N-Terminal Pro-B-Type Natriuretic Peptide and the Risk of Stroke: Results from the BiomarCaRE Consortium

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ABSTRACT

Background and Purpose: N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a risk factor for atrial fibrillation and a marker of cardiac function used in the detection of heart failure. Given the link between cardiac dysfunction and stroke, NT-proBNP is a candidate marker of stroke risk. Our aim was to evaluate the association of NT-proBNP with stroke and to determine the predictive value beyond a panel of established risk factors.

Methods: Based on the Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE)-Consortium, we analysed data of 58173 participants (50% men; mean age 52 yr) free of stroke from 6 community-based cohorts. NT-proBNP measurements were performed in the central BiomarCaRE laboratory. The outcomes considered were total stroke and subtypes of stroke (ischemic/haemorrhagic).

Results: During a median follow up time of 7.9 years, we observed 1550 stroke events (1176 ischemic). Increasing quarters of the NT-proBNP distribution were associated with increasing risk of stroke (P for trend <0.0001; multivariable Cox regression analysis adjusted for risk factors and cardiac diseases). Individuals in the highest NT-proBNP quarter (NT-proBNP >82.2 pg/mL) had more than two fold (95%CI: 75%-151%) greater risk of stroke than individuals in the lowest quarter (NT-proBNP <20.4 pg/mL). The association remained unchanged when adjusted for interim coronary events during follow-up, and though it was somewhat heterogeneous across cohorts, it was highly homogenous according to cardiovascular risk profile or subtypes of stroke. The addition of NT-proBNP to a reference model increased the C-index discrimination measure by 0.006 (P=0.0005), yielded a categorical net reclassification improvement of 2.0% in events and 1.4% in non-events and an integrated discrimination improvement of 0.007.

Conclusions: In European individuals free of stroke, levels of NT-proBNP are positively associated with risk of ischemic and haemorrhagic stroke, independently from several other risk factors and

conditions. The addition of NT-proBNP to variables of established risk scores improves prediction of stroke, with a medium effect size.

INTRODUCTION

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is the N-terminal fragment of the B-type natriuretic peptide (BNP), secreted by myocytes as a reaction to several stimuli including wall stretch (1). NT-proBNP has a central role in the regulation of blood pressure, blood volume, and sodium balance. Its levels increase with age, ventricular hypertrophy and in acute coronary syndromes, heart failure and atrial fibrillation (1,2). NT-proBNP is considered a valuable predictor in diagnosis and prognosis of patients with symptoms of heart failure, left ventricular dysfunction and acute coronary syndromes (3-8). Several studies have investigated the association of NT-proBNP with occurrence of cardiovascular or stroke events in general populations (9-10). A meta-analysis of 40 prospective studies has demonstrated a clear association between high levels of NT-proBNP and increased cardiovascular risk under a range of different conditions (10), but had insufficient power to assess whether NT-proBNP was associated differently with ischemic or haemorrhagic stroke or with fatal or non-fatal stroke. In the ARIC study (11) NT-proBNP was found to be associated with total stroke, non-lacunar ischemic, and especially cardioembolic stroke, but not with lacunar or haemorrhagic stroke. In a case–cohort study derived from the REGARDS cohort, the authors confirmed that the association of NT-proBNP with stroke was largest for cardioembolic stroke (12).

Using the harmonized database and biobank of the Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) project (FP7/2007–2013), (13) we centrally analysed individual NT-proBNP concentrations in 40 336 individuals of 6 population cohorts with the aims: (1) to achieve a precise characterization of the association of NT-proBNP with stroke in Europe; (2) to assess possible difference in the association of NT-proBNP with ischemic or haemorrhagic stroke or with fatal stroke; and (3) to determine the predictive value of NT-proBNP beyond classical risk factors for stroke.

METHODS

Study overview

Data, analytic methods, and study materials are not available to other researchers.

The present analysis is based on data from the BiomarCaRE consortium (http://www.biomarcare.eu), details of which have been described previously (13). BiomarCaRE is based on the MORGAM (MONICA Risk Genetics Archiving and Monograph) Project (14). The MORGAM/BiomarCaRE Data Center in Helsinki harmonized individual data from 15 population-based cohort studies with central storage of selected biological samples of almost 187736 participants in the BiomarCaRE laboratory at the University Heart Center, Hamburg. Current analyses include cohorts with available information on stroke status at baseline, with adjudication for stroke at follow-up and available data on NT-proBNP (n=6 cohort studies). All NT-proBNP levels included in the present study were measured centrally using the same assay. Statistical analyses were planned and conducted at the NEUROMED BiomarCaRE Center in Pozzilli, Italy. Our study complies with the Declaration of Helsinki, all participating studies were approved by local ethics review boards and written informed consent was obtained from individuals.

Study cohorts

Overall, the cohort consisted of 6 population-based studies involving 58173 individuals free of stroke at baseline (individuals with a positive history of stroke based on self-report and/or prior physician's diagnosis for stroke were excluded from analyses). The individual cohorts were FINRISK 1997, MONICA Northern Sweden, Prospective Epidemiological Study of Myocardial Infarction from

Belfast (PRIME), MATISS Rome, MONICA Brianza and Moli-sani. Each cohort is based on representative population samples. Full details of baseline data have been provided elsewhere (15-16). Cohort descriptions are provided in Supplementary material online, Table I. For each cohort the following harmonized variables were available at baseline: age, sex, body-mass-index (BMI), systolic and diastolic blood pressure, anti-hypertensive medication, smoking status, total and high-density lipoprotein cholesterol (HDL) and estimated glomerular filtration rate (eGFR) calculated by using of Chronic Kidney Disease Epidemiology Collaboration formula, history of diabetes, myocardial infarction, atrial fibrillation and heart failure. Data on atrial fibrillation or heart failure were not available for MATISS and MONICA Brianza. History of diabetes was defined as self-reported or documented diabetes. Data collection on risk factors followed a standardized protocol described in the MORGAM Manual (17).

Laboratory procedures

NT-proBNP levels were measured in the BiomarCaRE core laboratory using an electrochemiluminescence sandwich immunoassay (ECLIA, Roche Diagnostics) on either the ELECSYS 2010 or the Cobas e411 system. The analytical range was 5–35000 pg/mL. The study-specific intra- and inter-assay coefficients of variation are described in Supplementary material online, Table II.

Study outcome

Participants in each cohort were followed-up for first stroke (fatal or non-fatal) and death from other causes. Deaths were identified through record linkage with national or regional health information systems. Non-fatal strokes refer to survival at 28th day after onset, and were identified by linkage to population registers, hospital discharge data or direct contact with the participant.

Most centers adjudicated the events using MONICA diagnostic criteria. The MORGAM Manual gives further information about the event classifications (17). The procedure used for identification of stroke subtypes (ischemic or hemorrhagic) is described in details in Supplemental Methods. Briefly, a stroke is classified as a cerebral infarction (ischemic stroke) if at least one of the following is present: a) validation of recent brain infarction in necropsy; b) circumscribed hypodensity changes of recent origin in the brain parenchyma on computed tomography (CT); c) typical signs of infarct in the brain parenchyma on magnetic resonance imaging. The event was considered as cerebral infarction also if there was no validation as described above but the routine clinical or causes of death diagnoses indicated cerebral infarction (ICD-8 value of 432, 433 or 434, an ICD-9 value of 434 or an ICD-10 value of I63). To be accepted as a case of subarachnoid hemorrhage, at least one of the following must be present: a) necropsy - recent subarachnoid hemorrhage; b) CT-signs of blood in the subarachnoid cisterns or in cerebral ventricles; c) magnetic resonance imaging - signs of blood in the subarachnoid cisterns or in cerebral ventricles; d) cerebrospinal fluid (liquor) bloody and/or xanthochromic and the possibility of intracerebral hemorrhage excluded by necropsy or CT examination. To be accepted as a case of intracerebral hemorrhage in MORGAM, at least one of the following must be present: a) necropsy - recent intracerebral hemorrhage; b) CT-hyperdensity changes in the brain parenchyma.; c) magnetic resonance imaging - typical signs of bleeding in the brain parenchyma; d) cerebrospinal fluid (liquor) bloody in the presence of focal neurological signs at onset. The event was considered as hemorrhagic stroke also if there was no validation for subarachnoid or intracerebral hemorrhage as described above but the routine clinically recorded or officially registered causes of death diagnoses indicated hemorrhagic stroke (ICD-8 value of 430 or 431, an ICD-9 value of 430 or 431 or an ICD-10 value of I60 or I61).

Statistical analysis

N=4054 (7.0%) individuals had NT-proBNP values below the limit of detection (5 pg/mL); for these individuals NT-proBNP values have been imputed to NT-proBNP = 5 pg/mL. For 9.0% of the available population, one or more CVD risk factors or NT-proBNP levels were missing; in these cases, we used multiple imputation techniques (SAS PROC MI, n=10 imputed datasets; and PROC MIANALYZE) to maximize data availability.

The NT-proBNP distribution in the overall cohort was right-skewed (mean 82 pg/mL, standard deviation 232 pg/mL; median 43 pg/mL, coefficient of skewness 35.8). After a natural log transformation, the NT-proBNP distribution showed a Gaussian distribution (mean 3.7 log(pg/mL), standard deviation 1.1 log(pg/mL); median 3.7 log(pg/mL), coefficient of skewness 0.12). Hereafter, the natural log of NT-proBNP levels has been used. The correlation between log(NT-proBNP) and gender, examination age, total and HDL cholesterol, smoker status, hypertension, systolic and diastolic blood pressure, BMI, diabetes and estimated glomerular filtration rate, was assessed using Pearson's coefficient. To estimate the association between NT-proBNP and stroke outcome, we first derived sample quartiles for the marker in the pooled sample. Actually, because the log transformation is a monotonic transformation, quarters constructed by using log(NT-proBNP) values or by NT-proBNP values are identical. More importantly, the creation of quarters and the values of the quartiles are independent from the method of imputation for values under the limit of detection. Subsequently, we estimated the hazard ratios (with 95% confidence intervals) for stroke across increasing NT-proBNP quartiles from Cox proportional hazards models with age as the time scale, and adjusting for sex, study center (Model 1), smoking, BMI, diabetes, myocardial infarction at baseline, hypertension medication, total and HDL cholesterol (Model 2). We selected possible confounding variables for regression models based on previous analyses from the same populations (16). Additional adjustment was also made for baseline eGFR or for coronary heart disease, atrial fibrillation or heart failure as time-dependent variables, as these events occurred during follow-up

(Model 3). From this latter analysis, the MATISS Study and MONICA Brianza Study were excluded because data on atrial fibrillation or heart failure were not available. Associations of NT-proBNP with each stroke subtype were estimated after censoring participants when they developed stroke of another subtype. We reported the Cochran's Q test and the I^2 statistic to quantify heterogeneity among cohorts. The C-index, the categorical net reclassification improvement (NRI) and the absolute and relative integrated discrimination improvement (IDI) (18) were used to quantify the added predictive value of NT-proBNP beyond that from the reference model (Model 2). To estimate these metrics, the follow-up time was censored at 10 years (numbers of stroke events reduced to 948). The risk categories we chose for NRI calculation were: < 2%, 2% to <5%, 5% to <8%, ≥8%. These categories roughly correspond to low, intermediate-low, intermediate-high and high risk levels used in decisions to initiate treatment to prevent stroke in persons with atrial fibrillation (19). A two-sided P-value of <0.05 was considered statistically significant. All statistical methods were implemented in SAS statistical software for Windows, version 9.4.

RESULTS

Baseline Characteristics

Baseline characteristics for the overall study population are shown in Table 1. The sex ratio of the overall cohort was balanced with 50% males. The median age was 52 years (interquartile range 43 to 61 years). Study participants were slightly overweight (median BMI 27.3 kg/m^2). At baseline 24.7% of the individuals were daily smokers, 19.2% were prescribed anti-hypertensive medication, 5% had diabetes and less than 2.7% had a personal history of myocardial infarction, heart failure or atrial fibrillation (Table 1). Characteristics of the different cohorts are illustrated in Supplemental Table III.

NT-proBNP levels were lower in men (r=-0.24) and correlated positively with age (r=0.45). The correlation of log(NT-proBNP) levels with other cardiovascular risk factors and phenotypes was generally modest (Table 2).

We found slightly higher (+0.069, SD=0.008) log(NT-proBNP) age and sex adjusted levels in the northern Europe cohorts (Finland, Sweden and UK-Belfast) compared to the southern Europe cohorts (Italian cohorts; P<0.0001).

NT-proBNP concentrations and association with stroke outcomes

During a median follow up time of 7.9 years (interquartile range 4.2 to 13.8) n=1550 incident stroke events (n=249 fatal events) occurred. Of these, n=1176 were ischemic, n=330 were hemorrhagic (35% of them were subarachnoid and 65% intracerebral haemorrhages);and for n=44 there was insufficient information to determine the type of stroke (Table 1).

Table 3 displays fully adjusted hazard ratios across quarters of the NT-proBNP distribution indicating the associations with stroke. The risk of stroke in the top quarter was double that in the bottom quarter. The association was virtually unchanged when adjusted for eGFR and it was very slightly attenuated when further adjusted for occurrence of coronary events or atrial fibrillation or heart failure during follow-up (before the time of stroke event, for an individual who had a stroke) (Table 3, Model 3). These associations were of very similar magnitude for both ischemic and hemorrhagic and for fatal and nonfatal strokes (Table 3).

The adjusted hazard ratio (Model 2) for total, ischemic or hemorrhagic stroke associated with one standard deviation increase of log(NT-proBNP) concentration was 1.48 (95% CI: 1.40 to 1.57), 1.51 (95% CI: 1.41 to 1.61) and 1.45 (95% CI: 1.27 to 1.66), respectively.

The adjusted hazard ratio (Model 2) for case fatality (stroke fatal events among stroke events) for 4° vs 1°quarter of NT-proBNP was 1.16 (95%CI: 0.71 to 1.92).

Subgroup analysis of the NT-proBNP-associated risk

The distribution of stroke events in quarters of NT-proBNP across cohorts is reported in Supplemental Table III. Figures 1 and 2 display the adjusted hazard ratio (Model 2 as defined in Table 3) for total stroke for 4° vs 1°quarter of NT-proBNP across cohorts (Fig.1) and in subgroups of individuals with different cardiovascular risk profiles (Fig.2). The association of NT-proBNP with increased risk of total stroke was observed in all cohorts, with a negligible level of heterogeneity (Fig. 1; Cochran's Q=3.07, P=0.69; I^2=0%).

The effect of NT-proBNP was highly homogenous across CVD risk categories (Fig.2), with the exception of HDL (the association of NT-proBNP with stroke was greater in individuals with HDL>53 mg/dL). The relative risk of stroke for 4° vs 1° quarter of NT-proBNP was also similar in individuals free from cardiovascular disease at baseline (myocardial infarction, atrial fibrillation or heart failure, n=40507, n=1100 stroke events during follow up), HR=2.21 (95%CI: 1.79 to 2.72) when compared to individuals who did report a history of myocardial infarction or atrial fibrillation or heart failure, n=2313, n=212 stroke events) at baseline, HR=2.15 (95%CI: 0.93 to 4.99; P for difference = 0.73).

NT-proBNP and prediction of stroke

The addition of NT-proBNP to the base model increased the C-index discrimination measure by 0.006 (from 0.842 to 0.848; p-value for testing increment equal to zero: 0.0005), yielded a net

reclassification improvement of 2.0% in events and 1.4% in non-events, and an absolute and relative integrated discrimination improvement of 0.007 and 0.11, respectively.

DISCUSSION

Based upon harmonized individual level data and a centrally standardized NT-proBNP evaluation in more than 58000 individuals from 6 population based European studies, our analyses indicate that high levels of NT-proBNP (in particular in the upper quarter of the distribution, >82.2 pg/mL) are a risk factor for stroke, independent of conventional risk factors. This supports growing evidence associating high levels of natriuretic peptides with increased risk of stroke (8,10-12, 20-26).

Comparison to previous studies

Our findings are in agreement with a meta-analysis of 13 studies (10) including 2063 stroke events in 56764 individuals which found a relative risk of 1.93 (95% CI: 1.58 to 2.37) among individuals in the top third in comparison to the bottom third of the NT-ProBNP distribution. We also confirmed the association of NT-proBNP with ischemic stroke, as observed in the REGARDS study (12). Unfortunately, we were unable to distinguish cardio-embolic strokes and consequently cannot confirm the interesting findings of both the REGARDS study (12) and the ARIC study (11) which found that the associations of NT-proBNP with stroke events was strongest for cardio-embolic strokes.

NT-proBNP as a stroke risk factor

We found slightly higher NT-proBNP levels in populations from northern Europe compared to those in southern Europe. However, the difference represents only 6.3% of the standard deviation of the peptide distribution.

The association of NT-proBNP levels with stroke was absent when the second quarter was compared to the first, very modest when the third quarter was compared to the first and very evident when the top quarter is compared to the bottom. Our findings indicate that the critical value above which the risk of stroke becomes important is around NT-proBNP=80 pg/mL. This value accords well with the corresponding threshold observed in the ARIC study (80 pg/mL) (11) though it is lower than that observed in the REGARDS study (137 pg/mL) (12).

Interestingly, we observed an association of NT-proBNP with incidence of both ischemic and hemorrhagic stroke and with both fatal and non-fatal stroke. A link of NT-proBNP with risk of ischemic stroke is not unexpected given the correlation of NT-proBNP with cardiac function (1). To our knowledge, this is the first observation of a statistically significant association of high levels of NT-proBNP with risk of hemorrhagic stroke. In the ARIC study (11), higher levels of NT-proBNP were associated with an almost double risk of hemorrhagic stroke, but the small number of events (n=63) made the observation statistically imprecise. The association of NT-proBNP with different types of stroke may be due to shared risk factors, unidentified effects of NT-proBNP or unknown mechanisms for these strokes. Plasma brain natriuretic peptide levels have been shown to be elevated not only in acute ischemic stroke patients, but also in the acute phase of subarachnoid (27) and intracerebral hemorrhage (28).

Interestingly, growing evidence suggests causal relationships of natriuretic peptides to endothelial permeability (29), which might predispose not only to atherosclerosis (30), but to hemorrhages too. In fact, Lee et al. (31) demonstrated that salt-loaded stroke-prone spontaneous hypertensive rats

have increased vascular permeability at the site of subsequent intracerebral hemorrhage, and Lin et al. (32) demonstrated that elevated permeability predicted subsequent hemorrhagic transformation following ischemic stroke.

The stroke risk associated with elevated NT-proBNP levels is highly homogenous according to the presence or absence of other cardiovascular risk factors, suggesting that raised NT-proBNP levels affect risk for stroke over a broad spectrum of circumstances, and in particular, independent of the presence of hypertension.

The role of NT-proBNP as risk factor for stroke was comparable in individuals with or without cardiac diseases at baseline. In addition, adjustment for the presence of cardiovascular disease at baseline and during follow-up before the stroke event only slightly modified the association between NT-proBNP and total or ischemic stroke. These findings suggest that the association between NT-proBNP and stroke is not secondary to the occurrence of other cardiovascular disease that could associate with both NT-proBNP levels and stroke.

NT-proBNP is mainly released by cardiac myocytes and is only weakly associated with other CVD risk factors, except age and sex. In accord with the previous finding of a specific association with cardio-embolic stroke (11, 12), elevated NT-proBNP at baseline is most probably due to subclinical cardiac pathology which increases the risk of stroke events years later. In this case, an elevated NT-proBNP should prompt careful considerations and diagnosis of potential underlying cardiac problems, which if treated appropriately, may prevent future adverse events.

The addition of NT-proBNP on top of a number of stroke risk factors improved both discrimination and reclassification. The magnitude of improvement was comparable to that of troponin for coronary heart disease, as demonstrated in similar large collaborative studies of population-based cohorts (15). In addition, the relative IDI indicates that the strength of NT-proBNP is larger than the

average strength of risk factors in the reference model, according to the criterion suggested by Pencina et al. (33). Therefore, all considered, the effect size of NT-proBNP for stroke prediction in the general population can be considered to range from moderate to medium.

Limitations

Some strengths and limitations of the present study should be considered. Although our validation of stroke events was systematic and detailed, it was based, as is usually the case in most epidemiological studies, on medical reviews and not on standardized neurologic examinations or data from computed tomography or magnetic resonance imaging, especially for those cohorts with base-line enrollment in '80s and '90s. Moreover, information on the etiology of ischemic strokes, such as the presence of a cardiac source of embolism, would have been valuable for the analysis but unfortunately was not generally available. Because of the low number of hemorrhagic strokes (n=330) we decided to not conduct separate analyses for subarachnoid or intracerebral hemorrhages. Despite longstanding expertise in data harmonization in the MORGAM Data Centre in Helsinki since 1998, resulting in the best possible endpoint and co-variate validation, measurement error and lack of information on some other known cardio-metabolic risk factors (such as physical activity and diet), offers some room for residual confounding to affect the observed associations among the more than 58000 individuals investigated in these 6 European population-based cohort studies. On one hand, we present a large dataset of NT-proBNP values measured centrally with the same assay, but on the other, the differences in storage duration among the included cohorts may have led to differences and variability in NT-proBNP levels across cohorts, but given the broad homogeneity of the relationship with stroke risk, we see no reason why this would bias the observed associations. Further, since we had only single measures of NT-proBNP, we cannot correct for regression dilution bias. This could have led to an underestimation of our risk estimates.

We also cannot examine, with a single measure, how risk of stroke might vary when biomarker levels change over time.

CONCLUSIONS

In this the largest trans-national dataset with centrally measured NT-proBNP, we confirm NT-proBNP as a risk factor for ischemic stroke and also demonstrate an association with hemorrhagic stroke. NT-proBNP measurement might support the identification of those individuals at high risk for stroke, who would benefit most from preventive interventions.

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FIGURE LEGENDS

Figure 1 Hazard ratio and 95% CI (adjusted as in model 2, see Table 3) for 4° vs 1° quarter of NT-proBNP in overall and separate cohorts; all strokes.

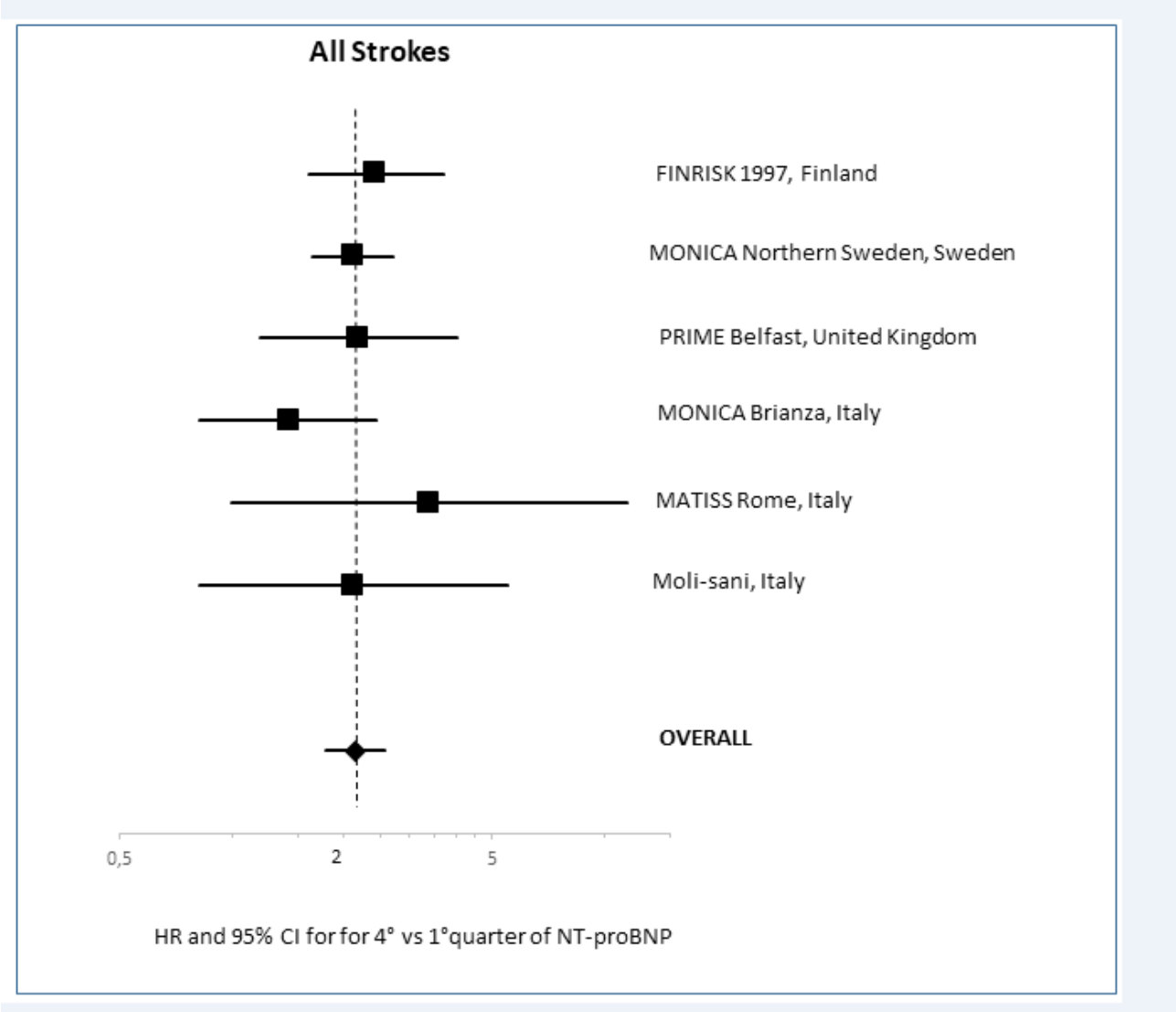

Figure 2 Hazard ratio and 95% CI (adjusted as in model 2, see Table 3) for 4° vs 1° quarter of NT-proBNP a with different cardiovascular risk factors; all strokes

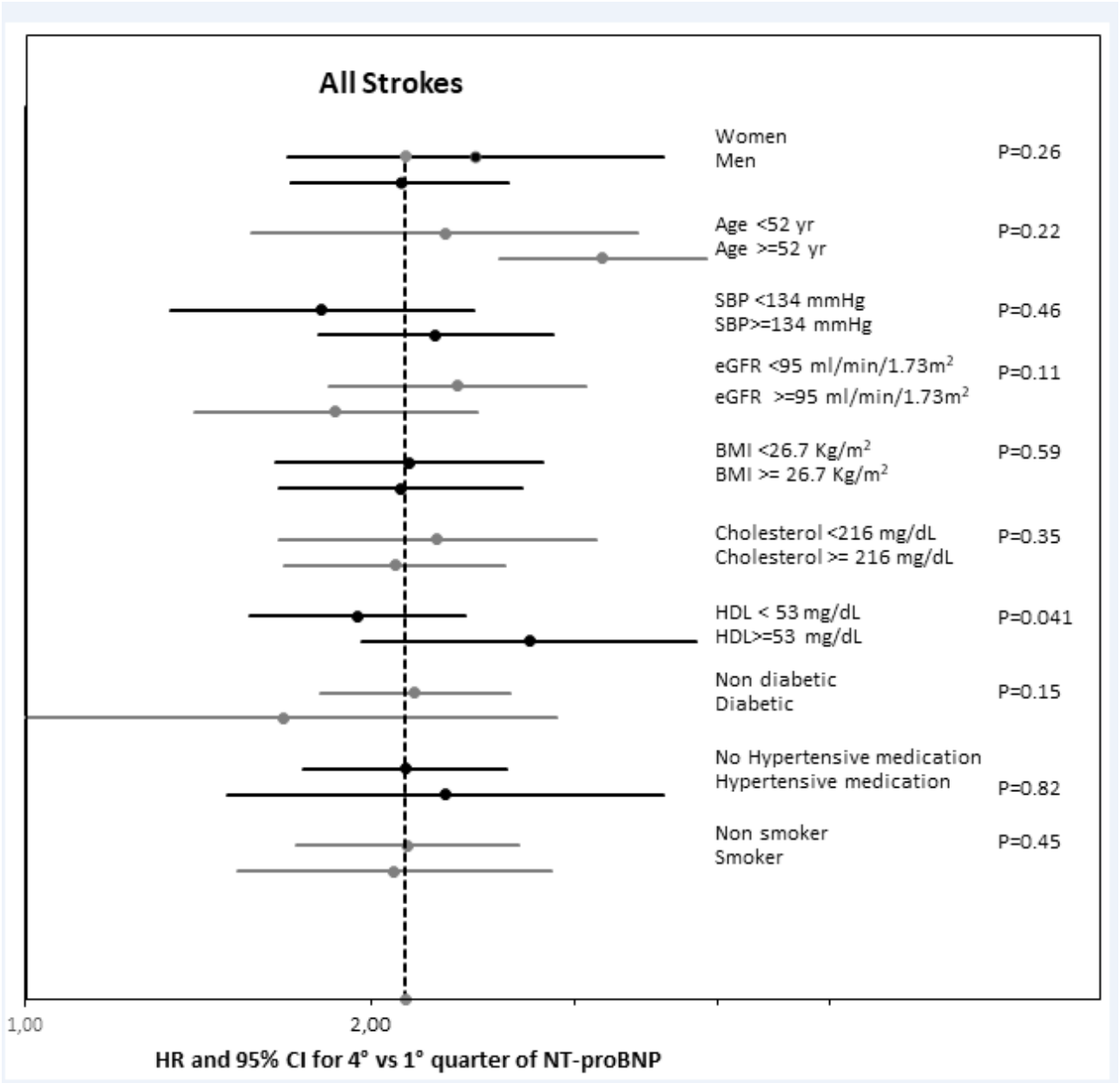

Table 1. General characteristics of the studied population

Baseline Characteristics	
Number of populations, No.	6
Number of individuals, No.	58173
Years of baseline examinations, range in years	1986−2008
Age at baseline examination, y	52 (13)
Men, No. (%)	29275 (50.32)
Myocardial infarction, (%)	1571 (2.70)
Heart failure, (%)	505/49009 (1.03)*
Atrial fibrillation, (%)	523/42532 (1.23)*
Stroke risk factors	
Daily smoker, No. (%)	14363 (24.69)
Diabetes, No. (%)	2888 (4.96)
Antihypertensive medication, No. (%)	11170 (19.20)
Body mass-index, kg/m²	27.3 (4.7)
Systolic blood pressure, mmHg	137 (21)
Total cholesterol, mg/dL	219 (44)
HDL cholesterol, mg/dL	55 (15)
Estimated Glomerular Filtration Rate, ml/min/1.73m^2	93 (18)
NT-ProBNP, pg/mL	82 (232)
Log(NT-ProBNP), log(pg/mL)	3.7 (1.1)
Endpoints during Follow-up	

Stroke (any type), No. (%)	1550 (2.66)
Ischemic Stroke, No. (%)†	1176 (2.02)
Haemorrhagic Stroke, No. (%)†	330 (0.57)
Fatal stroke (any type), No. (%)	249 (0.43)
Other events during Follow-up	
Myocardial infarction, No. (%)	776 (1.33)
Heart Failure, No. (%)	1431/46012 (3.11)**
Atrial fibrillation, No. (%)	1363/45775 (2.98)**

Characteristics are presented as absolute and relative frequencies for categorical variables, and mean value and standard deviation for continuous variables as well as ranges in years for years of baseline examinations

*History of heart failure at baseline was available for 49009 individuals. History of atrial fibrillation at baseline was available for 42532 individuals.

**Incidence of heart failure (atrial fibrillation) was not evaluated for 12161 (12398) individuals.

†N=44 strokes were unclassified

Table 2. Pearson correlations coefficient of (log) NT-ProBNP with stroke risk factors

With (log) NT-ProBNP	Sex (1=men)	Age	Total cholesterol	HDL cholesterol	Daily smoker	Hypertension medication	Systolic blood pressure	BMI	Diabetes	eGFR
Correlation coefficient	-0.24	0.45	-0.03	0.14	-0.09	0.25	0.26	0.04	0.09	-0.26
95% confidence interval	(-0.25 to -0.23)	(0.44 to 0.45)	(-0.04 to -0.02)	(0.13 to 0.14)	(-0.10 to -0.08)	(0.24 to 0.26)	(0.25 to 0.26)	(0.03 to 0.05)	(0.08 to 0.10)	(-0.27 to -0.25)
p-value	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

BMI stands for body mass index, eGFR for estimated glomerular filtration rate.

Table 3. Hazard ratios for different stroke outcomes, according to quarters of NT-ProBNP

	NT-ProBNP Quarters (pg/mL)			
	<20.4	**20.4-42.5**	**42.5-82.2**	**>82.2**
N	14543	14545	14541	14544
ALL STROKES (N=1550)				
Number of events	207	248	321	774
Model 1*	-1-	1.09	1.27	2.25
(95% CI)	*reference*	(0.89-1.32)	(1.05-1.53)	(1.88-2.68)
Model 2†	-1-	1.09	1.28	2.14
(95% CI)	*reference*	(0.90-1.32)	(1.06-1.54)	(1.79-2.57)
Model 3‡	-1-	1.09	1.27	2.06
(95% CI)	reference	(0.90-1.32)	(1.05-1.53)	(1.72-2.47)
ISCHEMIC STROKES (N=1176)				
Number of events	152	189	235	600
Model 2†	-1-	1.13	1.28	2.24
(95% CI)	*reference*	(0.91-1.42)	(1.02-1.60)	(1.82-2.76)
Model 3‡	-1-	1.12	1.25	2.12
(95% CI)	reference	(0.90-1.40)	(1.00-1.57)	(1.72-2.62)
HAEMORRHAGIC STROKES (N=330)				
Number of events	50	53	76	151
Model 2†	-1-	1.01	1.41	2.09
(95% CI)	*reference*	(0.67-1.51)	(0.96-2.06)	(1.42-3.09)
Model 3‡	-1-	1.02	1.42	2.09
(95% CI)	reference	(0.68-1.53)	(0.97-2.08)	(1.42-3.09)
INCIDENT STROKES THAT WERE FATAL (N=249)				
Number of events	25	34	48	142
Model 2†	-1-	1.08	1.09	2.15
(95% CI)	*reference*	(0.63-1.84)	(0.64-1.86)	(1.33-3.47)
Model 3‡	-1-	1.11	1.12	2.08

(95% CI)	reference	(0.65-1.88)	(0.66-1.90)	(1.29-3.37)
INCIDENT STROKES THAT WERE NON FATAL (N=1301)				
Number of events	182	214	273	632
Model 2†	-1-	1.10	1.32	2.15
(95% CI)	*reference*	(0.89-1.36)	(1.08-1.62)	(1.77-2.62)
Model 3‡	-1-	1.10	1.31	2.07
(95% CI)	reference	(0.89-1.35)	(1.07-1.61)	(1.70-2.52)

*Model 1: adjusted for age, sex and centre.

†Model 2: model 1 + smoking, BMI, diabetes, hypertension medication, systolic and diastolic blood pressure, total and HDL cholesterol, myocardial infarction at baseline

‡Model 3: model 2 + coronary heart disease, atrial fibrillation or heart failure as time-dependent variables as these events occurred during follow-up

SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Identification of stroke subtypes

According to MORGAM's criteria, a stroke is classified as a cerebral infarction (ischemic stroke) if at least one of the following is present:

- Validation of recent brain infarction in necropsy.
- Circumscribed hypodensity changes of recent origin in the brain parenchyma on computed tomography (CT). Ischemic stroke was also diagnosed if localized changes of recent origin were absent on CT, but the criteria for definite stroke were fulfilled (rapidly developed clinical signs of focal disturbance of cerebral function lasting more than 24 hours (unless interrupted by surgery, other medical intervention or death) with no apparent cause other than a vascular origin). Cases with small hematomas or diffuse bleedings occurring within a hypodense lesion were classified as hemorrhagic transformation and therefore coded as having cerebral infarction.
- Typical signs of infarct in the brain parenchyma on magnetic resonance imaging.

The event was considered as cerebral infarction in the data analysis also if there was no validation as described above but the routine clinical or causes of death diagnoses indicated cerebral infarction (ICD-8 value of 432, 433 or 434, an ICD-9 value of 434 or an ICD-10 value of I63).

To be accepted as a case of subarachnoid haemorrhage in MORGAM, at least one of the following must be present:

- Necropsy - recent subarachnoid haemorrhage
- CT - signs of blood in the subarachnoid cisterns or in cerebral ventricles
- Magnetic resonance imaging - signs of blood in the subarachnoid cisterns or in cerebral ventricles
- Cerebrospinal fluid (liquor) bloody and/or xanthochromic and the possibility of intracerebral haemorrhage excluded by necropsy or CT examination

To be accepted as a case of intracerebral haemorrhage in MORGAM, at least one of the following must be present:

- Necropsy - recent intracerebral haemorrhage
- CT - hyperdensity changes in the brain parenchyma. Note that small haematomas or diffuse bleedings occurring within a hypodense lesion (so-called haemorrhagic transformation) are not coded as intracerebral haemorrhage but as cerebral infarction (item CI below)
- Magnetic resonance imaging - typical signs of bleeding in the brain parenchyma
- Cerebrospinal fluid (liquor) bloody in the presence of focal neurological signs at onset.

An event of subarachnoid or intracerebral haemorrhage was classified as haemorrhagic stroke.

The event was considered as hemorrhagic stroke in the data analysis also if there was no validation for subarachnoid or intracerebral hemorrhage as described above but the routine clinical or causes of death diagnoses indicated hemorrhagic stroke (ICD-8 value of 430 or 431, an ICD-9 value of 430 or 431 or an ICD-10 value of I60 or I61).

Supplemental Table I. Overview and description of contributing studies

BiomarCaRE cohorts

Study/cohort	Country	Study/cohort full name and short description Reference
FINRISK 1997	Finland	The FINRISK study is a series of population-based cardiovascular risk factor surveys carried out every five years in five (or six in 2002) districts of Finland, including North Karelia, Northern Savo (former Kuopio), South-western Finland, Oulu Province, Lapland province (in 2002 only) and the region of Helsinki and Vantaa. A stratified random sample was drawn for each survey from the national population register; the age-range was 25-74 years. All individuals enrolled in the study received a physical examination, a self-administered questionnaire, and a blood sample was drawn. In 1997, altogether 11500 individuals were invited and 8444 (73%) participated in the clinical examination. During follow-up the National Hospital Discharge Register, the National Causes of Death Register and the National Drug Reimbursement Register were used to identify endpoints. At the moment, the follow-up extends until Dec. 31st, 2010, i.e., 14 years for the FINRISK 1997 cohort. The Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District approved the study, which followed the declaration of Helsinki. All subjects gave written informed consent. http://www.thl.fi/publications/morgam/cohorts/full/finland/fin-fina.htm. Accessed August 9, 2018. Borodulin K, Tolonen H, Jousilahti P, Jula A, Juolevi A, Koskinen S et al. Cohort Profile: The National FINRISK Study. [published online November 20, 2017]. Int J Epidemiol. 2017. https://academic.oup.com/ije/article/47/3/696/4641873. Accessed August 9, 2018.
Northern Sweden	Sweden	The Northern Sweden MONICA project was initiated as part of the WHO MONICA (Multinational MONItoring of trends and determinants in CArdiovascular disease) study in 1985. The six population-based surveys consist of individuals from the counties of Västerbotten and Norrbotten selected about every five years from 1986 to 2009. Individuals were randomly selected from population registers, stratified for 10-years age group (with age range from 25 to 64 years in 1986 and 1990, and 25 to 74 years from 1994 to 2009) and sex. Over all cohorts 10450 individuals participated, equaling a participation rate of 74.6 % to the random population samples. Follow-up was achieved through linkage with the national death register and the National registers at the National Board of Health and Welfare (Cause of Death Register, Inpatient Diagnosis Register, Cancer Register, and Medication Register) as well as the MONICA stroke event and myocardial

		infarction registers, with endpoint diagnosis based on MORGAM criteria. Follow-up is completed until December 31st 2011. http://www.thl.fi/publications/morgam/cohorts/full/sweden/swe-nswa.htm. Accessed August 9, 2018 http://www.org.umu.se/monica. Accessed August 9, 2018 Eriksson M, Forslund AS, Jansson JH, Söderberg S, Wennberg M, Eliasson M. Greater decreases in cholesterol levels among individuals with high cardiovascular risk than among the general population: the northern Sweden MONICA study 1994 to 2014. Eur Heart J 2016;37:1985-92.
Prospective Epidemiological Study of Myocardial Infarction (**PRIME**) Belfast	United Kingdom	The PRIME study examined the classic and putative cardiovascular risk factors to explain the large difference in heart disease incidence between Ireland and France. The study includes four cohorts of men aged 50-59; from Belfast, Northern Ireland (N=2745) and Lille (N=2633), Toulouse (N=2610) and Strasbourg (N=2612) in France. For the current analysis only the Belfast cohort was used. Baseline examinations took place in 1990-1993 and targeted cohorts which had broadly similar social class structures to the background population, initially sampling from industries and various employment groups, employment groups with more than 10% of their workforce of foreign origin were excluded. Follow up until 2004 (Toulouse, Strasbourg and Lille) and until 2012 (Belfast) was achieved through annual follow up questionnaires with verification against national death registers, medical records, hospital discharge diagnoses. Endpoints were validated by expert medical committee. http://www.thl.fi/publications/morgam/cohorts/full/uk/unk-bela.htm. Accessed August 9, 2018 Yarnell JW, Baker IA, Sweetnam PM, Bainton D, O'Brien JR, Whitehead PJ et al. Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies. Circulation. 1991;83:836-44
MATISS Study	Italy	The study started in 1983 as DiSCo - DIstretto Sezze controllo COmunitario - a demonstration project of non-communicable diseases conducted in Central Italy, in a rural area located 100 km southeast of Rome. In 1983-84 and in 1986-87 independent random samples of the general population, stratified by age and sex were drawn in 4 towns (Sezze, Roccagorga, Bassiano, and Priverno). Cardiovascular risk factors were measured in 1983-1984 (participation rate 67%) and in 1986-1987 (participation rate 41%). Since 1993 the project continued as prospective observational study with the name of MATISS. In 1993-96 a new cohort of 1 970

		persons (participation rate 60%) was examined together with the old cohorts (1983-84 and 1986-87). BiomarCaRE includes all data and biological specimens collected in 1993-96 for a total of 4489 persons aged 20-79 years, i.e. 72% of the re-examined old cohorts (only those with serum/plasma specimens) and the new cohort. Follow-up is available for all cohorts until December 2004. Vital status was checked at the municipalities of residence; mortality was performed by mortality registry and causes of deaths were collected from death certificates; suspected fatal and non-fatal coronary and stroke events, identified by the record linkage of mortality and hospital discharge diagnosis registries, were validated by the MONICA diagnostic criteria using the clinical records and other GPs information. The study is part of the CUORE Project of the Italian Public Health Institute and was approved by the Ethic Committee in 2008. http://www.cuore.iss.it/eng/assessment/procedures.asp. Accessed August 9, 2018 Giampaoli S, Urbinati GC, Menotti A, Ricci G. Short term changes in cardiovascular risk factors in the Di.S.Co. Intervention Project. Research Group of the DI.S.Co. Project. Eur J Epidemiol. 1991;7:372-9.
MONICA Brianza Study	Italy	The MONICA-Brianza Cohort Study is a prospective observational study of three cohorts of 25-64 years old residents in Brianza, a highly-industrialized area located between Milan and the Swiss border, Northern Italy. Gender- and ten-year age stratified samples were randomly drawn in 1986, 1990 and 1993, and cardiovascular risk factors were investigated at baseline following the procedures of the WHO MONICA Project. The overall participation rate was 69%. For all subjects whole blood and serum samples were stored in a biobank. The protocol was approved by the Monza Hospital Ethical Committee. Study participants were followed up for first coronary or stroke events, fatal and non-fatal, up to the end of 2008, for a median of 15 years. http://epimed.uninsubria.eu. Accessed August 9, 2018 Ferrario MM, Roncaioli M, Veronesi G, Holtermann A, Clays E, Borchini R, et al. Differing associations for sport versus occupational physical activity and cardiovascular risk. Heart. 2018;104:1165-1172
Moli-sani Study	Italy	The Moli-sani study is an ongoing, prospective, population-based cohort of 24,325 individuals (48% men, aged ≥35 years, mean age ± SD: 55.8±12.0 years) living in the Molise region in south-central Italy. Participants were randomly enrolled from town registries

between 2005 and 2010. Follow-up was performed through record linkage to national mortality registries and hospital discharge registers, while validation of events was achieved through hospital record linkage and doctors' medical records. The maximum follow-up time was 6.8 years. Exclusion criteria were pregnancy at the time of recruitment, lack of in understanding, current multiple trauma or coma, or refusal to sign the informed consent. Participation was 70%. The cohort was followed-up for a median of 4.2 years (maximum 6.5 years) at December 2011 and will be followed-up every 5 years.10 Follow up is achieved through record linkage to national mortality registries and hospital discharge registers, validation of events was achieved through hospital record linkage and doctors medical records using updated MORGAM criteria. http://www.moli-sani.org/Funding: The Moli-sani study was partially supported by research grants from Pfizer Foundation (Rome, Italy), the Italian Ministry of University and Research (MIUR, Rome, Italy)–Programma Triennale di Ricerca, Decreto n.1588 and Instrumentation Laboratory, Milan, Italy.

Di Castelnuovo A, de Curtis A, Costanzo S, Persichillo M, Olivieri M, Zito F, et al. Association of D-dimer levels with all-cause mortality in a healthy adult population: findings from the MOLI-SANI study. Haematologica. 2013;98:1476-80.

Supplemental Table II. NT-proBNP intra-assay and inter-assay coefficients of variation by cohort in the BiomarCaRE project

	Assay variation (%)	
Study/cohort	**Intra**	**Inter**
FINRISK 1997	2.58	1.38
Northern Sweden	1.48	5.88-8.70
PRIME, Belfast	2.58	1.38
MONICA Brianza Study	2.64	3.28-5.20
MATISS, Rome	3.23	6.3-8.8
Moli-sani Study	2.30	5.44-6.50