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Plasma mid-regional pro-atrial natriuretic peptide predicts cardiovascular events in patients with type 2 diabetes independently of subclinical organ damage

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Competing interests: the authors have no known competing interests.
Abstract

Aim: The aim of this study was to investigate the association between plasma MR-proANP and cardiovascular disease (CVD) in a middle-aged population with type 2 diabetes.

Methods: MR-proANP was measured in 690 patients with type 2 diabetes participating in the epidemiological study CARDIPP (Cardiovascular Risk Factors in Patients with Diabetes—a Prospective Study in Primary Care). The outcome variables were incident major adverse cardiovascular events (MACE) and all-cause mortality. Patients were followed using the national Swedish Cause of Death Registry and the Inpatient Register.

Results: During the mean follow-up period of 10.8 years, MACE occurred in 111 patients and 102 patients died. The hazard ratio for an increment of MR-proANP of 1pmol/l adjusted for sex, age, current smoking, previous CVD, HbA1c, serum cholesterol, eGFR, systolic blood pressure, C-reactive protein, aortic pulse wave velocity, left ventricular mass and intima media thickness in the carotid arteries was 1.007 (95% CI 1.000-1.013, P=0.042) for MACE and 1.008 (95% CI 1.001-1.014, P= 0.017) for all-cause mortality.

Conclusions: Elevated MR-proANP levels predict an increased risk for MACE and all-cause mortality in patients with type 2 diabetes independently of CVD risk factors and markers for subclinical organ damage.

Key words: Type 2 diabetes, MR-proANP, Cardiovascular events
1. Introduction

Type 2 diabetes is a major risk factor for the development of cardiovascular disease (CVD) [1-3]. Additionally, patients with type 2 diabetes have a notably increased mortality rate after a first myocardial infarction compared to patients without diabetes [4]. Considering the important clinical context, there is a growing interest in cardiac biomarkers that may contribute to improved cardiovascular risk stratification in patients with type 2 diabetes [5].

Atrial natriuretic peptide (ANP) is a vasoactive peptide, originated from the atrial myocytes, with multiple physiological effects including natriuresis, vasorelaxation and suppression of the renin-angiotensin-aldosterone system [6, 7]. Overall, ANP exerts cardioprotective effects, and the secretion is increased during myocardial strain to counteract the effects of volume and pressure overload [8]. Considering the short half-life of ANP in plasma, mid-regional pro atrial natriuretic peptide (MR-proANP), a stable fragment of the prohormone of ANP, can be used as a surrogate biomarker for ANP [9]. Previous studies have shown that low plasma levels of ANP predict impending onset of diabetes mellitus [10]. However, in patients with type 2 diabetes increased levels of MR-proANP are associated with cardiovascular events and mortality [11, 12]. MR-proANP being negative predictor of diabetes and positive predictor of diabetes complications is a clinical paradox not yet fully explained. Possibly, the levels of MR-proANP reflects separate pathophysiological processes in disparate patient groups. In adipose tissue natriuretic peptides are degraded through clearance receptors (NPR-C) [13]. Obesity, with an abundance of adipose tissue, is in turn highly correlation with type 2 diabetes [14]. On the other hand, in patients with already existing diabetes a proposed mechanism for the positive predictive role for diabetes complications is that hyperinsulinemia leads to impaired cardiac maturation of the natriuretic prohormones, resulting in high concentrations of natriuretic peptides in absence of peripheral effects [15].
Arterial stiffness measured by increased aortic pulse wave velocity (PVW), increased intima media thickness (IMT) in the carotid arteries and increased left ventricular mass are cardiovascular organ damage associated with future cardiovascular events [16-20]. Moreover, biomarkers of inflammation such as C-reactive protein (CRP), have considerable predictive value for cardiovascular events [21, 22].

There is a need for more accurate risk assessment tools for CVD in the general population and for patients with type 2 diabetes than what is currently available. Identification of patients at high risk for developing macrovascular complications by using novel biomarkers will enable individualized therapeutic targets for the prevention of CVD.

The aim of this study was to investigate if MR-proANP adjusted for commonly used cardiovascular risk factors, arterial stiffness, subclinical atherosclerosis in the carotid arteries and increased left ventricular mass (LVM) predicts major adverse cardiovascular events (MACE) and all-cause mortality in a middle-aged population with type 2 diabetes.

2. Methods

2.1 Participants

We analyzed data from the previously [17, 23, 24] described prospective observational cohort study CARDIPP (Cardiovascular Risk Factors in Patients with Diabetes—a Prospective Study in Primary Care, ClinicalTrials.gov Identifier: NCT01049737). The baseline examination was implemented as an extended annual follow up in patients with type 2 diabetes in 22 different primary health care centers in the counties of Östergötland and Jönköping in Sweden. Except for patients with severe physical or mental disease that prevents from participation, such as severe dementia or terminal cancer, all patients with type 2 diabetes mellitus, aged 55-65 years were eligible. A total of 761 patients were recruited by nurses specially trained in
diabetes care during 2005-2008. In this study, only patients with valid measurement of MR-proANP were included, yielding a study population of 690 participants.

2.2 Blood pressure

Office blood pressure was measured in sitting position after 5 min rest by specially trained nurses. At each measurement, the blood pressure values were rounded to the nearest 2 mmHg interval. Appropriately sized cuffs were used in relation to individual arm circumference. The reported office blood pressure values were composed of the mean values from three measurements recorded with one-minute intervals in each patient.

2.3 Aortic pulse wave velocity

PWV measurements were performed by electrocardiogram-gated pulse wave analyses of the carotid and femoral arteries with a Millar pressure tonometer and the SphygomoCor system (Model MM3, AtCor Medical, Sydney, Australia). Calculations of PWV were made by dividing the surface distance with the pulse wave transit time. The surface distance was defined as the distance between the suprasternal notch and the femoral measurement site, subtracted by the distance between the suprasternal notch and the carotid measurement site. Pulse wave transit time was determined by calculation of the difference in time measured between the R-wave and the two above mentioned measurement sites.

2.4 Left ventricular mass

Echocardiographic measurements were performed with the patient in the left lateral decubitus position. Assessment of the LVM was attained by applying the method described by Devereux & Reichek [25]. According to this method, measurements were made in M-mode of the dimensions of the left ventricle in diastole and systole, and interventricular septum thickness and posterior wall thickness in diastole. LVM was calculated according to the Penn convention.
2.5 *Intima media thickness in the carotid arteries*

The method of measuring IMT has been described previously [26]. In brief, B-mode ultrasound was used to measure the IMT in the carotid arteries. Scanning of the carotid artery in longitudinal section was performed with a digital ultrasound system (ATL HDI 5000, Bothell, WA, USA) equipped with a broadband linear transducer (L12-5). Mean values of IMT from both the right and the left sides were used for analyses.

2.6 *Laboratory analyses*

Blood specimens were drawn in the morning following a 10-hour over-night fast. The local laboratories were used for analyzing routine tests such as haemoglobin A1c (HbA1c) and serum lipids. HbA1c was analyzed according to the Swedish Mono-S HPLC standard and subsequently converted into the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) units (mmol/mol). Glomerular filtration rate (eGFR) was estimated by combining the creatinine values with age, sex and ethnicity in the Modification of Diet in Renal Disease (MDRD) formula. Blood samples were frozen for later analysis by high-sensitivity CRP immunoturbidimetric assays, Advia 1800, Siemens Diagnostic Medical Solutions, Erlangen, Germany, with a detection level of 0.12 mg/L at the Department of Laboratory Medicine at Linköping University Hospital. The coefficient of variation (CV) for CRP was 1.6%.

Plasma MR-proANP was analyzed by a high sensitive time-resolved amplified cryptate emission technology assay (B.R.A.H.M.S, KRYPTOR, AG, Hennigsdorf, Germany) with an analytical detection limit of 2.1 pmol/l. For MR-proANP the assay has a detection limit of 30.8 pmol/L and an inter-assay variability of 3.3%.

2.7 *Outcomes*
The outcome variables were incidence of major adverse cardiovascular events (MACE) and all-cause mortality. In the present study MACE was defined as the occurrence of any cardiovascular mortality, International Classification of Diseases (ICD)-10 codes: 100-199, or hospitalization for myocardial infarction, ICD-10: 121, or stroke, ICD-10: 160, 161, and 163. The first occurrence of any of these predefined events was classified as an endpoint event. Outcome variable data were retrieved by linkage of the study database with the Swedish Cause of Death Registry (The National Board of Health and Welfare, Stockholm, Sweden) and the Inpatient Register, using the Swedish national personal identification number for each patient. Patients were followed from inclusion until an event occurred, or until December 31st, 2018.

2.8 Statistics

For the statistical analyses, SPSS software (IBM SPSS Statistics 26, Chicago, IL, USA) was used. P-values <0.05 were regarded as statistically significant. Bivariate correlation analyses were performed to test the strength of correlations between numerical values and was presented as Spearmans´ correlation coefficients ($r$). The associations between the time to first end point event and values of MR-proANP were assessed with univariate and multivariate COX regression analyses, yielding hazard ratios with corresponding 95% confidence intervals (CI). Covariates in the adjusted multivariate model included sex, age, smoking status, previous CVD, HbA1c, total cholesterol, eGFR, office systolic blood pressure, CRP, PWV, LVM and IMT. The study population was then dichotomized with a cutoff at the 75th percentile in levels of MR-proANP. Hence, the cutoff value for MR-proANP was set at 86 pmol/l. Between group differences in baseline characteristics according to MR-proANP were analyzed with t-test for continuous variables with normal distribution and Mann-Whitney U test was used for data with skewed distribution. Chi-2 test was used for
categorical data. Kaplan-Meier curves were constructed and the significance of the difference in event-free survival was assessed with Log-Rank tests.

2.9 Ethics

All participants provided written informed consent. The merging of study data with other registries was approved by the National Board of Health and Welfare and by the Swedish Data Inspection Board in Linköping, Sweden. The study protocol followed the principles in the Declaration of Helsinki.

3. Results:

3.1 Baseline characteristics

Baseline characteristics for study participants with valid measurement of MR-proANP, stratified according to MR-proANP cutoff value of 86 pmol/l, are presented in Table 1. The median follow-up time was 10.8 (IQR 7.9-12.1) years. There were no patients lost to follow-up. The Spearman’s correlation analysis showed that MR-proANP correlated significantly with age (r=0.228, p>0.001), HbA1c (r=-0.130, p=0.001) previous CVD (r=0.118, p=0.002) and LVM (r=0.120, p=0.003). The range of MR-proANP was 17.0-307.1 pmol/l (SD 38.6).

3.2 MR-proANP and MACE

Death from cardiovascular disease or hospitalization for myocardial infarction or stroke occurred in 111 patients (16.1%). Separate univariate Cox regression analyses and multivariate Cox regression analysis including commonly used cardiovascular risk factors, MR-proANP, CRP, PWV, LVM and IMT were performed and the results are presented in Table 2. MR-proANP, sex, HbA1c and PWV remained statistically significant predictors of MACE in the multivariate model. For LVM and previous CVD the significance was lost after
adjustment, Table 2. Figure 2a shows Kaplan-Meier curves where patients in the strata with higher levels of MR-proANP, $\geq 86$ pmol/L, are compared with the patients with MR-proANP < 86 pmol/L. The log-rank test showed that the difference in event-free survival between the two strata was borderline significant ($P=0.058$), while Breslow and Tarone-Ware rendered significant $p$-values of 0.028 and 0.039 respectively.

### 3.3 MR-proANP and all-cause mortality

All-cause mortality occurred in 102 cases (14.8%) in the cohort during the follow-up period. In the univariable models, significant predictors of all-cause mortality were sex, current smoking, CRP, MR-proANP and LVM, but in the multivariable model the significance was lost for sex and LVM, Table 2. Figure 2b shows unadjusted Kaplan-Meier curves for subjects stratified by a cutoff level of MR-proANP 86pmol/l. The log-rank test was statistically significant ($P=0.012$) indicating a difference in time to all-cause mortality in the two strata.
4. Discussion

In this prospective cohort study of 690 patients with type 2 diabetes, we investigated the long-term predictive ability for cardiovascular events and all-cause mortality of plasma MR-proANP. MR-proANP showed a significant association with both MACE and all-cause mortality that remained after adjustment for markers for subclinical organ damage. Between group differences with a cut off of MR-proANP at 86 pmol/l was found using Log-rank test for all-cause mortality.

The clinical relevance of novel diagnostic tools and biomarkers is dependent on sufficient improvement in risk prediction to change the course of therapeutic strategy and improve clinical outcome. In order to find a biomarker with such abilities the challenge is to establish an adequate cut-off point. Therefore, a possible explanation for the lack of in-between group differences in the incidence of MACE during the follow-up period, could be due to a spurious cut-off value. Given that there are no established cut off value for MR-proANP, we a priori treated the 75th percentile point as elevated. This method of setting a cut off value has also been used in previous studies regarding MR-proANP’s prognostic abilities in different populations [27, 28]. Hamada et al. [29] showed that ANP is influenced by sex, age and hemoglobin levels in healthy subjects. Hence, individual cut-off values according to sex and/or age could be more appropriate. In addition, as the follow-up time varies with a large number of censored cases after around 3700 days, the Kapan-Meier curve could be considered unpredictable in the later time period. Accordingly, we believe that the results from of the cox proportional hazard ratios, which rendered significant results, are more relevant.

Our results are in accordance with earlier prospective studies of the cardiovascular predictive role of MR-proANP in a population with type 2 diabetes. In 2009, Maier et al. found that MR-proANP had a significant predictive ability for cardiovascular events after a shorter follow-up
time of 15 months as compared to our study [12]. In a study by Van Hateren et al. the follow-up time of 10 years was similar with the present study and showed a significant association between levels of MR-proANP and all-cause and cardiovascular mortality [11, 12].

The present study investigated the long-term predictive role of MR-proANP for both MACE and all-cause mortality adjusted for subclinical atherosclerosis in the carotid arteries, LVM and arterial stiffness in a population with type 2 diabetes. To our knowledge, no previous study has tested the robustness by integrating MR-proANP, PWV, LVM and IMT in the analyses. Measurement of PWV is acknowledged as the most simple, robust and reproducible method to determine arterial stiffness [30]. In a previous study, analyzing the CARDIPP cohort, PWV predicted MACE after a follow-up time of 8 years. This is in line with the present study, where PWV significantly predicted MACE in both the univariate and multivariate models after a follow-up time of 10 years. Klug et al. showed that increased PWV after primary coronary angioplasty in patients with STEMI was correlated with increased levels of MR-proANP at 4 months follow-up [31]. Development of left ventricle hypertrophy is a sign of subclinical organ damage and is highly correlated with hypertension. Santra et al. demonstrated that high LVM was associated with diabetes mellitus even in a normotensive population [32]. In a population of patients with end-stage renal disease, after adjusting for LVM and ejection fraction the independent predictive ability of ANP for all-cause and cardiovascular mortality was lost [33]. Increased IMT has been shown to predict cardiovascular events [34, 35]. However, Kozakova et al. analysed the association between increased IMT and CVD adjusting for Framingham risk score and found no significant association [36] which is in line with our results.

Our study renders additional knowledge about the association between MR-proANP and CVD by adjusting for several markers for subclinical cardiovascular organ damage as arterial stiffness, increased left ventricular mass and atherosclerosis in the carotid arteries in a
population with type 2 diabetes. This implicates that in a population with type 2 diabetes, MR-proANP plays an independent role in the risk prediction of cardiovascular events and all-cause mortality.

A strength of this study is the relatively large cohort that was recruited with broad inclusion criteria from their primary health care centers. We believe that the wide inclusion criteria for participation makes the population representative of the patient group seen in clinical practice. Moreover, no patient was lost to follow-up; we used clinically relevant end-points and the extensive baseline investigation allowed adjustments for several potential confounders such as PWV and left ventricle mass. Study limitations include an unintentional gender imbalance with predominance to males and the incapability to extrapolate our data to a non-Caucasian population since data on ethnicity was not collected at baseline.

5. Conclusion

In conclusion, higher levels of MR-proANP predicts an increased risk for MACE and all-cause mortality in patients with type 2 diabetes independently of traditional CVD risk factors and markers for subclinical organ damage. Along with previous studies in the field, our results support the use of MR-proANP in future risk algorithms for use in clinical practice for cardiovascular risk stratification of patients with type 2 diabetes.

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Author Contributions

Study concept design: Carl Johan Östgren and Fredrik H. Nyström (PI)

Data Collection: Carl Johan Östgren and Fredrik H. Nyström
Interpretation of results: Simona I. Chisalita, Emilia Gauffin, Carl Johan Östgren and Fredrik H Nyström

Drafting of manuscript: S.I. Chisalita, E. Gauffin, C.J. Östgren

Critical revision of manuscript for important intellectual content: Simona I. Chisalita, E. Gauffin, Carl Johan Östgren and Fredrik H Nyström and Jan Engvall
References


Figure 1: Flow chart illustrating the study population
Tables and figures

Table 1. Baseline characteristics of the CARDIPP study population divided according to MR-proANP cutoff level 86 pmol/L

<table>
<thead>
<tr>
<th>Variable</th>
<th>All n = 690</th>
<th>MRpro-ANP &lt;86 n = 512</th>
<th>MR-proANP ≥86 n = 178</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR-proANP (pmol/L)</td>
<td>73.4 ± 3.1</td>
<td>56.3 ± 15.7</td>
<td>122.8 ± 42.1</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>66</td>
<td>64</td>
<td>70</td>
<td>0.148</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.6 ± 3.1</td>
<td>60.4 ± 3.0</td>
<td>61.1 ± 3.2</td>
<td>0.002*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2 ± 4.7</td>
<td>30.1 ± 4.6</td>
<td>30.3 ± 4.9</td>
<td>0.730</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>104.5 ± 12.0</td>
<td>104.2 ± 11.8</td>
<td>105.1 ± 12.7</td>
<td>0.275</td>
</tr>
<tr>
<td>Current smoking</td>
<td>18.9</td>
<td>18.8</td>
<td>19.3</td>
<td>0.874</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>7.2 ± 6.2</td>
<td>7.2 ± 6.4</td>
<td>7.1 ± 5.4</td>
<td>0.801</td>
</tr>
<tr>
<td>Previous CVD</td>
<td>68.2</td>
<td>65.2</td>
<td>76.9</td>
<td>0.005*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136.9 ± 16.3</td>
<td>136.4 ± 15.9</td>
<td>138.1 ± 17.5</td>
<td>0.469</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80.0 ± 10.1</td>
<td>79.9 ± 10.0</td>
<td>80.3 ± 10.5</td>
<td>0.592</td>
</tr>
<tr>
<td>Thiazide diuretics</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>0.586</td>
</tr>
<tr>
<td>Loop diuretics</td>
<td>8</td>
<td>5</td>
<td>14</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>35</td>
<td>27</td>
<td>59</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ACE-I/ARB</td>
<td>44</td>
<td>41</td>
<td>53</td>
<td>0.004*</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>16</td>
<td>15</td>
<td>20</td>
<td>0.073</td>
</tr>
<tr>
<td>Serum Cholesterol (mmol/L)</td>
<td>4.7 ± 1.0</td>
<td>4.7 ± 1.0</td>
<td>4.6 ± 0.9</td>
<td>0.252</td>
</tr>
<tr>
<td>HbA1c NGSP (%)</td>
<td>6.1 (1.1)</td>
<td>6.1 (1.1)</td>
<td>6.0 (1.1)</td>
<td>0.055</td>
</tr>
<tr>
<td>HbA1c IFCC (mmol/mol)</td>
<td>(53.0 ± 11.6)</td>
<td>(53.4 ± 11.6)</td>
<td>(51.9 ± 11.7)</td>
<td>0.055</td>
</tr>
<tr>
<td>S-creatinine (µmol/L)</td>
<td>85.4 ± 16.3</td>
<td>84.4 ± 16.2</td>
<td>88.2 ± 16.4</td>
<td>0.008*</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>74.9 ± 16.4</td>
<td>75.5 ± 16.2</td>
<td>73.0 ± 17.0</td>
<td>0.052</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>3.6 ± 5.3</td>
<td>3.6 ± 5.4</td>
<td>3.5 ± 5.0</td>
<td>0.569</td>
</tr>
<tr>
<td>Pulse wave velocity (m/s)</td>
<td>10.3 ± 2.1</td>
<td>10.2 ± 2.1</td>
<td>10.6 ± 2.1</td>
<td>0.026*</td>
</tr>
<tr>
<td>Left ventricular mass (g/m²)</td>
<td>119.5 ± 28.9</td>
<td>116.7 ± 27.9</td>
<td>127.3 ± 30.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Intima media thickness (mm)</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.562</td>
</tr>
</tbody>
</table>

All linear variables are presented with mean ± standard deviation and categorical variables are presented as percentage (%).

Missing data for waist circumference was 4, for smoking status was 8, for diabetes duration was 44, for previous CVD was 21, for systolic blood pressure was 3, for diastolic blood
pressure was 3, for thiazide diuretics was 2, for loop diuretics was 2, for ACE-I/ARB was 1, for calcium channel blockers was 2, for serum cholesterol was 22, for HbA1c was 10, for S-creatinine and eGFR was 23, for CRP was 31, for pulse wave velocity was 53, for left ventricle mass was 84 and for Intima media thickness was 18.
Table 2. Crude and adjusted hazard ratios with 95% confidence intervals (CI) for MR-proANP, classical cardiovascular risk factors and markers for subclinical organ damage for the prediction of MACE and all-cause mortality.

<table>
<thead>
<tr>
<th></th>
<th>MACE</th>
<th>All-cause mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariable</td>
<td>Multivariable</td>
</tr>
<tr>
<td>MRproANP (pmol/L)</td>
<td><strong>1.008 (1.005-1.012)</strong>***</td>
<td><strong>1.007 (1.000-1.013)</strong>*</td>
</tr>
<tr>
<td>Male</td>
<td><strong>2.139 (1.352-3.386)</strong>***</td>
<td><strong>2.838 (1.339-6.014)</strong>**</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.025 (0.963-1.091)</td>
<td>0.935 (0.859-1.017)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>1.161 (0.727-1.855)</td>
<td>1.485 (0.799-2.759)</td>
</tr>
<tr>
<td>Previous CVD</td>
<td><strong>1.625 (1.029-2.567)</strong>*</td>
<td>1.545 (0.844-2.826)</td>
</tr>
<tr>
<td>HbA1c-IFCC (mmol/mol)</td>
<td><strong>1.028 (1.015-1.041)</strong>***</td>
<td><strong>1.021 (1.003-1.038)</strong>*</td>
</tr>
<tr>
<td>Serum Cholesterol (mmol/L)</td>
<td>1.023 (0.847-1.237)</td>
<td>1.106 (0.845-1.448)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>1.002 (0.990-1.014)</td>
<td>1.002 (0.988-1.016)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>1.010 (0.999-1.022)</td>
<td>1.004 (0.989-1.019)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.023 (0.994-1.052)</td>
<td>1.029 (0.983-1.078)</td>
</tr>
<tr>
<td>Pulse wave velocity (m/s)</td>
<td><strong>1.184 (1.091-1.284)</strong>***</td>
<td><strong>1.178 (1.060-1.309)</strong>**</td>
</tr>
<tr>
<td>Left ventricular mass (g/m²)</td>
<td>1.010 (1.004-1.017)**</td>
<td>1.002 (0.993-1.011)</td>
</tr>
<tr>
<td>Intima media thickness (mm)</td>
<td>1.381 (0.527-3.616)</td>
<td>0.966 (0.272 – 3.427)</td>
</tr>
</tbody>
</table>

* P ≤ 0.05  
** P ≤ 0.01  
*** P ≤ 0.001  

In the univariable columns, the unadjusted hazard ratios are presented for each variable. In the multivariable columns adjusted hazard ratios are presented based on multivariable Cox regression models where MR-proANP, sex, age, smoking status, previous cardiovascular disease (CVD), HbA1c, serum cholesterol, estimated glomerular filtration rate (eGFR), office systolic blood pressure, c-reactive protein (CRP), aortic pulse wave velocity (PWV) and left
ventricular mass were entered. A total of 500 patients with complete data for all the variables were included in the multivariate models.
**Figure 2a** – Proportions of patients without the composite outcome MACE in relation to the level of MR-proANP at baseline in 690 patients with type 2 diabetes. The blue line represents subjects with levels of MR-proANP lower than 86 pmol/L and the red line represents subjects with levels of MR-proANP equal to or higher than 86 pmol/L at baseline. A magnification of the graph with cropped y-axis is shown to the right.
Figure 2b – Proportions of patients surviving in relation to the level of MR-proANP at baseline in 690 patients with type 2 diabetes. The blue line represents subjects with levels of MR-proANP lower than 86 pmol/L and the red line represents subjects with levels of MR-proANP equal to or higher than 86 pmol/L at baseline. A magnification of the graph with cropped y-axis is shown to the right.

<table>
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<tr>
<th>Number at Risk</th>
<th>1000</th>
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<th>3000</th>
<th>4000</th>
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<tr>
<td>MRproANP&lt;86</td>
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<td>488</td>
<td>473</td>
<td>362</td>
<td>152</td>
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<tr>
<td>MRproANP≥86</td>
<td>171</td>
<td>163</td>
<td>156</td>
<td>116</td>
<td>45</td>
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</table>

<table>
<thead>
<tr>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Log Rank (Mantel-Cox)</td>
<td>0.013*</td>
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<tr>
<td>Breslow</td>
<td>0.004*</td>
</tr>
<tr>
<td>Tarone-Ware</td>
<td>0.009*</td>
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