Biological Variability of Estimated GFR and Albuminuria in CKD

Sushrut S. Waikar, Casey M. Rebholz, Zihe Zheng, Shelley Hurwitz, Chi-yuan Hsu, Harold I. Feldman, Dawei Xie, Kathleen D. Liu, Theodore E. Mifflin, John H. Eckfeldt, Paul L. Kimmel, Ramachandran S. Vasan, Joseph V. Bonventre, Lesley A. Inker, and Josef Coresh, on behalf of the Chronic Kidney Disease Biomarkers Consortium Investigators

Rationale & Objective: Determining whether a change in estimated glomerular filtration rate (eGFR) or albuminuria is clinically significant requires knowledge of short-term within-person variability of the measurements, which few studies have addressed in the setting of chronic kidney disease.

Study Design: Cross-sectional study with multiple collections over less than 4 weeks.

Setting & Participants: Clinically stable outpatients with chronic kidney disease (N = 50; mean age, 56.8 years; median eGFR, 40 mL/min/1.73 m^2; median urinary albumin-creatinine ratio (UACR), 173 mg/g).

Exposure: Repeat measurements from serially collected samples across 3 study visits.

Outcomes: Measurements of urine albumin concentration (UAC), UACR, and plasma creatinine, cystatin C, β_2-microglobulin (B2M), and beta trace protein (BTP).

Analytical Approach: We calculated within-person coefficients of variation (CV_w) values and corresponding reference change positive and negative (RCV_{pos} and RCV_{neg}) values using log-transformed measurements.

Results: Median CV_w (RCV_{pos}; RCV_{neg}) values of filtration markers were 5.4% (+16%; −14%) for serum creatinine, 4.1% (+12%; −11%) for cystatin C, 7.4% (+23%; −18%) for BTP, and 5.6% (+17%; −14%) for B2M. Results for albuminuria were 33.2% (+145%; −59%) for first-morning UAC, 50.6% (+276%; −73%) for random spot UAC, 32.5% (+141%; −58%) for first-morning UACR, and 29.7% (124%; −55%) for random spot UACR. CV_w values for filtration markers were comparable across the range of baseline eGFRs. CV_w values for UAC and UACR were comparable across the range of baseline albuminuria values.

Limitations: Small sample size limits the ability to detect differences in variability across markers. Participants were recruited and followed up in a clinical and not research setting, so some preanalytical factors could not be controlled.

Conclusions: eGFR markers appear to have relatively low short-term within-person variability, whereas variability in albuminuria appears to be high, making it difficult to distinguish random variability from meaningful biologic changes.

Complete author and article information (including a list of the Chronic Kidney Disease Biomarkers Consortium Investigators) provided before references.

Correspondence to S.S. Waikar (swaikar@partners.org)

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Abnormalities in glomerular filtration rate (GFR) and/or albuminuria define chronic kidney disease (CKD), a condition that affects ~10% of the US population.[1,2] GFR is estimated using endogenous plasma or serum filtration markers, most commonly creatinine.[3] Albuminuria is most commonly quantified by measuring urinary albumin concentration (UAC) or urinary albumin-creatinine ratio (UACR), which is UAC divided by urinary creatinine concentration. Reference ranges for estimated GFR (eGFR) and albuminuria in healthy individuals are well established.[4]

For individuals with CKD, clinicians and researchers measure and follow up serial changes in eGFR and albuminuria to assess disease progression, prognosis, and response to therapy.[5-8] Determining whether a change in eGFR or albuminuria is clinically significant requires knowledge of the expected variability in the absence of underlying clinical changes. For example, according to present guidelines, an increase in UACR from the 30-300 mg/g range to >300 mg/g denotes a transition from moderately to severely increased albuminuria, with consequences in clinical decision making. Similarly, an increase in serum creatinine (Scr) level by ≥ 0.3 mg/dL over 48 hours or ≥ 50% over 7 days defines acute kidney injury (AKI), a major complication in patients with and without underlying CKD.

Despite the clinical importance of assessing the significance of changes in measurements of kidney function, relatively little is known about the inherent biological variability of eGFR and UACR in the setting of CKD. We therefore conducted this study in clinically stable patients with CKD to provide estimates of the short-term within-person biological variability in measures of kidney function, including albuminuria (UAC and UACR) and plasma eGFR markers (creatinine and the newer markers cystatin C, β_2-microglobulin [B2M], and beta trace protein [BTP]).

Methods

Study Cohort

We collected urine and blood samples from individuals with CKD attending a nephrology subspecialty practice at

Brigham and Women's Hospital, a tertiary-care medical center that provides care for a racially and socioeconomically diverse population in eastern Massachusetts and the surrounding region. Patients provided written documentation of informed consent to participate in this study, which was approved by the local Institutional Review Board (2010P002703). Eligible participants had a diagnosis of CKD under the care of a nephrologist and were recruited as a convenience sample during a nephrology clinic visit between February 2011 and February 2014. Participants were excluded for any of the following reasons: inability or unwillingness to return for 2 additional study visits, recent hospitalization or an episode of AKI (> 50% increase in Scr over a 1-week period) within the past 3 months, active glomerulonephritis or history of kidney transplantation, reported or suspected urinary tract infection within the past 3 weeks, or a planned change by the attending nephrologist of the dose of a diuretic and/or antihypertensive medication during the study period.

Sample Collection

We collected urine and blood samples shortly after a clinic visit (8 AM to 5 PM) and then at 2 requested follow-up study visits within an approximate 2-week period (range, 1-4 weeks). At each of the 2 subsequent study visits, patients were asked to bring in a refrigerated first-void morning urine sample and also provide a fresh urine sample during the study visit. Thus, up to 3 blood specimens and up to 5 urine specimens were collected. Urine samples were centrifuged at 3,200*g* for 5 minutes and the supernatants were collected. Trained phlebotomists at Brigham and Women's Hospital collected blood samples according to standard clinical protocols. All plasma samples and supernatants of urine samples were frozen at −80°C within 4 hours of collection. We transferred or shipped frozen samples on dry ice to performance laboratories at Brigham and Women's Hospital for the measurement of total urine protein excretion and to the University of Minnesota for the measurement of plasma creatinine, cystatin C, B2M, BTP (as eGFR markers), urine albumin, and urine creatinine.

Assays

Plasma and urine creatinine were measured using the Roche enzymatic method on a Roche cobas 6000 chemistry analyzer, calibrated using the isotope-dilution mass spectrometry standard traceable to the fresh-frozen serum-based National Institute of Standards and Technology Standard Reference Material SRM 967. Plasma cystatin C and urinary albumin were measured on the Roche chemistry analyzer using a turbidimetric assay. Plasma B2M was measured using a latex agglutination assay. Plasma BTP was measured on a Siemens ProSpec nephelometer. All assays were performed over a 2-day period. Interassay coefficients of variation (CVs) of all plasma and urine markers were assessed using blind split-replicate samples from individuals with CKD and were < 3% for each marker. Published equations were used to estimate GFR from concentrations of filtration markers.[9-11]

Statistical Analysis

All biomarker measurements and GFR estimates were transformed onto the natural log-scale for analyses of within-person variability. For each participant, we calculated within-person CV (CV_w) values across repeat sample measurements (up to 5 plasma and up to 3 urine; missing values were not imputed) using the equation $CV_w = \sqrt{(e^{var}-1)}$ where *var* refers to within-participant variance. We then used median CV_w values to estimate 95% reference change values (RCVs) as:

$$RCV_{pos} = e^{\left(1.96 \times \sqrt{2} \times \sqrt{\ln(1+CVw^2)}\right)}$$

$$RCV_{neg} = e^{\left(-1.96 \times \sqrt{2} \times \sqrt{\ln(1+CVw^2)}\right)}$$

RCV_{pos} and RCV_{neg} refer to the increase or decrease that must be exceeded between 2 sequential results for a change to be considered different at a statistical significance level of 0.05.[12] For example, RCV_{pos} of +20% means that an increase from a 1.0 to 1.2 (arbitrary measurement) may be within the expected range of values.

Bootstrap with 1,000 iterations was used to calculate 95% confidence intervals (CIs) for median CV and differences in median CVs. Comparisons of median CVs between subgroups were conducted using Mann-Whitney tests and generalized Hodges-Lehmann median difference test, with Bonferroni-corrected $P < 0.005$ regarded as significant.

Results

Clinical Characteristics

The study cohort consisted of 50 individuals who provided a total of 139 plasma samples and 227 urine samples during the 4-week study period (Table 1). Mean age was 56.8 ± 15.8 (SD) years. The cohort was composed of 44% women, 32% African Americans, and 32% with diabetes mellitus. At enrollment, 36 (72%) were taking an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, and 42% were taking a diuretic. From samples obtained at the first timepoint, median eGFR using the CKD-EPI (CKD Epidemiology Collaboration) creatinine equation was 33 (range, 11-97) mL/min/1.73 m^2 and median random spot UACR was 143 (range, < lower limit of detection for the assay – 4,408) mg/g.

Within-Person Variability of eGFR Markers

Table 2 shows within-person variability results for the 4 filtration markers and eGFRs. Values for filtration markers at each time point from all participants (without exclusion of potential outliers) are listed in Table S1. Figure 1A to D shows scatterplots of CV_w values for each filtration marker against mean values for each marker. The 2 highest CV_w