The accuracy of predicting cardiovascular death based on one compared to several albuminuria values

Gudrun Hatlen[1,2], Solfrid Romundstad[1,3,4] and Stein I. Hallan[1,2]

[1]Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway; [2]Division of Nephrology, Department of Medicine, St Olav University Hospital, Trondheim, Norway; [3]Department of Internal Medicine, Levanger Hospital, Health Trust Nord-Trøndelag, Levanger, Norway and [4]HUNT Research Centre, Faculty of Medicine, Norwegian University of Science and Technology, Levanger, Norway

Albuminuria is a well-documented predictor of cardiovascular (CV) mortality. However, day-to-day variability is substantial, and there is no consensus on the number of urine samples required for risk prediction. To resolve this we followed 9158 adults from the population-based Nord-Trøndelag Health Study for 13 years (Second HUNT Study). The predictive performance of models for CV death based on Framingham variables plus 1 versus 3 albumin–creatinine ratio (ACR) was assessed in participants who provided 3 urine samples. There was no improvement in discrimination, calibration, or reclassification when using ACR as a continuous variable. Difference in Akaike information criterion indicated an uncertain improvement in overall fit for the model with the mean of 3 urine samples. Criterion analyses on dichotomized albuminuria information sustained 1 sample as sufficient for ACR levels down to 1.7 mg/mmol. At lower levels, models with 3 samples had a better overall fit. Likewise, in survival analyses, 1 sample was enough to show a significant association to CV mortality for ACR levels above 1.7 mg/mmol (adjusted hazard ratio 1.37; 95% CI 1.15–1.63). For lower ACR levels, 2 or 3 positive urine samples were needed for significance. Thus, multiple urine sampling did not improve CV death prediction when using ACR as a continuous variable. For cutoff ACR levels of 1.0 mg/mmol or less, additional urine samples were required, and associations were stronger with increasing number of samples.

Kidney International (2013) **85,** 1421–1428; doi:10.1038/ki.2013.500; published online 18 December 2013

KEYWORDS: albuminuria; cardiovascular; cardiovascular events; epidemiology and outcomes; risk factors; survival

Correspondence: *Gudrun Hatlen, Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, PO Box 8905, N-7491 Trondheim, Norway.*
E-mail: gudrun.hatlen@ntnu.no

Received 17 February 2013; revised 20 September 2013; accepted 3 October 2013; published online 18 December 2013

Albuminuria is a powerful and independent risk factor for adverse cardiovascular (CV) and renal outcomes.[1–9] However, in order to implement urine albumin-to-creatinine ratio (ACR) in clinical CV risk stratification, it is important to assess the number of samples required, as previous studies have almost exclusively used single urine samples.

The intraindividual day-to-day variation of urinary albumin excretion (UAE) might be substantial (25–50%) and transient elevations occur.[10–12] It is therefore often suggested that elevated UAE should be confirmed in at least two samples, but this is, even for diabetic patients, expert opinion based.[13] Recently, interpretation of proteinuria measurements in multiple- and single-urine samples were evaluated in a large laboratory-based cohort.[14] Results indicated that relying entirely on the first measurement led to lower estimates of absolute and relative risk for all-cause mortality and renal outcomes as compared with multiple measurements. However, predictive performance of one versus multiple urine samples was not evaluated. Furthermore, data indicate that UAE well below currently used cutoff values for high albuminuria (former 'microalbuminuria') predicts CV risk,[8,15,16] but even less is known about the number of samples needed for evaluation of risk at these low levels of UAE.

We conducted a population-based prospective long-term follow-up study, including individuals with known diabetes, hypertension, or randomly selected from the Second HUNT Study, Norway. We hypothesized that multiple urine samples are superior to single samples for predicting CV mortality, also at urine albumin levels below the currently used thresholds value for high albuminuria.

RESULTS

We followed 9158 individuals who all delivered three consecutive urine samples. Our cohort was overrepresented by individuals with known hypertension ($n = 5486$) and diabetes ($n = 1590$) compared with nonhypertensive/nondiabetic individuals ($n = 2082$). Table 1 shows the baseline characteristics of the participants. With increasing ACR

Table 1 | Baseline characteristics of participants stratified by subgroup or ACR percentile (mean of three urine samples)

	Subgroup				ACR percentile; based on non-HT/non-DM group					
	Pooled sample, $N=9158$	Non-HT/ non-DM, $N=2082$	Hypertension (without DM), $N=5486$	Diabetes mellitus, $N=1590$	<25th, $N=1957$	25th–49th, $N=1625$	50th–74th, $N=1781$	75th–89th, $N=1625$	90th–94th, $N=805$	⩾95th, $N=1365$
Age (years)	62.1 ± 14.1	49.2 ± 15.7	65.8 ± 11.4	66.2 ± 13.3	57.8 ± 14.2	58.1 ± 15.0	60.2 ± 15.1	64.9 ± 13.8	67.1 ± 12.1	69.0 ± 11.8
Female (%)	54.6	52.8	56.4	50.6	55.1	50.9	58.6	59.8	57.4	45.1
Prior CVD (%)	22.2	4.9	27.2	27.5	16.9	17.3	18.6	24.8	28.9	33.2
eGFR (ml/min per 1.73 m^2)	64.1 ± 13.3	71.9 ± 12.1	61.4 ± 12.6	63.5 ± 13.5	65.6 ± 12.3	67.3 ± 14.0	65.7 ± 12.7	63.8 ± 12.7	62.5 ± 12.8	58.9 ± 14.2
eGFR <60 (%)	38.2	15.4	46.3	40.1	35.4	30.5	33.6	40.2	41.5	53..2
Diabetes mellitus (%)	17.4	0	0	100.0	11.8	11.7	13.5	18.4	22.9	32.7
Hypertensive medication (%)	59.9	0	100.0	40.8	59.1	62.5	64.7	72.1	75.4	75.5
Systolic blood pressure (mm Hg)	151 ± 23.7	136 ± 20.8	156 ± 22.3	155 ± 23.9	144 ± 21.3	147 ± 22.1	148 ± 21.9	156 ± 24.1	159 ± 23.8	162 ± 24.5
Diastolic blood pressure (mm Hg)	86 ± 12.8	80 ± 11.7	88 ± 12.4	85 ± 13.4	83 ± 11.3	85 ± 12.1	84 ± 12.2	87 ± 13.4	89 ± 13.4	89 ± 14.2
BMI (kg/m^2)	28.1 ± 4.5	26.1 ± 3.8	28.6 ± 4.5	29.0 ± 4.8	28.1 ± 4.4	27.6 ± 4.3	27.9 ± 4.6	28.1 ± 4.5	28.6 ± 4.9	28.6 ± 4.8
Ever smoker (%)	51.8	54.4	51.1	50.6	49.6	52.9	50.9	49.7	51.2	57.4
Low education (%)	51.9	34.1	57.5	56.0	46.6	48.9	49.2	57.2	57.9	56.8
No/unknown physical activity (%)	32.7	14.4	30.6	34.9	20.8	21.0	24.7	32.3	36.6	38.5
Cholesterol (mmol/l)	6.3 ± 1.3	5.9 ± 1.2	6.5 ± 1.2	6.2 ± 1.3	6.2 ± 1.2	6.2 ± 1.3	6.2 ± 1.2	6.4 ± 1.3	6.4 ± 1.2	6.4 ± 1.3
HDL cholesterol (mmol/l)	1.3 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.2 ± 0.4
Triglycerides (mmol/l)	2.1 ± 1.3	1.7 ± 1.1	2.2 ± 1.2	2.4 ± 1.6	1.9 ± 1.1	2.0 ± 1.2	2.0 ± 1.2	2.1 ± 1.3	2.2 ± 1.3	2.4 ± 1.6
Glucose (mmol/l)	6.3 ± 2.6	5.3 ± 1.2	5.8 ± 1.3	9.6 ± 4.3	5.9 ± 1.9	6.0 ± 2.1	6.0 ± 2.0	6.4 ± 2.5	6.7 ± 2.9	7.5 ± 3.9
Mean ACR (mg/mmol)	3.1 ± 11.0	1.1 ± 2.7	3.0 ± 10.7	5.7 ± 16.7	0.4 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	1.3 ± 0.2	2.1 ± 0.3	15.4 ± 25.2

Abbreviations: ACR, albumin-to-creatinine ratio; BMI, body mass index; CVD, cardiovascular disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HT, hypertension.
Results are mean ± 1 s.d., unless otherwise noted. ACR cutoff levels from nonhypertensive/nondiabetic participants at the 25th (0.5 mg/mmol), 50th (0.7 mg/mmol), 75th (1.0 mg/mmol), 90th (1.7 mg/mmol), and 95th (2.7 mg/mmol) percentile were applied. Low education was defined as not attending upper secondary school. Baseline characteristics differed significantly across groups (all P for trend <0.001, besides cholesterol, $P=0.04$) except BMI ($P>0.05$). To convert ACR in mg/mmol to mg/g, multiply by 8.84.

levels, we typically observed increasing age, higher prevalence of cardiovascular disease, diabetes mellitus, elevated blood pressure, and lower estimated glomerular filtration rate (all P for trend <0.001), whereas the differences in cholesterol level and body mass index were minor. Mean ACR in the first, second, and third urine samples were statistically equal (3.05, 3.06, and 3.06 mg/mmol, $P>0.9$), but intraindividual variation was substantial (median coefficient of variation 20.4%, interquartile range 12.3–34.6). The first urine sample would have classified 93.6% of all subjects with normal albuminuria (defined as mean of three ACRs <1.0 mg/mmol) correctly. Correspondingly, 79.5% of those with mildly increased albuminuria (mean ACR 1.0–2.9 mg/mmol), 85.2% of those with moderately increased albuminuria (mean ACR 3.0–29.9 mg/mmol), and 90.1% of those with severely increased albuminuria (mean ACR ⩾30.0 mg/mmol) would have been classified correctly by using only the first sample.

During a median follow-up time of 13.1 years (0.1–14.4 years, 104,090 person-years), we observed 3096 deaths, and 1442 died of CV disease (730 women and 712 men). Cox proportional hazard survival analysis demonstrated the strong association between increasing levels of ACR and CV death starting at the 50th percentile (0.7 mg/mmol) with successively rising hazard ratios (HRs) (P for trend <0.001; Table 2). Crude analyses revealed a 5.3-fold risk (95% confidence interval (CI) 4.38–6.33; $P<0.001$) if mean ACR was above the 95th percentile (2.7 mg/mmol) as compared with the reference group (mean ACR <25th percentile). After adjusting for major CV risk factors used in the Framingham risk scores (that is, age, sex, diabetes, treated hypertension, smoking, total cholesterol, and high-density lipoprotein cholesterol), HR was reduced to 2.24 (95% CI 1.85–2.71; $P<0.001$). Further adjustment for estimated glomerular filtration rate did not change these estimates.

When using ACR as a continuous variable, overall diagnostic accuracy provided by albumin excretion did not differ significantly whether based on one, two, or three urine samples (area under the curve 0.658, 0.660, and 0.661, respectively, $P>0.05$ for all comparisons). Still, receiver operating characteristic analyses, displaying the sensitivity and specificity for all possible ACR cutoffs, showed that the curves for one versus three values diverged slightly in a limited range, corresponding to ACR cutoffs between 0.4 and 1.3 mg/mmol. The partial area under the curve in this range using one and three ACR measurements were 0.516 and 0.553 respectively ($P=0.016$), indicating that multiple ACRs were superior to single measurements within this lower range.

However, urine ACR measurements are not intended for predicting CV mortality by itself, thus the potential improvement of adding one versus three ACR values to the Framingham risk variables was evaluated. First, we displayed the distribution of predicted risk in subjects with and without future CV death ('cases' and 'controls') by three risk prediction models (Figure 1). Cases and controls clearly had overlapping risk predictions with the Framingham-like model (model 1), but there was a substantial mean risk