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Midregional Pro-Atrial Natriuretic Peptide and Outcome in Patients With Acute Ischemic Stroke

Mira Katan, MD,*† Felix Fluri, MD,† Philipp Schuetz, MD,* Nils G. Morgenthaler, MD, PHD,‡ Christian Zweifel, MD,§ Roland Bingisser, MD,‖ Ludwig Kappos, MD,† Andreas Steck, MD,† Stefan T. Engelter, MD,† Beat Müller, MD,*¶ Mirjam Christ-Crain, MD*

Basel and Aarau, Switzerland; and Hennigsdorf/Berlin, Germany

Objectives The purpose of this study was to examine the prognostic value of midregional pro-atrial natriuretic peptide (MR-proANP) in patients with acute ischemic stroke.

Background The rapid and reliable estimation of prognosis in acute ischemic stroke is pivotal to optimize clinical care. MR-proANP, a recently described, stable fragment of the ANP precursor hormone, may be useful in this setting.

Methods In a prospective observational study, we measured MR-proANP on admission in plasma of 362 consecutive patients presenting with acute ischemic stroke. The prognostic value of MR-proANP to predict mortality within 90 days and functional outcome (defined as a modified Rankin Scale of ≤2 or ≥3) was evaluated and compared with the National Institutes of Health Stroke Scale (NIHSS) score.

Results The discriminatory accuracy, calculated with the area under the curve (AUC) of the receiver operating characteristics curve, of MR-proANP to predict death was comparable to the NIHSS (AUC: 0.86 [95% confidence interval (CI): 0.82 to 0.90] and 0.85 [95% CI: 0.81 to 0.89; $p = 0.7$]). Combined, the accuracy significantly improved (0.92 [95% CI: 0.88 to 0.96; $p < 0.01$]). The AUC of MR-proANP to predict functional outcome was 0.70 (95% CI: 0.65 to 0.75), similar to the NIHSS (0.75 [95% CI: 0.70 to 0.80]; $p = 0.16$). The prognostic value of MR-proANP for both outcomes was independent of the NIHSS. Higher MR-proANP concentrations were found in stroke of cardioembolic etiology.

Conclusions MR-proANP is a prognostic marker in the acute phase of stroke, improving the discriminatory value of the NIHSS, independently predicting post-stroke mortality and functional outcome. (The "COSMOS"-Study [Copeptin in Osmoregulation and Stress Assessment]; NCT00390962) (J Am Coll Cardiol 2010;56:1045–53) © 2010 by the American College of Cardiology Foundation

Stroke is the third leading cause of death and the primary cause of long-term disability worldwide (1). The direct and indirect cost of stroke amounted to $65.5 billion in 2008 (2). Each year, over 5 million people die as a consequence of stroke, and at least 1 in 6 patients who survives a stroke will suffer another stroke within 5 years (3). An early risk assessment with estimate of the severity of disease and prognosis could facilitate optimized care and allocation of health care resources (4). Thus, there is the need to develop a credible evidence base of prognostic information for outcomes that are meaningful to patients, including mortality and level of independency. In this context, prognostic markers available during the initial phase after acute stroke would aid in the timely estimation of disease severity, functional outcome, and mortality.

See page 1054

A-type natriuretic peptide (ANP) is a family member of the natriuretic peptides. Its physiological role is mainly the regulation of blood pressure ascribed to its natriuretic,

From the *Department of Endocrinology, University Hospital Basel, Basel, Switzerland; †Department of Neurology, University Hospital Basel, Basel, Switzerland; ‡Research Department, BRAHMS AG, Hennigsdorf/Berlin, Germany; §Department of Neurosurgery, University Hospital Basel, Basel, Switzerland; ‖Department of Internal Medicine, University Hospital Basel, Basel, Switzerland; and the ¶Department of Internal Medicine, Kantonsspital, Aarau, Switzerland. The study was supported by in-house grants of the Department of Endocrinology, Diabetology, and Clinical Nutrition, research grants from the Swiss National Foundation PP00P3-123346 (to Dr. Christ-Crain) and in-house grants of the Department of Neurology of the University Hospital of Basel, Switzerland, as well as by a personal grant for young investigators to Dr. Katan from the University of Basel. Dr. Morgenthaler is an employee of BRAHMS, the manufacturer of the MR-proANP assay (BRAHMS MR-proANP LIA, BRAHMS AG, Hennigsdorf/Berlin, Germany). Drs. Müller and Schuetz have served as consultants and received payment from BRAHMS for meetings, speaking engagements, or research unrelated to this trial. Dr. Kappos has received payment from several companies regarding his research within the field of multiple sclerosis unrelated to this trial. All other authors have reported that they have no relationships to disclose. Drs. Müller and Christ-Crain are joint senior authors on this study.

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JACC Vol. 56, No. 13, 2010
September 21, 2010:1045–53

Abbreviations and Acronyms

AF = atrial fibrillation
ANP = A-type natriuretic peptide
AUC = area under the curve
AVP = arginine vasopressin
CE = cardioembolism
CI = confidence interval
IQR = interquartile range
LACS = lacunar syndrome
MR-proANP = midregional pro-atrial natriuretic peptide
mRS = modified Rankin Scale
NIHSS = National Institutes of Health Stroke Scale
OR = odds ratio
PACS = partial anterior circulation syndrome
POCS = posterior circulation syndrome
proBNP = pro-brain natriuretic peptide
ROC = receiver-operating characteristic curve
TACS = total anterior circulation syndrome

diuretic, and vasodilating action. ANP emerged as reliable prognostic marker for congestive heart failure and risk of cardiovascular events and death (5,6). In the acute phase of ischemic stroke, levels of ANP have been reported to be elevated (7) and to predict mortality (8). Immunohistochemical studies suggest that cerebral ischemia directly induces ANP secretion in brain tissue (9,10). Interestingly, individuals with a homozygous genotype for the ANP stop codon mutation have an increased risk of ischemic stroke (11). Thus, ANP is likely to play a role in the hemodynamic regulation during the acute phase of ischemic stroke.

Midregional pro-ANP (MR-proANP) derives from the precursor hormone of ANP. MR-proANP is released in an equimolar ratio to ANP. The present assay for MR-proANP was designed to detect the midregion of the prohormone, which is more stable than the N- or C-terminal part of the precursor (12). Other proANP assays may underestimate the release of the precursor due to an early degradation of crucial epitopes at the extreme ends of the molecule. The midregional fragment of proANP is also more stable in blood ex vivo, which renders it generally more applicable in clinical practice (13). Fragments of natriuretic prohormones (i.e., MR-proANP and pro-brain natriuretic peptide [proBNP], respectively) predict poor outcomes in patients after acute myocardial infarction and in patients with heart failure (14–18). We hypothesize that MR-proANP, measured in the acute phase after an ischemic stroke, is a good prognostic marker for functional outcome and mortality within 90 days.

Methods

Study design and setting. We conducted a prospective cohort study at the University Hospital Basel, Basel, Switzerland. From November 2006 to November 2007, consecutive patients presenting with an acute ischemic cerebrovascular event were included. The primary end point of this study was to evaluate prognostic biomarkers, particularly the arginine vasopressin (AVP) precursor (copeptin) and the precursor hormone of ANP (MR-proANP) to predict outcome in ischemic stroke. A complete description of copeptin and stroke outcome has been reported previously (19). In brief, after approval from the Ethics Committee of the University Hospital Basel was received, written informed consent was obtained from study participants or, if not feasible, from next of kin. A total of 605 consecutive patients with a suspicion of stroke within 72 h before admission at the emergency department were examined.

For the purpose of this study, we evaluated all patients with the final diagnosis of ischemic stroke (n = 362), confirmed by computed tomography and/or magnetic resonance imaging on admission. The other 243 patients had either transient ischemic attack, intracerebral hemorrhage, or other final diagnoses and were excluded from the final analysis. Neurological deficits were assessed using the National Institutes of Health Stroke Scale (NIHSS) prospectively on admission in all patients. Blood samples were collected within 0 to 3 h (n = 78), 3 to 12 h (n = 189), 12 to 24 h (n = 55), and 24 to 72 h (n = 40) from symptom onset. The clinical stroke syndrome was determined by applying the criteria of the Oxfordshire Community Stroke Project; that is, total anterior circulation syndrome (TACS), partial anterior circulation syndrome (PACS), lacunar syndrome (LACS), and posterior circulation syndrome (POCS) (20). Stroke etiology was determined according to the criteria of the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification (21), which distinguishes large artery arteriosclerosis, cardioembolism, small artery occlusion, other etiology, and undetermined etiology. At discharge from the hospital, each patient received an etiologic diagnosis of stroke.

Follow-up and end points. All patients underwent a structured telephone interview after 90 days in order to identify occurrence and timing of mortality of any cause and functional outcome. Ninety-day outcome was measured by the modified Rankin Scale (mRS), and a favorable outcome was defined as a mRS score of 0 to 2.

Assays. Blood was obtained from a venous catheter. Results of the routine blood analyses were recorded. Plasma was frozen at −70°C. MR-proANP was detected in plasma from all patients with a new sandwich immunoassay (BRAHMS AG, Hennigsdorf/Berlin, Germany), as described in detail elsewhere (13). In brief, the lower detection limit of the assay is 6.0 pmol/l. The intra-assay coefficient of variation was 10% for samples containing 23 to 3,000 pmol/l MR-proANP and 20% for samples containing 18 to 22.8 pmol/l. The interassay coefficient of variation was 10% at the concentration of 65 pmol/l MR-proANP and 20% at a concentration of 18 pmol/l MR-proANP. In 325 healthy individuals, the range of MR-proANP concentrations was 9.6 to 313 pmol/l. The median was 45 pmol/l (95% confidence interval [CI]: 43.0 to 49.1 pmol/l) (13).

Statistical analysis. Discrete variables are expressed as counts (percentage), and continuous variables as medians and interquartile ranges (IQRs). Two-group comparison of not normally distributed data was performed using Mann-Whitney *U* test and a Kruskal-Wallis 1-way analysis of

variance was used for multigroup comparisons. First, the relation of MR-proANP with outcomes (i.e., death and functional outcome in stroke patients) was assessed using logistic regression models. All baseline parameters were analyzed. Thereby, common logarithmic transformation (i.e., base 10) was performed to obtain normal distribution for skewed variables (i.e., MR-proANP concentrations) as the resulting model yielded a smaller Akaike Information Criterion, which was chosen to compare the results. We used crude models and only report odds ratios (ORs) of biological important or significant predictors in our tables. Multivariate models were adjusted for 3 biologically important outcome predictors for the end point mortality (n = 44) to avoid overfitting. For the end point functional outcome, we adjusted for all significant predictors, and then for the 4 main predictors. Note that the OR corresponds to an increase by 1 U in the explanatory variable. In terms of log-transformed MR-proANP values, the OR corresponds to a 10-fold increase.

Second, we compared the overall discriminatory ability of different predictors by calculating receiver-operating characteristic curve (ROC) analysis with the method by DeLong et al. (22). Thereby, the area under the ROC (AUC) is a summary measure over criteria and cut-point choices. The AUC summary equals the probability that the underlying classifier will score a randomly drawn positive sample higher than a randomly drawn negative sample. To test whether the MR-proANP level improves the performance of the NIHSS score, we compared ROCs of the logistic regression model combining the NIHSS score with MR-proANP with a ROC limited to the NIHSS. We also compared ROCs of MR-proANP and copeptin, which is a recently published prognostic biomarker in acute ischemic stroke.

Finally, to study the ability of MR-proANP to predict mortality, we calculated Kaplan-Meier survival curves and stratified patients by quartiles. All testing was 2-tailed, and p values <0.05 were considered to indicate statistical significance. All calculations were performed using STATA version 9.2 (Stata Corp, College Station, Texas).

Results

Baseline characteristics. Of the 362 consecutively enrolled patients with an ischemic stroke, 359 patients completed the 90-day follow-up and were analyzed; 2 patients were lost to follow-up, and 1 patient withdrew informed consent.

The median age of patients was 75 years (IQR 63 to 83 years), and 41% were women. Vital signs assessed on admission revealed a median systolic blood pressure of 160 mm Hg (IQR 140 to −180 mm Hg) and a body temperature of 37.0°C (IQR 36.5°C to 37.4°C). Regarding the neurological deficits on admission, the median NIHSS was 5 points (IQR 2 to 10 points). A total of 275 patients (77%) had a history of hypertension, 93 (26%) had hypercholesterolemia, 71 (20%) had a history of diabetes mellitus, 124 (35%) were smokers, 75 (21%) were diagnosed with atrial fibrillation (AF), 88 (25%) had a history of a previous vascular event, 91 (25%) had coronary heart disease, and 54 (15%) had a history of heart failure. According to the Oxfordshire Community Stroke Project classification, 162 (45%) had a PACS, 41 (11%) a TACS, 74 (21%) a LACS, and 83 (23%) a POCS. Baseline characteristics of the study population are provided in Table 1.

Outcome evaluation of the stroke population after 90 days showed a mortality rate of 12% (n = 44). The functional outcome assessment revealed a median mRS of 2 (IQR 1 to 4) in the whole stroke population, and 42% (n = 151) of patients had an unfavorable functional outcome defined as (mRS >2).

MR-proANP and severity of stroke on admission according to the NIHSS. MR-proANP concentrations increased with increasing severity of stroke as defined by the NIHSS (Fig. 1). MR-proANP levels in patients with a NIHSS of 0 to 6 points (n = 217) were 122.0 pmol/l (IQR 73.4 to 203.5 pmol/l), in patients with a NIHSS of 7 to 15 points (n = 90) 168.5 pmol/l (IQR 100.0 to 286.0 pmol/l), and in patients with a NIHSS >15 points (n = 55) 251.5 pmol/l (IQR 129.0 to 372.5 pmol/l) ($p < 0.0001$).

MR-proANP and death within 90 days. MR-proANP levels on admission in the 44 patients who subsequently died were about 3-fold increased as compared with survivors (345.0 pmol/l [IQR 232.0 to 465.0 pmol/l] vs. 130.5 pmol/l [IQR 78.2 to 216.5 pmol/l]; $p < 0.001$) (Fig. 2A). Univariate analysis identified MR-proANP concentrations, age, presence of TACS and POCS, history of heart failure, coronary artery disease, renal insufficiency, small vessel disease, and the NIHSS as predictors for death (Table 2). Thereby, the unadjusted OR of log-transformed MR-proANP was 128.7 (95% CI: 29.7 to 557.1), and for MR-proANP quartiles, it was 4.7 (95% CI: 2.8 to 8.0). After combining MR-proANP in bivariate logistic regression analysis with each predictor alone, it remained an independent predictor. In addition, multivariate analysis restricted to the 4 main predictors, age, the NIHSS, and TACS, MR-proANP remained an independent predictor for mortality with an adjusted OR of 61.01 (95% CI: 9.87 to 377.93); similarly after adjustment for age, the NIHSS, and heart failure, the adjusted OR was 62.83 (95% CI: 9.60 to 411.28) (Table 3). ROCs demonstrated a discriminatory accuracy (AUC) to predict mortality for MR-proANP of 0.86 (95% CI: 0.82 to 0.86), which was in the range of the NIHSS (AUC: 0.85 [95% CI: 0.78 to 0.91]) and copeptin (AUC: 0.82 [95% CI: 0.76 to 0.89]). MR-proANP had a better predictive value as compared with CRP (AUC: 0.70 [95% CI: 0.60 to 0.70]; $p < 0.05$) and glucose (AUC: 0.57 [95% CI: 0.47 to 0.66]; $p < 0.01$). The combination of MR-proANP and the NIHSS in a combined logistic model had a significantly higher discriminatory accuracy (AUC: 0.92 [95% CI: 0.88 to 0.96]) than the AUC of the NIHSS or MR-proANP alone ($p < 0.01$ and $p < 0.01$, respectively) (Fig. 3). Also,

JACC Vol. 56, No. 13, 2010
September 21, 2010:1045–53

Table 1 Baseline Characteristics

	All	Favorable Outcome (mRS 0–2)	Unfavorable Outcome (mRS 3–6)	p Value*
n	359	208	151	
Demographic data				
Age, yrs	75 (63–83)	71 (59–80)	80 (71–86)	<0.001
Female sex	41% (149)	35% (73)	49% (76)	<0.01
Stroke severity, NIHSS	5 (2–10)	4 (2–6)	8 (4–17)	<0.001
Laboratory findings				
MR-proANP, pmol/l†	141.5 (84.1–237.8)	119.0 (72.8–187.0)	213.0 (119.0–332.0)	<0.0001
Glucose level, mmol/l	6.1 (5.5–7.4)	6.0 (5.3–7.2)	6.3 (5.6–7.7)	<0.05
C-reactive protein, mmol/l	3.6 (3.0–9.9)	3.0 (3.0–6.5)	4.90 (3.0–19.9)	<0.001
Creatinine, μmol/l	76.0 (63.0–89.0)	76.0 (63.5–89.0)	76.0 (62.5–91.0)	NS
Vital parameters on admission				
Arterial pressure, mm Hg systolic	160 (140–180)	162 (143–180)	159 (132–180)	NS
Arterial pressure mm Hg diastolic	90 (80–100)	91 (81–102)	90 (79–98)	NS
Body temperature, °C	37.0 (36.5–37.4)	37.0 (36.7–37.5)	37 (36.4–37.4)	NS
Stroke etiology‡				
Small vessel occlusive	15% (55)	18% (38)	11% (17)	NS
Large vessel occlusive	18% (65)	18% (38)	18% (27)	NS
Cardioembolic	37% (131)	36% (75)	37% (56)	NS
Other	4% (16)	5% (11)	3% (5)	NS
Unknown	26% (92)	22% (46)	30% (46)	NS
Stroke syndrome				
TACS	11% (41)	5% (11)	20% (30)	<0.0001
PACS	45% (162)	44% (92)	47% (69)	NS
LACS	21% (74)	23% (47)	18% (27)	NS
POCS	23% (83)	28% (58)	16% (24)	<0.01
Comorbidities				
Charlson Index	1 (0–2)	0 (0–2)	1 (0–2)	**<0.0001**
Hypertension	77% (275)	73% (152)	81% (123)	**<0.05**
Atrial fibrillation	21% (75)	16% (34)	27% (41)	**<0.05**
Heart failure	15% (54)	10% (21)	21% (33)	**<0.05**
Renal insufficiency	11% (39)	7% (14)	17% (25)	**<0.05**
Smoking history	35% (124)	38% (79)	30% (45)	NS
Hypercholesterolemia	26% (93)	28% (58)	23% (35)	NS
Diabetes mellitus	20% (71)	19% (39)	21% (32)	NS
Coronary heart disease	25% (91)	23% (48)	28% (43)	NS
Prior stroke	25% (88)	23% (48)	26% (40)	NS
Therapies				
Antihypertensive (prior to admission)	59% (213)	57% (119)	62% (94)	NS
ASA (prior to admission)	37% (133)	36% (74)	39% (59)	NS
Clopidogrel (prior to admission)	5% (18)	3% (7)	7% (11)	NS
Anticoagulant (prior to admission)	11% (38)	9% (18)	13% (20)	NS
Statins (prior to admission)	22% (78)	23% (47)	21% (31)	NS
Thrombolysis (on admission)	16% (59)	17% (36)	15% (23)	NS

Values are median (interquartile range) or % (n). *p values were assessed using the Mann-Whitney *U* test; †9 patients had missing values for MR-proANP; ‡some patients had 2 etiologies at the same time, and because of rounding, percentages may not sum to 1. Bold values indicate statistical significance.

ASA = acetyl salicylic acid; IQR = interquartile range; LACS = lacunar syndrome; mRS = modified Rankin Scale; NIHSS = National Institutes of Health Stroke Scale; PACS = partial anterior circulation syndrome; POCS = posterior circulation syndrome; TACS = total anterior circulation syndrome.

the combination of the MR-proANP and copeptin improved the discriminatory accuracy of copeptin alone (AUC: 0.89 [95% CI: 0.84 to 0.93]; p = 0.04). Adding copeptin to the logistic model of MR-proANP and the NIHSS did not further improve the model's discriminatory ability and revealed an AUC of 0.92 (95% CI: 0.88 to 0.96).

The time to death in the 90-day follow-up was analyzed using Kaplan-Meier survival curves based on MR-proANP quartiles. As demonstrated in Figure 4, patients in higher MR-proANP quartiles had an increase in the risk of mortality.

MR-proANP and functional outcome of stroke patients within 90 days. MR-proANP levels in patients with an unfavorable outcome were about 2-fold higher compared with patients with a favorable outcome (213.0 pmol/l [IQR 119.0 to 333.0 pmol/l] vs. 119.0 pmol/l [IQR 72.8 to 187.0

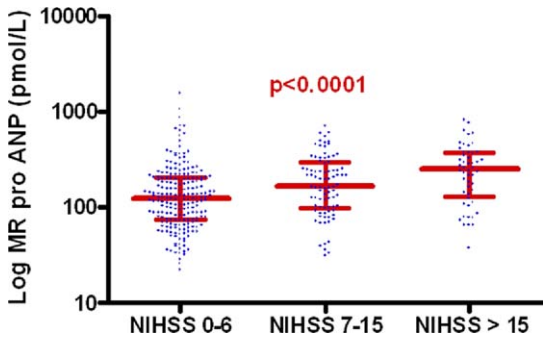

Figure 1 **MR-proANP Levels and Stroke Severity According to the NIHSS**

All data are medians and interquartile range (IQR) with dot plots representing all values. The p value refers to a Kruskal-Wallis 1-way analysis of variance. MR-proANP = midregional pro-atrial natriuretic peptide; NIHSS = National Institutes of Health Stroke Scale.

pmol/l], $p < 0.0001$) (Fig. 2B). In univariate logistic regression analysis, we calculated the predictive value of MR-proANP as compared with the NIHSS and other risk factors (Table 2). With an unadjusted OR of 10.06 (95% CI: 4.71 to 21.52), for log-transformed MR-proANP levels and 1.9 (95% CI: 1.6 to 2.4) for MR-proANP quartiles, MR-proANP was a strong predictor of outcome. Comparing MR-proANP with each significant outcome predictors in a bivariate regression model, MR-proANP remained an independent predictor (adjustment for the NIHSS revealed an OR of 5.78 [95% CI: 2.60 to 12.97; $p < 0.001$], for age an OR of 4.88 [95% CI: 2.08 to 11.46; $p < 0.001$], for female sex an OR of 9.30 [95% CI: 4.33 to 19.97; $p < 0.001$], for heart failure an OR of 9.07 [95% CI: 4.10 to 20.05; $p < 0.001$], for AF an OR of 10.08 [95% CI: 4.45 to 22.87; $p < 0.001$], for renal insufficiency an OR of 7.73 [95% CI: 3.44 to 17.35; $p < 0.001$], for CRP an OR of 6.47 [95% CI: 2.82 to 14.83; $p < 0.001$], for the Charlson Index an OR of 8.41 [95% CI: 3.84 to 18.41], and for the presence of TACS an OR of 9.34 [95% CI: 4.28 to 20.34; $p < 0.001$]). When combining all significant predictors in a multivariate model, only age, the Charlson score, and the NIHSS remained independent predictors for functional outcome.

With an AUC of 0.70 (95% CI: 0.65 to 0.75) to predict functional outcome, MR-proANP had a significantly higher prognostic discriminatory capacity as compared with TACS and sex, and was within the range of copeptin, the NIHSS, and age (Table 4). In addition, MR-proANP tended to improve the NIHSS with an AUC of the combined model of 0.79 (95% CI: 0.74 to 0.83; $p = 0.05$).

MR-proANP in different stroke etiologies. When dividing patients into 4 subgroups based on stroke etiology, 18% (n = 65) of patients were allocated to the large artery atherosclerosis group, 36% (n = 131) to the cardioembolism (CE) group, 15% (n = 55) to the small vessel occlusion group, 4% (n = 16) to the group of other etiologies (e.g., dissection), and 26% (n = 92) to the group with undetermined etiology. MR-proANP levels were highest in patients with CE etiology (206 pmol/l [IQR 119 to 326 pmol/l]), significantly higher as compared with other etiologies (124 pmol/l [IQR 73 to 207 pmol/l]; $p < 0.0001$). Logistic regression analysis revealed that MR-proANP was significantly associated with CE etiology (unadjusted OR of 7.1 [95% CI: 2.6 to 19.4]), independent of history of chronic heart failure (adjusted OR: 6.0 [95% CI: 2.3 to 13.9]) and atrial fibrillation (adjusted OR: 14.8 [95% CI: 1.2 to 6.1]) and hypertension (OR: 1.5 [95% CI: 0.80 to 2.91]). ROC analysis showed an AUC of 0.68 (95% CI: 0.62 to 0.74) for cardioembolic etiology. The optimal biomarker cutoff point for discriminating the presence or absence of a cardioembolic source was determined to be >180 pg/ml with a specificity of 60.3% (95% CI: 51.2 to 68.9), sensitivity of 71% (95% CI: 64.6 to 76.8), a positive likelihood ratio of 2.0, and a negative likelihood ratio of 0.6.

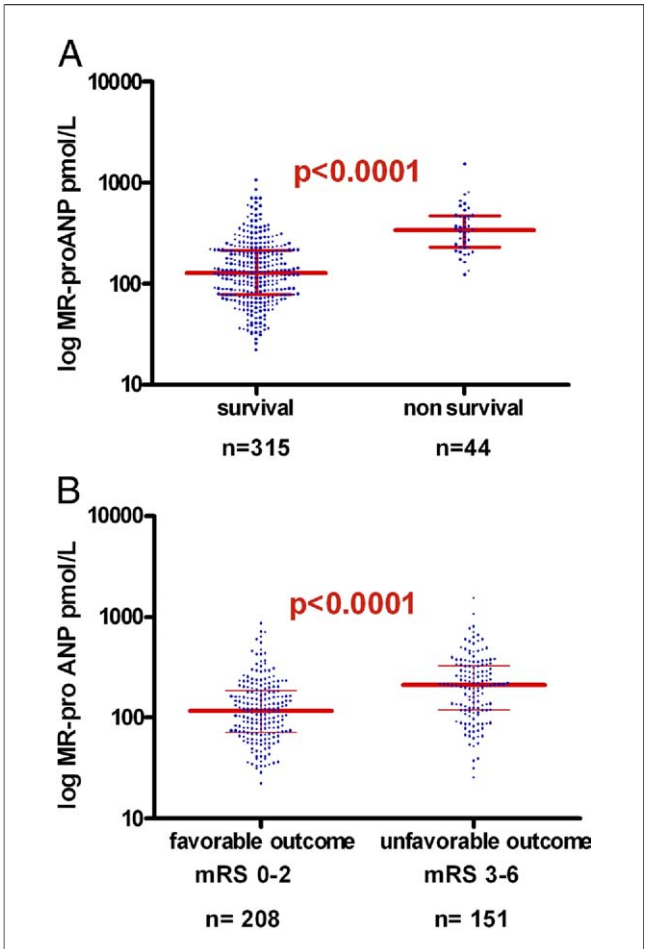

Figure 2 **Log MR-proANP Levels**

(A) Log MR-proANP levels in survivors and nonsurvivors of stroke. **(B)** Log MR-proANP levels in stroke patients with favorable and unfavorable functional outcome. All data are medians and IQR, with dot plots representing all values. The p value refers to a Mann-Whitney *U* test. Abbreviations as in Table 1.

JACC Vol. 56, No. 13, 2010
September 21, 2010:1045–53

Table 2 Univariate Analyses

	Mortality				Functional Outcome			
	Odds Ratio	R^2	95% CI	p Value	Odds Ratio	R^2	95% CI	p Value
Laboratory parameters								
MR-proANP*	128.31	0.25	29.71–554.22	**<0.0001**	10.07	0.09	4.71–21.52	**<0.0001**
C-reactive protein	1.10	0.03	0.99–1.21	0.05	1.01	0.02	1.00–1.02	0.01
Creatinine	1.00	0.0006	0.99–1.01	0.68	1.00	0.0006	0.99–1.00	0.59
Demographic data								
Age	1.09	0.12	1.05–1.14	**<0.0001**	1.06	0.09	1.04–1.08	**<0.0001**
Female sex	1.35	0.003	0.72–2.54	0.93	1.78	0.01	1.17–2.74	**0.01**
Stroke severity, NIHSS	1.20	0.28	1.14–1.25	**<0.0001**	1.16	0.15	1.12–1.21	**<0.0001**
Comorbidities								
Charlson Index	1.16	0.008	0.96–1.40	0.13	1.34	0.03	1.15–1.56	**<0.0001**
Heart failure	2.44	0.02	1.16–5.10	**0.02**	2.49	0.003	1.38–4.51	**0.003**
Atrial fibrillation	3.16	0.001	0.92–6.15	0.09	1.91	0.01	1.14–3.19	**0.01**
Coronary heart disease	2.07	0.017	1.07–4.00	**0.03**	1.33	0.003	0.82–2.14	0.25
Renal insufficiency	3.64	0.046	1.65–8.04	**0.001**	2.92	0.02	1.46–5.83	**0.002**
Stroke syndrome								
TACS	4.44	0.10	2.15–9.19	**<0.0001**	6.67	0.05	3.39–13.99	**<0.0001**
PACS	1.12	0.0001	0.74–1.69	0.74	0.81	0.0002	0.41–1.54	0.53
LACS	0.75	0.02	0.44–1.26	0.28	0.35	0.0001	0.12–1.02	0.54
POCS	0.49	0.006	0.29–0.83	**0.01**	0.50	0.01	0.20–1.23	0.20
Stroke etiology								
Small vessel occlusive	0.11	0.03	0.02–0.84	**0.03**	0.58	0.007	0.30–1.05	0.07
Large vessel occlusive	0.55	0.006	0.21–1.45	0.23	0.97	0.00001	0.56–1.68	0.93
Cardioembolic	1.54	0.007	0.82–2.92	0.43	1.05	0.0001	0.68–1.62	0.84
Other	0.47	0.002	0.06–3.64	0.46	0.61	0.001	0.21–1.80	0.37
Unknown	1.96	0.01	1.02–3.79	0.05	1.54	0.007	0.96–2.49	0.08

p values in **bold** indicate statistical significance. *Note that the odds ratio corresponds to a unit increase in the explanatory variable; for MR-proANP this corresponds to an increase per unit of the log transformation of MR-proANP (thus a log-transformed increase of 1 corresponds to a MR-proANP increase of 10 pmol/l).Bold values indicate statistical significance.

To estimate whether MR-proANP improved the diagnosis of CE etiology by already known clinical information, we calculated logistic models based on clinical information (age, known heart failure, and known AF on admission) and combined models based on clinical information plus MR-proANP concentrations. The model including MR-proANP (AUC: 0.81 [95% CI: 0.77 to 0.86]) was significantly better than the model based on clinical information alone (AUC: 0.76 [95% CI: 0.71 to 0.81]; $p < 0.001$).

MR-proANP levels in the subgroup of patients with heart failure or AF. In 54 (15%) patients with known chronic heart failure on admission, MR-proANP levels were higher as compared with patients without chronic heart failure (271 pmol/l [IQR 162.5 to 383.8] vs. 132.0 pmol/l (IQR 78.1 to 216.6]; $p < 0.001$). MR-proANP remained a significant predictor for both mortality and functional outcome after adjustment for heart failure with ORs of 148.83 (95% CI: 32.16 to 688.67) and 9.07 (95% CI: 4.10 to 20.05), respectively. In addition, ROC analysis showed a similar predictive value of MR-proANP for both

Table 3 Multivariate Analysis for Mortality

Predictors	Odds Ratio	95% CI	p Value
MR-proANP	62.83	9.60–411.28	**<0.001**
Age	1.04	0.99–1.09	*0.07*
Stroke severity, NIHSS	1.19	1.12–1.26	**<0.001**
Chronic heart failure	0.76	0.26–2.23	*0.60*

p values in **bold** indicate statistical significance; p values in *italics* indicate nonsignificance. Abbreviations as in Tables 1 and 2.

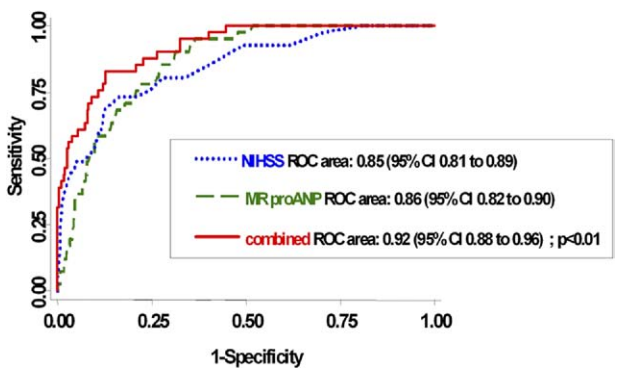

Figure 3 ROC Area to Predict Mortality

Area under the receiver-operating characteristics curve (ROC) of the NIHSS and MR-proANP, and the combined AUC. The combined model (AUC of the NIHSS and AUC of MR-proANP) was more accurate to discriminate survivors from non-survivors as compared with the clinical score (NIHSS) or the biomarker (MR-proANP) alone ($p < 0.01$ and $p < 0.01$, respectively). Abbreviations as in Figure 1.

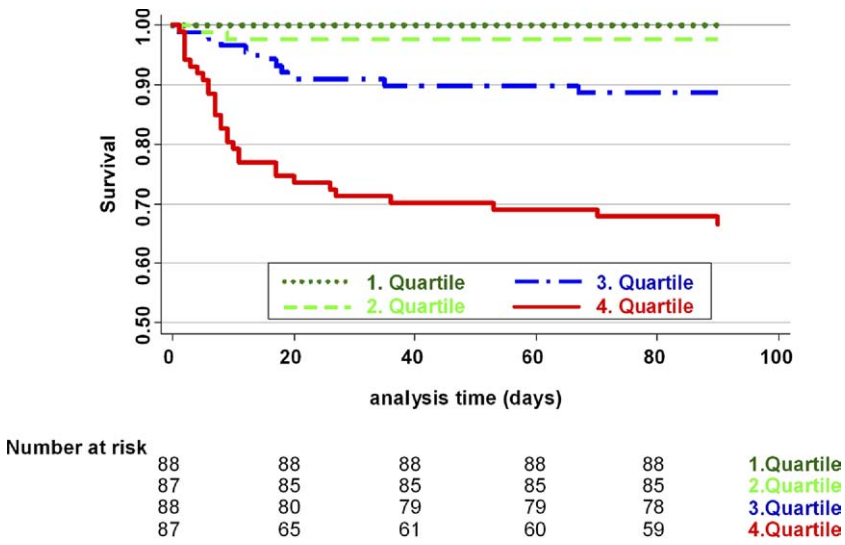

Figure 4 Kaplan-Meier Survival Curves

Estimates by midregional pro-atrial natriuretic peptide (MR-proANP) quartiles and numbers at risk. Note that in 9 patients, we had missing values for MR-proANP.

outcomes in patients with and without heart failure (data not shown).

Similarly, in 75 (21%) patients with AF, MR proANP levels were higher (258.5 pmol/l [IQR 181.8 to 380.0 pmol/l]) as compared with patients without AF (123.5 pmol/l [IQR 74.2 to 213.0 pmol/l]; $p < 0.001$). Again, MR-proANP remained a significant predictor for both outcomes after adjustment for AF. ROC analysis showed a similar predictive value of MR-proANP for both outcomes in patients with and without AF (data not shown).

Discussion

MR-proANP levels on admission were increased in stroke patients, with particularly prominently elevated levels in patients with cardioembolic strokes. MR-proANP proved to accurately predict 90-day mortality and functional outcome, independent of other comorbidities such as chronic heart failure and AF, age, and clinical scores. Importantly, MR-proANP improved the prognostic value of the NIHSS. To our knowledge, this is the first prospective study evaluating MR-proANP levels in a large cohort of stroke patients.

Table 4 ROC Analysis of Functional Outcome

Parameter	AUC	95% CI	p Value*
MR-proANP	0.71	0.65–0.75	
Copeptin	0.73	0.67–0.78	0.62
NIHSS	0.75	0.70–0.80	0.21
Age	0.70	0.65–0.75	0.88
Charlson score	0.63	0.58–0.69	0.06
Sex	0.58	0.52–0.63	**0.002**
Combined score (Copeptin and MR-proANP)	0.73	0.68–0.80	0.78
Combined score (NIHSS and MR-proANP)	0.79	0.74–0.83	**0.016**

*p values indicate significance of area under the curve (AUC) between predictors and MR-proANP. p values in **bold** indicate statistical significance.

ROC = receiver-operating characteristic curve; other abbreviations as in Tables 1 and 2.

Elevated concentrations of natriuretic peptides, especially BNP, have been shown to be of prognostic value in patients with congestive heart failure and myocardial ischemia (assessed by electrocardiographic changes) (18,23,24). Recently, MR-proANP levels were also evaluated in patients with heart failure showing comparable prognostic capabilities to BNP values with respect to 1-year all-cause mortality (25). Hence, higher natriuretic peptide levels reflect a greater degree of hemodynamic dysfunction and explain the increased mortality in patients with acute cardiac disease. A role of natriuretic peptides in the hemodynamic regulation has also been shown during the acute phase of ischemic stroke (7,26–28). Clinical studies have revealed elevated levels of natriuretic peptides in the acute phase of ischemic stroke (8,28–30). One study (8) reported elevated levels of BNP and ANP in 51 ischemic stroke patients compared with healthy controls and higher levels of natriuretic peptides in patients who died as compared with those who survived. BNP and its N-terminal peptide (NT-proBNP) has been demonstrated to be excellent markers for vascular mortality and re-events in stroke (6) although NT-proBNP levels on admission were not significantly associated with functional outcome within 3 months (30) if adjusted for vascular risk factors in another study. Estrada et al. (7) investigated ANP concentrations in 37 patients with acute ischemic stroke. Compared with healthy controls, concentration of ANP increased immediately after stroke and remained elevated for 7 days. We

found no published data on ANP or its precursor protein on the independent predictive value concerning mortality as well as functional outcome after an acute ischemic stroke.

The relation of natriuretic peptides and stroke prognosis seems not to be monocausal and is complex. First, it has been demonstrated that a higher level of NT-proBNP in stroke patients is associated with increased sympathetic activation. Sympathetic activation by itself is a prognostic determinant after an acute thromboembolic stroke (31,32). Second, previous studies have shown that heart failure is associated with dependency after stroke (27) and that it is an independent predictor for mortality after first cerebral infarction (33–35).

In our study, unadjusted and adjusted logistic regression analysis revealed that levels of MR-proANP were predictors for 90-day mortality and functional outcome, independently of the presence of heart failure or AF. The pathophysiological mechanism explaining this independent predictive value of MR-proANP observed in our study remains to be clarified. It may be hypothesized that high MR-proANP concentrations indicate the presence of a profound neurohormonal dysfunction, and thus a worse outcome. Furthermore, increased MR-proANP levels might mirror, not only manifest heart failure, but also a beginning cardiac pathology (e.g., subclinical heart failure) in which intracardial thrombus development might be more likely. This would explain the additional diagnostic value of MR-proANP levels to differentiate CE etiologies from others. It is essential to identify the CE etiology in stroke because recurrent stroke occurs within 2 weeks in up to 12% of patients who initially experience embolic stroke from cardiac sources (36). AF might be no longer present when patients are examined by 24-h electrocardiography monitoring. Thus, anticoagulant therapy might be delayed even when the neurologist suspects an embolic origin due to distinct patterns of lesions. Using biomarkers may be a reasonable strategy to improve the identification of cardioembolic stroke already in the acute phase, thus rapidly point to need of other diagnostic tests and accelerating the start of optimal secondary prevention (26).

Other known mediators involved in the ischemic cascade are markers for neuronal cell death, such as protein S-100β (37), inflammation markers, such as interleukin-6 (38), or markers for oxidative stress and blood-brain barrier disruption, such as matrix metalloproteinase-9 (39,40). Although these biomarkers reliably mirror the initial stroke severity (NIHSS and lesion size) and some even bear an association with outcome, they were not able to independently predict functional outcome and death within 3 months as well as to improve the NIHSS. To our knowledge, only 1 circulation biomarker, copeptin, the c-terminal part of the AVP prohormone, was able to significantly improve prediction of clinical outcome after stroke for death and functional outcome (19). Copeptin is released in an equimolar ratio to AVP and is more stable in the circulation and easier to determine as AVP (41). Interestingly, MR-proANP seems to be a better predictor for mortality, whereas copeptin had a higher prognostic accuracy to predict functional outcome. One reason for the absence of a "unique" biomarker preeminently mirroring all aspects of prognosis might be that the complexity of brain ischemia and recovery capacities is less amenable to the use of a single biochemical marker. In the difficult task of outcome prediction, it seems, therefore, reasonable to rely on several parameters, each mirroring different pathophysiological aspects. The approach of using a multiple biomarker panel has already been well established in the clinical setting of patients with cardiovascular disease (6,42). A model combining biomarkers and clinical parameters might also be the most promising approach to predict outcome in stroke patients.

Study limitations. We are aware of the following limitations. First, our study is a single-center study and should be externally validated in a large cohort of patients. Second, we did not directly compare the prognostic value of MR-proANP with other natriuretic peptides, especially B-type natriuretic peptides. However, this study shows promising data for a further multicenter trial including MR-proANP in a prognostic biomarker panel.

Conclusions

The present study shows that MR-proANP is a new, valuable marker for the prognosis in patients with ischemic stroke. It also seems to act as a diagnostic marker by differentiating patients with a CE etiology of stroke from other etiologies, especially when combining it with clinical information. Early risk assessment in acute ischemic stroke may allow for better and earlier intervention and improved care strategies to effectively change the dismal natural history of stroke.

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Reprint requests and correspondence: Dr. Mira Katan, University Hospital Basel, Petersgraben, 4031 Basel, Switzerland. E-mail: katanm@uhbs.ch or mk3270@columbia.edu.

REFERENCES

1. Lees KR, Zivin JA, Ashwood T, et al. NXY-059 for acute ischemic stroke. N Engl J Med 2006;354:588–600.
2. Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics—2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008;117:e25–146.
3. Hankey GJ, Warlow CP. Treatment and secondary prevention of stroke: evidence, costs, and effects on individuals and populations. Lancet 1999;354:1457–63.

4. Johnston SC. Clinical practice. Transient ischemic attack. N Engl J Med 2002;347:1687–92.
5. Wang TJ, Larson MG, Levy D, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. N Engl J Med 2004;350:655–63.
6. Wang TJ, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med 2006;355:2631–9.
7. Estrada V, Tellez MJ, Moya J, Fernandez-Durango R, Egido J, Fernandez Cruz AF. High plasma levels of endothelin-1 and atrial natriuretic peptide in patients with acute ischemic stroke. Am J Hypertens 1994;7:1085–9.
8. Makikallio AM, Makikallio TH, Korpelainen JT, et al. Natriuretic peptides and mortality after stroke. Stroke 2005;36:1016–20.
9. Giuffrida R, Bellomo M, Polizzi G, Malatino LS. Ischemia-induced changes in the immunoreactivity for endothelin and other vasoactive peptides in the brain of the Mongolian gerbil. J Cardiovasc Pharmacol 1992;20 Suppl 12:S41–4.
10. Nogami M, Shiga J, Takatsu A, Endo N, Ishiyama I. Immunohistochemistry of atrial natriuretic peptide in brain infarction. Histochem J 2001;33:87–90.
11. Rubattu S, Stanzione R, Di Angelantonio E, et al. Atrial natriuretic peptide gene polymorphisms and risk of ischemic stroke in humans. Stroke 2004;35:814–8.
12. Ala-Kopsala M, Magga J, Peuhkurinen K, et al. Molecular heterogeneity has a major impact on the measurement of circulating N-terminal fragments of A- and B-type natriuretic peptides. Clin Chem 2004;50:1576–88.
13. Morgenthaler NG, Struck J, Thomas B, Bergmann A. Immunoluminometric assay for the midregion of pro-atrial natriuretic peptide in human plasma. Clin Chem 2004;50:234–6.
14. Omland T, Persson A, Ng L, et al. N-terminal pro-B-type natriuretic peptide and long-term mortality in acute coronary syndromes. Circulation 2002;106:2913–8.
15. Omland T, de Lemos JA, Morrow DA, et al. Prognostic value of N-terminal pro-atrial and pro-brain natriuretic peptide in patients with acute coronary syndromes. Am J Cardiol 2002;89:463–5.
16. Gegenhuber A, Mueller T, Dieplinger B, Poelz W, Pacher R, Haltmayer M. B-type natriuretic peptide and amino terminal proBNP predict one-year mortality in short of breath patients independently of the baseline diagnosis of acute destabilized heart failure. Clin Chim Acta 2006;370:174–9.
17. Gegenhuber A, Struck J, Poelz W, et al. Midregional pro-A-type natriuretic peptide measurements for diagnosis of acute destabilized heart failure in short-of-breath patients: comparison with B-type natriuretic peptide (BNP) and amino-terminal proBNP. Clin Chem 2006;52:827–31.
18. Christ M, Thuerlimann A, Laule K, et al. Long-term prognostic value of B-type natriuretic peptide in cardiac and non-cardiac causes of acute dyspnoea. Eur J Clin Invest 2007;37:834–41.
19. Katan M, Fluri F, Morgenthaler N, et al. Copeptin a novel, independent prognostic marker in patients with ischemic stroke. Ann Neurol 2009;66:799–808.
20. Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. Lancet 1991;337:1521–6.
21. Adams HP, Jr., Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24:35–41.
22. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–45.
23. Maisel AS, Krishnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002;347:161–7.
24. Staub D, Jonas N, Zellweger MJ, et al. Use of N-terminal pro-B-type natriuretic peptide to detect myocardial ischemia. Am J Med 2005; 118:1287.
25. Gegenhuber A, Struck J, Dieplinger B, et al. Comparative evaluation of B-type natriuretic peptide, mid-regional pro-A-type natriuretic peptide, mid-regional pro-adrenomedullin, and Copeptin to predict 1-year mortality in patients with acute destabilized heart failure. J Card Fail 2007;13:42–9.
26. Montaner J, Perea-Gainza M, Delgado P, et al. Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. Stroke 2008;39: 2280–7.
27. Appelros P, Nydevik I, Viitanen M. Poor outcome after first-ever stroke: predictors for death, dependency, and recurrent stroke within the first year. Stroke 2003;34:122–6.
28. Giannakoulas G, Hatzitolios A, Karvounis H, et al. N-terminal pro-brain natriuretic peptide levels are elevated in patients with acute ischemic stroke. Angiology 2005;56:723–30.
29. Nakagawa K, Yamaguchi T, Seida M, et al. Plasma concentrations of brain natriuretic peptide in patients with acute ischemic stroke. Cerebrovasc Dis 2005;19:157–64.
30. Etgen T, Baum H, Sander K, Sander D. Cardiac troponins and N-terminal pro-brain natriuretic peptide in acute ischemic stroke do not relate to clinical prognosis. Stroke 2005;36:270–5.
31. Klingelhofer J, Sander D. Cardiovascular consequences of clinical stroke. Baillieres Clin Neurol 1997;6:309–35.
32. Sander D, Winbeck K, Klingelhofer J, Etgen T, Conrad B. Prognostic relevance of pathological sympathetic activation after acute thromboembolic stroke. Neurology 2001;57:833–8.
33. James P, Ellis CJ, Whitlock RM, McNeil AR, Henley J, Anderson NE. Relation between troponin T concentration and mortality in patients presenting with an acute stroke: observational study. Bmj 2000;320:1502–4.
34. Petty GW, Brown RD, Jr., Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO. Survival and recurrence after first cerebral infarction: a population-based study in Rochester, Minnesota, 1975 through 1989. Neurology 1998;50:208–16.
35. Moulin T, Tatu L, Vuillier F, Berger E, Chavot D, Rumbach L. Role of a stroke data bank in evaluating cerebral infarction subtypes: patterns and outcome of 1,776 consecutive patients from the Besancon stroke registry. Cerebrovasc Dis 2000;10:261–71.
36. Cardiogenic brain embolism. Cerebral Embolism Task Force. Arch Neurol 1986;43:71–84.
37. Foerch C, Wunderlich MT, Dvorak F, et al. Elevated serum S100B levels indicate a higher risk of hemorrhagic transformation after thrombolytic therapy in acute stroke. Stroke 2007;38:2491–5.
38. Smith CJ, Emsley HC, Gavin CM, et al. Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome. BMC Neurol 2004;4:2.
39. Rosell A, Alvarez-Sabin J, Arenillas JF, et al. A matrix metalloproteinase protein array reveals a strong relation between MMP-9 and MMP-13 with diffusion-weighted image lesion increase in human stroke. Stroke 2005;36:1415–20.
40. Rosell A, Cuadrado E, Ortega-Aznar A, Hernandez-Guillamon M, Lo EH, Montaner J. MMP-9-positive neutrophil infiltration is associated to blood-brain barrier breakdown and basal lamina type IV collagen degradation during hemorrhagic transformation after human ischemic stroke. Stroke 2008;39:1121–6.
41. Struck J, Morgenthaler NG, Bergmann A. Copeptin, a stable peptide derived from the vasopressin precursor, is elevated in serum of sepsis patients. Peptides 2005;26:2500–4.
42. Zethelius B, Berglund L, Sundstrom J, et al. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. N Engl J Med 2008;358:2107–16.

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