Immune-unreactive urinary albumin as a predictor of cardiovascular events: the Hortega Study

Fernando Martínez1,2,3, Gernot Pichler1,2, Adrian Ruiz1,2, Juan C. Martín-Escudero4, Felipe J. Chaves5,6, Veronica Gonzalez-Albert5, Maria Tellez-Plaza2,7, Hiddo J.L. Heerspink8, Dick D.E. Zeeuw8 and Josep Redon1,2,3,9

1Internal Medicine Department, Clinical Hospital of Valencia, Valencia, Spain, 2Area of Cardiometabolic and Renal Risk, Biomedical Research Institute Hospital Clinic of Valencia (INCLIVA), Valencia, Spain, 3Department of Medicine, University of Valencia, Valencia, Spain, 4Department of Internal Medicine, University Hospital Rio Hortega, Valladolid, Spain, 5Genetic and Genomic Unit, Research Institute of the Clinical Hospital of Valencia (INCLIVA), Valencia, Spain, 6CIBER of Diabetes and Associated Metabolic Diseases (CIBERDEM), Institute of Health Carlos III, Madrid, Spain, 7Department of Environmental Health Sciences, Johns Hopkins Medical Institutions, Baltimore, MD, USA, 8Department of Clinical Pharmacy and Pharmacology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands and 9CIBER 03/06 Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, Madrid, Spain

Correspondence and offprint requests to: Fernando Martínez; E-mail: fermar23@uv.es

ABSTRACT

Background. We aimed to determine if immune-unreactive albumin excretion (IURAE) is associated with cardiovascular (CV) events in a representative sample of a general population from Spain.

Methods. We included 1297 subjects (mean age ± standard error 48.0 ± 0.2 years, 48% females), who participated in the Hortega Follow-Up Study. The primary endpoint was incidence of fatal and non-fatal CV events. Urinary albumin excretion (UAE) was measured in spot voided urine, frozen at –80°C, by immunonephelometry [immune-reactive albumin excretion (IRAE)] and by high-performance liquid chromatography (HPLC) [total albumin excretion (AE)]. IURAE was calculated as the difference between HPLC measurements and IRAE. We estimated fully adjusted hazard ratios (HRs) of CV incidence by Cox regression for IRAE, IURAE and total AE.

Results. After an average at-risk follow-up of 13 years, we observed 172 CV events. urinary albumin to creatinine ratio (UACR) of ≥30 mg/g assessed by IRAE, IURAE or total AE concentrations was observed in 74, 273 and 417 participants, respectively. Among discordant pairs, there were 49 events in those classified as micro- and macroalbuminuric by IRAE, but normoalbuminuric by IURAE. Only the IRAE was a significant independent factor for the incidence of CV events [HR (95% confidence interval) 1.15 (1.04–1.27)]. The association of UAE with CV events was mainly driven by heart failure (HF) [HR 1.33 (1.15–1.55) for IRAE; HR 1.38 (1.06–1.79) for IURAE; HR 1.62 (1.22–2.13) for total AE]. Those subjects who were micro- and macroalbuminuric by both IRAE and IURAE had a significant increase in risk for any CV event, and especially for HF.

Conclusions. IRAE, IURAE and AE were associated with an increased risk for CV events, but IRAE offered better prognostic assessment.

Keywords: cardiovascular events, immune-reactive albumin excretion, urinary albumin excretion

INTRODUCTION

Microalbuminuria is a well-known marker for cardiovascular (CV) and renal disease [1]. Initially tested in diabetic patients, its prognostic value in hypertensives and in different subgroups of the general population has been well established [2–13]. Microalbuminuria is routinely assessed by immunologic methods: radioimmunoassay (RIA), immunonephelometry (INF) or immune-reactive (IR) strips [immune-reactive albumin excretion (IRAE)] [14]. Conventional immunoassays, however, may underestimate albuminuria by not detecting a mildly denatured, unfragmented form of albumin, immune-unreactive to conventional antibodies or albumin aggregates [immune-unreactive albumin excretion (IURAE)] [15, 16].

Excretion of this denatured albumin has been a matter of controversy over the last decade. Based on a small number of studies, the value of IURAE in CV risk prediction beyond that of IRAE, as determined by conventional methods, is unclear. In diabetics [17], an increment in total albumin excretion (AE), measured by high-performance liquid chromatography (HPLC), has been suggested to precede the development of IRAE [18]. In stroke patients, IURAE was more tightly associated with the severity of stroke and with oxidative stress than IRAE [19]. The association of total albumin excretion with CV
risk, but not specifically of IURAE, was tested in the Heart Outcomes Prevention Evaluation (HOPE) study in a population with high CV risk. There was a positive association for both total albumin and IRAE, but the risk was higher for urinary albumin excretion (UAE) determined by RIA than that measured by HPLC, although the threshold levels needed for microalbuminuria with each method were different [20, 21]. There was no difference between both methods regarding the predictive value for CV events [20]. The role of IURAE in predicting CV risk in the general population is unknown.

Therefore, our objective was to determine if IURAE, measured by HPLC, was associated with CV events in a representative sample of a general population from Spain. We also aimed to determine if IURAE was superior to IRAE or total AE in the prediction of CV events.

**MATERIALS AND METHODS**

The Hortega Study is a population-based, multistage complex survey of adults residing in the catchment area of the University Hospital Rio Hortega (UHRH) (Health Department of Eastern Valladolid, Spain) in 1997–2003. The objective of the study was to investigate genetic, environmental and lifestyle risk factors of chronic diseases, with particular focus on CV disease, in a representative sample of the general population. Population selection and methodology were as previously described [22, 23]. The study was approved by the local Ethics Committee and all participants gave written consent to participate. In the Hortega Follow-up Study, which began in 2015, incident health endpoints and mortality during follow-up of 1502 participants from the Hortega Phase III Study were assessed by reviewing electronic health records, including primary care, hospital and mortality registries, in collaboration with physicians and epidemiologists from UHRH and the Research Institute of the Clinical Hospital of Valencia (INCLIVA). Prediction models based on electronic health registries (EHR), rather than on relatively small selected study cohorts, are promising tools for improved clinical decision-making [24]. These data will be prospectively updated throughout follow-up of health status on the electronic health registries.

In this study, we only included those individuals with no previous history of stroke, coronary heart disease (CHD) and heart failure (HF) at the beginning of the follow-up period.

**Procedures**

Demographic data were collected, along with anthropometric parameters [body weight (kg), height (cm) and abdominal circumference (cm)]. Information on CV risk factors and the presence of established CV diseases were also recorded. A non-fasting blood sample was obtained for measuring plasma glucose and creatinine levels, as well as lipid profiles. If the glucose levels were over the normal range (≥140 mg/dL), a second sample under fasting conditions was obtained to assess if the patient was diabetic or not.

Systolic (SBP) and diastolic (DBP) blood pressures (BPs) were measured in the sitting position using an OMRON® Hem-711C automated device (OMRON Healthcare, Inc. 300 Lakeview ParkwayVernon Hills, Illinois, USA). Two BP recordings, at least 3 min apart, were taken. The average of both values was recorded as the true BP of the patient. More readings were taken if there were discrepancies of >5 mmHg between the first two measurements. The presence of hypertension (HTN) was further confirmed by using 24-h ambulatory blood pressure monitoring in those subjects with elevated BP. Subjects were divided into three groups, according to the BP results and the presence or absence of a prior diagnosis of HTN: (i) normotensive subjects (BP not elevated and no prior diagnosis of HTN); (ii) known hypertensive subjects (subjects with a prior diagnosis of HTN or under antihypertensive treatment, irrespective of whether their BP was controlled or not); and (iii) patients with elevated BP, but no prior diagnosis of HTN. Subjects with BP values of <140/90 mmHg were considered normotensive. Likewise, subjects were considered as having diabetes if they had a record of type 2 diabetes diagnosis or use of antidiabetic medications in their clinical history, if fasting plasma glucose levels were ≥126 mg/dL or if HbA1c was ≥6.5%. Smoking status was recorded as current, previous or never.

**UAE**

UAE, corrected for creatinine excretion [albumin:creatinine ratio (ACR) (mg/g)], was measured in spot first-morning void urine samples, stored at –80°C, by INF (Behring Nephelometer, USA) and HPLC (Accumin® HPLC Assay, AusAm BiotechnologiesT, New York, NY, USA). IURAE was assessed as the difference between values measured by HPLC, which measures total UAE, and values measured by INF (IRAE). A subject was considered normoalbuminuric if his/her urinary albumin levels were <30 mg/g creatinine, microalbuminuric if these values were ≥30 and <300 mg/g and macroalbuminuric if these levels were ≥300 mg/g.

**CV endpoints**

The primary study outcome was CV incidence, including both fatal and non-fatal events. Only patients with no CV disease at the initial time of follow-up were eligible. For those individuals with more than one CV event, only the first event was considered in the Cox’s regression analysis. Detailed definitions of fatal and non-fatal CV events were in accordance with the 2014 guidelines by the American College of Cardiology and American Heart Association Task Force of Clinical Data Standards Committee [25]. Incident CHD was defined as the first occurrence of definite fatal myocardial infarction, sudden death due to CHD, non-fatal myocardial infarction or definite non-fatal CHD. Incident cases of stroke were defined as the first occurrence of definite or possibly fatal or definite non-fatal stroke. The composite endpoint of all CV diseases was defined as the occurrence of CV death or definite non-fatal CHD, stroke or HF. Time to event was calculated as the time interval between the date of the baseline examination and the date of the CV event or the date of death or 30 November 2015 (date of administrative censoring), whichever occurred first. Records were reviewed by a committee composed of two physician reviewers who assigned incident events.
**Statistical analysis**

The descriptive statistics and most of the statistical analysis, including the Cox’s proportional hazards model, were performed by taking into account the survey design characteristics. Quantitative variables were expressed as the mean ± standard error whereas qualitative variables were expressed as proportions. The pooled cohort risk assessment equation variables [age, sex, SBP, type 2 diabetes, current smoking, total cholesterol, high-density lipoprotein (HDL) cholesterol and anti-HTN treatment] [26] were used to adjust the Cox’s regression models, although different models were considered according to the significance of the variables within the models. C-statistic, integrated discrimination improvement (IDI) index and the category-less net reclassification improvement (NRI) index were used to compare different risk prediction models after inclusion of the different types of albuminuria to the pooled cohort risk assessment equation and in addition to the standard determination of IRAE. Censoring was adjusted by the inverse probability censoring weight. Proportional hazards models were used as working models.

Before their inclusion into the Cox’s regression model, IURAE, IRAE and total AE were logarithmically transformed using base 2 logarithms due to the non-normal distribution. The different categories of albuminuria using IURAE and IRAE were combined to study the prognostic value of the combination and to determine the concordance and discordance pairs for the categories of microalbuminuria. Pearson’s coefficient was used to assess the linear correlation between IURAE, IRAE and total AE, after excluding those individuals with proteinuria (≥500 mg/g of creatinine). Bland–Altman plots were used to analyse the agreement between these variables. All statistical analyses were carried out using StataMP 14 (StataCorp 4905 Lakeway Drive, College Station, TX, USA), and IDI and NRI analyses were carried out using the package ‘survIDINRI’ [27, 28] in R v. 3.3.3.

**RESULTS**

**Cohort description**

The sample size included 1297 subjects [mean age 48.0 ± 0.2 years, 48% females, mean body mass index (BMI) 26 ± 0.1 kg/m²], with no history of CV events at initial assessment. The main characteristics of the study population are shown in Table 1. After an average at-risk follow-up of 13 years (15 637 person-years of follow-up), there were 172 CV incidence events in 144 individuals: 63 cases of stroke (eight fatal stroke events), 51 cases of CHD (four fatal CHD events) and 58 cases of HF (seven fatal HF hospitalization events). In total, 118 subjects had only one CV event, whereas 24 subjects had two CV events and two subjects had three different CV events.

**Relationship among different UAE variables**

With regard to total AE, approximately three quarters corresponded to IURAE and one quarter to IRAE. As expected, there were significant correlations between IRAE and total AE and between IURAE and total AE (r = 0.61 and 0.91 for IRAE and IURAE, respectively, P < 0.001) and between IRAE and IURAE (r = 0.55, P < 0.001). The Bland–Altman plot for IRAE and IURAE, after logarithmic transformation, is shown in Figure 1.

The number of individuals with an UAE within the microalbuminuria range were 74, 273 and 417 participants for IRAE, IURAE or total AE, respectively. There were 807 subjects who were normoalbuminuric by both IRAE and IURAE; 387 subjects who were micro- or macroalbuminuric by IRAE, but normoalbuminuric by IURAE; 18 subjects who were micro- or macroalbuminuric by IRAE, but normoalbuminuric by IURAE; and 85 individuals who were micro- or macroalbuminuric by both IRAE and IURAE. The number and percentage of events for each one of these categories are shown in Figure 2.
Association of IRAE, IURAE and AE as quantitative trait with CV events

The prognostic value of IRAE, IURAE and AE in predicting the risk of developing CV disease was determined using UAE as a quantitative trait in the total adjusted Cox's regression model. For the risk for total CV events, only IRAE, and not IURAE or total AE, was significantly associated [hazard ratio (HR) (95% confidence interval) 1.15 (1.04–1.27), P = 0.006]. Concerning the risk for HF, both IRAE and IURAE, as well as total AE, were significantly associated [HR 1.33 (1.15–1.55), P < 0.001 for IRAE; HR 1.38 (1.06–1.79), P = 0.013 for IURAE; HR 1.62 (1.22–2.13), P = 0.001 for total AE]. In contrast, there was no association with the risk for CHD, stroke or total mortality.

We also performed separate analyses for total CV events in hypertensives (n = 519). As expected, a higher number of CV events occurred in hypertensives and diabetics, compared with normotensives and non-diabetics (21.2% of hypertensives versus 4.4% of normotensives; 32.3% of diabetics versus 9.5% of non-diabetics). A significant association was obtained only in hypertensives, in terms of all the quantitative variables of albuminuria, i.e. IRAE, IURAE and total AE [HR 1.15 (1.03–1.28), P = 0.012 for IRAE; HR 1.19 (1.00–1.43), P = 0.048 for IURAE; HR 1.23 (1.02–1.48), P = 0.024 for total AE]. Although there was a trend towards significance for all the UAE variables in diabetics, only IRAE in non-diabetics reached statistical significance [HR 1.16 (1.02–1.32), P = 0.024].

Association of IRAE, IURAE and AE as qualitative trait with CV events

The prognostic value of IRAE, IURAE and AE in predicting the risk of developing CV disease was analysed using UAE as a qualitative trait (normo, micro- and macroalbuminurics). For total CV events, the survival curves (Figure 3) clearly showed an increase in risk according to the categories of UAE for IRAE, IURAE and AE (log rank <0.01), but only macroalbuminuria, and not microalbuminuria, was significantly associated in the fully adjusted Cox's regression model [HR 3.36 (1.53–7.40), P < 0.01 for IRAE; HR 1.96 (1.19–3.21), P < 0.01 for IURAE; HR 3.88 (1.52–9.90), P < 0.01 for total AE]. However, for HF, both micro- and macroalbuminuria, as determined by IRAE, IURAE and total AE, were highly significant in the fully adjusted Cox's regression model, with an increase in risk from micro- to macroalbuminuria [HR 2.98 (1.34–6.64), P < 0.01 and HR 6.0 (1.70–21.2), P < 0.01 for micro- and macroalbuminuria, respectively, by IRAE; HR 2.15 (1.08–4.30), P < 0.05 and HR 3.23 (1.54–6.80), P < 0.01 for micro- and macroalbuminuria,
respectively, by IURAE; HR 2.20 (1.17–4.15), P < 0.05 and HR 8.22 (1.71–39.35), P < 0.01 for micro- and macroalbuminuria, respectively, by total AE]. The survival curves in the case of HF are shown in Figure 4.

The presence of macroalbuminuria, but not microalbuminuria, as determined by whichever method, was associated with the risk for stroke and total mortality (data not shown). In the case of coronary events, only macroalbuminuria by IRAE reached statistical significance [HR 3.70 (1.01–13.48), P < 0.05], with the survival curves significantly different only in the case of IRAE and a decrease in survival across all categories of UAE.

Similar to using albuminuria as a quantitative trait, an association was only obtained in the hypertensive group, and not in the normotensive group. IRAE, IURAE and total AE were significantly associated with total CV events with HR of 1.7, 1.4 and 1.5, respectively. In the case of type 2 diabetes, there was a significant association only for IRAE in non-diabetics [HR 2.0 (1.3–3.0), P = 0.002].

We used different statistics to assess the predictive value, at 10 years, of the different AE measurements, in addition to the pooled cohort risk assessment equation, as well as the predictive value of adding IURAE or total AE to the fully adjusted model including the IRAE. The C-statistic for all the models was found to be similar at around 0.85, with delta for the C-statistic, the continuous NRI and the IDI being low (Supplementary data, Table S1).

Concordance analysis between IRAE and IURAE

In order to clarify if IURAE could add valuable information to the commonly used IRAE, we analysed the relationship between concordance or discordance pairs and CV events. These results are shown in Figure 5. According to this analysis, subjects who were micro- or macroalbuminuric by both IRAE and IURAE had a significant increase in risk for any CV events, particularly so for HF and less so for the combined endpoint [HR 2.18 (1.16–4.06), P = 0.014 for total CV events; HR 5.44 (2.32–12.77), P < 0.001 for HF]. In subjects who were micro-albuminuric by IURAE and normoalbuminuric by IRAE, the risk for CV events was similar to that for normoalbuminuric subjects by both methods. The HR for subjects who were microalbuminuric only by IRAE, but not by IURAE, was 1.00 [(0.22–4.52), P = 0.99], although there were only 22 subjects in this particular subgroup. Similar results were obtained if we considered the concordance for total AE and IRAE, with those individuals with micro- and macroalbuminuria by both total AE and IRAE being at higher risk for total CV events and HF, compared with those with normoalbuminuria by both total AE and IRAE and those with microalbuminuria by total AE only [HR 1.81 (0.95–3.44), P = 0.069 for total CV events; HR 5.09 (2.17–11.84), P < 0.001 for HF].

Renal function and albuminuria during follow-up

During follow-up, no differences were observed by subgroups of IURAE or AE in the risk for developing microalbuminuria by IRAE, although there was a trend towards developing more microalbuminuria by IRAE in those with microalbuminuria by IURAE at baseline (2% versus 4.3%, P = 0.098). A total of 116 patients developed chronic kidney...
FIGURE 5: Survival curves for the Cox proportional hazards regression, with covariates at their mean, for categories of concordance for normo-, micro- and macroalbuminuria between IRAE and IURAE and total CV events, stroke, HF and CHD. Micro- and macroalbuminuria only by IRAE are not shown due to the small number of subjects within that category. The log-rank test was highly significant for CV events and HF ($P < 0.001$), non-significant for stroke and close to significance for CHD ($P = 0.09$).
Urinary immune-unreactive albumin and cardiovascular events

Disease, defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min, and the percentage of patients with new eGFR of <60 mL/min was higher in the IRAE microalbuminuric group, as compared with the normoalbuminuric group (13.7% versus 8.9%, P = 0.391).

DISCUSSION

The present study was performed in a representative sample of a general population with low CV risk and no previous history of CV events. The results support the role of UAE as a prognostic factor for CV events, not only in selected populations with CV risk factors, but also in the general population. The strongest prognostic value was in the risk for HF, with average prognostic value in the risk for CHD and minimal value in the risk for stroke. Total AE and IURAE were significant risk markers, although not superior to IRAE.

Albumin is filtered by the kidneys and is biochemically modified by lysosomal enzymes, upon uptake into tubular cells which then is mediated by cubulin and megalin, resulting in the excretion of 1% of intact albumin and 99% of small-sized albumin-derived fragments (<10 kDa) [15, 16]. Conventional methods are only able to detect IR albumin, polymer albumin aggregates and fragments of albumin of >12 kDa. Thus, a large proportion of albumin is not currently detectable by these methods [29]. Albumin, or even intact albumin, can therefore appear in the urine in different forms, as IR or immune-unreactive albumin and as albumin-forming monomers or dimers [30]. The exact underlying mechanism of IURAE is not known but may involve a conformational change of the albumin epitope as a result of incomplete activation of UAE. To the best of our knowledge, there is no other protein that is not currently detectable by these methods [29]. Albumin determinations are quite small [17]. However, potential contaminants, which could interfere with the albumin determination, are quite small [17].

In the present study, both IRAE and IURAE, and therefore total AE as determined by HPLC, were significant predictors of CV events. In the HOPE trial [20], the adjusted risk for the ACR with a threshold of 29 mg/g was 1.52 by HPLC and 1.81 by RIA. In our study, the risk associated with base 2 logarithmic transformations in the fully adjusted model was 1.15 for IRAE and the same for HPLC. These differences can be explained, at least in part, by differences in baseline population characteristics, different composite endpoints and the inclusion of different co-variables into the Cox’s regression model. As observed in the HOPE and PREVEND studies [20, 21], the C-statistic did not vary significantly regarding the type of albuminuria in the unadjusted or adjusted model. It has been suggested that albuminuria, as determined by HPLC, could provide additional information on mortality prediction, compared with IRAE [33]. In our study, this has also been shown with the survival curves for CV mortality, as well as for HF, which were more significantly different for HPLC than for IRAE or IURAE.

The fact that a proportion of UAE is not detected by conventional methods may have implications on the prognostic value of UAE. To the best of our knowledge, there is no other prospective study that has investigated the association between CV events and IRAE, IURAE and total AE individually. In this study, we also evaluated if combinations of these variables could provide additional information of prognostic value for CV events, compared with each individual variable alone. The CV events were analysed as a composite endpoint, as well as separately. However, as expected in a general population, the number of events for each category of albuminuria was small, which could have influenced the power of the study. The same issue was applied to the macro-albuminuric group, which was quite small for some analyses. We also observed that the association was particularly significant in those subjects with CV risk factors such as HTN. The apparently conflicting results that were found in diabetics could be explained by the small number of diabetics in our population. Larger studies are needed to clarify the potential usefulness of different AE measurements in clinical practice. At the present time, however, the main guidelines from scientific societies recommend to measure the ACR from morning spot urine samples using conventional immunologic methods in clinical practice [34–36].

From a clinical point of view, it has been suggested that elevated UAE, determined by HPLC, can precede the development of microalbuminuria measured by conventional methods (IRAE), and therefore it could be used for early detection [18, 37]. Moreover, some studies have suggested that it could help identify more people at risk of death or CV disease than conventional methods [33, 38]. However, questions still remain to be answered. For example, do both types of albumin predict the same or different CV risk? Does IURAE always manifest before IRAE? Which type of patients benefit most from the determination of IRAE?

It is worthwhile to comment on the necessity of having different thresholds to define microalbuminuria, depending of the type of albumin, IRAE or IURAE, or the sum of both. In the Australian Diabetes, Obesity, and Lifestyle Cohort (AusDiab) Study [39], also conducted in a general population, the prevalence of microalbuminuria by HPLC was four times higher than that determined by INF. In our study, the prevalence of microalbuminuria by HPLC was 6-fold higher, compared with the ACR determined by INF. In contrast to the AusDiab Study [39], where large discrepancies between HPLC and the ACR were observed as the degree of albuminuria decreased, our Bland–Altman plot showed larger differences as the albuminuria level increased, and these differences were apparent even at very low levels of UAE. In agreement with the AusDiab Study [39], Wang et al. [40] found good correlation between the ACR by HPLC and by INF at levels of albuminuria of >100 mg/g de creatinine. Their study assessed prospectively the association of albuminuria, determined by these two methods, with renal and non-renal mortality in Australian Aborigines. Both measurements of albuminuria were strong predictors of mortality and no differences between the methods were found as continuous variable or in an age-sex tertiles analysis [40]. Besides, this study further supports what was previously commented on the optimal thresholds to define risk according to the type of
albuminuria and reflects the difficulties of finding differences with highly correlated variables.

In the case of IURAE and IRAE, using the same threshold as that for total albumin determined by HPLC is likely not appropriate, as previously discussed by others [16]. Since this issue has not been clarified yet, we used the standard threshold to study the difference among methods regarding CV risk prediction. However, it has been shown that the CV risk increases even at what is considered to be normal levels of IRAE in diabetic and non-diabetic subjects [9]. In our study, the threshold to detect individuals who were microalbuminuric by IURAE, but normoalbuminuric by IRAE, could be as low as 8.9 mg/g, which is the average of the ACR in those classified as microalbuminuric by IURAE. It has also been suggested that the relationship with CV events could be stronger for microalbuminuria, whereas macroalbuminuria could be more tightly associated with renal endpoints [41, 42]. According to our data, in general, the risk for CV disease increases from micro- to macroalbuminuria, as expected for a continuous variable. The exact mechanisms underlying the association between low levels of urinary albumin and CV disease are unclear. Endothelial dysfunction and an increase in von Willebrand factor levels have been postulated among potential contributors [43–45]. Also albuminuria has been consistently associated with a worse clinical risk factor profile [46–48].

Our results should be considered in light of the study strengths and limitations. The present study is population-based, being representative of the general population in Spain with a low rate of external admission. Moreover, the study was conducted over a long observational period of over 13 years of follow-up. Concerning study weaknesses, although measurements were made in the same laboratory using state-of-the-art methodology [14–18], it has been suggested that cryopreservation of samples at −80°C could lead to a decrease in urinary albumin levels. This is especially relevant for HPLC, and less so for INF [49]. This implies that the differences and IURAE levels could be even higher than observed. We did not measure IURAE directly, but rather calculated it by subtracting the IRAE from the AE determined by HPLC. The same strategy was also used in another study that included 98 patients with ischaemic stroke, which showed the IURAE was more strongly associated, compared with the IRAE, with oxidative stress and stroke severity [19]. The authors hypothesized that IURAE through an increase in endothelial dysfunction could indicate a higher CV risk, including the risk for stroke [19]. In our study, only macroalbuminuria, but not microalbuminuria, as determined by either INF or HPLC, was clearly associated with the risk for stroke. Our study has also other methodological issues such as UAE measurements only on one day, since day-to-day variability in UAE can result in possible misclassification.

In summary, the association of UAE with CV incidence was similar for IURAE, as compared to IRAE. Total albumin determined by HPLC did not add better prognostic information, compared with each of its individual components, at least concerning the commonly used thresholds for micro- and macroalbuminuria. Until larger studies are available to address the use of, and thresholds for, the IURAE, and hence for total albumin, the ACR will remain the standard approach in clinical care. Also, the presence of subtle differences in the evaluated markers suggests the need for mechanistic studies to evaluate its biological implications.

**SUPPLEMENTARY DATA**

Supplementary data are available at ndt online.

**FUNDING**

The study was undertaken with grants PI11/00726, PI12/02615, PI14/00031, PI14/00874 and PI16/01402 of the Institute Carlos III of Madrid provided from the FEDER funds.

**CONFLICT OF INTEREST STATEMENT**

None declared. The material has not been previously published and is not under consideration for publication elsewhere, and all authors approve of the material submitted for publication.

**REFERENCES**


F. Martinez et al.

Received: 26.11.2017; Editorial decision: 3.3.2018