DR. ARTUR FEDOROWSKI (Orcid ID : 0000-0002-5352-6327)

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Biomarkers of microvascular endothelial dysfunction predict incident dementia: a population-based prospective study

H. Holm[1, 2], K. Nägga[3], ED. Nilsson[3], F. Ricci[4, 5], O. Melander[1, 2], O. Hansson[3], E. Bachus[1, 2], M. Magnusson[1, 6] *, and A. Fedorowski[1, 6]*.

* Shared senior authorship.

Affiliations:

[1] Department of Clinical Sciences, Lund University, Clinical Research Center, Malmö, Sweden

[2] Department of Internal Medicine, Skåne University Hospital, Malmö, Sweden

[3] Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden

[4] Institute of Cardiology, "G. d'Annunzio" University, Chieti, Italy.

[5] Institute for Advanced Biomedical Technologies, Department of Neuroscience and Imaging, "G. d'Annunzio" University, Chieti, Italy.

[6] Department of Cardiology, Skåne University Hospital, Malmö, Sweden

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Corresponding author: Artur Fedorowski, MD, Assoc. Prof; Dept. of Cardiology, Inga Marie Nilssons gata 46, Skåne University Hospital, 205 02 Malmö, Sweden. Fax: +46 40 33 62 25, Phone: +46 40 33 10 00. Email: artur.fedorowski@med.lu.se

Abstract

Background

Cerebral endothelial dysfunction occurs in a spectrum of neurodegenerative diseases. Whether biomarkers of microvascular endothelial dysfunction can predict dementia is largely unknown. We explored the longitudinal association of midregional pro-atrial natriuretic peptide (MR-proANP), C-terminal endothelin-1 (CT-proET-1) and midregional pro-adrenomedullin (MR-proADM) with dementia and subtypes among community-dwelling older adults.

Methods

A population-based cohort of 5,347 individuals (men, 70%; age, 69±6 years) without prevalent dementia provided plasma for determination of MR-proANP, CT-proET-1 and MR-proADM. Three-hundred-and-seventy-three patients (7%) were diagnosed with dementia (120 Alzheimer's disease, 83 vascular, 102 mixed, and 68 other aetiology) over a period of 4.6±1.3 years. Relations between baseline biomarker plasma concentrations and incident dementia were assessed using multivariable Cox regression analysis.

Results

Higher levels of MR-proANP were significantly associated with increased risk of all-cause and vascular dementia (hazard ratio [HR] per 1 SD: 1.20, 95% confidence interval [CI], 1.07-1.36; p=0.002, and 1.52; 1.21-1.89; p<0.001, respectively). Risk of all-cause dementia increased across the quartiles of MR-proANP (p for linear trend=0.004; Q4, 145-1681pmol/L vs. Q1, 22-77pmol/L: HR: 1.83; 95%CI, 1.23-2.71), and was most pronounced for vascular type (p for linear trend=0.005: HR: 2.71; 95%CI, 1.14-6.46). Moreover, the two highest quartiles of CT-proET-1 predicted vascular dementia with a cut-off value at 68 pmol/L (Q3-Q4, 68-432pmol/L vs. Q1-Q2,4-68pmol/L; HR: 1.94; 95%CI, 1.12-3.36). Elevated levels of MR-proADM indicated no increased risk of developing dementia after adjustment for traditional risk factors.

Conclusions

Elevated plasma concentration of MR-proANP is an independent predictor of all-cause and vascular dementia. Pronounced increase in CT-proET-1 indicates higher risk of vascular dementia.

Keywords: dementia; biomarkers; atrial natriuretic peptide; adrenomedullin; endothelin; endothelial dysfunction.

Introduction

Dementia is a comprehensive term for central nervous system disorders associated with failure of cognitive functions including memory, mental speed, executive functions, and speech [1]. The prevalence of dementia increases exponentially with advancing age [2], while its prevention and treatment constitute a serious global health issue [3]. Cardiovascular (CV) risk factors such as hypertension, smoking, dyslipidaemia, and prevalent CV disease have been associated with increased incidence of dementia [4]. Further, long-term blood pressure variability has also been associated with increased risk of dementia [5].

Although prevention and treatment of CV disease have made substantial advances in the last decades, a better understanding of the role of modifiable CV risk factors in the development of dementia is eagerly awaited. Recently, regulators of endothelial function and vasodilation including atrial natriuretic peptide (ANP), endothelin-1 (ET-1) and adrenomedullin (ADM) have been proposed as markers of microvascular pathology and increased CV risk in both healthy individuals and patients with established atherosclerotic disease [6, 7]. While bioactive peptides are very difficult to measure, recently developed assays detect their stable precursor fragments, i.e. midregional pro-atrial natriuretic peptide (MR-proANP), C-terminal endothelin-1 precursor (CT-pro ET1) and midregional proadrenomedullin (MR-proADM). Elevated CT-proET-1 has been associated with higher incidence of heart failure and myocardial infarction [8, 9], whereas elevated MR-proADM and MR-proANP herald higher prevalence of hypertension and increased mortality in heart failure [10-12]. The cross-sectional association between elevated levels MR-proANP, CT-proET-1 and MR-proADM with Alzheimer's disease has also been reported [13, 14]. Given the known impact of MR-proANP, CT-proET-1 and MR-proADM on vascular function [15], we hypothesized that these three vasoactive neurohormones would predict the development of incident dementia, in particular of vascular type. To test this hypothesis, we prospectively assessed the incidence of all-cause dementia and its main subclasses in relation to baseline levels of MR-proANP, CT-proET-1 and MR-proADM in a population-based cohort of older individuals.

Methods

Study design and population

The Malmö Preventive Project (MPP) was funded in the mid 1970s to explore CV risk factors in general population, and enrolled 33,346 individuals living in Malmö [16]. Between 2002 and 2006, a total of 18,240 original participants responded to the invitation (participation rate, 70.5%) and were screened including a comprehensive physical examination and collection of blood samples[17]. The re-examination in MPP is in the present study regarded as the baseline. Plasma levels of MR-proANP, CT-proET-1 and MR-proADM were measured in a random and representative subset of 5,418 participants. Seventy-one individuals who were either diagnosed with dementia before the screening procedure (n=64) or had missing values (n=7) were excluded yielding a study sample of 5,347 individuals. For the valid diagnosis of incident dementia, we assumed a period of at least 30 days after the screening examination and blood sampling. An informed consent was obtained from all participants and the Ethical Committee of Lund University, Lund, Sweden, approved the study protocol.

Dementia diagnosis

Information about dementia diagnoses was requested from the Swedish National Patient Register (SNPR) and covered the period from MPP baseline through Dec 31, 2009. The diagnoses in the register were collected according to different revisions of the International Classification of Diseases (ICD) codes 290, 293 (ICD-8), 290, 331 (ICD-9) or F00, F01, F03, G30 (ICD-10). Since 1987, SNPR includes all in-patient care in Sweden and, in addition, contains data on outpatient visits including day surgery and psychiatric care from both private and public caregivers recorded after 2000. All-cause dementia was diagnosed according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-III revised edition, while the DSM-IV criteria were applied for the Alzheimer's disease and vascular dementia diagnoses. Diagnoses were validated by a thorough review of medical records as well as neuroimaging data when available. A research physician assigned the final diagnosis for each patient and a geriatrician specialized in cognitive disorders was consulted in

unresolved cases. Four-hundred-and-seventy-one individuals had a dementia diagnosis registered in SNPR. Of these, 373 cases were validated and included 120 Alzheimer's disease, 83 vascular dementia, 102 mixed type, 35 Lewy-body dementia/Parkinson's dementia, 4 fronto-temporal dementia, and 29 unspecified type.

Statistical methods

Group differences in continuous variables between dementia-positive and -negative individuals were compared using One-Way ANOVA test, whereas categorical variables were compared using Pearson's chi-square test. The distribution of all three biomarkers was right-skewed and log-transformation was performed. Cox regression model was applied and log-transformed and standardized values of MR-proANP, CT-proET-1 and MR-proADM were entered as independent variables. The adjusted model was built by entering age, gender, systolic blood pressure (SBP), antihypertensive treatment, smoking, diabetes, plasma-high-density lipoprotein (HDL) and prevalent stroke as covariates. Further, the biomarkers were stratified into quartiles and used for Kaplan-Meier survival analysis. Then, the quartiles were used as an independent variable for Cox regression analysis in order to test the risk increment across the quartiles of tested biomarkers. The time variable was calculated as follow-up time between screening and date of dementia diagnosis, death, or end of follow-up through Dec 31, 2009. All analyses were performed using IBM SPSS Statistics version 23 (SPSS Inc., Chicago, IL, USA). All tests were two-sided, whereby $p<0.05$ was considered statistically significant.

Results

The mean duration of follow-up period from screening to dementia diagnosis or end of follow-up was 4.6±1.3 years. Study participants who developed dementia (n= 373) were older, more likely to be female, had higher plasma concentration of HDL and higher proportion of statin treatment, and had lower SBP compared with individuals free from dementia, while proportion of antihypertensive treatment was slightly but non-significantly higher in the dementia-positive group (43% vs. 39%, p=0.104) (Table 1). Plasma concentrations of MR-proANP, CT-proET-1 and MR-proADM were

higher in participants who developed dementia.

Risk of dementia and levels of MR-proANP, CT- proET-1 and MR-proADM

In the multivariable-adjusted Cox proportional hazard models, higher levels of MR-proANP were significantly associated with increased risk of all-cause and vascular dementia (hazard ratio (HR) per 1SD: 1.20, 95% confidence interval (CI), 1.07-1.36; p=0.002, and 1.52; 1.21-1.89; p<0.001, respectively) (Table 2).

None of the biomarkers showed significant association with Alzheimer's disease, but higher MR-proANP tended to predict mixed dementia type (HR: 1.25; 95%CI, 0.99-1.58, p=0.057). Further adjustment for baseline education level and exclusion of incident heart failure cases that were recorded during follow-up period (n=151) did not substantially change our results.

Risk of dementia and quartiles of MR-proANP, CT- proET-1, and MR-proADM

The risk of both all-cause and vascular dementia increased across quartiles of MR-proANP (Figures 1 and 2, and Table 3).

The risk of vascular dementia increased across quartiles of CT-proET-1 (p for linear trend = 0.028; Table 3) with a distinct cut-off point between second and third quartile. The two highest quartiles of CT-proET-1 predicted vascular dementia with a cut-off value at 68 pmol/L (Q3-Q4, 68-432 pmol/L vs. Q1-Q2, 4-68 pmol/L; HR: 1.94; 95%CI, 1.12-3.36) (Figure 3). The highest quartile of MR-proADM was associated with risk of all-cause dementia in the unadjusted model, but not after controlling for traditional risk factors (Table 3).

Discussion

In this study, we have found that in community-dwelling older adults elevated plasma concentrations of MR-proANP are independently associated with higher risk of incident all-cause and vascular dementia. Moreover, we have observed that distinctly elevated CT-proET-1 level independently predicts vascular dementia.

The increased incidence of dementia has been previously associated with the increasing prevalence of major CV risk factors and history of CV disease [4]. However, studies appointing the linkage between circulating CV biomarkers and the risk of dementia are very sparse. Here we present the longitudinal relationship between increased plasma concentrations of biomarkers associated with microcirculatory dysfunction and dementia development in older individuals.

MR-proANP

ANP is a cardiac hormone released by the atrium in response to wall stretch [18]. Through inhibition of the renin–angiotensin–aldosterone system and stimulation of natriuresis and vasodilatation, the role of ANP in blood pressure regulation is now fairly well understood [18]. In the present study, individuals with all-cause dementia had lower systolic and diastolic blood pressure at baseline examination. This finding is in accordance with previous reports, where blood pressure declines within the years before clinical appearance of dementia [19]. In contrast, elevated BP in midlife has been implicated as a crucial risk factor for dementia development [20]. Hence, the elevated plasma levels of MR-proANP observed in individuals with incident dementia may reflect a feedback mechanism to induce salt and water excretion and lower BP in the presence of hypertension. However, while BP reduction entails CV protective effects in healthy older individuals, it may also lead to cerebral hypoperfusion, an important factor involved with progression of both vascular dementia and Alzheimer's disease [21]. In contrast to previous studies, we did not observe any association between increased plasma concentrations of MR-proANP and Alzheimer's disease [13]. Increased levels of ANP seen in individuals with incident dementia may also counteract the oxidative stress [22], which has previously been indicated as a crucial contributor for development of vascular dementia [23]. Among individuals with mild cognitive decline, MR-proANP has been demonstrated as an important predictor of the effect of antihypertensive treatment on conversion to manifest Alzheimer dementia[24]. Protective effect of antihypertensive therapy is clearly demonstrated in younger individuals, and early initiation of a BP lowering therapy appears to be a crucial factor. Alternatively, elevated MR-proANP levels at younger age might define subgroup of patients with marked endothelial dysfunction that would benefit most from the therapy.

CT-proET-1

Endothelin 1 (ET-1) is a potent vasoconstrictive peptide which is mainly synthetized by endothelial and vascular smooth muscle cells and is considered to be a crucial contributor in the progression of endothelial dysfunction and CV disease since it stimulates inflammation and proliferation [25, 26]. Elevated levels of ET-1 have been observed in atherosclerosis, hypertension, chronic heart failure, and myocardial infarction [13].

In a cross sectional study of older individuals, elevated levels of ET-1 has been associated with worsening of psychomotor speed and language [27]. In addition, higher ET-1 levels have been found in brains of patients with Alzheimer's disease compared with control tissues [14]. Down regulation of ET-1 in presence of white matter lesions have been argued to be a protective mechanism to reduce vasoconstriction, increase cerebral blood flow and minimize the risk of ischaemic damage [28]. Conversely, up regulation of CT-proET-1 expression in patients with orthostatic hypotension [29], a well-known condition associated with chronic cognitive impairment and dementia development, might represent a counter-regulatory response to chronic hypotension aimed to preserve adequate cerebral perfusion.

Lipid levels and dementia

Higher HDL but not LDL plasma concentration among dementia-positive participants deserves a commentary. One possible explanation is that participants who developed dementia were more frequently treated with statins at baseline (27 vs. 19%, p=0.001). On the other hand, LDL levels were not different between the groups. This unexpected observation warrants further epidemiological studies in independent population for verification.

Clinical implications

These relatively novel CV biomarkers of endothelial dysfunction may be employed in the stratification of middle-aged patients into high vs. low-risk groups for developing dementia, in particular of the vascular type. Individuals who are in the highest quartiles of both MR-proANP and CT-proET-1 could be targeted for a specific therapy in order to reduce or prevent the neurodegenerative effects of vascular aging[30].

Limitations

First, as primary care is not covered by the SNPR, an underestimation of dementia cases cannot be excluded. Second, study participants were predominantly of European ancestry; hence results cannot be generalized to other ethnic groups.

Conclusions

Elevated plasma concentrations of MR-proANP predict all-cause and vascular dementia while pronounced increase in CT-proET-1 indicates higher risk of vascular dementia. This observation may be used in stratification of middle-aged and older adults into different risk groups of developing dementia and targeted interventions.

Conflict of interest

No conflicts of interest were declared.

The manuscript has been handled by an external editor, Professor Sam Schulman, Thrombosis Service, McMaster Clinic, HHS - General Hospital, Canada

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LEGEND TO FIGURES

Figure 1. Kaplan-Meier curves for all-cause dementia cumulative incidence from re-examination (2002-2006) to the end of follow-up (31st Dec 2009) among 5,344 participants in MPP stratified according to quartiles of MR-proANP.

Figure 2. Kaplan-Meier curves for vascular dementia cumulative incidence from re-examination (2002-2006) to the end of follow-up (31st Dec 2009) among 5,344 participants in MPP stratified according to quartiles of MR-proANP.

Figure 3. Kaplan-Meier curves for vascular dementia cumulative incidence from re-examination (2002-2006) to the end of follow-up (31st Dec 2009) among 5,337 participants in MPP stratified according to quartiles of CT-proET-1.

Table 1. Characteristics of the study population (*n* = 5347)

Characteristics	Dementia positive (n=373)	Dementia negative (n= 4974)	P value
Age (years)	73±5	69±6	<0.001
Sex, (% male)	58	71	<0.001
Current smoker, n (%)	48 (13)	795 (16)	0.062
Supine systolic BP (mmHg)	143±21	146 ±21	0.006
Supine diastolic BP (mmHg)	81±11	84±11	<0.001
Heart rate (bpm)	71±12	71±12	0.54
Antihypertensive treatment, n (%)	159 (43)	1948 (39)	0.10
Statin treatment, n (%)	99 (27)	957 (19)	0.001
Prevalent stroke, n (%)	1 (0.3)	4 (0.1)	0.30
Plasma cholesterol (mmol/l)	5.6±1.1	5.5±1.1	0.67
Plasma LDL (mmol/l)	3.6±1	3.6±1	0.66
Plasma HDL (mmol/l)	1.44±0.4	1.37±0.4	<0.001
Diabetes, n (%)	47 (13)	620 (13)	0.50
MR-proANP (pmol/l)	151± 85	123±82	<0.001
MR-proADM (nmol/l)	0.80± 0.23	0.74±0.23	<0.001
CT-proET-1 (pmol/l)	75± 21	71±19	<0.001

BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; MR-proANP: midregional pro-atrial natriuretic peptide; MR-proADM: midregional pro-adrenomedullin; CT-proET-1: C-terminal endothelin-1.

Table 2. Relations between microcirculatory biomarkers (MR-proANP, CT-proET-1, and MR-proADM) and risk of dementia in multivariable-adjusted Cox regression model.

Biomarkers	Type of dementia	
	aHR (95%CI)	P value
	All-cause dementia (n=373)	
MR-proANP	1.20(1.07-1.36)	0.002
CT-proET-1	1.04(0.93-1.17)	0.51
MR-proADM	1.00(0.89-1.14)	0.91
	Alzheimer dementia (n=120)	
MR-proANP	0.97 (0.77-1.21)	0.77
CT-proET-1	0.94 (0.77-1.16)	0.56
MR-proADM	0.87 (0.70-1.07)	0.19
	Vascular dementia (n=83)	
MR-proANP	1.52 (1.21-1.89)	<0.001
CT-proET-1	1.22 (0.98-1.53)	0.073
MR-proADM	1.20 (0.94-1.52)	0.14

	Mixed dementia (n=102)	
MR-proANP	1.25 (0.99-1.58)	0.057
CT-proET-1	1.07 (0.85-1.34)	0.57
MR-proADM	0.94 (0.74-1.19)	0.63

CI: confidence interval; CT-proET-1: C-terminal endothelin-1; aHR: adjusted hazard ratio; MR-proADM: midregional pro-adrenomedullin; MR-proANP: midregional pro-atrial natriuretic peptide; CI; confidence interval. Adjusted for age age, gender, systolic blood pressure, anti-hypertensive treatment, smoking, diabetes, and plasma-HDL, and prevalent stroke. All biomarker plasmatic concentrations were log-transformed.

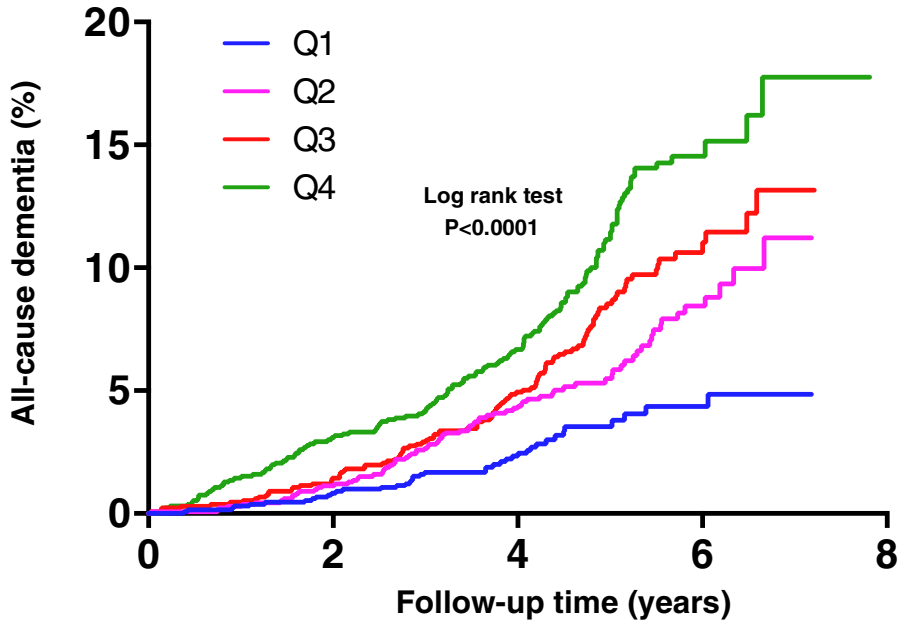
Table 3. Risk of incident dementia across quartiles of microcirculatory biomarkers (MR-proANP, CT-proET-1, and MR-proADM) in multivariable-adjusted Cox regression model.

Biomarker quartiles	**n**	**All-cause dementia**		**Vascular dementia**	
		aHR (95% CI) **p (linear trend)**	P value	aHR (95% CI) **p (linear trend)**	P value
MR-proANP		**p =0.004**		**p=0.005**	
Q1 (22-77 pmol/l)	1334	*Reference*		*Reference*	
Q2 (77-104 pmol/l)	1332	1.48 (1.01-2.19)	0.048	1.19 (0.46-3.09)	0.72
Q3 (104-145 pmol/l)	1333	1.59 (1.08-2.34)	0.018	1.91 (0.79-4.62)	0.15

Q4 (145-1681 pmol/l)	1331	1.83 (1.23-2.71)	0.003	2.71 (1.14-6.46)	0.024
CT-proET-1		**p =0.14**		**p =0.028**	
Q1 (4-60 pmol/l)	1331	*Reference*		*Reference*	
Q2 (60-68 pmol/l)	1332	0.85 (0.60-1.20)	0.36	1.20 (0.48-2.99)	0.70
Q3 (68-78 pmol/l)	1332	1.17 (0.85-1.61)	0.34	2.12 (0.93-4.81)	0.073
Q4 (78-432 pmol/l)	1328	1.16 (0.84-1.62)	0.37	2.18 (0.96-4.94)	0.062
MR-proADM		**p =0.80**		**p =0.051**	
Q1 (0.12-0.61 nmol/l)	1335	*Reference*		*Reference*	
Q2 (0.61-0.70 nmol/l)	1333	0.97 (0.69-1.35)	084	1.24 (0.51-3.01)	0.63
Q3 (0.70-0.83 nmol/l)	1329	0.83 (0.60-1.17)	0.29	1.21 (0.51-2.84)	0.66
Q4 (0.83-4.38 nmol/l)	1332	1.06 (0.76-1.48)	0.75	2.02 (0.89-4.60)	0.092

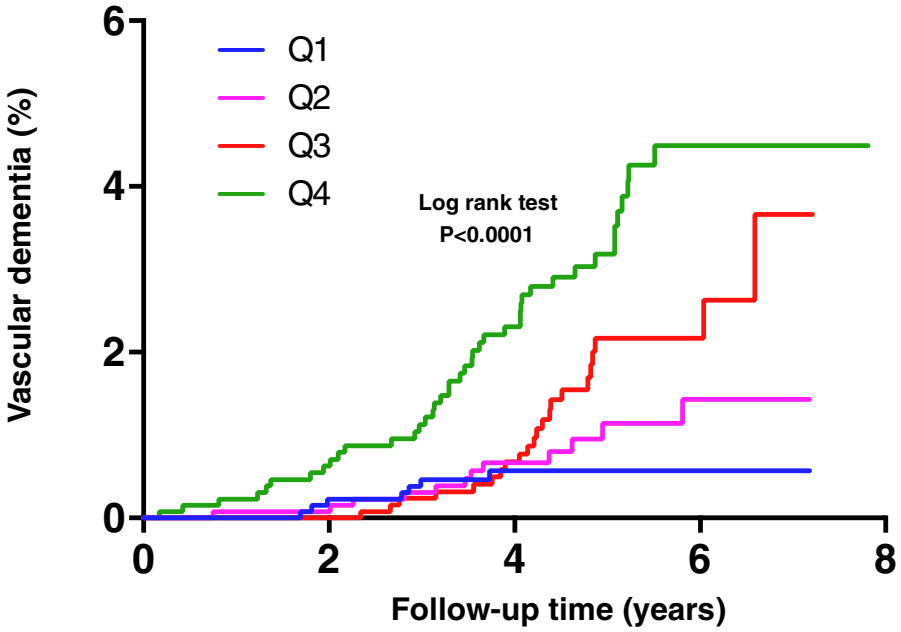
CI: confidence interval; CT-proET-1: C-terminal endothelin-1; aHR: adjusted hazard ratio; MR-proADM: midregional pro-adrenomedullin; MR-proANP: midregional pro-atrial natriuretic peptide. Adjusted for age, gender, systolic blood pressure, anti-hypertensive treatment, smoking, diabetes, plasma-LDL and prevalent stroke.

Figure 1.



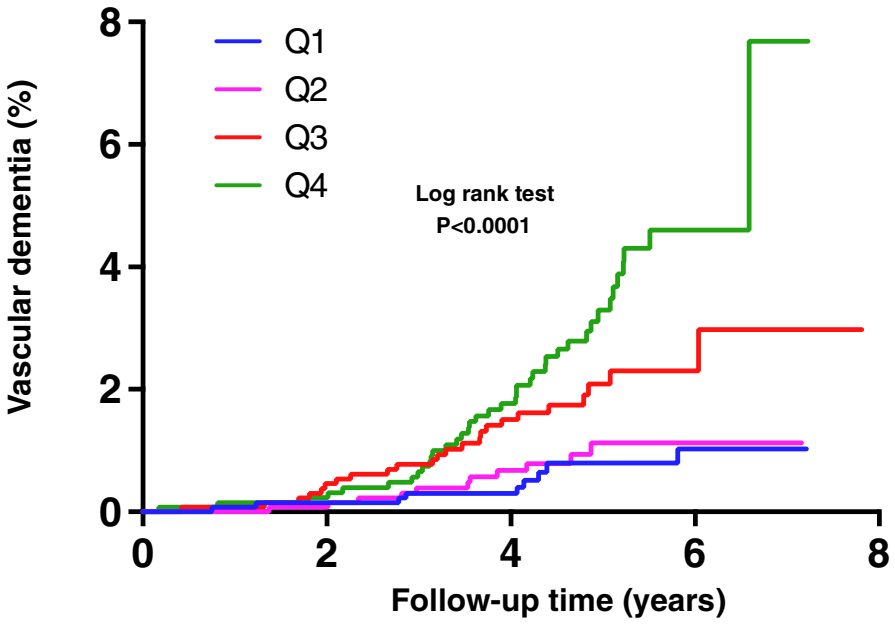
Q1	1336	1305	871	198	0
Q2	1336	1303	1036	255	0
Q3	1336	1284	1090	214	0
Q4	1336	1219	1028	145	0

Figure 2.



Q1	1336	1305	871	198	0
Q2	1336	1303	1036	255	0
Q3	1336	1284	1090	214	0
Q4	1336	1219	1028	145	0

Figure 3.



Q1	1334	1306	1020	336	0
Q2	1334	1297	1011	239	0
Q3	1335	1283	998	153	0
Q4	1334	1218	988	71	0