

Evaluation of a Rapid Screening Test for Microalbuminuria with a Spot Measurement of Urine Albumin-Creatinine Ratio

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Abstract

Introduction: Microalbuminuria has been established as a marker of incipient diabetic nephropathy, and a regular screening programme has been advocated in patients with diabetes mellitus. We investigated if urine albumin levels normalised by creatinine give results comparable to the urinary albumin excretion rate (UAER) with a timed 24-h urine for detecting microalbuminuria. **Materials and Methods:** Morning urine specimens and 24-h collections were obtained from 65 diabetic patients. Albumin and creatinine levels in the spot urine specimens were measured in a single rapid (7 min) assay format using the Bayer DCA2000+™ desktop system. Results of albumin/creatinine ratio and urine albumin concentration were then compared to the reference laboratory method of measuring UAER with the timed 24-h urine sample. **Results:** The determination of the albumin/creatinine ratio gave good performance characteristics for diagnosis of microalbuminuria (defined as >30 mg/g creatinine) with sensitivity of 71.4% and specificity of 98.0%. With urine albumin concentration alone, sensitivity was 64.3% and specificity was 96.1%. Receiver operating characteristic (ROC) curves, however, suggest similar diagnostic usefulness for screening microalbuminuria with albumin levels expressed in concentration units or as a ratio of creatinine compared to the reference method. Analysis also indicated that lowering the established cut-off values in general, improves diagnostic performance. **Conclusions:** Measurement of microalbuminuria with a spot morning specimen using the DCA2000+™ desktop system that simultaneously measures albumin and creatinine levels, provides a rapid and reliable method for incipient diabetic nephropathy in clinical practice.

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Key words: Diabetic nephropathy, Receiver operating characteristic curve

Introduction

In 1989, the St Vincent Declaration was formulated by all European countries to address the growing problem of diabetes, with the general goals of improving health care and quality of life for the diabetic patient. One of the targets in the declaration was the need to implement effective measures for reducing the number of people entering end-stage renal failure.

Early kidney damage in diabetes can be detected by screening for microalbuminuria. Preventive measures instituted at this incipient nephropathic stage have been shown to retard the development of renal failure. In Type 1 patients, 50% to 80% of patients with microalbuminuria progress to overt nephropathy in 10 years time. Corresponding figures of 20% to 40% are observed for patients with Type 2 diabetes.¹ It is recommended that screening be carried out for microalbuminuria on a yearly basis. However, there is considerable debate on the type of urine specimen acceptable for detecting early damage to the kidneys in diabetic patients.

Screening for microalbuminuria can be performed by using three methods of urine sampling; i) the 24-h collection, the 'gold standard'; ii) a timed collection, either 4 h or overnight; and iii) the random spot sample. The spot urine sample method is probably the most convenient to the patient, and the preferred sample is the first-void or morning specimen. Both the 24-h and timed urine collections require proper patient instruction and compliance for accurate urine collections. Estimation of urinary albumin has been expressed in concentration units (mg/L) or to reduce the influence of urine flow rate, as a ratio (mg/g) with creatinine excretion. Recent consensus statements define microalbuminuria as 30 to 300 mg/24-h [urinary albumin excretion rate (UAER)] in 24-h collections and 30 to 300 mg/g creatinine in random specimens.²

Our study evaluated the suitability of using spot morning specimens for the detection of microalbuminuria with a rapid test formatted for use with the Bayer DCA2000+™, a desktop system currently available for the HbA1c test. The analyser accepts a single cartridge containing all

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reagents for measuring HbA1c or microalbumin/creatinine. The operational steps for the microalbumin/creatinine test are simple. A capillary holder containing 40 µl urine is first inserted into the cartridge and placed into the analyser. A plunger is immediately depressed into the holder to discharge the sample, the reagent tab pulled away and followed by the closure of the analyser lid. In a short period of 7 min, the assay determines simultaneously albumin and creatinine levels. Results of urine albumin were given as (i) a ratio of creatinine or (ii) in concentration units. Further assessment of performance characteristics by the receiver operating characteristic (ROC) approach was performed to describe the overall test performance and also to compare the test's discriminatory ability using albumin concentration or the albumin/creatinine ratio.

Materials and Methods

Seventy-one consecutive patients with diabetes who were undergoing an annual outpatient screening protocol for diabetic complications which includes testing for incipient nephropathy, participated in this study. The patients were asked to provide a 24-h urine collection the day before their clinic appointments. At the clinic the next morning, ca. 8 am, these patients were also asked to provide a morning urine specimen.

These participants comprised of 38 men (mean age 52.6 ± 14.0 y) and 33 women (45.3 ± 13.4 y) with a mean age of 49.2 ± 14.1 y. Most (70%) were non-insulin-dependent diabetic patients on oral hypoglycaemic drugs or diet treatment.

Albumin Determination

The 24-h urine collections were tested for albuminuria by the hospital's reference laboratory facilities, which use the Beckman Array System with a sensitivity of 2.0 mg/L. Specimens with albumin concentration below the sensitivity level were assigned a value of 1.0 mg/L. Definition of microalbuminuria was when the UAER range is 30 to 300 mg/24-h, with lower values indicating normoalbuminuria.

Aliquots of the 24-h collection and the morning specimen were tested for albuminuria by the DCA2000+™ microalbumin/creatinine assay system that provides a measure of albumin concentrations (measuring range: 5 to 300 mg/L) as well as the creatinine levels (15 to 500 mg/dL). The system measures albumin by immunoturbidimetric direct antibody-antigen aggregation and for creatinine, colorimetrically using the Benedict-Behre reaction. The DCA2000+™ also provides a calculated albumin/creatinine ratio (1 to 2000 mg/g) result. Specimens with albumin levels below detection limits were given a value of 4.0 mg/L.

Receiver Operating Characteristic (ROC)

The ROC curve of a test is a graphical representation of

the true positive rates (sensitivity) versus the false positive rates (100-specificity) calculated for each cut-off point^{3,4} with respect to the reference method (UAER in this study). This ROC curve approach was applied to the different expressions of albumin levels; in concentration units (mg/L) or as a ratio of creatinine (mg/g). Typical performance characteristics were derived at 2 cut-off points. The first point chosen selects 100% sensitivity. The second point representing the overall best diagnostic performance is at the apex of the upper left portion of the curve.

Statistical Analysis

Data were analysed by SAS 6.12 (SAS Institute, USA). Values were given as mean $\pm$ SD. The ROC curve was generated by logistic regression modelling. Differences between ROC curves of each expression of albumin concentration as depicted by the c-statistic (area under the curve), were tested by the Mann-Whitney U test⁵ with the computation routine written in SAS language. The level of significance was set at 0.05.

Results

Of the 71 subjects participating in the study, 6 were tested to have macroalbuminuria (>300 mg/24-h) and were excluded from the analyses. The remaining 65 subjects with mean age 49.1 ± 14.0 y, consisted of 36 men (52.9 ± 13.8 y) and 29 women (44.3 ± 13.0 y). The mean albumin concentration was 13.4 mg/L with only one specimen having less than 2.0 mg/L (range 1.0 to 128 mg/L). Fifty-one (78.5%) urine samples were tested negative for microalbuminuria (<30 mg/24-h), 14 samples were deemed to have microalbuminuria (30 to 300 mg/24-h) giving a mean of 88 ± 82.8 mg/24-h (range 34 to 300 mg/24-h).

Linear regression analysis of albumin levels determined by DCA2000+™ and by the reference laboratory showed a close correlation giving a Pearson moment coefficient of 0.997 and a slope of 0.93.

With the established cut-off value of 30 mg/g for the albumin/creatinine ratio and 20 mg/L for albumin concentration, the performance characteristics for identifying microalbuminuria can be calculated for the morning specimen compared to the UAER (Table I). Morning specimens showed 2% (1/65) false positives and 6% (4/65) false negatives with mg/g creatinine as the basis of identifying microalbuminuria. The sensitivity was 71.4% with a specificity of 98.0%. An increase in false determinations was obtained when values were expressed in mg/L—3% (2/65) positives and 8% (5/65) negatives with sensitivity of 64.3% and specificity of 96.1%.

By comparing the areas under the ROC curves (the c-statistic), the diagnostic usefulness based on albumin levels in mg/g creatinine or in mg/L was equally applicable for

TABLE I: PERFORMANCE FOR MORNING URINE SPECIMENS AT ESTABLISHED CUT-OFF VALUES

	Microalbuminuria by reference (UAER) method		
	Normal	Abnormal	Total
Microalbuminuria by DCA2000+™			
Normal	50 (49)	4 (5)	54
Abnormal	1 (2)	10 (9)	11
Total	51	14	65

Number of patients assessed as normal or abnormal for microalbuminuria are given based on the UAER results and for DCA2000+™ results based on 30 mg/g creatinine or (20 mg/L) cut-offs.

TABLE II: PERFORMANCE FOR MORNING URINE SPECIMENS BY ROC CURVE ANALYSIS

Cut-off point	Albumin-creatinine ratio (mg/g)		Albumin (mg/L)	
	14*	21 [#]	11.1*	13.3 [#]
Sensitivity (%)	100	92.9	100	85.7
Specificity (%)	86.3	94.1	86.3	86.3
Positive PV (%)	66.7	81.3	66.7	63.2
Negative PV (%)	100	98.0	100	95.7

* The first cut-off point is taken at 100% sensitivity.

[#] The second cut-off point is nearest to the intersection of the curve at the 100%-to-100% diagonal.

ROC: receiver operating characteristic; PV: predictive value

microalbuminuria. The c-statistic for performance based on mg/g was 0.978 [standard error (SE) 0.0507] and for that on mg/L was 0.964 (SE 0.0681); a value of 1.0 means total agreement with the reference (UAER) method and a value of 0.5 indicates no diagnostic value.

Figure 1 shows that for albumin levels expressed in mg/g creatinine, 100% sensitivity was obtained at 14 mg/g cut-off (Table II). Selecting the threshold level at 21 mg/g gave optimal performance values closer to that obtained with 30 mg/g, the established cut-off value. Similarly at a lower cut-off of 11 mg/L, 100% sensitivity and 86% specificity were obtained for the morning specimens but with lower performance characteristics.

Discussion

Screening for microalbuminuria with early intervention by various measures has been shown to delay the development of nephropathy by as much as 20 years.¹ Accurate diagnosis of microalbuminuria with spot urine specimen measuring the albumin/creatinine ratio (normalisation of albumin levels) provides an important advantage in clinical practice, as it obviates the need to collect timed urine samples.^{2,6,7}

Albumin concentrations measured by the DCA2000+™

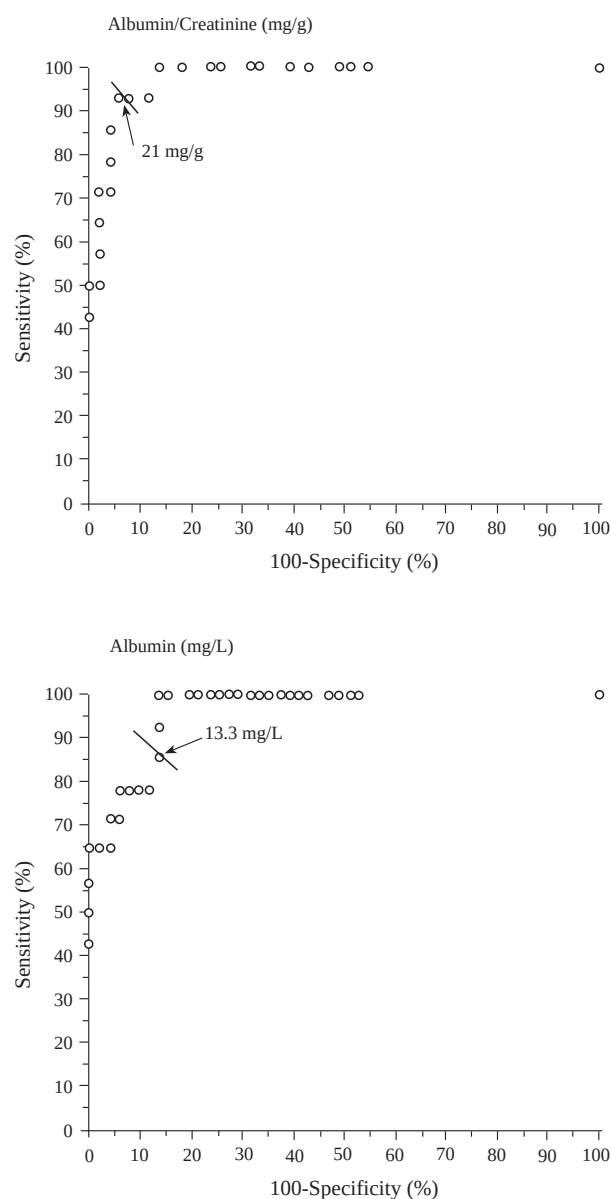


Fig. 1. ROC curves for morning urine specimens in the screening of microalbuminuria. Results based on albumin/creatinine ratio and mg/L albumin (cut-off values given at 100%-to-100% diagonal).

were comparable to the reference laboratory method. Creatinine was not however, determined in the 24-h urine collection by the reference laboratory as it was not needed for determining the UAER. The DCA2000+™ desktop device permits decentralised testing with a significant feature being that within the same 7-min test period, a simultaneous measurement of urinary creatinine is also performed on the sample. There is no requirement for any pre-dilution of sample, calibration with solutions or reagent preparation. The in-built calculation of the albumin/creatinine ratio allows an immediate appreciation of the albuminuria status.

ROC curves illustrate the sensitivity and specificity

relationship as the decision thresholds are adjusted. Comparison of the area under the curve (the c-statistic) in the evaluation of two or more assays or specimen types allows the selection of the best diagnostic test. Our analysis indicates no significant differences in diagnostic usefulness with albumin levels expressed as a ratio of creatinine or in concentration units, but improved data were obtained for results based on mg/g creatinine. In a previous study, we had also shown that morning or random urine specimens could be used for screening microalbuminuria using a semi-quantitative dipstick test, albeit with lower performance.⁸ Zelmanovitz and associates⁹ studied 95 Type 2 diabetic patients, measuring urinary albumin in morning specimens and 24-h collections and then compared areas under the ROC curves. They showed that random urine specimens were valid for screening, and albumin concentration or albumin/creatinine ratios were equally suitable. It has also been reported in a recent study of 40 patients with Type 1 diabetes that spot urine specimens measured by the DCA2000+TM could reliably replace 24-h or timed overnight urine collections when the albumin/creatinine ratio was used.¹⁰ The investigators reported figures of 96.9% sensitivity, 87.5% specificity, 96.9% positive predictive value and 97.5% negative predictive value with spot urine specimens. Our own results support this conclusion with indications of better specificity but lower sensitivity.

Our study confirmed that screening for microalbuminuria in morning specimens could be based on albumin concentrations compensated for fluctuations in urine flow rates, i.e. mg/g creatinine. As is the recommendation, sustained microalbuminuria will require confirmation from 2 or 3 more tests over a period of time. In contrast to a 24-h urine collection, it is readily apparent that applying a single spot urine sample in a reagent cartridge for two measurements (albumin and creatinine) will make it much easier and convenient for the patients to be assessed of their albuminuria status. This test format should encourage

frequent and regular monitoring and provides an acceptable alternative method for screening of microalbuminuria.

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