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Reproducibility of albuminuria in type 2 diabetic subjects. Findings from the Renal Insufficiency And Cardiovascular Events (RIACE) study

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Abstract

Background. Measurement of urinary albumin excretion (UAE) shows important intra-individual variability suggesting the need for multiple assessments. This study aimed at investigating the reproducibility of UAE in type 2 diabetes.

Methods. UAE was obtained from two to three samples collected in a 3- to 6-month period from 4062 of the 15 773 type 2 diabetic subjects participating in the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicentre Study in 2007–08. UAE was assessed as albumin excretion rate (AER) in 24-h urine collections from 833 subjects and albumin:creatinine ratio (A/C) in early-morning urine samples from 3229 patients. Albuminuria was measured by immunonephelometry or immunoturbidimetry.

Results. The median coefficient of variation (CV) was 32.5% (interquartile range: 14.3–58.9). Concordance rate between a single UAE and the geometric mean of multiple measurements was 94.6% for normoalbuminuria, 83.5% for microalbuminuria, 91.1% for macroalbuminuria and 90.6% for albuminuria (micro + macro). CV was significantly higher ($P < 0.01$) for AER measurement than for A/C and with immunoturbidimetry than with immunonephelometry, whereas concordance rates were similar between the two modalities of urine collection and the two assay methods. Receiver-operating characteristic (ROC) plots demonstrated a good performance of single UAE in predicting the geometric mean of multiple measures at the cut-off level of both microalbuminuria (ROC_{AUC} 0.926; 95% confidence interval: 0.915–0.937) and macroalbuminuria (ROC_{AUC} 0.950; 95% confidence interval: 0.927–0.973).

Conclusions. Data from this large cohort indicate that, in type 2 diabetic subjects, a single UAE value, thought to be encumbered with high intra-individual variability, is an accurate predictor of nephropathy stage for clinical and epidemiological purposes.

Keywords: albuminuria; diabetic nephropathy; type 2 diabetes

Introduction

Measurement of urinary albumin excretion (UAE) is considered the main tool for the screening of diabetic nephropathy, though estimated glomerular filtration rate (eGFR), blood pressure and funduscopic changes should also be assessed [1]. Moreover, a relationship between UAE and cardio-renal risk, with no clearly defined lower or upper threshold, is widely recognized [2]. However, pre-analytical, intra-individual biological variability of UAE ranges from 4 to 103%, with a central tertile of 28–47% [3]. Because of this, almost all guidelines and recommendations [3–5] claim the need to carry out three UAE measurements within a 3- to 6-month period and that two of three values should be abnormal before contemplating that a patient has crossed one of the diagnostic thresholds defining UAE abnormalities [3–5]. However, these suggestions are not routinely followed in current clinical practice, due to the difficulty in obtaining multiple UAE measurements, and are mainly based on expert consensus or clinical experience.

This study was aimed at assessing the reproducibility of UAE in a large cohort of subjects with type 2 diabetes participating in the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicentre Study.

Materials and methods

Study population

The RIACE Italian Multicentre Study (registered at http://ClinicalTrials.gov, NCT00715481; URL http://clinicaltrials.gov/ct2/show/NCT00715481) is an observational, prospective cohort study on reduced eGFR as an

independent predictor of cardiovascular disease in type 2 diabetes. The RIACE cohort consists of 15 773 patients with type 2 diabetes (American Diabetes Association criteria), visiting consecutively 19 hospital-based Diabetes Clinics of the National Health Service throughout Italy (see online appendix) in 2007–08. Exclusion criteria were dialysis or renal transplantation. Anamnestic, clinical, laboratory and instrumental data were collected in a single database using a dedicated computer software developed by Client-Server.net (Belluno, Italy). The study protocol was approved by the locally appointed ethics committees.

Albuminuria

One UAE measurement was obtained from all patients during enrolment (UAE_{ENR}). In 4062 (25.8%) and 2310 (14.6%) individuals, one and two additional measurements, respectively, within 3–6 months from enrolment were available from five centres. In this case, the geometric mean of 2 or 3 values (UAE_{GMEAN}) was calculated to assign each patient to one of the following classes of albuminuria:normoalbuminuria (<30 mg/24 h or mg/g), microalbuminuria (30–299 mg/24 h or mg/g) or macroalbuminuria (≥300 mg/24 h or mg/g).

UAE was assessed as albumin excretion rate (AER) in 24-h urine collections ($n = 833$; 20.5%) or albumin:creatinine ratio (A/C) in early morning, first-voided urine samples ($n = 3229$; 79.5%). Patients were instructed to discharge urine at start and to save urine for the next 24 h or to collect midstream urine for AER and A/C, respectively. Albumin concentration was measured in fresh urine samples by immunonephelometry ($n = 2643$; 65.1%) or immunoturbidimetry ($n = 1419$; 34.9%), in the absence of symptoms and signs of urinary tract infection or other interfering clinical conditions. As recommended [6], the analytical coefficient of variation (CV) of both methods was largely <15%. At the reference laboratory of the coordinating centre, analytical CVs across a working range of 5–300 mg/L was of 2.1–5.2% for immunonephelometry versus 3.4–8.1% for immunoturbidimetry, with a detection limit of 1.7 and 3.0 mg/L, respectively, in keeping with previous reports [7, 8]. As an external quality control of urinary albumin assays, 50 samples from each centre were reanalysed at the reference laboratory using the immunonephelometry method to verify that the CVs between the peripheral and the central values were <15% at least in the relevant clinical range of 15–500 mg/L, which was the case for 94% of samples. Urinary creatinine concentration was measured by the modified Jaffe method.

Statistical analysis

Intra-individual CV of UAE from multiple measurements was calculated and the Kruskal–Wallis test was used as appropriate. The Spearman rank-order correlation between measurements was also computed. Receiver-operating characteristic (ROC) curves were plotted and areas under the curve (AUC) were then calculated to assess predictivity of albuminuria staging. The performance of UAE_{ENR} was determined according to the AUC, sensitivity (percentage of subjects with raised albuminuria with a positive test), specificity (percentage of subjects without raised albuminuria with a negative test), positive predictive value and negative predictive value. Difference between UAE_{ENR} and the geometric mean of two UAE measurements ($UAER_{GTWO}$) in ROC_{AUC} and diagnostic efficiency (the proportion of patients correctly classified at each cut-point) was calculated by the Hanley and McNeil's method and the chi-square test, respectively.

Statistical analysis was performed using the SPSS software package version 10.0 (SPSS Inc., Chicago, IL). A P-value of <0.05 was considered statistically significant.

Results

Age, gender, diabetes duration and control, other cardiovascular risk factors and prevalence of complications in the 4062 subjects with at least two measurements (Table 1) were similar to those observed in the whole RIACE cohort (data not shown), including distribution of UAE_{ENR} (Figure 1) and distribution among classes of UAE_{GMEAN} (normoalbuminuria 71.9%, microalbuminuria 23.2% and macroalbuminuria 4.9%).

Intra-individual CV was 32.5% (14.3–58.9), with no difference among subjects with normo (31.6%; 13.2–59.1), micro (34.1%; 17.1–59.2) and macroalbuminuria (34.2%; 17.9–53.2).

CV was significantly higher ($P < 0.01$) for AER (35.3%; 16.4–62.0) than for A/C ratio (31.5%; 13.9–58.4) as well as for immunoturbidimetry (43.0%; 25.1–65.9) than for immunonephelometry (25.5%; 10.7–54.2).

Table 1. Clinical characteristics of the 4062 subjects from the RIACE cohort with at least two UAE measurements[a]

Variable	Value
Age, years	66 (59–72)
Male gender, *n* (%)	2420 (59.6%)
Smoking, *n* (%)	
No	2141 (52.7%)
Ex	1248 (30.7%)
Yes	673 (16.6%)
Diabetes duration, years	10 (5–18)
HbA_{1c}, %	7.29 (6.59–8.17)
BMI, kg/m^2	
Males	27.93 (25.43–30.82)
Females	28.96 (25.80–32.91)
Triglycerides, mg/dL	122.0 (89.0–171.0)
Total cholesterol, mg/dL	182.0 (159.0–205.0)
HDL cholesterol, mg/dL	
Males	47.0 (39.0–56.0)
Females	54.0 (45.0–63.0)
LDL cholesterol, mg/dL	103.0 (83.0–123.7)
Lipid-lowering treatment, *n* (%)	1963 (48.3%)
Dyslipidemia, *n* (%)	3618 (89.1%)
Systolic BP, mmHg	140 (130–150)
Diastolic BP, mmHg	80 (70–83)
Antihypertensive treatment, *n* (%)	2850 (70.2%)
ACE-I/ARB treatment, *n* (%)	2425 (59.7%)
Hypertension, *n* (%)	3391 (83.5%)
Serum creatinine, mg/dL	0.90 (0.76–1.07)
eGFR MDRD, mL/min/1.73 m^2	81.8 (67.6–97.6)
Albuminuria, *n* (%)	2921 (71.9%)
Normo	943 (23.2%)
Micro	198 (4.9%)
Macro	
CKD, *n* (%)	
No CKD	2537 (62.5%)
CKD stage 1	301 (7.4%)
CKD stage 2	508 (12.5%)
CKD stage 3	658 (16.2%)
CKD stages 4–5	58 (1.4%)
Retinopathy, *n* (%)	
No	3107 (76.5%)
Non-advanced	715 (17.6%)
Advanced	240 (5.9%)
Major acute CVD events, *n* (%)	
Any	812 (20.0%)
AMI	424 (10.4%)
Coronary revascularization	357 (8.8%)
Stroke	132 (3.2%)
Carotid revascularization	78 (1.9%)
Foot ulcer/gangrene	124 (3.1%)
Lower limb revascularization	70 (1.7%)

[a]HbA_{1c}, glycated haemoglobin; BMI, body mass index; BP, blood pressure; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; MDRD, Modification of Diet in Renal Disease; CKD, chronic kidney disease; CVD, cardiovascular disease; AMI, acute myocardial infarction.

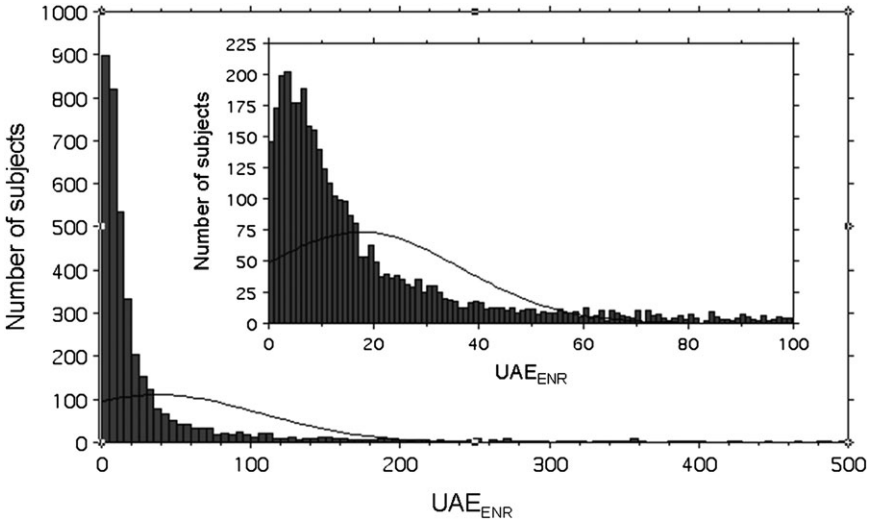

Fig. 1. Distribution of UAE$_{ENR}$, i.e. UAE obtained at the enrolment visit. The larger picture illustrates distribution of UAE$_{ENR}$ in the range of 0–500 mg/24 h (or mg/g) leaving to the right 142 subjects with UAE values >500 mg/24 h (or mg/g). The smaller picture illustrates distribution of UAE$_{ENR}$ in the interval of 0–100 mg/24 h (or mg/g) leaving to the right 514 subjects with UAE values >100 mg/24 h (or mg/g). For both picture, the black line represents normal distribution.

A strong linear correlation emerged between UAE$_{ENR}$ and UAE$_{GMEAN}$ in the 4062 subjects with at least two UAE results ($r = 0.910$, $r^2 = 0.827$, $P < 0.0001$). Correlation was slightly, though significantly ($P < 0.001$), lower in the 2310 individuals with three UAE results ($r = 0{,}863$, $r^2 = 0.744$, $P < 0.0001$). No correlation was observed between UAE$_{GMEAN}$ and CV ($r = 0.011$, $P = 0.47$).

Concordance rate between UAE$_{ENR}$ and UAE$_{GMEAN}$ was 94.6% for normoalbuminuria, 83.5% for microalbuminuria, 91.1% for macroalbuminuria and 90.6% for albuminuria (micro or macro) in subjects with at least two UAE measures and 94.6, 84.2, 86.8 and 90.8%, respectively, in subjects with three UAE measurements. Concordance rates for normoalbuminuria, microalbuminuria, macroalbuminuria and albuminuria were similar between the two modalities of urine collection (AER: 92.6, 84.0, 89.6 and 90.8%, respectively; A/C: 95.0, 83.2, 92.1 and 90.5%, respectively) and the two assay methods (immunonephelometry: 94.2, 83.6, 91.1 and 90.9%, respectively; immunoturbidimetry: 95.3, 83.3, 91.2 and 89.8%, respectively). Results with immunonephelometry and immunoturbidimetry are shown separately in Supplementary Tables 1 and 2, respectively. Performance was also similar in subgroups where urine creatinine concentration is expected to be different, i.e. male versus female, young versus old, low versus high body mass index, and no centre effect was detected (data not shown).

ROC analysis demonstrated the good performance of UAE$_{ENR}$ in predicting UAE$_{GMEAN}$ at the cut-off level for both micro and macroalbuminuria in subjects with at least two and three measurements. The improvement obtained by UAE$_{GTWO}$ in predicting UAE$_{GMEAN}$ was statistically significant at the cut-off level for microalbuminuria but not for macroalbuminuria. Sensitivity and specificity were high, with a low chance for a false positive or negative result, at the cut-off level for both micro and macroalbuminuria. The diagnostic efficiency of UAE$_{GTWO}$ was higher than that of UAE$_{ENR}$ for both microalbuminuria and macroalbuminuria (Figure 2 and Table 2).

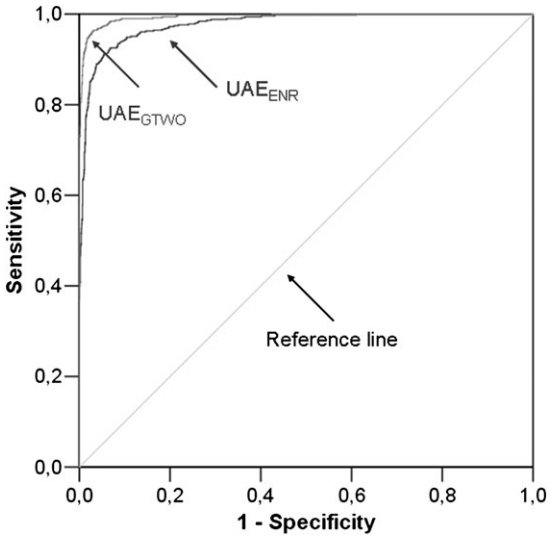

Fig. 2. Predictive performance of UAE$_{ENR}$ (UAE obtained at the enrolment visit) and UAE$_{GTWO}$ (geometric mean of two UAE measurements) for UAE$_{GMEAN}$ (geometric mean of three UAE values) in 2310 type 2 diabetic subjects who performed three urine collections according to the ROC curves. Areas under the specificity-sensitivity curves (AUC) are 0.927 (95% confidence interval: 0.911–0.943) and 0.966 (95% confidence interval: 0.954–0.977) for UAE$_{ENR}$ and UAE$_{GTWO}$, respectively (Hanley and McNeil test for ROC curves comparison, $P = 0.0001$).

Discussion

UAE and eGFR are front-line tests in evaluating diabetic nephropathy from a research and clinical viewpoint. Though urinary albumin shows important pre-analytical intra-individual variability [3], the performance of a single UAE value in staging nephropathy in type 2 diabetes has been poorly explored. This is the first large-cohort study assessing the reproducibility of UAE in subjects with type 2 diabetes.

The intra-individual CV of UAE in 4062 subjects with type 2 diabetes from the RIACE cohort was high, as expected.

Table 2. Results of ROC analysis for UAE_{ENR} and UAE_{GTWO} in assessing albuminuria staging in the whole cohort of type 2 diabetic patients irrespective of albumin assay method[a]

	ROC area	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Diagnostic efficiency, %
N: 4062 subjects with at least two UAE measures						
1. UAE_{GMEAN} cut-off level: 30 mg/24 h or 30 mg/g; positive subjects 26.0% (*n*, 1057)						
UAE_{ENR}	0.926 (0.915, 0.937)	90.6 (88.7, 92.3)	94.6 (93.7, 95.4)	85.5 (83.3, 87.5)	96.6 (95.9, 97.2)	93.6
2. UAE_{GMEAN} cut-off level: 300 mg/24 h or 300 mg/g; positive subjects 5.0% (*n*, 203)						
UAE_{ENR}	0.950 (0.927, 0.973)	91.1 (86.1, 94.5)	98.9 (98.5, 99.2)	81.4 (75.7, 86.2)	99.5 (99.2, 99.7)	98.5
N: 2310 subjects with three UAE measures						
1. UAEGMEAN cut-off level: 30 mg/24 h or 30 mg/g; positive subjects 22.1% (*n*, 510)						
UAE_{ENR}	0.927 (0.911, 0.943)	90.8 (87.8, 93.1)	94.6 (93.4, 95.6)	82.3 (79.2, 85.7)	97.3 (96.4, 98.0)	93.8
UAE_{GTWO}	0.966 (0.954, 0.977)	95.5 (93.2, 97.0)	97.6 (96.8, 98.2)	91.2 (89.1, 94.0)	98.7 (98.0, 99.1)	97.1
	Hanley and McNeil P = 0.0001					$\chi 2 = 30.3$, $P < 0.0001$
2. UAE_{GMEAN} cut-off level: 300 mg/24 h or 300 mg/g: positive subjects 3.9% (*n*, 91)						
UAE_{ENR}	0.930 (0.889, 0.971)	86.8 (77.7, 92.7)	99.1 (98.6, 99.5)	80.6 (71.1, 87.6)	99.4 (99.0, 99.7)	98.6
UAE_{GTWO}	0.975 (0.951, 1.000)	95.6 (88.5, 98.6)	99.4 (99.0, 99.7)	87.9 (79.4, 93.3)	99.8 (99.5, 99.9)	99.3
	Hanley and McNeil P = 0.0628					$\chi^2 = 4.84$, $P < 0.05$

[a]The 95% confidence intervals are reported in parenthesis. PPV, positive predictive value; NPV, negative predictive value.

Nevertheless, the performance of a single UAE value in predicting albuminuria staging as assessed by multiple UAE measures was quite good at both the microalbuminuria and macroalbuminuria thresholds. As compared with results of the two largest studies addressing these issues in the general population, the intra-individual CV of UAE in 4062 subjects with type 2 diabetes was almost similar to that reported in 4680 individuals randomly selected from 17 population samples by Dyer *et al.* [9], and the performance of a single UAE value in predicting albuminuria staging was better than that reported in 1241 subjects from the NHANES III cohort (84 versus 63% for microalbuminuria) [10]. This latter finding might be due to the higher prevalence of renal disease in subjects with type 2 diabetes than in individuals from the general population, with consequently a lower chance for false-positive results.

Our data may have important implications for the screening, diagnosis and staging of nephropathy in subjects with type 2 diabetes. In fact, the good performance of a single UAE in predicting albuminuria staging would argue against the need for multiple UAE measurements in these patients, provided that interfering clinical conditions are carefully excluded. The higher diagnostic efficiency of the combination of two UAE versus a single UAE value might be not meaningful for classification of patients with type 2 diabetes in epidemiological studies and, more importantly, for diagnosis and staging of these subjects in clinical settings, where repeated measurements are difficult to obtain and avoidance would result in significant cost and time savings. However, this may not apply to UAE assessment for evaluating disease progression and efficacy of renoprotective treatment with blockers of the renin–angiotensin system.

Limitations of this study include variations due to the different methods for the assessment of albuminuria (AER versus A/C and immunonephelometry versus immunoturbidimetry), which may have influenced the results since reproducibility was better for A/C and immunonephelometry than for AER and immunoturbidimetry, respectively. Nevertheless, performance of UAE was similar with the two modalities of urine sampling and assay techniques. The similar performance of immunoturbidimetry, despite the higher CV, as compared with immunonephelometry, suggests that variability associated with this assay method is attributable predominantly to the higher analytical CV [7, 8]. Moreover, the higher CV for AER is likely to be dependent on the lower accuracy of the 24-h urine collection modality, as compared with early-morning sampling for A/C, which is in fact the preferred urine sampling method for UAE assessment [5].

Data from the large RIACE cohort indicates that a single UAE value, thought to be encumbered with high intra-individual variability, is an accurate predictor of the stage of nephropathy in subjects with type 2 diabetes, thus suggesting that multiple UAE measurements may not be necessary for classification purposes in both clinical and epidemiological settings.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org/

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Conflict of interest statement. None declared.

Trial Registration. NCT00715481; http://clinicaltrials.gov/ct2/show/NCT00715481.

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