Microalbuminuria and cardiovascular autonomic dysfunction are independently associated with cardiovascular mortality: evidence for distinct pathways. The Hoorn Study.

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Objective – Microalbuminuria is associated with cardiovascular mortality, particularly among individuals with type 2 diabetes, but the mechanisms underlying this association are not completely understood. Microalbuminuria is known to be associated with cardiovascular autonomic dysfunction (C-AD), and C-AD in turn is associated with cardiovascular mortality. The purpose of this study therefore was to investigate whether C-AD can explain the relationship between microalbuminuria and cardiovascular mortality.

Research design and methods – We studied 490 individuals from a population–based cohort of individuals aged 50-75 years who were followed for a median period of 13.6 years. Microalbuminuria was defined as an albumin-to-creatinine ratio ≥2.0 mg/mmol in an early morning spot urine sample. Ten parameters reflecting different aspects of cardiovascular autonomic function were measured, and compiled into a total score of C-AD (mean of separate z-scores). The association between C-AD and microalbuminuria was estimated by multiple linear regression and relative risks (RR) for cardiovascular mortality were estimated by Cox proportional hazard analyses.

Results – After adjustments for age, sex, glucose tolerance status and other risk factors, C-AD was associated with microalbuminuria (β=0.16, 95%CI: -0.01; 0.33), and both microalbuminuria [RR=2.09 (1.07-4.08)] and C-AD [RR=1.74 (1.04-2.89)] were associated with cardiovascular mortality. These associations did not change after further mutual adjustment for C-AD [RR=2.13 (1.09-4.17)] or microalbuminuria [RR=1.76 (1.05-2.94)], respectively.

Conclusions – Both microalbuminuria and C-AD are independently associated with cardiovascular mortality, and the excess mortality attributable to microalbuminuria cannot be explained by C-AD.
**Microalbuminuria** is associated with an increased risk of cardiovascular disease and mortality (1). This association is independent of other known cardiovascular risk factors such as hypertension, dyslipidaemia, obesity, smoking, and impaired renal function (1,2). Several mechanisms, notably endothelial dysfunction and low-grade inflammation, have been proposed to explain, at least in part, the increased risk of cardiovascular mortality in individuals with microalbuminuria (3). Cardiovascular autonomic dysfunction (C-AD) could potentially constitute another such mechanism.

Indeed, we, as others, have previously shown that C-AD is associated with microalbuminuria, especially in individuals with impaired glucose metabolism (IGM) and type 2 diabetes (DM2) (4-6). Two proposed mechanisms explaining this association are, firstly, a disturbance in glomerular arteriolar autoregulation, which in turn may result in an inability to counteract glomerular hypertension (7), and, secondly, a reduced drop in nightly blood pressure due to C-AD, both of which may result in microalbuminuria (8). In addition, C-AD is associated with cardiovascular mortality (9,10) and can potentially link microalbuminuria to cardiovascular mortality by arrhythmogenic or atherogenic effects, for example by promoting vascular calcification and arterial stiffness (11).

In view of these considerations, we investigated, in a prospective cohort study, whether C-AD can explain the relationship between microalbuminuria and cardiovascular mortality or, alternatively, whether both microalbuminuria and C-AD are independently associated with cardiovascular mortality. These hypotheses have never been investigated in the general population and could have clinical relevance as the first hypothesis suggests that C-AD should be targeted to decrease mortality risk in individuals with microalbuminuria. On the other hand, the second hypothesis suggests that both microalbuminuria and C-AD can be used for estimating risk of cardiovascular mortality.

**RESEARCH DESIGN AND METHODS**

**Subjects:** We used data from the Hoorn Study, a population-based cohort study on glucose metabolism and other cardiovascular risk factors in a general Caucasian population, which has been described in detail previously (12). Briefly, men and women aged 50-75 years were randomly selected from the population register of the town of Hoorn, the Netherlands; 2484 subjects participated (response rate 71%). Baseline examinations were conducted from October 1989 until February 1992. All subjects had a 75-g oral glucose tolerance test, except those previously diagnosed with DM2. An extensive metabolic and cardiovascular investigation was performed in an age-, sex- and glucose tolerance-stratified, random sub-sample of 631 participants (89% of those invited), which is used in the present study (12).

Participants without representative urine samples available (n=45) and/or who did not complete at least seven out of the ten autonomic function tests (n=31), had a history of neurological disease (n=5), were using ACE-inhibitors (n=33) and/or drugs known to influence autonomic nerve function (namely anti-parkinson drugs, phenytoin, antihistamines, parasympatholytic, parasympathomimetic and sympathomimetic drugs) (n=49) were excluded from the analyses. The present study therefore consisted of 490 individuals: 305 with normal glucose metabolism, 71 with IGM (including those with impaired fasting glucose and/or impaired glucose tolerance) and 114 with DM2.
The Hoorn study was approved by the Ethical Review Committee of VU University Medical Centre, Amsterdam, the Netherlands. Informed consent was obtained from all participants.

**Baseline measurements:** Urinary albumin concentration was measured in a first voided sample by rate nephelometry (Array protein system, Beckman, Ireland) with a detection threshold of 6.2 mg/l (intra- and inter-assay coefficients of variation of 5% and 8%, respectively). Urinary creatinine was measured with a modified Jaffé method. Microalbuminuria was present if the albumin-to-creatinine ratio (ACR) was in the range of 2.0-30 mg/mmol (5). The average value of the ACR was used if two representative samples (n=154) were available. Excluding subjects with an ACR >30 mg/mmol (i.e. macroalbuminuria, n=4) did not materially affect any of the results, which are therefore presented for the entire group.

Cardiovascular autonomic function tests were performed as described in detail elsewhere (9). Briefly, participants were asked to refrain from smoking and drinking coffee for at least two hours prior to the assessments. A light meal >1 hour before the measurements was allowed. Tests took place between 8:30 AM and 4:00 PM in a quiet ambience with room temperature of 19-22 ºC after a resting period of at least 10 minutes. Ten parameters of cardiac autonomic function were derived from the R-R interval and finger systolic blood pressure (SBP) continuous recordings by a computerized data analyses system developed locally by the Department of Medical Physics, VU University, Amsterdam, the Netherlands (13). Tests were performed under three conditions: during spontaneous breathing over 3 minutes in the supine position (MeanNN, SDNN, LF-power, HF-power, LF/(LF+HF)), during six deep breaths over 1 minute in the supine position (EI-difference, BRS) and during an active change from lying-to-standing (SBP-difference, RRmax, RRmax/min) (details provided in Online Appendix, Table A1 at http://care.diabetesjournals.org). We thus obtained results in four tests which reflect heart rate or blood pressure changes due to certain ‘maneuvers’, in this case deep breathing or standing up (i.e. EI-difference, RRmax, RRmax/min, SBP-difference) (14), five tests of spectral analyses of heart rate variability (HRV) (i.e. MeanNN, SDNN, LF-power, HF-power, LF/(LF+HF)) (15), and one baroreflex sensitivity (BRS) measurement (16). Additionally, these tests were grouped into those representing predominantly parasympathetic (i.e. EI-difference, RR-max, HF-power), sympathetic (i.e. SBP-difference) or both functions (i.e. Mean-NN, SDNN, LF-power, LF/(LF+HF), BRS, RRmax/min) (15,16).

Body mass index, waist-to-hip ratio, systolic (SBP) and diastolic blood pressure (DBP), levels of fasting plasma glucose, HbA1c, insulin, total- HDL- and LDL-cholesterol, triglycerides, creatinine and smoking status were measured as described elsewhere (5,9,12). Glomerular filtration rate (eGFR) was estimated by the short Modification of Diet in Renal Disease equation. Hypertension was defined as SBP≥140 and/or DBP≥90 mmHg and/or the use of anti-hypertensive drugs. Prior CVD was defined when individuals had (any of the following): a history of myocardial infarction, abnormalities on a resting ECG (Minnesota codes 1.1-1.3, 4.1-4.3, 5.1-5.3 or 7.1), undergone coronary bypass surgery or angioplasty, peripheral arterial bypass or non-traumatic amputation, and an ankle-brachial index of <0.9 in either leg.

**Follow-up:** Data on the participants’ vital status up to January 1st 2005, were collected from the mortality register of the municipality of Hoorn. Information on cause of death was extracted from the medical records of the general practitioners and the local hospital and coded according to the
International Classification of Diseases, Injuries and Causes of Death (ICD-9). Cardiovascular mortality, including sudden death, was defined by the ICD-9 codes 390-459 and 798. Information on cause of death could not be obtained for 20 of the deceased individuals. All subjects were followed until death or end of follow-up, at which time they were censored.

**Statistical analyses:** Baseline characteristics between survivors and non-survivors were compared with the use of Student’s t- or Chi-squared tests. We computed z-scores [(individual’s observed value – population mean)/SD] for each of the ten measurements of C-AD. All z-scores (except SBP-difference) were inverted and averaged into a total score (‘C-AD total score’), so that higher values reflect greater C-AD.

The association between microalbuminuria and C-AD total score was investigated with multiple linear regression analyses. Kaplan-Meier survival curves were plotted for cardiovascular mortality among individuals with normo- vs. microalbuminuria, and similarly across tertiles of the C-AD total score, and differences between groups were tested with a log-rank test. Cox-proportional hazard regression models were used to calculate the crude and adjusted relative risks (RRs) and respective 95%CI of microalbuminuria (vs. normoalbuminuria) and of the C-AD total score [per standard deviation (=0.605) increase] for cardiovascular and all-cause mortality. A two-sided p-value of <0.05 was considered statistically significant. All analyses were performed with SPSS, version 15.0.

**RESULTS**

Subjects excluded from the analyses (n=141) were older, more often had diabetes and had a worse risk profile than the ones included (n=490) (data not shown). Median duration of the follow-up was 13.6 (range 0.52-15.19) years. During the follow-up period, 141 (28.8%) participants died of whom 53 (37.6%) of CVD.

At baseline, individuals who died more often had microalbuminuria and had a worse C-AD total score, were older, more often were men and had DM2 and hypertension, and had a higher HbA1c and WHR as compared to the survivors (Table 1).

**Association between microalbuminuria and C-AD:** C-AD total score was associated with microalbuminuria [β=0.33 (95%CI: 0.16; 0.51)] (Table 2, model 1). After adjustment for potential confounding factors, C-AD total score was still borderline associated with microalbuminuria [β=0.16 (-0.01; 0.33)] (model 3). Comparable results were obtained when tests were combined on the basis of their methodology or on the part of the autonomic nervous system they predominately represent. All individual C-AD tests, except LF/(LF+HF), were positively associated with microalbuminuria (though not all statistically significantly so) (data not shown).

**Association of microalbuminuria and C-AD total score with cardiovascular and all-cause mortality:** Both microalbuminuria [RR=3.49 (95%CI: 1.87-6.53)] and C-AD total score [RR=2.54 (1.60-4.04)] were associated with cardiovascular mortality (Table 3; model 1, Figure 1). These associations were attenuated after adjustment for age, sex and GTS: RR=2.23 (1.16-4.29) and RR=1.81 (1.11-2.94), respectively (model 2). Further adjustment for other cardiovascular risk factors only slightly further attenuated these RRs (model 3) and when the C-AD total score or microalbuminuria were added to these models, the RR attributable to microalbuminuria remained practically unchanged and both microalbuminuria [RR=2.13 (1.09-4.17)] and C-AD total score [RR=1.76 (1.05-2.94)] were independently
associated with cardiovascular mortality (model 4).

Both microalbuminuria and C-AD total score were also associated with all-cause mortality (model 1). However, after adjustment for age, sex, GTS and other cardiovascular risk factors, microalbuminuria [RR=1.33 (0.83-2.13)], in contrast to C-AD total score [RR=1.52 (1.11-2.09)], was not independently associated with all-cause mortality (model 3).

**Additional analyses:** The associations described above were also investigated by examining autonomic function tests combined on the basis of their methodology or on the part of the autonomic nervous system they predominantly represent (Online Appendix, Table A2). These additional analyses showed that: overall, all different groups of tests were associated with increased mortality risk; these associations were attenuated when further adjusted for age, sex, GTS and other risk factors; and that none of the specific group of tests explained the association between microalbuminuria and mortality.

The associations reported in Table 3 did not materially change after additional adjustment for use of lipid- or blood pressure-lowering drugs and the presence of leukocytes in urine (tested by microscopy and scored as positive whenever >5 leukocytes per high power field), or when the dichotomous variable hypertension used in those models was replaced by either SBP or pulse pressure (a marker of arterial stiffness), or when analyses were adjusted for prior coronary heart disease instead of prior CVD (data not shown).

Finally, we found no evidence of effect modification by GTS in the association between cardiovascular mortality and C-AD total score or microalbuminuria (p-value for interaction >0.4 in both cases).

**CONCLUSIONS**

In this prospective study we found that both microalbuminuria and C-AD (estimated from the mean of 10 standardized tests) were independently associated with cardiovascular mortality in a cohort of elderly, Caucasian subjects with and without DM2. Therefore, C-AD does not explain why microalbuminuria is related to cardiovascular mortality in either the general population or in DM2 subjects.

Recently, HRV was found not to predict the decline of GFR but was independently associated with cardiovascular mortality in middle-aged type 1 diabetic subjects with overt nephropathy (17). In another study, comparing the independent predictive role of GFR, microalbuminuria and cardiovascular autonomic neuropathy, showed the latter to also be associated with all-cause and cardiovascular mortality in subjects with DM2 (18). These findings are in line with the ones reported herein. Hitherto, no other prospective studies examining the relation of microalbuminuria and C-AD with cardiovascular mortality have been reported. The independent association of both microalbuminuria and C-AD with cardiovascular mortality reported herein indicates that they are linked to cardiovascular mortality by different biological pathways. Several mechanisms linking C-AD to cardiovascular mortality have been proposed (e.g. QT-interval prolongation and silent myocardial ischemia) (19). Microalbuminuria may be linked to CVD by a common pathophysiological process (such as endothelial dysfunction or chronic low-grade inflammation) (3). However, the possibility that microalbuminuria reflects the presence of a set of undiscovered factors that are causally related to CVD remains to be further elucidated. Our study shows that C-AD is an unlikely candidate in this respect.

Microalbuminuria is an established risk indicator for CVD. Furthermore, aggressive
treatment of microalbuminuria by inhibiting the renin-angiotensin-aldosterone system has been shown to decrease renal and cardiovascular risk in individuals with DM2 (20). There is some evidence that C-AD is also a useful risk indicator for CVD, especially in subjects at high risk (diabetes, hypertension, or prior CVD) (9,21) and this study reinforces this observation. However, whether the improvement of C-AD, e.g. with exercise training or blood pressure or lipid-lowering drugs) (22-24), leads to a better prognosis is unknown. In our study, additional adjustment for use of beta blockers or lipid lowering drugs had no effect on the associations reported. Therefore the clinical relevance of treating C-AD in the general population remains unclear and needs to be further examined.

The strengths of our study are the relatively large, truly population-based and prospective design with a long follow-up period. Additionally, we characterized subject’s C-AD as comprehensively as possible by conducting ten autonomic function tests, which are thought to reflect all aspects of cardiovascular autonomic function. Previous, cross-sectional, studies evaluating the relationship between microalbuminuria and C-AD, employed less extensive autonomic function tests (4,6). Because we included all three groups of GTS, we were able to analyze (but found none) possible effect modification of GTS in the relationship between the main determinants, C-AD and microalbuminuria, and cardiovascular mortality.

There are several limitations to our study. Firstly, each of the test parameters of autonomic function were measured with moderate levels of reproducibility (reliability coefficient around 50%) (13). Therefore, the results were combined into a ‘C-AD total score’, which enabled us to circumvent, at least partially, this problem. Specifically, the results hereby reported with the C-AD total score were more powerful, than when obtained on the basis of each individual test (separately), because these results would be more strongly affected by misclassification (likely to be random) resulting in an underestimation of the strength of the associations. Indeed, the associations between each individual test result with cardiovascular mortality were in accordance, though with lower strength, with those as reported herein with the C-AD total score (data not shown). In addition, this approach had the advantage of circumventing the problem of multiple testing (Type I errors) that would have occurred when analyzing the associations with each test separately. However, because each test may represent different parts of autonomic function, some overlapping but others complementary, our C-AD total score does not allow the distinction of potential different contributions of sympathetic and parasympathetic functions to cardiovascular mortality. Therefore, we examined the autonomic function tests combined on the basis of their methodology or on the part of the autonomic nervous system they predominantly represent in the additional analyses. The results of these alternative combinations of autonomic function tests were comparable to those of the C-AD total score (Online Appendix, Table A2), but we acknowledge that these analyses may have been not specific enough, as most authors agree that a clear distinction between parasympathetic or sympathetic dysfunction cannot be made on the basis of these tests. Altogether, we feel that the advantages (i.e. reduced misclassification and avoidance of type I errors without impairment of etiological validity) of the approach used by us outweigh its disadvantages.

Secondly, the spectral analyses were performed during 3 minutes. Measuring HRV during a longer period may be more reliable, although measurements obtained in time periods as short as 2 minutes correlate highly
with 24h measurements (25). Thirdly, evaluation of subjects’ C-AD was conducted at baseline only, and therefore we were not able to evaluate deterioration of autonomic function during follow-up. Fourthly, for most participants microalbuminuria was defined on an ACR measured in one urine sample only. As microalbuminuria is quite variable from day-to day we cannot fully exclude the possibility that some subjects were misclassified. However, we tried to avoid this by collecting overnight urine and expressing albumin excretion as ACR. In addition, analyses in the 154 participants of whom two samples were available yielded essentially similar results (data not shown). Finally, we studied an elderly, Caucasian population and it is unknown if our results can be generalized to other ethnic groups or to younger individuals.

In conclusion, we have shown that both microalbuminuria and C-AD are independently associated with cardiovascular mortality in an elderly, Caucasian population of individuals with NGM, IGM and DM2. These results suggest that microalbuminuria and C-AD are related to cardiovascular mortality by different biological pathways. Therefore it might be useful to treat not only microalbuminuria but also C-AD in populations at high risk of cardiovascular mortality.

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Table 1 - Baseline characteristics of survivors vs. non survivors

| Characteristics                        | Survivors (n=349) | Non-survivors (n=141) | All-cause mortality (n=141) | Cardiovascular mortality (n=53) |
|----------------------------------------|-------------------|-----------------------|----------------------------|--------------------------------|
| Age (years)                            | 62.4 (6.8)        | 67.1(6.4)*            | 67.7(6.7)*                 |                                 |
| Male sex (%)                           | 43.6              | 53.9*                 | 50.9                       |                                 |
| Glucose tolerance status (%)           |                   |                       |                            |                                 |
| NGM                                    | 66.5              | 51.8                  |                           |                                 |
| IGM                                    | 14.0              | 15.6                  |                           |                                 |
| DM                                     | 19.5              | 32.6*                 | 39.6*                      |                                 |
| HbA1c (%)                              | 5.7 (1.1)         | 6.2 (1.4)*            | 6.4 (1.6)*                 |                                 |
| Systolic blood pressure (mmHg)         | 136.2 (18.5)      | 142.8 (19.6)*         | 147.2 (21.6)*              |                                 |
| Diastolic blood pressure (mmHg)        | 82.0 (9.3)        | 83.3 (11.2)           | 84.4 (10.8)                |                                 |
| Use of blood-pressure-lowering drugs (%)| 20.9              | 31.9*                 | 32.1*                      |                                 |
| Hypertension (%)                       | 46.4              | 65.2*                 | 69.8*                      |                                 |
| History of CVD (%)                     | 19.8              | 27.0                  | 30.2                       |                                 |
| (Ever) smokers (%)                     | 59.6              | 71.6*                 | 73.6                       |                                 |
| WHR                                    | 0.90 (0.08)       | 0.94 (0.08)*          | 0.95 (0.08)*               |                                 |
| BMI (kg/cm²)                           | 26.8 (3.7)        | 27.2 (3.9)            | 27.4 (3.8)                 |                                 |
| Serum creatinine (μmol/l)              | 89.5 (16.1)       | 95.0(23.0)            | 97.6 (30.6)                |                                 |
| eGFR (ml/min)                          | 69 (11)           | 66 (13)               | 64 (15)                    |                                 |
| Cholesterol (mmol/l)                   | 6.61 (1.13)       | 6.73(1.31)            | 6.86 (1.19)                |                                 |
| HDL (mmol/l)                           | 1.31 (0.37)       | 1.26 (0.34)           | 1.23 (0.31)                |                                 |
| LDL (mmol/l)                           | 4.51 (0.99)       | 4.60 (1.13)           | 4.77 (1.05)                |                                 |
| Use of lipid-lowering drugs (%)         | 1.7               | 1.4                   | 1.9                       |                                 |
| Triglycerides (mmol/l)                 | 1.50 [1.10-2.10]  | 1.70 [1.20-2.40]      | 1.80 [1.25-2.40]           |                                 |
| EL-difference (ms)                     | 162 [107-237]     | 131 [83-213]*         | 113 [79-183]*              |                                 |
| RRmax (ms)                             | 254 (96)          | 217 (88)              | 223 (91)                   |                                 |
| RRmax/min                              | 1.27 (0.16)       | 1.19(0.14)            | 1.21(0.13)                 |                                 |
| HF-power (ms²)                         | 183 [85-438]      | 151[67-363]           | 145 [77-330]               |                                 |
| LF-power (ms²)                         | 239 [123-525]     | 160 [68-390]*         | 152 [63-307]               |                                 |
| LF/(LF+HF)                             | 0.56 (0.19)       | 0.52(0.21)            | 0.50 (0.21)                |                                 |
| BRS (ms/mmHg)                          | 9.01 (4.96)       | 7.91 (4.20)           | 7.84 (4.14)                |                                 |
| SDNN (ms)                              | 36 (16)           | 33 (19)               | 32 (19)                    |                                 |
| Mean NN (ms)                           | 966 (147)         | 927 (156)             | 935 (157)                  |                                 |
| SBP-difference (mmHg)                  | -6.42 (14.82)     | -6.32 (15.17)         | -6.45 (16.51)              |                                 |
| C-AD total score                       | -0.08 (0.58)      | 0.22 (0.61)*          | 0.22 (0.65)*               |                                 |
| Microalbuminuria (%)                   | 7.7               | 16.3*                 | 24.5*                      |                                 |

Data are presented as frequencies (%), means (±SD) or medians [inter-quartile range (IQR)]

*p <0.05 vs. survivors; NGM: normal glucose metabolism; IGM: impaired glucose metabolism; DM: type 2 diabetes mellitus; CVD: cardiovascular disease; WHR: waist-to-hip ratio; BMI: body mass index; eGFR: estimated glomerular filtration rate; HDL: high density lipoprotein; LDL: low density lipoprotein; C-AD: cardiovascular autonomic dysfunction

For detailed explanation of autonomic function tests and their abbreviations, see Table A1.

* these include diuretics, alpha-blockers, beta-blockers, calcium channel blockers and other blood pressure lowering drugs but exclude ACE-inhibitors (because subjects using these drugs were excluded from the analyses).
Table 2 - Association between microalbuminuria and C-AD

| Dependent variable | Model | β     | 95% CI    | p-value |
|--------------------|-------|-------|-----------|---------|
| C-AD total score   | 1     | 0.33  | 0.16; 0.51| <0.001  |
|                    | 2     | 0.21  | 0.04; 0.38| 0.016   |
|                    | 3     | 0.16  | -0.01; 0.33| 0.067   |
| C-AD maneuvers      | 1     | 0.34  | 0.15; 0.53| 0.001   |
|                    | 2     | 0.17  | -0.01; 0.35| 0.061   |
|                    | 3     | 0.12  | -0.07; 0.30| 0.209   |
| C-AD BRS           | 1     | 0.40  | 0.08; 0.71| 0.014   |
|                    | 2     | 0.23  | -0.08; 0.54| 0.149   |
|                    | 3     | 0.21  | -0.11; 0.53| 0.194   |
| C-AD HRV           | 1     | 0.31  | 0.12; 0.51| 0.001   |
|                    | 2     | 0.22  | 0.03; 0.41| 0.025   |
|                    | 3     | 0.16  | -0.04; 0.35| 0.110   |
| C-AD parasympathetic | 1  | 0.45  | 0.22; 0.69| <0.001  |
|                     | 2    | 0.28  | 0.05; 0.51| 0.017   |
|                     | 3    | 0.23  | -0.01; 0.46| 0.055   |
| C-AD sympathetic   | 1     | 0.14  | -0.18; 0.44| 0.394   |
|                    | 2     | 0.13  | -0.19; 0.44| 0.433   |
|                    | 3     | 0.11  | -0.22; 0.44| 0.503   |
| C-AD both          | 1     | 0.32  | 0.14; 0.49| <0.001  |
|                    | 2     | 0.23  | 0.06; 0.41| 0.001   |
|                    | 3     | 0.18  | 0.01; 0.36| 0.041   |

β, indicates difference in C-AD score between individuals with micro- vs. normoalbuminuria;
Model 1: univariate analysis;
Model 2: adjusted for sex, age and glucose tolerance status;
Model 3: model 2 plus adjustments for hypertension, WHR, eGFR, LDL, HDL, triglycerides, smoking and prior CVD;

Table 3 - Association of microalbuminuria or C-AD with cardiovascular and all-cause mortality

| Main determinant | Model | Cardiovascular mortality | All-cause mortality |
|------------------|-------|--------------------------|---------------------|
|                  |       | RR [95% CI]              | RR [95%CI]          |
| Microalbuminuria | 1     | 3.49 [1.87-6.53]         | 2.12 [1.36-3.21]    |
|                  | 2     | 2.23 [1.16-4.29]         | 1.46 [0.92-2.31]    |
|                  | 3     | 2.09 [1.07-4.08]         | 1.32 [0.83-2.12]    |
|                  | 4     | 2.13 [1.09-4.17]         | 1.33 [0.83-2.13]    |
| C-AD total score | 1     | 2.54 [1.60-4.04]         | 2.11 [1.58-2.81]    |
|                  | 2     | 1.81 [1.11-2.94]         | 1.62 [1.20-2.20]    |
|                  | 3     | 1.74 [1.04-2.89]         | 1.52 [1.11-2.08]    |
|                  | 4     | 1.76 [1.05-2.94]         | 1.52 [1.11-2.09]    |

Model 1: univariate analysis;
Model 2: adjusted for sex, age and glucose tolerance status;
Model 3: model 2 plus adjustments for hypertension, WHR, eGFR, LDL, HDL, triglycerides, smoking and prior CVD;
Model 4: model 3, with additional adjustment for respectively C-AD or microalbuminuria;
RR: relative risk of micro- vs. normoalbuminuria, or per standard deviation (=0.605) increase in C-AD total score.
Figure 1

A. Survival curve showing a significant difference between normoalbuminuria and microalbuminuria (Log rank test: p<0.001).

B. Survival curve for different tertiles with tertile 1 as the reference (Log rank test: p=0.045).