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Original Investigation

Day-to-Day Variability in Spot Urine Protein-Creatinine Ratio Measurements

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Background: Accurate measurement of proteinuria is important in the diagnosis and management of chronic kidney disease (CKD). The reference standard test, 24-hour urinary protein excretion, is inconvenient and vulnerable to collection errors. Spot urine protein-creatinine ratio (PCR) is a convenient alternative and is in widespread use. However, day-to-day variability in PCR measurements has not been evaluated.

Study Design: Prospective cohort study of day-to-day variability in spot urine PCR measurement.

Setting & Participants: Clinically stable outpatients with CKD (n = 145) attending a university hospital CKD clinic in Australia between July 2007 and April 2010.

Index Test: Spot urine PCR.

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

Measurements: Spot PCRs were measured from urine samples collected at 9:00 AM on consecutive days and 24-hour urinary protein excretion was collected concurrently.

Results: Paired results were analyzed from 145 patients: median age, 56 years; 59% men; and median 24-hour urinary protein excretion, 0.7 (range, 0.06-35.7) g/d. Day-to-day variability was substantial and increased in absolute terms, but decreased in relative terms with increasing baseline PCR. For patients with a low baseline PCR (20 mg/mmol [177 mg/g]), a change greater than ±160% (repeatability limits, 0-52 mg/mmol [0-460 mg/g]) is required to indicate a real change in proteinuria status with 95% certainty, whereas for those with a high baseline PCR (200 mg/mmol [1,768 mg/g]), a change of ±50% (decrease to <100 mg/mmol [<884 mg/g] or increase to >300 mg/mmol [>2,652 mg/g]) represents significant change.

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

Conclusions: Changes in PCR observed in patients with CKD, ranging from complete resolution to doubling of PCR values, could be due to inherent biological variation and may not indicate a change in disease status. This should be borne in mind when using PCR in the diagnosis and management of CKD.

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INDEX WORDS: Chronic kidney disease (CKD); diagnostic test; protein-creatinine ratio; variability.

Proteinuria (protein excretion >0.15 g/d) is a hallmark of chronic kidney disease (CKD)[1] and a marker of increased cardiovascular risk.[2] The magnitude of protein excretion is associated linearly with subsequent decrease in glomerular filtration rate (GFR) and risk of end-stage kidney disease[3,4] and therefore is an important indicator of prognosis and response to therapy. Consequently, reliable measurement of proteinuria is an important aspect of clinical practice.

The optimal method for detecting proteinuria in the clinic is yet to be defined. Point-of-care tests such as dipsticks are semiquantitative, and although potentially useful as a screening tool, suboptimal sensitivity and specificity limit their usefulness for informing prognosis and monitoring therapy in the clinic.[5] A quantitative 24-hour urine collection for total protein excretion is the reference standard test to quantify proteinuria. However, this is cumbersome and subject to collection errors.[6] Measurement of spot urinary protein-creatinine ratio (PCR) is convenient to the patient and is recommended in US guidelines.[1,6]

Spot PCR has been shown to correlate well with 24-hour protein excretion in many studies, although there are differences in correlation levels at different magnitudes of protein excretion,[6,7] and to be a superior predictor of disease progression in one longitudinal cohort.[7] However, existing studies are largely retrospective, used various analytical techniques to

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measure protein, and have determined correlation rather than agreement between tests.[6,7] Diurnal variation in proteinuria occurs in health and in CKD; thus, timing of spot urine collections may be important.[8] The extent of day-to-day variability in PCRs has not been documented in the existing literature, but may be important in determining whether a change in the quantity of proteinuria over time indicates a change in disease status, response to therapy, or simple test variability. We performed a prospective cohort study to quantify day-to-day variability in spot urine PCR.

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.[9] The Sydney South West Area Health Service Ethics Review Committee approved this study, protocol no. X06-0196.

Patient Recruitment and Consent

Patients were recruited from the hospital's CKD and kidney transplant clinics. Eligible individuals identified from an electronic database were adults (aged ≥18 years) with albuminuria (albumin-creatinine ratio >3.5 mg/mmol) or proteinuria (24-hour urine total protein >150 mg/d) with stable kidney function (outpatients with less than ±15% variation in proteinuria and estimated GFR [eGFR] during the preceding 3 months). Patients were excluded if they were on dialysis therapy, known to be pregnant or less than 3 months post partum, 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

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. They 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 voided urine into a clean container at 9:00 AM and, using a syringe, collected a 10-mL aliquot of this urine into a spot container and stored it at 1°C-4°C. On the next 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 in the intervening 24 hours was collected into the 24-hour bottle. Specimens were returned to the hospital that day and analyzed in the hospital's accredited centralized laboratory within 48 hours. No specimen was frozen.

Participants underwent a blood test for hemoglobin, urea, and creatinine when urine specimens were returned; blood pressure, height, weight, medication, and relevant medical history were recorded; and standard demographic information was collected from all participants. eGFR was derived using the isotope-dilution mass spectrometry–traceable 4-variable MDRD (Modification of Diet in Renal Disease) Study equation.[10] The data were deidentified 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.

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.[11] The spot specimens were analyzed for protein (grams per liter) and creatinine (millimoles per liter), and PCR was derived by dividing the protein concentration by the creatinine concentration. The ratio was expressed as milligrams per millimoles. Urine protein was measured by immunoturbidimetry using a Roche Hitachi modular analyzer (Roche Diagnostics, www.roche-australia.com). The analytical detection sensitivity limit for the urine protein assay was 0.04-2 g/L. Urine protein concentrations >2 g/L were diluted before measurement. The laboratory within-run and between-run coefficients of variation for urine protein were 5.2% and 3.8%, 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, the laboratory within-run and between-run coefficients of variation were 1.1% and 1.2%, respectively.

Statistical Analyses

The statistical significance of the mean difference between day-1 and day-2 PCRs was determined using paired *t* tests, with 95% confidence intervals (CIs) and significance level at 0.05. Data were not geometrically transformed. Correlation between day-1 and day-2 PCRs was measured using Spearman ρ.

We constructed Bland Altman plots in which the difference in measurements is plotted against the average of 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 the methods described by Bland and Altman.[12,13] First, we performed a regression of the absolute difference (D) between measurements against the average (A) of the methods. There was a small number of observations (n = 6) with an average PCR >600 mg/mmol (all in the range of 626-1,341 mg/mmol [5,534-11,855 mg/g]). Because of the lack of data in this range, we excluded these observations from the regression models. Thus, we restricted analysis to the 139 observations with PCR ≤600 mg/mmol [≤5,304 mg/g]. Because the absolute difference between measurements depended on the level of measurement in a nonlinear manner, we used fractional polynomials in the regression. We fitted a fractional polynomial with 2 fractional polynomial terms, but this had only marginally better fit than a model with a single term ($P = 0.826$). Therefore, we used the simpler model for ease of explanation. The fractional polynomial model was $|D| = -7.095 + 3.441\sqrt{A}$. However, to avoid problems with negative standard deviations (SDs) at small values of the measurement, we refitted the model without the constant term, which gave $|D| = 2.868\sqrt{A}$ (this model had almost identical fit). We also bootstrapped the final regression model using 10,000 replicates to obtain estimates of uncertainty around the regression coefficient. These gave a 95% CI for the regression coefficient of 2.270-3.509. The SD of the differences is then given by $SD = \sqrt{\pi/2} \times 2.868\sqrt{A} = 3.594\sqrt{A}$. The 95% repeatability limits are then given by $\pm 1.96 \times 3.594 \times \sqrt{A} = 7.045\sqrt{A}$. In other words, 95% of repeated measurements should lie within $7.0\sqrt{A}$ of the original measurements.

We tested whether the repeatability limits differed by a fixed amount across the levels of age (<55 vs ≥55 years), sex, and