NT-proBNP (N-Terminal Pro-B-Type Natriuretic Peptide) and the Risk of Stroke

Results From the BiomarCaRE Consortium

Augusto Di Castelnuovo, PhD; Giovanni Veronesi, PhD; Simona Costanzo, PhD; Tanja Zeller, PhD; Renate B. Schnabel, PhD; Amalia de Curtis, BSc; Veikko Salomaa, PhD; Rossana Borchini, MD; Marco Ferrario, PhD; Simona Giampaoli, MD; Frank Kee, MD; Stefan Söderberg, PhD; Teemu Niiranen, MD; Kari Kuulasmaa, PhD; Giovanni de Gaetano, PhD; Maria Benedetta Donati, PhD; Stefan Blankenberg, MD; Licia Iacoviello, MD; on behalf of the BiomarCaRE Investigators

Background and Purpose—NT-proBNP (N-terminal pro-B-type natriuretic peptide) 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-Consortium, we analyzed data of 58 173 participants (50% men; mean age 52 y) free of stroke from 6 community-based cohorts. NT-proBNP measurements were performed in the central Biomarkers for Cardiovascular Risk Assessment in Europe laboratory. The outcomes considered were total stroke and subtypes of stroke (ischemic/hemorrhagic).

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 2-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 nonevents 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 hemorrhagic 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. **(*Stroke*. 2019;50:610-617. DOI: 10.1161/STROKEAHA.118.023218.)**

Key Words: atrial fibrillation ■ biomarkers ■ brain ■ epidemiology ■ stroke

NT-proBNP (N-terminal pro-B-type natriuretic peptide) is the N-terminal fragment of the B-type natriuretic peptide, 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

Received August 10, 2018; final revision received December 7, 2018; accepted January 15, 2019.

From the Department of Epidemiology and Prevention, IRCCS NEUROMED, Pozzilli (IS), Italy (A. Di Castelnuovo, S.C., A. de Curtis, G.d.G., M.B.D., L.I.); Department of Medicine and Surgery, Research Centre in Epidemiology and Preventive Medicine, University of Insubria, Varese, Italy (G.V., R.B., M.F., L.I.); Department for General and Interventional Cardiology, University Heart Center Hamburg, Germany (T.Z., R.B.S., S.B.); German Center for Cardiovascular Research (DZHK), Partner Site Hamburg/Luebeck/Kiel, Hamburg, Germany (T.Z., R.B.S., S.B.); National Institute for Health and Welfare, Helsinki, Finland (V.S., T.N., K.K.); Department of Cardiovascular, Dysmetabolic and Ageing-Associated Diseases, Istituto Superiore di Sanità, Rome, Italy (S.G.); UKCRC Centre of Excellence for Public Health, Queens University of Belfast, Belfast, Northern Ireland (F.K.); Department of Public Health and Clinical Medicine, and Heart Centre, Umeå University, Sweden (S.S.); and Department of Internal Medicine, Turku University Hospital and University of Turku, Finland (T.N.).

The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.118.023218.

Correspondence to Licia Iacoviello, MD, PhD, Department of Epidemiology and Prevention, IRCCS NEUROMED, Via dell'Elettronica, 86077 Pozzilli (Isernia), Italy. Email licia.iacoviello@moli-sani.org

syndromes.[3–8] Several studies have investigated the association of NT-proBNP with an 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 hemorrhagic stroke or with fatal or nonfatal stroke. In the ARIC study (The Atherosclerosis Risk in Communities Study),[11] NT-proBNP was found to be associated with total stroke, nonlacunar ischemic, and especially cardioembolic stroke but not with lacunar or hemorrhagic stroke. In a case-cohort study derived from the REGARDS cohort (Reasons for Geographic and Racial Differences in Stroke), 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 analyzed individual NT-proBNP concentrations in 40 336 individuals of 6 population cohorts with the aims: to achieve a precise characterization of the association of NT-proBNP with stroke in Europe[1]; to assess possible difference in the association of NT-proBNP with ischemic or hemorrhagic stroke or with fatal stroke[2]; and to determine the predictive value of NT-proBNP beyond classical risk factors for stroke.[3]

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 MONICA Risk Genetics Archiving and Monograph (MORGAM) 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 187 736 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 58 173 individuals free of stroke at baseline (individuals with a positive history of stroke based on self-report 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 Table I in the online-only Data Supplement. For each cohort the following harmonized variables were available at baseline: age, sex, body mass index, systolic and diastolic blood pressure, antihypertensive medication, smoking status, total and HDL (high-density lipoprotein) cholesterol and estimated glomerular filtration rate calculated by using of Chronic Kidney Disease Epidemiology Collaboration formula, history of diabetes mellitus, 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 mellitus was defined as self-reported or documented diabetes mellitus. 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 to 35 000 pg/mL. The study-specific intra-assay and interassay coefficients of variation are described in Table II in the online-only Data Supplement.

Study Outcome

Participants in each cohort were followed-up for first stroke (fatal or nonfatal) and death from other causes. Deaths were identified through record linkage with national or regional health information systems. Nonfatal 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 Methods in the online-only Data Supplement. Briefly, a stroke is classified as a cerebral infarction (ischemic stroke) if at least one of the following is present: (1) validation of recent brain infarction in necropsy; (2) circumscribed hypodensity changes of recent origin in the brain parenchyma on computed tomography (CT); and (3) 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 (*International Classification of Diseases, Eighth Revision* [*ICD-8*] value of 432, 433, or 434; an *International Classification of Diseases, Ninth Revision* [ICD-9] value of 434; or an *International Classification of Diseases, Tenth Revision* [ICD-10] value of I63). To be accepted as a case of subarachnoid hemorrhage, at least one of the following must be present: (1) necropsy—recent subarachnoid hemorrhage; (2) CT-signs of blood in the subarachnoid cisterns or in cerebral ventricles; (3) magnetic resonance imaging—signs of blood in the subarachnoid cisterns or in cerebral ventricles; and (4) cerebrospinal fluid (liquor) bloody and 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: (1) necropsy—recent intracerebral hemorrhage; (2) CT-hyperdensity changes in the brain parenchyma; (3) magnetic resonance imaging—typical signs of bleeding in the brain parenchyma; and (4) 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 cardiovascular disease (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, SD 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], SD 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 sex, examination age, total and HDL cholesterol, smoker status, hypertension, systolic and diastolic blood pressure, body mass index, diabetes mellitus, and estimated glomerular filtration rate was assessed using Pearson 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 (HRs; with 95% CI) 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, body mass index, diabetes mellitus, 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 estimated glomerular filtration rate 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 Q test and the I^2 statistic to quantify heterogeneity among cohorts. The C-index, the categorical net reclassification improvement and the absolute and relative integrated discrimination improvement[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 net reclassification improvement calculation were: <2%, 2% to <5%, 5% to <8%, and ≥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 2-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 43–61 years). Study participants were slightly overweight (median body mass index 27.3 kg/m^2). At baseline 24.7% of the individuals were daily smokers, 19.2% were prescribed antihypertensive medication, 5% had diabetes mellitus, and <2.7% had a personal history of myocardial infarction, heart failure, or atrial fibrillation (Table 1). Characteristics of the different cohorts are illustrated in Table III in the online-only Data Supplement.

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 with the southern Europe cohorts (Italian cohorts; P<0.0001).

Table 1. General Characteristics of the Studied Population

Baseline Characteristics	
No. of populations, N	6
No. of individuals, N	58 173
Years of baseline examinations, range in years	1986–2008
Age at baseline examination, y	52 (13)
Men, N (%)	29 275 (50.32)
Myocardial infarction, N (%)	1571 (2.70)
Heart failure, N (%)	505/49 009 (1.03)*
Atrial fibrillation, N (%)	523/42 532 (1.23)*
Stroke risk factors	
Daily smoker, N (%)	14 363 (24.69)
Diabetes mellitus, N (%)	2888 (4.96)
Antihypertensive medication, N (%)	11 170 (19.20)
Body mass index, kg/m^2	27.3 (4.7)
Systolic blood pressure, mm Hg	137 (21)
Total cholesterol, mg/dL	219 (44)
HDL cholesterol, mg/dL	55 (15)
Estimated glomerular filtration rate, mL/min per 1.73 m^2	93 (18)
NT-ProBNP, pg/mL	82 (232)
Log(NT-ProBNP), log(pg/mL)	3.7 (1.1)
End points during follow-up	
Stroke (any type), N (%)	1550 (2.66)
Ischemic stroke, N (%)†	1176 (2.02)
Hemorrhagic stroke, N (%)†	330 (0.57)
Fatal stroke (any type), N (%)	249 (0.43)
Other events during follow-up	
Myocardial infarction, N (%)	776 (1.33)
Heart failure, N (%)	1431/46 012 (3.11)‡
Atrial fibrillation, N (%)	1363/45 775 (2.98)‡

Characteristics are presented as absolute and relative frequencies for categorical variables, and mean value and SD for continuous variables as well as ranges in years for years of baseline examinations. HDL indicates high-density lipoprotein; and NT-proBNP, N-Terminal Pro-B-type natriuretic peptide.

*History of heart failure at baseline was available for 49 009 individuals. History of atrial fibrillation at baseline was available for 42 532 individuals.

†N=44 strokes were unclassified.

‡Incidence of heart failure (atrial fibrillation) was not evaluated for 12 161 (12 398) individuals.

NT-proBNP Concentrations and Association With Stroke Outcomes

During a median follow-up time of 7.9 years (interquartile range 4.2–13.8) 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 hemorrhages), and for n=44 there was insufficient information to determine the type of stroke (Table 1).

Table 3 displays fully adjusted HRs across quarters of the NT-proBNP distribution indicating the associations with

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 Mellitus	eGFR
Correlation coefficient (95% CI); *P* value	–0.24 (–0.25 to –0.23); *P*<0.001	0.45 (0.44 to 0.45); *P*<0.001	–0.03 (–0.04 to –0.02); *P*<0.001	0.14 (0.13 to 0.14); *P*<0.001	–0.09 (–0.10 to –0.08); *P*<0.001	0.25 (0.24 to 0.26); *P*<0.001	0.26 (0.25 to 0.26); *P*<0.001	0.04 (0.03 to 0.05); *P*<0.001	0.09 (0.08 to 0.10); *P*<0.001	–0.26 (–0.27 to –0.25); *P*<0.001

BMI indicates body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; and NT-proBNP, N-Terminal Pro-B-type natriuretic peptide.

stroke. The risk of stroke in the top quarter was double that in the bottom quarter. The association was virtually unchanged when adjusted for estimated glomerular filtration rate and it was 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 similar magnitude for both ischemic and hemorrhagic and for fatal and nonfatal strokes (Table 3).

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

The adjusted HR (Model 2) for case fatality (stroke fatal events among stroke events) for 4° versus 1° quarter of NT-proBNP was 1.16 (95% CI, 0.71–1.92).

Subgroup Analysis of the NT-proBNP-Associated Risk

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

The effect of NT-proBNP was highly homogenous across CVD risk categories (Figure 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° versus 1° quarter of NT-proBNP was also similar in individuals free from CVD at baseline (myocardial infarction, atrial fibrillation, or heart failure, n=40 507, n=1100 stroke events during follow-up), HR=2.21 (95% CI, 1.79–2.72) when compared with 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, 0.93–4.99; *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 nonevents, and an absolute and relative integrated discrimination improvement of 0.007 and 0.11, respectively.

Discussion

Based on harmonized individual-level data and a centrally standardized NT-proBNP evaluation in >58 000 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 56 764 individuals which found a relative risk of 1.93 (95% CI, 1.58–2.37) 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 cardioembolic 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 were strongest for cardioembolic strokes.

NT-proBNP as a Stroke Risk Factor

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

The association of NT-proBNP levels with stroke was absent when the second quarter was compared with the first, modest when the third quarter was compared with the first and evident when the top quarter is compared with 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 nonfatal 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

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)				
No. of events	207	248	321	774
Model 1* (95% CI)	-1- reference	1.09 (0.89–1.32)	1.27 (1.05–1.53)	2.25 (1.88–2.68)
Model 2† (95% CI)	-1- reference	1.09 (0.90–1.32)	1.28 (1.06–1.54)	2.14 (1.79–2.57)
Model 3‡ (95% CI)	-1- reference	1.09 (0.90–1.32)	1.27 (1.05–1.53)	2.06 (1.72–2.47)
Ischemic strokes (N=1176)				
No. of events	152	189	235	600
Model 2† (95% CI)	-1- reference	1.13 (0.91–1.42)	1.28 (1.02–1.60)	2.24 (1.82–2.76)
Model 3‡ (95% CI)	-1- reference	1.12 (0.90–1.40)	1.25 (1.00–1.57)	2.12 (1.72–2.62)
Hemorrhagic strokes (N=330)				
No. of events	50	53	76	151
Model 2† (95% CI)	-1- reference	1.01 (0.67–1.51)	1.41 (0.96–2.06)	2.09 (1.42–3.09)
Model 3‡ (95% CI)	-1- reference	1.02 (0.68–1.53)	1.42 (0.97–2.08)	2.09(1.42–3.09)
Incident strokes that were fatal (N=249)				
No. of events	25	34	48	142
Model 2† (95% CI)	-1- reference	1.08 (0.63–1.84)	1.09 (0.64–1.86)	2.15 (1.33–3.47)
Model 3‡ (95% CI)	-1- reference	1.11 (0.65–1.88)	1.12 (0.66–1.90)	2.08 (1.29–3.37)
Incident strokes that were nonfatal (N=1301)				
No. of events	182	214	273	632
Model 2† (95% CI)	-1- reference	1.10 (0.89–1.36)	1.32 (1.08–1.62)	2.15 (1.77–2.62)
Model 3‡ (95% CI)	-1- reference	1.10 (0.89–1.35)	1.31 (1.07–1.61)	2.07 (1.70–2.52)

BMI indicates body mass index; HDL, high-density lipoprotein; and NT-proBNP, N-Terminal Pro-B-type natriuretic peptide.

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

†Model 2: model 1 + smoking, BMI, diabetes mellitus, 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.

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 because of 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 CVD 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 CVD 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 cardioembolic stroke,[11,12] elevated NT-proBNP at baseline is the most probably because of subclinical cardiac pathology which increases the risk of stroke events years later. In this case, an elevated NT-proBNP should

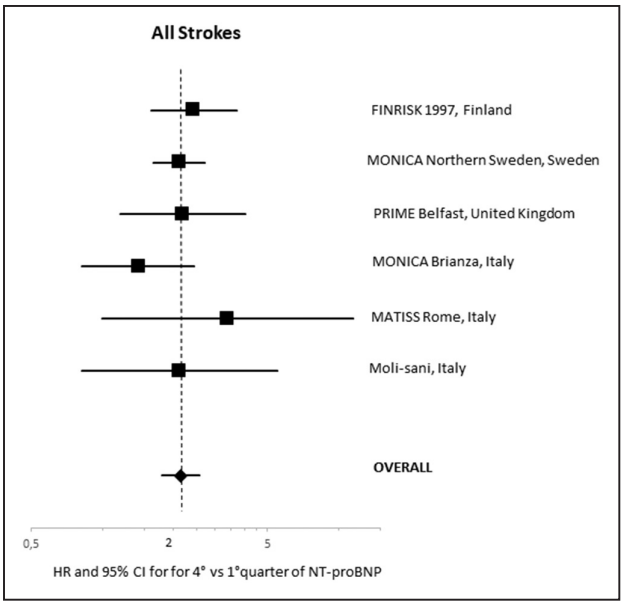

Figure 1. Hazard ratio and 95% CI (adjusted as in model 2, see Table 3) for 4° vs 1° quarter of NT-proBNP (N-Terminal Pro-B-type natriuretic peptide) in overall and separate cohorts; all strokes.

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 several 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 integrated discrimination improvement 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 neurological examinations or data from CT or magnetic resonance imaging, especially for those cohorts with baseline enrollment in 1980s and 1990s. Moreover, information on the cause 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 end point and covariate 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 58 000 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

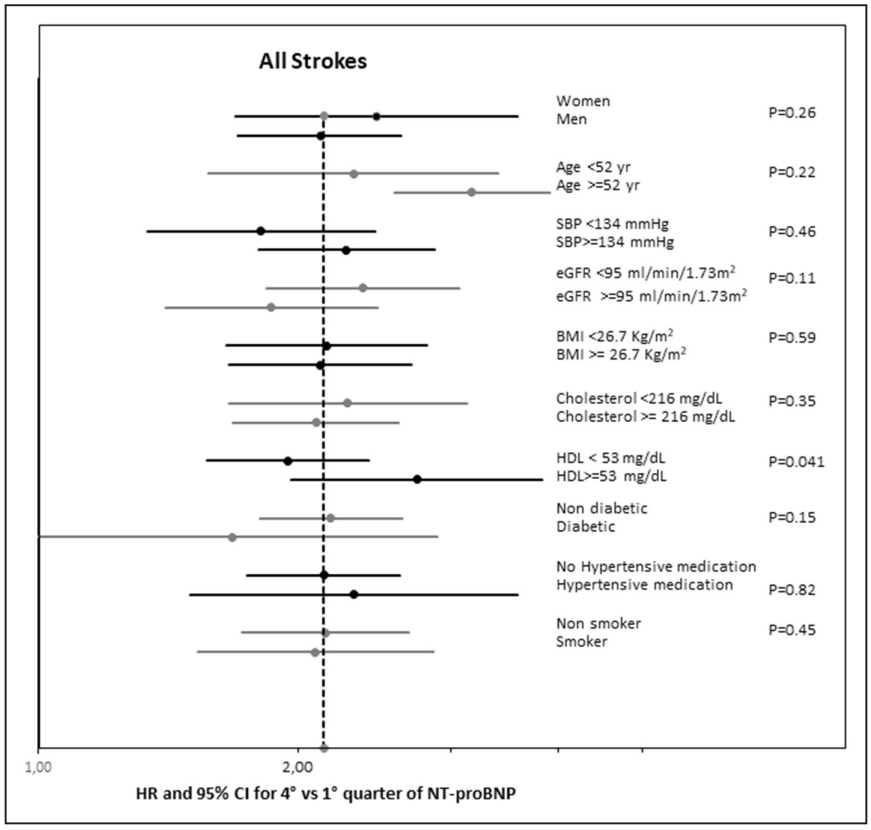

Figure 2. Hazard ratio and 95% CI (adjusted as in model 2, see Table 3) for 4° vs 1° quarter of NT-proBNP (N-Terminal Pro-B-type natriuretic peptide) a with different cardiovascular risk factors; all strokes.

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 transnational 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.

Sources of Funding

The BiomarCaRE Project is funded by the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement No. HEALTH-F2-2011–278913. The activities of the MORGAM Data Centre have been sustained also by recent funding from European Union FP 7 project CHANCES (HEALTH-F3-2010–242244). A part of the biomarker determinations in the population cohorts was funded by the Medical Research Council London (G0601463, identification No. 80983: Biomarkers in the MORGAM Populations). This project has received funding from the European Research Council under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 648131) (Dr Schnabel), German Ministry of Research and Education (BMBF 01ZX1408A) (Dr Zeller and Dr Schnabel) and German Center for Cardiovascular Research (DZHK e.V.) (81Z1710103) (Dr Schnabel) and 81Z1710101 (Dr Zeller). The more recent activities of the MONICA Brianza Study have been funded by the Health Administration of the Lombardia Region (grants no. 17155/2004 and 10800/2009). The follow-up was partially supported with grants from the Italian Ministry of Health (grant 2012/597) and it was performed in collaboration with the Department for Cardiovascular Dysmetabolic and Ageing—Associated Diseases of the Istituto Superiore di Sanità in Rome. Dr Salomaa was supported by the Finnish Foundation for Cardiovascular Research.

Disclosures

Dr Salomaa has participated in a conference trip sponsored by Novo Nordisk and received a modest honorarium from the same source for participating in an advisory board meeting. Dr Blankenberg reports investigator-initiated grants from SIEMENS, Abbott Diagnostics, and Thermofisher. Dr Schnabel has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 648131), German Ministry of Research and Education (BMBF 01ZX1408A), and German Center for Cardiovascular Research (DZHK e.V.) (81Z1710103). Dr Kee has received funding from the National Institute of Health Research United Kingdom. The other authors report no conflicts.

References

1. Daniels LB, Alan S. Maisel, natriuretic peptides. *J Am Coll Cardiol*. 2007;50:2357–2368.
2. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC Jr. Plasma brain natriuretic peptide concentration: impact of age and gender. *J Am Coll Cardiol*. 2002;40:976–982.
3. Arakawa N, Nakamura M, Aoki H, Hiramori K. Plasma brain natriuretic peptide concentrations predict survival after acute myocardial infarction. *J Am Coll Cardiol*. 1996;27:1656–1661.
4. de Lemos JA, Morrow DA, Bentley JH, Omland T, Sabatine MS, McCabe CH, et al. The prognostic value of B-type natriuretic peptide in patients with acute coronary syndromes. *N Engl J Med*. 2001;345:1014–1021. doi: 10.1056/NEJMoa011053
5. Fonarow GC, Peacock WF, Phillips CO, Givertz MM, Lopatin M; ADHERE Scientific Advisory Committee and Investigators. Admission B-type natriuretic peptide levels and in-hospital mortality in acute decompensated heart failure. *J Am Coll Cardiol*. 2007;49:1943–1950. doi: 10.1016/j.jacc.2007.02.037
6. James SK, Lindahl B, Siegbahn A, Stridsberg M, Venge P, Armstrong P, et al. N-terminal pro-brain natriuretic peptide and other risk markers for the separate prediction of mortality and subsequent myocardial infarction in patients with unstable coronary artery disease: a Global Utilization of Strategies To Open occluded arteries (GUSTO)-IV substudy. *Circulation*. 2003;108:275–281. doi: 10.1161/01.CIR.0000079170.10579.DC
7. Kragelund C, Grønning B, Køber L, Hildebrandt P, Steffensen R. N-terminal pro-B-type natriuretic peptide and long-term mortality in stable coronary heart disease. *N Engl J Med*. 2005;352:666–675. doi: 10.1056/NEJMoa042330
8. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med*. 2004;350:655–663. doi: 10.1056/NEJMoa031994
9. Welsh P, Doolin O, Willeit P, Packard C, Macfarlane P, Cobbe S, et al. N-terminal pro-B-type natriuretic peptide and the prediction of primary cardiovascular events: results from 15-year follow-up of WOSCOPS. *Eur Heart J*. 2013;34:443–450. doi: 10.1093/eurheartj/ehs239
10. Di Angelantonio E, Chowdhury R, Sarwar N, Ray KK, Gobin R, Saleheen D, et al. B-type natriuretic peptides and cardiovascular risk: systematic review and meta-analysis of 40 prospective studies. *Circulation*. 2009;120:2177–2187. doi: 10.1161/CIRCULATIONAHA.109.884866
11. Folsom AR, Nambi V, Bell EJ, Oluleye OW, Gottesman RF, Lutsey PL, et al. Troponin T, N-terminal pro-B-type natriuretic peptide, and incidence of stroke: the atherosclerosis risk in communities study. *Stroke*. 2013;44:961–967. doi: 10.1161/STROKEAHA.111.000173
12. Cushman M, Judd SE, Howard VJ, Kissela B, Gutiérrez OM, Jenny NS, et al. N-terminal pro-B-type natriuretic peptide and stroke risk: the reasons for geographic and racial differences in stroke cohort. *Stroke*. 2014;45:1646–1650. doi: 10.1161/STROKEAHA.114.004712
13. Zeller T, Hughes M, Tuovinen T, Schillert A, Conrads-Frank A, Ruijter Hd, et al. BiomarCaRE: rationale and design of the European BiomarCaRE project including 300,000 participants from 13 European countries. *Eur J Epidemiol*. 2014;29:777–790. doi: 10.1007/s10654-014-9952-x
14. Kulathinal S, Niemela M, Niiranen T, Saarela O, Palosaari T, Tapanainen H et al. Contributors from Participating Centres, for the MORGAM Project. Description of MORGAM Cohorts. MORGAM Project. http://www.thl.fi/publications/morgam/manual/contents.htm. Accessed August 9, 2018.
15. Blankenberg S, Salomaa V, Makarova N, Ojeda F, Wild P, Lackner KJ, et al; BiomarCaRE Investigators. Troponin I and cardiovascular risk prediction in the general population: the BiomarCaRE consortium. *Eur Heart J*. 2016;37:2428–2437. doi: 10.1093/eurheartj/ehw172
16. Ferrario MM, Veronesi G, Kee F, Chambless LE, Kuulasmaa K, Jørgensen T, et al; MORGAM Project. Determinants of social inequalities in stroke incidence across Europe: a collaborative analysis of 126 635 individuals from 48 cohort studies. *J Epidemiol Community Health*. 2017;71:1210–1216. doi: 10.1136/jech-2017-209728
17. MORGAM Project. MORGAM Manual. MORGAM Project e-publications. http://www.thl.fi/publications/morgam/manual/contents.htm. Accessed August 9, 2018.
18. Pencina MJ, D'Agostino RB Sr, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med*. 2011;30:11–21. doi: 10.1002/sim.4085
19. Zimetbaum PJ, Thosani A, Yu HT, Xiong Y, Lin J, Kothawala P, et al. Are atrial fibrillation patients receiving warfarin in accordance with stroke risk? *Am J Med*. 2010;123:446–453. doi: 10.1016/j.amjmed.2009.11.015
20. Rutten JH, Mattace-Raso FU, Steyerberg EW, Lindemans J, Hofman A, Wieberdink RG, et al. Amino-terminal pro-B-type natriuretic peptide improves cardiovascular and cerebrovascular risk prediction in the population: the Rotterdam study. *Hypertension*. 2010;55:785–791. doi: 10.1161/HYPERTENSIONAHA.109.143313
21. Kistorp C, Raymond I, Pedersen F, Gustafsson F, Faber J, Hildebrandt P. N-terminal pro-brain natriuretic peptide, C-reactive protein, and urinary albumin levels as predictors of mortality and cardiovascular events in older adults. *JAMA*. 2005;293:1609–1616. doi: 10.1001/jama.293.13.1609

22. Winkler K, Wanner C, Drechsler C, Lilienthal J, März W, Krane V; German Diabetes and Dialysis Study Investigators. Change in N-terminal-pro-B-type-natriuretic-peptide and the risk of sudden death, stroke, myocardial infarction, and all-cause mortality in diabetic dialysis patients. *Eur Heart J*. 2008;29:2092–2099. doi: 10.1093/eurheartj/ehn278
23. Takahashi T, Nakamura M, Onoda T, Ohsawa M, Tanno K, Itai K, et al. Predictive value of plasma B-type natriuretic peptide for ischemic stroke: a community-based longitudinal study. *Atherosclerosis*. 2009;207:298–303. doi: 10.1016/j.atherosclerosis.2009.04.029
24. Omland T, Sabatine MS, Jablonski KA, Rice MM, Hsia J, Wergeland R, et al; PEACE Investigators. Prognostic value of B-Type natriuretic peptides in patients with stable coronary artery disease: the PEACE Trial. *J Am Coll Cardiol*. 2007;50:205–214. doi: 10.1016/j.jacc.2007.03.038
25. Doi Y, Ninomiya T, Hata J, Hirakawa Y, Mukai N, Ikeda F, et al. N-terminal pro-brain natriuretic peptide and risk of cardiovascular events in a Japanese community: the Hisayama study. *Arterioscler Thromb Vasc Biol*. 2011;31:2997–3003. doi: 10.1161/ATVBAHA.111.223669
26. Hijazi Z, Oldgren J, Andersson U, Connolly SJ, Ezekowitz MD, Hohnloser SH, et al. Cardiac biomarkers are associated with an increased risk of stroke and death in patients with atrial fibrillation: a Randomized Evaluation of Long-term Anticoagulation Therapy (RE-LY) substudy. *Circulation*. 2012;125:1605–1616. doi: 10.1161/CIRCULATIONAHA.111.038729
27. Tung PP, Olmsted E, Kopelnik A, Banki NM, Drew BJ, Ko N, et al. Plasma B-type natriuretic peptide levels are associated with early cardiac dysfunction after subarachnoid hemorrhage. *Stroke*. 2005;36:1567–1569. doi: 10.1161/01.STR.0000170699.59783.d6
28. Shibazaki K, Kimura K, Sakai K, Aoki J, Sakamoto Y. Plasma brain natriuretic peptide is elevated in the acute phase of intracerebral hemorrhage. *J Clin Neurosci*. 2014;21:221–224. doi: 10.1016/j.jocn.2013.02.035
29. Kuhn M. Endothelial actions of atrial and B-type natriuretic peptides. *Br J Pharmacol*. 2012;166:522–531. doi: 10.1111/j.1476-5381.2012.01827.x
30. Cannone V, Huntley BK, Olson TM, Heublein DM, Scott CG, Bailey KR, et al. Atrial natriuretic peptide genetic variant rs5065 and risk for cardiovascular disease in the general community: a 9-year follow-up study. *Hypertension*. 2013;62:860–865. doi: 10.1161/HYPERTENSIONAHA.113.01344
31. Lee JM, Zhai G, Liu Q, Gonzales ER, Yin K, Yan P, et al. Vascular permeability precedes spontaneous intracerebral hemorrhage in stroke-prone spontaneously hypertensive rats. *Stroke*. 2007;38:3289–3291. doi: 10.1161/STROKEAHA.107.491621
32. Lin K, Kazmi KS, Law M, Babb J, Peccerelli N, Pramanik BK. Measuring elevated microvascular permeability and predicting hemorrhagic transformation in acute ischemic stroke using first-pass dynamic perfusion CT imaging. *AJNR Am J Neuroradiol*. 2007;28:1292–1298. doi: 10.3174/ajnr.A0539
33. Pencina MJ, D'Agostino RB, Pencina KM, Janssens AC, Greenland P. Interpreting incremental value of markers added to risk prediction models. *Am J Epidemiol*. 2012;176:473–481. doi: 10.1093/aje/kws207