ORIGINAL ARTICLE

Clinical severity of ischemic stroke and neural damage biomarkers in the acute setting: the STROke MArkers (STROMA) study

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ABSTRACT

Background. Stroke is a leading cause of long-term morbidity and mortality affecting several hundred-thousand people annually in the Western Countries. Various panels of biomarkers of neural damage have been developed and validated. The primary objective of this investigation was to measure the correlation between the clinical severity of stroke and the serum/plasma concentrations of neural damage biomarkers.

Methods. A prospective investigation was conducted on a panel of biomarkers composed of S100β, matrix metalloproteinase-9 (MMP-9), N-terminal pro-B-type natriuretic peptide (NT pro-BNP) and D-dimer at admission and after 24 hours, in a cohort patients with a confirmed diagnosis of stroke in an emergency setting (STROke-MArkers STROMA).

Results. A total of 58 consecutive patients were enrolled, no participant was excluded; according to clinical severity measured by National Institute of Health Stroke Scale (NIHSS) there were 29 minor strokes, 24 moderate, 3 moderate-severe, 2 severe. The Spearman's rank correlation test was used to assess the relationship between the baseline NIHSS value and the concentrations of the four biomarkers: all the studied biomarkers showed a statistically significant correlation with baseline NIHSS at 24 hours. A multivariate ordinal regression model was used to analyze the correlation of markers with stroke severity, stratified, according to NIHSS score: MMP-9 and S100β showed a statistically significant correlation after 24 hours.

Conclusion. MMP-9, S100β, NT pro-BNP and D-dimer showed a good correlation with the clinical severity of stroke which may become an additional resource in the acute patient evaluation and potentially follow-up. *(Minerva Anestesiol 2013;79:750-7)*

Key words: Stroke - Biological markers - Matrix Metalloproteinase 9 - S-100 calcium-binding protein beta subunit - Pro-brain natriuretic peptide (1-76) - Fibrin fragment D.

Stroke is a leading cause of long-term morbidity and mortality affecting several hundred-thousand people annually in the Western Countries,[1-3] where it becomes not only a medical but also a social challenge that requires a timely assistance and carefully planned investments to maximize the effectiveness of available treatment options and offer a sustainable patient management.

Thrombolytic therapy is, at present, the intervention of choice in ischemic stroke and its effectiveness is time dependent and maximal within three hours from symptom onset;[4] fibrinolysis however is often underused due to various obstacles,[5] one of which may be diagnostic

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uncertainty. It is thus important to improve the diagnostic process degree of reliability without prolonging the time necessary to complete clinical evaluation.

The routine approach to a patient presenting at the Department of Emergency with symptom compatible with stroke includes: focused medical history to highlight possible risk factors and onset characteristics, physical examination to uncover neurological signs associated, complete blood count, coagulation testing, determination of electrolytes and glucose and brain imaging.[6] Although magnetic resonance imaging-based techniques have demonstrated a greater sensitivity in early diagnosis of stroke,[7] they are not widely available, while non-contrasted head computerized tomography (CT), that can be performed rapidly at most institutions, can rule out with an acceptable degree of accuracy intracranial hemorrhage, subdural hematoma and mass lesions but it is almost insensitive to early ischemic lesions.[8]

Similarly to what has been done for early triage and evaluation of cardiac symptom compatible with acute myocardial infarction,[9] another setting where treatment is highly time-sensitive, various panels of biomarkers of neural damage [10] have been developed and validated [11] as there is not a single molecule capable of identifying brain damage in all its forms.

If diagnosis is the necessary prerequisite to any further medical action, the second most important aspect in the evaluation of a neurological patient is the assessment of the severity neural damage, which is traditionally and effectively achieved with physical examination aided by neuroimaging studies, while a biomarker based approach, that could explore the pathophysiology of neural damage, is less studied.

The primary objective of this investigation is to measure the correlation between stroke severity and the following markers: S100β,[12, 13] a calcium binding protein found in astrocytes, matrix metalloproteinase-9 (MMP-9), a gelatinase present in neural extracellular matrix and activated during inflammation,[14, 15] N-terminal pro-B-type natriuretic peptide (NT pro-BNP), a marker of heart failure, elevated also during brain damage,[16-18] and D-dimer, the end-product of fibrinolytic process,[19, 20] at admission and after 24 hours, in a cohort patients with a confirmed diagnosis of stroke in an emergency setting.

Materials and methods

The prospective investigation STROMA (STROke MArkers) sought to measure the levels of four biomarkers of neuronal damage and thrombosis on a cohort of patients with a confirmed diagnosis of stoke at the admission at the Department of Neurology (Ramazzini Hospital, Carpi, Italy) and after 24 hours; approval from Institutional Ethics Committee (Comitato Etico Provinciale di Modena) was obtained prior to study initiation. Patients were enrolled in the STROMA study from September 2010 to September 2011, if they were older than 18 years of age and were admitted to the Department of Neurology with a confirmed diagnosis of stroke within 12 hours from the onset of new neurological symptoms. Written formal consent was obtained from the study participants or legal designate/relative. Demographic, clinical, laboratory and radiographic data were collected by a standardized protocol.

The final diagnosis of stroke was rendered by one of eight board-certified Neurologists (GG, SA, MB, MC, MD, CS, LV), who evaluated each case with symptoms compatible with stroke. A non-contrasted head CT was routinely performed to rule out intracranial hemorrhage, subdural hematoma or mass lesion. All on site clinicians were blinded to biomarkers results. Stroke was defined as persistent neurological deficit lasting for more than 24 hours, presumably of vascular etiology, associated with compatible imaging studies, transitory ischemic attack (TIA) as a focal neurological deficit with a likely vascular cause but lasting less than 24 hours. The size of cerebral infarctions was reported according to Oxford Community Stroke Project classification [21] and their baseline clinical severity was measured by National Institute of Health Stroke Scale (NIHSS),[22] which guided also their stratification into: minor (NIHSS 1-4), moderate (5-15), moderate-severe (16-20), severe (21-42).

Immunoassays

Blood samples were obtained within one hour from admission to the Department of Neurology and after 24 h by venous puncture. Plasma samples were collected into EDTA- tubes for D-dimer and NT pro-BNP detection; serum samples were collected for S100β and MMP-9 detection. Blood samples were centrifuged at 1500 x g within 60 minutes from collection. Each serum or plasma sample was subdivided into 2 CryoVials™ and stored at -80°C.

Serum MMP9 was quantified by a commercially available MMP-9 (human) ELISA kit (DRG Diagnostics, DRG Instruments GmbH, Marburg, Germany). Serum S100β and plasma NT pro-BNP were quantified with appropriate fully automated Electrochemiluminescence ImmunoAssay ECLIA (Cobas, Roche Diagnostics GmbH, Mannheim, Germany) in accordance with the manufacturer's instructions. Plasma D-dimer concentration was measured using a fully automated Tina-quant D-dimer D-DI2 test (Cobas, Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturer's instructions. The lower limit of sensitivity of the MMP9 assay was 0.05 ng/mL while the analytic range for D-dimer, S100β and NT pro-BNP was 150-9000 ng/mL, 0,00539 µg/L and 535000 pg/mL respectively.

Statistical analysis

Statistical analysis was performed using Stata 10.0 (StataCorp, Texas, USA). Descriptive statistics, including median and interquartile range (IQR) were obtained for demographic variables, Wilcoxon rank-sum test was used to compare the distributions of continuous variables, the Wilcoxon sing-rank test for paired data and χ^2 test for categorical variables. The Spearman's rank correlation test was used to assess the relationship between two interval variables: the NIHSS value and the concentrations of the four biomarkers at admission and 24 hours later. A multivariate ordinal regression model was applied to study the possible relation of the dependent variables (at admission and after 24 hours) with the severity of stroke.

Results

A total of 58 consecutive patients were enrolled in the STROMA study, no participant was excluded. The clinical severity of stroke was gauged by NIHSS: median NIHSS with IQR was 5 (from 3 to 7), according to this classification there were 29 minor strokes, 24 moderate, 3 moderate-severe, 2 severe. The demographics and risk factors of stroke for minor and moderate strokes are summarized in Table I; only one

Table I.—*Patient demographics for the study cohort divided into three groups according to the stroke severity measured by baseline National Institute of Health Stroke Scale (NIHSS). NIHSS and age are expressed as median with interquartile range (IQR); for the categorical variables data are reported as percentages.*

	Minor (N.=29)	Moderate (N.=24)	Moderate/severe & severe (N.=5)
NIHSS	3 (2-3)	6.5 (5-10)	20 (17-21)
Age (years)	76 (71-80)	78 (72-82)	79(79-81)
Sex (males)	62.1%	70.8%	40.0%
Tobacco smoke	17.2%	16.7%	0%
Alcohol (>2 glasses of wine per day)	34.8%	11.76%	0%
Hypercholesterolemia	58.62%	25.0%	80%
Diabetes	27.6%	37.5%	40%
Hypertension	79.3%	79.2%	100%
AF	20.7%	37.5%	60%
TIA	17.2%	8.3%	0%
Stroke	6.9%	16.7%	60%

AF: atrial fibrillation; TIA: transitory ischemic attack.

Table II.—*Median levels with interquartile range (IQR) of the four biochemical markers involved in neuronal damage and thrombosis of the whole studied cohort at admission and after 24 hours.*

Biomarker	Admission	24 hours	P
MMP-9 (ng/mL)	838	804	0.5105
IQR	617-1244	503-1314	
S100β (μg/L)	0.065	0.076	0.0339
IQR	0.052-0.112	0.054-0.169	
NT pro-BNP (pg/mL)	410	434	0.2198
IQR	132-977	148-934	
D-dimer (ng/mL)	685	873	0.8073
IQR	336-1697	411-1662	

death occurred during hospital stay. There was a statistically significant difference between NT-pro-BNP median levels in patients with atrial fibrillation and without this condition: at admission 1134 pg/mL *vs.* 277 pg/mL, P-value 0.0002 and after 24 hours 875 pg/mL *vs.* 225 pg/mL, P-value 0.0001.

The median levels with interquartile range of the four biochemical markers involved in neuronal damage and thrombosis of the whole studied cohort are summarized in Table II: there were no statistically significant differences between the levels of the biomarkers at admission and after 24 hours with the exception of S100β.

The Spearman's rank correlation test was used to assess the relationship between the baseline NIHSS value and the concentrations of the four biomarkers included in this panel at admission and 24 hours. Figure 1 shows the relationship between NIHSS and each biomarker and reports correlation coefficients at admission and at 24 hours. All the studied biomarkers showed a statistically significant correlation with baseline NIHSS at 24 hours, while this finding was confirmed only for NT pro-BNP and D-dimer at admission.

A multivariate ordinal regression model was used to analyze the correlation of markers with stroke severity, stratified, according to NIHSS score, as minor, moderate and greater than moderate, at admission, and after 24 hours. D-dimer was the only marker that maintained a statistically significant correlation with stroke severity at admission with a coefficient of 0.017 for 100 ng/mL increments (SE 0.008, P-value 0.039) while MMP-9 and S100β showed a statistically significant correlation after 24 hours as summarized in Table III.

Cerebral infarctions were classified according to Oxford Community Stroke Project classification as follows: 4 total anterior circulation infarcts (TACI), 17 posterior circulation infarcts (POCI), 22 partial anterior circulation infarcts (PACI), 10 lacunar infarcts (LACI) and 5 transitory ischemic attacks (TIA); the neuroimaging studies demonstrated the presence of one hemorrhagic stroke. The Kruskal-Wallis equality-of-populations rank test was used to compare the median values of the studied biomarkers, at admission and at 24 hours, with infarct size. The only variable significantly associated with infarct size was S100β at 24 hours (Table IV).

Discussion

At present the absence of a readily available, single, diagnostic test for acute stroke makes the evaluation and treatment of the disease potentially more difficult: in the current study a biomarker panel, assessing MMP-9, S100β, NT pro-BNP and D-dimer, that demonstrated a good diagnostic accuracy in the diagnosis of acute ischemic stroke [11, 23, 24] was used in the evaluation of the clinical severity of stroke.[25]

Knowing the peculiar nature of cerebrovascular accidents and the narrow time window for an effective thrombolytic therapy, when applicable, there is a clinical need for supplementary information to refine and support a clinical diagnosis in the acute setting. In fact, a better, more precise evaluation of the clinical severity of stroke can become an additional resource in medical decisions, integrating with existing patient data.

The correlation between the serum/plasma concentrations of specific biomarkers with the

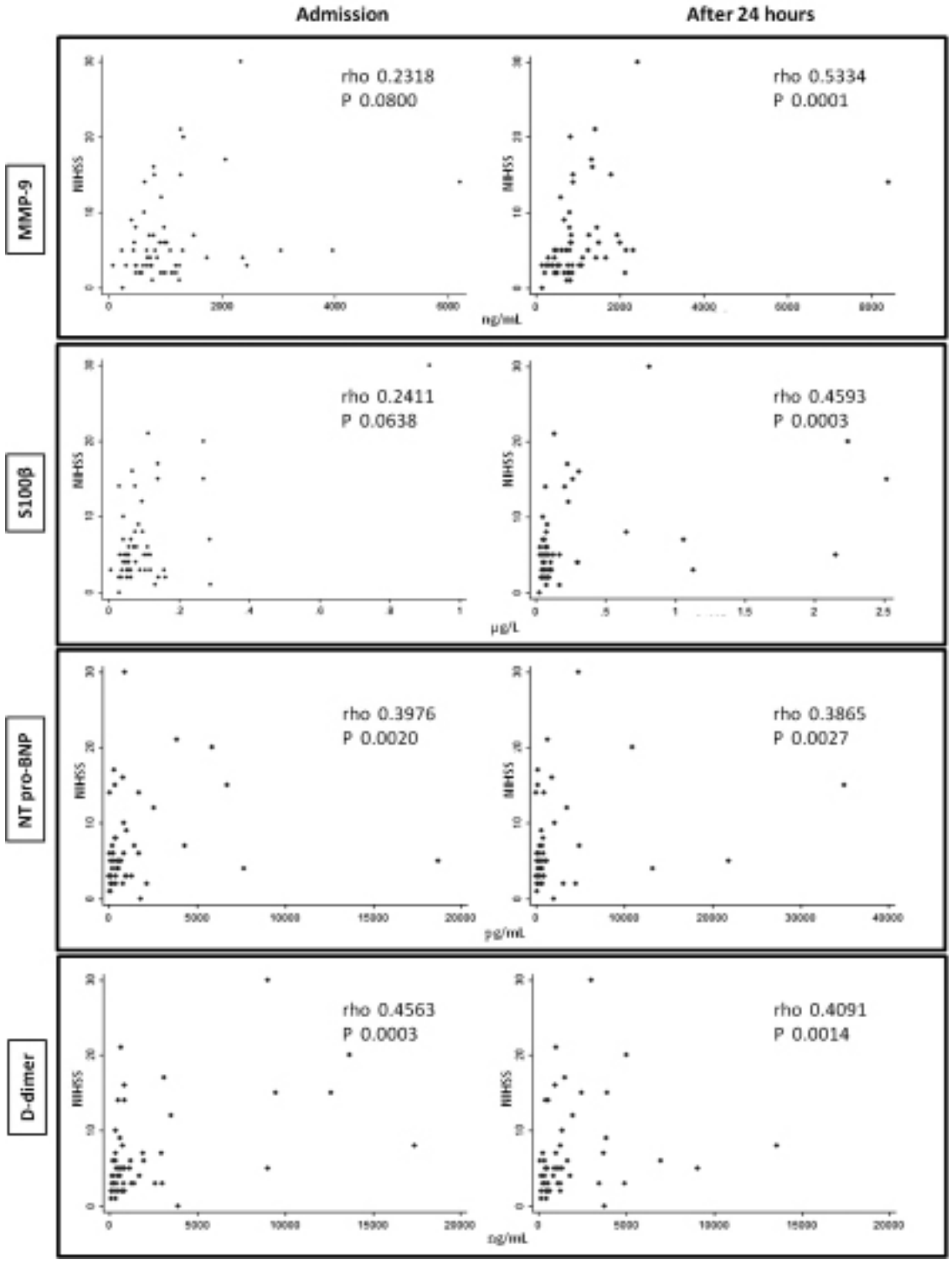

Figure 1.—Correlation between baseline National Institute of Health Stroke Scale (NIHSS) value and the serum/plasma concentrations of MMP-9, S100β, NT pro-BNP and D-dimer at admission to the Department of Neurology and after 24 hours. Data have been analyzed by Spearman's rank correlation test.

Table III-*Multivariate ordinal regression model for the severity of stroke, stratified, according to National Institute of Health Stroke Scale, as minor, moderate and greater than moderate, incorporating MMP-9, S100β, NT-proBNP and D-dimer. For model calculation were considered S100β increments of 0.01 μg/L, MMP-9 of 10 ng/mL and D-dimer of 100 ng/mL.*

Parameter	Coef.	SE	P	Lower 95% CI	Higher 95% CI
MMP-9 (ng/mL)	0.006	0.003	0.023	$8{\cdot}10^{-5}$	0.012
S100β (μg/L)	0.002	0.001	0.047	$3{\cdot}10^{-5}$	0.004
NT-proBNP (pg/mL)	-0.0008	0.0001	0.382	-0.0003	0.0001
D-dimer (ng/mL)	0.020	0.010	0.057	$-6{\cdot}10^{-6}$	0.0004

Coef.: Coefficient; SE: standard error; CI: confidence interval.

Table IV.—*Median S100β levels with interquartile range (IQR) after 24 hours from admission in correlation with cerebral infarction extension (Kruskal-Wallis equality-of-populations rank test). Cerebral infarctions were classified according to Oxford Community Stroke Project classification as follows: total anterior circulation infarcts (TACI), posterior circulation infarcts (POCI), partial anterior circulation infarcts (PACI), lacunar infarcts (LACI) and transitory ischemic attacks (TIA).*

Biomarkers	TACI	POCI	PACI	LACI	TIA	P
S100β (μg/L) IQR	0.563 From 0.220 to 1.483	0.061 From 0.045 to 0.077	0.086 From 0.057 to 0.169	0.070 From 0.053 to 0.298	0.106 From 0.054 to 0.166	0.0493

clinical severity of stroke is of peculiar pathophysiological interest as it links two distinct aspects of neurological damage: the functional and the biochemical side. This correlation might contribute to better understand the mechanisms of neural damage and the factors that contribute to determine its clinical outcome. So their alterations can become indicators of acute damage and contribute to better, more promptly, respond to a variation of neurological symptom. The availability of a biomarker panel of neural damage, independent from physical examination, may contribute to the assessment of stroke and its evolution in cases where symptoms may be unclear or masked by confounding factors, such as preexisting neurological pathologies, contributing to decision making by adding an element for the clinicians' evaluation.

In the present study we evaluated a panel of four biomarkers: D-dimer, NT pro-BNP, S100β and MMP-9.

All the studied biomarkers showed a direct correlation between their levels at 24 hours and the clinical severity of stroke, measured by NIHSS, while only NT pro-BNP and D-dimer were significantly correlated to severity in the admission samples. This finding may give a challenging insight into neural damage pathophysiology and how it affects patients' degree of disability. D-dimer is the less specific marker, as its elevation reflects the activation of the coagulation cascade during cerebral infarcts, while NT pro-BNP lies in the intersection between the central nervous system and the cardiovascular system regulating volemia and consequently tissue perfusion deeply affected by stroke: BNP has already been involved in stroke severity assessment.[26] S100β is released from astrocytes, a cellular population that is vital to neuronal trophic support being involved into most repair and apoptosis processes. MMP-9 is less neuron specific as it is a marker of inflammation, present as active and inactive form, the former being more abundant within the blood-brain barrier indicating, at high concentration, damage at this level. Koh S *et al.*[27] reported a similar behavior in MMP-9 levels in lacunar stokes.

As highlighted by the ordinal regression model S100β and MMP-9 showed a proportional correlation with the severity of strokes so, with further validation and more accurate calibration, their concentration or more likely their trend could be used as estimates of clinical manifestations of cerebral infarctions when neurological examination is not possible, such as during a sedation whose suspension is not recommended. These data are in contrast with what Worthmann H *et al.*[28] reported regarding the correlation between MMP-9 and stroke severity: the gelatinase

peripheral levels, however, rise during inflammation and damage of the blood-brain barrier,[29] events that accompany neural damage. Further research, on a larger cohort of patients should be performed to better understand the role of this biomarkers during stroke.

These biomarkers could potentially be integrated into a severity score that combines both clinical and laboratory data to produce a more accurate diagnostic/prognostic tool in order to improve not only patients' evaluation in the acute setting but also the monitoring of neural damage evolution in the subsequent days.

The only biomarker that correlated with infarct extension was S100β at 24 hours: the higher concentration was reached in the TACI class but, interestingly, in TIAs its concentrations were similar to those observed in true cerebral infarcts. This result could indicate that stroke and cerebral ischemia are, from a biomarker perspective, two clinically distinct entities that lay on a *continuum* of neuronal damage, that several factors, such as location and duration, contribute to determine.

One of the main limitations to this study was the relative small number of patients enrolled in a single center, that may increase the risk of selection bias due to a specific case mix admitted to our Department of Neurology, which also reflects the net prevalence of minor and moderate strokes on the whole cohort. No data was available on the time of onset of symptoms, which may bias results in cases of considerable delay between stroke and hospitalization. There was, moreover, a considerable degree of overlap between the levels of biomarkers and NIHSS score, which may reduce the clinical usefulness of the single biomarker sample and call for the analysis only as a panel. The presence of atrial fibrillation was associated to higher levels of NT-proBNP, which may bias the diagnostic accuracy of this test, however, the presence of this cardiac arrhythmia is a risk factor for cardio-embolic stroke. Further confounding factors which may have influenced the levels of MMP-9 and D-dimer, such as systemic inflammatory state or infection were not recorded. NIHSS at discharge and follow-up information were not included into the data collection.

Conclusions

When compared with basic medical information, MMP-9, S100β, NT pro-BNP and D-dimer showed a good correlation with the clinical severity stroke at 24 hours. This ability to estimate severity through the peripheral levels of a panel of biomarkers offers an interesting insight into the pathophysiology of neural damage and may become an additional resource in the patient evaluation and potentially follow-up.

Key messages

— MMP-9, S100β, NT pro-BNP and D-dimer have a good diagnostic accuracy in the diagnosis of acute ischemic stroke.

— A biomarker-based approach in ischemic stroke can contribute to early evaluation of symptom severity in the acute setting integrating the results of physical examination and imaging techniques.

— MMP-9, S100β, NT pro-BNP and D-dimer showed a good correlation with the clinical severity stroke at 24 hours which may become an additional resource in the patient evaluation.

— These biomarkers could potentially be integrated into a severity score that combines both clinical and laboratory data to produce a more accurate diagnostic/prognostic tool in order to improve not only patients' evaluation in the acute setting but also the monitoring of neural damage evolution in the subsequent days.

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