Prediction and monitoring of chronic kidney disease

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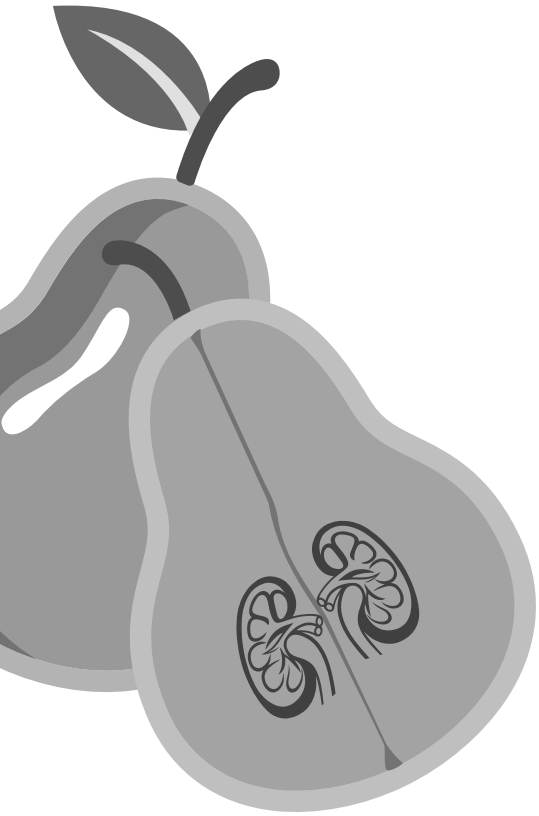
Chapter 7

Estimating GFR using creatinine, cystatin C, beta-2-microglobulin or beta-trace protein to assess change in kidney function over time



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Abstract

Background: Kidney function is generally assessed by means of creatinine based eGFR equations. New filtration markers, including cystatin C, Beta-2-Microglobulin (B2M) and Beta-Trace protein (BTP) have been proposed for more accurate GFR estimation. We aimed to investigate which filtration marker or combination of filtration markers performs best for monitoring eGFR.

Methods: We performed a post-hoc analysis of the SUN-MACRO trial in 849 patients with type 2 diabetes and kidney disease in whom filtration markers were measured at least twice during 18 months follow-up. eGFR was calculated with the CKD-EPI equations. Linear mixed models were used to calculate eGFR slopes per individual for each filtration marker. Reliability of eGFR slopes was assessed by comparison of sum of squares of the eGFR measures per individual (SOQ, within-individual variability), comparison of standard deviations of the mean eGFR slopes (SD, inter-individual variability), and by comparing associations of baseline established CKD risk markers with eGFR slopes (R^2, biological plausibility).

Results: $eGFR_{btp}$ slopes yielded the lowest SOQ (lowest within-individual variability; $P<0.05$ versus SOQ $eGFR_{creat}$) and lowest SDs (lowest between-individual variability; $P<0.001$ vs $eGFR_{creat}$). Multivariable linear regression showed, however, that $eGFR_{cysc}$ slopes reached the highest adjusted R^2 value (highest biological plausibility), but this was not significantly different from the R^2 of the model with $eGFR_{creat}$ alone.

Conclusions: None of the filtration markers, alone or in combination, consistently outperformed creatinine to monitor eGFR over time. These data suggest that in patients with diabetes and kidney disease there is yet no value to replace creatinine by novel filtration markers.

Introduction

The glomerular filtration rate of individual patients is generally estimated by means of creatinine based eGFR equations. Although these estimation equations are widely applicable, they are less reliable in patients with extremes of muscle mass for a given age and gender, and their use is limited by interference of biological substances and drugs with the creatinine assay.[1] Therefore, several alternative glomerular filtration markers have been proposed, including cystatin C, Beta-2-Microglobulin (B2M) and Beta-Trace protein (BTP).[2,3]

Cystatin C has been validated as an alternative filtration marker for eGFR assessment, but has not been shown to outperform creatinine for estimation of GFR.[2] More recently developed filtration markers include B2M and BTP. Both are low molecular weight proteins that are freely filtered by the glomerulus and their serum levels increase with decreasing GFR.[4,5] B2M is an amino acid protein component of class I major histocompatibility molecules and is found on the surface of nucleated cells.[4] BTP is an amino acid glycoprotein enzyme produced in the central nervous system.[5] Recently, new GFR estimating equations were developed using either B2M or BTP, or both markers combined.[3]

These novel filtration markers and their respective eGFR equations are generally validated using cross-sectional comparisons with measured GFR.[2,3] To determine their clinical usefulness, validation should also involve assessment of their ability to assess longitudinal changes in GFR (i.e. eGFR slopes), since in clinical practice as well as in clinical trials change in eGFR over time is used to monitor progression of disease or to assess drug efficacy. Only a limited number of longitudinal studies has been conducted to compare creatinine based eGFR slopes with mGFR.[6-8] Moreover, there is limited to no information on the performance of eGFR based on cystatin C, B2M or BTP to assess change in eGFR over time.[9,10] We therefore first investigated which marker, or combination of markers, is best for monitoring changes in kidney function over time. For this study we used data of SUN-MACRO trial, that included patients with type 2 diabetes and diabetic kidney disease. In the absence of a gold standard measurement technique to assess GFR, we applied three methodological approaches to identify how these novel filtration markers perform in comparison to creatinine to assess change in kidney function over time, i.e. the best filtration marker, or combination of filtration markers, should yield eGFR slopes with the lowest within- and between-individual variability and the strongest association with established CKD risk factors.

Subjects and Methods

Study population

SUN-MACRO was a randomized placebo controlled clinical trial investigating the effect of sulodexide in delaying the progression of kidney function decline in patients with diabetes mellitus type 2 and diabetic kidney disease.[11] The trial was terminated early after a median follow up time of 11 months because sulodexide did not decrease albuminuria. In addition, sulodexide had no effect on change in kidney function. The study design and results have been published previously.[11,12] In short, patients aged ≥18 years with type 2 diabetes, overt proteinuria (≥0.9 g/24h) and increased serum creatinine

(1.5 to 3.0 mg/dL in men and 1.3 and 3.0 mg/dL in women) were eligible for inclusion. The most important exclusion criteria were type 1 diabetes and non-diabetic kidney disease. Patients were enrolled between August 2005 and February 2008. A total of 849 patients were available for analysis.

Measurements

During the study, serum was collected from patients at baseline, and after 3, 6, 12 and 18 months of follow-up. All serum samples were stored at -80°C. The preparation of the samples included one freeze-thaw cycle before measurement of serum creatinine, cystatin C, Beta-2-Microglobulin, and Beta-Trace protein. Others have shown that serum levels of these markers remain stable after freezing and thawing.[13-15] All filtration markers, except creatinine, were measured by nephelometry using a BN II analyzer (Siemens, Germany). Creatinine was measured enzymatically and isotope dilution mass spectrometry (IDMS) traceable with a Cobas 8000 analyzer (Roche Diagnostics, IN, USA). All samples were analysed between November 2014 and May 2015. All markers were measured within 24 hours after thawing. For each patient, all samples were analysed in the same run, thereby excluding inter-assay variability when assessing eGFR slope over time within an individual patient. Additionally, at each measurement day two samples from a plasma pool were analysed to calculate intra- and inter-assay coefficients of variation for each marker. The intra-assay coefficient of variation for creatinine was 6.1%, for cystatin C 3.5%, for B2M 3.9%, and for BTP 7.9%. The inter-assay coefficient of variation for creatinine was 7.6%, for cystatin C 4.8%, for B2M 4.3%, and for BTP 14.5%. Samples with missing creatinine values were excluded from the analyses (n=221).

eGFR estimation equations

Estimated GFR (eGFR) expressed in ml/min/1.73m^2 per time point was calculated using 6 equations developed by the CKD-EPI collaboration: for creatinine, cystatin C, creatinine +cystatin C, B2M, BTP, and B2M+BTP.[2,3,16] The equations are included in the supplemental data file.

Statistical analysis

Continuous data are presented as mean with standard deviation (SD) or as median and interquartile range (IQR) in case of skewed distribution. Categorical data are presented as count and percentage. Patients were divided in quartiles of $eGFR_{creat}$ slopes to assess differences in baseline characteristics across slope quartiles. We used linear regression analysis to derive a p for trend. Baseline characteristics were also assessed according to quartiles of $eGFR_{cysc}$, $eGFR_{b2m}$, $eGFR_{btp}$, $eGFR_{creat/cysc}$ and $eGFR_{b2m/btp}$.

We compared the 4 markers and 6 GFR estimating equations using 3 methods. First, we compared the within-individual variability in eGFR slopes per filtration marker using sums of squares to determine which marker resulted in the lowest median within-individual sum of squares. The within-individual sum of squares for each of the (combined) markers was computed using linear regression analysis and the medians of the total sums of squares were compared using the Wilcoxon signed rank test.

Second, we compared the between-individual variability of eGFR slopes per equation and assessed which filtration marker provided the smallest standard deviation in the average eGFR slope over time. We used linear mixed models to calculate individual eGFR slopes for all subjects who had

at least 2 creatinine measurements available. The mean eGFR slope and SD was derived from these models. Levene's test was used to compare the SD's of the mean slopes for each of the (combined) markers.

Third, we compared the biological plausibility of eGFR slopes by investigating the strength of the associations of eGFR slopes with established CKD progression risk factors to identify the filtration marker that yielded eGFR slopes with the strongest association with these risk factors. We performed univariable and multivariable linear regression analysis of the eGFR slopes with all covariates. We bootstrapped the multivariable regression models with 1000 repetitions to obtain p-values for the difference in R^2 between the models. Established kidney risk factors were chosen as covariates a priori. These included: age, gender, current smoking, body mass index (BMI), systolic blood pressure (SBP), serum total cholesterol, HbA1c, and urinary albumin creatinine ratio (UACR).

Several sensitivity analyses were performed. First, all analyses except the analysis of between-individual variability were repeated with z-scores of the respective marker(s) instead of eGFR, to rule out the effect of the non-kidney components incorporated in the respective GFR equations that were used (i.e. the variables sex, age and race). To this end, the values of the markers were inversed (1/marker) and then expressed as a z-score for each individual per visit. Z scores were calculated as follows: (individual value-population mean)/SD of population mean. Subsequently, for each individual a series of composite z-scores for combinations of markers (creatinine plus cystatin C, B2M plus BTP, and the four markers combined) was calculated for each measurement as follows: ((z-score marker 1+zscore marker 2+zscore marker 3+zscore marker4)/number of markers). Second, multivariable linear mixed models were computed with the repeated eGFR measurements as outcome for each filtration marker instead of linear regression analysis with slopes as outcome. The 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 used to indicate statistical significance.

Results

Baseline characteristics

Baseline characteristics for all 849 patients with at least 2 creatinine measurements are shown in Table 1. Patients with the steepest $eGFR_{creat}$ decline (Q1, eGFR decline >5.9 ml/min/1.73m^2 per year) were less often Caucasians, more often male and smokers, had higher blood pressure and UACR, and lower serum creatinine, cystatin C, B2M and BTP at baseline (P for trend <0.001). Supplemental baseline tables organized by quartile of eGFR decline of the other filtration markers showed largely similar results (Supplemental Tables 1-5). Interestingly, there was no significant trend of UACR across quartiles of eGFR slopes for either marker, with the exception of $eGFR_{creat}$ and $eGFR_{creat/cysc}$.

Within individual variability in eGFR slopes

Mean eGFR per time point for all (combined) markers is shown in Table 2. There was a difference in eGFR across the filtration markers of 1 to 4 ml/min/1.73m^2 at all time points. $eGFR_{cysc}$ slopes yielded the lowest eGFR and the $eGFR_{b2m}$ slopes the highest. Table 3 shows the calculated median and

Table 1 Baseline characteristics

	All	eGFR change per year (ml/min/1.73m²)*				P-trend
		Q1	Q2	Q3	Q4	
		≤-5.9	-5.8 to -5.2	-5.1 to -4.7	≥-4.6	
N	849	212	212	212	213	
Age (years)	63.1±9.1	60.1±9.0	64.0±9.2	64.1±9.2	64.2±8.2	**<0.001**
Male (n(%))	649 (76)	174 (82)	178 (84)	147 (69)	150 (70)	**<0.001**
Caucasian race (n(%))	580 (68)	127 (60)	155 (73)	144 (68)	154 (72)	**0.01**
Smoking (current) (n(%))	125 (15)	42 (20)	29 (14)	35 (17)	19 (9)	**0.003**
BMI (kg/m²)	32.1±6.4	31.6 ±6.3	31.6±5.5	32.1±6.5	33.0±6.8	**0.04**
SBP (mmHg)	138±14	137±15	139±13	141±13	136±15	0.7
DBP (mmHg)	73±10	75±9	73±11	74±9	71±10	**0.002**
Total Cholesterol (mg/dL)	177±50	182±60	175±44	178±46	175±50	0.2
Glucose (mg/dL)	159±70	164±74	155±72	162±68	153±65	0.2
HbA1c (%)	8.0±1.6	8.1±1.7	7.9±1.6	8.1±1.6	7.9±1.4	0.5
Serum Creatinine (mg/dL)	2.3±0.7	2.0±0.5	2.2±0.6	2.4±0.5	2.6±0.6	**<0.001**
Baseline eGFR (ml/min/1.37m²)*	30.1±9.4	37.0±8.6	31.9±7.9	26.8±7.5	25.6±8.8	**<0.001**
Cystatin C (mg/L)	2.2±0.6	2.0±0.5	2.1±0.6	2.4±0.6	2.5±0.7	**<0.001**
Beta 2 Microglobuline (mg/L)	6.2±2.3	5.5±1.8	5.8±2.2	6.7±2.4	6.9±2.5	**<0.001**
Beta Trace Protein (mg/L)	2.0 ±0.7	1.8±0.7	1.8±0.6	2.1±0.6	2.1±0.7	**<0.001**
UACR (mg/g)	1387 (650 – 2407)	1684 (853 – 2933)	1634 (790 – 2583)	1452 (667 – 2465)	814 (484 – 1601)	**<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

Conversion factors for units: serum creatinine in mg/dL to µmol/L, ×88.4; Cholesterol in mg/dL to mmol/L , x0.2586; Glucose in mg/DL to mmol/L, x0.05551

interquartile range of the total sums of squares for the eGFR slopes per filtration marker. As expected, the sums of squares increased with the number of available eGFR measurements per individual. For each subgroup of available eGFR measurements per individual (3, 4 or 5 available measurements), the sum of squares was consistently lowest for $eGFR_{btp}$. Overall, the sums of squares were also lowest for $eGFR_{btp}$ (mean sum of squares 24.5 (IQR 9.1-52.6), P<0.05 versus $eGFR_{creat}$). The sum of squares for the combination of all 4 filtration markers was not lower than for $eGFR_{btp}$ alone. This indicates that the within-individual overall eGFR slope calculation error was lowest for this filtration marker.

Table 2 Mean eGFR per time point for the various filtration markers (ml/min/1.73m^2)

	Time (months)				
eGFR equation	0	3	6	12	18
Creatinine	30.0±9.3	29.1±9.6	28.5±9.8	24.4±9.4	23.7±9.6
Cystatin C	29.2±11.4	27.5±11.1	27.5±11.8	22.1±11.0	22.5±11.3
B2M	30.4±9.1	29.9±9.2	29.3±8.9	25.2±8.9	25.5±9.4
BTP	31.9±8.8	31.4±8.6	30.6±8.6	27.1±8.3	27.5±9.4
Creatinine/Cystatin C	28.5±9.7	27.5±9.6	26.9±10.1	22.1±9.5	22.0±9.8
B2M/BTP	29.5±29.5	29.0±8.5	28.3±8.9	24.3±8.4	24.7±8.9

Between individual variability in eGFR slopes

Table 4 shows the mean eGFR slopes and SD for all 6 eGFR equations. The steepest eGFR slope was obtained for $eGFR_{CysC}$ slopes, but it did not significantly differ from the mean $eGFR_{creat}$ slope (mean slope -6.1 ml/min/1.73m^2 per year for $eGFR_{CysC}$ versus -5.3 for $eGFR_{creat}$, P=0.4). Overall, there were no substantial differences in the distribution of eGFR slopes for each marker (Supplemental Figure 1). Pairwise comparisons of the standard deviations of the mean eGFR slope for each of the markers showed that $eGFR_{B2M/BTP}$ slopes had the smallest SD, and thus the lowest between individual variability, as compared to the other markers, with exception of $eGFR_{B2M}$ slopes (P=0.1 vs $eGFR_{B2M/BTP}$).

Associations of covariates with eGFR slopes

Univariable and multivariable associations of eGFR slopes with established risk factors for CKD progression are shown in Table 5. In the univariable analysis, the eGFR slopes of creatinine, cystatin C and creatinine/cystatin C had significant associations with more risk factors than eGFR slopes of B2M, BTP and B2M/BTP. In the multivariable analysis the numerically highest R^2 value was observed for $eGFR_{cysc}$ (adjusted R^2=0.084). However, this R^2 value was not significantly different from the one for $eGFR_{creat}$ (adjusted R^2=0.066, P=0.5 vs. $eGFR_{cysc}$, Supplemental Table 6). In more detail, the variance of $eGFR_{cysc}$ slopes was mostly explained by age, sex, BMI, systolic blood pressure and total cholesterol (all P≤0.05), whereas for $eGFR_{creat}$ slope the variance was explained by age, sex, BMI and UACR (all P≤0.01).

Table 3 Within-individual variability: median sum of squares of eGFR slopes per filtration marker

No of measurements (N)	$eGFR_{creat}$	$eGFR_{cysc}$	$eGFR_{b2m}$	$eGFR_{btp}$	$eGFR_{creat/cysc}$	$eGFR_{b2m/btp}$
Overall	38.1 (16.4-96.0)	49.9 (18.9-125.0)*	36.3 (14.2-79.0)*	24.5 (9.1-52.6)*	38.3 (15.0-90.8)	32.1 (12.4-66.0)*
3 (274)	22.5 (7.4-48.1)	28.3 (10.0-64.2)	19.8 (6.8-43.5)	13.6 (3.9-31.0)	19.9 (7.2-45.7)	18.7 (4.4-37.5)
4 (261)	48.7 (21.0-120.0)	71.9 (34.1-153.6)	45.7 (20.6-91.8)	29.9 (12.7-55.3)	52.6 (20.8-119.3)	55.3 (19.4-71.2)
5 (132)	73.1 (43.0-142.0)	93.2 (48.9-188.5)	64.2 (33.4-114.6)	41.6 (24.2-82.2)	79.5 (148.5-64.2)	28.1 (28.1-94.0)

Mean 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

*=P<0.05 versus $eGFR_{creat}$

Table 4 Between individual variability: mean slopes and their standard deviation for eGFR Creatinine, Cystatin C, Creatinine + Cystatin C, B2M, BTP and B2M+BTP and pairwise comparisons of the SDs

	Mean slope±SD	$eGFR_{creat}$	$eGFR_{cysc}$	$eGFR_{b2m}$	$eGFR_{btp}$	$eGFR_{creat/cysc}$
$eGFR_{creat}$	-5.26±6.7					
$eGFR_{cysc}$	-6.12[a]±6.9	0.4				
$eGFR_{b2m}$	-4.89[a]±5.9	**<0.001**	**<0.001**			
$eGFR_{btp}$	-4.15[a]±6.0	**0.001**	**<0.001**	0.6		
$eGFR_{creat/cysc}$	-5.67[a]±6.6	0.7	0.2	**<0.001**	**0.006**	
$eGFR_{b2m/btp}$	-4.76[a]±5.6	**<0.001**	**<0.001**	0.1	**0.04**	**<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; b2m, Beta-2-Microglobuline; btp, Beta-Trace protein; CI, confidence interval.
a= P<0.05 for difference of slope versus $eGFR_{creat}$ slope

Sensitivity analyses

Three sets of sensitivity analyses were performed. First, the within-individual variability and associations with risk factors were investigated using z-scores and z-score slopes of the markers instead of eGFR and eGFR slopes. For the within-individual variability, the sensitivity analysis using z-scores instead of eGFR showed that as in the main analysis, BTP had the lowest sum of squares (P<0.05 versus creatinine) (Supplemental Table 7). For the association with CKD risk factors, using z-scores instead of eGFR slopes for the univariable and multivariable regression analysis also yielded similar results to the main analysis. Even the combination of 4 markers did not further increase the R^2 compared to single filtration markers (Supplemental Table 8). Second, full linear mixed models for the repeated eGFR measures of each filtration marker showed that $eGFR_{creat}$ and $eGFR_{cysc}$ had the strongest associations with CKD risk factors (Supplemental Table 9). Third, eGFR slopes, regardless of the marker they were derived from, did not differ between patients who received sulodexide or placebo, indicating that sulodexide had no effect on GFR (Supplemental Table 10).

Table 5 Associations of established chronic kidney disease progression risk factors with eGFR slopes for the various filtration markers.

		$eGFR_{creat}$		$eGFR_{cysc}$		$eGFR_{b2m}$		$eGFR_{btp}$		$eGFR_{creat/cysc}$		$eGFR_{b2m/btp}$	
		Univar	Multivar	Univar	Multivar	Univar	Multivar	Univar	Multivar	Univar	Multivar	Univar	Multivar
Adjusted R^2			0.066		0.084		0.040		0.033		0.063		0.029
Age	Stand β	0.140	0.127	0.128	0.130	0.087	0.068	0.044	-0.006	0.123	0.113	0.063	0.034
	P-value	**<0.001**	**0.002**	**0.001**	**0.001**	**0.01**	0.1	0.2	0.9	**<0.001**	**0.005**	0.1	0.4
Female	β	0.246	0.227	0.820	0.833	0.335	0.340	0.565	0.632	-0.548	0.541	0.366	0.390
	P-value	0.01	0.04	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Smoking	β	-0.183	-0.079	0.035	0.206	-0.015	-0.012	0.112	0.010	-0.102	0.019	0.022	-0.031
	P-value	0.1	0.6	0.8	0.2	0.7	0.9	0.5	1.0	0.4	0.9	0.8	0.8
BMI	Stand β	0.076	0.052	0.074	0.078	0.065	0.038	0.031	-0.013	0.090	0.070	0.054	0.011
	P-value	**0.04**	0.2	**0.05**	**0.05**	0.8	0.4	0.4	0.8	**0.02**	0.1	0.1	0.8
SBP	Stand β	-0.014	0.015	0.075	0.037	0.038	0.037	0.053	0.078	0.035	0.030	0.038	0.049
	P-value	0.7	0.7	**0.03**	0.3	0.3	0.3	0.1	**0.05**	0.3	0.4	0.3	0.2
HbA1c	Stand β	-0.028	-0.008	-0.046	-0.03	-0.036	-0.019	-0.060	-0.052	-0.032	-0.018	-0.043	-0.029
	P-value	0.4	0.8	0.2	0.5	0.3	0.6	0.1	0.2	0.4	0.6	0.2	0.5
Total Cholesterol	Stand β	-0.064	-0.033	-0.087	-0.083	-0.040	-0.047	-0.014	-0.107	-0.067	-0.053	-0.023	-0.062
	P-value	0.1	0.4	**0.02**	**0.03**	0.3	0.2	0.7	**0.007**	0.1	0.2	0.5	0.1
Ln(UACR)	Stand β	-0.231	-0.120	0.009	0.004	-0.061	-0.078	-0.048	-0.054	-0.130	-0.112	-0.060	-0.073
	P-value	**<0.001**	**<0.001**	0.8	0.9	0.1	0.1	0.2	0.2	**<0.001**	**0.005**	0.1	0.1

Abbreviations are: eGFR, estimated glomerular filtration rate; creat, creatinine; cysc, cystatin C; b2m, Beta-2-Microglobulin; btp, Beta-Trace protein; Univar, univariable; Multivar, multivariable; Stand, standardized; BMI, body mass index; SBP, systolic blood pressure; ACR, albumin to creatinine ratio

Discussion

In this study we performed a head-to-head comparison of longitudinal changes in kidney function using eGFR equations based on creatinine, cystatin C, B2M, BTP, creatinine plus cystatin C, and B2M plus BTP to assess which filtration marker alone or in combination performs best for monitoring changes in eGFR over time. To our knowledge, this is the first study to compare eGFR slopes for these novel filtration markers.[3] We used three approaches to find the best marker for eGFR slope monitoring. First, we showed that BTP based slopes yielded the lowest sum of squares, suggesting a more precise eGFR slope within an individual with repeated BTP measurements. Second, the comparison of between-individual eGFR slopes, as assessed by the standard deviation of the average eGFR slope in the overall population, suggested that B2M-based eGFR slopes performed best. Third, we found that established CKD progression risk factors had the strongest association with $eGFR_{cysc}$ slopes, and the weakest with $eGFR_{BTP}$ and $eGFR_{B2M}$ slopes. However, the total explained variability in eGFR slopes for the novel filtration markers, either alone or in combination, was not significantly better than for creatinine. In combination, these data suggest that in patients with diabetic kidney disease eGFR slopes based on neither novel filtration marker consistently outperformed creatinine based eGFR.

The three statistical methods we used address different methodological aspects of selecting the best filtration marker for slope analysis. First, we addressed the value of the various markers to monitor change in kidney function over time within an individual. In clinical practice, high within-individual precision, represented by a low within-individual sum of squares, is generally preferred for obtaining correct individual eGFR slopes. However, it should be noted that the within-individual sum of squares for eGFR slopes is influenced by four sources of variation: true variation in GFR over time, laboratory variation in the measurement of the filtration marker, biological non-kidney variation in filtration marker concentration, and inclusion of other factors incorporated in the GFR equations that are studied. We excluded inter-assay variability as a source of variation since all measurements for each individual were conducted in a single run. Interestingly, the intra-assay variability was highest for BTP, as is the within-subject biological variation in BTP that is known from literature (for creatinine 6.0%, cystatin C 5.0%, B2M 5.9%, and for BTP 11.6%[17,18]). Therefore, one would expect that BTP would by default yield a larger within-individual variability of eGFR slopes. Yet in our study, BTP showed the lowest within-individual variability. This suggests that assay and biological variability of the marker only has a small effect on the within-individual variability. Our analyses using just the filtration markers (z-scores) instead of GFR estimation equations that also incorporate other variables yielded similar results. This excludes a significant effect of differences in the non-filtration variables that are incorporated in the GFR equations. Taken together, BTP appears to be the most precise.

Second, we assessed the performance of the eGFR slopes with regards to between-individual variability, by calculating the standard deviation of the average eGFR slopes for each filtration marker. The between-individual (population-level) variation of eGFR slopes is subject to the same sources of variation as the within-individual slope. Since the eGFR slopes for each marker were measured in the same group of patients, the variation in GFR decline should be the same for each filtration marker, which was not the case in our study. The filtration marker with the lowest variability is expected

to have the smallest standard deviation for the average rate of eGFR decline. However, a narrow standard deviation of the GFR slope can also be caused by non-GFR factors. For example, if these non-GFR factors have more impact on the serum concentration of the marker than the GFR has, it can cause a falsely small SD. In our study, the filtration marker with the smallest between-individual variability was B2M.

Third, since established CKD progression risk factors, especially albuminuria, are associated with the incidence of hard end points such as ESRD, these risk factors should also be associated with eGFR slopes. It is thus biologically plausible that the filtration marker and eGFR slope that shows the strongest association with CKD progression risk factors is the most reliable. Remarkably, we found that $eGFR_{cysc}$ slopes, although having the highest intra- and inter-individual variability, showed the highest R^2 value. However, the differences in R^2 values for the models explaining slopes based on the various filtration markers were not statistically significant and $eGFR_{cysc}$ slopes did not have a significant association with albuminuria, the major risk factor for eGFR decline.

Although, as reasoned above, each of the three methods that were used to assess reliability of eGFR slopes may have shortcomings, the combination of the three indicates that none of the novel filtration markers consistently outperforms creatinine based eGFR slopes to assess change in kidney function over time. As far as we know, five studies have compared slopes of (eGFR-) creatinine and cystatin C.[7,8,19-21] One study, performed in patients with type 1 diabetes with preserved kidney function, found that eGFR slopes based on cystatin C were more accurate than eGFR slopes based on creatinine when compared to mGFR slopes.[19] At the time of publication, the CKD-EPI equations to estimate $eGFR_{cysc}$ and $eGFR_{creat/cysc}$ were not yet available. Consequently, the authors used an alternative equation to calculate eGFR from cystatin C, which limits the external validity of these findings. A more recent study that did include the CKD-EPI equations found that $eGFR_{creat/cysc}$ slopes were most accurate.[7] This study also found that slopes of $eGFR_{cysc}$ and $eGFR_{creat/cysc}$ had the strongest association with blood pressure and HbA1c, which is consistent with our findings. To our knowledge, our study is the first to compare slopes of $eGFR_{btp}$, $eGFR_{b2m}$ and $eGFR_{btp/b2m}$ to creatinine and cystatin C based eGFR slopes.

In addition to comparing single filtration markers, we also assessed the performance of combinations of filtration markers to monitor eGFR over time. None of the combinations of markers were consistently superior to $eGFR_{creat}$ alone, even when all four filtration markers were combined into one model. Since there is no eGFR estimation equation for the four filtration markers combined, we used z-scores for this analysis, that did not demonstrate an improvement when compared to single filtration markers.

Some limitations of the present study need to be addressed. The most obvious limitation is the absence of a gold standard measurement of GFR (mGFR). However, others have shown that a single eGFR, and also a short-term change in eGFR, had a stronger association with incidence of ESRD and mortality than (change in) mGFR .[8,22,23] These observations, in combination with known methodological shortcomings in mGFR assessment,[24] question whether mGFR is really a gold standard. Furthermore, assessment of mGFR is a cumbersome and expensive procedure, which is hardly feasible in a large scale study with repeated measurements, such as the clinical trial we analyzed. Second, this is a post-hoc analysis of a clinical trial, with specific inclusion criteria leading

to a cohort of subjects with a relatively narrow range of eGFR and albuminuria. This may limit the generalizability of our results and may have weakened the association of albuminuria with eGFR slopes. Of note, the SUN-trial failed to demonstrate a renoprotective effect of sulodexide and we were therefore unable to assess which eGFR marker can best be used to show an effect of intervention in a clinical trial setting. Future studies should assess and compare treatment effects on eGFR slopes based on the various filtration markers. Lastly, due to the short study duration with consequently a low number of incident ESRD and mortality events, we were unable to investigate the associations of eGFR slopes with these hard end points.

To conclude, this first comparison of eGFR slopes based on serial creatinine, cystatin C, B2M and BTP measurements, shows that new filtration markers may be preferred with regards to within- and between- individual variability. However, none of the novel filtration markers that can be used to calculate slopes of eGFR over time, either alone or when used in combination, consistently outperformed creatinine. These data suggest that in patients with diabetes and kidney disease, there is yet no value to replace creatinine by (combinations of) novel filtration markers.

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Supplemental Data

eGFR equations as proposed by the CKD-EPI collaboration:

eGFR creatinine:

Females with Cr≤0.7 mg/dL: eGFR = (144 + 22 if black) x (Cr/0.7)^-0.329 x 0.993^age

Females with Cr>0.7 mg/dL: eGFR = (144 + 22 if black) x (Cr/0.7)^-1.209 x 0.993^age

Males with Cr≤0.9 mg/dL: eGFR = (141 + 22 if black) x (Cr/0.9)^-0.411 x 0.993^age

Males with Cr>0.9 mg/dL: eGFR = (141 + 22 if black) x (Cr/0.9)^-1.209 x 0.993^age

eGFR cystatin C:

If cystatin C ≤0.8 mg/L: eGFR= 133 × (Scys/0.8)−0.499 × 0.996Age [× 0.932 if female]

If cystatin C >0.8 mg/L: eGFR= 133 × (Scys/0.8)−1.328 × 0.996Age [× 0.932 if female]

eGFR creatinine/cystatin C:

If female, creatinine ≤0.7 mg/dL and cystatin C:	≤0.8 mg/L eGFR=130 × (Scr/0.7)− 0.248 × (Scys/0.8)−0.375 × 0.995Age [× 1.08 if black]
	>0.8 mg/L eGFR=130 × (Scr/0.7) −0.248 × (Scys/0.8)−0.711 × 0.995Age [× 1.08 if black]
If female, creatinine >0.7 mg/dL and cystatin C:	≤0.8 mg/L eGFR=130 × (Scr/0.7) −0.601 × (Scys/0.8)−0.375 × 0.995Age [× 1.08 if black]
	>0.8 mg/L eGFR=130 × (Scr/0.7) −0.601 × (Scys/0.8)−0.711 × 0.995Age [× 1.08 if black]
If male, creatinine ≤0.9 mg/dL and cystatin C:	≤0.8 mg/L eGFR=135 × (Scr/0.9) −0.207 × (Scys/0.8)−0.375 × 0.995Age [× 1.08 if black]
	>0.8 mg/L eGFR=135 × (Scr/0.9) −0.207 × (Scys/0.8)−0.711 × 0.995Age [× 1.08 if black]
If male, creatinine >0.9 mg/dL and cystatin C	≤0.8 mg/L eGFR=135 × (Scr/0.9) −0.601 × (Scys/0.8)−0.375 × 0.995Age [× 1.08 if black]
	>0.8 mg/L eGFR=135 × (Scr/0.9) −0.601 × (Scys/0.8)−0.711 × 0.995Age [× 1.08 if black]

eGFR Beta-2-Microglobulin:

eGFR=133*b2m^(-0.852)

eGFR Beta-Trace protein:

eGFR=55*btp^(-0.695)*0.998^age*0.899 if female

eGFR=55*btp^(-0.695)*0.998^age if male

eGFR Beta-Trace protein+Beta-2-Microglobulin

eGFR=96*btp^(-0.278)*b2m^(-0.588)

Supplemental Table 1 Baseline characteristics per quartile of $eGFR_{cysc}$ slope

	$eGFR_{cysc}$ change per year (ml/min/1.73m²)*				P-trend
	Q1	Q2	Q3	Q4	
	≤-6.8	-6.7 to -6.0	-5.9 to -5.3	≥-5.2	
N	212	212	212	213	
Age (years)	60.8±8.7	64.0±8.9	63.8±9.5	63.9±8.8	**<0.001**
Male (n(%))	190 (90)	181 (85)	150 (71)	128 (60)	**<0.001**
Caucasian race (n(%))	121 (57)	147 (69)	154 (73)	158 (74)	<0.001
Smoking (current) (n(%))	28 (13)	34 (16)	29 (14)	34 (16)	0.5
BMI (kg/m²)	31.5±6.0	31.6±5.8	31.6±6.2	33.6±7.3	**0.003**
SBP (mmHg)	136±14	139±14	139±15	139±14	0.05
DBP (mmHg)	75±10	74±10	73±10	71±9	**0.001**
Total Cholesterol (mg/dL)	183±63	178 ±44	174±45	177±48	0.1
Glucose (mmol/L)	9.1±3.8	8.7±3.8	8.9±4.0	8.5±4.0	0.2
HbA1c (%)	8.2±1.8	7.9±1.5	8.0±1.6	7.9±1.5	0.1
Serum Creatinine (mg/dL)	1.9±0.4	2.2±1.4	2.4±0.6	2.7±0.6	**<0.001**
Baseline eGFR (ml/min/1.37m²)*	42.7±10.7	30.6±5.1	25±4.9	19.6±6.1	**<0.001**
Cystatin C (mg/L)	1.6±0.3	2.0±0.3	2.3±0.3	2.9±0.6	<0.001
Beta 2 Microglobuline (mg/L)	4.4±1.1	5.4±1.3	6.6±1.6	8.3±2.5	**<0.001**
Beta Trace Protein (mg/L)	1.5±0.5	1.8±0.5	2.1±0.6	2.4±0.7	**<0.001**
UACR (mg/g)	1360 (650-2325)	1291 (609-2352)	1511 (717-2712)	1415 (578-2348)	0.7[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 cystatin C; a: p for natural log of UACR

Supplemental Table 2 Baseline characteristics per quartile of $eGFR_{b2m}$ slope

	$eGFR_{b2m}$ change per year (ml/min/1.73m^2)*				
	Q1	Q2	Q3	Q4	p-trend
	≤-5.8	-5.7 to -4.8	-4.7 to -4.4	≥-4.3	
N	212	212	212	213	
Age (years)	61.9±9.1	63.2±9.2	63.5±9.7	63.9±8.2	**0.02**
Male (n(%))	191 (88)	178 (83)	148 (71)	133(63)	**<0.001**
Caucasian race (n(%))	120 (56)	156 (73)	150 (72)	156 (74)	**<0.001**
Smoking (current) (n(%))	34 (16)	32 (15)	34 (16)	25 (12)	0.4
BMI (kg/m^2)	31.6±6.1	32.0±6.1	32.0±6.5	32.8±6.9	0.07
SBP (mmHg)	137±14	139±15	139±14	138±15	0.4
DBP (mmHg)	74±11	74±9	73±10	72±10	**0.006**
Total Cholesterol (mg/dL)	183±59	173±44	176±43	177±49	0.2
Glucose (mmol/L)	8.6±3.6	9.3±4.3	8.6±3.7	8.5±3.6	0.6
HbA1c (%)	8.0±1.7	8.1±1.6	8.0±1.5	7.8±1.4	0.1
Serum Creatinine (mg/dL)	2.0±0.5	2.2±0.5	2.4±0.6	2.6±0.7	**<0.001**
Baseline eGFR (ml/min/1.37m^2)*	39.5±8.0	33.2±5.9	27.1±5.3	23.9±7.1	**<0.001**
Cystatin C (mg/L)	1.7±0.4	2.0±0.4	2.4±0.4	2.8±0.7	**<0.001**
Beta 2 Microglobuline (mg/L)	4.4±1.1	5.3±1.2	6.8±1.4	8.3±2.7	**<0.001**
Beta Trace Protein (mg/L)	1.6±0.6	1.7±0.5	2.2±0.6	2.3±0.8	**<0.001**
UACR (mg/g)	1225 (679-2304)	1440 (622-2330)	1621 (841-2690)	1108 (565-2211)	1.0[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 B2M; a: p for natural log of UACR

Supplemental Table 3 Baseline characteristics per quartile of $eGFR_{btp}$ slope

	$eGFR_{btp}$ change per year (ml/min/1.73m^2)*				
	Q1	Q2	Q3	Q4	P-trend
	≤-4.9	-4.8 to -4.0	-3.9 to -3.3	≥-3.2	
N	212	212	212	213	
Age (years)	62.3±8.5	62.6±9.9	64.7±8.9	62.9±8.8	0.2
Male (n(%))	183 (86)	173 (82)	159 (75)	133 (63)	**<0.001**
Caucasian race (n(%))	134 (64)	140 (66)	160 (75)	145 (68)	0.1
Smoking (current) (n(%))	28 (13)	34 (16)	32)15)	31 (15)	0.7
BMI (kg/m^2)	32.2±6.4	32.0±5.6	31.5±6.8	32.7±6.7	0.7
SBP (mmHg)	136±15	138±14	140±6.8	139±14	**0.03**
DBP (mmHg)	74±10	74±9	73±10	72±10	0.1
Total Cholesterol (mg/dL)	181±49	177±44	175±45	175±48	0.1
Glucose (mmol/L)	8.9±3.8	8.8±4.6	8.7±3.5	8.8±3.5	0.7
HbA1c (%)	8.1±1.7	8.2±1.6	7.9±1.6	7.9±1.5	0.07
Serum Creatinine (mg/dL)	2.1±0.6	2.2±0.5	2.3±0.6	2.6±0.7	**<0.001**
Baseline eGFR (ml/min/1.37m^2)*	40.0±10.8	32.9±5.6	29.0±5.1	26.6±6.3	**<0.001**
Cystatin C (mg/L)	1.9±0.5	2.1±0.5	2.3±0.5	2.6±0.7	**<0.001**
Beta 2 Microglobuline (mg/L)	5.1±1.7	5.7±1.9	6.5±2.0	7.6±2.7	<0.001
Beta Trace Protein (mg/L)	1.4±0.5	1.8±0.5	2.1±0.5	2.4±0.7	**<0.001**
UACR (mg/g)	1184 (612 – 2183)	1550 (743 – 2465)	1472 (740 – 2513)	1211 (459 – 2489)	0.8[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 BTP; a: p for natural log of UACR

Supplemental Table 4 Baseline characteristics per quartile of $eGFR_{creat/cysc}$ slope

	$eGFR_{creat/cysc}$ change per year (ml/min/1.73m²)*				
	Q1	Q2	Q3	Q4	p-trend
	≤-6.3	-6.2 to -5.6	-5.5 to -5.0	≥4.9	
N	212	212	212	213	
Age (years)	60.8±8.8	63.5±9.4	64.4±9.4	63.8±8.3	**<0.001**
Male (n(%))	186 (88)	180 (85)	150 (71)	133 (62)	**<0.001**
Caucasian race (n(%))	129 (61)	149 (70)	152 (72)	150 (70)	**0.03**
Smoking (current) (n(%))	37 (18)	28(13)	34 (16)	26 (12)	0.2
BMI (kg/m²)	31.2±6.1	31.8±6.1	31.8±6.1	33.4±7.1	**0.002**
SBP (mmHg)	137±14	139±13	139±15	138±15	0.4
DBP (mmHg)	75±10	74±9	73±11	71±9	**<0.001**
Total Cholesterol (mg/dL)	184±65	176±42	177±44	175±49	0.06
Glucose (mmol/L)	9.2±3.9	9.0±4.2	8.4±3.6	8.6±3.7	0.1
HbA1c (%)	8.1±1.7	8.0±1.6	7.9±1.6	8.0±1.4	0.3
Serum Creatinine (mg/dL)	1.9±0.4	2.1±0.4	2.5±0.6	2.7±0.7	**<0.001**
Baseline eGFR (ml/min/1.37m²)*	37.9±9.0	30.8±6.5	24.9±5.9	21.1±6.8	**<0.001**
Cystatin C (mg/L)	1.7±0.4	2.0±0.4	2.4±0.4	2.8±0.7	**<0.001**
Beta 2 Microglobuline (mg/L)	4.8±1.4	5.5±1.7	6.6±1.8	7.9±2.6	**<0.001**
Beta Trace Protein (mg/L)	1.6±0.6	1.8±0.6	2.0±0.5	2.3±0.8	**<0.001**
UACR (mg/g)	1478 (705 – 2482)	1582 (640 – 2469)	1456 (736 – 2560)	1037 (519 – 1983)	**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 and cystatin C; a: p for natural log of UACR

Supplemental Table 5 Baseline characteristics per quartile of $eGFR_{b2m/btp}$ slope

	$eGFR_{b2m/btp}$ change per year (ml/min/1.73m²)*				
	Q1	Q2	Q3	Q4	p-trend
	≤-5.3	-5.2 to -4.7	-4.6 to -4.2	≥-4.1	
N	214	216	210	209	
Age (years)	62.0±8.9	63.4±9.7	63.3±9.4	63.8±8.3	**0.04**
Male (n(%))	186 (87)	180 (83)	151 (72)	132 (63)	**<0.001**
Caucasian race (n(%))	126 (59)	153 (71)	148 (70)	154 (74)	**0.001**
Smoking (current) (n(%))	32 (15)	38 (18)	23 (11)	32 (15)	0.7
BMI (kg/m²)	32.0±5.9	31.7±6.1	32.0±6.9	32.6±6.7	0.3
SBP (mmHg)	137±14	138±15	138±15	139±14	0.2
DBP (mmHg)	74±11	74±9	73±10	72±10	**0.008**
Total Cholesterol (mg/dL)	181±48	173±41	176±44	176±49	0.3
Glucose (mmol/L)	8.7±3.6	9.1±4.4	8.6±3.7	8.7±3.6	0.8
HbA1c (%)	8.0±1.7	8.1±1.6	7.8±1.5	7.9±1.4	0.2
Serum Creatinine (mg/dL)	2.0±0.5	2.2±0.5	2.4±0.6	2.5±0.6	**<0.001**
Baseline eGFR (ml/min/1.37m²)*	37.5±8.7	32.2±5.7	26.3±5.3	23.9±6.6	**<0.001**
Cystatin C (mg/L)	1.8 v 0.4	2.0±0.4	2.4±0.5	2.7±0.7	**<0.001**
Beta 2 Microglobuline (mg/L)	4.5±1.2	5.3±1.4	6.7±1.6	8.1±2.7	**<0.001**
Beta Trace Protein (mg/L)	1.5±0.5	1.8±0.5	2.1±0.5	2.4±0.8	<0.001
UACR (mg/g)	1367 (679-2278)	1376 (628-2375)	1526 (762-2678)	1216 (567-2309)	0.8[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 B2M and BTP; a: p for natural log of UACR

Supplemental Table 6 Pairwise comparison of bootstrapped R^2 values derived from Table 5 (Associations of established chronic kidney disease progression risk factors with eGFR slopes for the various filtration markers)

	R^2	$eGFR_{creat}$	$eGFR_{cysc}$	$eGFR_{b2m}$	$eGFR_{btp}$	$eGFR_{creat/cysc}$
$eGFR_{creat}$	0.066					
$eGFR_{cysc}$	0.084	0.4				
$eGFR_{b2m}$	0.040	0.1	**0.003**			
$eGFR_{btp}$	0.033	0.1	**0.009**	0.6		
$eGFR_{creat/cysc}$	0.063	0.8	0.1	0.05	0.1	
$eGFR_{b2m/btp}$	0.029	**0.04**	**<0.001**	0.1	0.8	**0.01**

P-values for difference in R-square

Abbreviations are: eGFR, estimated GFR; creat, creatinine; cysc, cystatin C; b2m, Beta-2-Microglobuline; btp, Beta-Trace protein; CI, confidence interval.

a= P<0.05 for difference of slope versus $eGFR_{creat}$ slope

Supplemental Table 7 Within-individual variability: mean sum of squares of eGFR slope per z-score of each (combined) marker

No of measurements (N)	Creatinine	Cystatin C	B2M	BTP	Creat/CysC	B2M/BTP	All markers
Overall	0.45 (0.18-0.99)	0.45 (0.18-1.03)	0.38 (0.15-0.85)	0.26 (0.10-0.64)*	0.40 (0.15-0.92)*	0.30 (0.12-0.67)*	0.33 (0.13-0.72)*
3 (316)	0.25 (0.83-0.56)	0.25 (0.08-0.55)	0.20 (0.08-0.48)	0.14 (0.05-0.36)	0.20 (0.07-0.45)	0.17 (0.04-0.38)	0.17 (0.06-0.37)
4 (271)	0.57 (0.23-1.22)	0.61 (0.31-1.27)	0.49 (0.21-1.03)	0.32 (0.15-0.70)	0.54 (0.19-1.15)	0.36 (0.16-0.78)	0.42 (0.18-0.87)
5 (142)	0.90 (0.58-1.38)	0.96 (0.43-1.55)	0.73 (0.36-1.23)	0.45 (0.26-1.13)	0.91 (0.52-1.37)	0.63 (0.27-1.07)	0.75 (0.34-1.08)

*=P<0.05 compared to creatinine

Supplemental Table 8 Associations of established chronic kidney disease progression risk factors with respective z-score slopes

		Creatinine		Cys C		B2M		BTP		Creatinine/CysC		B2M/BTP		All markers	
		Univar	Multivar	Univar	Multivar	Univar	Multivar	Univar	Multivar	Univar	Multivar	Univar	Multivar	Univar	Multivar
Adjusted R^2			0.08		0.04*		0.06		0.03		0.06		0.03*		0.03*
Age	Stand β	0.10	0.09	0.04	0.03	0.10	0.08	0.02	-0.03	0.06	0.05	0.06	0.02	0.05	0.04
	P-value	**0.004**	**0.02**	0.2	0.4	**0.004**	0.04	0.6	0.4	0.06	0.2	0.1	0.6	0.1	0.4
Female	β	0.00	0.001	0.04	0.05	0.05	0.05	0.05	0.06	0.01	0.01	0.05	0.06	0.02	0.03
	P-value	1.0	0.9	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.005**	**0.002**	0.4	0.3	**<0.001**	**<0.001**	**0.01**	**0.02**
Smoking	β	-0.01	0.005	0.004	0.01	-0.02	-0.001	0.03	-0.001	-0.01	0.01	0.01	0.001	0.001	0.01
	P-value	0.5	0.7	0.7	0.3	0.5	0.9	0.2	1.0	0.6	0.7	0.6	0.9	0.9	0.7
BMI	Stand β	0.07	0.05	0.09	0.06	0.06	0.05	-0.01	-0.05	0.08	0.05	003	0.003	0.08	0.05
	P-value	**0.04**	0.2	**0.02**	0.1	0.08	0.2	0.7	0.3	**0.03**	0.2	0.4	0.9	**0.03**	0.2
SBP	Stand β	-0.08	-0.01	0.01	0.01	0.08	0.04	0.08	0.08	-0.06	-0.01	0.07	0.06	-0.01	0.02
	P-value	**0.03**	0.7	0.8	0.7	0.02	0.3	**0.03**	**0.04**	0.1	0.8	0.06	0.1	0.7	0.7
HbA1c	Stand β	-0.01	-0.002	-0.02	-0.02	-0.07	-0.04	-0.05	-0.07	-0.006	-0.01	-0.06	-0.05	-0.03	-0.03
	P-value	0.8	0.9	0.5	0.6	0.04	0.3	0.1	0.1	0.9	0.9	0.1	0.2	0.4	0.5
Total Cholesterol	Stand β	-0.06	-0.03	-0.05	-0.04	-0.04	-0.07	-0.02	-0.14	-0.05	-0.02	-0.02	-0.10	-0.03	-0.04
	P-value	0.08	0.5	0.1	0.3	0.3	0.1	0.6	**<0.001**	0.1	0.6	0.6	**0.01**	**0.4**	0.3
Ln(UACR)	Stand β	-0.27	-0.26	-0.10	-0.11	0.04	0.02	0.04	0.05	-0.23	-0.23	0.01	0.003	-0.15	-0.16
	P-value	<0.001	<0.001	0.004	**0.007**	0.3	0.5	0.2	0.2	**<0.001**	**<0.001**	0.8	0.9	**<0.001**	**<0.001**

Abbreviations are: Cys C; Cystatin C; b2m, beta 2 microglobulin; btp, beta trace protein; BMI, body mass index; SBP, systolic blood pressure; ACR, albumin to creatinine ratio

*=P<0.05 vs creatinine

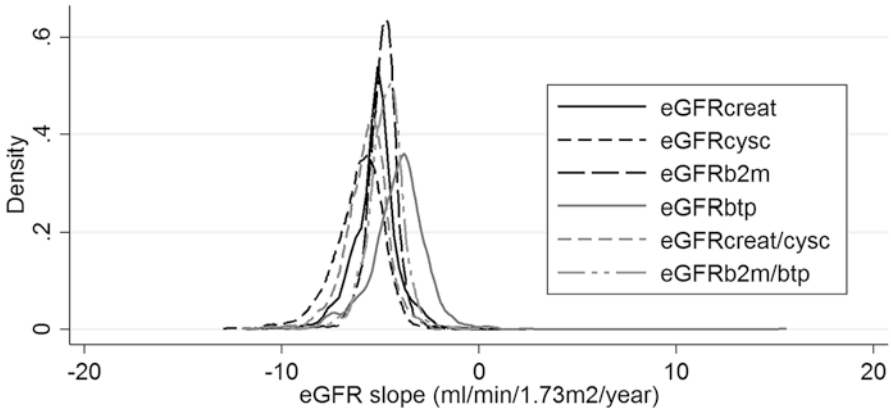
Supplemental Table 9 Linear mixed model of repeated eGFR slopes with established chronic kidney disease progression risk factors (multivariable models)

Multivariable		$eGFR_{creat}$	$eGFR_{cysc}$	$eGFR_{b2m}$	$eGFR_{btp}$	$eGFR_{creat/cysc}$	$eGFR_{b2m/btp}$
Age	β	0.102	-0.052	0.035	-0.010	0.071	0.018
	P-value	**0.001**	0.2	0.2	0.7	**0.02**	0.5
Female	β	1.130	2.571	1.526	1.478	1.978	1.488
	P-value	0.06	**<0.001**	**0.003**	**0.003**	**0.001**	**0.002**
Smoking	β	-0.098	0.900	0.359	0.110	0.442	0.405
	P-value	0.9	0.3	0.6	0.9	0.5	0.5
BMI	β	0.074	0.070	0.031	0.020	0.074	0.029
	P-value	0.07	0.1	0.4	0.6	0.07	0.4
SBP	β	-0.004	0.002	0.005	0.022	-0.002	0.008
	P-value	0.8	0.9	0.8	0.1	0.9	0.6
HbA1c	β	0.013	-0.029	0.057	-0.090	-0.022	0.004
	P-value	0.3	0.9	0.7	0.5	0.9	1.0
Total Cholesterol	β	-0.006	-0.005	0.003	-0.011	-0.005	-0.006
	P-value	0.2	0.3	0.4	**0.03**	0.3	0.2
Ln(UACR)	β	-1.820	-1.462	-1.297	-0.925	-1.626	-1.125
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations are: eGFR, estimated glomerular filtration rate; cysc; Cystatin C; b2m, beta 2 microglobulin; btp, beta trace protein; Stand, standardized; BMI, body mass index; SBP, systolic blood pressure; ACR, albumin to creatinine ratio

Supplemental Table 10 Comparison of the mean eGFR slope for each marker between the sulodexide and placebo group

	Mean slope (ml/min/1.73m²/year)		
eGFR slope based on:	Sulodexide	Placebo	P
Creatinine	-5.23	-5.28	0.6
Cystatin C	-6.07	-6.16	0.3
Beta-2-Microglobulin	-4.87	-4.92	0.3
Beta-Trace-Protein	-4.16	-4.14	0.9
Creatinine/Cystatin C	-5.64	-5.71	0.4
Beta-2-Microglobulin/Beta-Trace protein	-4.73	-4.79	0.3



Supplemental Figure 1 Kernel density plot of the distribution of eGFR slopes for creatinine (black, solid), cystatin C (black, dash), Beta-2-microglobuline (black, long dash), Beta-trace protein (gray, solid), creatinine/cystatin C (light gray, dash) and Beta-2-microglobuline/Beta-trace protein (gray, long dash short dash).