Original Article

Moderate Levels of N-Terminal Pro-B-Type Natriuretic Peptide is Associated with Increased Risks of Total and Ischemic Strokes among Japanese

The Circulatory Risk in Communities Study

Kenji Ebihara[1], Kazumasa Yamagishi[1, 2, 3], Mitsumasa Umesawa[1, 2, 4], Isao Muraki[2, 5], Renzhe Cui[5], Hironori Imano[2, 5], Yasuhiko Kubota[2], Mina Hayama-Terada[2, 6], Yuji Shimizu[2], Tetsuya Ohira[7], Tomoko Sankai[8], Takeo Okada[2], Akihiko Kitamura[2, 9], Masahiko Kiyama[2], Hiroyasu Iso[1, 5], for the CIRCS Investigators

[1]Department of Public Health Medicine, Faculty of Medicine, and Health Services Research and Development Center, University of Tsukuba, Ibaraki, Japan.
[2]Osaka Center for Cancer and Cardiovascular Disease Prevention, Osaka, Japan.
[3]Ibaraki Western Medical Center, Ibaraki, Japan.
[4]Department of Public Health, Dokkyo Medical University School of Medicine, Tochigi, Japan.
[5]Public Health, Department of Social Medicine, Osaka University Graduate School of Medicine, Osaka, Japan.
[6]Yao Public Health Center, Yao City Office, Osaka, Japan.
[7]Department of Epidemiology, Fukushima Medical University School of Medicine, Fukushima, Japan.
[8]Department of Public Health and Nursing, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan.
[9]Research Team for Social Participation and Community Health, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan.

Aim: N-terminal pro-B-type natriuretic peptide (NT-proBNP), frequently used as a biochemical marker for detecting and monitoring heart failure, is also a risk marker for development of coronary heart disease and total stroke. However, studies that explore subtypes of ischemic stroke with regard to NT-proBNP are scarce. Here, we examined NT-proBNP and its impact upon subtypes of ischemic stroke (lacunar stroke, large-artery occlusive stroke and embolic stroke) among Japanese.

Methods: We measured NT-proBNP and categorized 4,393 participants of the Circulatory Risk in Communities Study into four groups (<55, 55-124, 125-399, and ≥ 400 pg/ml). We used a multivariable Cox proportional hazards model to examine association with risks of stroke and subtypes.

Results: During 4.7 years of follow-up, we identified 50 strokes, including 35 ischemic (15 lacunar, 6 large-artery occlusive, 10 embolic strokes) and 14 hemorrhagic strokes. NT-proBNP was associated with stroke risk: the multivariable hazard ratio of total strokes was 7.29 (2.82-18.9) for the highest and 2.78 (1.25-6.16) for the second highest NT-proBNP groups compared with the lowest group. The respective hazard ratios for the highest NT-proBNP group were 9.37 (3.14–28.0) for ischemic stroke and 6.81 (1.11–41.7) for lacunar stroke. Further adjustment for atrial fibrillation did not attenuate these associations. The associations were similarly observed for large-artery occlusive and embolic strokes.

Conclusion: We found that even moderate serum levels of NT-proBNP were associated with the risk of total and ischemic strokes among Japanese whose NT-proBNP levels were relatively low compared with Westerners.

See editorial vol. 27: 749-750

Key words: Follow-up study, Epidemiology, Biomarker, Risk factor

Introduction

Pro-brain natriuretic peptide (proBNP) is a cardiac natriuretic hormone secreted by myocytes upon stretching tension. It is activated by cleavage of the N-terminal, resulting in the formation of brain natriuretic peptide (BNP), an active part, and N-terminal pro-B-type natriuretic peptide (NT-proBNP), an inac-

tive fragment. As these non-reactive fragments are known to persist in the bloodstream longer than BNP, they serve as a useful proxy marker for it[1]. Clinically, NT-proBNP is frequently used as a biochemical means to detect and gage heart failure severity as well as a risk marker to predict cardiovascular diseases, including stroke[2-13]. Increased NT-proBNP is associated with a poor prognosis after stroke[14-16]; however, these studies were mostly conducted in the Western populations where the proportion of stroke subtypes is largely different from the Asian populations. The proportions of each subtype for total stroke among the Japanese populations are 40% for lacunar stroke, 20% for large-artery occlusive stroke, and 20%–30% for intraparenchymal hemorrhage, whereas the respective proportions among Western populations are 20%, 50%, and 10%–20%[17]. As evidence of any link between NT-proBNP and stroke subtypes in the Japanese are scarce, we therefore examined the association between NT-proBNP and incident stroke with respect to its subtypes (lacunar stroke, large-artery occlusive stroke, embolic stroke, and hemorrhagic stroke) in the Japanese people.

Methods

Study Population

The Circulatory Risk in Communities Study (CIRCS) is an ongoing, dynamic, community-based prospective study based on five communities in Japan. Details of the CIRCS protocol have been described elsewhere[18]. There were 4,404 participants, aged 20–95 years, who participated in annual health check-ups from 2010 to 2012 at the CIRCS Ikawa site and from 2010 to 2013 at the CIRCS Kyowa site. Exclusion criteria were those with a history of stroke at baseline ($n=11$). A total of 4,393 participants were enrolled according to these criteria. Participants were monitored from baseline to whichever of the following dates came first: date of incident stroke/death or December 31, 2014 in Kyowa or December 31, 2016 in Ikawa.

Risk Factor Measurements

Lifestyle-related factors, such as smoking, alcohol consumption, medication for hypertension, medication for diabetes, medication for hyperlipidemia, and history of stroke and heart disease were recorded from face-to-face interviews. We included angina and myo-

cardial infarction in heart disease. Blood samples were collected into siliconized vacuum tubes and left to stand for 10 to 30 min at room temperature (to clot) before being centrifuged at 1,300 to $1,500 \times g$ for approximately 15 min. NT-proBNP was measured on a Cobas 8000 analyzer (Roche Diagnostics Corporation, Indianapolis, United States) via electro-chemiluminescence immunoassay. Total cholesterol, high-density lipoprotein (HDL) cholesterol, blood glucose and creatinine were measured using standardized protocols at the laboratory of the Osaka Center for Cancer and Cardiovascular Disease Prevention (for Ikawa) and the Ibaraki Health Service Association (for Kyowa). Serum HDL and total cholesterols were measured using enzymatic methods. Serum non-HDL cholesterol was defined as serum values of total cholesterol minus HDL cholesterol. Blood glucose was measured using the hexokinase method and serum creatinine was assayed enzymatically. Diabetes mellitus was defined as having a fasting blood glucose ≥ 126 mg/dl (less than 8 hours postprandial) or non-fasting blood glucose ≥ 200 mg/dl (8 hours or more postprandial) and/or medication for diabetes. Sitting blood pressure was measured after a 5-minute rest and the measurement was repeated if the systolic blood pressure level was 130 mmHg or more and/or the diastolic level was 85 mmHg or more. The values of this second reading, if applicable, were used in place of the first. We calculated the estimated glomerular filtration rate (eGFR) as follows: (ml/min per 1.73 m^2) = 194 × (serum creatinine)$^{-1.094}$ × (age)$^{-0.287}$ (× 0.739 for women)[19]. Electrocardiography was conducted in a supine position. Two physicians independently coded each electrocardiograph based on the Minnesota Codes. If the codes agreed, they were accepted, while disputed codes were discussed by the two physicians and, if necessary, a third experienced physician arbitrated. The electrocardiography implementation rate was 81.1%.

Determination of Incident Stroke

We identified incident stroke events occurring between baseline (2010–2012 in Ikawa or 2010–2013 in Kyowa) and December 31, 2016 in Ikawa and December 31, 2014 in Kyowa. Information on incident strokes was based on the CIRCS community stroke registration system that systematically covered all incident strokes in these communities[18]. Non-symptomatic stroke was not included as a stroke case. For the survey, trained physicians reviewed medical

Table 1. Age- and sex-adjusted baseline characteristics according to the clinical category of N-terminal pro-B type natriuretic peptide levels among 4,393 Japanese men and women, CIRCS 2010–2016

| Number of subjects | N-terminal pro-B type natriuretic peptide (pg/ml) | | | | |
	<55 (N=2,617)	55–124 (N=1,218)	125–399 (N=457)	≥ 400 (N=101)	P for trend
Age[¶], years	56.9	64.6	70.4	71.4	<0.001
Male sex[§], %	46.8	30.4	32.7	48.8	0.29
Current smoker, %	14.1	17.4	18.8	18.4	0.11
Current drinker, %	36.5	39.5	38.5	32.1	0.46
Body mass index, kg/m^2	23.8	23.3	23.0	23.5	0.06
Systolic blood pressure, mmHg	124.5	126.8	128.6	123.6	0.16
Diastolic blood pressure, mmHg	75.1	75.1	74.1	72.4	0.008
Hypertension medication use, %	25.1	28.6	36.9	43.8	<0.001
Total cholesterol, mg/dl	211	203	195	188	<0.001
Non-high density lipoprotein cholesterol, mg/dl	149	140	132	127	<0.001
High-density lipoprotein cholesterol, mg/dl	62	63	62	61	0.83
Cholesterol lowering medication use, %	15.5	12.8	14.2	12.3	0.11
Estimated glomerular filtration rate, ml/min/1.73 m^2	79.1	78.6	76.5	70.2	<0.001
Diabetes mellitus, %	5.1	5.0	5.1	4.9	0.80
Atrial fibrillation, %	0.07	0.2	1.5	51.9	<0.001
History of heart disease, %	15.9	18.0	23.6	24.2	0.03

[¶]sex-adjusted
[§]age-adjusted

records from local clinics and hospitals while photocopied neuroimaging was available for 88.0% of stroke cases. Diagnosis of incident strokes were based on the modified criteria of the National Survey of Stroke[20] and subtypes were classified by computed tomography and/or magnetic resonance imaging based on the modified criteria of the Nurse's Health Study[21]. Cases were classified as ischemic stroke (lacunar stroke, large-artery occlusive stroke and embolic stroke) or hemorrhagic stroke (intraparenchymal hemorrhage and subarachnoid hemorrhage). We defined lacunar strokes, large-artery occlusive strokes, embolic strokes and unclassified ischemic strokes as "ischemic stroke."

Statistical Analysis

We categorized NT-proBNP into four groups using clinical cutoff points suggested by prior reports[9, 11, 22, 23]. Baseline characteristics with respect to NT-proBNP categories were tested using analysis of covariance, and trends of risk factors across NT-proBNP levels were tested by regression analysis. Cox proportional hazards model was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) by SAS 9.4 (SAS Institute, Cary, NC, USA). For missing values for potential confounding variables (<3.4% of each variable), we set dummy variables and included them as another group in the models. There

were no missing values for continuous variables. Our Cox analyses were stratified by area (Ikawa or Kyowa), adjusted for sex and age (Model 1), and further adjusted for body mass index (quartiles), diastolic blood pressure (continuous), antihypertensive medication use, non-HDL cholesterol (quartiles), and eGFR (continuous) (Model 2). Model 3 HRs were further adjusted for atrial fibrillation. All probability values for the statistical tests were two-tailed, and probability values below 0.05 were considered significant. Since there were no interactions between sex and NT-proBNP in relation to stroke, we combined the results for both men and women.

Ethical Considerations

The ethics committees of the Osaka Center for Cancer and Cardiovascular Disease Prevention, Osaka University and the University of Tsukuba approved this study. Informed consent was obtained verbally and an opt-out option was provided to all participants.

Results

Table 1 shows age and sex-adjusted baseline characteristics according to the clinical cutoff points of NT-proBNP levels. NT-proBNP levels were positively correlated with age, antihypertension medica-

tion use, atrial fibrillation and history of heart disease, but inversely correlated with total and non-HDL cholesterol, eGFR and diastolic blood pressure.

During a mean of 4.7 years of follow-up, 50 incident strokes were confirmed of which 35 were ischemic strokes (15 lacunar, 6 large-artery occlusive and 10 embolic strokes) and 14 were hemorrhagic strokes (7 intraparenchymal and 7 subarachnoid hemorrhages).

As shown in **Table 2**, the highest levels of NT-proBNP (≥ 400 pg/ml) were strongly associated with an increased risk of total stroke (**Fig. 1**) and its subtypes. The Model 2 HRs (adjusted for cardiovascular risk factors) were 7.29 (95% CI: 2.82-18.9) for total stroke and 9.37 (3.14-28.0) for ischemic stroke. Although the case numbers were small, similarly enhanced HRs were observed for both lacunar and large-artery occlusive strokes. Further adjustment for atrial fibrillation (Model 3) did not attenuate these associations. The HRs associated with a 1-SD increment of log-transformed NT-proBNP levels (Model 2) were 1.89 (1.46-2.46) for total stroke, 1.90 (1.40-2.57) for ischemic stroke, 1.57 (0.94-2.61) for lacunar stroke, 1.87 (0.89-3.90) for large-artery occlusive stroke, and 2.25 (1.30-3.90) for embolic stroke. Hemorrhagic stroke was not found to be associated with NT-proBNP.

These results did not alter materially when excluding persons with atrial fibrillation (**Table 3**), history of heart disease (**Supplemental Table 1**), chronic kidney disease (eGFR <60 ml/min per 1.73 m²) (**Supplemental Table 2**) or hypertension (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg or antihypertensive medication use) (**Supplemental Table 3**).

Kaplan–Meier curves for total stroke stratified by category of NT-proBNP levels were shown in Figure. Statistically significant differences in cumulative incidence were observed in persons with NT-proBNP of 125–399 and ≥ 400 compared with those of <50 pg/ml.

Discussion

We confirmed that NT-proBNP was associated with increased risk of ischemic strokes, lacunar strokes, and large-artery occlusive strokes in our Japanese study population. This positive association was also observed for embolic stroke. Notably, we found two-to three-fold higher risks for total and ischemic strokes at the second highest category of NT-proBNP levels (125–399 pg/ml) compared with the lowest category (<55 pg/ml). This finding suggests the need for the measurement of NT-proBNP in health checkups and careful monitoring for potential heart failure with moderate NT-proBNP elevation and also the management of major cardiovascular risk factors such as hypertension, diabetes, and smoking[24] at clinical practice. However, cost-effectiveness should be carefully examined.

To the best of our knowledge, two American cohort studies have examined links between NT-proBNP and risk of ischemic stroke subtypes[8, 13]. The Atherosclerosis Risk in Communities (ARIC) Study of 10,902 African–American and white men and women, aged 45-64, in 4 communities showed that NT-proBNP was associated with increased risk of total, ischemic, non-lacunar, and cardioembolic strokes during an 11.3 year follow-up period[13]. Another study, the Reasons for Geographic and Racial Differences in Stroke case-cohort study of 1,502 African–American and White men and women showed that NT-proBNP was associated with increased risk of total, ischemic, large vessel, and cardioembolic strokes during a 5.4 year follow-up period[8]. Both studies found trends toward the positive association of NT-proBNP with lacunar stroke although they were not statistically significant. Our study is therefore the first to find that NT-proBNP is significantly associated with increased risk of lacunar stroke, which is indicative of its more common prevalence in the Asian populations compared with the Western populations[17]. In our study of the Japanese populations, the distributions of NT-proBNP levels were much lower than those in the above-mentioned American studies as the quintile distribution of NT-proBNP for persons aged 45–64 years was ≤ 18, 19–28, 29–43, 44–68, and ≥ 69 pg/ml in the CIRCS versus ≤ 27.2, 27.3–51.9, 52.0–87.3, 87.4–155.1, and ≥ 155.2 pg/ml in the ARIC Study[13]. In line with the Hisayama Study[11], we also found a significantly excessive risk of ischemic stroke for the ≥ 400 pg/ml category of NT-proBNP levels compared with the <55 pg/ml category. Although the number of cases was small, we also performed analyses for stroke subtypes and found an increased risk of lacunar and large-artery occlusive strokes was associated with NT-proBNP levels of ≥ 400 pg/ml.

NT-proBNP levels are a strong predictor of atrial fibrillation, which contributes to embolic stroke[25, 26]. The exact mechanism by which NT-proBNP is associated with the risk of lacunar and large-artery occlusive strokes is unknown but may be due to different mechanisms in underlying vascular pathology. NT-proBNP has been correlated with retinal microvascular damage related to arteriosclerosis[27], which was associated with lacunar stroke risk[28] while also correlating with the presence of carotid plaques[29] that increase the risk for large-artery occlusive strokes[30].

Table 2. Adjusted HRs (95% CI) for incident stroke according to the clinical category of N-terminal pro-B type natriuretic peptide (NT-proBNP) levels

	NT-proBNP (pg/ml)					1-SD increment of log-transformed NT-proBNP
	<55	55–124	125–399	≥ 400		
Number of subjects	2,617	1,218	457	101	*p* for trend	
Person years	11,730	5,474	2,149	468		
Total stroke, *n*	19	9	14	8		
Age- and sex-adjusted and area-stratified HR (95%CI) (Model 1)	1.00	0.83 (0.36–1.89)	2.70 (1.24–5.87)	5.62 (2.26–14.0)	<0.001	1.75 (1.38–2.24)
Multivariable-adjusted HR (95%CI)[†] (Model 2)	1.00	0.84 (0.36–1.92)	2.78 (1.25–6.16)	7.29 (2.82–18.9)	<0.001	1.89 (1.46–2.46)
Multivariable-adjusted HR (95%CI)[‡] (Model 3)	1.00	0.85 (0.37–1.94)	2.77 (1.25–6.13)	8.45 (2.85–25.0)	<0.001	1.94 (1.44–2.61)
Ischemic stroke, n	13	6	9	7		
Age- and sex-adjusted and area-stratified HR (95%CI) (Model 1)	1.00	0.79 (0.29–2.15)	2.28 (0.88–5.91)	6.01 (2.15–16.8)	<0.001	1.72 (1.30–2.29)
Multivariable-adjusted HR (95%CI)[†] (Model 2)	1.00	0.87 (0.32–2.38)	2.58 (0.97–6.88)	9.37 (3.14–28.0)	<0.001	1.90 (1.40–2.57)
Multivariable-adjusted HR (95%CI)[‡] (Model 3)	1.00	0.88 (0.32–2.43)	2.53 (0.95–6.70)	9.45 (2.67–33.4)	<0.001	1.83 (1.30–2.58)
Lacunar stroke, *n*	7	3	3	2		
Age and sex-adjusted and area-stratified HR (95%CI) (Model 1)	1.00	0.76 (0.18–3.10)	1.50 (0.34–6.65)	3.23 (0.59–17.8)	0.13	1.35 (0.85–2.13)
Multivariable-adjusted HR (95%CI)[†] (Model 2)	1.00	0.88 (0.21–3.67)	1.64 (0.34–7.83)	6.81 (1.11–41.7)	0.02	1.57 (0.94–2.61)
Multivariable-adjusted HR (95%CI)[‡] (Model 3)	1.00	0.90 (0.22–3.77)	1.67 (0.35–7.91)	13.5 (2.17–84.2)	0.003	1.52 (0.93–2.49)
Large-artery occlusive stroke, *n*	3	1	0	2		
Age- and sex-adjusted and area-stratified HR (95%CI) (Model 1)	1.00	0.52 (0.05–5.38)	-	6.95 (0.87–55.8)	0.02	1.61 (0.79–3.29)
Multivariable-adjusted HR (95%CI)[†] (Model 2)	1.00	0.72 (0.07–7.65)	-	15.5 (1.54–155)	0.005	1.87 (0.89–3.90)
Multivariable-adjusted HR (95%CI)[‡] (Model 3)	1.00	0.71 (0.07–7.53)	-	26.9 (2.92–248)	<0.001	2.07 (0.95–4.54)
Embolic stroke, *n*	2	2	4	2		
Age and sex-adjusted and area-stratified HR (95%CI) (Model 1)	1.00	1.63 (0.22–12.3)	5.62 (0.84–37.6)	9.13 (1.06–78.3)	0.05	2.28 (1.39–3.73)
Multivariable-adjusted HR (95%CI)[†] (Model 2)	1.00	1.54 (0.20–12.1)	5.14 (0.75–35.4)	7.83 (0.78–78.6)	0.10	2.25 (1.30–3.90)
Multivariable-adjusted HR (95%CI)[‡] (Model 3)	1.00	1.59 (0.20–12.3)	4.53 (0.65–31.7)	2.51 (0.12–50.9)	0.71	2.03 (1.05–3.94)
Hemorrhagic stroke, *n*	6	3	4	1		
Age and sex-adjusted and area-stratified HR (95%CI) (Model 1)	1.00	0.87 (0.21–3.69)	2.78 (0.67–11.6)	3.15 (0.33–29.7)	0.18	1.64 (0.97–2.78)
Multivariable-adjusted HR (95%CI)[†] (Model 2)	1.00	0.75 (0.17–3.26)	2.45 (0.57–10.5)	2.36 (0.23–24.0)	0.32	1.59 (0.91–2.78)
Multivariable-adjusted HR (95%CI)[‡] (Model 3)	1.00	0.74 (0.17–3.21)	2.44 (0.57–10.5)	4.67 (0.46–47.6)	0.10	1.85 (1.00–3.42)

[†]Adjusted further for body mass index, diastolic blood pressure, antihypertensive medication use, non-high-density lipoprotein cholesterol, and estimated glomerular filtration rate.
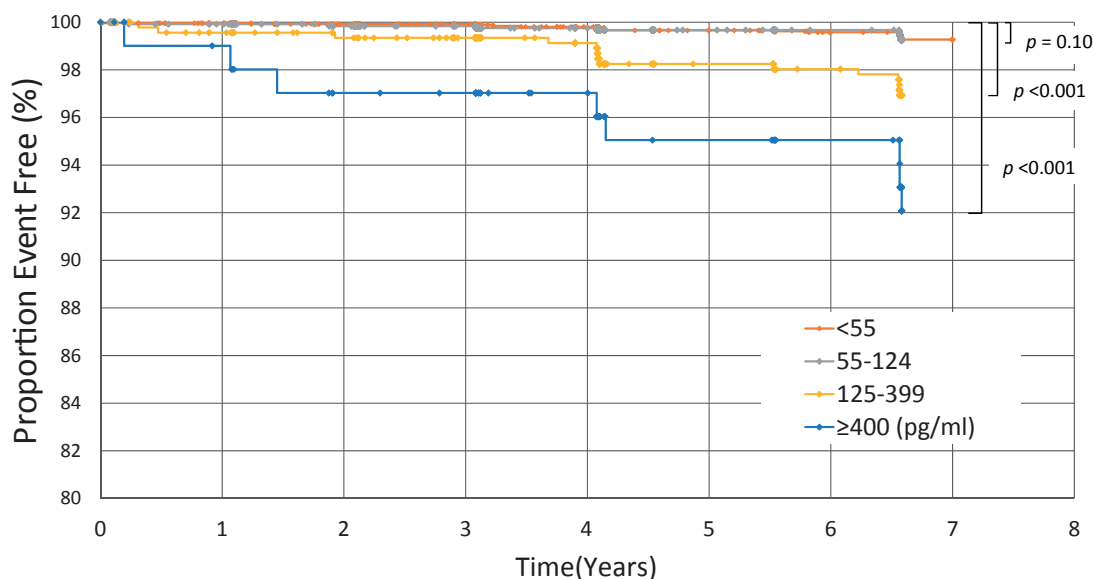[‡]Adjusted further for atrial fibrillation.

Fig. 1. Kaplan-Meier curves for total stroke

Kaplan–Meier curves for total stroke stratified by category of NT-proBNP levels was shown in Figure. Log rank *p* value between 55 pg/ml and 55–124 pg/ml was 0.10. Log rank *p* value between 55 pg/ml and 125–400 pg/ml was <0.001. Log rank *p* value between 55 pg/ml and ≥ 400 pg/ml was <0.001.

Table 3. Adjusted HRs (95% CI) for incident stroke according to the clinical category of N-terminal pro-B type natriuretic peptide (NT-proBNP) levels after the exclusion of persons with atrial fibrillation at baseline

	NT-proBNP (pg/ml)					1-SD increment of
	< 55	55–124	125–399	≥ 400	*P* for	log-transformed
Number of subjects	2,617	1,217	452	59	trend	NT-proBNP
Person years	11,730	5,467	2,125	257		
Total stroke, *n*	19	9	14	5		
Multivariable-adjusted HR (95%CI)	1.00	0.83 (0.36–1.90)	2.78 (1.25–6.18)	9.23 (3.07–27.8)	<0.001	1.95 (1.44–2.65)
Ischemic stroke, *n*	13	6	9	4		
Multivariable-adjusted HR (95%CI)	1.00	0.86 (0.31–2.36)	2.60 (0.97–6.97)	11.0 (3.02–40.4)	<0.001	1.86 (1.30–2.66)
Lacunar stroke, *n*	7	3	3	2		
Multivariable-adjusted HR (95%CI)	1.00	0.88 (0.21–3.66)	1.64 (0.34–7.78)	14.4 (2.34–88.3)	0.002	1.74 (1.02–2.98)
Large-artery occlusive stroke, *n*	3	1	0	2		
Multivariable-adjusted HR (95%CI)	1.00	0.70 (0.07–7.42)	-	28.8 (3.21–258)	<0.001	2.16 (0.99–4.72)
Embolic stroke, *n*	2	2	4	0		
Multivariable-adjusted HR (95%CI)	1.00	1.17 (0.15–9.21)	3.96 (0.56–27.8)	–	0.93	1.80 (0.85–3.84)
Hemorrhagic stroke, *n*	6	3	4	1		
Multivariable-adjusted HR (95%CI)	1.00	0.75 (0.17–3.23)	2.43 (0.57–10.4)	4.40 (0.44–44.2)	0.11	1.84 (1.00–3.38)

Adjusted for age, sex, body mass index, diastolic blood pressure, antihypertensive medication use, non-high-density lipoprotein cholesterol and estimated glomerular filtration rate and area-stratified.

Several limitations should be noted. First, the follow-up period was 4.7 years on average, ranging from 0.01 to 7.0 years. However, the Ohasama Study showed that predictive ability decreased at follow-up periods of more than 5 years after NT-proBNP measurement[9]. Second, the numbers of lacunar, large-artery occlusive, and embolic stroke cases were small so that further studies are necessary to confirm our findings. Third, because we had only a single measurement of NT-proBNP, this could lead to an underesti-

mation of our risk estimates due to regression dilution bias. However, Spearman's correlation between the baseline and 1- to 5-year post-measurement values of NT-proBNP was 0.77 in the subsample of 1,446 persons in the present study. Thus, the impact of regression dilution, if any, may not be large. Fourth, we did not collect information use of antiplatelet and anticoagulant drugs at and after baseline, which should have impacted the development of both ischemic and hemorrhagic strokes. However, most potential users of antiplatelet and anticoagulant drugs should have had heart disease or atrial fibrillation, and excluding these patients did not alter the results, implying that the impact of these drugs on the results may be small.

Conclusion

We found that even moderate serum levels of NT-proBNP were associated with increased risk of total and ischemic strokes among the Japanese whose NT-proBNP levels were relatively low compared with the Westerners.

Acknowledgments

Notice of Grant Support

Conflict of Interest

No conflicts of interest to declare. The funding agencies for this study made no decisions regarding study design, data collection, data analysis, manuscript preparation or decision to publish.

References

1) Hall C: NT-proBNP: the mechanism behind the marker. J Card Fail, 2005; 11: 81-83
2) Campbell DJ, Gong FF, Jelinek MV, Castro JM, Coller JM, McGrady M, Boffa U, Shiel L, Wan BH, Liew D, Wolfe R, Stewart S, Owen AJ, Krum H, Reid CM and Prior DL: Prediction of incident heart failure by serum amino-terminal pro-B-type natriuretic peptide level in a community-based cohort. Eur J Heart Fail, 2019; 21: 449-459
3) Hijazi Z, Verdecchia P, Oldgren J, Andersson U, Reboldi G, Di Pasquale G, Mazzotta G, Angeli F, Eikelboom JW, Ezekowitz MD, Connolly SJ, Yusuf S and Wallentin L: Cardiac biomarkers and left ventricular hypertrophy in relation to outcomes in patients with atrial fibrillation: experiences from the RE - LY trial. J Am Heart Assoc, 2019; 8: e010107
4) Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, Wolf PA and Vasan RS: Plasma natriuretic peptide levels and the risk of cardiovascular events and death. N Engl J Med, 2004; 350: 655-663
5) Di Angelantonio E, Chowdhury R, Sarwar N, Ray KK, Gobin R, Saleheen D, Thompson A, Gudnason V, Sattar N and Danesh J: B-type natriuretic peptides and cardiovascular risk: systematic review and meta-analysis of 40 prospective studies. Circulation, 2009; 120: 2177-2187
6) Rutten JH, Mattace-Raso FU, Steyerberg EW, Lindemans J, Hofman A, Wieberdink RG, Breteler MM, Witteman JC and van den Meiracker AH: Amino-terminal pro-B-type natriuretic peptide improves cardiovascular and cerebrovascular risk prediction in the population: the Rotterdam study. Hypertension, 2010; 55: 785-791
7) Kistorp C, Raymond I, Pedersen F, Gustafsson F, Faber J and 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
8) Cushman M, Judd SE, Howard VJ, Kissela B, Gutierrez OM, Jenny NS, Ahmed A, Thacker EL and Zakai NA: 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
9) Satoh M, Murakami T, Asayama K, Hirose T, Kikuya M, Inoue R, Tsubota-Utsugi M, Murakami K, Matsuda A, Hara A, Obara T, Kawasaki R, Nomura K, Metoki H, Node K, Imai Y and Ohkubo T: N-terminal pro-B-type natriuretic peptide is not a significant predictor of stroke incidence after 5 years- the Ohasama study. Circ J, 2018; 82: 2055-2062
10) Di Castelnuovo A, Veronesi G, Costanzo S, Zeller T, Schnabel RB, de Curtis A, Salomaa V, Borchini R, Ferrario M, Giampaoli S, Kee F, Soderberg S, Niiranen T, Kuulasmaa K, de Gaetano G, Donati MB, Blankenberg S and Iacoviello L; BiomarCaRE Investigators: NT-proBNP (N-terminal pro-B-type natriuretic peptide) and the risk of stroke. Stroke, 2019; 50: 610-617
11) Doi Y, Ninomiya T, Hata J, Hirakawa Y, Mukai N, Ikeda F, Fukuhara M, Iwase M and Kiyohara Y: 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
12) Hijazi Z, Oldgren J, Andersson U, Connolly SJ, Ezekowitz MD, Hohnloser SH, Reilly PA, Vinereanu D, Siegbahn A, Yusuf S and Wallentin L: 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

13) Folsom AR, Nambi V, Bell EJ, Oluleye OW, Gottesman RF, Lutsey PL, Huxley RR and Ballantyne CM: Troponin T, N-terminal pro-B-type natriuretic peptide, and incidence of stroke: the atherosclerosis risk in communities study. Stroke, 2013; 44: 961-967

14) Otaki Y, Watanabe T, Sato N, Shirata T, Tsuchiya H, Wanezaki M, Tamura H, Nishiyama S, Arimoto T, Takahashi H, Shishido T, Morikane K and Watanabe M: Direct comparison of prognostic ability of cardiac biomarkers for cardiogenic stroke and clinical outcome in patients with stroke. Heart Vessels, 2019; 34: 1178-1186

15) Rost NS, Biffi A, Cloonan L, Chorba J, Kelly P, Greer D, Ellinor P and Furie KL: Brain natriuretic peptide predicts functional outcome in ischemic stroke. Stroke, 2012; 43: 441-445

16) Yang J, Zhong C, Wang A, Xu T, Bu X, Peng Y, Wang J, Peng H, Li Q, Ju Z, Geng D, Zhang Y and He J; on behalf for the CATIS Investigation Groups: Association between increased N-terminal pro-brain natriuretic peptide level and poor clinical outcomes after acute ischemic stroke. J Neurol Sci, 2017; 383: 5-10

17) Iso, H: A Japanese health success story: Trends in cardiovascular diseases, their risk factors, and the contribution of public health and personalized approaches. EPMA J, 2011; 2: 49-57

18) Yamagishi K, Muraki I, Kubota Y, Hayama-Terada M, Imano H, Cui R, Umesawa M, Shimizu Y, Sankai T, Okada T, Sato S, Kitamura A, Kiyama M and Iso H: The Circulatory Risk in Communities Study (CIRCS): A long-term epidemiological study for lifestyle-related disease among Japanese men and women living in communities. J Epidemiol, 2018; 29: 83-91

19) Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H and Hishida A; Collaborators developing the Japanese equation for estimated GFR: Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis, 2009; 53: 982-992

20) WHO expert committee on arterial hypertension and ischaemic heart disease & World Health Organization. Arterial hypertension and ischaemic heart disease: preventive aspects, report of an expert committee [meeting held in Geneva from 16 to 23 October 1961]. World Health Organization, 1962; Technical Report Series No. 231

21) Iso H, Rexrode K, Hennekens CH and Manson JE: Application of computer tomography-oriented criteria for stroke subtype classification in a prospective study. Ann Epidemiol, 2000; 10: 81-87

22) Seino Y, Ogawa A, Yamashita T, Fukushima M, Ogata K, Fukumoto H and Takano T: Application of NT-proBNP and BNP measurements in cardiac care: a more discerning marker for the detection and evaluation of heart failure. Eur J Heart Fail, 2004; 6: 295-300

23) Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P; ESC Scientific Document Group: 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J, 2016; 37: 2129-2200

24) Kinoshita M, Yokote K, Arai H, Iida M, Ishigaki Y, Ishibashi S, Umemoto S, Egusa G, Ohmura H, Okamura T, Kihara S, Koba S, Saito I, Shoji T, Daida H, Tsukamoto K, Deguchi J, Dohi S, Dobashi K, Hamaguchi H, Hara M, Hiro T, Biro S, Fujioka Y, Maruyama C, Miyamoto Y, Murakami Y, Yokode M, Yoshida H, Rakugi H, Wakatsuki A, Yamashita S and committee for epidemiology and clinical management of atherosclerosis: Japan atherosclerosis society (JAS) guidelines for prevention of atherosclerotic cardiovascular diseases 2017. J Atheroscler and Thromb, 2018; 25: 846-984

25) Svennberg E, Lindahl B, Berglund L, Eggers KM, Venge P, Zethelius B, Rosenqvist M, Lind L and Hijazi Z: NT-proBNP is a powerful predictor for incident atrial fibrillation - Validation of a multimarker approach. Int J Cardiol, 2016; 223: 74-81

26) Schnabel RB, Larson MG, Yamamoto JF, Sullivan LM, Pencina MJ, Meigs JB, Tofler GH, Selhub J, Jacques PF, Wolf PA, Magnani JW, Ellinor PT, Wang TJ, Levy D, Vasan RS and Benjamin EJ: Relations of biomarkers of distinct pathophysiological pathways and atrial fibrillation incidence in the community. Circulation, 2010; 121: 200-207

27) Mutlu U, Ikram MA, Hofman A, de Jong PT, Klaver CC and Ikram MK: N-terminal pro-B-type natriuretic peptide is related to retinal microvascular damage: the Rotterdam Study. Arterioscler Thromb Vasc Biol, 2016; 36: 1698-1702

28) Yatsuya H, Folsom AR, Wong TY, Klein R, Klein BE and Sharrett AR; ARIC Study Investigators: Retinal microvascular abnormalities and risk of lacunar stroke: Atherosclerosis Risk in Communities Study. Stroke, 2010; 41: 1349-1355

29) Sinning C, Kieback A, Wild PS, Schnabel RB, Ojeda F, Appelbaum S, Zeller T, Lubos E, Schwedhelm E, Lackner KJ, Debus ES, Munzel T, Blankenberg S and Espinola-Klein C: Association of multiple biomarkers and classical risk factors with early carotid atherosclerosis: results from the Gutenberg Health Study. Clin Res Cardiol, 2014; 103: 477-485

30) Nagai Y, Kitagawa K, Yamagami H, Kondo K, Hougaku H, Hori M and Matsumoto M: Carotid artery intima-media thickness and plaque score for the risk assessment of stroke subtypes. Ultrasound Med Biol, 2002; 28: 1239-1243

Supplemental Table 1. Adjusted HRs (95% CI) for incident stroke according to the clinical category of N-terminal pro-B type natriuretic peptide (NT-proBNP) levels after the exclusion of patients with history of heart disease at baseline

	NT-proBNP (pg/ml)					1-SD increment of log transformed
	<55	55-124	125-399	≥ 400		
Number of subjects	2,252	979	326	70	P for trend	NT-proBNP
Person years	10,475	4,650	1,651	353		
Total stroke, _n_	17	6	9	8		
Multivariable-adjusted HR (95%CI)	1.00	0.69 (0.26-1.83)	2.09 (0.82-5.30)	9.39 (3.50-25.2)	<0.001	1.98 (1.47-2.65)
Ischemic stroke, _n_	12	3	7	7		
Multivariable-adjusted HR (95%CI)	1.00	0.55 (0.15-2.02)	2.39 (0.81-7.03)	12.9 (4.22-39.4)	<0.001	2.08 (1.49-2.89)
Lacunar stroke, _n_	6	2	3	2		
Multivariable-adjusted HR (95%CI)	1.00	0.80 (0.15-4.31)	2.07 (0.40-10.7)	13.7 (2.18-86.4)	0.002	1.88 (1.06-3.34)
Large-artery occlusive stroke, _n_	3	0	0	2		
Multivariable-adjusted HR (95%CI)	1.00	-	-	21.0 (1.78-248)	0.003	1.94 (0.89-4.25)
Embolic stroke, _n_	2	1	3	2		
Multivariable-adjusted HR (95%CI)	1.00	1.06 (0.09-13.3)	5.47 (0.67-44.4)	12.5 (1.20-131)	0.03	2.61 (1.44-4.75)
Hemorrhagic stroke, _n_	5	3	2	1		
Multivariable-adjusted HR (95%CI)	1.00	0.94 (0.20-4.38)	1.51 (0.23-9.75)	2.70 (0.23-31.4)	0.38	1.54 (0.81-2.93)

Adjusted for age, sex, body mass index, diastolic blood pressure, antihypertensive medication use, non high-density lipoprotein cholesterol, and estimated glomerular filtration rate and area-stratified.

Supplemental Table 2. Adjusted HRs (95% CI) for incident stroke according to the clinical category of N-terminal pro-B type natriuretic peptide (NT-proBNP) levels after the exclusion of persons with chronic kidney disease (eGFR <60 ml/min per 1.73 m^2) at baseline.

	NT-proBNP (pg/ml)					1-SD increment of log transformed
	<55	55-124	125-399	≥ 400		
Number of subjects	2,441	1,089	364	59	P for trend	NT-proBNP
Person years	11,025	4,942	1,710	284		
Total stroke, _n_	17	7	13	6		
Multivariable-adjusted HR (95%CI)	1.00	0.77 (0.31-1.93)	3.35 (1.46-7.66)	8.64 (3.01-24.8)	<0.001	2.10 (1.57-2.79)
Ischemic stroke, _n_	11	4	9	6		
Multivariable-adjusted HR (95%CI)	1.00	0.74 (0.23-2.41)	3.56 (1.30-9.72)	14.5 (4.53-46.7)	<0.001	2.23 (1.60-3.10)
Lacunar stroke, _n_	6	2	3	2		
Multivariable-adjusted HR (95%CI)	1.00	0.71 (0.13-3.75)	2.05 (0.41-10.3)	12.5 (1.98-79.5)	0.003	1.82 (1.05-3.17)
Large-artery occlusive stroke, _n_	2	1	0	2		
Multivariable-adjusted HR (95%CI)	1.00	1.30 (0.11-15.8)	-	59.6 (4.72-754)	<0.001	2.97 (1.35-6.54)
Embolic stroke, _n_	2	1	4	1		
Multivariable-adjusted HR (95%CI)	1.00	0.84 (0.07-9.91)	5.96 (0.88-40.4)	2.20 (0.11-42.4)	0.57	2.00 (1.05-3.81)
Hemorrhagic stroke, _n_	6	3	3	0		
Multivariable-adjusted HR (95%CI)	1.00	0.72 (0.16-3.22)	1.99 (0.41-9.74)	-	0.78	1.42 (0.75-2.70)

Adjusted for age, sex, body mass index, diastolic blood pressure, antihypertensive medication use, non high-density lipoprotein cholesterol, and estimated glomerular filtration rate and area-stratified.

Supplemental Table 3. Adjusted HRs (95% CI) for incident stroke according to the clinical category of N-terminal pro-B type natriuretic peptide (NT-proBNP) levels after the exclusion of persons with hypertension (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg or antihypertensive medication use) at baseline

| | NT-proBNP (pg/ml) | | | | | 1-SD increment of log transformed |
| | <55 | 55-124 | 125-399 | ≥ 400 | | |
Number of subjects	2,011	792	227	41	P for trend	NT-proBNP
Person years	8,943	3,483	1,032	192		
Total stroke, n	9	4	5	4		
Multivariable-adjusted HR (95%CI)	1.00	1.05 (0.30-3.62)	3.59 (1.01-12.7)	19.1 (4.83-75.8)	<0.001	2.49 (1.66-3.73)
Ischemic stroke, n	6	3	3	4		
Multivariable-adjusted HR (95%CI)	1.00	1.45 (0.33-6.47)	3.81 (0.74-19.6)	29.7 (6.59-134)	<0.001	2.54 (1.63-3.98)
Lacunar stroke, n	3	2	1	1		
Multivariable-adjusted HR (95%CI)	1.00	2.56 (0.28-23.8)	5.80 (0.29-114)	46.5 (2.54-854)	0.008	2.35 (1.07-5.19)
Large-artery occlusive stroke, n	1	1	0	1		
Multivariable-adjusted HR (95%CI)	1.00	6.63 (0.25-176)	-	102 (2.30-4513)	0.01	3.76 (1.36-10.4)
Embolic stroke, n	1	0	1	1		
Multivariable-adjusted HR (95%CI)	1.00	-	4.57 (0.10-205)	36.8 (0.41-3268)	0.07	5.64 (1.11-28.6)
Hemorrhagic stroke, n	3	1	2	0		
Multivariable-adjusted HR (95%CI)	1.00	0.45 (0.04-4.97)	2.51 (0.27-23.7)	-	0.99	1.81 (0.70-4.68)

Adjusted for age, sex, body mass index, diastolic blood pressure, non high-density lipoprotein cholesterol, and estimated glomerular filtration rate and area-stratified.