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Original Investigation

Day-to-Day Variability in Spot Urine Albumin-Creatinine Ratio

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Background: Accurate quantification of albuminuria is important in the diagnosis and management of chronic kidney disease. The reference test, a timed urinary albumin excretion, is cumbersome and prone to collection errors. Spot urine albumin-creatinine ratio (ACR) is convenient and commonly used, but random day-to-day variability in ACR measurements has not been assessed.

Study Design: Prospective cohort study of day-to-day variability in spot urine ACR measurements.

Setting & Participants: Clinically stable outpatients (N = 157) attending a university hospital clinic in Australia between July 2007 and April 2010.

Outcomes: Spot urine ACR variability was assessed and repeatability limits were determined using fractional polynomials.

Measurements: ACRs were measured from spot urine samples collected at 9:00 AM on consecutive days and 24-hour urine albuminuria was measured concurrently.

Results: Paired ACRs were obtained from 157 patients (median age, 56 years; 60% men; median daily albumin excretion, 226 [range, 2.5-14,000] mg/d). Day-to-day variability was substantial and increased in absolute terms, but decreased in relative terms, with increasing baseline ACR. For patients with normoalbuminuria (ACR < 3 mg/mmol [<27 mg/g]), a change greater than ±467% (0-17 mg/mmol [0-150 mg/g]) is required to indicate a significant change in albuminuria status with 95% certainty; for those with microalbuminuria (ACR of 3-30 mg/mmol [27-265 mg/g]), a change of ±170% (0-27 mg/mmol [0-239 mg/g]) is required; for those with macroalbuminuria (ACR > 30 mg/mmol [>265 mg/g]), a change of ±83% (5-55 mg/mmol [44-486 mg/g]) is required; and for those with nephrotic-range proteinuria (ACR > 300 mg/mmol [>2,652 mg/g]), a change of ±48% (158-443 mg/mmol [1,397-3,916 mg/g]) is needed to represent a significant change.

Limitations: These study results need to be replicated in other ethnic groups.

Conclusions: Changes in chronic kidney disease status attributed to therapy or disease progression, when based solely on a change in ACR, may be incorrect unless the potential for day-to-day biological variation has been considered. Only relatively large changes are likely to indicate a change in disease status.

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INDEX WORDS: Albuminuria; albumin-creatinine ratio; proteinuria; chronic kidney disease.

Albuminuria (albumin excretion > 30 mg in a 24-hour period) is a marker of kidney disease in both diabetic and nondiabetic populations.[1] It also is a risk factor for cardiovascular events and cardiovascular and all-cause mortality.[2] Therapeutic strategies that decrease albumin excretion have been shown to delay the progression of kidney disease and lower the risk of cardiovascular mortality and morbidity.[3,4] Consequently, an accurate measurement of albumin excretion is crucial to stratify cardiorenal risk and monitor disease progression.

Semiquantitative tests to measure albuminuria, such as dipsticks, have suboptimal test specificity and sensitivity, which limit their clinical utility in the management of chronic kidney disease (CKD).[5] The reference test is albumin excretion measured from a 24-hour urine sample, which is cumbersome and subject to collection errors. A spot urine albumin-creatinine ratio (ACR) is a quick and convenient alternative and currently is advocated by key guideline groups.[1,6,7]

Spot urine ACR has been found to correlate well with 24-hour albumin excretion[8]; however, the extent of day-to-day variability in ACR at various magnitudes of albumin excretion is unclear.[9-11] When caring for patients with CKD, it is critical to know whether changes in ACR reflect biological variability in albumin excretion or a true change in disease status. We previously have reported that substantial day-to-day variability in spot urine protein-creatinine ratio (PCR) exists in individuals with CKD.[12] To quantify the

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day-to-day variability in spot urine ACR, we conducted a prospective study evaluating ACR measurements in paired samples obtained over 2 consecutive days in a cohort of patients with stable CKD.

METHODS

Study Design

We performed a study between July 2007 and April 2010 at a metropolitan tertiary-care teaching hospital in Sydney, Australia, which was designed and reported using the STARD (Standards for Reporting of Diagnostic Accuracy) guidelines.[13] The Sydney South West Area Health Service Ethics Review Committee approved this study, protocol number X06-0196.

Patient Recruitment and Consent

Patients were recruited from the hospital's CKD and kidney transplantation clinics. Eligible individuals identified from an electronic database were adults (aged ≥ 18 years) with albuminuria (ACR > 3.5 mg/mmol [>31 mg/g]) or proteinuria (24-hour urine total protein excretion >150 mg/d) with stable kidney function (outpatients with less than ±15% variation in estimated glomerular filtration rate [eGFR] over the preceding 3 months). Patients were excluded if they were on dialysis therapy, were known to be pregnant or less than 3 months postpartum, had symptomatic urinary tract infection, were treated for sepsis or hospitalized within the past 2 weeks, had overt cardiac failure, were menstruating, or were unable to provide informed consent. Participants provided written consent, and no financial incentives were provided.

Specimen Collection and Storage

Patients were given a urine collection kit containing 2 spot containers, a 24-hour urine collection (5-L) bottle, a sterile 10-mL plastic syringe, and written instructions for urine collection and storage. Participants were advised to continue their usual lifestyle, diet, and medications during the study period without changes, restrictions, or exclusions, in accordance with usual clinical practice.

Participants voided urine into a clean container at 9:00 AM and, using a syringe, transferred a 10-mL aliquot of this urine into a spot container and stored it at 1°C-4°C. On the following day at 9:00 AM, another spot urine collection was performed and stored using the same methods. The spot collections at 9:00 AM on both days were not first morning voids. All urine passed during the intervening 24 hours was collected in the 5-L sample bottle. Specimens were returned to the hospital the following day and analyzed in the hospital's centralized laboratory within 48 hours. No specimen was frozen. Participants underwent a blood test for hemoglobin, urea, and creatinine when urine specimens were returned. eGFR was derived using the isotope-dilution mass spectrometry–traceable 4-variable MDRD (Modification of Diet in Renal Disease) Study equation.[14]

The participant's blood pressure, height, weight, medications, and relevant medical history were recorded, and standard demographic information was collected from all participants. The data were de-identified before analysis and 10% of the entered data was randomly audited for accuracy of data entry.

Specimen Assay

The 24-hour specimens were assessed for adequacy. Any specimen with creatinine excretion <15 mg/kg/d in men and <12 mg/kg/d in women was regarded as incomplete and excluded from the study analysis.

The spot specimens were analyzed for albumin (milligrams per liter) and creatinine (millimoles per liter). ACR was derived by dividing the albumin concentration by the creatinine concentration, and the ratio was expressed as milligrams per millimole. Urine albumin was measured by a chemiluminescent enzyme immunoassay using an Immulite 2000 analyzer (Siemens). The analytical detection sensitivity limit for the urine albumin assay was 1 μg/mL. Laboratory within- and between-run coefficients of variation for urine albumin were 6% and 4.5%, respectively. Urine creatinine was measured by the kinetic Jaffé method on a Roche Hitachi modular analyzer. The detection sensitivity limit for urine creatinine was 360-57,500 mmol/L. For urine creatinine at concentrations of 5.39 mmol/L, laboratory within- and between-run coefficients of variation were 1.1% and 1.2%, respectively. Spot urine samples were not routinely cultured to detect bacteriuria because there is no convincing evidence that the presence of asymptomatic urinary tract infection significantly alters protein excretion rates.[15]

Statistical Analyses

The statistical significance of the mean difference between ACRs collected on consecutive days was determined using paired *t* tests, with 95% confidence intervals (CIs) and significance level at 0.05. Correlation between ACRs collected on consecutive days was measured using Spearman ρ.

We constructed Bland-Altman plots in which the difference of the measurements is plotted against the average of the measurements. We then calculated repeatability limits; that is, lower and upper limits in which 95% of the differences between 2 measurements on the same person should lie, using methods derived from those described by Bland and Altman.[16,17] First, we performed a regression using fractional polynomials of the absolute difference between measurements against the average of the methods. There was a small number of observations (n = 5) with an average ACR > 600 mg/mmol (all in the range of 600-1,500 mg/mmol [5,304-13,260 mg/g]). Because of the paucity of data in this range, we excluded these observations from the regression models. Thus, we restricted analysis to the 152 observations with ACR < 600 mg/mmol. Because of the possibility that the absolute difference between measurements may have depended on the level of measurement in a nonlinear manner, we used fractional polynomials in the regression.

We first fitted a fractional polynomial model with 2 powers, but because this was not significantly better than a model with a single power ($P = 0.06$), we used the model with single power. This model was $4\,|D| = 5.019 + 0.177 \times A$, where D denotes the difference of the 2 measurements and A denotes the average. The standard deviation (SD) of the differences is then given by multiplication by $\sqrt{(\pi/2)}$, which gives $SD = 6.290 + 0.222 \times A$; multiplying by 1.96 gives 95% repeatability limits of $\pm(12.328 + 0.434 \times A)$. This model provided reasonable fit, with 140 of 152 (92.1%; 95% CI, 87%-96%) of the observations lying within the repeatability limits (compared to an expected 95%, or 144 observations).

We tested whether the repeatability limits varied with age (stratified around a threshold of 55 years), sex, and eGFR category (<30, 30-<60, and ≥60 mL/min/1.73 m^2) by including a term for each of these variables in the regression equations.

The repeatability limits for test results were statistically extrapolated at different baseline ACR thresholds, if 2 or 3 repeat test results were available. Data were analyzed using Stata, version 12.1 (StataCorp LP).

We have previously published a similar analysis of day-to-day variability in spot urinary PCR.[12] We used those data to compare the day-to-day variability of ACR with PCR among 141 patients who were common to both analyses. We examined the correlation between ACR1:ACR2 and PCR1:PCR2 for each