01**	0.1	**<0.001**	0.9	<0.001	0.02
BMI	Stand β	-0.03	0.05	-0.20	-0.07	-0.09	0.01
	P-value	**0.03**	**0.001**	**<0.001**	**<0.001**	**<0.001**	0.4
SBP	Stand β	-0.12	-0.08	-0.27	-0.09	-0.20	-0.10
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Glucose	Stand β	-0.11	-0.08	-0.18	-0.06	-0.16	-0.09
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total Cholesterol	Stand β	-0.01	0.04	-0.11	0.04	-0.04	0.05
	P-value	0.4	**0.002**	**<0.001**	**0.002**	**<0.001**	**<0.001**
Ln(UACR)	Stand β	-0.14	-0.08	-0.23	-0.11	-0.19	-0.11
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: creat, creatinine; cysc, cystatin C; BMI, body mass index; SBP, systolic blood pressure; UACR, urinary albumin to creatinine ratio; stand, standardized

Supplemental Table 3 Cox regression analysis of z-score slopes between baseline and follow-up visit 1 with death, cardiovascular disease events and the composite of death and cardiovascular disease events

	Creatinine		Cystatin C		Creatinine/Cystatin C	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Death						
Model 1	0.24 (0.10-0.60)	**0.002**	0.09 (0.03-0.22)	**<0.001**	0.20 (0.10-0.41)	**<0.001**
Model 2	0.30 (0.13-0.73)	**0.01**	0.23 (0.08-0.63)	**0.005**	0.35 (0.18-0.69)	**0.002**
Model 3	0.39 (0.16-0.97)	**0.04**	0.32 (0.11-0.94)	**0.04**	0.44 (0.22-0.89)	**0.02**
Model 4	0.42 (0.17-1.03)	0.06	0.35 (0.12-1.01)	0.052	0.47 (0.23-0.94)	**0.03**
Model 5	0.48 (0.20-1.18)	0.1	0.41 (0.14-1.22)	0.1	0.53 (0.26-1.07)	0.08
Fatal and non-fatal CVE						
Model 1	0.46 (0.18-1.18)	**0.1**	0.50 (0.19-1.32)	0.2	0.50 (0.23-1.05)	0.07
Model 2	0.34 (0.13-0.92)	**0.03**	0.89 (0.31-2.60)	0.8	0.51 (0.23-1.09)	0.08
Model 3	0.44 (0.17-1.13)	0.09	1.42 (0.49-4.15)	0.5	0.66 (0.32-1.38)	0.3
Model 4	0.49 (0.19-1.25)	0.1	1.60 (0.55-4.64)	0.4	0.73 (0.35-1.52)	0.4
Model 5	0.51 (0.20-1.31)	0.2	1.70 (0.59-4.91)	0.3	0.76 (0.36-1.59)	0.5

Abbreviations are: CVE, cardiovascular events; HR, hazard ratio; CI, confidence interval.

Hazard ratio is given per 1 SD change of the respective z-score per year

Model 1: crude model; model 2: as model 1 + age and gender; model 3: as model 2 + bmi, smoking and systolic blood pressure; model 4: as model 3 + glucose and cholesterol; model 5: as model 4 + ln(UACR)