# Prediction and monitoring of chronic kidney disease

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# Chapter 5

## Creatinine for monitoring of kidney function over time: a comparison of routine versus single run measurement and Jaffe versus enzymatic assays

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## Abstract

**Introduction:** During studies and clinical trials, creatinine is routinely measured from fresh samples. It is unknown whether single-run measurement of frozen samples can improve monitoring of kidney function decline. Furthermore, it is not yet known whether eGFR slopes obtained from repeated creatinine measures are more reliable when obtained with an enzymatic assay instead of a Jaffe assay.

**Methods:** We performed a post hoc analysis of the SUN-MACRO study in 795 patients with type 2 diabetes and diabetic kidney disease in whom creatinine was measured at least twice. Creatinine was measured routinely during the trial with a Jaffe assay. We performed single run measurement of creatinine using both a Jaffe and an enzymatic assay on samples that had been stored at -80°C. Linear mixed models were used to calculate eGFR slopes per individual for each method. 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 slopes obtained with routine creatinine measurement yielded the lowest SOQ (lowest within-individual variability; $P<0.05$ versus single run enzymatic), but this was not significantly different from the SOQ obtained in single run with a Jaffe assay. The between individual variability was identical for each method. Multivariable linear regression showed that eGFR slopes from routine measurement reached the highest adjusted $R^2$ value (highest biological plausibility).

**Conclusion:** This study shows that single run creatinine measurement, using plasma samples stored frozen, with a Jaffe or enzymatic assay, does not outperform routine creatinine measurement in fresh plasma samples for assessing eGFR slopes.

# Introduction

In epidemiological studies and clinical trials creatinine is routinely measured from fresh samples by either a Jaffe or an enzymatic assay and then GFR is estimated at each patient visit. An alternative option is to store all samples collected during the study, to perform a single run analysis of all samples per individual at the end of the study under the same analytical circumstances, thereby eliminating the effect of day-to-day measurement variability. Others have shown that day-to-day variability of creatinine and cystatin C, another marker used to estimate GFR, can significantly impact the marker level and subsequent estimation of eGFR.[1,2] This measurement error could in turn cause inaccurate determination of eGFR slopes in an individual.

It has been shown previously that creatinine levels can be more accurately measured with an enzymatic assay than with a Jaffe assay, mostly due to the fact that Jaffe assays suffer from non-specificity bias caused by compounds such as albumin and glucose.[3-5] However, all data comparing enzymatic and Jaffe-based creatinine assays have been obtained with cross-sectional studies, and it is unknown whether the enzymatic method also outperforms the Jaffe method for the monitoring of creatinine based eGFR slopes over time.

We therefore investigated two research questions: first, is single run or routine creatinine measurement better for obtaining eGFR slopes, and second, is an enzymatic or a Jaffe-based creatinine assay better to monitor change of eGFR over time? In the absence of a gold standard measurement technique to assess GFR, we applied three methodological approaches to identify the best method for obtaining repeated measures of eGFR over time. We hypothesized that creatinine measured in a single run with an enzymatic assay would provide the best eGFR slopes, defined as the lowest within- and between individual variability and the highest biological plausibility. For this study we used data from the SUN-MACRO trial.



# Methods

## Study population

SUN-MACRO was a large, randomized placebo controlled clinical trial investigating the effect of sulodexide in delaying the progression kidney function decline in 1179 patients with diabetes mellitus type 2 and diabetic kidney disease.[6] 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.[6,7] 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.

## Measurements

During the study, serum was collected from patients at baseline, and after 3, 6, 12 and 18 months of follow-up. During the original study serum creatinine was routinely measured using a modified Jaffe, rate-blanked, alkaline picrate method, incorporated on the automated Roche/Hitachi Modular System (Roche Diagnostics, IN, USA). All serum samples were subsequently stored at -80°Celsius and were analyzed in two series for the current analyses. Per patient all samples (baseline and follow-up) were analyzed in the same run. The preparation of the samples included one freeze-thaw cycle prior to the first analysis round which took place between November 2014 and May 2015. Creatinine was measured enzymatically and isotope dilution mass spectrometry (IDMS)-traceable with a Cobas 8000 analyzer (Roche Diagnostics, IN, USA). At each measurement day two samples from a plasma pool were analyzed to obtain variation coefficients. The intra- and inter- assay coefficient of variation (CV) were 2.2% and 3.4%. The second single-run measurement of creatinine was performed between February and March 2016 using an IDMS-traceable modified Jaffe method on the same Cobas 8000 analyzer. For this analysis, the intra- and inter- assay CV were 6.1% and 7.6%. Samples that had missing information to estimate GFR (creatinine, age or gender) for any of the laboratory methods (n=627 samples, 11%) and/or subjects that did not have follow-up eGFR available to estimate change in eGFR (n=384 subjects, 33%) were excluded from the analyses. A total of 795 patients with at least 2 eGFR measurements were available for analysis.

## Statistical analysis

The outcome measure for the present analyses was slope of eGFR expressed in ml/min/1.73m$^2$ per year, with GFR estimated using the CKD-EPI equation.[8] 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 single run enzymatic method based eGFR slopes to assess differences in baseline characteristics across slope quartiles. We used linear regression to derive a p for trend.

We compared the three creatinine measurement methods using three statistical approaches: First, we compared the within-individual variability of eGFR slopes per method using sums of squares to determine which method and respective eGFR slope resulted in the lowest median within-individual sum of squares. We calculated the total sum of squares for each individual using within-patient linear regression. The means of the sums of squares were then compared using the Wilcoxon singed rank test.

Second, we compared the between-individual variability of eGFR slopes per method to assess which method 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 were derived from these models. Leven's test was used to compare the standard deviations of the mean slopes for the three methods.

Third, we compared the biological plausibility of eGFR slopes by investigating the strength of the associations of eGFR slopes with CKD progression risk factors to identify the method that resulted in the strongest association with these risk factors. We performed a univariable and multivariable linear regression analysis of the eGFR slopes with all covariates. We bootstrapped the multivariable

regression analyses with 1,000 repetitions to obtain p-values for the difference in $R^2$ between the models. Established CKD progression risk factors were chosen as covariates a priori. Covariates were: age, gender, current smoking, body mass index (BMI), systolic blood pressure (SBP), serum total cholesterol, HbA1c, and urinary albumin to creatinine ratio (UACR).

Two sensitivity analyses were performed. First, all analyses except the analysis of between-individual variability were repeated with z-scores of the creatinine results of the respective assay instead of eGFR, to rule out the effect of the non-kidney components incorporated in the GFR estimating equation (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. Second, multivariable linear mixed models were computed with the repeated eGFR measurements as outcome for each assay instead of linear regression analysis with slopes as outcome. 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 used to indicate statistical significance.



# Results

## Baseline data

Baseline characteristics for all 795 patients with at least 2 eGFR values are listed in Table 1. The overall mean age was 63.0±9.1 years, 76% were male and 69% were Caucasian. Patients with the steepest eGFR decline (Q1, eGFR decline of more than 4.2 ml/min/1.73m$^2$ per year) were more often male and smokers, less often Caucasians, and had higher baseline single run creatinine and UACR levels (P for trend <0.05).

**Table 1** Baseline characteristics, with subjects stratified according to eGFR slopes based on routine, Jaffe-based, creatinine

| | All | eGFR change per year (ml/min/1.73m²)* | | | | P-trend |
|---|---|---|---|---|---|---|
| | | Q1 | Q2 | Q3 | Q4 | |
| | | <-4.2 | -4.2 to -3.50 | -3.49 to -2.8 | >-2.8 | |
| N | 795 | 198 | 199 | 199 | 199 | |
| Age (years) | 63.0±9.1 | 60.3±8.9 | 64.2±8.7 | 64.7±8.6 | 62.8±9.3 | <0.01 |
| Male (n(%)) | 603 (76) | 144 (72) | 153 (77) | 152 (76) | 154 (77) | 0.3 |
| Caucasian race (n(%)) | 544 (69) | 123 (62) | 140 (70) | 140 (70) | 141 (71) | 0.1 |
| Smoking (current) (n(%)) | 114 (14) | 34 (17) | 30 (15) | 25 (13) | 25 (13) | 0.2 |
| BMI (kg/m²) | 32.1±6.5 | 31.5±6.9 | 31.9±6.1 | 32.3±6.3 | 32.8±6.7 | 0.04 |
| SBP (mmHg) | 138±14.4 | 139±14 | 142±13 | 137±14 | 135±16 | <0.001 |
| DBP (mmHg) | 73±14 | 75±10 | 74±10 | 73±10 | 72±10 | 0.002 |
| Total Cholesterol | 178±51 | 185±65 | 180±43 | 170±41 | 177±50 | 0.1 |
| Glucose (mmol/L) | 8.8±3.9 | 9.1±4.4 | 8.9±1.6 | 9.0±3.9 | 8.3±3.4 | 0.1 |
| HbA1c (%) | 8.0±1.6 | 8.1±1.7 | 7.9±1.6 | 8.0±1.5 | 8.0±1.6 | 0.6 |
| Serum Creatinine (mg/dL) | | | | | | |
| Routine – Jaffe | 2.2±0.5 | 2.2±0.6 | 2.3±0.5 | 2.1±0.5 | 2.1±0.5 | 0.1 |
| Single run - Jaffe | 2.4±0.8 | 2.6±1.2 | 2.5±0.6 | 2.4±0.6 | 2.3±0.6 | 0.01 |
| Single run – Enzymatic | 2.3±0.6 | 2.4±0.7 | 2.4±0.6 | 2.3±0.6 | 2.2±0.6 | 0.01 |
| UACR (mg/g) | 1326 (693-2406) | 2097 (1220-3153) | 1249 (639-2645) | 1283 (650-2124) | 753 (482-1557) | <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 slopes of Single run - enzymatic; a: p for natural log of UACR

## Within-individual variability in eGFR slopes

The mean eGFR per time point for each creatinine measurement method is shown in Table 2 and Figure 1. The single-run Jaffe-based eGFR was highest, both at baseline and during follow-up (P<0.05 compared to routine Jaffe and single run enzymatic). The sum of squares for each eGFR slope is shown in Table 3. Overall, the sum of squares and thus the within-individual variability was lowest for the routine Jaffe eGFR slopes, but this was not significantly different from the single run Jaffe-based eGFR slopes. Single run enzymatic eGFR slopes had the highest within-individual variability (p<0.001 versus both methods)

**Table 2** Mean eGFR per time point for each creatinine measurement method

| | Time (months) | | | | |
|---|---|---|---|---|---|
| eGFR | 0 | 3 | 6 | 12 | 18 |
| N | 747 | 796 | 754 | 476 | 241 |
| Routine – Jaffe | 32.1±9.1[a] | 30.9±9.6[a] | 30.6±10.2[a] | 28.0±9.9[a] | 27.5±10.2[a] |
| Single run – Jaffe | 28.7±8.8[a] | 27.5±8.9[a] | 27.0±9.4[a] | 22.8±8.7[a] | 22.1±9.0[a] |
| Single run – Enzymatic | 30.5±9.5[a] | 29.2±9.7[a] | 28.6±9.9[a] | 24.3±9.4[a] | 23.4±9.6[a] |

a: P<0.001 vs all other methods per time point (paired t-test)

**Table 3** Within-individual variability: median sum of squares of eGFR slope for each method

| No of measurements (N) | Routine – Jaffe | Single run – Jaffe | Single run – Enzymatic |
|---|---|---|---|
| Overall | 28.8 (11.0-68.2)* | 31.8 (12.0-83.4)* | 34.4 (13.2-93.6) |
| 3 (282) | 17.7 (6.7-42.9) | 18.1 (6.9-40.7) | 21.1 (7.4-45.9) |
| 4 (224) | 35.4 (16.3-88.8) | 47.7 (17.9-108.6) | 52.2 (21.4-124.4) |
| 5 (99) | 63.0 (32.4-113.2) | 71.1 (34.9-112.7) | 78.9 (34.9-143.6) |

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

*P<0.001 versus single run - Enzymatic

## Between-individual variability in eGFR slopes

The mean eGFR slope was -3.5±7.2 ml/min/1.73m$^2$/year for routine Jaffe, -5.3±7.2 ml/min/1.73m$^2$/year for single run Jaffe and -5.6±7.2 ml/min/1.73m$^2$/year for single run enzymatic. All slopes differed significantly from each other (P<0.05). However, the standard deviation was equal for all three methods, and thus there was no difference in between-individual variability between any of the methods.

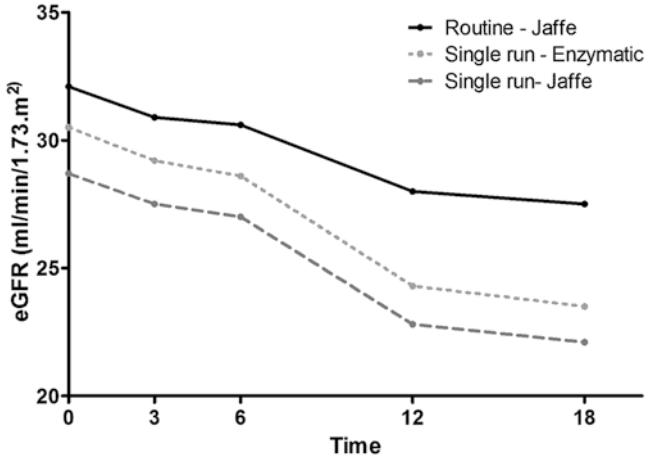


**Figure 1** eGFR trajectories over time for the three creatinine measurement methods: routine Jaffe (black/solid), single run enzymatic (light grey/dotted) and single run Jaffe (dark grey/dashed)

## Associations of established CKD progression risk factors with eGFR slopes

Univariable and multivariable associations of eGFR slopes per creatinine measurement method with established risk factors for CKD progression are shown in Table 4. The univariable analysis showed no major differences between the creatinine measurement methods. In the multivariable analysis we found that routine Jaffe-based eGFR slopes had the strongest association with CKD progression risk factors ($R^2$ 0.10, P<0.05 versus both other methods), and that there was no difference between the Jaffe and enzymatic method within the single run methods (both $R^2$ 0.05). For all three methods, most of the explained variance of the eGFR slopes was explained by UACR.

## Sensitivity analyses

Two sensitivity analyses were performed. First, the within-individual variability and biological plausibility were investigated using z-scores and z-score slopes of the markers instead of eGFR and eGFR slopes. Both analyses showed similar results as the main analysis, indicating that our results were driven by the marker, and not influenced by the GFR estimating equation. (Supplemental Table 1 and 2). Second, multivariable linear mixed models with the repeated eGFR measurements as outcome also found that the routine Jaffe method had the overall strongest associations with established CKD progression risk factors (Supplemental Table 3).

**Table 4** Associations of established chronic kidney disease progression risk factors with eGFR slopes for each method.

| | | Routine – Jaffe | | Single run – Jaffe | | Single run – Enzymatic | |
|---|---|---|---|---|---|---|---|
| | | Univar | Multivar | Univar | Multivar | Univar | Multivar |
| Adjusted $R^2$ | | | 0.10 | | 0.05* | | 0.05* |
| Age | Stand β | 0.07 | 0.05 | 0.10 | 0.10 | 0.15 | 0.14 |
| | P-value | **0.05** | 0.2 | **0.003** | **0.01** | **<0.001** | **0.001** |
| Female | β | -0.15 | -0.11 | 0.23 | 0.17 | 0.29 | 0.25 |
| | P-value | 0.2 | 0.4 | **0.02** | 0.2 | **<0.001** | **0.007** |
| Smoking | β | -0.13 | -0.00 | -0.25 | -0.15 | -0.23 | -0.15 |
| | P-value | 0.3 | 1.0 | **0.04** | 0.3 | **0.02** | 0.2 |
| BMI | Stand β | 0.08 | 0.03 | 0.08 | 0.06 | 0.08 | 0.07 |
| | P-value | 0.04 | 0.4 | **0.04** | 0.1 | **0.03** | 0.1 |
| SBP | Stand β | -0.13 | -0.05 | -0.06 | -0.04 | -0.01 | -0.01 |
| | P-value | **<0.001** | 0.2 | 0.1 | 0.3 | 0.9 | 0.9 |
| HbA1c | Stand β | -0.00 | 0.02 | 0.00 | 0.01 | -0.01 | 0.02 |
| | P-value | 0.9 | 0.5 | 0.9 | 0.8 | 0.8 | 0.7 |
| Total | Stand β | -0.09 | -0.03 | 0.04 | 0.07 | -0.04 | -0.03 |
| Cholesterol | P-value | **0.02** | 0.4 | 0.3 | 0.1 | 0.3 | 0.5 |
| Ln(UACR) | Stand β | -0.32 | -0.29 | -0.21 | -0.18 | -0.18 | -0.13 |
| | P-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.002 |

P-values <0.05 are highlighted in bold

*Abbreviations are:* eGFR, estimated glomerular filtration rate; Stand, standardized; BMI, body mass index; SBP, systolic blood pressure; UACR, urine albumin to creatinine ratio

*: P<0.05 vs Routine – Jaffe

## Discussion

In this study we performed comparisons of eGFR slopes obtained using three creatinine measurement methods to answer two research questions: is single run or routine creatinine measurement better for obtaining eGFR slopes, and is an enzymatic or a Jaffe-based creatinine assay better to monitor change of eGFR over time? We found that eGFR slopes based on creatinine values measured per individual in one run, did not outperform slopes based on routinely obtained creatinine with regards to within-individual variability and biological plausibility. The comparison of Jaffe and enzymatic measured creatinine, both measured in a single run, showed that both methods performed equally with regards to within- and between-individual variability and biological plausibility.

The three statistical methods we used aimed to address different aspects of eGFR slopes. First, we investigated the within-individual variability in eGFR slopes using the sum of squares. Within-individual variability in eGFR slopes can have four sources: change in kidney function, biological

variability of the marker, assay variability, and the eGFR equation used. Since we used the same samples, the same filtration marker and the same GFR estimation equation in our analyses, only day-to-day variability could have differed between the creatinine measurement methods, and could have only affected the eGFR slopes based on routinely measured creatinine. Yet despite this fact, eGFR slopes calculated from routinely measured creatinine had the lowest within-individual variability.

Second, we investigated the between-individual variability by comparing the standard deviations of the mean eGFR slopes. As the eGFR slope for each method was measured in the same participants, the variation of eGFR decline should be the same for each method. The slope with the smallest SD has the highest precision, and theoretically may be expected to be closest to the true eGFR slope. In our study, we found no difference in between-individual variability between the methods.

Third, we investigated biological plausibility by investigating the association of the eGFR slopes with established CKD progression risk factors. We found that routine measurement of creatinine yielded the strongest association with CKD progression risk factors, both in models with eGFR slopes as well as with z-score slopes. There was no difference in biological plausibility between eGFR slopes obtained with creatinine measured with a Jaffe or an enzymatic assay in a single run.

Taken together, these results suggest that in contrast to our hypothesis, single run analysis of frozen samples does not outperform routine measurement of fresh plasma samples, and that eGFR slope estimation does not improve when an enzymatic instead of a Jaffe assay is used to obtain repeated measures of creatinine. How can these unexpected findings be explained? To our knowledge, no other studies have compared eGFR slopes derived with different creatinine assays. A possible explanation for our findings is that prolonged freezing of the samples for approximately 10 years as well as freeze-thaw cycles may have negatively influenced the quality of the samples. However, others have shown that serum creatinine levels are stable up to 35 years when stored at -25°Celsius and up to 10 freeze-thaw cycles.[9,10] Furthermore, there was a non-significant decrease of creatinine over time in these studies, whereas our frozen samples showed an increase in creatinine compared to the results from the routine measurement. In addition, during the SUN MACRO trial, standardization of creatinine assays to IDMS-traceable standards was not yet available. Yet despite the fact that our single run analyses were indeed performed with IDMS-traceable assays, these methods did not outperform the results obtained from routine analysis of plasma samples. Therefore, evaporation or sample handling issues of the frozen samples could be a key explanation for this failure to outperform routine analysis.

Our second major finding was that the use of an enzymatic creatinine assay did not outperform the Jaffe method with regards to eGFR slopes. Use of the Jaffe method is no longer supported because of its non-specificity bias, caused by chemical interference of several compounds, such as albumin and glucose.[4] However, the levels of most of these interfering compounds probably remain stable within a patient over time. This could explain why we found that Jaffe based eGFR slopes are not outperformed by slopes derived with an enzymatic method. Therefore, it is likely that correct eGFR slopes can also be obtained from creatinine measured with a Jaffe assay. This could be of particular interest to researchers in countries where enzymatic methods are too expensive. We

emphasize however that all studies should consistently use the same creatinine assay and device throughout the study, as changing methods during the study could introduce unacceptable bias, regardless of the assay that is used. Others have shown that significant bias occurs when the same samples are analyzed at different laboratories with different creatinine assays or devices.[2,4]

Some limitations of this study need to be addressed. First, a gold standard measurement of GFR was not available. 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.[11,12,13] These observations, in combination with known methodological shortcomings in mGFR assessment,[14] 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 patients with a relatively narrow range of eGFR and albuminuria. This may have limited the generalizability of our results. This could have weakened the associations with CKD progression risk factors, and could also be partly responsible for the overall low adjusted $R^2$ values we found in our analyses. 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.

In conclusion, in this study we found that for monitoring of eGFR decline, single run measurement of creatinine is not superior to routine measurement of fresh serum samples with regards to within- and between-individual variability and biological plausibility. Furthermore, we found that eGFR slopes obtained with both an enzymatic and a Jaffe creatinine assay did not differ.

This study shows that single run creatinine measurement after prolonged storage of samples at -80°C, either with a Jaffe or enzymatic assay, does not outperform routine creatinine measurement for assessing eGFR slopes. Therefore there is no reason for clinical trials and epidemiological studies with repeated eGFR measurements to replace routine measurement of creatinine by a single run measurement at the end of the study.

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# Supplemental Tables

**Supplemental Table 1** Within-individual variability: Median sum of squares of creatinine z-score slope per assay

| No of measurements (N) | Routine – Jaffe | Single run – Jaffe | Single run – Enzymatic |
|---|---|---|---|
| Overall | 0.36 (0.14-0.80)* | 0.33 (0.10-1.04) | 0.41 (0.16-0.94) |
| 3 (282) | 0.21 (0.09-0.54) | 0.17 (0.05-0.54) | 0.24 (0.08-0.50) |
| 4 (224) | 0.40 (0.19-0.90) | 0.55 (0.15-1.31) | 0.59 (0.22-1.23) |
| 5 (91) | 0.72 (0.39-1.26) | 0.90 (0.33-2.19) | 0.85 (0.53-1.35) |

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

*=P<0.05 versus both other methods



**Supplemental Table 2** Associations of established chronic kidney disease progression risk factors with z-score slopes for each method.

| | | Routine – Jaffe | | Single run – Jaffe | | Single run – Enzymatic | |
|---|---|---|---|---|---|---|---|
| | | Univar | Multivar | Univar | Multivar | Univar | Multivar |
| Adjusted $R^2$ | | | 0.12 | | 0.11 | | 0.05 |
| Age | Stand β | 0.10 | 0.09 | -0.12 | -0.14 | 0.08 | 0.08 |
| | P-value | **<0.001** | **<0.001** | **0.001** | **0.001** | **0.02** | 0.07 |
| Female | β | 0.01 | 0.01 | -0.03 | -0.05 | 0.002 | 0.002 |
| | P-value | 0.3 | 0.2 | 0.6 | 0.4 | 0.8 | 0.8 |
| Smoking | β | 0.02 | 0.01 | 0.02 | -0.12 | -0.01 | -0.002 |
| | P-value | **0.01** | 0.4 | 0.7 | 0.1 | 0.2 | 0.8 |
| BMI | Stand β | 0.08 | 0.03 | -0.08 | -0.09 | 0.08 | 0.06 |
| | P-value | **<0.001** | 0.06 | **0.03** | **0.03** | **0.03** | 0.2 |
| SBP | Stand β | -0.14 | -0.07 | 0.13 | 0.08 | -0.08 | -0.03 |
| | P-value | **<0.001** | **<0.001** | **0.001** | **0.03** | **0.03** | 0.4 |
| HbA1c | Stand β | 0.001 | 0.02 | 0.03 | 0.04 | 0.02 | 0.02 |
| | P-value | 1.0 | 0.3 | 0.5 | 0.3 | 0.6 | 0.6 |
| Total Cholesterol | Stand β | -0.07 | -0.03 | -0.09 | -0.18 | -0.04 | -0.02 |
| | P-value | **<0.001** | 0.1 | **0.01** | **<0.001** | 0.2 | 0.6 |
| Ln(UACR) | Stand β | -0.33 | -0.30 | -0.26 | -0.25 | -0.24 | -0.21 |
| | P-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

*Abbreviations are:* eGFR, estimated glomerular filtration rate; Stand, standardized; BMI, body mass index; SBP, systolic blood pressure; UACR, urine albumin to creatinine ratio

All differences between $R^2$ values were non-significant

**Supplemental Table 3** Linear mixed models for established CKD progression risk factors with repeated eGFR measurements of each method (multivariable model)

| Multivariable | | Routine – Jaffe | Single run - Jaffe | Single run – Enzymatic |
|---|---|---|---|---|
| Age | β | 0.08 | 0.08 | 0.10 |
| | P-value | 0.01 | 0.02 | <0.001 |
| Female | β | 0.77 | 0.84 | 1.22 |
| | P-value | 0.2 | 0.2 | 0.06 |
| Smoking | β | -0.43 | -0.45 | -0.37 |
| | P-value | 0.6 | 0.6 | 0.6 |
| BMI | β | 0.06 | 0.08 | 0.09 |
| | P-value | 0.2 | 0.08 | 0.06 |
| SBP | β | -0.03 | -0.28 | -0.02 |
| | P-value | 0.1 | 0.2 | 0.4 |
| HbA1c | β | 0.09 | 0.07 | 0.09 |
| | P-value | 0.6 | 0.7 | 0.6 |
| Total Cholesterol | β | -0.01 | -0.01 | -0.00 |
| | P-value | 0.1 | 0.2 | 0.3 |
| Ln(UACR) | β | -2.19 | -1.60 | -1.52 |
| | P-value | <0.001 | <0.001 | <0.001 |