

# The Midregional Fragment of Pro-A-Type Natriuretic Peptide, Blood Pressure, and Mortality in a Prospective Cohort Study of Patients With Type 2 Diabetes (ZODIAC-25)

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**OBJECTIVE**—Evidence that midregional fragment of pro-A-type natriuretic peptide (MR-proANP) is a marker of mortality in patients with type 2 diabetes is limited. Therefore, we aimed to investigate the capabilities of MR-proANP in predicting mortality. We also investigated whether MR-proANP influences the relationship between blood pressure and mortality in old age.

**RESEARCH DESIGN AND METHODS**—In 1998, 1,143 primary care patients with type 2 diabetes participated in the ZODIAC study. Because blood was drawn for 867 patients (76%) and confounders were missing for 19 patients, the final study sample comprised 848 patients. After a follow-up time of 10 years, we used Cox proportional hazard models to evaluate the relationship between MR-proANP and (cardiovascular) mortality. Harrell C statistic was used to compare models with and without MR-proANP. The regression analyses were repeated without MR-proANP for patients aged older than 75 years.

**RESULTS**—Median MR-proANP in the total study sample was 75 pmol/L (interquartile range, 48–124 pmol/L). During follow-up, 354 (42%) out of 848 patients had died, of whom 152 (43%) deaths were attributable to cardiovascular factors. MR-proANP was independently associated with all-cause and cardiovascular mortality, irrespective of age. During old age, there was a significant inverse relationship between blood pressure and mortality. This relationship did not change after adjustment for MR-proANP.

**CONCLUSIONS**—MR-proANP is independently associated with mortality in patients with type 2 diabetes. MR-proANP did not influence the inverse relationship between blood pressure and mortality in elderly patients.

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**N**atriuretic peptides are important cardiac hormones that have both diagnostic and prognostic values in patients with heart failure (1,2). A-type natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are derivatives of

precursor hormones, which are split into the biologically active peptides (ANP and BNP) as well as the biologically inactive N-terminal fragments (N-terminal pro-A-type natriuretic peptide [NT-proANP] and N-terminal pro-B-type

natriuretic peptide [NT-proBNP]). Because of variable accessibility and fragmentation of the detected antigen, assays for NT-proANP have performed disappointingly (3,4). A novel immunoassay has been developed that measures a more stable fragment of proANP, the midregional fragment of proANP (MR-proANP).

Although there are conflicting results with respect to the value of biomarkers, there is still great interest in developing new assays, including MR-proANP, to optimize identifying those patients at increased risk for development of cardiovascular complications (5). Head-to-head comparisons between MR-proANP and NT-proBNP have shown that both peptides are comparable in predicting cardiovascular events and mortality, and in diagnosing heart failure (6–9). Among patients with chronic heart failure, measurement of MR-proANP even provided prognostic information with respect to mortality independent of NT-proBNP (10,11). There is only one study that has specifically investigated MR-proANP in a cohort of patients with type 2 diabetes (12). This study showed that higher serum levels of MR-proANP were related to the composite end point of cardiovascular events and deaths. Because heart failure is highly prevalent in patients with type 2 diabetes, and because its prevalence increases with advancing age (13), one may hypothesize that MR-proANP is an important risk factor in elderly patients with type 2 diabetes. Lower prognostic properties of traditional cardiovascular risk factors in elderly (older than 75 years) patients, compared with younger ones (14–18), underline the importance of finding new cardiovascular risk factors in this specific population.

In a previous study by our group, we found that blood pressure was inversely related to mortality in type 2 diabetic patients aged older than 75 years (17). We hypothesized that heart failure may be a possible explanation for the inverse

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relationship. The primary goal of the current study was to investigate the relationship between MR-proANP and mortality in patients with type 2 diabetes, with a special focus on the oldest elderly. We also investigated whether adjustment for MR-proANP, as a measure of heart failure, influenced the inverse relationship of blood pressure with mortality in elderly patients with type 2 diabetes, as published in our Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC)-12 study (17).

## **RESEARCH DESIGN AND METHODS**

### **Study sample**

This study is part of the ZODIAC study; the design and details of this study have been presented elsewhere (19). In this project, general practitioners are assisted by hospital-based nurses specialized in diabetes in their care of patients with type 2 diabetes. The patients consult with the nurses once per year. During the first year (1998) of the ZODIAC study, 1,664 patients were assessed for eligibility. A total of 338 patients that were already treated in the secondary care for their diabetes were excluded from participation. Another 57 patients were excluded because of a very short life expectancy (including patients with active cancer) or insufficient cognitive abilities. Eventually, 1,269 eligible patients were invited to participate. Of those, 1,143 patients agreed to participate in the study.

### **Data collection**

Baseline data, collected in 1998, consisted of a full medical history, including macrovascular complications, medication use, and tobacco consumption. Patients were considered to have 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, such as lipid profile, creatinine levels, urinary albumin/creatinine ratio, blood pressure, weight, and height were collected annually. Blood pressure was measured twice with a Welch Allyn sphygmomanometer in the supine position after at least 5 min of rest. For each visit, the mean blood pressure of two recordings was calculated.

### **Measure of heart failure**

The MR-proANP was used as a measure of heart failure. Previous studies found that

the prognostic properties of MR-proANP are comparable with those of NT-proBNP (6–9). MR-proANP was measured using an automated sandwich immunoassay in 867 out of the 1,143 (75.9%) patients using plasma collected at baseline and kept frozen at  $-80^{\circ}\text{C}$  until analysis according to the instructions of the manufacturer (B.R.A.H.M.S. MR-proANP KRYPTOR; B.R.A.H.M.S. GmbH, Hennigsdorf/Berlin, Germany). The limit of quantitation was 4.5 pmol/L. The interassay coefficient of variation was  $<6.5\%$  for all MR-proANP concentrations  $>10$  pmol/L (4).

### **Clinical end points**

We examined two clinical end points in this study: all-cause mortality and cardiovascular mortality. In early 2009, the vital status and cause of death were retrieved from records maintained by the hospital and the general practitioners. The causes of death were coded according to the International Classification of Diseases, 9th revision (ICD-9).

### **Statistical analyses**

We used SPSS version 16.0 (SAS Institute, Cary, NC) and STATA version 11 (StataCorp, College Station, TX) for statistical analyses. Continuous variables are represented as mean ( $\pm$  SD) for normally distributed values and as median (interquartile range) for the non-normally distributed variables. Variables with a skewed distribution were logarithmically transformed before analysis. Because of missing confounders in 19 patients, all analyses were performed in 848 out of 867 patients (98%) whose MR-proANP values were determined.

**MR-proANP and mortality.** Cox proportional hazard models were used to investigate the association between MR-proANP and mortality with adjustment for selected confounders. The following variables were selected for possible confounding effects: age, sex, smoking (dichotomous), BMI, systolic blood pressure (SBP), duration of diabetes, serum creatinine level, cholesterol-to-HDL ratio, macrovascular complications (dichotomous), albuminuria (dichotomous), and the use of lipid-lowering and antihypertensive medications (dichotomous). We used three different models: a crude model, an age-adjusted and sex-adjusted model, and a model in which we additionally adjusted for all these mentioned variables. Analyses were performed for MR-proANP as a continuous variable (Log MR-proANP). STATA ph test was

used to test the assumption of proportional hazards for baseline predictors. All *P* values for the ph test were nonsignificant, meaning that no substantial deviations were observed.

In case of a significant association between MR-proANP and (cardiovascular) mortality, the following analyses were performed. Calibration was investigated using the Groennesby and Borgan test, assessing the goodness of fit (20). Calibration is a measure of how well predicted probabilities agree with actual observed risk. When the average predicted risk within subgroups of a prospective cohort matches the proportion that actually develops disease, the model is considered well-calibrated. Harrell C statistic was used to compare how well the presence of MR-proANP in the different models used predicts mortality (21). Harrell C value is a rank-based measure (more or less comparable with the area under the receiver-operating characteristic curve). The higher the value, the better the model predicts mortality. Furthermore, the integrated discrimination improvement (IDI) was calculated (22). The IDI can be interpreted as the difference between model-based probabilities for events and nonevents for the models with and without MR-proANP.

**Blood pressure and mortality.** The multivariate model as described was repeated without MR-proANP for patients aged older than 75 years to investigate whether the relationship between blood pressure and mortality differs between the models with and without MR-proANP. Analyses were performed for MR-proANP as a continuous variable and for MR-proANP as a categorical variable. For the categorical variable, we used a cut-off value of 122 pmol/L. This cut-off value was chosen because in patients with known chronic heart failure, this value had the highest diagnostic accuracy for the detection of a left ventricular ejection fraction  $<40\%$  (10). All analyses were performed for SBP, diastolic blood pressure, and pulse pressure.

Additional analyses, in which we used updated mean blood pressure values were performed to adjust for changes in blood pressure over time. This technique is similar to the one used in the UK Prospective Diabetes Study (UKPDS) (23). For example, at 2 years the updated mean of SBP is the average of baseline, 1-year, and 2-year values. The relationship between MR-proANP, blood pressure, and mortality was illustrated by using a

Kaplan Meier curve, for which all patients were categorized into four categories: 1) MR-proANP less than the median and SBP greater than the median; 2) MR-proANP and SBP less than the median; 3) MR-proANP and SBP greater than the median; and 4) MR-proANP greater than the median and SBP less than the median. All hazard ratios of the blood pressure indices refer to a pressure increase of 10 mm Hg.

### Ethics statement

The ZODIAC study and the informed consent procedure were approved by the local medical ethics committee of the Isala Clinics, Zwolle, the Netherlands. Verbal informed consent was obtained for all patients by the participating diabetes specialist nurses and the consent was documented in the patients' records. According to Dutch law, written informed consent was not necessary for this type of study in 1998. All data were analyzed anonymously.

**RESULTS**—The baseline characteristics of the study sample are shown in Table 1. Median MR-proANP in the total study sample was 75 pmol/L (interquartile range, 48–124 pmol/L). For patients older than 75 years, the median MR-proANP value was 122 (80–184) pmol/L. After follow-up for 10 years, 354 out of 848 patients (41.7%) had died. The number of deaths attributable to cardiovascular causes was 152 (42.9%).

Results of the Cox regression analyses for the total study sample as well as the analyses stratified according to age are presented in Table 2. In the overall sample and in both age strata, higher levels of Log MR-proANP were related to increased all-cause and cardiovascular mortality. Harrell C values were the lowest for the patients older than 75 years. The highest IDI values and the largest increases in Harrell C values were observed for adding MR-proANP to the age-adjusted and sex-adjusted models. Adding MR-proANP to the multivariate model resulted in smaller increases in the Harrell C values and in lower IDI values. In patients older than 75 years, no significant IDI values were found for cardiovascular mortality.

Rates of cardiovascular mortality according to different levels of SBP and MR-proANP in patients older than 75 years are shown in Fig. 1. The number of cardiovascular deaths was the highest in patients with MR-proANP levels greater than the median and SBP less than the

**Table 1—Baseline characteristics**

Characteristic	Total (n = 848)	Older than 75 years (n = 225)
Age, years	70 (60–76)	79 (77–82)
Male	366 (43%)	85 (38%)
BMI, kg/m <sup>2</sup>	29.0 (4.8)	27.8 (4.5)
Systolic blood pressure, mm Hg	154.6 (24.9)	156.5 (23.1)
Diastolic blood pressure, mm Hg	84.0 (10.8)	81.8 (10.4)
Pulse pressure, mm Hg	70.5 (20.1)	74.6 (18.2)
Current smoking	157 (19%)	23 (10%)
Albuminuria present	372 (44%)	122 (54%)
Cholesterol/HDL ratio	5.2 (1.5)	4.8 (1.5)
HbA <sub>1c</sub> , %	7.5 (1.3)	7.4 (1.2)
MR-proANP, pmol/L	75 (48–124)	122 (80–184)
Serum creatinine, $\mu$ mol/L	92 (81–104)	97 (85–113)
eGFR, MDRD, mL/min/1.73 m <sup>2</sup>	65 (55–75)	57 (48–67)
Macrovascular complications present	296 (35%)	100 (44%)
Treatment of type 2 diabetes		
Diet only	92 (11%)	24 (11%)
Oral glucose-lowering agents	607 (72%)	164 (73%)
Insulin	149 (18%)	37 (16%)
Receiving antihypertensive treatment	415 (49%)	141 (63%)
ACE inhibitor	193 (23%)	60 (27%)
Beta-blocker	161 (19%)	41 (18%)
Receiving lipid-lowering treatment	94 (11%)	11 (5%)
Duration of type 2 diabetes, years	6 (3–11)	8 (4–13)

eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease. Data are means (SD), medians (interquartile range), or n (%).

median. The lowest number was observed in patients with MR-proANP levels less than the median and SBP greater than the median. Hazard ratios for all-cause and cardiovascular mortality according to different measures of blood pressure are shown in Table 3. All blood pressure measures were inversely related to all-cause mortality. For cardiovascular mortality, we observed inverse relationships for SBP and pulse pressure. Adjustment for MR-proANP did not affect the relationship for any of the blood pressure measures.

Using updated mean blood pressure values and exclusion of deaths in the first 2 years of follow-up did not relevantly change the results (data not shown). The Groennesby and Borgan tests showed that all models were well-calibrated.

**CONCLUSIONS**—Three main conclusions can be drawn from this study. First, higher serum levels of MR-proANP were associated with increased all-cause and cardiovascular mortality in patients with type 2 diabetes, irrespective of age. Second, the consistently lower Harrell C values in old age showed that the predictive capabilities of MR-proANP diminish with advancing age. Third, the inverse

relationship between blood pressure and mortality in old age was not affected by additional adjustment for baseline serum levels of MR-proANP used as a surrogate marker of heart failure.

Adding MR-proANP to models with age, sex, and other cardiovascular risk factors resulted in increased predictive capabilities, as measured with the Harrell C and IDI statistics, of both all-cause and cardiovascular mortality. Although MR-proANP has prognostic value for cardiovascular events in patients from the general population, results from previous studies showed conflicting results concerning the association with cardiovascular mortality (5,9,10). In a recently published study in the general population, significant associations were only found for all-cause mortality (9). However, based on the width of the 95% CI, a relevant association between MR-proANP and cardiovascular mortality could not be excluded. In a study with chronic heart failure patients, the relationship between MR-proANP and cardiovascular mortality was similar to the study with all-cause mortality (10).

The predictive capabilities of the various models, as measured with the Harrell C statistics, increased by adding more

**Table 2—Results of the Cox regression analyses of the logarithmically transformed midregional fragment of pro-A-type natriuretic peptide, the comparison of predictive capability for mortality and cardiovascular events as determined by the Harrell C statistic, and the integrated discrimination improvement for adding the peptide to models 2 and 3, respectively**

	HR (95% CI)	Harrell C (95% CI)	Harrell C (95% CI)*	IDI, % (95% CI)
Total study sample				
All-cause mortality				
Model 1	4.16 (3.53–4.91)	0.74 (0.72–0.77)	NA	NA
Model 2	2.43 (2.01–2.95)	0.79 (0.77–0.82)	0.77 (0.75–0.79)	4.9 (3.3–6.5)
Model 3	2.23 (1.78–2.79)	0.80 (0.78–0.82)	0.79 (0.76–0.81)	2.7 (1.5–3.9)
Cardiovascular mortality				
Model 1	4.79 (3.73–6.16)	0.76 (0.72–0.80)	NA	NA
Model 2	3.29 (2.46–4.40)	0.79 (0.75–0.82)	0.75 (0.71–0.78)	4.6 (2.5–6.7)
Model 3	2.42 (1.74–3.38)	0.82 (0.78–0.85)	0.80 (0.77–0.84)	1.2 (0.0–2.4)
Sample 75 years or younger				
All-cause mortality				
Model 1	4.14 (3.27–5.25)	0.73 (0.70–0.77)	NA	NA
Model 2	2.90 (2.21–3.80)	0.76 (0.73–0.80)	0.73 (0.69–0.77)	6.5 (4.0–8.9)
Model 3	2.36 (1.71–3.24)	0.78 (0.74–0.81)	0.76 (0.72–0.80)	2.7 (1.1–4.4)
Cardiovascular mortality				
Model 1	5.62 (3.98–7.94)	0.77 (0.72–0.82)	NA	NA
Model 2	4.54 (3.09–6.70)	0.78 (0.74–0.83)	0.71 (0.65–0.77)	8.5 (4.7–12.3)
Model 3	2.95 (1.88–4.63)	0.83 (0.78–0.87)	0.80 (0.75–0.85)	2.8 (0.5–5.1)
Sample older than 75 years				
All-cause mortality				
Model 1	2.30 (1.75–3.01)	0.62 (0.58–0.67)	NA	NA
Model 2	2.06 (1.57–2.69)	0.66 (0.61–0.70)	0.61 (0.56–0.65)	7.6 (3.8–1.4)
Model 3	2.07 (1.49–2.86)	0.69 (0.65–0.74)	0.68 (0.64–0.72)	5.4 (1.8–9.0)
Cardiovascular mortality				
Model 1	2.35 (1.54–3.58)	0.62 (0.55–0.69)	NA	NA
Model 2	2.22 (1.45–3.40)	0.63 (0.56–0.70)	0.56 (0.48–0.63)	1.7 (0.0–3.4)
Model 3	1.82 (1.08–3.05)	0.73 (0.67–0.78)	0.71 (0.65–0.77)	0.1 (0.0–0.7)

HR, hazard ratio; IDI, integrated discrimination improvement; NA, not applicable. \*Harrell C values for the models without MR-proANP. Model 2 is adjusted for age and sex. Model 3 is adjusted for age, sex, smoking (dichotomous), BMI, systolic blood pressure, duration of diabetes, serum creatinine level, cholesterol-to-HDL ratio, macrovascular complications (dichotomous), albuminuria (dichotomous), and the use of lipid-lowering and antihypertensive medications (dichotomous);

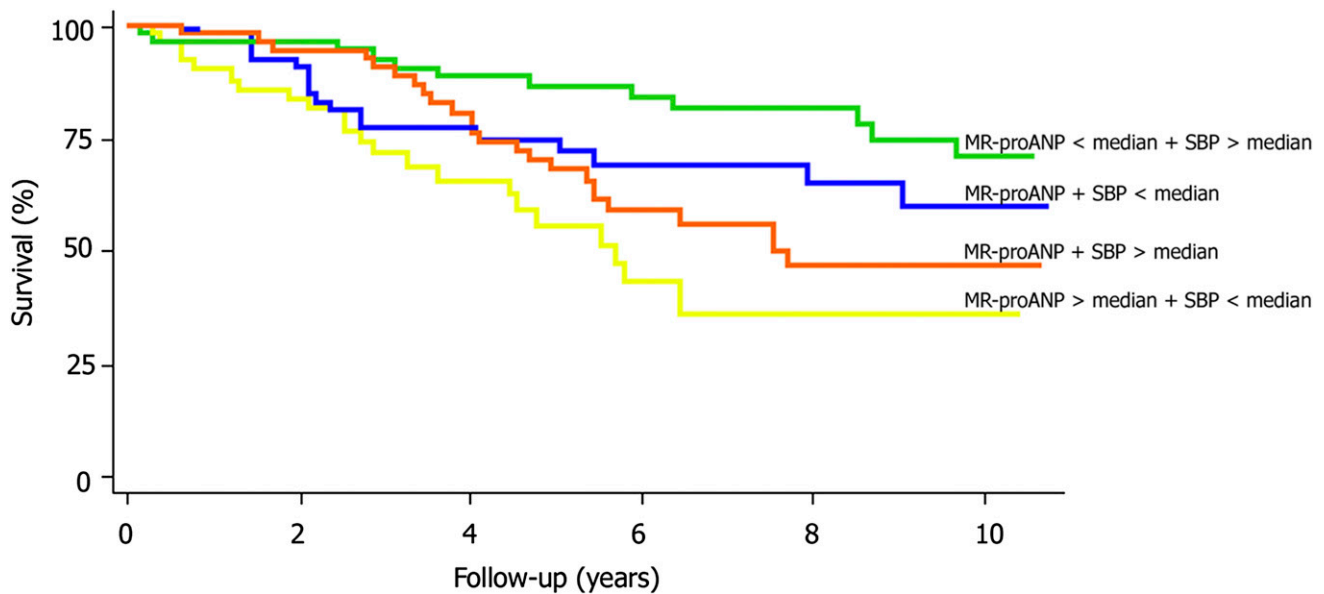
variables to the models. Adding MR-proANP to the models with all conventional risk factors resulted in small increases in the C values. The lowest C values were observed in the group of elderly patients, indicating that the prognostic properties of MR-proANP diminish with advancing age. The IDI for adding MR-proANP to the fully adjusted models in the sample 75 years of age or older was significant for both all-cause and cardiovascular mortality. For elderly patients, improvements in the IDI were only observed for the models investigating all-cause mortality. A plausible explanation for the diminishing properties with advancing age may be the phenomenon of competing risks: the effect of a certain risk factor on mortality declines with advancing age because

other risk factors, including age itself, become more important with advancing age (24).

In the current study we had no data on NT-proBNP; therefore, we were not able to perform a head-to-head comparison. Previous studies, however, have shown that MR-proANP has comparable prognostic and diagnostic capabilities as NT-proBNP, and it can be considered as an alternative for NT-proBNP (6–9). Although our study showed that MR-proANP is an independent risk factor for mortality, its practical implications still remain to be determined. Based on the small improvements in Harrell C values when adding biomarkers to the adjusted models, one may conclude that the additional value of MR-proANP in risk

prediction seems rather limited. However, it is important to realize that it is difficult to achieve further improvements in risk prediction by adding a biomarker to models with all conventional risk factors (5). In the current study, the C values of the fully adjusted models without MR-proANP in the overall population were 0.79 and 0.80 for all-cause and cardiovascular mortality, respectively. These values leave little room for further improvements in risk prediction.

To our knowledge, this is the first study investigating whether adjustment for natriuretic peptides influences the inverse relationship between blood pressure and mortality in old age. It is important to emphasize that because of the observational design of our study, no conclusions about causality can be drawn. The results from Fig. 1, in which the highest mortality rate was observed in patients with the highest MR-proANP levels and the lowest SBP levels, suggest that heart failure may explain the inverse relationship. However, these results were not confirmed by the Cox regression analyses. Even though we tried to adjust for comorbidities and frailty in our analyses, it is not unlikely that these factors still account for the inverse relationship (residual confounding). Odden et al. (25) showed that the relationship between mortality and blood pressure is influenced by walking speed, which can be regarded as a marker of frailty. Higher blood pressure was only associated with increased mortality among faster walkers and no relationship was found for slow walkers (25). A subgroup analysis of the ADVANCE trial, in which predominantly healthy elderly patients were included, showed beneficial results of antihypertensive treatment in patients with type 2 diabetes aged older than 75 years (26). These results confirm the hypothesis that blood pressure is especially an important target for treatment in healthy elderly patients with a low level of frailty. Side effects of antihypertensive medication and excessive lowering of blood pressure also should be considered as explanations for the inverse relationships observed. For example, in the International Verapamil SR-trandolapril trial, a study among patients with type 2 diabetes and coronary artery disease, SBP <115 mm Hg was associated with increased mortality (27). Because the majority of our elderly population had hypertension (84%), and because ~64% received antihypertensive treatment, confounding by



**Figure 1**—Kaplan-Meier curve showing cardiovascular mortality. All elderly patients (n = 225) were divided into four categories. From top to bottom, the lines represent the following: 1) MR-proANP < median and SBP > median (green); 2) MR-proANP and SBP < median (blue); 3) MR-proANP and SBP > median (orange); and 4) MR-proANP > median and SBP < median (yellow).

indication also may have led to the inverse relationships.

The main limitation of our study is its observational design. Also, the results of our blood pressure analyses should be used as hypothesis-generating only, because the use of baseline serum MR-proANP levels as a surrogate measure of heart failure has several limitations. First, because we only adjusted for a single baseline MR-proANP value, we were not able to adjust for heart failure that has

developed during the follow-up period or for potential variability in concentrations. Second, the evidence for which cut-off values should be used in the diagnosis of chronic heart failure is limited. The cut-off value we used was established in a study that compared the prognostic properties of MR-proANP and NT-proBNP (10). Third, levels of natriuretic peptides tend to increase with older age, which makes it more problematic to use these peptides as a marker of heart failure.

However, we did observe increased mortality with higher levels of MR-proANP. Another limitation is that ranges of meaningful improvements are not established for the IDI (22). Furthermore, the IDI originally had not been developed for censored data such as ours. Therefore, the results are difficult to interpret and caution is needed when basing conclusions on the IDI. Strengths of our study were its prospective design, the high number of deaths after the 10-year follow-up period, and the additional analyses, including the updated mean method, we performed.

We conclude that this study shows that MR-proANP is independently associated with all-cause and cardiovascular mortality in patients with type 2 diabetes, and that its predictive capabilities decrease with advancing age.

**Table 3**—Hazard ratios of blood pressure indices for all-cause and cardiovascular mortality in patients older than 75 years

	All-cause mortality	Cardiovascular mortality
Systolic blood pressure		
Multivariate	0.86 (0.80–0.93)	0.90 (0.79–1.00)
With log MR-proANP	0.85 (0.78–0.92)	0.89 (0.78–0.99)
With ANP 122 pmol	0.86 (0.79–0.93)	0.89 (0.79–1.00)
Diastolic blood pressure		
Multivariate	0.85 (0.73–0.99)	0.82 (0.64–1.03)
With log MR-proANP	0.83 (0.71–0.96)	0.80 (0.64–1.01)
With ANP 122 pmol	0.83 (0.71–0.97)	0.79 (0.62–1.04)
Pulse pressure		
Multivariate	0.85 (0.78–0.93)	0.90 (0.78–1.04)
With log MR-proANP	0.84 (0.76–0.92)	0.90 (0.78–1.04)
With ANP 122 pmol	0.85 (0.77–0.93)	0.90 (0.78–1.04)

The hazard ratios (95% CI) refer to a pressure increase of 10 mm Hg. In the multivariate model, sex, smoking (yes or no), BMI, duration of diabetes, serum creatinine level, cholesterol-to-HDL ratio, macrovascular complications (yes or no), albuminuria (yes or no), the use of lipid-lowering and antihypertensive medications (yes or no), and age were selected as potential confounders. In the other models, we additionally adjusted for either log MR-proANP as a continuous variable or MR-proANP with 122 pmol/L as a cut-off value.

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K.J.J.v.H., N.K., K.H.G., and H.J.G.B. designed the study. K.J.J.v.H., J.S., S.J.L.B., and H.J.G.B. acquired the data used in this study.

K.J.J.v.H. and K.H.G. analyzed the data and performed the statistical analyses. K.J.J.v.H. drafted the manuscript. K.J.J.v.H., G.W.D.L., N.K., K.H.G., J.S., G.J.N., S.J.L.B., S.T.H., K.v.d.M., and H.J.G.B. participated in interpretation of the data and in revision of the manuscript. N.K., S.T.H., S.J.L.B., K.v.d.M., and H.J.G.B. supervised the study. All authors had full access to all of the data. K.J.J.v.H. 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

- de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. *Lancet* 2003;362:316–322
- Porapakham P, Porapakham P, Zimmet H, Billah B, Krum H. B-type natriuretic peptide-guided heart failure therapy: A meta-analysis. *Arch Intern Med* 2010;170:507–514
- Ala-Kopsala M, Magga J, Peuhkurinen K, Leipälä J, Ruskoaho H, Leppälüoto, Vuolteenaho O. Molecular heterogeneity has a major impact on the measurement of circulation N-terminal fragments of A- and B-type natriuretic peptides. *Clin Chem* 2004;50:1576–1588
- Morgenthaler NG, Struck J, Thomas B, Bergmann A. Immunoluminometric assay for the midregion of pro-atrial natriuretic peptide in human plasma. *Clin Chem* 2004;50:234–236
- Melander O, Newton-Cheh C, Almgren P, et al. Novel and conventional biomarkers for prediction of incident cardiovascular events in the community. *JAMA* 2009;302:49–57
- von Haehling S, Jankowska EA, Morgenthaler NG, et al. Comparison of midregional pro-atrial natriuretic peptide with N-terminal pro-B-type natriuretic peptide in predicting survival in patients with chronic heart failure. *J Am Coll Cardiol* 2007;50:1973–1980
- Khan SQ, Dhillon O, Kelly D, et al. Plasma N-terminal B-Type natriuretic peptide as an indicator of long-term survival after acute myocardial infarction: comparison with plasma midregional pro-atrial natriuretic peptide: the LAMP (Leicester Acute Myocardial Infarction Peptide) study. *J Am Coll Cardiol* 2008;51:1857–1864
- Potocki M, Breidhardt T, Reichlin T, et al. Comparison of midregional pro-atrial natriuretic peptide with N-terminal pro-B-type natriuretic peptide in the diagnosis of heart failure. *J Intern Med* 2010;267:119–129
- Van Hateren KJ, Alkhalaf A, Kleefstra N, et al. Comparison of the capabilities of a midregional fragment of the pro-A-type natriuretic peptide and the N-terminal pro-B-type natriuretic peptide for predicting mortality and cardiovascular events. *Clin Chem* 2012;58:293–297
- Moertl D, Berger R, Struck J, et al. Comparison of midregional pro-atrial and B-type natriuretic peptides in chronic heart failure: influencing factors, detection of left ventricular systolic dysfunction, and prediction of death. *J Am Coll Cardiol* 2009;53:1783–1790
- Masson S, Latini R, Carbonieri E, et al; GISSI-HF Investigators. The predictive value of stable precursor fragments of vasoactive peptides in patients with chronic heart failure: data from the GISSI-heart failure (GISSI-HF) trial. *Eur J Heart Fail* 2010;12:338–347
- 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
- Cohen-Solal A, Beauvais F, Logeart D. Heart failure and diabetes mellitus: epidemiology and management of an alarming association. *J Card Fail* 2008;14:615–625
- de Ruijter W, Westendorp RG, Assendelft WJ, et al. Use of Framingham risk score and new biomarkers to predict cardiovascular mortality in older people: population based observational cohort study. *BMJ* 2009;338:a3083
- Rönnback M, Isomaa B, Fagerudd J, et al; Botnia Study Group. Complex relationship between blood pressure and mortality in type 2 diabetic patients: a follow-up of the Botnia Study. *Hypertension* 2006;47:168–173
- van Hateren KJ, Landman GW, Kleefstra N, et al. The lipid profile and mortality risk in elderly type 2 diabetic patients: a ten-year follow-up study (ZODIAC-13). *PLoS ONE* 2009;4:e8464
- van Hateren KJ, Landman GW, Kleefstra N, et al. Lower blood pressure associated with higher mortality in elderly diabetic patients (ZODIAC-12). *Age Ageing* 2010;39:603–609
- van Hateren KJ, Landman GW, Kleefstra N, et al. Glycaemic control and the risk of mortality in elderly type 2 diabetic patients (ZODIAC-20). *Int J Clin Pract* 2011;65:415–419
- Ubink-Veltmaat LJ, Bilo HJG, Groenier KH, Rischen RO, Meyboom-de Jong B. Shared care with task delegation to nurses for type 2 diabetes: prospective observational study. *Neth J Med* 2005;63:103–110
- 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
- Harrell FE Jr, Lee KL, Mark DB. Multi-variable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996;15:361–387
- 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
- Adler AI, Stratton IM, Neil HA, et al. Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study. *BMJ* 2000;321:412–419
- Welch HG, Albertsen PC, Nease RF, Bubolz TA, Wasson JH. Estimating treatment benefits for the elderly: the effect of competing risks. *Ann Intern Med* 1996;124:577–584
- Odden MC, Peralta CA, Haan MN, Covinsky KE. Rethinking the association of high blood pressure with mortality in elderly adults: the impact of frailty. *Arch Intern Med* 2012;172:1162–1168
- Ninomiya T, Zoungas S, Neal B, et al; ADVANCE Collaborative Group. Efficacy and safety of routine blood pressure lowering in older patients with diabetes: results from the ADVANCE trial. *J Hypertens* 2010;28:1141–1149
- Cooper-DeHoff RM, Gong Y, Handberg EM, et al. Tight blood pressure control and cardiovascular outcomes among hypertensive patients with diabetes and coronary artery disease. *JAMA* 2010;304:61–68