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Midregional Fragment of Proadrenomedullin, New-Onset Albuminuria, and Cardiovascular and All-Cause Mortality in Patients With Type 2 Diabetes (ZODIAC-30)

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OBJECTIVE

The midregional fragment of proadrenomedullin (MR-proADM) is a marker of endothelial dysfunction and has been associated with a variety of diseases. Our aim was to investigate whether MR-proADM is associated with new-onset albuminuria and cardiovascular (CV) and all-cause mortality in patients with type 2 diabetes treated in primary care.

RESEARCH DESIGN AND METHODS

Patients with type 2 diabetes participating in the observational Zwolle Outpatient Diabetes Project Integrating Available Care (ZODIAC) study were included. Cox regression analyses were used to assess the relation of baseline MR-proADM with new-onset albuminuria and CV and all-cause mortality. Risk prediction capabilities of MR-proADM for new-onset albuminuria and CV and all-cause mortality were assessed with Harrell's C and the integrated discrimination improvement.

RESULTS

In 1,243 patients (mean age 67 [±12] years), the median follow-up was 5.6 years (interquartile range 3.1–10.1); 388 (31%) patients died, with 168 (12%) CV deaths. Log_2 MR-proADM was associated with CV (hazard ratio 1.96 [95% CI 1.27–3.01]) and all-cause mortality (1.78 [1.34–2.36]) after adjusting for age, sex, BMI, smoking, systolic blood pressure, cholesterol-to-HDL ratio, duration of diabetes, HbA_{1c}, ACE inhibitor/angiotensin receptor blocker, history of CV diseases, log serum creatinine, and log albumin-to-creatinine ratio. MR-proADM slightly improved mortality risk prediction. The age- and sex-adjusted, but not multivariate-adjusted, MR-proADM levels were associated with new-onset albuminuria.

CONCLUSIONS

MR-proADM was associated with CV and all-cause mortality in patients with type 2 diabetes after a median follow-up of 5.6 years. There was no independent relationship with new-onset albuminuria. In the availability of an extensive set of risk factors, there was little added effect of MR-proADM in risk prediction of CV and all-cause mortality.

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Endothelial dysfunction in patients with type 2 diabetes is associated with cardiovascular (CV) complications (1). The peptide hormone adrenomedullin (ADM) appears to have a role in the pathophysiology of endothelial dysfunction (2–4). ADM has a role as a circulating hormone as well as a paracrine regulator of cell function (5). Endothelial cells highly express the mRNA of ADM and actively synthesize and secrete ADM (6). Increased plasma levels of ADM lead to a number of physiological effects, such as induction of vasodilation and hypotension, increased glomerular filtration rate, fractional sodium excretion, and increased cardiac output (7,8). ADM could exert a protective effect against cardiac hypertrophy and fibrosis in ADM-knockout mice by attenuating remodeling (9). ADM infusion increases renin levels and decreases plasma aldosterone levels in patients with heart failure (10), and a search for drugs targeting ADM is ongoing (11–15).

The nonfunctional midregional fragment of the prohormone of ADM (MR-proADM) can be used as a surrogate marker of ADM and predicts early CV mortality (16). ADM synthesis is increased in response to vascular injury and counterbalances negative processes, and unlike ADM, MR-proADM is stable and the longer half-life results in a 1,000-fold higher concentration than ADM (4,17). Increased plasma MR-proADM levels are also associated with the development of CV morbidity and have a role in predicting adverse outcomes in patients with coronary artery disease (14,16) and heart failure (15,17) and patients presenting to the emergency department with dyspnea (18). MR-proADM may also be useful for excluding the diagnosis of left ventricular failure (19), predicting the response to antimicrobials and estimating prognosis in patients with sepsis (20,21). Few studies exist with long-term follow-up (22). In patients with type 2 diabetes, increased levels of MR-proADM have been associated with an increased risk for CV events after short-term follow-up (23).

There are no studies investigating the relationship between plasma MR-proADM levels and mortality after long-term follow-up in patients with type 2 diabetes, nor are there studies investigating the relationship with new-onset albuminuria, another marker of endothelial dysfunction. This study aimed to investigate the association with and predictive capabilities of MR-proADM and new-onset albuminuria and CV and all-cause mortality in patients with type 2 diabetes (24–26).

RESEARCH DESIGN AND METHODS

Study Group

The Zwolle Outpatient Diabetes Project Integrating Available Care (ZODIAC) study was initiated in 1998 in the Zwolle region of the Netherlands. The design and details of this study have been published previously (27). Patients with a very short life expectancy (including patients with active cancer) or insufficient cognitive abilities were excluded from participation in this study. In the first year, 1,143 patients with type 2 diabetes were included in this prospective cohort study. In 2001, 546 patients with type 2 diabetes were additionally enrolled, resulting in a combined cohort of 1,689 patients, treated exclusively in primary care (28). Of the 1,689 included patients, 1,374 samples were eligible for further analyses to measure plasma MR-proADM. Plasma MR-proADM values could be measured in 1,243 available samples. The ZODIAC study was approved by the local medical ethics committee, and all patients provided informed consent.

Data Collection and Measurements

Baseline data were collected in 1998 and 2001, consisting of a full medical history, including a history of CV diseases (CVDs), use of medication, and tobacco consumption. Patients were considered to have a history of CVD if they had a history of angina pectoris, myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, stroke, or transient ischemic attack. Laboratory and physical assessment data were collected at baseline and included nonfasting lipid profile, HbA_{1c}, serum creatinine (SCr), urinary albumin-to-creatinine ratio (ACR), and blood pressure. SCr was measured by a kinetic colorimetric Jaffe method (Modular P Analyzer; Roche, Almere, the Netherlands), ACR was measured using immunonephelometry (Behring Nephelometer; Behring, Mannheim, Germany), and blood pressure was measured twice with a Welch Allyn sphygmomanometer in supine position after at least 5 min of rest. Albuminuria was defined as an ACR $>$2.5 mg/mmol for men and $>$3.5 mg/mmol for women.

MR-proADM was measured in plasma samples collected at baseline. Plasma MR-proADM was measured using a commercial assay in the chemiluminescence/coated tube format (MR-proADM LIA; B.R.A.H.M.S GmbH, Thermo Fisher Scientific, Hennigsdorf, Germany) (17). The lower limit of detection of the assay is 0.08 nmol/L. The functional assay sensitivity (defined as the lowest concentration detectable with an interassay coefficient of variability of 20%) is 0.11 nmol/L. The intra-assay coefficients of variability at 0.5 and 5 nmol/L are 3 and 3.5%, respectively; the interassay coefficients of variability at 0.5 and 5 nmol/L are 8.5 and 6.5%. Prolonged frozen storage and repeated freeze-thaw cycles have no effect on MR-proADM values (17). MR-proADM is produced in equimolar masses (4,17). MR-proADM follows a Gaussian distribution with a mean (SD) of 0.33 nmol/L (0.07) (17). There is a trend to higher MR-proADM values in older individuals. Values are very stable during the day and not influenced by food or water intake.

Clinical End Points and Follow-up

The end points of this study were *1*) new-onset albuminuria, *2*) CV mortality, and *3*) all-cause mortality. Patients were regarded as new-onset albuminuria when they *1*) had normoalbuminuria at baseline and developed albuminuria in two consecutive follow-up years; *2*) had normoalbuminuria at baseline and showed albuminuria in at least one single follow-up year, followed by initiation of an ACE inhibitor (ACEi) or angiotensin-II antagonist treatment in the same year; and *3*) were normoalbuminuric on ACEi/angiotensin-II antagonist treatment at baseline and developed albuminuria in at least one of

the follow-up years. In 2009, vital status and cause of death were retrieved from records maintained by the hospital and the general practitioners for the first 1,143 patients. For the additional 546 patients, vital status and cause of death were retrieved in 2005. Causes of death were coded according to the ICD-9. CV death was defined as death in which the principal cause of death was CV in nature, using ICD-9 codes 390–459.

Table 1—Baseline patient characteristics of the study population (*n* = 1,243) presented as tertiles of MR-proADM concentration

	Tertile 1	Tertile 2	Tertile 3	*P* value
MR-proADM (nmol/L)	<0.425	0.425–0.526	>0.526	
n	415	413	415	
Deceased (*n*, %)	70 (17)	110 (27)	208 (50)	
Follow-up time (years)	9.8	6.0	6.1	
Age (years)	60 ± 11	68 ± 10	67 ± 10	0.00
Smoking (%)	22	19	16	0.40
History of CVD (%)	23	30	51	0.00
BMI (kg/m^2)	28 ± 4	29 ± 5	29 ± 5	0.00
Systolic blood pressure (mmHg)	148 ± 24	155 ± 24	154 ± 24	0.00
Use of ACEi/ARB (%)	18	24	40	0.00
Cholesterol-to-HDL ratio	4.9 ± 1.5	4.7 ± 1.4	4.7 ± 1.4	0.31
Use of lipid-lowering drugs (%)	14	16	15	0.32
Duration of diabetes (years)	4 (2–9)	4 (2–9)	4 (2–9)	0.16
HbA_{1c} (%)	7.3 ± 1.3	7.3 ± 1.4	7.3 ± 1.4	0.29
ACR (mg/mmol)	1.5 (0.8–3.8)	1.9 (0.9–5.7)	1.9 (0.9–5.8)	0.00
SCr (μmol/L)	89 ± 15	89 ± 15	89 ± 15	0.00

Data are expressed as mean ± SD or median (IQR), unless otherwise specified.

Statistical Analyses

Cox regression analyses were used to analyze the risk of new-onset albuminuria and CV and all-cause mortality during follow-up. MR-proADM and ACR followed a non-Gaussian distribution, and $\log_2$ transformations were applied so the hazard ratios (HRs) derived were expressed as an increase in risk per doubling of baseline MR-proADM values.

Four models were used: *1*) a crude model, *2*) a model adjusted for age, sex, and MR-proADM, *3*) a fully adjusted model (BMI, smoking, systolic blood pressure, cholesterol-to-HDL ratio, duration of diabetes, HbA_{1c}, history of CVD, use of ACEi/angiotensin receptor blocker [ARB], log SCr, and log ACR) with MR-proADM, and *4*) a fully adjusted model without MR-proADM. The assumption of proportional hazards for baseline predictors was investigated by inspecting the Schoenfeld residuals. Cox regression analyses were used to test whether an association existed between the presence or absence of a MR-proADM measurement and new-onset albuminuria and CV and all-cause mortality. The effect on the fit of the fully adjusted models by including MR-proADM in the models was tested using the likelihood ratio test.

The possible additional value of MR-proADM for the risk prediction of new-onset albuminuria and CV and all-cause mortality was assessed with the receiver operating characteristic analysis (using Harrell's C) and the integrated discrimination improvement (IDI). The Harrell's C and the IDI were used to investigate the predictive capability of each model. The IDI can be interpreted as the difference between model-based probabilities for events and nonevents for the models with and without MR-proADM, although the IDI has been criticized (29). Calibration, a measure to evaluate how well predicted probabilities agree with observed risks, was assessed using the Grønessby and Borgan "goodness-of-fit" likelihood-ratio

Table 2—HRs and additional value of baseline $\log_2$ MR-proADM concentrations in risk prediction compared with established CV risk markers

	Model 1	Model 2	Model 3	Model 4
New-onset albuminuria				
HR (95% CI)	1.83 (1.32–2.54)	1.46 (1.01–2.10)	1.40 (0.94–2.10)	NA
Harrell's C (95% CI)	0.58 (0.53–0.63)	0.65 (0.61–0.69)	0.70 (0.66–0.74)	0.70 (0.66–0.74)
Grønnesby and Borgan test	0.17	0.77	0.64	0.63
IDI % (*P* value)	NA	0.006 (0.01)	0.003 (0.25)	NA
CV mortality				
HR (95% CI)	5.89 (4.43–7.99)	3.68 (2.59–5.23)	1.96 (1.27–3.01)	NA
Harrell's C (95% CI)	0.72 (0.68–0.77)	0.78 (0.74–0.82)	0.82 (0.78–0.85)	0.81 (0.78–0.85)
Grønnesby and Borgan test	0.04	0.86	0.01	0.08
IDI % (*P* value)	NA	0.03 (*P* = 0.0001)	0.002 (*P* = 0.3)	NA
All-cause mortality				
HR (95% CI)	4.49 (3.65–5.52)	2.35 (1.85–2.98)	1.78 (1.34–2.36)	NA
Harrell's C (95% CI)	0.70 (0.67–0.73)	0.79 (0.76–0.81)	0.81 (0.78–0.83)	0.80 (0.78–0.82)
Grønnesby and Borgan test	0.04	0.99	0.40	0.18
IDI % (*P* value)	NA	0.009 (*P* = 0.005)	0.002 (*P* = 0.16)	NA

Model 1, crude, only MR-proADM; model 2, as in model 1 and also adjusted for age and sex; model 3, as in model 2 and also adjusted for BMI, smoking, systolic blood pressure, cholesterol-to-HDL ratio, duration of diabetes, HbA_{1c}, history of CVD, use of ACEi/ARB, log SCr, and log ACR; model 4, as in model 3 but without MR-proADM.

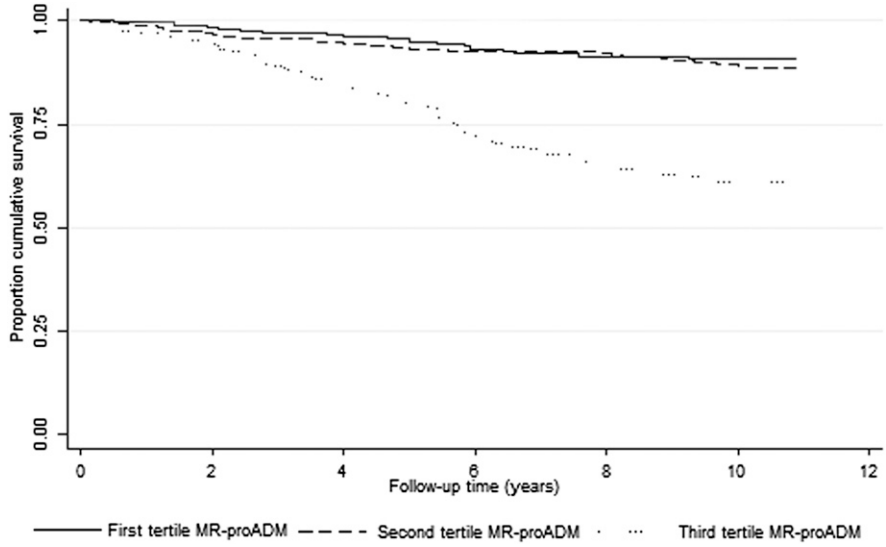

Figure 1—Kaplan-Meier survival curves (CV mortality) for tertiles of MR-proADM.

test; a nonsignificant result means an acceptable calibration (30). Statistical analyses were performed using SPSS version 20.0 for Windows (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY) and STATA version 11 (StataCorp LP, College Station, TX). Results were expressed as mean ± SD or median (interquartile range [IQR]) for normally distributed and nonnormally distributed data, respectively. A two-sided $P < 0.05$ was considered significant.

RESULTS

From 1,243 patients with baseline measurements of MR-proADM, 1,194 (96%) had complete data on all confounders. Two outliers were excluded, one patient with a MR-proADM value of 5.5 nmol/L and one patient with an undetectable level. Ultimately, 1,192 patients were included in the multivariate analyses. Baseline characteristics are presented in Table 1. Variables that were relevantly different between tertiles of MR-proADM concentrations were age, history of CVD, systolic blood pressure, ACR, and use of ACEi/ARB (see Table 1). The median MR-proADM concentration was 0.49 nmol/L (IQR 0.39–0.63). Besides a higher SCr among patients without MR-proADM measurements (98.0 ± 20.9 vs. 95.0 ± 21.0 μmol/L, $P = 0.02$), there were no other significant differences between baseline characteristics of patients with and without MR-proADM measurements or from patients with and without complete baseline variables.

MR-proADM and New-Onset Albuminuria and Mortality

The median follow-up period was 5.6 years (IQR 3.1–10.1), 9.7 years for the patients entering the study in 1998, and 3.1 years for those included in 2001. Of the 1,243 included patients, 388 (31%)

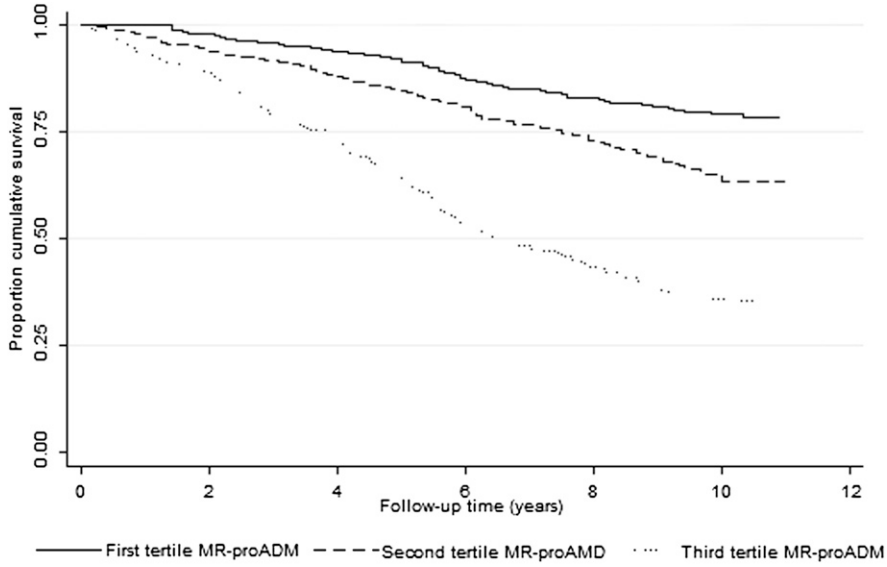

Figure 2—Kaplan-Meier survival curves (all-cause mortality) for tertiles of MR-proADM.

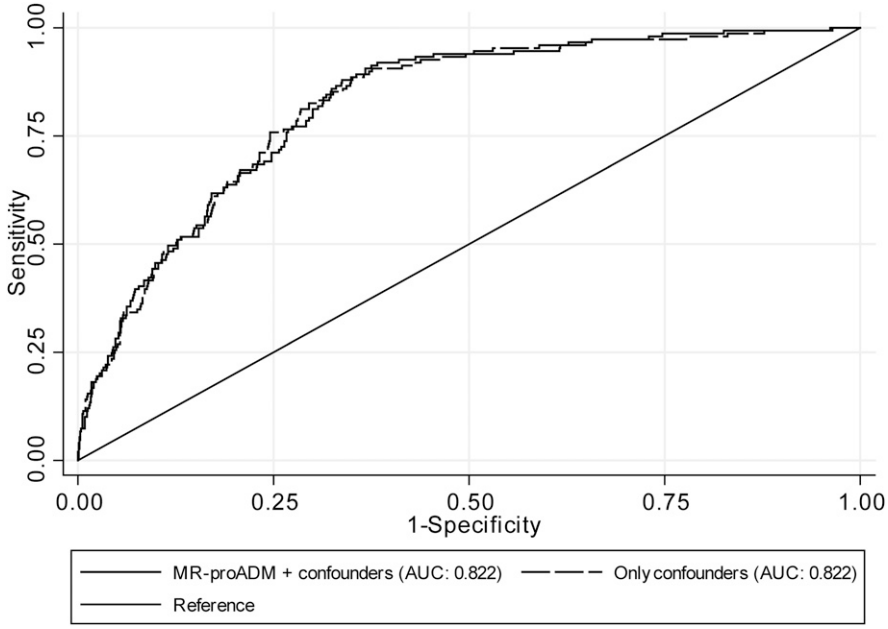

Figure 3—Receiver operating characteristic curves at the end of the follow-up period for CV mortality.

had died, with 168 (12%) deaths attributable to CV causes. The median baseline MR-proADM concentration of 0.45 nmol/L in survivors was significantly lower than the median MR-proADM level of 0.59 nmol/L in nonsurvivors ($P < 0.001$). From the 681 patients with normoalbuminuria at baseline, 182 (26.7%) developed albuminuria.

In the Cox regression analyses, $\log_2$ MR-proADM was significantly associated with new-onset albuminuria, CV mortality, and all-cause mortality, except for new-onset albuminuria in model 3 (see Table 2 and Figs. 1 and 2). If model 3 is compared with model 4 (without MR-proADM), the model fit was not significantly better for new-onset albuminuria ($P = 0.089$), whereas for CV mortality and all-cause mortality, including MR-proADM in the fully adjusted model improved the model fit significantly ($P = 0.002$ and $P < 0.001$, respectively). Harrell's C values were not different for new-onset albuminuria and only marginally higher for CV mortality and all-cause mortality (see Figs. 3 and 4). In addition, the IDI values were <1% for all models. The IDI values for model 2

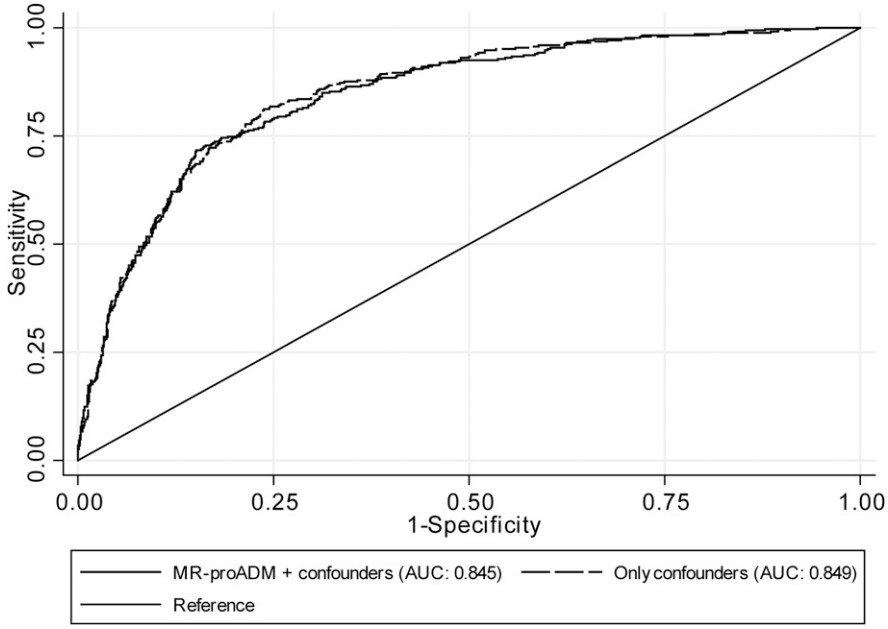

Figure 4—Receiver operating characteristic curves at the end of the follow-up period for all-cause mortality.

predicting CV and all-cause mortality were significant, indicating that MR-proADM had additional value on top of age and sex. In the fully adjusted models, the IDI values were not significant. The Grønessby and Borgan *P* values in Table 2 indicate that since predicted probabilities correspond well with observed risks (except for the crude model and model 3 predicting CV mortality), all models were well calibrated.

Adding estimated glomerular filtration rate to models 3 and 4 instead of SCr produced similar results. The Schoenfeld residuals showed no substantial deviations, supporting the assumption of proportional hazards. The association between the presence or absence of a MR-proADM measurement and new-onset albuminuria and CV and all-cause mortality in the combined cohort of 1,689 patients was also tested in a multivariable Cox regression analysis; no significant associations with the presence or absence of a MR-proADM measurement were present.

CONCLUSIONS

Increased plasma MR-proADM levels were associated with CV and all-cause mortality in patients with type 2 diabetes after long-term follow-up. No independent associations were found between MR-proADM and new-onset albuminuria. This is the first study evaluating the relationship between MR-proADM and mortality in patients with type 2 diabetes and the first to evaluate the relationship with new-onset albuminuria (13,16,19,20,24–26,31).

The age- and sex-adjusted MR-proADM levels were associated with new-onset albuminuria, although there was no significant relationship after adjusting for multiple risk factors. MR-proADM levels were also associated with several baseline risk factors (i.e., BMI, systolic blood pressure, and SCr). Although there was no independent relationship, the development of new-onset albuminuria was related to the same risk factors, e.g., blood pressure, factors that also increase MR-proADM levels. Thus, MR-proADM could possibly act as a unified marker for several known risk factors.

Previous observations showed that increased levels of MR-proADM were associated with endothelial dysfunction (6); through this mechanism, the association with CV and all-cause mortality could also be explained. Despite mutual correlations between MR-proADM and baseline risk factors, MR-proADM, corrected for an extensive set of risk factors, was independently associated with CV and all-cause mortality. Adding an extensive set of risk factors to the age- and sex-corrected MR-proADM levels increased the Harrell's C from 0.78 to 0.82, indicating that the age- and sex-corrected serum levels of MR-proADM were able to predict CV mortality to some degree. The improvement in risk prediction by adding MR-proADM to a fully adjusted model was significant, as measured by the improvement in model fit. Despite the fact that the model fit significantly improved, the relevancy of this small effect on risk prediction, expressed by the change in Harrell's C, is not clear. This small improvement in risk prediction seems to be in line with results from previous biomarker studies, in which addition of biomarkers to a comprehensive model, with overlapping risk factors, has only a little beneficial effect. Important risk factors, even lipid abnormalities, show only small or no improvements in Harrell's C when added to a combination of other known risk factors (32).

MR-proADM levels were measured in serum plasma, and from these data, it remains unclear whether on a cellular level ADM, expressed by the serum level of MR-proADM, acts in a paracrine fashion or plays a relevant role in plasma through endocrine effects (5).

The strengths of this study were a relative long-term follow-up and the number and completeness of confounders used in the multivariate model. This study also has several limitations. First, selection bias may have occurred, because patients whose MR-proADM had not been measured were excluded from statistical analysis. However, no relevant differences were found in Cox regression analysis between groups in which MR-proADM had or had not been measured. Second, MR-proADM was measured only once, without correction for potential variability in concentrations. Fortunately, the intra-assay and interassay variability are known to be low and values are stable (17). Third, unlike the statistically significant increase in fit of the fully adjusted models for CV and all-cause mortality, Harrell's C values did not change much and the value of the IDI was not significant when MR-proADM was added to the fully adjusted model. Also, the results of the IDI values need to be interpreted with caution, ranges of meaningful improvements are not established, values are strongly dependent on the number of events, the IDI was not yet developed in the context of censored data, and the value of the IDI can, by accident or deliberately, be inflated (29). Fourth, the relatively small sample size in the analysis of the relationship between MR-proADM levels and new-onset albuminuria does not fully exclude the presence of an independent relationship.

In conclusion, ADM is increasingly being studied for its prognostic properties in a variety of disease states. This is the first report showing an independent association between increased plasma MR-proADM levels and CV and all-cause mortality after years of follow-up in patients with type 2 diabetes who were treated in primary care. In this study, an extensive set of known risk factors predicted mortality to a high degree. When combining MR-proADM with these risk factors, there was little added benefit of using MR-proADM in risk prediction. Future studies are needed to clarify the role of increased plasma MR-proADM levels on a cellular level and to establish whether MR-proADM could have a role in predicting adverse outcomes, including mortality, in individual patients with type 2 diabetes.

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Duality of Interest. J.S. was previously employed by B.R.A.H.M.S, a company that manufactures and holds patent rights on the MR-proADM assay. No other potential conflicts

of interest relevant to this article were reported.

Author Contributions. G.W.D.L. and P.R.v.D. researched data and wrote the manuscript. I.D., K.J.J.v.H., J.S., R.O.B.G., H.J.G.B., S.J.L.B., and N.K. contributed to discussion and reviewed the manuscript. K.H.G. researched data, contributed to discussion, and reviewed the manuscript. G.W.D.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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