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Department of Biomedical, Metabolic
and Neural Sciences

From biomarkers of neural damage to neuro-protection

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Introduction

Brain is a complex entity, that ultimately defines and characterizes an individual. The importance and fascination of this organ lies in its volatility – neurons have a limited possibility to regenerate and repair large losses of tissue.

The brain receives about 20% of the cardiac output so it is almost completely dependent from a constant fresh supply of oxygen and nutrients. The survival time is, in fact, short: resuscitation guidelines report that irreversible lesions can appear after just 8 minutes of cardiac arrest.

During the Doctorate in Clinical and Experimental Medicine I focused my research on understanding and identifying neural damage to conclude with the study of potential protective treatments.

My first paper analyzed the evolution of neural damage after stroke and its correlation with clinical severity. I measured a panel of bio-markers and followed its variation over the first 24 hours of hospital stay.

I then tested the same panel on a cohort of patients undergoing carotid surgery to identify the technique than offered the best profile in terms of neural protection.

Finally, I studied potential neuroprotective strategies and in particular hypothermia for comatose patients after cardiac arrest.

Abstracts

Article 1

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.

Article 2

BACKGROUND: Carotid endarterectomy (CEA) is the gold standard for treating severe carotid artery stenosis, whereas carotid artery stenting (CAS) represents an endovascular alternative. The objective of this study was to assess the potential neural damage following open or endovascular carotid surgery measured by peripheral blood concentration of 3 biomarkers: S100β, matrix metalloproteinase-9 (MMP-9), and d-dimer.

METHODS: Data for this prospective investigation were obtained from the Carotid Markers study (January 2010-2011), which sought to measure the levels of specific biomarkers of neuronal damage and thrombosis on candidates to CEA or CAS presenting at the Department of Vascular Surgery of the Nuovo Ospedale

S. Agostino Estense of Modena (Italy) at baseline and at 24 hr after surgery. Relevant medical comorbidities were noted.

RESULTS: A total of 113 consecutive patients were enrolled in the study, 41 in the endarterectomy group and 72 in the endovascular group. The baseline levels of the studied biomarkers did not show any statistically significant difference between the groups with the exception of MMP-9, which showed higher concentrations in the endovascular group (median 731 vs. 401, P = 0.0007), while 24 hr after surgery the endarterectomy group featured significantly higher peripheral blood concentrations of MMP-9, S100β, and d-dimer. Conversely, no significant difference was detected in the endovascular group except the d-dimer level.

CONCLUSIONS: Neural damage biomarkers demonstrated a substantial difference between open and endovascular carotid surgery, which, if performed in selected patients, may become a less invasive alternative to CEA.

Study

BACKGROUND: Out-of-hospital cardiac arrest is a leading cause of permanent disability and death in high-income countries. Therapeutic hypothermia is recommended by international guidelines as a neuro-protective strategy in the

management of acute trauma; however, there is currently no consensus on the target temperature that is most effective in optimizing clinical outcomes. There is, however, strong evidence that supports its adoption after cardiac arrest as it has demonstrated to improve survival and neurological outcome. Consequently, the objective of the current investigation was to measure the effect of therapeutic hypothermia of after cardiac arrest in comatose patients on neurological outcome and survival.

METHODS: all the patients, that underwent therapeutic hypothermia after cardiac arrest presenting at Nuovo Ospedale S. Agostino Estense (NOCSAE, Modena) Intensive Care Unit over a 24-month period (01/01/2013 – 31/12/2014), were analyzed for this retrospective observational trial. Exclusion criteria were failure to complete at least 6 consecutive hours of therapeutic hypothermia. Demographic a clinical data of each participant were obtained from the patient records during ICU stay. Body temperature monitoring data were recorded. A one year follow-up was established for each participant.

RESULTS: A total of 39 comatose patients, who received therapeutic hypothermia after cardiac arrest, were enrolled in the study, 2 were excluded. The average age of the cohort was 66.7±2.4 years and 30-day mortality was 54.0%, while one-year mortality reached 78.4%. Patients were divided into two groups according to body temperature during the first 6 hours (A<36.0°C, B≥36.0°C). The median GCS at

admission did not show any statistically significant differences between Group A and B (3.5 and 3.4 respectively). However, reaching a body temperature lower than 36.0°C within the first 6 hours of therapeutic hypothermia was correlated to a better neurological outcome measured by GCS during follow-up. There was a statistically significant difference between GCS of group A and B at follow-up: 9.9 vs. 5.7 p-value 0.0192. The Cox regression model showed a protective effect of early hypothermia on survival (Hazard Ratio -0.39, Standard Error -0.16, 95% Confidence Interval from -0.17 to -0.89, p-value 0.026)

DISCUSSION: This study showed a substantial improvement of GCS and mortality in a cohort of patients admitted to ICU after resuscitation due to the early application of mild hypothermia, which stresses the importance of temperature control in critically ill patients with an acute brain damage as a consequence of cardiac arrest.

Article 1

CLINICAL SEVERITY OF ISCHEMIC STROKE AND NEURAL DAMAGE BIOMARKERS IN THE ACUTE SETTING: THE STROke-MArkers (STROMA) STUDY

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This research was carried out at the Department of Neurology of the Ramazzini Hosptial in Carpi (Modena, Italy).

BACKGROUND

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 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, 0,005-39 µg/L and 5-35000 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 1; only one 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.

Demographics

	Minor (n=29)	Moderate (n=24)	Moderate/Severe & Severe (n=5)
NIHSS	3 (2-3)	6.5 (5-9.5)	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%

Table 1 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. Abbreviations: AF – atrial fibrillation; TIA – transitory ischemic attack.

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 2: there were no statistically significant differences between the levels of the biomarkers at admission and after 24 hours with the exception of S100β.

Biomarker levels

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

Table 2 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

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.

Figure 1 Correlation between NIHSS and biomarker levels

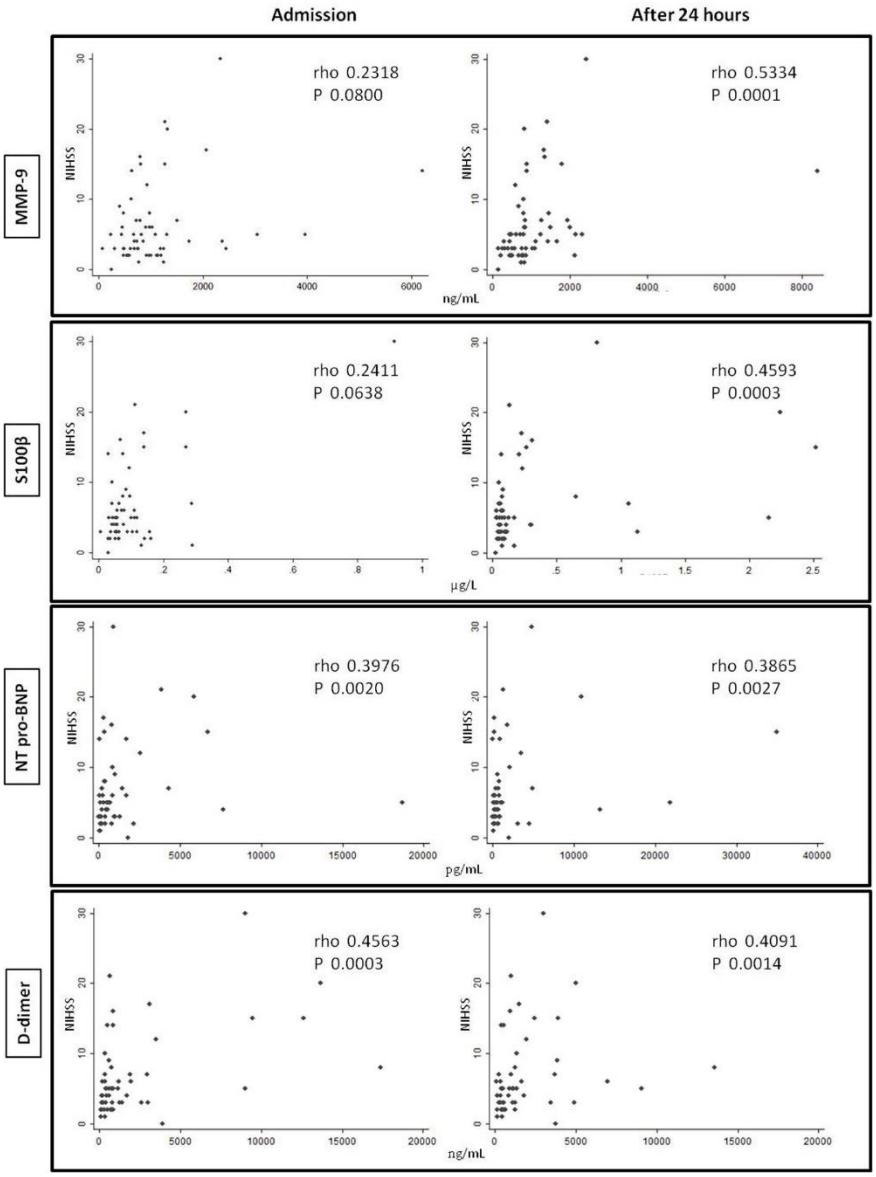

Figure 1 Correlation between baseline National Institute of Health Stroke Scale (NIHSS) value and the serum/plasma concentrations of MMP-9, S100β, NT pro-BNP and D-dimer at admission to the Department of Neurology and after 24 hours. Data have been analyzed by Spearman's rank correlation test.

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 3.

Multivariate ordinal regression model for stroke severity and biomarkers at 24 hours

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

Table 3 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. Abbreviations: Coef.- Coefficient , SE - standard error, CI - confidence interval.

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 (tab. 4).

S100β levels after 24 hours and the extension of stroke

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

Table 4 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).

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 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. and colleagues [27] reported a similar behavior in MMP9 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 and colleagues [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-pro-BNP, 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.

REFERENCES

1. Sarti C, Rastenyte D, Cepaitis Z, et al. International trends in mortality from stroke, 1968 to 1994. *Stroke* 2000; 31: 1588-1601.

2. Feigin VL, Lawes CM, Bennett DA, et al. Stroke epidemiology: a review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. *Lancet Neurol* 2003; 2: 43-53.

3. Centre for Disease Control and Prevention (CDC). Prevalence of Stroke – United States, 2006-2010. *Morb Mortal Wkly Rep* 2012; 61: 379-382.

4. Wang DZ, Rose JA, Honings DS, et al. Treating acute stroke patients with intravenous tPA. The OSF Stroke Network experience. *Stroke* 2000; 31: 77-81.

5. Barber PA, Zhang J, Demchuk AM, et al. Why are stroke patients excluded from tPA therapy? An analysis of patient eligibility. *Neurology* 2001; 56: 1015-1020.

6. Adams HP Jr, del Zoppo G, Alberts MJ, et al. Guidelines for the early management of adults with ischemic stroke. *Stroke* 2007; 38: 1655-1711.

7. Fiebach JB, Schellinger PD, Jansen O, et al. CT and diffusion-weighted MR imaging in randomized order: diffusion-weighted imaging results in higher

accuracy and lower interrater variability in the diagnosis of hyperacute ischemic stroke. *Stroke* 2002; 33: 2206-2210.

8. Amar AP. Brain and vascular imaging of acute stroke. *World Neurosurg* 2011; 76: S3-8.

9. Yiadom MY. Acute coronary syndrome clinical presentation and diagnostic approaches in the emergency department. *Emerg Med Clin North Am* 2011; 29: 689-697.

10. Whiteley W, Tseng MC, Sandercock P. Blood biomarkers in the diagnosis of ischemic stroke a systematic review. *Stroke* 2008; 39: 2902-2909.

11. Laskowitz DT, Kasner SE, Saver J, et al. Clinical usefulness of a biomarker-based diagnostic test for acute stroke: The Biomarker Rapid Assessment in Ischemic Injury (BRAIN) Study. *Stroke* 2009; 40: 77-85.

12. Elting JW, de Jager AE, Teelken AW, et al. Comparison of serum S-100 protein levels following stroke and traumatic brain injury. *J Neurol Sci* 2000; 181: 104-110.

13. Heizmann CW, Fritz G, Schäfer BW. S100 proteins: structure, functions and pathology. *Front Biosci* 2002; 7: d1356-1368.

14. Yong VW. Metalloproteinases: mediators of pathology and regeneration in the CNS. *Nat Rev Neurosci* 2005; 6: 931-944.

15. Vukasovic I, Tesija-Kuna A, Topic E, et al. Matrix metalloproteinases and their inhibitors in different acute stroke subtypes. *Clin Chem Lab Med* 2006; 44: 428-434.

16. Powner DJ, Hergenroeder GW, Awili M, et al. Hyponatremia and comparison of NT-pro-BNP concentrations in blood samples from jugular bulb and arterial sites after traumatic brain injury in adults: a pilot study. *Neurocrit Care* 2007; 7: 119-123.

17. Kirchhoff C, Stegmaier J, Bogner V, et al. Intrathecal and systemic concentration of NT-proBNP in patients with severe traumatic brain injury. *J Neurotrauma* 2006; 23: 943-946.

18. Whiteley W, Wardlaw J, Dennis M, et al. Blood Biomarkers for the Diagnosis of Acute Cerebrovascular Diseases: A Prospective Cohort Study. *Cerebrovasc Dis* 2011; 32: 141-147.

19. Skoloudík D, Bar M, Sanák D, et al. D-dimers increase in acute ischemic stroke patients with the large artery occlusion, but do not depend on the time of artery recanalization. *J Thromb Thrombolysis* 2010; 29: 477-482.

20. Haapaniemi E, Tatlisumak T. Is D-dimer helpful in evaluating stroke patients? A systematic review. *Acta Neurol Scand* 2009; 119: 141-150.

21. Bamford J, Sandercock P, DennisM, et al. Classification and natural history of clinical subtypes of cerebral infarction. *Lancet* 1991; 337: 1521–1526.

22. Brott T, Adams HP Jr, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 1989; 20: 864-870.

23. Sibon I, Rouanet F, Meissner W, et al. Use of the Triage Stroke Panel in a neurologic emergency service. *Am J Emerg Med* 2009; 27: 558-562.

24. Vanni S, Polidori G, Pepe G, et al. Use of biomarkers in triage of patients with suspected stroke. *J Emerg Med* 2011; 40: 499-505.

25. Worthmann H, Tryc AB, Goldbecker A, et al. The temporal profile of inflammatory markers and mediators in blood after acute ischemic stroke differs depending on stroke outcome. *Cerebrovasc Dis* 2010; 30: 85-92.

26. Montaner J, García-Berrocoso T, Mendioroz M, et al. Brain Natriuretic Peptide Is Associated with Worsening and Mortality in Acute Stroke Patients but Adds No Prognostic Value to Clinical Predictors of Outcome. *Cerebrovasc Dis* 2012; 34: 240-245.

27. Koh SH, Park CY, Kim MK, et al. Microbleeds and free active MMP-9 are independent risk factors for neurological deterioration in acute lacunar stroke. *Eur J Neurol* 2011; 18: 158–164.

28. Worthmann H, Tryc AB, Goldbecker A, et al. The temporal profile of inflammatory markers and mediators in blood after acute ischemic stroke differs depending on stroke outcome. *Cerebrovasc Dis* 2010; 30: 85-92.

29. Cata JP, Abdelmalak B, Farag E. Neurological biomarkers in the perioperative period. *Br J Anaesth*. 2011;107: 844-858.

Article 2

NEURAL DAMAGE BIOMARKERS DURING CAROTID SURGERY

OPEN VS. ENDOVASCULAR APPROACH

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INTRODUCTION

Stroke is one of the leading causes of death and permanent disability in high-income countries[1]. Significant carotid artery stenosis may be a predisposing factor in stroke, so the surgical treatment of this condition can reduce the risk of recurrent stroke in patients with severe carotid stenosis[2].

Carotid endarterectomy (CEA) is the gold standard for treating severe carotid artery stenosis, whereas carotid artery stenting (CAS) represent its endovascular alternative[3, 4]. Open surgery has demonstrated, on one hand, a lower periprocedural risk of death and stroke, with, on the other hand, a higher risk of acute myocardial infarction (AMI) and cranial nerve injury, making PTA and CAS second line treatment options for carotid artery stenosis. However no long-term differences concerning the outcome of stroke or death were demonstrated in the meta-analysis by Meier and colleagues[5].

This meta-analysis and several trials, CREST[6, 7], CAVATAS[8] and SPACE[9], have shifted the balance in favor of CEA[10], due to the lower incidence of cerebrovascular complications[11], reserving endovascular techniques to selected cases.

The diagnosis of periprocedural stroke relies on clinical parameters and neuroimaging techniques[12], as with all other forms of acute stroke; similarly to

what has been done for early triage and evaluation of cardiac symptoms compatible with AMI, various panels of biomarkers of neural damage[13-15] have been developed and validated in recent years; the necessity of adopting a multiple markers approach is based on the absence of a single molecule capable of identifying brain damage in all its forms. S100β[16] a calcium binding protein is released from astrocytes, a cellular population that is vital to neuronal trophic support being involved into most repair and apoptosis processes while matrix metalloproteinase-9 (MMP-9)[17, 18], a gelatinase, 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. D-dimer[19] is the end-product of fibrinolytic process. These three markers are part of the triage stroke panel[13], the brain natriuretic peptide, present in the original version, was excluded from the perioperative assays as it could be biased by surgery related variables not correlated to neural damage, such as fluid therapy and cardiac function.

A biomarker-based approach can be proposed to asses potential periprocedural neurological damage whose effect are still present at 24 hours; this could help to identify more subtle variations and uncover sub-clinical, active injuries that might contribute to the development of long-term effects. In fact, an acute elevation of these biomarkers could result solely from minor surgical alterations to cerebral

perfusion and inflammation while a lasting elevation at 24 hours may be correlated to a more substantial lesion.

The main objective of this investigation was to assess the potential neural damage following open vs. endovascular carotid surgery measured by peripheral blood concentration of three biomarkers (S100β, MMP-9, D-dimer) at baseline and 24 hours after surgery.

METHODS

Data for this prospective pilot investigation came from the Carotid Markers (CARMA) study (January 2010-January 2011), which sought to measure the levels of specific biomarkers of neuronal damage and thrombosis on candidates to CEA or CAS, presenting at the Department of Vascular Surgery of the Nuovo Ospedale S.Agostino Estense (Baggiovara, Modena, Italy) at baseline and at 24 hours after surgery. Approval from Institutional Ethics Committee of Modena was obtained prior to study initiation. Patients were included in the analysis if CEA or CAS were performed according to the below described techniques. Written formal consent was obtained from the study participants or legal designate. Demographic, clinical, laboratory and radiographic data were collected by a standardized protocol. The American Society of Anesthesiologists (ASA) physical status

classification system was adopted as a measure of the overall physical health of the patient before surgery.

Patient initial assessment was performed by echo-color-duplex scan and angio-computed tomography (CT) of the neck and brain, in order to define the anatomical characteristics of the aortic arch, supraaortic vessels and intracranial circulation. Asymptomatic patients with stenosis >80% and/or ulcerated lesions >50% and symptomatic patients with stenosis >60% and/or ulcerated lesions >50% were considered for treatment. Patients over 65 years of age were primarily considered for CAS at this center when favorable anatomies were documented at angio-CT studies. Suitable anatomic criteria for CAS included bovine and normal arches (type I, II and III) and adequate femoral artery access. Endovascular treatment was withhold in cases of stenosis of the brachiocephalic artery or at the origin of the left common carotid artery (CCA) and calcification of the aortic arch. Patients were considered for CAS when unsuitable for traditional surgery because of clinically significant cardiac disease, severe pulmonary disease, contralateral laryngeal nerve palsy, restenosis >80% after CEA, previous neck radiation exposure or radical neck surgery, and high carotid bifurcation or intracranial extension of a carotid lesion. Finally, CAS was performed in patients at high risk of cerebral ischaemia during carotid clamping (i.e. occlusion of the contralateral internal carotid artery and anomalies of the circle of Willis).There is no maximum

age threshold at this center, but patients aged under 65 years, who were suitable for surgery, were preferably treated with CEA.

All new neurological deficits, defined as previously not documented focal or general neurological signs or symptoms presented by the patient during and after surgery were recorded by a consultant neurologist.

SURGICAL TECHNIQUES

CEA

The eversion CEA technique was performed through an oblique transection of the internal carotid artery (ICA) from the CCA, endarterectomy by eversion of the ICA, endarterectomy of the carotid bifurcation and of the external carotid artery, and re-implantation of the ICA on the CCA. Conventional CEA was performed through a longitudinal arteriotomy from the CCA bifurcation to the ICA on the anterior surface of the artery. Endarterectomy was carried out after careful identification of the cleavage plane. Arteriotomy was routinely closed with a prosthetic patch (Finesse Fine, Maquet, NJ).

The procedure was performed during conscious sedation: after the cannulation of a peripheral vein with a large bore catheter and radial artery with a 20G catheter for invasive monitoring of blood pressure, general anaesthesia was induced by a standard protocol, the endotracheal tube was positioned and mechanical

ventilation initiated. Under remifentanil continuous infusion at a rate of 0.1 µg/kg/min the patient regained consciousness with the ability to tolerate orotracheal intubation and mechanically assisted ventilation. Cerebral blood flow adequacy, after carotid clamping, was measured by monitoring the ability of the patient to execute simple orders (squeeze test) issued by the Anaesthesiologist at regular intervals, of at least five minutes or more frequently if necessary, with the hemisoma contralateral to the surgical site: inability to perform this task, in the absence of other possible causes such as systemic hypotension, was the indication for carotid shunt placement.

CAS

An access was obtained by percutaneous puncture of the common femoral artery under local anaesthesia with mepivacaine 5 mg/kg. The Piton GC® carotid guide catheter (Medtronic Invatec, Frauenfeld, Switzerland) and Mo.Ma®[20, 21] a proximal cerebral embolic protection device (Medtronic Invatec) were used in all cases. Pre-dilation was selectively performed with a non compliant coronary balloon (2.5-3.5 mm in diameter) in case of pre-occlusive calcified stenosis (a stenosis of at least 90%) which impeded stent deployment. Self-expanding nitinol stents were used in the study. The diameter of the stent was chosen according to a 1-2 mm oversizing with a length of 30 or 40 mm. In the case of lesions longer than 40 mm, two stents were inserted, with an overlapping of 2-5 mm. Double stenting

was employed for longer lesions and not for stent design reinforcement (in 1 case double stenting was employed to avoid plaque prolapse). Stents used in this study include X-act® (Abbott Vascular, Redwood City, CA,USA), Vivexx® (CR Bard; Murray Hill, New Jersey, USA), Vascuflex® (B.Braun Medical,Boulogne Cedex, France), Cristallo Ideale Carotid Stent System® (Medtronic Invatec). Post-dilation was performed with a 5x20 mm non compliant balloon (range 4-5.5 mm) at 8 atm, with inflation and deflation performed slowly (1 atm/2 sec). After the final aspiration, when there were no signs of clamping intolerance, endovascular flushing to the external carotid artery (ECA) was performed. Prior to the removal of the protection device, the post-dilation balloon was re-introduced into the ICA and re-inflating at a low pressure (4 atm) to further remodel the debris or detached protruding plaque. The balloons in the ECA and CCA were then deflated, allowing passage of the reinstated hematic current into the ECA for 5-10 seconds (cerebral flow is blocked by the inflated post-dilation balloon). Then balloons were re-inflated and the post-dilation balloon was deflated and removed. A second aspiration was performed, checking for the absence of debris prior to reinstating blood flow and removing the device. This procedure was intended to mobilize the unstable protruding plaque whilst the cerebral flow was still blocked and redirect it into the ECA, achieving a spreading effect of any protruding plaque. An intra- and extra-cranial angiography post intervention were performed to assess stent

patency and eventual residual stenosis (≤ 20% is accepted) and to visualize and assess any potential intra-cranial embolization.

The choice of the stent used was based on both anatomical and plaque-related criteria: closed cells were preferred in linear vessels with soft plaques, open cells in cases of tortuous anatomies with calcified plaques while hybrid cells were used in soft plaques.

POSTOPERATIVE MEDICAL THERAPY

All candidates to CAS received aspirin 100mg and clopidogrel 75mg daily for the three days preceding the operation. During both procedures a standard dose of heparin was administered and from the first postoperative day an antiplatelet regimen with aspirin 100mg, associated for 30 days to clopidogrel in case of CAS. Statins were added as plaque stabilizers, when not absolutely contraindicated.

IMMUNOASSAYS

Blood samples were obtained at admission to the Department of Vascular Surgery and after 24 hours by either venous puncture or a catheter placed in the radial artery for the invasive monitoring of blood pressure. Plasma samples were collected into EDTA- tubes for D-dimer; 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 was 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 and S100β was 150-9000 ng/mL and 0.005-39 µg/L respectively.

FOLLOW-UP

All included patients were scheduled for follow-up one week after the procedure, one and six months afterwards: on these occasions a carotid ultrasound scan was acquired by a vascular surgeon, who also evaluated potential surgical complications, while a neurologist performed a detailed neurological examination to detect potential lesions correlated to surgery and follow their evolution.

STATISTICAL ANALYSIS

Statistical analysis was performed using Stata 10.0 (StataCorp, Texas, USA). Descriptive statistics, including median and interquartile range 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.

RESULTS

A total of 113 consecutive patients were enrolled in the CARMA study, 41 in the endarterectomy group and 72 in the endovascular group. Figure 1 reports the enrollment flowchart: 82.9% of the subjects in the endarterectomy group were treated with patch angioplasty, 17.1% with eversion.

Enrollment flowchart

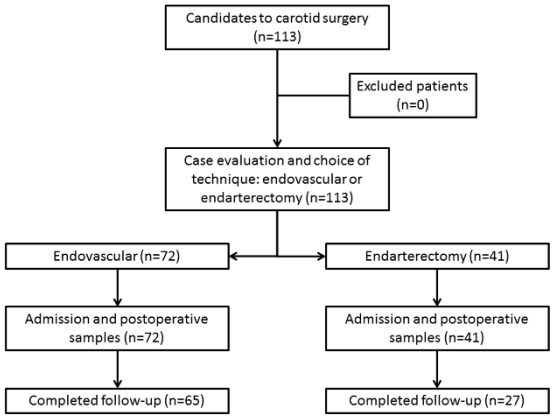

Figure 1 Enrollment flow-chart

The demographics for the study population did not show statistically significant differences between groups as regards to sex, vascular risk factors and relevant past medical history, with the exception of age and peripheral artery disease (PAD) (Table 1); the median degree of preoperative carotid stenosis, measured by echo-color-duplex scan as percentage of lumen reduction in the vessel to be operated, was 75% in both groups (p-value 0.1961). No deaths were reported during hospital stay, two patients died during the follow-up period (1.6%): one for the complications of a stroke at day 24 in the endovascular group and one after a cerebral hemorrhage at day 150 in the endarterectomy group.

Demographics

	Endarterectomy (n=41)	Endovascular (n=72)	*P*
Age (years)	71.0±1.3	75.5±0.9	0.0047
Sex (males)	70.7%	59.7%	0.242
Tobacco smoke	24.4%	18.1%	0.421
Dyslipidemia	56.1%	43.1%	0.182
Diabetes	17.1%	27.8%	0.199
Hypertension	70.7%	81.9%	0.167
AMI	14.6%	25.0%	0.195
TIA	19.5%	16.7%	0.703
Stroke	34.1%	20.8%	0.119
PAD	14.6%	36.1%	0.015

Table 1 Patient demographics for Endarterectomy and Endovascular groups. Age is expressed as mean±SD; for the categorical variables, percentages are given as a proportion of the patients that had the characteristics. Abbreviations: AMI – acute myocardial infarction, TIA – transitory ischemic attack, PAD – peripheral arterial disease.

The mean duration of the perioperative period of the endarterectomy group was 95.8±9.0 minutes while for CAS it lasted on average 52.7±17.6 minutes (p-value <0.0001). There were 1 ASA 1, 25 ASA 2 and 15 ASA 3 patients in the

endarterectomy group compared to 25 ASA 2, 45 ASA 3, 2 ASA 4 patients in the endovascular group; the anesthesiologic and surgical characteristic of the studied cohort presented statistically significant differences of ASA classification and duration of carotid flow arrest while the proportion of episodes of new neurological deficits during surgery measured as inability to perform squeeze test did not reach the level of significance (Table 2). One stroke was reported during postoperative period in each group with documented ischemic lesions at brain imaging and permanent sensory and motor deficit at one hemisoma.

Surgical Characteristics

		Endarterectomy (n=41)	Endovascular (n=72)	*P*
ASA	Median	2	3	0.0038
	Range	2 – 3	2 – 3	
Clamp	Median	41.5	7.5	<0.0001
(minutes)	Range	29.5 – 50	5 – 10	
Squeeze test failure (%)		21.9%	12.5%	0.158

Table 2 Surgical characteristics of the studied cohort: ASA and clamp are expressed as median and interquartile range; for the categorical variables, percentages are given as a proportion of the patients that had the characteristics.

There were 1 ASA 1, 25 ASA 2 and 15 ASA 3 patients in the endarterectomy group compared to 25 ASA 2, 45 ASA 3, 2 ASA 4 patients in the endovascular group. In 7 out of 9 patients that failed to perform the squeeze in the endarterectomy group was used a shunt to partially restore carotid blood flow. The differences of the distributions of the continuous variables were assessed by the Wilcoxon's rank-sum test while for the categorical variables χ^2 test was used. Abbreviations: ASA – American Society of Anesthesiologist physical status classification assessment, clamp – duration of carotid flow arrest, Squeeze test failure – percentage of patients that failed the squeeze test.

Table 3 summarizes the complications related to surgery identified during the follow-up, no statistically significant differences of prevalence were present between the two groups.

Follow-up

Groups	Follow-up One week (number of cases)	One month (number of cases)	Six months (number of cases)
Endovascluar (n=72)	Reduction of muscle power of the controlateral upper limb (1) Bleeding with hematoma of puncture site (1)	Death due to ischemic stroke (1)	Death due to sepsis (1)
Endarterectomy (n=41)	Hypoglossal nerve involvement (2) Upper limb palsy with thrombosis of the operated vessel (1) *	Ischemic stroke (1) *	Death due to cerebral hemorrhage (1)

* Same patient

Table 3. Main complications reported during the follow-up period for each group, no statistically significant difference in the prevalence of complications was present between groups.

The baseline levels of the studied biomarkers did not show any statistically significant difference between groups with the exception of MMP9, that showed higher concentrations in the endovascular group (median 731 vs. 401, p-value 0.0007), while 24 hours after surgery the endarterectomy group featured significantly higher peripheral blood concentrations of MMP-9, S100β and D-

dimer. Conversely, no significant difference was detected in the endovascular group except the D-dimer level (Figure 2).

Figure 2 Biomarkers in the perioperative phase

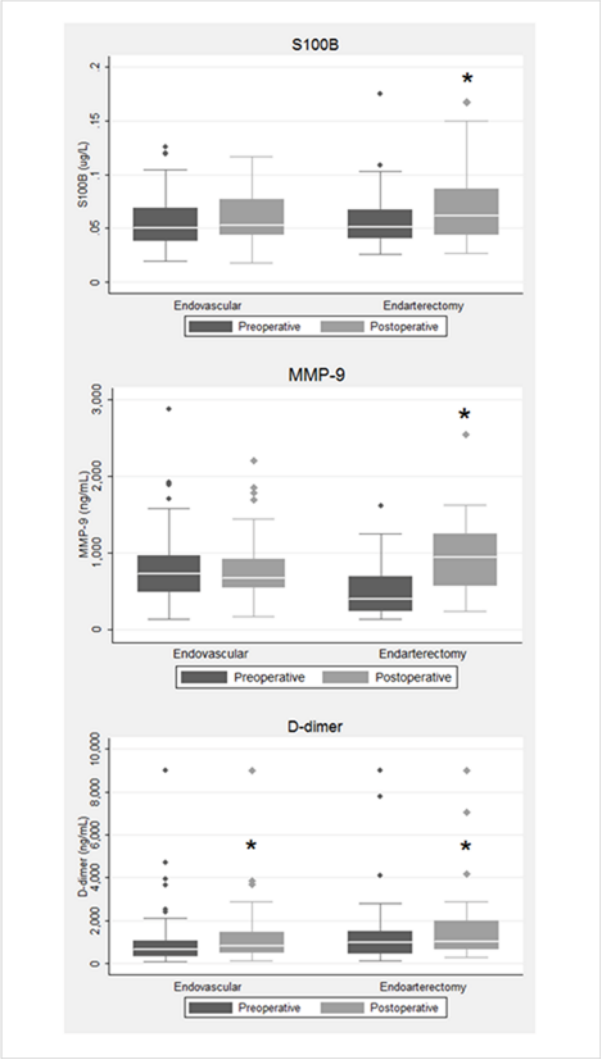

Figure 2 Panel of biomarkers: data regarding S100β protein, matrix metalloproteinase-9 (MMP-9), D-dimer peripheral blood levels, at baseline

(preoperative) and 24 hours after surgery (postoperative), are reported as median with interquartile range for the endovascular and endarterectomy groups.

A Wilcoxon rank sum test was performed to assess the differences of concentration of the studied biomarkers in the individuals who failed the squeeze test within each group: there was a statistically significant difference between D-dimer postoperative levels in the endarterectomy group (median in failure subgroup 1886 ng/mL vs. 818 ng/mL, p-value 0.0304). Similarly a statistically significant difference was shown between postoperative S100β levels in those subjects where shunt was used during endarterectomy (0.083 µg/mL in the shunt subgroup vs. 0.055 µg/mL, p-value 0.0066).

The Spearman's rank correlation test was used to assess the relationship between the level of the studied biomarkers at 24 hours and the duration of carotid flow arrest: no statistically significant correlations were observed between the concentrations of the three biomarkers included in this panel at 24 hours and the duration of carotid flow arrest.

DISCUSSION

Carotid surgery is one of the main vascular interventions. CEA is considered the gold standard technique for symptomatic stenosis while CAS is indicated only for selected cases as its generalized application is controversial[3,4]. In fact, randomized trials have demonstrated an excess in complications for the endovascular treatment when compared to open surgery[10, 11]. However the former is characterized by shorter duration of the procedure and the potential for reduced invasivity. Factors that may influence the outcome of CAS are the experience of the operator, anatomical and clinical characteristics of the patient, that make them suitable for an endovascular approach: randomization in fact could lead to attempting an stent placement in a subject with unfavorable anatomical characteristics that would probably lead to complications both during the procedure and in the postoperative period. CAS still remains a technique for selected cases[22] but a careful preoperative assessment can extend its application to larger portion of patients, who may benefit from the reduced invasivity and duration of an endovascular approach when compared to open surgery.

The rate of reported complications seems comparable between groups, especially when considering the endovascular procedures[11].

Contrary to what are the actual indications to CAS, the present endovascular group was characterized by older age and an higher prevalence of PAD, as a less invasive approach was preferred in higher comorbidity patients with suitable anatomy, as reflected also by the ASA physical status classification assessment. The shorter duration of the procedure, the feasibility with local anaesthesia, so without the need for mechanical ventilation, were considered beneficial factors in the perioperative management of these individuals, as an attempt to reduce the impact on the homeostasis of the elderly vascular patient. In fact, in these subjects complications can be related to factors not directly linked to the surgical procedure.

The studied biomarkers showed a significant increase from baseline in the open surgery group, even if this was, apparently, not related to the duration of carotid flow arrest. D-dimer, that reflects the activation of the coagulation cascade, raised in both groups likely as a consequence of carotid atherosclerosis and activation of the fibrinolytic cascade[23] during and after surgery

The correlation between the serum/plasma concentrations of a panel of specific biomarkers with neural damage has already been demonstrated. Here we propose that their application to vascular surgery, especially to carotid interventions, can contribute to detect subclinical lesions that may not have direct consequences in the short term but can manifest as neurological status deterioration on a longer

term. Postoperative cognitive impairment is associated to carotid surgery due to possible emboli that may detach from the carotid plaque during the procedure and the underlying clinical condition of the patient that may predispose him to the development of cerebrovascular pathologies.

S100β peripheral levels, although characterized by a half-life of 25 minutes, have shown a good correlation with cerebral lesions 24 hours after cardiac surgery[24]; elevated levels of this protein have been described after carotid endarterectomy probably as consequence of transitory episodes of cerebral hypoperfusion[25] and carotid clamping[26]. Contrary to what reported by Brightwell and colleagues[25] our data show that the treatment modality affected S100β peripheral levels 24 hours after the operation only in CEA group. These findings were not related to the length of the carotid flow arrest, so they may be dependent from a more effective proximal cerebral protection strategy, which, however, calls for further assessment.

MMP-9, a gelatinase, peaks during CEA in correlation to vessel occlusion[27]: it is a marker of inflammation and damage of the blood-brain barrier therefore its postoperative increase could be related to microembolization and/or transient brain tissue damage.

Interestingly in this study the levels of MMP-9 were significantly higher in the CAS group. This suggests a higher ‘baseline’ inflammatory state or silent areas of damage associated with subclinical blood-brain barrier dysfunction in this group of patients. Nevertheless, the incidence of neurological deficits did not differ in the two groups both during surgery and the six-month follow-up.

The present data show that the endovascular approach determined a minor release of neural damage biomarkers than traditional open surgery for factors independent from carotid flow arrest duration but probably related to intrinsic characteristics of the procedure somehow entailing an improved cerebral protection, as shown by Montorsi and colleagues with a reduced rate of microembolization associated with the use of a proximal protection device[21]. However the statistically shorter duration of carotid flow arrest experienced during the endovascular procedures may have presumably led to a reduced neural damage, as highlighted by the biomarkers trend.

LIMITATIONS

The relatively small group of patients studied, enrolled in a single center, may limit the extent of the conclusions. Randomization was not implemented due to the substantially different surgical characteristics peculiar to patients suitable to

endovascular surgery, that would bias the outcome of the procedures. The panel of markers could have comprised other neuron specific markers and integrated their levels with imaging data to improve the diagnostic accuracy.

CONCLUSIONS

Peripheral neural damage biomarkers, an auxiliary diagnostic tool in the detection of subclinical lesions, demonstrated a substantial difference between open and endovascular carotid surgery, that, if performed in selected patients, may become a less invasive alternative to CEA. Data from this study do not support previous literature results obtained in randomized trials showing an excess in complications for the endovascular treatment when compared to open surgery. These findings would call for further investigation to better assess the relationship between open and endovascular surgery and clinical neural damage, both in the acute phase and during follow-up.

REFERENCES

1. Murray CJ, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2013; 380: 2197-2223.

2. Dickerson LM, Carek PJ, Quattlebaum RG. Prevention of recurrent ischemic stroke. *Am Fam Physician*. 2007; 76: 382-388.

3. Erickson KM, Cole DJ. Carotid artery disease: stenting vs endarterectomy. *Br J Anaesth*. 2010; 105: i34-49.

4. Skerritt MR, Block RC, Pearson TA, et al. Carotid endarterectomy and carotid artery stenting utilization trends over time. *BMC Neurol*.2012; 12: 17.

5. Meier P, Knapp G, Tamhane U, et al. Short term and intermediate term comparison of endarterectomy versus stenting for carotid artery stenosis: systematic review and meta-analysis of randomised controlled clinical trials. *BMJ*. 2010; 340: c467.

6. Mantese VA, Timaran CH, Chiu D, et al. The Carotid Revascularization Endarterectomy versus Stenting Trial (CREST): stenting versus carotid endarterectomy for carotid disease. *Stroke*. 2010; 41: S31-34.

7. Hill MD, Brooks W, Mackey A, et al. Stroke After Carotid Stenting and Endarterectomy in the Carotid Revascularization Endarterectomy Versus Stenting Trial (CREST). *Circulation.* 2012; 126: 3054-3061.

8. Bonati LH, Ederle J, McCabe DJ, et al. Long-term risk of carotid restenosis in patients randomly assigned to endovascular treatment or endarterectomy

in the Carotid and Vertebral Artery Transluminal Angioplasty Study (CAVATAS): long-term follow-up of a randomised trial. *Lancet Neurol*. 2009; 8: 908-917.

9. Eckstein HH, Ringleb P, Allenberg JR, et al. Results of the Stent-Protected Angioplasty versus Carotid Endarterectomy (SPACE) study to treat symptomatic stenoses at 2 years: a multinational, prospective, randomised trial. *Lancet Neurol*. 2008; 7: 893-902.

10. International Carotid Stenting Study investigators, Ederle J, Dobson J, et al. Carotid artery stenting compared with endarterectomy in patients with symptomatic carotid stenosis (International Carotid Stenting Study): an interim analysis of a randomised controlled trial. *Lancet.* 2010; 375: 985-997.

11. Bonati LH, Lyrer P, Ederle J, et al. Percutaneous transluminal balloon angioplasty and stenting for carotid artery stenosis. *Cochrane Database Syst Rev*. 2012; 9: CD000515.

12. Perry JM, McCabe KK. Recognition and initial management of acute ischemic stroke. *Emerg Med Clin North Am*. 2012; 30: 637-657.

13. Laskowitz DT, Kasner SE, Saver J, et al. Clinical usefulness of a biomarker-based diagnostic test for acute stroke: the Biomarker Rapid Assessment in Ischemic Injury (BRAIN) study. *Stroke.* 2009; 40: 77-85.

14. Sibon I, Rouanet F, Meissner W, et al. Use of the Triage Stroke Panel in a neurologic emergency service. *Am J Emerg Med*. 2009; 27: 558-562.

15. Ahmad O, Wardlaw J, Whiteley WN. Correlation of levels of neuronal and glial markers with radiological measures of infarct volume in ischaemic stroke: a systematic review. *Cerebrovasc Dis.* 2012; 33: 47-54.

16. Cata JP, Abdelmalak B, Farag E. Neurological biomarkers in the perioperative period. *Br J Anaesth*. 2011; 107: 844-858.

17. Ramos-Fernandez M, Bellolio MF, Stead LG. Matrix metalloproteinase-9 as a marker for acute ischemic stroke: a systematic review. *J Stroke Cerebrovasc Dis.* 2011; 20: 47-54.

18. Graham CA, Chan RW, Chan DY, et al. Matrix metalloproteinase 9 mRNA: an early prognostic marker for patients with acute stroke. *Clin Biochem.* 2012; 45: 352-355.

19. Hasan N, McColgan P, Bentley P, et al Towards the identification of blood biomarkers for acute stroke in humans: a comprehensive systematic review. *Br J Clin Pharmacol.* 2012; 74: 230-240.

20. Diederich KW, Scheinert D, Schmidt A, et al. First clinical experiences with an endovascular clamping system for neuroprotection during carotid stenting. *Eur J Vasc Endovasc Surg*. 2004; 28: 629-633.

21. Montorsi P, Caputi L, Galli S, et al. Microembolization during carotid artery stenting in patients with high-risk, lipid-rich plaque. A randomized trial of proximal versus distal cerebral protection. *J Am Coll Cardiol.* 2011; 58: 1656-1663.

22. Gahremanpour A, Perin EC, Silva G. Carotid artery stenting versus endarterectomy: a systematic review. *Tex Heart Inst J.* 2012; 39: 474-487.

23. Georgiadis D, Berger A, Kowatschev E, et al. Predictive value of S-100beta and neuron-specific enolase serum levels for adverse neurologic outcome after cardiac surgery. *J Thorac Cardiovasc Surg*. 2000; 119: 138–147.

24. Krupinski J, Catena E, Miguel M, et al. D-dimer local expression is increased in symptomatic patients undergoing carotid endarterectomy. *Int J Cardiol.* 2007; 116: 174-179.

25. Brightwell RE, Sherwood RA, Athanasiou T, et al. The neurological morbidity of carotid revascularisation: using markers of cellular brain injury to compare CEA and CAS. *Eur J Vasc Endovasc Surg*. 2007; 34: 552–560.

26. Falkensammer J, Oldenburg WA, Hendrzak AJ, et al. Evaluation of subclinical cerebral injury and neuropsychologic function in patients undergoing carotid endarterectomy. *Ann Vasc Surg*. 2008; 22: 497–504

27. Bicknell CD, Peck D, Alkhamesi NA, et al. Relationship of matrix metalloproteinases and macrophages to embolization during endoluminal carotid interventions. *J Endovasc Ther*. 2004; 11: 483–493.

Clinical Study

HYPOTHERMIA AFTER CARDIAC ARREST

TIMING AND OUTCOMES

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This research was carried out in the Intensive Care Unit of the NOCSAE Hosptial in Baggiovara (Modena, Italy).

BACKGROUND

Out-of-hospital cardiac arrest is a leading cause of permanent disability and death in high-income countries [1] and prompt cardiopulmonary resuscitation and defibrillation are the basis of its treatment [2]. Acute therapeutic hypothermia can be effective in improving survival and neurologic outcome of comatose patients after a cardiac arrest, as shown by a recent Cochrane review [3].

International guideline recommend therapeutic hypothermia as a neuro-protective strategy in the treatment of acute neurologic events; however, there is no agreement on the most effective target temperature [4, 5]. A recent large case control study [6] stressed the importance of temperature management over simple hypothermia in comatose patients after cardiac arrest: the study showed no additional benefits in terms of mortality and neurological outcome with cooling to 33°C vs 36°C [6].

The protective effect of hypothermia is the consequence of its action on multiple damage pathways [7]:

- Apoptosis – through a modulation of caspases and calcium homeostasis
- Free radical production – through the reduction of the metabolic rate and oxygen consumption; it has also a potent anti-inflammatory effect further reducing the energetic substrates expenditure and oxidative stress;

- Excitotoxicity – via the reduction of glutamate excitosis from synaptic vescicles
- Fever – by direct lowering of tissue temperature, which is an independent risk factor of poor outcome, as by raising temperature all previously described mechanisms are enhanced.

There are evidences of a beneficial effect of therapeutic hypothermia in the reduction of intra-cranial pressure, which could improve tissue perfusion and contribute to recovery, even if its overall effect on cerebral blood flow is still under debate [8].

Current cooling systems are split into two categories: intravascular and extravascular. Intravascular methods use cooling catheters or cold fluids infused directly into the blood stream [9] to reduce body temperature, while surface cooling methods use cooling pads, ice packs, or immersion into a cold medium [10]. With the exception of application of ice packs and cold fluids, other methods of cooling cannot easily be deployed in an out-of-hospital scenario. In fact, various studies have failed to identify a clear benefit of early on-site hypothermia in unconscious survivors of cardiac arrest [11, 12].

Although the effectiveness of on-site hypothermia is still under debate, the application of this treatment after cardiac arrest is supported by strong evidence as

it improves survival and neurological outcome. The objective of the current investigation was to measure the effect of therapeutic hypothermia of after cardiac arrest in comatose patients on neurological outcome and survival.

METHODS

After approval by the local Institutional Ethics Committee (Comitato Etico Provinciale di Modena) the consecutive records of all the patients, that underwent therapeutic hypothermia after cardiac arrest presenting at Nuovo Ospedale S. Agostino Estense (NOCSAE, Modena) Intensive Care Unit (ICU) over a 24-month period (01/01/2013 – 31/12/2014), were analyzed for this retrospective observational trial.

Inclusion criteria were undergoing therapeutic hypothermia after the presentation to NOCSAE ICU with diagnosis of successfully resuscitated cardiac arrest in a comatose patient.

Exclusion criteria were failure to complete at least 6 consecutive hours of therapeutic hypothermia.

Therapeutic hypothermia was defined as an actively induced body temperature under 36.0°C, independently from the technique used to achieve this state.

Demographic a clinical data of each participant were obtained from the patient records during ICU stay:

- Relevant medical conditions, habits or comorbidities: tobacco smoke, obesity, diabetes, hypertension, heart failure, atrial fibrillation, peripheral

vascular disease, acute or chronic kidney disease, chronic obstructive pulmonary disease and obstructive sleep apnea syndrome.

- Relevant medication: beta-blockers, diuretics, oral inotropes, sartans, statins, anti-diabetics (insulin or oral antidiabetic treatments), antiplatelets and anticoagulants.

Possible conditions accompanying the cardiac arrest were also investigated and divided into four categories: acute myocardial infarction (AMI), stroke, traumatic brain injury and polytrauma.

The setting of the cardiac arrest being out-of-hospital or in-hospital was noted as an indicator of the average rescue times. Moreover the presence of a witness and early cardiopulmonary resuscitation (CPR) was obtained from medical records. Presentation cardiac rhythms were divided into shockable and non-shockable.

Post-resuscitation onset of acute cardiac failure, respiratory failure and septic shock was recorded as possible negative side-effects of the acute cardiac event.

The neurological condition of each patient was assessed by Glasgow Coma Scale (GCS) at admission to the ICU, at discharge and at the end of follow-up. The latter was considered the primary outcome measure of the study. All subjects, in fact, were studied for the duration of 12 months after the acute event through the available medical records.

Core body temperature and several key vital parameters and indicators were also monitored during therapeutic hypothermia such as: saturation of peripheral oxygen (SpO_2), mean arterial pressure (MAP), blood pH, concentration of bicarbonate (HCO_3^-) and potassium (K^+), urine output. They are more likely than others affected by reduced body temperature.

Patients were finally divided into two groups: Group A that reached hypothermia within 6 hours form admission and Group B that failed to reach this goal. The effect of the rapid induction of hypothermia on neurological outcome and mortality was calculated.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) and numbers (percentages).

Skewness and kurtosis test for normality was applied to the distribution of GCS scores. The median values for each time interval were calculated for group A and B. GCS score median distributions were compared by Wilcoxon rank-sum test in each studied group.

A Cox regression model was calculated to assess the effect of the rapid induction of hypothermia on survival so a Kaplan-Meier survival curve was created.

All p values are two-tailed and a p value of <0.05 was considered significant. Statistical analysis was performed on a personal computer using STATA (10.0)© software.

RESULTS

A total of 39 comatose patients, who received therapeutic hypothermia after cardiac arrest, were enrolled in the study, 2 were excluded as death occurred within 10 hours from ICU admission. Figure 1 reports the enrollment flow-chart.

Figure 1 Enrollment flowchart

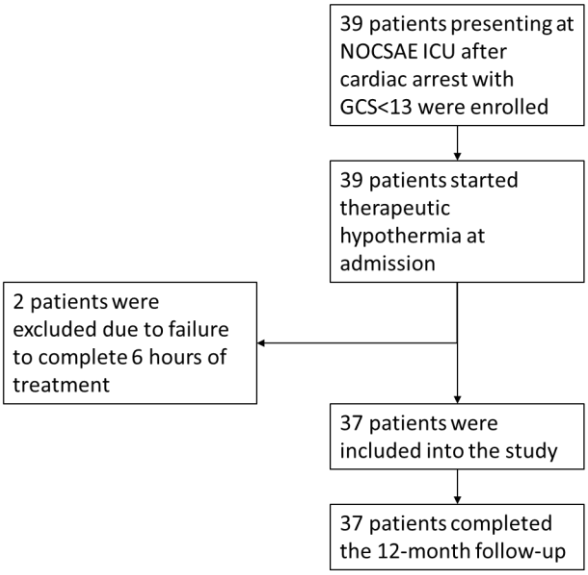

Figure 1 Enrollment Flowchart: 39 comatose patients presenting at Nuovo Ospedale S. Agostino Estense (NOCSAE) Intensive Care Unit (ICU) successfully resuscitated after cardiac arrest were included. The comatose condition was defined as a Glasgow Coma Scale (GCS) score <13/15. A total of 2 patients were excluded as they failed to undergo 6 hour of hypothermia due to death, 37 were included and completed the 12-month follow-up period.

The average age of the cohort was 66.7±2.4 years and 30-day mortality was 54.0%, while one-year mortality reached 78.4%.

The medical history for relevant comorbidities, such as tobacco smoke, obesity, diabetes, hypertension, heart failure, atrial fibrillation, peripheral vascular disease, acute or chronic kidney disease, chronic obstructive pulmonary disease and obstructive sleep apnea syndrome, is summarized in table 1. The prevalence of hypertension was 56.8%, peripheral vascular disease 40.5%, diabetes 32.4%.

Table1 Medical History

Medical condition	Percentage
Tobacco smoke	16.2%
Obesity	10.8%
Diabetes	32.4%
Hypertension	56.8%
Heart failure	18.9%
Atrial fibrillation	18.9%
Peripheral vascular disease	40.5%
Acute or chronic kidney disease	13.5%
Chronic obstructive pulmonary disease	24.3%
Obstructive sleep apnea syndrome	2.7%

Table 1 Medical history for relevant comorbidities: tobacco smoke, obesity, diabetes, hypertension, heart failure, atrial fibrillation, peripheral vascular disease, acute or chronic kidney disease, chronic obstructive pulmonary disease and obstructive sleep apnea syndrome. Data are reported as percentages of subjects who have the condition.

The therapeutic regimens for the abovementioned medical conditions were noted (table 2) and in particular were included therapies with: beta-blockers, diuretics, oral inotropes, sartans, statins, anti-diabetics (insulin or oral antidiabetic treatments), antiplatelets and anticoagulants. The 40.5% of the patients was taking diuretics, 35.1% anti-platelets/anticoagulants and 32.4% sartans.

Table 2 Chronic Therapeutic Regimens

Type of medication	Percentage
Beta-blockers	18.9%
Diuretics	40.5%
Oral inotropes	10.8%
Sartans	32.4%
Statins	29.7%
Anti-diabetics	21.6%
Anti-platelet / anticoagulant	35.1%

Table 2 Chronic medications for relevant medical conditions: tobacco smoke, obesity, diabetes, hypertension, heart failure, atrial fibrillation, peripheral vascular disease, acute or chronic kidney disease, chronic obstructive pulmonary disease and obstructive sleep apnea syndrome). Therapies are grouped in seven categories: beta-blockers, diuretics, oral inotropes, sartans, statins, anti-diabetics (insulin or oral antidiabetic treatments), anti-platelets/anticoagulants. Data are reported as percentages of subjects who have the condition.

The cardiac arrest was associated to AMI in 40.5% of the cases, to stroke and traumatic brain injury in one case respectively (2.7%). The large majority of events occurred in an out-of-hospital setting (73.0%) in the presence of a witness (86.5%)

but only 45.9% of the subjects received early CPR (several data are missing regarding the exact timing of resuscitation).

Shockable cardiac rhythms were present in 59.5% of the subjects. After resuscitation 27.0% of patients developed acute cardiac failure, 35.1% respiratory failure and 5.4% septic shock.

Neurological status was measured by GCS after the acute event, during hypothermia, at ICU discharge and at the end of follow-up. Table 3 reports mean GCS values ± SD.

Table 3 Neurological condition

GCS	Mean ± SD
Admission	3.5 ± 1.5
ICU Discharge	7.6 ± 4.2
Follow-up	8.4 ± 5.3

Table 3 Glasgow Coma Scale (GCS) scores at admission to Intensive Care Unit (ICU), at discharge from ICU. GCS was also measured at the end of the follow-up period. Data are expressed as mean ± standard deviation (SD).

A set of vital parameters (HR, MAP, SpO_2, urine output, blood pH, K^+ concentration and HCO_3^- concentration) were monitored during hypothermia for all patients (figure 2).

Figure 2 Vital parameters

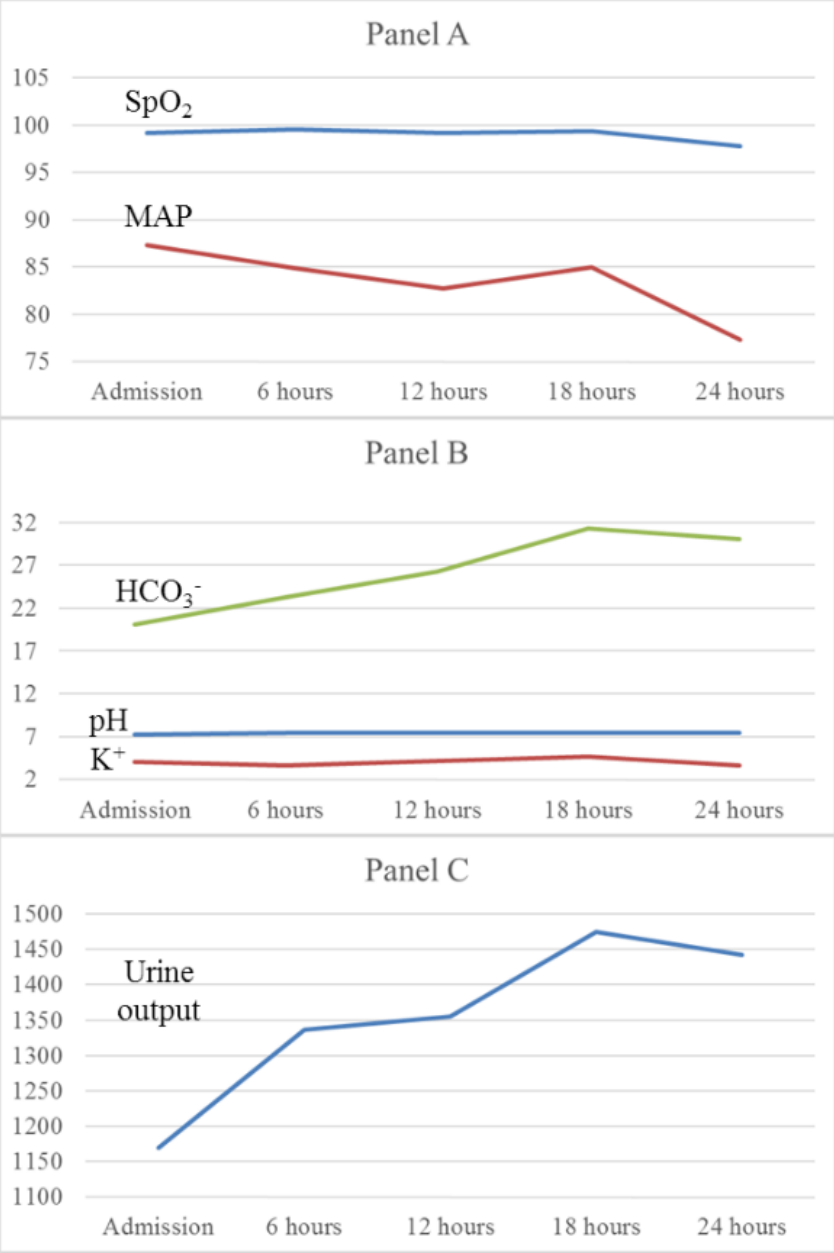

Figure 2 Trends of a set of vital parameters over the course of 24 hours of therapeutic hypothermia. Panel A Saturation of peripheral oxygen (SpO_2), expressed as average percentage of haemoglobin saturation, and mean arterial pressure (MAP), expressed as average pressure in mmHg. Panel B Average blood pH (pH), mean concentration of potassium (K^+) and bicarbonate (HCO_3^-) in mEq/L. Panel C Average urine output in ml during 6 hours of observation.

The mean temperature during the 24-hour therapeutic hypothermia was 35.6°C ± 1.0°C. Figure 3 shows the average body temperature values during this period. A total of 13 patients reached and maintained a body temperature lower than 36°C during the first 6 hours of hypothermia (Group A).

The median GCS at admission did not show any statistically significant differences between Group A and B (3.5 and 3.4 respectively). However, reaching a body temperature lower than 36.0°C within the first 6 hours of therapeutic hypothermia was correlated to a better neurological outcome measured by GCS during follow-up. There was a statistically significant difference between GCS of group A and B at follow-up: 9.9 vs. 5.7 p-value 0.0192.

Figure 3 Body temperature

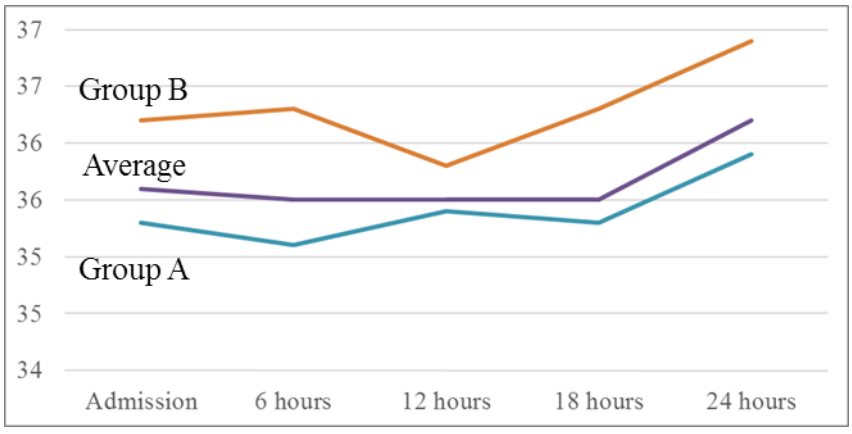

Figure 3 Body temperature trend during a 24-hour therapeutic hypothermia. Curves are displayed for Group A, (body temperature <36°C with 6 hours from admission and Group B that did not reach this goal. Values are expressed as mean body temperatures in °C.

The Cox regression model showed a protective effect of early hypothermia on survival (Hazard Ratio -0.39, Standard Error -0.16, 95% Confidence Interval from -0.17 to -0.89, p-value 0.026) (figure 4).

Figure 4 Survival Curve

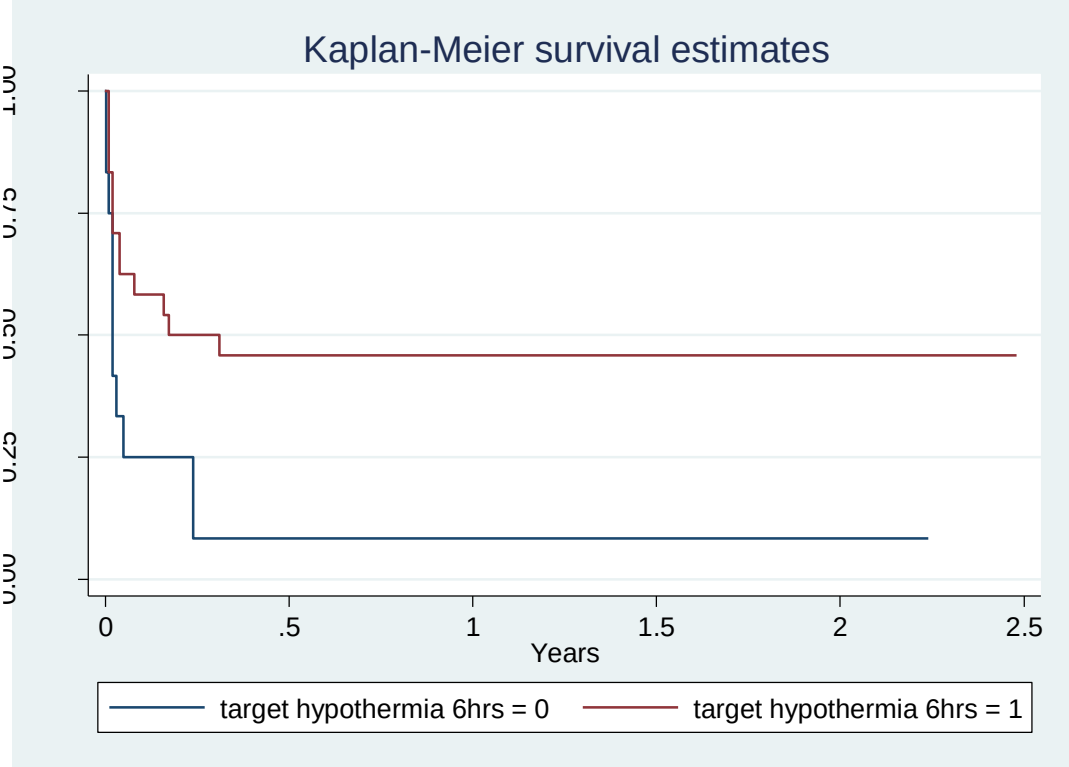

Figure 4 Kaplan Meyer survival curve for reaching hypothermia (body temperature <36.0°C) after cardiac arrest within 6 hours from Intensive Care Unit admission. Hazard Ratio -0.39, Standard Error -0.16, 95% Confidence Interval from -0.17 to -0.89, p-value 0.026. Group A is target hypothermia 6hrs=1, Group B is target hypothermia 6hrs=0

DISCUSSION

The present work suggests that rapid reduction of body temperature below 36.0°C can be beneficial in comatose patients after cardiac arrest admitted to the ICU for supportive therapy.

The study cohort was characterized by older age, at the peak of incidence of acute cardiac event, during the 6th decade of life. There was a high prevalence of cardio-vascular risk factors and mainly hypertension, peripheral vascular disease and diabetes[13]. These are common comorbidities associated to high-income countries life style, whose socio-economical burden on healthcare system is becoming unbearable. They are, in fact, correlated not only to mortality but also to permanent disability. According to the World Health Organization, the global healthcare expenditures for brain injuries from all causes in 2010 represent 5.2% of the total ($333.5 billion) – subdivided into 41% in the Unites States, 26% in Europe, 3% in Italy, 30% in other countries – with growing costs related to assistance and pensions/insurances [14].

Moreover, most of the subjects were already taking chronic medications for the abovementioned comorbidities, which further stresses how an acute event, like sudden cardiac death, is likely preceded by the deterioration of the health status. The population at risk for major cardiac events should be closely monitored [15].

Educational campaigns on early CPR are a cornerstone of an effective recovery. Even if in most of the cases there was a witness to the acute event only half of the patients received on-site resuscitation, which further stresses the importance of a social intervention on the general population to raise the awareness on these life-saving maneuvers [2]. Cardiac arrest was often associated to AMI [13], recognizing the signs and symptoms of acute coronary syndrome could lead to a more timely activation of the emergency system.

Patients were, in fact, mostly in an out-of-hospital setting, which lengthens rescue times and leads to a poor outcome also in terms of mortality. Cardiac arrest is a lethal complication with high mortality [16]: in this cohort the overall mortality was almost 80%.

Rapidly reaching a core body temperature of 36.0°C, that some authors define as controlled normothermia, seems to offer similar advantages of lower body temperatures with fewer side effects [6]. In Group A, in fact, there was a lower mortality and a better medium term neurological outcome. Group B did not reach a state a mild hypothermia within the first 6 hours from ICU admission possibly leaving room to damage processes to determine permanent lesions. In a time sensitive context, like emergency neuroprotection, it is vital to preserve as much as possible the vitality of tissues, especially if they lack substantial regeneration capabilities, such as the brain, leading to significant cognitive impairment [1].

Our study has several limitations, which must be considered when designing future studies aiming to test the effectiveness of early hypothermia. Foremost, the retrospective design limits the extent and strength of the conclusions. GCS does not adequately portray the complexity of neural damage evaluation. Patients admitted to ICU are a subgroup of all the subjects who experienced a cardiac arrest – early hypothermia should be tested also in the out-of-hospital emergency rescue to fully understand its protective potential. Patients in Group B had a baseline temperature higher than patients in Group A which could have contributed to failing to reach 36°C within the first 6 hours from ICU admission.

The potential beneficial action, moreover, in contrasting high intra-cranial pressure [8], which is a severe complication of neural tissue injuries, has to be carefully evaluated assessing the real metabolic effects of potentially improved blood flow and perfusion.

In conclusion, this study showed a substantial improvement of GCS and mortality in a cohort of patients admitted to ICU after resuscitation due to the early application of mild hypothermia, which stresses the importance of temperature control in critically ill patients with an acute brain damage as a consequence of cardiac arrest.

REFERENCES

1. Moulaert VR, Verbunt JA, van Heugten CM, et al. Cognitive impairments in survivors of out-of-hospital cardiac arrest: a systematic review. *Resuscitation* 2009; 80: 297-305.
2. Murakami Y, Iwami T, Kitamura T, et al. Outcomes of out-of-hospital cardiac arrest by public location in the public-access defibrillation era. *J Am Heart Assoc* 2014; 3: e000533.
3. Arrich J, Holzer M, Havel C, et al. Hypothermia for neuroprotection in adults after cardiopulmonary resuscitation. *Cochrane Database Syst Rev* 2012; 9:CD004128.
4. Lopez-de-Sa E, Rey J, Armada E, et al. Hypothermia in comatose survivors from out-of-hospital cardiac arrest: pilot trial comparing 2 levels of target temperature. *Circulation* 2012; 126: 2826-2833.
5. Bro-Jeppesen J, Hassager C, Wanscher M, et al. Targeted temperature management at 33°C versus 36°C and impact on systemic vascular resistance and myocardial function after out-of-hospital cardiac arrest: a sub-study of the Target Temperature Management Trial. *Circ Cardiovasc Interv* 2014; 7: 663-672.
6. Nielsen N, Wetterslev J, Cronberg T, et al. Targeted temperature management at 33°C versus 36°C after cardiac arrest. *N Engl J Med* 2013; 369: 2197-2206.

7. Polderman K. Induced Hypothermia for Neuroprotection: Understanding the Underlying Mechanisms. In: Vincent JL. Intensive Care Medicine Annual Update 2006. New York, USA: Springer New York, 2006, pp 328-346.
8. Flynn L, Rhodes J, Andrews P. Therapeutic Hypothermia Reduces Intracranial Pressure and Partial Brain Oxygen Tension in Patients with Severe Traumatic Brain Injury: Preliminary Data from the Eurotherm3235 Trial. *Ther Hypothermia Temp Manag* 2015 [Epub ahead of print].
9. Chmayssani M, Stein N, McArthur D, et al. Therapeutic intravascular normothermia reduces the burden of metabolic crisis. *Neurocrit Care* 2015; 22: 265-272.
10. Uray T, Mayr F, Stratil P, et al. Prehospital surface cooling is safe and can reduce time to target temperature after cardiac arrest. *Resuscitation* 2015; 87: 51-56.
11. Kim F, Nichol G, Maynard C, et al. Effect of prehospital induction of mild hypothermia on survival and neurological status among adults with cardiac arrest: a randomized clinical trial. *JAMA* 2014; 311: 45-52.
12. Bernard S, Smith K, Cameron P, et al. Induction of therapeutic hypothermia by paramedics after resuscitation from out-of-hospital ventricular fibrillation cardiac arrest: a randomized controlled trial. *Circulation* 2010; 122: 737-742.

13. Hayashi M, Shimizu W, Albert C. The spectrum of epidemiology underlying sudden cardiac death. *Circ Res*. 2015; 116:1887-1906.
14. World Health Organization. Annual Report 2010. Geneve, Switzeland: WHO Press, 2011.
15. Deyell M, Krahn A, Goldberger J. Sudden cardiac death risk stratification. *Circ Res*. 2015;116: 1907-1918.
16. Kuriachan V, Sumner G, Mitchell L. Sudden cardiac death. *Curr Probl Cardiol* 2015;40: 133-200.

Conclusions

The challenge of managing brain injuries in the acute setting involves many processes from diagnosis to treatment, that have to be finely coordinated to maximize their effectivenss.

Assessing neural damage is dependent on the clinical evaluation of the physician, as in not every context adequate imaging technology is available. A biomarker-based approach, just like for acute myocardial infarction, would provide a useful tool in the ruling out possible confounding diagnosis. There is, however, no single reliable marker for the brain, so a panel based approach has been adopted internationally. Although much has been done in choosing the right markers a consensus has not been reached.

Our studies show that bio-markers may have a role in identifying and evaluating neural damage especially if it is subclinical, like in the case of carotid surgery, helping healthcare professionals to better tailor their decisions.

Therapy, on the other hand, offers multiple options for the treatment of the patient in the acute phase. Hypothermia is a promising approach as it affects multiple damage pathways with an overall protective effect on neurons. Its application in clinical practice requires the coordinated effort of multiple actors to ensure the patient may benefit from the treatment. Our study stresses the importance of

thermal management and timing in the acute care of patients with a brain damage following cardiac arrest. Being able to reach rapidly a temperature target grants a significant benefit on the medium to long-term.

Critical Care Medicine combines like few other medical specialization resource management, diagnosis and treatment – in this medical orchestra new tools are needed to keep offering patients the high standard of care they deserve.