Plasma COOH-Terminal Proendothelin-1

A marker of fatal cardiovascular events, all-cause mortality, and new-onset albuminuria in type 2 diabetes? (ZODIAC-29)

Iefke Drion, md[1]
Nanne Kleefstra, md, phd[1,2,3]
Gijs W.D. Landman, md[1]
Alaa Alkhalaf, md[1,3,4]
Joachim Struck, phd[5]
Klaas H. Groenier, phd[1,6]
Stephan J.L. Bakker, md, phd[3]
Henk J.G. Bilo, md, phd, frcp[1,3,4]

OBJECTIVE—The aim of this study was to investigate the association between plasma COOH-terminal proendothelin-1 (CT-proET-1) and fatal cardiovascular events, all-cause mortality, and new-onset albuminuria in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS—A total of 1,225 patients with type 2 diabetes participated in this prospective observational study of two combined cohorts. Three clinical end points were studied: fatal cardiovascular events, all-cause mortality, and new-onset albuminuria. After a median follow-up of 3 or 10 years, Cox proportional hazard modeling was used to investigate the association between CT-proET-1 and the end points. Harrell C statistic, the Groennesby and Borgan test, the integrated discrimination improvement (IDI), and the net reclassification improvement (NRI) were used to evaluate whether CT-proET-1 is of additional value compared with classic cardiovascular and renal risk factors.

RESULTS—During follow-up, 364 (30%) patients died, 150 (42%) of whom died of cardiovascular disease; 182 (26.7%) of 688 patients with normoalbuminuria at baseline developed albuminuria. CT-proET-1 was associated with fatal cardiovascular events, all-cause mortality, and new-onset albuminuria with hazard ratios of 1.59 (95% CI 1.15–2.20), 1.41 (95% CI 1.14–1.74), and 1.48 (95% CI 1.10–2.01), respectively. Addition of CT-proET-1 to a model containing traditional risk factors leads only to improved prediction of fatal cardiovascular events. The IDI appeared significant for fatal cardiovascular events (0.82 [0.1–1.54]) and all-cause mortality (0.4 [0.05–0.92]), but not for new-onset albuminuria.

CONCLUSIONS—CT-proET-1 has additional value for the prediction of fatal cardiovascular events and new-onset albuminuria in patients with type 2 diabetes, compared with conventional risk factors, but not for all-cause mortality.

Diabetes Care 35:2354–2358, 2012

Hypertension and diabetes mellitus (DM) increase the risk of alterations in endothelial structures and function, leading to vascular alterations, with increased risk of cardiovascular morbidity, mortality, and glomerulosclerosis (1,2). In previous studies, a pathophysiologic role for increased levels of endothelin-1 (ET-1) has been suggested (3,4).

ET-1 is mainly generated in endothelial cells (5,6). Alongside of having inotropic, chemotactic, and mitogenic properties, it has peripheral vasoconstrictor properties, stimulates the renin–angiotensin–aldosteron axis as well as the sympathetic nervous system, and influences the salt and water homeostasis (7). As a consequence, ET-1 potentially is an important mediator of essential hypertension and (cardiovascular) complications such as coronary atherosclerosis (7). Because rapid degradation makes the measurement of plasma concentrations of this peptide cumbersome, assessment of the more stable cleavage product of its preprotein COOH-terminal proendothelin-1 (CT-proET-1) has been propagated as an alternative (8,9).

Previous cross-sectional studies in patients with DM consistently found elevated levels of plasma ET-1 (3,10–13). To the best of our knowledge, only one study has prospectively evaluated a potential association of CT-proET-1 with cardiovascular morbidity and mortality in patients with type 2 diabetes. This study did not find a significant association, possibly due to relatively short follow-up of 1 year with a small number of events (14). Therefore, we aimed to investigate the predictive capability of plasma CT-proET-1 on fatal cardiovascular events and all-cause mortality in a large prospective Dutch cohort of patients with type 2 diabetes with long-term follow-up. Moreover, because albuminuria is an important risk factor for mortality and development of end-stage renal disease, while being interrelated with endothelial dysfunction (15,16), we aimed to investigate whether CT-proET-1 is associated with the future development of albuminuria.

RESEARCH DESIGN AND METHODS

Study population

In 1998, the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) Study was initiated in the Netherlands. In the first year, 1,143 patients with type 2 diabetes were included in this prospective observational study. This study has been described in detail previously (17). Briefly, the objective was to investigate the effects of a shared-care project for type 2 diabetes. Sixty-one general practitioners participated. In 2001, another 546 unique patients with DM entered the study, adding up to a combined cohort of 1,689 patients (18). The ZODIAC Study was approved by the local medical ethics committee, and all patients gave informed consent.

Data collection

Data consisted of a full medical history including macrovascular complications,

From the [1]Diabetes Centre, Isala Clinics, Zwolle, the Netherlands; the [2]Langerhans Medical Research Group, Zwolle, the Netherlands; the [3]Department of Internal Medicine, University of Groningen and University Medical Center Groningen, Groningen, the Netherlands; the [4]Department of Internal Medicine, Isala Clinics, Zwolle, the Netherlands; the [5]Research Department, B.R.A.H.M.S GmbH/Thermo Fischer Scientific, Hennigsdorf, Germany; and the [6]Department of General Practice, University Medical Center Groningen, Groningen, the Netherlands.
Corresponding author: Iefke Drion, i.drion@isala.nl.
Received 27 December 2011 and accepted 14 May 2012.
DOI: 10.2337/dc11-2526

medication use, diabetes duration, and tobacco consumption. Patients were considered to have a history of macrovascular complications when 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 annually and included glycated hemoglobin (HbA_{1c}), nonfasting lipid profile, serum creatinine (SCr, a kinetic colorimetric Jaffé method [Modular P Analyzer; Roche, Almere, the Netherlands] was used), albumin-to-creatinine ratio (ACR) using immunonephelometry (Behring Nephelometer; Behringwerke, Mannheim, Germany), blood pressure (measured twice with a Welch Allyn sphygmomanometer in the supine position after at least 5 min of rest), weight, and height. Information on CT-proET-1 and confounders was complete for 1,225 patients. A total of 481 patients already had albuminuria at baseline. Regarding new-onset albuminuria, data were available for 688 patients. Data were missing for 44 (3.6%) patients, and 12 patients died within a year after baseline.

Plasma levels of CT-proET-1

CT-proET-1 was measured in 2010 in plasma collected at baseline and kept frozen at −80°C until analysis using a fully automated sandwich immunoassay according to the instructions of the manufacturer (B.R.A.H.M.S CT-proET-1 KRYPTOR; B.R.A.H.M.S, Hennigsdorf, Germany) (8,9,19). CT-proET-1 has a stable plasma half-life ex vivo (8). Because the performance of one freeze-thaw cycle is without consequence for assessed CT-proET-1 concentrations, and the study samples underwent one freeze-thaw cycle, no influence of frozen storage on assessed concentration is to be expected. The analytical detection limit for plasma CT-proET-1 was 2.94 pmol/L. The intra- or interassay coefficients of variation of CT-proET-1 are: <6 and <20% for the range 15–40 pmol/L, <5 and <11% for the range 40–150 pmol/L, and <3 and <8%, respectively, for >150 pmol/L.

Clinical end points

We examined three clinical end points in this study: fatal cardiovascular events, all-cause mortality, and newly developed albuminuria.

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. The causes of death were coded according to ICD-9.

Albuminuria was defined as an ACR >2.5 mg/mmol for men and >3.5 mg/mmol for women. We considered patients to have newly developed albuminuria if: *1*) patients had normoalbuminuria at baseline and developed albuminuria in 2 consecutive follow-up years; *2*) patients had normoalbuminuria at baseline and developed albuminuria in 1 single follow-up year, followed by initiation of an ACE inhibitor (ACEi) or angiotensin-II antagonist (AIIA) treatment in the same year; and *3*) patients were normoalbuminuric on ACEi/AIIA treatment at baseline and developed albuminuria in one of the follow-up years.

Statistical analyses

We used SPSS version 16.0 (SPSS Inc., Chicago, IL) and STATA version 11 (StataCorp, College Station, TX) for statistical analyses. Continuous variables are represented as mean (± SD) for normally distributed values and as median (interquartile range) for nonnormally distributed variables. Variables with a skewed distribution were logarithmically transformed before analysis. One-way ANOVA or the Kruskal-Wallis test were used as appropriate to test for differences between groups. In order to investigate possible interactions, we included interaction terms between all confounders with a *P* value <10% and CT-proET-1. None of these interactions showed a statistically significant result. For this reason, we did not perform a separate analysis in those subgroups. Cox proportional hazard analyses were used to investigate the association between the logarithm (base2) of CT-proET-1, fatal cardiovascular events, mortality, and new-onset albuminuria with adjustment for selected confounders. The same analysis was used to test whether an association existed between the presence or absence of a CT-proET-1 measurement and all end points. Age, sex, smoking (dichotomous), BMI, log SCr, log ACR, systolic blood pressure, a history of macrovascular complications (dichotomous), diabetes duration, HbA_{1c}, receiving ACEi/AIIA treatment (dichotomous), receiving lipid-lowering therapy (dichotomous), and the total cholesterol/HDL ratio at baseline were selected as possible confounders. The logarithm of CT-proET-1 was used in the proportional hazard analysis, because the original measure showed a nonlinear relationship with survival, whereas the log-transformed value showed a linear relationship. Furthermore, the base 2 log was used to facilitate the interpretation of the hazard ratio. One unit change in the base 2 log is equivalent to a doubling of the original value of CT-proET-1. Four different models were assessed: model 1 was the univariate model for log CT-proET-1, model 2 included age and sex as confounders, model 3 included all selected confounders, and model 4 contained all selected confounders without log CT-proET-1.

The ph-test that was used to test the assumption of proportional hazards for baseline predictors showed nonsignificant *P* values, meaning that no substantial deviations were observed. Harrell C statistic, a rank-based measure, was used to compare how well the different models predict fatal cardiovascular events, all-cause mortality, and new-onset albuminuria (20). Calibration, a measure to evaluate how well predicted probabilities agree with actual observed risks, was investigated using the Groennesby and Borgan test; a nonsignificant result means an acceptable calibration (21). When the average predicted risk matches the proportion that actually develops disease within subgroups of a prospective cohort, the model is considered well-calibrated. The higher the value, the better the model predicts mortality. Furthermore, two measures of discrimination improvement—the integrated discrimination improvement (IDI) and the net reclassification improvement (NRI)—were calculated (22). The IDI can be interpreted as the difference between model-based probabilities for events and nonevents for the models with and without CT-proET-1. The NRI is calculated by assessing the net improvement in risk classification (<10, 10–20, 20–30, and >30%) separately for events and nonevents. Survival curves for tertiles of CT-proET-1 were used for initial prospective evaluation and illustration.

RESULTS—Baseline characteristics according to tertiles of CT-proET-1 are presented in Table 1. Patients in the highest tertile were older, more frequently female, more frequently current smokers, had lower HbA_{1c}, lower cholesterol/HDL ratio, higher prevalence of macrovascular complications, lower estimated glomerular filtration rate, and higher ACR. No significant association was found in mortality and new-onset albuminuria

Table 1—*Baseline characteristics of study population according to tertiles of CT-proET-1*

	Tertile 1	Tertile 2	Tertile 3	*P* value
CT-proET-1	23 (20–25)	33 (30–35)	47 (42–57)	
n	408	409	408	
Age (years)	64 (11)	66 (11)	71 (11)	<0.001
Male sex [*n* (%)]	188 (46)	195 (48)	158 (39)	<0.01
BMI (kg/m^2)	29 (4)	29 (5)	29 (5)	0.52
Smoking [*n* (%)]	96 (23)	70 (17)	64 (16)	0.02
Diabetes duration (years)	5 (2–9)	4 (2–9)	5 (2–9)	0.43
HbA_{1c} (%)	7.4 (1.3)	7.3 (1.4)	7.2 (1.2)	0.02
Systolic blood pressure (mmHg)	152 (24)	152 (25)	152 (24)	0.98
Receiving ACEi and/or AIIA [*n* (%)]	97 (24)	94 (23)	133 (33)	0.03
Cholesterol/HDL ratio	5.1 (1.5)	4.9 (1.4)	4.8 (1.5)	0.03
Receiving lipid-lowering treatment [*n* (%)]	49 (12)	60 (15)	63 (15)	0.33
Macrovascular complications (%)	121 (30)	126 (31)	171 (43)	<0.001
MDRD (mL/min/1.73 m^2)	70 (61–78)	67 (58–74)	57 (48–66)	<0.001
Albuminuria [*n* (%)]	160 (39)	145 (36)	183 (45)	0.02

Data are means (± SD), medians (interquartile range), or *n* (%). One-way ANOVA or the Kruskal-Wallis test were used as appropriate to test for differences between groups. MDRD, modification of diet in renal disease formula.

between groups in which CT-proET-1 had been measured or not. For total mortality, there was a significant interaction between CT-proET-1 and systolic blood pressure.

Mortality and albuminuria

After a follow-up period of 10 years for the patients entering the study in 1998 and 3 years for those included in 2001 (resulting in 8,002 person-years total analysis time at risk), 364 of 1,225 (29.7%) patients had died, with 154 (42.3%) attributable to fatal cardiovascular events. From the 681 patients with normoalbuminuria at baseline, 182 (26.7%) developed albuminuria. The median baseline plasma value (interquartile range) of CT-proET-1 in survivors was lower than that of patients who died during follow-up: 32 (25–40) versus 42 (28–57), respectively ($P < 0.001$).

Kaplan-Meier curves showing fatal cardiovascular events, all-cause mortality, and new-onset albuminuria according to tertiles of CT-proET-1 are shown in Fig. 1. The first two tertiles do not differ significantly in all figures, with the highest tertile deviating from the other two in the direction of poorer survival ($P < 0.001$, $P < 0.001$, and $P = 0.007$ for Fig. 1*A–C*, respectively). In univariate Cox regression analyses, log CT-proET-1 was associated with all selected clinical end points (Table 2). After adjustment for potential confounders, the association remained significant.

Harrel *C* values in Table 2 show that the more confounders we adjusted for, the better the model predicts fatal cardiovascular events, all-cause mortality, and new-onset albuminuria. However, if model 3 is compared with model 4, Harrel C values are only slightly better for fatal cardiovascular events and new-onset albuminuria and equivalent for all-cause mortality.

The goodness-of-fit analyses indicate that all models are well-calibrated. So, the number of deceased patients as estimated by the model is in line with the number of patients that actually died.

Furthermore, the IDI values appeared <1% for all models predictive of fatal cardiovascular events, except model 2, all-cause mortality, and for new-onset albuminuria. NRI only showed a substantial value of 18.4% for model 2 in the prediction of fatal cardiovascular events.

CONCLUSIONS—In this prospective cohort of patients with type 2 diabetes, CT-proET-1 was found to be associated with fatal cardiovascular events, all-cause mortality, and new-onset albuminuria. CT-proET-1 was found to have additional value for the prediction of fatal cardiovascular events and new-onset albuminuria compared with conventional risk factors in patients with type 2 diabetes. However, this was not the case for all-cause mortality.

To our knowledge, previous studies investigating the relationship between CT-proET-1 and fatal cardiovascular events and/or all-cause mortality in patients with DM are scarce. Because endothelin increases vascular tone and blood pressure, an association between CT-proET-1 and fatal cardiovascular events may be expected. Moreover, in patients with DM, the structure of the endothelium may be altered, leading to atherosclerosis and cardiovascular morbidity and mortality in this patient group (3,4,13).

In a study from 2009 with mean baseline CT-proET-1 levels of 74.6 ± 1.0 pmol/L (14), no statistical significant association was found between CT-proET-1 and the composite end point cardiovascular morbidity or mortality. However, the follow-up in this study was short (median of 15 months), participants were significantly younger than the patients in our cohort, and the numbers of events was lower. Unfortunately, no hazard ratios were reported, so it cannot be judged whether the association, although not significant, was in the same direction and order of magnitude as in our study.

CT-proET-1 has been investigated in several other patient categories. Values in healthy subjects as measured by B.R.A.H.M.S had a median value of 45.1 pmol/L (95th percentile, 67.8). In patients with stable coronary artery disease and a preserved left ventricular ejection fraction, CT-proET-1 was found to be associated with cardiovascular death and heart failure risk (23). Moreover, two prospective studies have been performed in patients with heart failure (24,25). In these studies, CT-proET-1 was found to be an independent additional predictor of death after, respectively, 12 and 24 months of follow-up.

Albuminuria and CT-proET-1

As far as we know, the current study is the only study evaluating the association between the development of new-onset albuminuria and CT-proET-1 in a prospective cohort. It is important to diagnose albuminuria in an early stage, because early treatment with intensive antihypertensive therapy can slow down renal function loss. Previous studies have mostly focused on the association between ET-1 and urinary albumin excretion and found an independent association of plasma ET-1 levels with urinary albumin excretion and suggested that early abnormalities of ET-1 production possibly precede the albuminuric phase of diabetic nephropathy (15). Moreover, it often concerns studies containing a small number and selected patients, which makes it difficult to generalize the results.

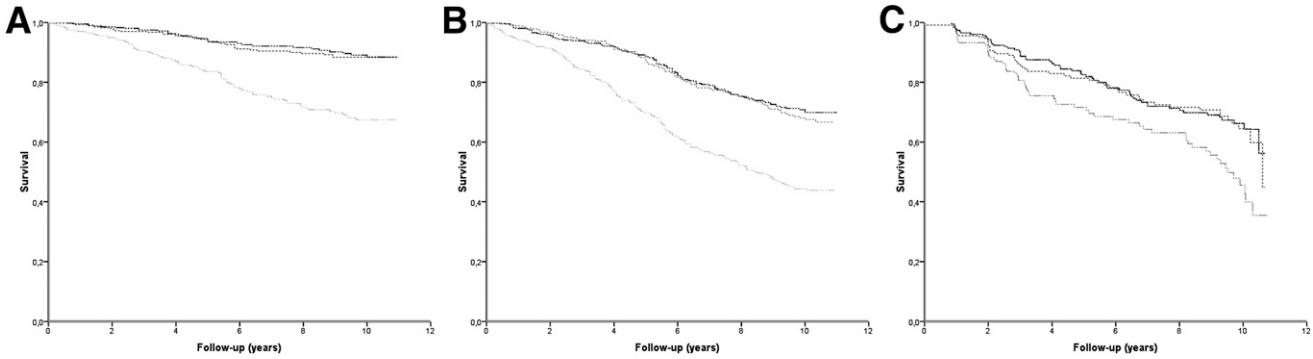

Figure 1—*Kaplan-Meier curves showing the association between CT-proET-1 and fatal cardiovascular events* (A)*, all-cause mortality* (B)*, and new-onset albuminuria* (C)*. The black line shows tertile 1, the dark gray line displays tertile 2, and the light gray line shows tertile 3.*

As we show in the current study also with the more stable cleavage product of ET-1's preprotein, an association exists between CT-proET-1 and albuminuria. However, CT-proET-1 turned out to be of little, if any, additional predictive value when added to traditional risk factors (26,27).

Strengths and limitations

To our knowledge, this is the first study of CT-proET-1 in a large group of patients with type 2 diabetes with long-term follow-up. However, some limitations need to be mentioned. First, selection bias may have occurred, because patients whose CT-proET-1 had not been measured were excluded from statistical analysis. However, in Cox regression analysis, no significant association in mortality and new-onset albuminuria was found between groups in which CT-proET-1 had or had not been measured. Second, CT-proET-1 was measured only once, without a correction for potential variability in concentrations. Whether sequential measurements of CT-proET-1 will result in a better prediction of mortality in patients with diabetes remains to be investigated. Third, the presence of C-reactive protein, fasting lipid values, and mortality data ranging up to 2009 for the whole population would have been of additional value. Moreover, based on the results of this study, we cannot comment on the applicability of CT-proET-1 as a risk marker in individual diabetes patients. Future research should be performed to clarify which patient population might benefit from a risk determination using CT-proET-1.

Finally, the results of the IDI and NRI values need to be interpreted with caution, because ranges of meaningful improvements are not established for them, and the values are strongly dependent on the prevalence of the number of events

Table 2—*Association of log^2 CT-proET-1 with end points and the additional value in risk prediction compared with classic cardiovascular risk markers*

	Model 1	Model 2	Model 3	Model 4
Fatal cardiovascular events				
Hazard ratio (95% CI)	3.36 (2.61–4.36)	2.45 (1.84–3.26)	1.59 (1.15–2.20)	NA
Harrell C (95% CI)	0.68 (0.63–0.73)	0.77 (0.74–0.81)	0.82 (0.78–0.85)	0.81 (0.78–0.85)
Groennesby and Borgan test	0.07	0.43	0.07	0.21
IDI (%) (95% CI)	NA	2.57 (1.28–3.87)	0.82 (0.10–1.54)	NA
NRI (%) (95% CI)	NA	18.42 (8.18–28.66)	−1.75 (−9.08 to 5.58)	NA
All-cause mortality				
Hazard ratio (95% CI)	2.49 (2.09–2.97)	1.67 (1.38–2.01)	1.41 (1.14–1.74)	NA
Harrell C (95% CI)	0.63 (0.60–0.67)	0.78 (0.76–0.80)	0.80 (0.78–0.82)	0.80 (0.77–0.82)
Groennesby and Borgan test	0.08	0.28	0.18	0.15
IDI (%) (95% CI)	NA	0.87 (0.24–1.51)	0.4 (0.05–0.92)	NA
NRI (%) (95% CI)	NA	1.77 (−1.93 to 5.46)	3.75 (0.19–7.30)	NA
New-onset albuminuria				
Hazard ratio (95% CI)	1.60 (1.21–2.11)	1.42 (1.06–1.91)	1.48 (1.10–2.01)	NA
Harrell C (95% CI)	0.56 (0.51–0.61)	0.64 (0.60–0.68)	0.70 (0.66–0.74)	0.69 (0.65–0.73)
Groennesby and Borgan test	0.17	0.28	0.59	0.86
IDI (%) (95% CI)	NA	0.03 (0.00–0.19)	0.24 (0.00–0.67)	NA
NRI (%) (95% CI)	NA	0.56 (−3.39 to 4.51)	−1.19 (−5.74 to 3.36)	NA

Hazard ratios (95% CI) for fatal cardiovascular events, all-cause mortality, and new-onset albuminuria using four different models: model 1: log^2 CT-proET-1; model 2: log^2 CT-proET-1 adjusted for age and sex; model 3: log^2 CT-proET-1 adjusted for age, sex, log SCr, systolic blood pressure, log ACR, diabetes duration, HbA_{1c}, receiving lipid-lowering treatment (dichotomous), receiving ACEi/AIIA (dichotomous), macrovascular complications (dichotomous), smoking (dichotomous), BMI, and cholesterol/HDL ratio; and model 4: all confounders (without CT-proET-1). Comparison of performance of models for mortality risk prediction in type 2 diabetes as determined by the Harrel C, the Groennesby and Borgan test, IDI, and NRI. NA, not applicable.

and not yet developed in the context of censored data (22).

Conclusion

The biomarker CT-proET-1 is associated with fatal cardiovascular events, all-cause mortality, and new-onset albuminuria and has additional value for the prediction of fatal cardiovascular events and new-onset albuminuria compared with conventional risk factors, but not for all-cause mortality in patients with type 2 diabetes. Although our data suggest that measurement of CT-proET-1 levels may aid in predicting mortality, additional research is needed to evaluate the screening characteristics of this biomarker. Because previous studies have shown that reducing the levels of established risk factors decreases the risk of cardiovascular disease in type 2 diabetes patients, it would be interesting in further studies to evaluate whether blockade of ET-1 retards or decreases mortality.

Acknowledgments—S.J.L.B. received support from the Netherlands Heart Foundation, Dutch Diabetes Research Foundation, and Dutch Kidney Foundation, together participating in the framework of the Center for Translational Molecular Medicine (www.ctmm.nl) project PREDICCt (Grant 01C-104-07). J.S. is employed by B.R.A.H.M.S, a company that manufactures and holds patent rights on the CT-proET-1 assay. No other potential conflicts of interest relevant to this article were reported.

I.D. researched data, contributed to the discussion, and wrote the manuscript. N.K. researched data, reviewed and edited the manuscript, and contributed to the discussion. G.W.D.L., A.A., and J.S. reviewed and edited the manuscript. K.H.G. researched data and reviewed and edited the manuscript. S.J.L.B. and H.J.G.B. contributed to the discussion and reviewed and edited the manuscript. I.D. 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.

References

1. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 1998;339:229–234
2. Fernández-Real JM, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. Endocr Rev 2003;24:278–301
3. Haak T, Jungmann E, Felber A, Hillmann U, Usadel KH. Increased plasma levels of endothelin in diabetic patients with hypertension. Am J Hypertens 1992;5:161–166
4. Perfetto F, Tarquini R, Tapparini L, Tarquini B. Influence of non-insulin-dependent diabetes mellitus on plasma endothelin-1 levels in patients with advanced atherosclerosis. J Diabetes Complications 1998;12:187–192
5. Inoue A, Yanagisawa M, Kimura S, et al. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci USA 1989;86:2863–2867
6. Inoue A, Yanagisawa M, Takuwa Y, Mitsui Y, Kobayashi M, Masaki T. The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression. J Biol Chem 1989;264:14954–14959
7. Agapitov AV, Haynes WG. Role of endothelin in cardiovascular disease. J Renin Angiotensin Aldosterone Syst 2002;3:1–15
8. Papassotiriou J, Morgenthaler NG, Struck J, Alonso C, Bergmann A. Immunoluminometric assay for measurement of the C-terminal endothelin-1 precursor fragment in human plasma. Clin Chem 2006;52:1144–1151
9. Struck J, Morgenthaler NG, Bergmann A. Proteolytic processing pattern of the endothelin-1 precursor in vivo. Peptides 2005;26:2482–2486
10. Takahashi K, Ghatei MA, Lam HC, O'Halloran DJ, Bloom SR. Elevated plasma endothelin in patients with diabetes mellitus. Diabetologia 1990;33:306–310
11. Mather KJ, Mirzamohammadi B, Lteif A, Steinberg HO, Baron AD. Endothelin contributes to basal vascular tone and endothelial dysfunction in human obesity and type 2 diabetes. Diabetes 2002;51:3517–3523
12. Güvener N, Aytemir K, Aksöyek S, Gedik O. Plasma endothelin-1 levels in non-insulin dependent diabetes mellitus patients with macrovascular disease. Coron Artery Dis 1997;8:253–258
13. Schneider JG, Tilly N, Hierl T, et al. Elevated plasma endothelin-1 levels in diabetes mellitus. Am J Hypertens 2002;15:967–972
14. Maier C, Clodi M, Neuhold S, et al. Endothelial markers may link kidney function to cardiovascular events in type 2 diabetes. Diabetes Care 2009;32:1890–1895
15. Zanatta CM, Gerchman F, Burttet L, et al. Endothelin-1 levels and albuminuria in patients with type 2 diabetes mellitus. Diabetes Res Clin Pract 2008;80:299–304
16. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. Diabetes 2002;51:1157–1165
17. Ubink-Veltmaat LJ, Bilo HJ, Groenier KH, Houweling ST, Rischen RO, Meyboom-de Jong B. Prevalence, incidence and mortality of type 2 diabetes mellitus revisited: a prospective population-based study in The Netherlands (ZODIAC-1). Eur J Epidemiol 2003;18:793–800
18. Lutgers HL, Graaff R, Links TP, et al. Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes. Diabetes Care 2006;29:2654–2659
19. Krüger S, Ewig S, Giersdorf S, Hartmann O, Suttorp N, Welte T; German Competence Network for the Study of Community Acquired Pneumonia (CAPNETZ) Study Group. Cardiovascular and inflammatory biomarkers to predict short- and long-term survival in community acquired pneumonia. Am J Respir Crit Care Med 2010;182:1426–1434
20. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med 1996;15:361–387
21. May S, Hosmer DW. A simplified method of calculating an overall goodness-of-fit test for the Cox proportional hazards model. Lifetime Data Anal 1998;4:109–120
22. Pencina MJ, D'Agostino RB, Vasan RS. Statistical methods for assessment of added usefulness of new biomarkers. Clin Chem Lab Med 2010;48:1703–1711
23. Sabatine MS, Morrow DA, de Lemos JA, et al. Evaluation of multiple biomarkers of cardiovascular stress for risk prediction and guiding medical therapy in patients with stable coronary disease. Circulation 2012;125:233–240
24. Jankowska EA, Filippatos GS, von Haehling S, et al. Identification of chronic heart failure patients with a high 12-month mortality risk using biomarkers including plasma C-terminal pro-endothelin-1. PLoS ONE 2011;6:e14506
25. Adlbrecht C, Hülsmann M, Strunk G, et al. Prognostic value of plasma midregional pro-adrenomedullin and C-terminal-pro-endothelin-1 in chronic heart failure outpatients. Eur J Heart Fail 2009;11:361–366
26. Cook NR. Methods for evaluating novel biomarkers - a new paradigm. Int J Clin Pract 2010;64:1723–1727
27. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157–172; discussion 207–212