

B-Type Natriuretic Peptides Help in Cardioembolic Stroke Diagnosis

Pooled Data Meta-Analysis

Víctor Llombart, MSc; Albert Antolin-Fontes, MSc; Alejandro Bustamante, MD; Dolores Giralt, BSc; Natalia S. Rost, MD, MPH; Karen Furie, MD, MPH; Kensaku Shibazaki, MD; Murat Biteker, MD; José Castillo, MD, PhD; Manuel Rodríguez-Yáñez, MD, PhD; Ana Catarina Fonseca, MD, PhD; Tetsu Watanabe, MD, PhD; Francisco Purroy, MD; Wu Zhixin, MD; Thorleif Etgen, MD; Naohisa Hosomi, MD, PhD; Scott Reza Jafarian Kerman, MD; Jagdish C. Sharma, FRCP; Carolin Knauer, PhD; Estevo Santamarina, MD, PhD; George Giannakoulas, MD; Teresa García-Berrocso, PhD; Joan Montaner, MD, PhD

Background and Purpose—Determining the underlying cause of stroke is important to optimize secondary prevention treatment. Increased blood levels of natriuretic peptides (B-type natriuretic peptide/N-terminal pro-BNP [BNP/NT-proBNP]) have been repeatedly associated with cardioembolic stroke. Here, we evaluate their clinical value as pathogenic biomarkers for stroke through a literature systematic review and individual participants' data meta-analysis.

Methods—We searched publications in PubMed database until November 2013 that compared BNP and NT-proBNP circulating levels among stroke causes. Standardized individual participants' data were collected to estimate predictive values of BNP/NT-proBNP for cardioembolic stroke. Dichotomized BNP/NT-proBNP levels were included in logistic regression models together with clinical variables to assess the sensitivity and specificity to identify cardioembolic strokes and the additional value of biomarkers using area under the curve and integrated discrimination improvement index.

Results—From 23 selected articles, we collected information of 2834 patients with a defined cause. BNP/NT-proBNP levels were significantly elevated in cardioembolic stroke until 72 hours from symptoms onset. Predictive models showed a sensitivity >90% and specificity >80% when BNP/NT-proBNP were added considering the lowest and the highest quartile, respectively. Both peptides also increased significantly the area under the curve and integrated discrimination improvement index compared with clinical models. Sensitivity, specificity, and precision of the models were validated in 197 patients with initially undetermined stroke with final pathogenic diagnosis after ancillary follow-up.

Conclusions—Natriuretic peptides are strongly increased in cardioembolic strokes. Future multicentre prospective studies comparing BNP and NT-proBNP might aid in finding the optimal biomarker, the best time point, and the optimal cutoff points for cardioembolic stroke identification. (*Stroke*. 2015;46:1187-1195. DOI: 10.1161/STROKEAHA.114.008311.)

Key Words: biomarker ■ etiology

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From the Neurovascular Research Laboratory, Institut de Recerca Vall d'Hebron, Neurovascular Unit, Department of Neurology, Universitat Autònoma de Barcelona, Barcelona, Spain (V.L., A.A.-F., A.B., D.G., T.G.-B., J.M.); Acute Stroke Services, Massachusetts General Hospital, J. Philip Kistler Stroke Research Center, Boston (N.S.R.); Department of Neurology, Rhode Island Hospital, Alpert Medical School of Brown University, Providence (K.F.); Department of Stroke Medicine, Kawasaki Medical School, Kurashiki, Okayama, Japan (K.S.); Department of Cardiology, Haydarpaşa Numune Education and Research Hospital, Istanbul, Turkey (M.B.); Department of Neurology, Clinical University, Hospital University of Santiago de Compostela, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain (J.C., M.R.-Y.); Department of Neurosciences (Neurology), Hospital de Santa Maria, University of Lisbon, Lisbon, Portugal (A.C.F.); Department of Cardiology, Pulmonology and Nephrology, Yamagata University School of Medicine, Yamagata, Japan (T.W.); Department of Neurology, Stroke Unit, Hospital Universitari Arnau de Vilanova, Grup Neurociències Clínicas IRBLleida, Lleida, Spain (F.P.); Department of Emergency Medicine, Foshan Hospital of Traditional Chinese Medicine, Foshan, Guangdong, China (W.Z.); Department of Neurology, Kliniken Südostbayern-Klinikum Traunstein, Traunstein, Germany; Department of Psychiatry and Psychotherapy, Technische Universität München, München, Germany (T.E.); Department of Clinical Neuroscience and Therapeutics, Hiroshima University Graduate School of Biomedical and Health Sciences, Hiroshima, Japan (N.H.); Tehran University of Medical Sciences and Health Services, Tehran, Iran (S.R.J.K.); Stroke and Parkinson's Disease Services, Lincoln County Hospital, University of Nottingham, Nottingham, United Kingdom (J.C.S.); Department of Neurology, University of Ulm, Ulm, Germany (C.K.); Stroke Unit, Department of Neurology, Hospital Vall d'Hebron, Barcelona, Spain (E.S.); and Cardiology Department, AHEPA University Hospital, Thessaloniki, Greece (G.G.).

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Correspondence to Joan Montaner, MD, PhD, Neurovascular Research Laboratory, Vall d'Hebron Institute of Research (VHIR), Vall d'Hebron University Hospital, Pg. Vall d'Hebron 119-129, 08035 Barcelona, Spain. E-mail 31862jmv@comb.es

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Ischemic stroke is one of the most important neurological disorders.¹ An accurate pathogenic classification of ischemic stroke is essential to prescribe the most suitable secondary treatment to prevent recurrences.² Patients with cardioembolic stroke are treated with anticoagulant drugs, whereas antiplatelet agents are the treatment of choice for patients with large artery atherosclerosis (LAA) stroke and small vessel disease (SVD).³ Cardioembolic strokes are generally more severe and more prone to recurrence than LAA or SVD and account for approximately one fifth of ischemic strokes.⁴

However, in spite of the importance of an accurate etiopathogenic classification, the cause of $\approx 35\%$ of patients remains undetermined, even after complete evaluation.⁵ This group of patients presents a rate of recurrence of $\approx 30\%$ during the first year after the event, partly explained by an inappropriate secondary prevention treatment.⁶ Stroke of undetermined cause is an heterogeneous group that includes patients with 2 or more potential causes of stroke, patients with $<50\%$ of stenosis and patients with a negative diagnostic workup.⁷ From the latter, a negative diagnostic might be caused by a transitory or reversible condition which is difficult to detect, such as atrial fibrillation (AF). AF is a frequent cardiac-rhythm disorder which detection is essential for cardioembolic stroke diagnosis. However, AF can be paroxysmal and thus challenging to detect by standard cardiac monitoring.⁸ Recently, a new clinical construct has been described called embolic stroke of undetermined cause, defined as a nonlacunar stroke (based on neuroimaging), with absence of proximal arterial stenosis or major risk cardioembolic sources.⁹ In these patients, thromboemboli originated in left atrium because of paroxysmal AF and other dysrhythmias that promote stasis-related clot formation are considered important contributors to embolic strokes of undetermined cause.¹⁰

ECG monitoring during 24 hours or more is suggested by current guidelines to rule out AF, but recent clinical trials have demonstrated that the rate of detection of AF increases with longer and continuous monitoring (during 30 days to 12 months).^{11,12} The diagnosis of the underlying cause of stroke might be complemented with the use of blood biomarkers, with an important saving of time and resources, especially in these challenging scenarios in which the cause of stroke is an intermittent condition difficult to detect with conventional follow-up.¹³

B-type natriuretic peptide (BNP) is a cardiac hormone that plays an antifibrotic role in the heart and is produced after the cleavage of the precursor pro-BNP, releasing equimolar amounts of the inactive N-terminal peptide (NT-proBNP).¹⁴ Pro-BNP is released from the myocyte because of stretch stimulation in response to increased wall tension and volume/pressure overload, and also in stages of hemodynamic stress.¹⁵ This relation between pro-BNP and atrial dilatation explains the clear association of elevated BNP/NT-proBNP with the presence of AF¹⁵ and cardioembolic stroke.^{16–23} Both natriuretic peptides have been associated with cardioembolic stroke in several, but generally small studies,^{16–23} reporting different cutoff points to distinguish cardioembolic from noncardioembolic stroke and considering different time points from symptoms onset. A clear association of BNP/NT-proBNP with cardioembolic stroke cause would allow to complement and

speedup the diagnosis of cardioembolic stroke especially in the most challenging cases (ie, paroxysmal AF) and might aid to reduce the rate of undetermined strokes.

We aimed to increase the certainty of the relation between cardioembolic stroke and BNP/NT-proBNP circulating levels, to assess their value to complement clinical information to identify cardioembolic stroke origin, and to validate the cardioembolic predictive models in a subset of patients with undetermined cause, through a systematic review and individual participants' data (IPD) meta-analysis.

Methods

Methods are registered and available at PROSPERO database (CRD42013005924).²⁴

Inclusion Criteria and Search Strategy

All included studies were original articles that determined BNP or NT-proBNP blood levels among different subtypes in patients with established ischemic stroke or transient ischemic attack. After the search on PubMed database till November 12, 2013, 4 independent reviewers performed the selection of articles. The terms used for searching were (b-type natriuretic peptides OR brain natriuretic peptide OR BNP) AND stroke AND (Cardioembolic OR atherothrombotic OR lacunar OR subtype OR etiology). Duplicates were only considered once. References from selected studies and published reviews were manually screened to find other relevant studies.

Data Collection

Three reviewers independently checked the quality of the selected studies using a 15-point quality questionnaire.²⁵ Corresponding authors of all selected studies were contacted by e-mail and were asked to share their data for IPD analysis. A template form was fulfilled with IPD, including BNP/NT-proBNP levels (pg/mL), a methodology used for BNP/NT-proBNP measurement, time of sample collection since stroke symptoms onset, age, sex, pathogenesis classification system used, diagnosed cause of stroke, type of stroke (established or transient ischemic attack), and presence of risk factors, such as hypertension, diabetes mellitus, dyslipidemia, AF, ischemic cardiomyopathy, other embolic cardiopathy, or tobacco and alcohol consumption. Baseline neurological severity was collected as National Institutes of Health Stroke Scale (NIHSS) score. When Scandinavian Stroke Scale was used, we corrected it to NIHSS score by $NIHSS = 25.68 - 0.43 \times \text{Scandinavian Stroke Scale}$ before inclusion in the IPD analysis.²⁶

Individual data were compiled in a single database for statistical analysis. We derived our cause predictive model only among patients with known stroke subtype, based on Trial of Org 10172 in Acute Stroke Treatment classification (cardioembolic, LAA, or SVD). Patients with undetermined stroke were excluded in this step.

Once the model was derived, we then applied it to the subset of patients whose stroke was initially of undetermined cause. Our goal was to determine how well our model could predict the stroke cause that was ultimately established. This would help to answer the question of whether a model including natriuretic peptides can predict the stroke cause at admission that would ultimately be determined after delayed evaluations. These evaluations included ECG, transesophageal cardiography, and transcranial Doppler sonography complemented with chest radiography. Twenty-four-hour Holter monitoring was conducted in 3 out of 5 cohorts of patients with undetermined stroke, the first months after stroke, and any of these patients did not undergo long-term monitoring. To that end, we applied the previously developed predictive models to the undetermined patients database and as a result, we obtained probabilities of being cardioembolic according to our models. Finally, we compared the model-predicted cause (considering as cardioembolic for those patients with $>60\%$ of probabilities) with the cause reported on the corresponding article, which was identified after complete clinical screening. The rest of

the clinical variables considered in these models were obtained at admission. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were given.

All included cohorts identified stroke cause independently of biomarker levels.

IPD Analysis

SPSS statistical package 15.0 (SPSS Inc, Chicago, IL) was used, unless contrary is stated.

BNP and NT-proBNP were non-normally distributed (assessed by Kolmogorov–Smirnov test) and Mann–Whitney *U* or Kruskal–Wallis test was used. Median and interquartile range are reported. Because of heterogeneity on BNP/NT-proBNP levels among different cohorts, we standardized BNP/NT-proBNP levels calculating the *Z* score value of BNP or NT-proBNP in each subject as previously reported.²⁷ We compared standardized levels of BNP/NT-proBNP among cardioembolic, LAA, and SVD, and *P* values were adjusted by Bonferroni correction. In the univariate analysis, differences between cardioembolic and noncardioembolic patients were assessed by Pearson χ^2 test for categorical variables. Correlations were checked through Spearman test for continuous variables.

The highest and the lowest quartiles for BNP/NT-proBNP levels were established as cutoff points to build the predictive models. Because standardized values were used, we were not able to identify cutoff points expressed in units of concentration (ie, pg/mL). Sensitivity, specificity, PPV, and NPV were determined for each cutoff used.

A clinical predictive model was built using basic variables. We forced age, sex, and NIHSS score at admission in a logistic regression analysis using Enter method, and odds ratio, 95% confidence interval, and *P* value are given. A second clinical predictive model was built by adding AF to the previous model. Afterward, we added BNP or NT-proBNP to each model, dichotomized by the cutoff points described above, and the corresponding probabilities were obtained.

We performed bootstrap calculation for odds ratio and 95% confidence interval, using a modified version of Car R-package.²⁸ The added value of each biomarker to each clinical model was assessed using receiving operating characteristic curves and compared the area under the curve among models by DeLong's Method using MedCalc v.12.3 (Mariakerke, Belgium). For that same purpose, we used R software v.2.15.0 (R Development Core Team 2012; Vienna, Austria; Hmisc and PredictABEL packages)²⁹ for Integrated Discrimination Improvement (IDI) index calculation.

Results

A total of 651 articles fitted the search criteria. After bibliography checking, 27 articles were included. Because of unavailability of the requested data or lack of response from authors, we finally selected 23 articles (Figure 1).^{16–23,30–44} The median quality score was 9 (minimum=5 and maximum=11; data not shown). These 23 included articles corresponded to 18 different cohorts of patients, 16 of which had data from patients with a defined cause (cardioembolic, LAA, or SVD) and 2 cohorts containing data of undetermined patients only. These 16 cohorts were included in the IPD (Table I in the online-only Data Supplement) excluding patients with undetermined cause at this step. All patients from 16 cohorts underwent ECG (12-lead ECG, 24-hour ECG, or long-term ECG) to determine cardioembolic cause of cerebral infarction and 11 cohorts underwent specific cardiac monitoring. Echocardiography was performed in 12 cohorts.

In total, we compiled individual data from 2834 patients with defined stroke cause. We found significantly increased levels of NT-proBNP compared with BNP, as previously reported,²⁵ and consequently we considered both markers

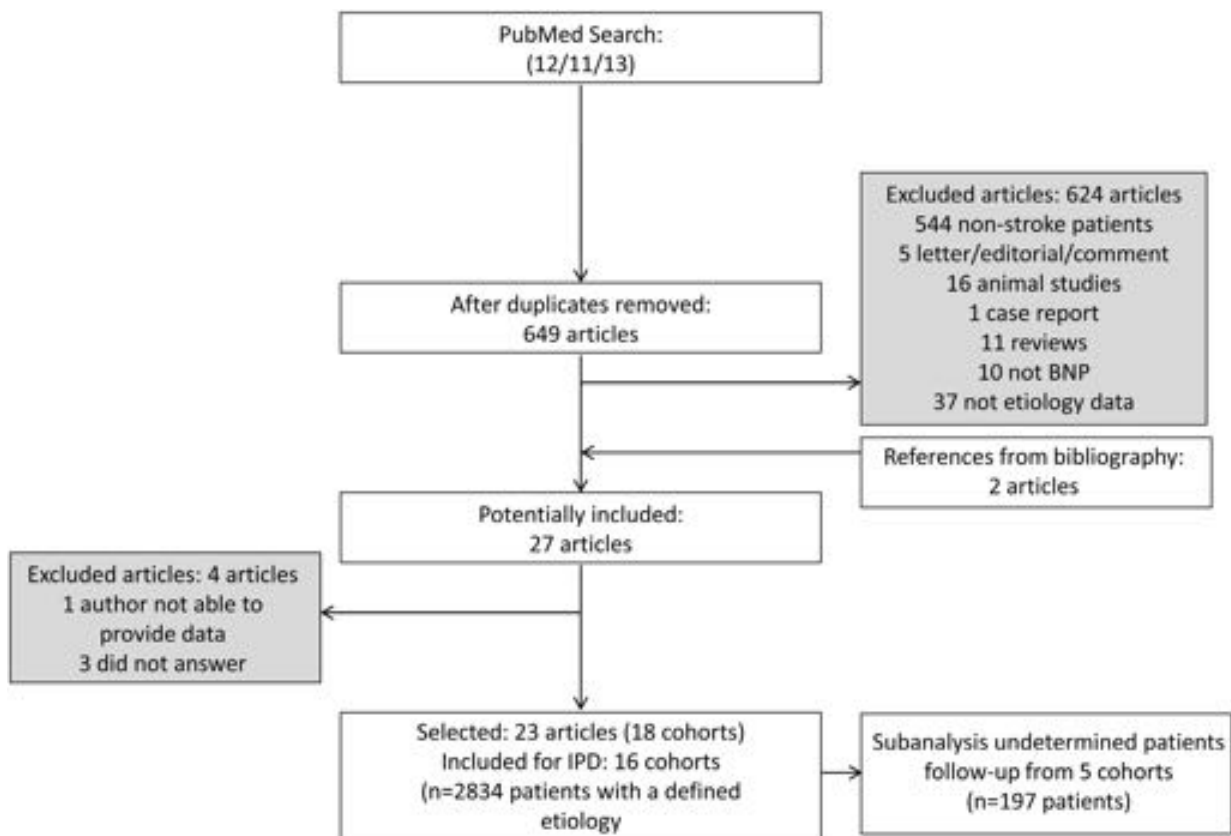


Figure 1. Flow chart diagram. BNP indicates B-type natriuretic peptide; and IPD, individual participants' data.

separately. BNP was analyzed in a cohort of 1570 patients and NT-proBNP in a cohort of 1264 patients.

We found higher BNP/NT-proBNP values in patients with cardioembolic stroke than in patients with LAA ($P<0.0001$) and SVD ($P<0.0001$; Figure 2A and 2B). For BNP, these differences were significant until 72 hours after symptoms onset, whereas NT-proBNP levels remained significantly higher in cardioembolic strokes during 1 week (Figure 2C and 2D). After univariate analysis, patients with cardioembolic stroke were remarkably older, mostly women had more severe stroke. They also showed more cardiac disorders than noncardioembolic patients (Table 1).

Taking the highest quartile for BNP as cutoff point, we were able to distinguish cardioembolic stroke from other causes with 42.3% sensitivity, 90.7% specificity, a PPV of 80%, and a NPV of 37%. Similarly, NT-proBNP highest quartile showed 40% sensitivity, 90.1% specificity for cardioembolic stroke, with a PPV of 59.3% and a NPV of 88%. When the lowest quartile was considered as cutoff point, we obtained 93.68% sensitivity, 41.94% specificity, a PPV of 80%, and a NPV of 40% for BNP; and 86.3% sensitivity, 36.2% specificity, a PPV of 57.3%, and a NPV of 72.8% for NT-proBNP.

In the multivariate logistic regression analysis, we considered the previous cutoff points aiming to obtain a highly specific and a highly sensitive model for each biomarker. In all the predictive

models obtained, BNP and NT-proBNP remained as the main predictors of cardioembolic stroke cause after adjusting by age, sex, and NIHSS score at admission (Table 2). Even when AF was added to each model, BNP and NT-proBNP remained as independent predictors with barely altered predictive values (Table II in the online-only Data Supplement). The discrimination of patients with cardioembolic stroke measured by the area under the curves and IDI index was significantly greater when we added BNP or NT-proBNP to clinical data (Table 2). Similar results were obtained when the predictive models were developed considering the presence of AF as the end point, instead of cardioembolic subtype. Unfortunately, we only disposed of enough data of the diagnosis of cardioembolic stroke in the undetermined cohort to attempt the validation of the predictive models; thus, we were not able to validate predictive models of AF (Table III in the online-only Data Supplement).

We attempted to verify the usefulness of the generated predictive models in patients with undetermined cause at baseline ($n=83$ for BNP; $n=114$ for NT-proBNP), but with a defined cause after complete diagnostic workup. The predictive models that included NT-proBNP showed greater sensitivity and specificity for cardioembolic stroke cause than those that included BNP. About accuracy, higher area under the curves were also found with NT-proBNP included in the predictive model (Figure 3; Table 3).

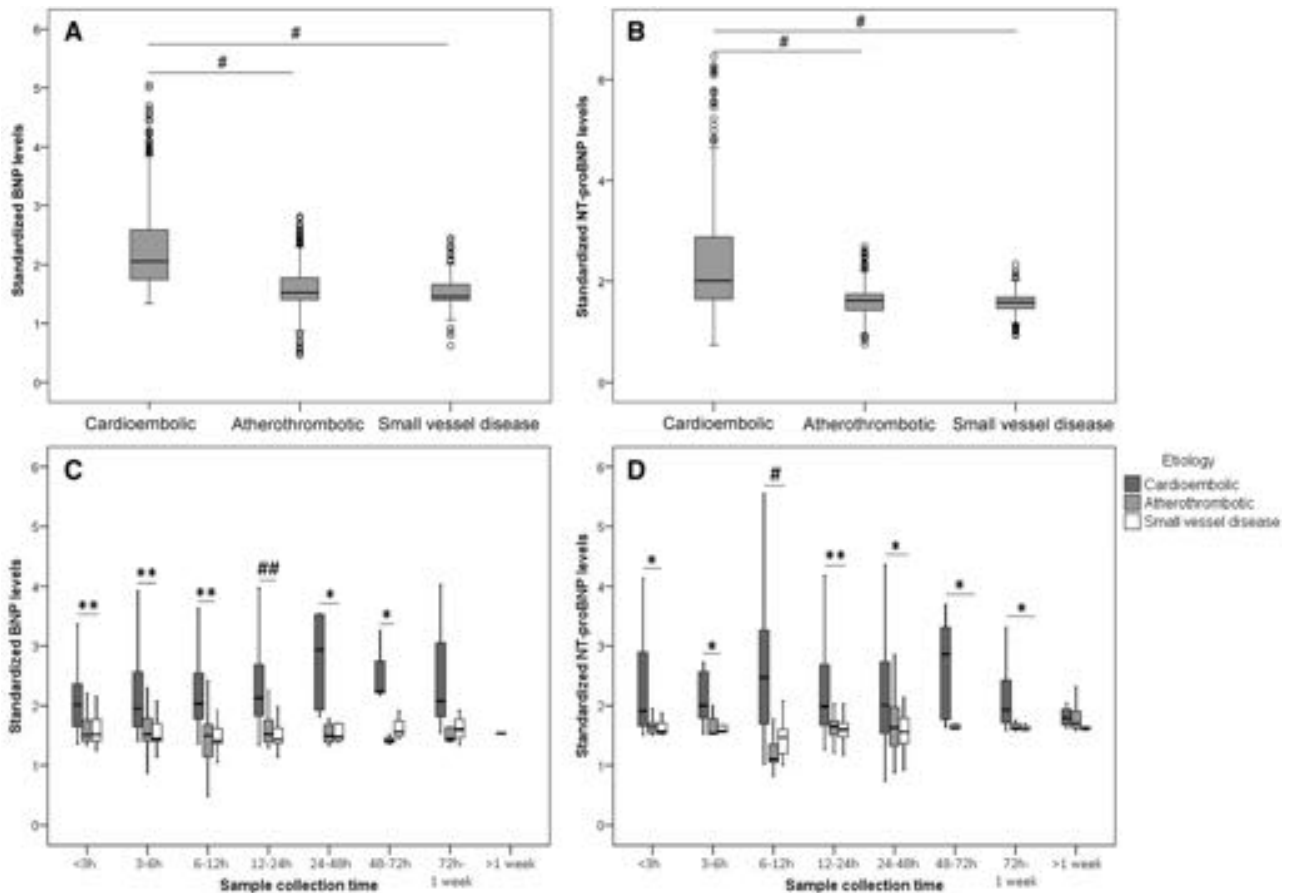


Figure 2. B-type natriuretic peptide/N-terminal pro-BNP (BNP/NT-proBNP) levels among ischemic stroke causes. **A** and **B**, Standardized values of BNP and NT-proBNP among patients with cardioembolic stroke, atherothrombotic stroke, and small vessel disease. **C** and **D**, BNP/NT-proBNP levels depending on sample collection time. * $P<0.05$, ** $P<0.0001$, # $P<1\times 10^{-10}$, and ## $P<1\times 10^{-20}$.

Table 1. Univariate Analysis

	BNP			NT-ProBNP		
	Non-CE	CE	P Value	Non-CE	CE	P Value
Age, y	72 (62–78)	77 (69–83)	<0.0001	68 (59–76)	72 (62–79)	<0.0001
Sex, female	324 (39.1%)	406 (52.9%)	<0.0001	242 (37.9%)	308 (48.7%)	<0.0001
Admission NIHSS	5 (2–10)	11 (4–18)	<0.0001	6 (2–11)	10 (4–17)	<0.0001
Arterial hypertension	590 (73.1%)	474 (61.9%)	<0.0001	433 (67.8%)	396 (62.6%)	0.051
Diabetes mellitus	262 (32.5%)	185 (24.2%)	<0.0001	191 (29.9%)	111 (17.5%)	<0.0001
Dyslipidemia	262 (32.5%)	158 (20.6%)	<0.0001	213 (33.3%)	205 (32.4%)	0.719
Smoker	286 (35.4%)	194 (25.3%)	<0.0001	272 (42.7%)	207 (33%)	<0.0001
Alcohol consumption	53 (11%)	82 (15.1%)	0.056	172 (30.6%)	144 (26.3%)	0.114
Ischemic cardiomyopathy	108 (14.8%)	136 (24.6%)	<0.0001	29 (10.6%)	40 (14.2%)	0.198
Embolic cardiomyopathy	1 (0.2%)	56 (12.6%)	<0.0001	4 (1.6%)	65 (23.7%)	<0.0001
Atrial fibrillation	43 (6.8%)	490 (74.7%)	<0.0001	29 (5%)	273 (48.8%)	<0.0001
Standardized BNP/NT-proBNP	1.493 (1.400–1.714)	2.054 (1.742–2.586)	<0.0001	1.598 (1.404–1.714)	2.006 (1.641–2.844)	<0.0001

For categorical variables n (%) is given and for continuous variables median and interquartile range are indicated, in each group. *P*<0.05 was considered significant. BNP indicates B-type natriuretic peptide; CE, cardioembolic; NIHSS, National Institutes of Health Stroke Scale; and NT-proBNP, N-terminal pro-BNP.

Discussion

Currently, the use of biomarkers in the stroke field is only recommended for research purposes, with the exception of phospholipase A2 in the prediction of stroke risk.⁴⁵ However, stroke biomarkers might aid in different scenarios such as

stroke diagnosis, screening high-risk subjects, predicting outcome, or detecting the cause of stroke.¹³

Pro-BNP is released from the myocyte because of stretch. Interestingly, despite being produced equimolarly, NT-proBNP has a longer half-life than BNP and fewer fluctuations on its

Table 2. Comparison Between Clinical Predictive Models and Predictive Models Adding BNP or NT-proBNP for Cardioembolic Stroke

	Clinical Model in BNP Cohort	Clinical Model+BNP		Clinical Model in NT-Pro-BNP Cohort	Clinical Model+NT-proBNP	
		Highest Quartile	Lowest Quartile		Highest Quartile	Lowest Quartile
Logistic regression (OR with 95% CI and <i>P</i> value)						
Age, y	1.03 (1.02–1.04), <i>P</i> <0.0001	1.02 (1.01–1.03), <i>P</i> =0.0003	1.01 (1–1.03), <i>P</i> =0.017	1.01 (1–1.02), <i>P</i> =0.267	0.99 (0.98–1), <i>P</i> =0.117	0.1 (0.99–1.01), <i>P</i> =0.890
Sex, female	1.4 (1.1–1.77), <i>P</i> =0.006	1.36 (1.06–1.74), <i>P</i> =0.016	1.33 (1.03–1.71), <i>P</i> =0.027	1.42 (1.1–1.8), <i>P</i> =0.007	1.44 (1.11–1.89), <i>P</i> =0.007	1.32 (1.02–1.71), <i>P</i> =0.037
Admission NIHSS	1.09 (1.07–1.11), <i>P</i> <0.0001	1.08 (1.06–1.1), <i>P</i> <0.0001	1.08 (1.06–1.1), <i>P</i> <0.0001	1.06 (1.04–1.08), <i>P</i> <0.0001	1.05 (1.03–1.07), <i>P</i> <0.0001	1.05 (1.03–1.07), <i>P</i> <0.0001
BNP/NT-proBNP	...	4.49 (3.26–6.2), <i>P</i> <0.0001	7.1 (4.98–10.12), <i>P</i> <0.0001	...	6.17 (4.31–8.84), <i>P</i> <0.0001	3.34 (2.44–4.59), <i>P</i> <0.0001
IDI statistics						
IDI for cardioembolic	...	0.026	0.042	...	0.067	0.049
IDI for noncardioembolic	...	0.038	0.053	...	0.073	–0.011
Total IDI	...	0.064	0.095	...	0.140	0.038
<i>P</i> value	Reference	<0.0001	<0.0001	Reference	<0.0001	<0.0001
ROC curve						
AUC (95% CI)	0.71 (0.68–0.74)	0.75 (0.73–0.78)	0.77 (0.75–0.8)	0.63 (0.6–0.66)	0.71 (0.68–0.74)	0.7 (0.67–0.73)
<i>P</i> value	Reference	<0.0001	<0.0001	Reference	<0.0001	<0.0001

BNP and NT-proBNP were included in the models as standardized values dichotomized by the highest or the lowest quartile cutoff point. OR is indicated in the logistic regression with 95% CI and *P* value. Bootstrapping gives 95% CI for OR of BNP highest quartile cutoff (3.26–6.45) and BNP lowest quartile cutoff (4.08–7.94); and for OR of NT-proBNP highest quartile cutoff (4.42–9.22) and NT-proBNP lowest quartile (2.44–4.55). AUC indicates area under the curve; BNP, B-type natriuretic peptide; CI, confidence interval; IDI, integrated discrimination improvement index; NIHSS, National Institutes of Health Stroke Scale score; NT-proBNP, N-terminal pro-BNP; OR, odds ratio; and ROC, receiver operating characteristic.

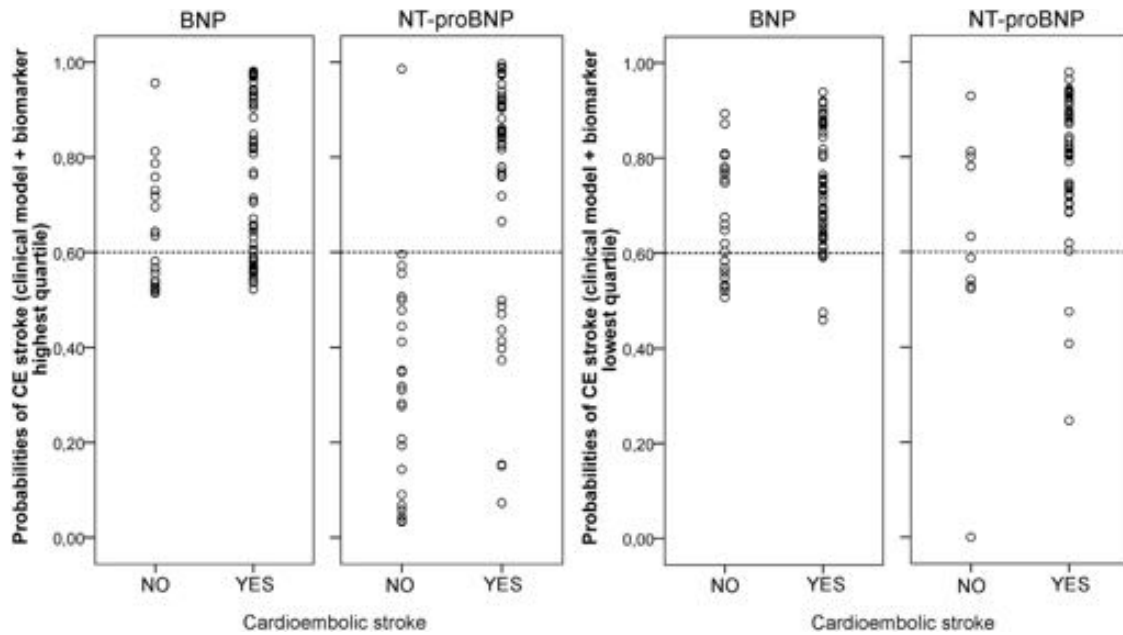


Figure 3. Probabilities of cardioembolic stroke. Patients with undetermined cause reclassified as cardioembolic or noncardioembolic after follow-up. **A**, Probabilities of being cardioembolic corresponding to the predictive model conformed by age, sex, National Institutes of Health Stroke Scale (NIHSS) at admission, and B-type natriuretic peptide/N-terminal pro-BNP (BNP/NT-proBNP) dichotomized by the highest quartile. **B**, Probabilities of being cardioembolic corresponding to the predictive model conformed by age, sex, NIHSS at admission and BNP/NT-proBNP dichotomized by the lowest quartile. Leader line indicates 60% of probabilities of being cardioembolic.

circulating levels.⁴⁶ This phenomenon might explain the fact that higher amounts of NT-proBNP are found in blood compared with BNP.²⁵ For this reason, our IPD analysis was performed separately on each of these markers.

Literature-based meta-analyses are considered basic for the elaboration of guidelines and recommendations for disease management.⁴⁷ However, they represent some limitations in front of IPD meta-analyses. IPD meta-analyses allow the analysis of multiple individual factors and their combination, the generation of predictive models and the development of sub-analysis different from those previously reported in the literature.⁴⁸ During the development of this study, a literature-based meta-analysis showed the association of BNP/NT-proBNP with cardioembolic origin of stroke.⁴⁹ Our results strongly support these associations, and following the IPD strategy, we were able to perform further subanalysis to study the predictive value of BNP/NT-proBNP in depth. We could evaluate differences in BNP/NT-proBNP among causes at different

time points, develop 2 different predictive models, and validate their usefulness in a cohort of undetermined patients. We found increased levels of BNP/NT-proBNP in patients with cardioembolic stroke than in noncardioembolic and these differences were significant until 72 hours after symptoms onset. Although, there is no clear evidence of the optimal time point to initiate anticoagulation,⁵⁰ an early identification of patients with cardioembolic stroke through BNP/NT-proBNP testing might aid in speeding up the decision-making process.

On clinical daily practice, patients diagnosed with ischemic stroke are evaluated, when indicated, with cardiac ancillary tests, such as ECG, echocardiography, or Holter monitoring to identify possible cardiac sources of embolism. However, some causes of ischemic stroke are transitory (ie, paroxysmal AF) and, as a consequence, might not be detected during diagnostic workup. In these cases, prolonged cardiac monitoring is necessary to increase the percentage of detection of arrhythmias,⁵¹ challenging the diagnosis of cardioembolic stroke. In

Table 3. Application of the Predictive Models in Undetermined Strokes With Complete Diagnosis Follow-Up

Predictive Model	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	AUC (95% CI)
Clinical variables+BNP highest quartile	74.58%	56.52%	81.48%	46.43%	0.758 (0.644–0.873)
Clinical variables+NT-proBNP highest quartile	78.79%	95.83%	96.3%	76.67%	0.879 (0.810–0.947)
Clinical variables+BNP lowest quartile	91.53%	39.13%	79.41%	64.29%	0.674 (0.536–0.811)
Clinical variables+NT-proBNP lowest quartile	93.94%	79.17%	86.11%	90.48%	0.896 (0.832–0.959)

Clinical variables include age, sex, and National Institutes of Health Stroke Scale score at admission. AUC indicates area under curve; BNP, B-type natriuretic peptide; CI, confidence interval; and NT-proBNP, N-terminal pro-BNP.

this study, both BNP and NT-proBNP were found to predict cardioembolic stroke independently of age, sex, and NIHSS score at admission. We created a high sensitive model for cardioembolic stroke that might ease the selection of suitable subjects for prolonged monitoring aiming to identify cardiac disorders undetected in earlier ancillary tests. Additionally, we built up a predictive model with an elevated specificity for cardioembolic stroke subtype that allows the identification of patients who would benefit from receiving anticoagulation as secondary prevention therapy. Even when AF (one of the strongest predictors of cardioembolic stroke) was included in these predictive models, they showed similar levels of precision than those without AF, maintaining BNP and NT-proBNP significant independent predictors of cardioembolic stroke. This supports that a basic clinical predictive model conformed by easily determinable variables, such as age, NIHSS, and sex, and complemented with circulating BNP/NT-proBNP levels, might be suitable to diagnose cardioembolic stroke, mainly in those cases in which AF detection is difficult, such as the recently described embolic strokes of undetermined cause.¹⁰ In addition, IDI results indicate that both peptides aid to better discriminate cardioembolic from noncardioembolic strokes. It is also important to notice that, although they were not determined in the same patients, NT-proBNP showed a higher predictive value and IDI index than BNP for the specific model, suggesting that NT-proBNP is a more valuable biomarker for cardioembolic cause discrimination. In contrast, BNP showed higher odds ratio and IDI index for cardioembolism when considered in the more sensitive models.

The rate of recurrence in patients with undetermined stroke has been reported to be 14% to 20% during the first 2 years after stroke^{5,52} mainly attributable to an inappropriate secondary prevention. They also present poorer functional outcome after 3 months and a higher cumulative death rate after 3-year follow-up.⁵³ The use of biomarkers has been considered as a good approach to reclassify the cause of patients with undetermined stroke. A combined assessment of proatrial natriuretic peptide, creatine kinase-MB, and NT-proBNP has been previously reported to reclassify 41% of undetermined patients as likely CE.³⁴ NT-proBNP has also shown its usefulness to identify patients with undetermined stroke having risk of developing AF.³⁵ We followed a similar approach in this study to correctly identify patients likely to be cardioembolic and found higher sensitivity and specificity when NT-proBNP was measured compared with BNP.

Our analysis has been conducted in a wide spectrum of individuals with a defined cause (gold standard) and enabling the assessment of appropriate tests of diagnosis accuracy. Hence, according to evidence classification scheme for a diagnostic measure, as BNP/NT-proBNP showed high sensitivity/specificity for cardioembolic stroke cause, they could be considered to diagnose cardioembolic stroke with a high level of evidence (Class II).⁴⁷

Our study has some limitations. First, although the included studies share the same end point, it is important to notice that the mechanism identification and pathogenic workup of the included articles have been performed in different centers from many countries and during the past 9 years; thus, the reliability of pathogenic subtyping might be uncertain and

could be considered as a source of bias. Second, because of BNP/NT-proBNP values standardization, we were not able to provide a cutoff point to differentiate cardioembolic strokes expressed in units of concentration (ie, pg/mL). Third, although the predictive models are based on a considerable number of participants, the subsets of patients with cryptogenic stroke used in the application of these models are sparse. Consequently, the results of this application of the model should be interpreted with caution. In addition, time of follow-up of these undetermined patients was unavailable. In the fourth place, apart from AF, we were not able to include other cardiac disorders, such as embolic cardiopathies, ischemic cardiomyopathy, or previous heart failure in the predictive models because of a drastic reduction in sample size that compromised the statistical power. Finally, we could not establish a direct comparison between BNP and NT-proBNP because we could not avoid interindividual variability.

Next steps to study in depth the applicability of BNP/NT-proBNP in clinical daily practice for cardioembolic stroke identification should include a simultaneous analysis of BNP and NT-proBNP in the same patients in a multicentre prospective study with a validated measurement device that would allow a direct comparison between both peptides. This might aid in finding the optimal biomarker and the best time point to determine its blood levels, as well as the optimal cutoff points for cardioembolic stroke identification. The evaluation and addition of other cardiac disorders in the predictive models would probably aid in increasing sensitivity/specificity of predictive models of future studies. Finally, a clinical trial might clarify whether BNP/NT-proBNP-directed anticoagulation is clinically significant. Taken together, this would increase the level of evidence to definitely implement the clinical use of NT-proBNP or BNP.

In conclusion, our IPD support the role of natriuretic peptides for the identification of cardioembolic origin of ischemic stroke. Their implementation on clinical daily practice might serve as a better trigger for anticoagulation than performing invasive transesophageal echocardiography or waiting weeks to months to detect an episode of AF. Simultaneously, the use of natriuretic peptides may also be useful to effectively rule out the possibility of underlying cardioembolism and therefore obviate the need for transesophageal echocardiography or intensive heart-rhythm monitoring.

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SUPPLEMENTAL MATERIAL

B-type natriuretic peptides help in cardioembolic stroke diagnosis: a pooled data meta-analysis

Víctor Llombart¹, MSc; Albert Antolin-Fontes¹, MSc; Alejandro Bustamante¹, MD; Dolores Giralt¹, BSc; Natalia S Rost², MD, MPH, FAAN; Karen Furie³, MD, MPH; Kensaku Shibazaki⁴, MD; Murat Biteker⁵, MD; José Castillo⁶, MD, PhD; Manuel Rodríguez-Yáñez⁶, MD, PhD; Ana Catarina Fonseca⁷, MD, PhD; Tetsu Watanabe⁸, MD, PhD; Francisco Purroy⁹, MD; Wu Zhixin¹⁰, MD; Thorleif Etgen¹¹, MD; Naohisa Hosomi¹², MD, PhD; Scott Reza Jafarian Kerman¹³, MD; Jagdish C Sharma¹⁴, FRCP; Carolin Knauer¹⁵, PhD; Estevo Santamarina¹⁶, MD, PhD; George Giannakoulas¹⁷, MD; Teresa García-Berrocoso¹, PhD; Joan Montaner¹, MD, PhD.

- 1- Neurovascular Research Laboratory, Institut de Recerca Vall d'Hebron. Neurovascular Unit. Department of Neurology. Universitat Autònoma de Barcelona, Barcelona, Spain.
- 2- Acute Stroke Services, Massachusetts General Hospital, J. Philip Kistler Stroke Research Center, Boston, USA.
- 3- Department of Neurology, Rhode Island Hospital, Alpert Medical School of Brown University, Providence, USA.
- 4- Department of Stroke Medicine, Kawasaki Medical School, Kurashiki City, Okayama, Japan.
- 5- Department of Cardiology, Haydarpas,a Numune Education and Research Hospital, Istanbul, Turkey.
- 6- Departament of Neurology, Clinical University Hospital University of Santiago de Compostela, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain.
- 7- Department of Neurosciences (Neurology), Hospital de Santa Maria, University of Lisbon, Lisbon, Portugal.
- 8- Department of Cardiology, Pulmonology and Nephrology, Yamagata University School of Medicine, Yamagata, Japan.
- 9- Stroke Unit, Hospital Universitari Arnau de Vilanova, Grup Neurociències Clínicas IRBLleida, Lleida, Spain.
- 10- Department of Emergency Medicine, Foshan Hospital of Traditional Chinese Medicine, Foshan City, Guangdong Province, China.
- 11- Department of Neurology, Kliniken Südostbayern-Klinikum Traunstein, Germany; Department of Psychiatry and Psychotherapy, Technische Universität München, München, Germany.
- 12- Department of Clinical Neuroscience and Therapeutics Hiroshima University Graduate School of Biomedical & Health Sciences, Hiroshima, Japan.
- 13- Research Associate, Tehran University of Medical Sciences and Health Services, Tehran, Iran.

- 14- Stroke & Parkinson's Disease Services, Lincoln County Hospital, University of Nottingham, Nottingham, UK.
- 15- Department of Neurology, University of Ulm, Ulm, Germany.
- 16- Stroke Unit and Department of Neurology, Hospital Vall d'Hebron, Barcelona, Spain
- 17- Cardiology Department, AHEPA University Hospital, Thessaloniki, Greece.

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Supplemental table I: Main demographic characteristics of the cohorts included in the Individual Participants' Data analysis

Cohort N ^o , (ref)	Sample size	Age (years)	NIHSS adm	Gender (female) (% , n)	Smokers (% ,n)	AHT (% ,n)	DM (% ,n)	DL (% ,n)	AF (% , n)	IC (% ,n)	EC (% ,n)	Alcohol (% ,n)	CE (% , n)	Biomarker	Method
1 (1)	133	70.6±12.2	-	38.3(51)	13.5(18)	64.7 (86)	21.8 (29)	30.8 (41)	30.1 (40)	21.8 (29)	13.5 (18)	3.8 (5)	40.6 (54)	NT-proBNP	-
2 (2)	108	69.1±12.2	16±5.6	52.8(57)	25(27)	68.5 (74)	37 (40)	36.1 (39)	32.4 (35)	-	-	0 (0)	52.8 (57)	NT-proBNP	Elecsys2010
3 (3)	283	71.8±11.4	11.8±6.2	48.8(138)	25.4(72)	71.4 (202)	27.6 (78)	15.5 (44)	-	34.6 (98)	-	-	38.9 (110)	BNP	-
4 (4)	126	69.7±11.9	-	52.4(66)	16.7(21)	77 (97)	31.7 (40)	24.6 (31)	19 (24)	22.2 (28)	6.3 (8)	4.8 (6)	28.6 (36)	BNP	Triage
5 (5)	168	62.9±12.2	8.2±7.1	44(74)	22(37)	69.6 (117)	24.4 (41)	44 (74)	18.5 (31)	14.9 (25)	25 (42)	13.7 (23)	56 (94)	NT-proBNP	Elecsys2010
6 (6,7)	291	74.1±11.7	10.7±8.7	43.3(126)	46(134)	65.6 (191)	25.1 (73)	24.4 (71)	66 (192)	-	-	35.4 (103)	73.2 (213)	BNP	Immunoassay (Shionogi)
7 (8)	371	67.3±13.8	5.5±6.4	40.4(150)	63.7(232)	62 (230)	22.1 (82)	39.1 (145)	25.3 (94)	-	-	62.8 (231)	50.4 (187)	NT-proBNP	Immunoassay (Biomedica Gruppe)
8 (9)	156	70.8±11.5	5.1±5.6	35.3(55)	57.7(90)	78.2 (122)	31.4 (49)	46.8 (73)	45.5 (71)	0 (0)	0 (0)	-	32.7 (51)	BNP	Radioimmuno assay
9 (10)	95	72.8±11.4	6.4±6.3	48.4(46)	16.8(16)	80 (76)	26.3 (25)	46.3 (44)	33.7 (32)	18.9 (18)	4.2 (4)	1.1 (1)	40 (38)	BNP	Triage
10 (11)	45	60.7±14.5	-	40(18)	22.2(10)	51.1 (23)	8.9 (4)	22.2 (10)	15.6 (7)	0 (0)	13.3 (6)	22.2 (10)	57.8 (26)	NT-proBNP	Elecsys2010
11 (12)	180	70.8±11.6	10.6±6	43.3(78)	22.2(40)	58.9 (106)	21.1 (38)	21.1 (38)	30 (54)	1.1 (2)	1.7 (3)	15.6 (28)	55.6 (100)	NT-proBNP	Elecsys2010
12 (13,14)	111	70.2±10.3	-	32.4(36)	57.7(64)	64 (71)	28.8 (32)	24.3 (27)	44.1 (49)	7.2 (8)	7.2 (8)	-	55.9 (62)	BNP	Radioimmuno assay

13 (15)	508	72.8±12.3	8.2±6.9	49.2(263)	16.2(83)	59.7 (305)	29.4 (150)	25.4 (130)	32.3 (165)	18 (92)	7.2 (37)	4.9 (25)	48.2 (258)	BNP	Immunoassay (Biosite)
14 (16)	104	73.3±12.5	10.8±5.7	52.9(55)	44.2(46)	61.5 (64)	14.4 (15)	23.1 (24)	28.8 (30)	-	-	17.3 (18)	30.8 (32)	NT-proBNP	Modular Analytics E170
15 (17)	125	69.3±10	9.8±7.5	39.8(51)	46.1(59)	76.6 (98)	32 (41)	25 (32)	-	-	-	-	57.8 (74)	NT-proBNP	Elecsys2010
16 (18)	30	73.9±7.9	10.5±4.5	46.7(14)	26.7(8)	86.7 (26)	40 (12)	46.7 (14)	33.3 (10)	43.3 (13)	-	-	26.7 (8)	NT-proBNP	Immunoassay (Biomedica)

AF, atrial fibrillation; AHT, arterial hypertension; CE, Cardioembolic; DL, dyslipidemia, DM, diabetes mellitus; EC, embolic cardiopathy; IS, ischemic cardiomyopathy; NIHSS, National Institute of Health Stroke Scale score, at admission.

Supplemental table II: Comparison between clinical predictive models and predictive models adding BNP or NT-proBNP for cardioembolic stroke, when AF was considered.

	Clinical model in BNP cohort	Clinical model + BNP		Clinical model in NT-proBNP cohort	Clinical model + NT-proBNP	
		Highest quartile	Lowest quartile		Highest quartile	Lowest quartile
Logistic Regression (OR with 95% CI and p-value)						
Age, years	0.988 (0.973-1.003), p=0.103	0.981 (0.966-0.997), p=0.018	0.976 (0.960-0.991), p=0.003	0.979 (0.968-0.991), p=0.001	0.969 (0.956-0.981), p<0.0001	0.975 (0.964-0.987), p<0.0001
Gender, (female)	2.086 (1.449-3.004), p<0.0001	2.066 (1.418-3.010), p=0.0002	2.012 (1.376-2.942), p<0.0001	1.522 (1.123-2.064), p=0.007	1.536 (1.119-2.108), p=0.008	1.431 (1.049-1.952), p=0.024
NIHSS score at admission	1.110 (1.080-1.141), p<0.0001	1.099 (1.068-1.130), p<0.0001	1.101 (1.070-1.133), p<0.0001	1.046 (1.023-1.069), p<0.0001	1.038 (1.014-1.062), p=0.002	1.043 (1.019-1.067), p<0.0001
Atrial Fibrillation	39.537 (25.767-60.666), p<0.0001	37.314 (24.106-57.759), p<0.0001	33.940 (21.859-52.696), p<0.0001	18.294 (11.352-29.481), p<0.0001	15.298 (9.373-24.970), p<0.0001	16.242(10.040-26.277), p<0.0001
BNP/NT-proBNP	-	3.122 (1.953-4.992), p<0.0001	4.283 (2.667-6.878), p<0.0001	-	4.920 (3.241-7.464), p<0.0001	2.566 (1.804-3.650), p<0.0001
IDI statistics						
IDI for CE	-	0.003	0.007	-	0.028	0.013
IDI for non-CE	-	0.015	0.020	-	0.028	0.013
Total IDI	-	0.018	0.028	-	0.056	0.026
p-value	ref	<0.0001	<0.0001	ref	<0.0001	<0.0001
ROC curve						
AUC (95%CI)	0.899 (0.879-0.919)	0.908 (0.889-0.928)	0.913 (0.894-0.930)	0.771 (0.741-0.802)	0.809 (0.781-0.837)	0.797 (0.770-0.823)
p-value	ref	0.0076	0.001	ref	0.0002	0.0034

BNP and NT-proBNP were included as standardized values dichotomized by the highest and the lowest quartile cut-off point. Bootstrapping gives 95% CI for OR of BNP highest quartile cut-off (1.87-5.42) and BNP lowest quartile cut-off (2.51-5.96); and for OR of NT-proBNP highest quartile cut-off (3.38-7.62) and NO-proBNP lowest quartile (1.78-3.8).

AUC: area under curve; IDI: integrated discrimination improvement index; NIHSS: National Institutes of Health Stroke Scale

Supplemental table III: Comparison between clinical predictive models and predictive models adding BNP or NT-proBNP for atrial fibrillation

	Clinical model in BNP cohort	Clinical model + BNP		Clinical model in NT-proBNP cohort	Clinical model + NT-proBNP	
		Highest quartile	Lowest quartile		Highest quartile	Lowest quartile
Logistic Regression (OR with 95% CI and p-value)						
Age, years	1.061 (1.047-1.076); p<0.001	1.055 (1.041-1.070), p<0.001	1.048 (1.033-1.063), p<0.001	1.060 (1.044-1.076), p<0.001	1.048 (1.032-1.065), p<0.01	1.055 (1.039-1.072), p<0.001
Gender, (female)	0.948 (0.715-1.256), p=0.709	0.913 (0.684-1.219), p=0.538	0.921 (0.688-1.234), p=0.582	1.265 (0.920-1.739), p=0.148	1.253 (0.9-1.745), p=0.181	1.156 (0.835-1.602), p=0.382
NIHSS score at admission	1.071 (1.051-1.092), p<0.001	1.066 (1.045-1.087), p<0.001	1.065 (1.044-1.86), p<0.001	1.050 (1.027-1.073), p<0.001	1.039 (1.015-1.063), p<0.001	1.044 (1.021-1.068), p<0.001
BNP/NT-proBNP	-	2.922 (2.099-4.070), p<0.001	5.865 (3.821-9.002), p<0.001	-	3.778 (2.681-5.324), p<0.001	4.135 (2.534-6.748), p<0.001
IDI statistics						
IDI for AF	-	0.021	0.038	-	0.051	0.033
IDI for non-AF	-	0.015	0.028	-	0.018	0.011
Total IDI	-	0.037	0.066	-	0.068	0.044
p-value	ref	<0.0001	<0.0001	ref	<0.0001	<0.0001
ROC curve						
AUC (95%CI)	0.725 (0.693-0.756)	0.755 (0.725-0.784)	0.772 (0.743-0.8)	0.722 (0.687-0.758)	0.770 (0.737-0.804)	0.762 (0.727-0.796)
p-value	ref	0.0004	<0.0001	ref	0.0001	<0.0001

BNP and NT-proBNP were included as standardized values dichotomized by the highest and the lowest quartile cut-off point. Bootstrapping gives 95% CI for OR of BNP highest quartile cut-off (2.077-4.238) and BNP lowest quartile cut-off (3.877-9.574); and for OR of NT-proBNP highest quartile cut-off (2.68-5.419) and NO-proBNP lowest quartile (2.639-7.486). AUC: area under curve; IDI: integrated discrimination improvement index; NIHSS: National Institutes of Health Stroke Scale

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