CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector

Comparative study on carotid revascularization (endarterectomy vs stenting) using markers of cellular brain injury, neuropsychometric tests, and diffusion-weighted magnetic resonance imaging

Laura Capoccia, MD,[a] Francesco Speziale, MD,[a] Marianna Gazzetti, MD,[a] Paola Mariani, MD,[b] Annarita Rizzo, MD,[a] Wassim Mansour, MD,[a] Enrico Sbarigia, MD,[a] and **Paolo Fiorani, MD,[a]** *Rome, Italy*

Objective: **Subclinical alterations of cerebral function can occur during or after carotid revascularization and can be detected by a variety of standard tests. This comparative study assessed the relationship among serum levels for two biochemical markers of cerebral injury, postoperative diffusion-weighted magnetic resonance imaging (DW-MRI), and neuropsychometric testing in patients undergoing carotid endarterectomy (CEA) or carotid artery stenting (CAS) for high-grade asymptomatic carotid stenosis.**

Methods: **Forty-three consecutive asymptomatic patients underwent carotid revascularization by endarterectomy (CEA, 20) or stenting (CAS, 23). They were evaluated with DW-MRI and the Mini-Mental State Examination (MMSE) test preoperatively and ≤24 hours after carotid revascularization. Venous blood samples to assess serum levels of neuron-specific enolase (NSE) and S100β protein were collected for each patient preoperatively and five times in a 24-hour period postoperatively and assayed using automated commercial equipment. The MMSE test was repeated at 6 months. The relationship between serum marker levels and neuropsychometric and imaging tests and differences between the two groups of patients were analyzed by χ^2 test, with significance at $P < .05$.**

Results: **No transient ischemic attacks or strokes were clinically observed. CAS caused more new subcortical lesions at postoperative DW-MRI and a significant decline in the MMSE postoperative score compared with CEA ($P = .03$). In CAS patients, new lesions at DW-MRI were significantly associated with a postoperative MMSE score decline >5 points ($P = .001$). Analysis of S100β and NSE levels showed a significant increase at 24 hours in CAS patients compared with CEA patients ($P = .02$). The MMSE score at 6 months showed a nonsignificant increase vs the postoperative score in both groups.**

Conclusions: **Biochemical markers measurements of brain damage combined with neuropsychometric tests and DW-MRI can be used to evaluate silent injuries after CAS. The mechanisms of rise in S100β and NSE levels at 24 hours after CAS may be due to increased perioperative microembolization rather than to hypoperfusion. Further studies are required to assess the clinical significance of those tests in carotid revascularization. (J Vasc Surg 2010;51:584-92.)**

Carotid endarterectomy (CEA) is an evidence-based effective means of preventing strokes in symptomatic and asymptomatic patients.[1] Ongoing trials will definitely state the role of carotid artery stenting (CAS) compared with CEA in terms of stroke prevention and low rates of perioperative neurologic events.[2] Neurologic risk assessment in major studies is based on the detection of transient ischemic attack (TIA) or stroke, but little is known about subclinical neurologic morbidity.

Diffusion-weighted magnetic resonance imaging (DW-MRI) is effective in detecting new microembolic brain lesions occurring during or after carotid revascularization.[3] Recent studies have focused on the role of microembolization after revascularization as causing neurocognitive decline that can be detected by the use of neuropsychometric tests.[4-8] Subclinical neurologic ischemic events can also be detected by measuring serum markers of brain injury. A variety of biochemical markers of brain injury have been described. Among them, neuron-specific enolase (NSE) and the calcium-binding protein S100β have been demonstrated to be markers of stroke in animal models[9-10] and in humans.[11-17]

In 1965 Moore isolated a subcellular fraction from bovine brain, which was thought to contain nervous-system specific proteins. This fraction was called S100 because the constituents were soluble in 100% saturated ammonium sulfate at neutral pH. Subsequent studies demonstrated that this fraction contained predominantly two polypeptides, S100Al and S100β, which have molecular weights of approximately 10,000 Da and contain two high-affinity EF-hand calcium-

From Vascular Surgery Division[a] and Clinical Pathology Division,[b] Department of Surgery "Paride Stefanini," Policlinico Umberto I, "Sapienza" University of Rome.

Competition of interest: none.

Presented at the 2009 Vascular Annual Meeting, Denver, Colo, Jun 11-14, 2009.

Additional material for this article may be found online at www.jvascsurg.org.

Reprint requests: Laura Capoccia, Via Antonio Labranca 44, 00123 Rome, Italy (e-mail: lauracapoccia@yahoo.it).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a competition of interest.

0741-5214/$36.00

Copyright © 2010 by the Society for Vascular Surgery.

doi:10.1016/j.jvs.2009.10.079

binding domains. S100β has also been called "NEF" because in the mid-1980s, Kligman and Marshak identified a purified protein molecule that exhibited neurite extension factor (NEF) activity when applied to primary chick cortical neuron cultures as S100β.[18] The estimated biologic half-life of S100β is about 2 hours, and maximum levels of the protein can be detected as early as 20 minutes after brain injury.[19]

Neuron-specific enolase (NSE) is a glycolytic enzyme that is found mainly in the cytoplasm of neurons and cells of neuroendocrine origin.[20] It has also been found in erythrocytes and platelets, although in smaller concentrations, but the isoforms γγ and αγ are released from neurons after infarction. They have a molecular weight of 78 kDa and a biologic half-life in serum of 20 hours.[21,22]

The aim of this preliminary comparative study was to assess the relationship between serum levels of S100β and NSE and postoperative DW-MRI and Mini-Mental State Examination (MMSE) score in two groups of patients undergoing carotid revascularization by CEA or CAS.

METHODS

All patients who participated in this study gave informed, written consent. The study protocol was approved by the local Ethical Research Committee.

Patients. Between April and September 2008, 43 consecutive asymptomatic patients undergoing elective carotid revascularization were recruited to participate in this prospective study. Inclusion criteria were the presence of a carotid stenosis ≥70% (European Carotid Surgery Trial stenosis evaluation criteria),[23] with no previous neurologic symptoms referred in the medical history and the absence of a previous brain ischemic lesion detected at DW-MRI. Patients with symptomatic carotid lesions, previous ischemic lesions detected at DW-MRI, or inability to give consent were excluded from the study.

All patients underwent duplex ultrasound imaging and computed tomography (CT) angiography to assess anatomic suitability for CEA or CAS and were clinically evaluated by independent neurologists and cardiologists before and after treatment. Patients were allocated in the two treatment groups according to clinical and anatomic criteria.

Exclusion criteria for CAS were tortuous or highly calcified arteries, ostial lesion of the common carotid or brachiocephalic artery, poor entry points at the femoral artery, length of the target lesion requiring more than one stent, presence of intraluminal thrombus or hypo-anechoic plaque composition, history of bleeding disorder, or intracranial aneurysm or hemorrhage. Exclusion criteria for CEA were clinically significant cardiac disease (congestive heart failure, abnormal stress test, or need for open-heart surgery), severe pulmonary disease, contralateral carotid occlusion, contralateral laryngeal-nerve palsy, previous radial neck surgery or radiotherapy to the neck, or recurrent stenosis.

Demographic and clinical characteristics such as age, gender, hypertension, smoking habits, diabetes, hyperlipemia, coronary artery disease (documented by the presence of previous myocardial infarction, coronary artery bypass grafting, or history of angina), chronic obstructive pulmonary disease, side of carotid lesion, contralateral carotid occlusion, stenosis percentage, and plaque composition, were recorded and compared between the two treatment groups. Plaque composition was evaluated by gray scale median score.[24] Demographic and clinical data are listed in Table I.

Table I. Demographic and clinical data for carotid endarterectomy and stenting groups

Variable	*CEA*	*CAS*	P
Patients, No.	20	23	
Age, mean ± SD, y	70.1 ± 7.2	71.7 ± 7.2	.67
Sex			.16
Males, No. (%)	14 (70)	13 (56.5)	
Females	6	10	
Left side stenosis, No. (%)	11 (55)	12 (52.2)	.54
Contralateral occlusion, No. (%)	0	4 (17.4)	.07
Stenosis, mean ± SD %	77 ± 7.32	73.9 ± 4.99	.11
Hyperechoic plaque, No. (%)	16 (80)	22 (95.7)	.11
Hypertension, No. (%)	17 (85)	20 (87)	.33
Smoking, No. (%)	12 (60)	10 (43.5)	.21
Diabetes, No. (%)	8 (40)	13 (56.5)	.13
Hyperlipemia, No. (%)	10 (50)	8 (34.8)	.24
CAD,[a] No. (%)	4 (20)	12 (52.2)	.03
COPD, No. (%)	6 (30)	12 (52.2)	.12

CAD, coronary artery disease; *CAS,* carotid artery stenting; *CEA,* carotid endarterectomy; *COPD,* Chronic obstructive pulmonary disease; *SD,* standard deviation.
Boldface type MMSE scores obtained in CAS patients presenting with new ischemic lesions at DW-MRI.
[a]Includes previous ischemic myocardial infarction, coronary artery bypass grafting, and history of angina.

Carotid endarterectomy. A locoregional cervical block involving the rami of C2 to C4 was used for CEA in all 20 cases. Routine physiologic monitoring included pulse oximetry, electrocardiogram, and invasive blood pressure measurement through the radial artery. Before the internal carotid artery (ICA) was clamped, heparin (5000 ± 2500 IU depending on body weight) was injected intravenously. Neurologic status assessment was performed by using the contralateral "hand grip" test and consciousness assessment throughout the entire procedure, avoiding any sedative use.

CEA was performed by standard surgical protocol. The patient was placed supine with the neck turned to the opposite side and extended. A 7- to 8-cm incision was made along the anterior border of the sternocleidomastoid, equidistant from the mastoid and the sternoclavicular joint and extending caudal or cephalad depending on the site of the bulb. The platysma muscle and intervening connective tissue were incised using electrocautery. The sternocleidomastoid muscle and the internal jugular vein were retracted laterally to expose the carotid artery. The internal (ICA), common (CCA), and external carotid arteries (ECA) were dissected circumferentially beyond the level of the plaque. After the ECA and the superior thyroid artery were clamped, the CCA was clamped to assess neurologic func-

tion, thus leaving the backflow from the ICA. After the neurologic status assessment, the ICA was clamped and full-thickness circumferential mobilization of the CCA, bulb, and ICA was performed. A longitudinal arteriotomy on the posterior-lateral side of the bifurcation was performed, and the plane for endarterectomy was developed longitudinally and circumferentially.

The plaque was elevated and transacted in the middle. The plaque on the ECA was removed by traction and partial eversion; the plaque on the CCA was then extracted by eversion and transection of the plaque flush with the everted edge. On the ICA, the plaque was pinched and removed from the vessel. The end point was carefully inspected for semi-adherent fibers, which were easily recognized by irrigating with heparinized saline.

Before the arteriotomy was closed, a temporary "flashing" from the ICA was made, thus allowing the backflow to drive out loose debris. Dacron patch angioplasty was performed in 80% of cases using 5-0 polypropylene suture in a semi-continuous fashion, and direct closure was made in a continuous fashion in 20%. Near closure, the clamps were temporarily released, thereby flashing the vessel of loose debris. On completion of the anastomosis, the ECA was opened to allow debris from the CCA to enter this system rather than the ICA system. A shunt was used in two patients (10%). Patients were maintained postoperatively under their scheduled aspirin (100 mg) or ticlopidine (250 mg) medication.

Carotid artery stenting. CAS was performed with local anesthesia with continuous control of heart frequency, blood pressure, and partial pressure of arterial oxygen in all 23 cases. Blood pressure was measured during the entire procedure by a sphygmomanometer with automated cuff inflation. Neurologic status was monitored using the contralateral "hand grip" test during and after all catheter manipulations and avoiding the use of any sedatives before or during the procedure. Patients received aspirin (100 mg) and clopidogrel (75 mg) at least 24 hours preoperatively and on the day of the procedure.

A transfemoral approach through a 6F to 7F arterial sheath was established. Angiography was performed using diluted nonionic contrast media (70% contrast, 30% saline solution) and digital subtraction. An initial intravenous heparin bolus (5000 IU) was administered, followed by a continuous intra-arterial infusion of heparinized saline solution (5000 IU in 1000 mL of saline) through the guiding catheter.

The common carotid artery was entered with a 6F to 7F guiding catheter, followed by an angled 0.035-inch hydrophilic guidewire. A selective angiogram of the carotid artery and its intracranial branches was obtained to document length and degree of the stenosis and carotid arteries diameters. A FilterWire (Boston Scientific, Natick, Mass) embolic protection device and Wallstent (Boston Scientific) were used in all patients. Stent size was chosen according to an estimation of the carotid artery diameters and the kind of lesion at ultrasound detection and confirmed by CT angiography. Stent deployment was not proceeded by predilation and was routinely followed by dilation of the lesion using a 5- to 6-mm-diameter balloon catheter and a pressure of 6 to 10 atm for a maximum of 5 seconds. Intravenous atropine (1 mg) was administered immediately before balloon inflation to prevent reflex bradycardia or asystole.

Selective control angiograms were obtained after stenting to confirm a final adequate dilation of the stenotic segment and no occurrence of local complications such as dissection and to evaluate the patency of the intracranial arteries. Technical success was achieved in all cases. Patients were maintained under lifelong aspirin (100 mg) and additional clopidogrel (75 mg) for 6 weeks after the procedure.

Neuroradiologic examination. All patients underwent DW-MRI preoperatively and at 24 hours postoperatively. No patient in this series showed ischemic lesions at the preoperative DW-MRI.

Neuropsychometric assessment. Patients were assessed with the MMSE test (Appendix, online only) before the operation, ≤24 hours postoperatively, and at the 6-month follow-up visit. The research assistant (A. R.) responsible for performing the MMSE preoperatively and postoperatively in all patients was trained to administer and score the test. Downgrading in the postoperative examination, such as from normal to some cognitive impairment (1 step) or a difference ≥5 in the postoperative score compared with the preoperative value was considered significant. No psychotropic or sedative medications were administered to the patients before performing tests.

Serum biomarkers of brain injury. Venous blood samples were obtained for each patient preoperatively (basal sample), at 5 minutes after declamping the ICA or embolic protection device retrieval, and at 2, 6, 12, and 24 hours after the end of the procedure. Samples were allowed to clot. After centrifugation (1800*g* for 6 minutes) ≤20 minutes from collection, serum was stored at −80°C for later analysis. S100β and NSE proteins were analyzed by the use of automated immunoluminometric assays (S100 Elecsys test, Roche Diagnostics GmbH, Mannheim, Germany; ELSA-NSE, CIS Bio International, Gif-sur-Yvette Cedex, France). The S100 test measures the β-subunit of protein S100 as defined by three monoclonal antibodies with a detection limit of 0.02 μg/L. NSE measurement is based on monoclonal antibodies that bind to the γ-subunit of the enzyme with a minimal measurable concentration of 0.3 μg/L. The biochemist (P. M.) who performed the blood samples analysis was blinded to the treatment group and imaging test data.

Statistical analysis. The χ^2 test, unpaired *t* test for multiple comparisons, and the Fisher exact test (95% confidence interval) were used to assess differences in demographic and clinical data, MMSE scores, presence of new lesions on DW-MRI, and serum biomarkers levels between the two treatment groups. Continuous values were expressed as mean ± standard deviation.

For each treatment group we identified patients with new lesions on DW-MRI or a significant (1-step) decrease on the postoperative MMSE score and analyzed the correlation between new lesions on DW-MRI, MMSE score

decline, and the increase in biomarker levels. Analysis within and between groups was performed with the χ^2 test and the Fisher exact test for categoric data. Analysis of continuous values of brain injury markers was performed with the adjusted *t* test for multiple comparisons. We primarily considered the within variation in markers of brain injury (S100β and NSE) in patients by comparing each value with the basal sample and 24-hour values with the 12-hour values. We considered significant an increase of ±25% from the reference value. Results were subsequently stratified as stable, increased, or decreased in each patient and then analyzed as belonging to these three groups for within-group and between-group (CAS vs CEA) variation analysis. The threshold for significance was set at $P < .05$.

RESULTS

Descriptive results of study population. Twenty patients underwent CEA and 23 underwent CAS, with a mean age of 70.1 ± 7.22 and 71.7 ± 7.21 years, respectively. Among preoperative data, coronary artery disease was significantly more frequent in CAS patients (52.2%) vs CEA patients (20%; $P = .03$). No patients died in the perioperative period. Neurologic morbidity was 5% (1 temporary cranial nerve lesion) in the CEA group and 0% in the CAS group. Two groin hematomas (8.7%) were recorded in the CAS group.

Imaging and neuropsychometric tests. No preoperative ischemic lesions were detected by preoperative DW-MRI. In five CAS patients (21%), new ischemic lesions were detected at the 24-hour postoperative DW-MRI, with no lesion encountered in CEA patients ($P = .035$; Table II). The mean preoperative MMSE scores were 26.1 ± 3.46 and 25.6 ± 4.46 in the CEA and CAS groups, respectively, and postoperative scores were 25.6 ± 3.27 and 22.9 ± 4.54. Within-group analysis revealed a significant decrease in the MMSE score in the CAS group that was not observed in the CEA group ($P = .045$ and $P = .67$, respectively). Between-group analysis showed a significant decrease in the postoperative score in CAS patients with respect to CEA patients ($P = .03$), with a >5-point decrease in seven CAS patients (30%) and one CEA patient (5%).

New lesions at DW-MRI in CAS patients were significantly associated with the MMSE score decline >5 points ($P = .001$); the MMSE scores in those patients were significantly decreased compared with patients with negative results on postoperative DW-MRI ($P < .001$). At the 6-month follow-up, the MMSE score showed an improvement in CAS patients and it was effectively stable in the CEA group, with a mean score 23.7 ± 4.58 in CAS and 25.9 ± 3.43 in CEA patients (within- and between-group analysis, P = NS). DW-RMI results and MMSE scores are listed in Table II.

Table II. Diffusion-weighted magnetic resonance imaging and Mini-Mental State Examination evaluations preoperatively, postoperatively, and at 6 months

Coronary artery stenting						*Carotid endarterectomy*					
	DW-MRI		*MMSE score*				*DW-MRI*		*MMSE score*		
Pt	*Pre*	*Post*	*Pre*	*Post*	*6 mon*	*Pt*	*Pre*	*Post*	*Pre*	*Post*	*6 mon*
1	−	−	24	19	20	1	−	−	27	25	25
2	−	−	15	13	13	2	−	−	23	27	26
3	−	−	30	27	27	3	−	−	30	28	30
4	−	+	**25**	**19**	**18**	4	−	−	25	26	26
5	−	−	30	28	28	5	−	−	21	25	25
6	−	−	25	26	23	6	−	−	30	28	28
7	−	−	29	27	27	7	−	−	26	26	25
8	−	+	**29**	**24**	**27**	8	−	−	27	23	22
9	−	+	**27**	**17**	**25**	9	−	−	16	18	18
10	−	−	28	28	30	10	−	−	28	30	28
11	−	−	26	18	22	11	−	−	26	24	27
12	−	+	**30**	**24**	**20**	12	−	−	25	25	27
13	−	−	21	21	21	13	−	−	27	27	26
14	−	−	16	15	15	14	−	−	29	28	30
15	−	−	28	28	28	15	−	−	23	18	18
16	−	−	26	22	25	16	−	−	27	25	25
17	−	−	30	28	28	17	−	−	30	29	30
18	−	−	23	23	23	18	−	−	30	30	30
19	−	+	**27**	**21**	**22**	19	−	−	25	27	27
20	−	−	30	26	28	20	−	−	27	24	25
21	−	−	24	24	24						
22	−	−	28	28	30						
23	−	−	18	20	21						
Mean			**25.6**	**22.9**	**23.7**				26.1	**25.6**	**25.9**

−, Negative result; +, positive result; *DW-MRI,* diffusion-weighted magnetic resonance imaging; *MMSE,* Mini-Mental State Examination; *Pre,* preoperative; *Post,* ≤24 hours postoperatively.
Boldface type MMSE scores obtained in CAS patients presenting with new ischemic lesions at DW-MRI.

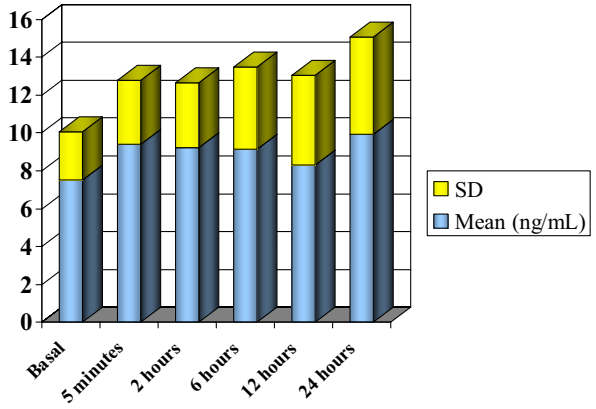


Fig 1. Mean neuron-specific enolase values in carotid artery stenting patients are shown with the standard deviation *(SD)*.

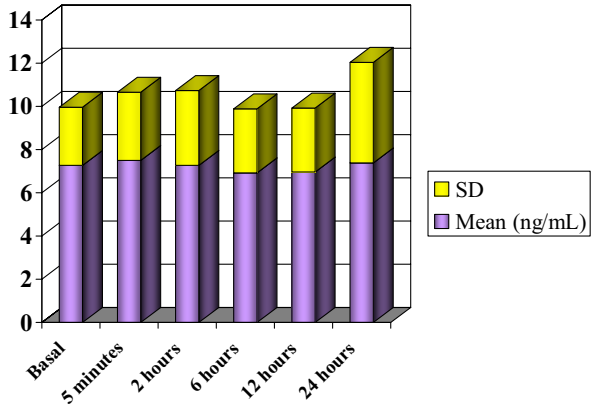


Fig 2. Mean neuron-specific enolase values in carotid endarterectomy patients are shown with the standard deviation *(SD)*.

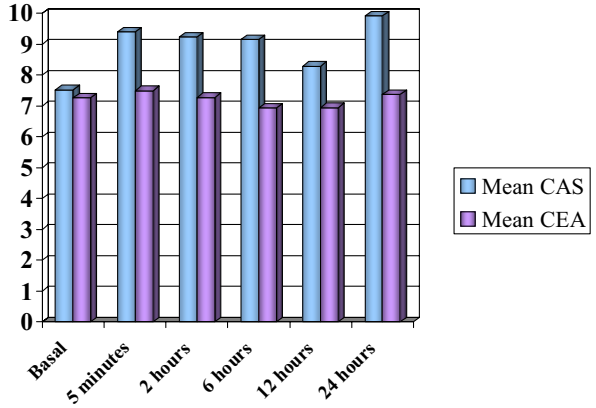


Fig 3. Mean neuron-specific enolase values are shown in carotid artery stenting *(CAS)* and carotid endarterectomy *(CEA)* patients.

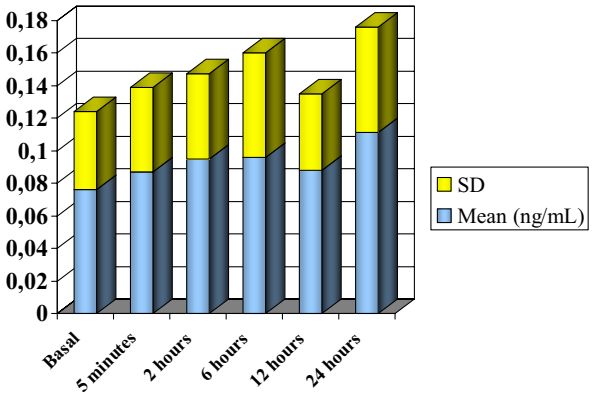


Fig 4. Mean S100β levels in carotid artery stenting patients are shown with the standard deviation *(SD)*.

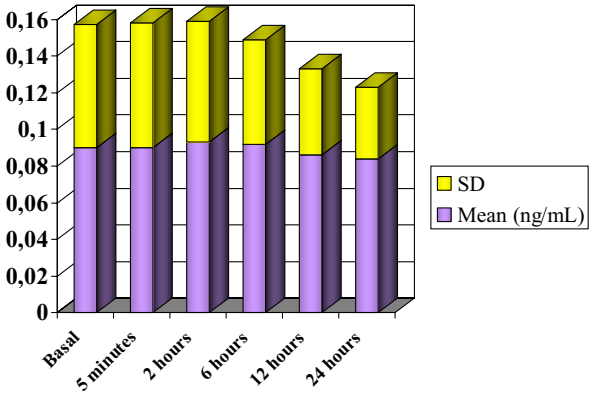


Fig 5. Mean S100β levels in carotid endarterectomy patients are shown with the standard deviation *(SD)*.

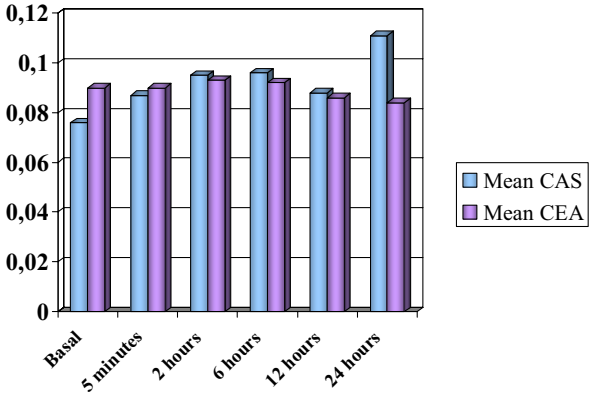


Fig 6. Mean S100β levels are shown in carotid artery stenting *(CAS)* and carotid endarterectomy *(CEA)* patients.

Serum biomarkers of brain injury. Figs 1 to 6 show the NSE and S100β levels from basal through 24 hours after intervention. Basal NSE and S100β levels were the same for each group ($P = .77$ and .12, respectively). We considered the variation in the S100β and NSE markers of brain injury primarily in patients (variation within) and then between different groups (variation between). Analysis on continuous values after intervention in CAS group showed an increasing trend for all S100β and NSE levels compared

with the basal value and for the 24-hour value compared with the 12-hour level ($P = .072$ and $P = .38$, respectively, for S100β and $P = .004$ and $P = .581$ for NSE at within-group analysis performed by unpaired t test adjusted for multiple comparisons). This trend was not confirmed in CEA patients ($P = .875$ and $P = .824$, respectively, for all values compared with basal value and for the 24-hour value vs the 12-hour level for S100β, and $P = .139$ and $P = .292$ for NSE). Analysis on continuous values between groups showed no statistically significant difference except for 6 hours NSE levels ($P = .042$).

Then we divided patients in each treatment group according to the variation of markers encountered by comparing each value with the basal sample and 24-hour value with 12-hour value. We considered significant an increase of ±25% from the basal value. Results were subsequently stratified as stable, increased, or decreased in each patient and then analyzed as belonging to these three groups for between-groups variation analysis.

In the CAS group, S100β increased by ≥25% at 12 hours vs baseline and by ≥25% at 24 hours vs 12 hours in 78% and 83%, respectively. Likewise, such increases in NSE occurred in 56% and 70% of CAS patients, respectively. In CEA patients, such S100β increases occurred in 45% and 50% of patients respectively, and ≥25% NSE increases occurred in 55% and 35%, respectively. Analysis between the groups showed a significant number of CAS patients with increasing 24-hour values vs 12-hour levels of S100β and NSE compared with CEA patients ($P = .02$). All CAS patients with new lesions on postoperative DW-MRI and significant decline in the postoperative MMSE score had a nonsignificant increase in the 24-hour S100β level compared with the basal value.

DISCUSSION

After an ischemic stroke, DW-MRI is highly sensitive to the changes occurring in the lesion.[25] It is speculated that increases in restriction (barriers) to water diffusion, as a result of cytotoxic edema (cellular swelling), is responsible for the increase in signal on a DWI scan. The DWI enhancement appears within 5 to 10 minutes of the onset of stroke symptoms and remains for up to 2 weeks. Coupled with imaging of cerebral perfusion, it can highlight regions of "perfusion/diffusion mismatch" that may indicate regions capable of salvage by reperfusion therapy. DWI is able to detect subclinical neurologic injuries from the very early onset and has been shown to be positive within minutes of brain injury in both animals and humans.[25,26]

CAS for carotid revascularization has been introduced as an alternative to CEA, which rarely is accompanied by new lesions on DWI. Some authors[27,28] have shown that new lesions are rarely seen on DWI after CEA. In a comparison of CAS and CEA, Roh et al[29] found that both neurologic events and new lesions on DWI were far more common with CAS. In their study, Rapp et al[3] reported a series of 48 patients undergoing 54 CAS procedures with excellent clinical outcomes but a concerning number of new lesions on DW-MRI. These subclinical brain injuries did not occur during the procedure but in the ensuing 48 hours, when transcranial Doppler studies had confirmed an ongoing number of embolic events. Although in the short-term period new subtle cerebral lesions seem to have no measurable consequences and by 6 months post-CAS most have resolved without residual effects, repetitive embolic injuries, nevertheless, may have a cumulative effect.[30] In our study no ischemic lesions were detected at the preoperative DW-MRI; however, new ischemic lesions were detected at the 24-hour postoperative DW-MRI in 21% of CAS patients, with no lesion encountered in CEA group.

Neuropsychometric tests were developed to detect subtle changes in higher cortical functioning. The MMSE, or Folstein test, is a brief 30-point questionnaire that is used to screen for cognitive impairment and is commonly used to screen for dementia. It is also used to estimate the severity of cognitive impairment at a given point in time and to monitor the course of cognitive changes in an individual over time, thus making it an effective way to document an individual's response to treatment. In the time span of about 10 minutes, it samples various functions, including arithmetic, memory, and orientation. The MMSE was introduced by Folstein et al[31] in 1975. The standard MMSE form that is currently published by Psychological Assessment Resources is based on its original 1975 conceptualization, with minor subsequent modifications by the authors.

Various other tests are also used, such as the Hodkinson abbreviated mental test score[32] as well as longer formal tests for deeper analysis of specific deficits. The MMSE test includes simple questions and problems in a number of areas: the time and place of the test, repeating lists of words, arithmetic, language use and comprehension, and basic motor skills; for example, one question asks the individual to copy a drawing of two pentagons. The test has a potential score of 30, and any score >27 is effectively normal. Below this, 20 to 26 indicates some cognitive impairment, 10 to 19 indicates moderate to severe cognitive impairment, and <10 indicates very severe cognitive impairment. The raw score may also be corrected for degree of schooling and age.[33] Low to very low scores correlate closely with the presence of dementia, although other mental disorders can also lead to abnormal findings on MMSE testing.

A decline in neuropsychometric test performance can be reasonably due to clinically silent hypoperfusion or microembolization occurring during or after carotid revascularization. Although controversial results have been reported by several studies that have addressed the effect of CEA on cognitive function,[4,7,34,35] little is known of the effect of CAS on neuropsychometric test results. There are no clear guidelines for judging what is a significant improvement or decline when performing a neuropsychometric test, and patients significantly improve their score with repeated performances.[36]

Some patients can experience an improvement in neuropsychometric test performance after CEA. Owens et al[37] demonstrated that if patients with >50% stenosis had normal CT scans and computerized radionuclide angiograms before and after CEA, they showed cognitive improvement

as measured by a battery of neuropsychometric tests in the immediate 3 to 10 days after the procedure but deteriorated in their cognitive performance if these tests demonstrated evidence of small infarcts or if there was clinical evidence of a stroke. Only those with small infarcts improved at later follow-up testing at 3 to 6 months.[37] In our series we noted a significant decline in the postoperative MMSE score in CAS patients, with some improvement in the 6-month follow-up score. This finding can probably be related to the kind of training developed by patients with repeated performances.

Furthermore, we reported a significant association between new ischemic lesions on postoperative DW-MRI and MMSE score decline in CAS patients compared with preprocedural values and with patients with no postoperative ischemic lesions. This may suggest a postprocedural microembolic mechanism involvement in those patients, as stated in previous reports.[3,29,30] Jordan et al[38] observed that the percutaneous angioplasty with stenting procedure is associated with more than eight times the rate of microemboli seen during CEA when evaluated with transcranial Doppler monitoring.

We found increased levels of postoperative S100β and NSE in CAS patients, and all CAS patients with new lesions on postoperative DW-MRI and significant decline in postoperative MMSE score showed an increase of S100β value at 24 hours compared with the basal value. S100β and NSE proteins are considered markers of cerebral injuries.[5,6,8-22] Because of the S100β short half-life of about 2 hours, the sustained elevated levels observed in the serum of stroke patients likely represent ongoing release of the marker by perishing tissue as the densely ischemic infarction core expands and recruits marginally viable penumbral tissues into the enlarging necrotic region so that sustained S100β release occurs with the death of the penumbral tissues.[39] As a consequence, peak S100β serum levels can be sustained for long periods and can be demonstrated on days 2 to 4 after middle cerebral artery infarctions.[11] Because more extensive cerebral injuries are associated with higher serum S100β levels with relatively late peak times,[17,19] patients with subclinical cerebral tissue death exhibit lower and progressively earlier peak serum levels. Kilminster et al[39] demonstrated that a mild decline in neuropsychometric test performance after coronary artery bypass grafting was correlated with increased S100β levels measured at 5 hours after the procedure.

Serum NSE levels have been demonstrated to increase after cerebral infarctions in patients. Experimental data gained from middle cerebral artery occlusion in a rat model showed a significant increase of NSE starting 2 hours after focal ischemia.[9] NSE serum levels correspond to the ischemia-induced cytoplasmic loss of NSE in neurons and are detectable before irreversible neuronal damage takes place.[5,9,10] The first NSE peak ≤7 to 18 hours after stroke onset may reflect the initial damage of neuronal tissue, whereas a second increase may be attributed to secondary mechanisms of neuronal damage due to edema and an increase of intracranial pressure. The correlation among NSE levels, cerebral infarction volume, and early neurobehavioral outcomes remains controversial.[5,13,14,39,40]

We analyzed S100β and NSE values as continuous and categoric (belonging to increased, stable or decreased groups) data and found that to some degree they can be considered related to subclinical brain injuries. Our observations have shown CAS is associated with a higher number of new ischemic lesions on DW-MRI and an important decrease in the MMSE score. In CAS patients presenting with new subclinical injuries, S100β and NSE values experienced an increasing trend that was not noticed in CEA patients. These findings, together with time of presentation, could be related to microembolization rather than to procedural embolization phenomena in CAS patients despite routine use of embolic protection devices.[41]

Study limitations. This study assessed the relationship between serum levels of S100β and NSE and postoperative DW-MRI and MMSE scores in two groups of patients who underwent carotid revascularization by CEA or CAS. Because of the small number of patients assigned in each treatment group, our results might be considered preliminary. The ongoing recruitment of patients will strengthen the statistical power of our results or will confute them. Allocation of patients between the two treatment groups was not randomized according to our inclusion and exclusion criteria for carotid revascularization by CEA or CAS.

CONCLUSIONS

Because DW-MRI is mainly accepted as the gold standard in revealing subclinical brain injuries in patients undergoing carotid revascularization from their very early onset, the role of neuropsychometric tests and markers of brain injuries in detecting subclinical ischemic lesions needs to be assessed in larger series to better understand their clinical relevance. Nevertheless, our preliminary study suggests a subclinical but measurably higher rate of microembolic events in CAS procedures. Our findings should be further evaluated in a larger randomized trial.

AUTHOR CONTRIBUTIONS

Conception and design: LC
Analysis and interpretation: LC, PM
Data collection: MG, AR, WM
Writing the article: LC
Critical revision of the article: ES
Final approval of the article: FS, PF
Statistical analysis: LC
Obtained funding: Not applicable
Overall responsibility: FS

REFERENCES

1. Sacco RL, Adams R, Albers G, Alberts MJ, Benavente O, Furie K, et al. Guidelines for prevention of stroke in patients with ischemic stroke or transient ischemic attack. Stroke 2006;37:577-617.
2. Ederle J, Featherstone RL, Brown MM. Randomized controlled trials comparing endarterectomy and endovascular treatment for

carotid artery stenosis: a Cochrane systematic review. Stroke 2009; 40:1373-80.
3. Rapp JH, Wakil L, Sawhney R, Pan XM, Yenari MA, Glastonbury C, et al. Subclinical embolization after carotid artery stenting: new lesions on diffusion-weighted magnetic resonance imaging occur postprocedure. J Vasc Surg 2007;45:867-74.
4. Heyer EJ, Adams DC, Solomon RA, Todd GJ, Quest DO, McMahon DJ, et al. Neuropsychometric changes in patients after carotid endarterectomy. Stroke 1998;1110-15.
5. Wunderlich MT, Ebert AD, Kratz T, Goertler M, Jost S, Herrmann M. Early neurobehavioral outcome after stroke is related to release of neurobiochemical markers of brain damage. Stroke 1999;30:1190-5.
6. Connolly ES, Winfree CJ, Rampersad A, Sharma R, Mack WJ, Mocco J, et al. Serum S100B protein levels are correlated with subclinical neurocognitive declines after carotid endarterectomy. Neurosurgery 2001; 49:1076-83.
7. Pearson S, Maddern G, Fitridge R. Cognitive performance in patients after carotid endarterectomy. J Vasc Surg 2003;38:1248-53.
8. Palombo D, Lucertini G, Mambrini S, Zettin M. Subtle cerebral damage after shunting vs non shunting during carotid endarterectomy. Eur J Vasc Endovasc Surg 2007;34:546-51.
9. Barone FC, Clark RK, Price WJ, White RF, Feuerstein GZ, Storer BL, et al. Neuron-specific enolase increases in cerebral and systemic circulation following focal ischemia. Brain Res 1993;623:77-82.
10. Hardemark H-G, Ericsson N, Kotwica Z, Rundstrom G, Mendel-Hartvig I, Olsson Y, et al. S-100 protein and neuron-specific enolase in CSF after experimental traumatic or focal ischemic brain damage. J Neurosurg 1989;71:727-31.
11. Buttner T, Weyers S, Postert T, Sprengelmeyer R, Kuhn W. S-100 protein: serum marker of focal brain damage after ischemic territorial MCA infarction. Stroke 1997;28:1961-5.
12. Cunningham RT, Watt M, Winder J, McKinstry S, Lawson JT, Johnston CF, et al. Serum neuron-specific enolase as an indicator of stroke volume. Eur J Clin Invest 1996;26:298-303.
13. Missler U, Wiesmann M, Friedrich C, Kaps M. S-100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke. Stroke 1997;28: 1956-60.
14. Persson L, Hardemark H-G, Gustafsson J, Rundstrom G, Mendel-Hartvig I, Esscher T, et al. S-100 protein and neuron-specific enolase in cerebrospinal fluid and serum: markers of cell damage in human central nervous system. Stroke 1987;18:911-8.
15. Sindic CJM, Chalon MP, Cambiaso CL, Laterre EC, Masson PL. Assessment of damage to the central nervous system by determination of S-100 protein in the cerebrospinal fluid. J Neurol Neurosurg Psychiatry 1982;45:1130-5.
16. Biberthaler P, Mussack T, Wiedermann E, Gilg T, Soyka M, Koller J, et al. Elevated serum levels of S-100B reflect the extent of brain injury in alcohol intoxicated patients after mild head trauma. Shock 2001;16:97-101.
17. Jonsson H, Johnsson P, Alling C, Backstrom M, Bergh C, Blomquist S, et al. S100beta after coronary artery surgery: release pattern, source of contamination, and relation to neuropsychometric outcome. Ann Thorac Surg 1999;68:2202-8.
18. Zimmer DB, Cornwall EH, Landar A, Song W. The S100 protein family: history, function and expression. Brain Res Bull 1995;37: 417-29.
19. Jonsson H, Johnsson P, Hoglund P, Alling C, Blomquist S. Elimination of S-100B and renal function after cardiac surgery. J Cardiothorac Vasc Anesth 2002;14:698-701.
20. Jauch EC. Diagnosis of stroke: the use of serum markers. Adv Emerg Card Neurovasc Care 2002;39-45.
21. Cooper EH. Neuron-specific enolase. Int J Biol Markers 1994;9: 205-10.
22. Ingebrigsten T, Romner B. Biochemical serum markers for brain damage: a short review with emphasis on clinical utility in mild head injury. Restorative Neurology and Neuroscience 2003;21:171-6.
23. Staikov IN, Arnold M, Mattle HP, Remonda L, Sturzenegger M, Baumgartner RW, et al. Comparison of the ECST, CC, and NASCET grading methods and ultrasound for assessing carotid stenosis. European Carotid Surgery Trial. North American Symptomatic Carotid Endarterectomy Trial. J Neurol 2000;247:681-6.
24. Tegos TJ, Stavropoulos P, Sabetai MM, Khodabakhsh P, Sassano A, Nicolaides AN. Determinants of carotid plaque instability: echoicity versus heterogeneity. Eur J Vasc Endovasc Surg 2001;22:22-30.
25. Moseley ME, Cohen Y, Mintorovitch J, Chileuitt L, Shimizu H, Kucharczyk J, et al. Early detection of regional cerebral ischemia in cats: comparison of diffusion-and T2-weighted MRI and spectroscopy. Magn Reson Med 1990;14:330-46.
26. Yoneda Y, Tokui D, Hanihara T, Kitagaki H, Tabuchi M, Mori E. Diffusion-weighted magnetic resonance imaging: detection of ischemic injury 39 minutes after onset in a stroke patient. Ann Neurol 1999;45: 794-7.
27. Feiwell RJ, Besmertis L, Sardar R, Saloner DA, Rapp JH. Detection of clinically silent infarcts after carotid endarterectomy by use of diffusion-weighted imaging. Am J Neuroradiol 2001;22:646-9.
28. Forbes KP, Shill KA, Britt PM, Zabramski HM, Spetzler RF, Heiserman HE. Assessment of silent embolism from carotid endarterectomy by use of diffusion-weighted imaging. Am J Neuroradiol 2001;22:650-3.
29. Roh HG, Byun HS, Ryoo JW, Na DG, Moon WJ, Lee BB, et al. Prospective analysis of cerebral infarction after carotid endarterectomy and carotid artery stent placement by using diffusion-weighted imaging. Am J Neuroradiol 2005;26:376-84.
30. Hauth EA, Jansen C, Drescher R, Schwartz M, Forsting M, Jaeger HJ, et al. MR and clinical follow-up of diffusion-weighted cerebral lesions after carotid artery stenting. Am J Neuroradiol 2005;26:2336-41.
31. Folstein MF, Folstein SE, McHugh PR. Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-98.
32. Hodkinson HM. Evaluation of a mental test score for assessment of mental impairment in the elderly. Age and ageing 1972;1:233-8.
33. Crum RM, Anthony JC, Bassett SS, Folstein MF. Population-based norms for the Mini-Mental State Examination by age and educational level. JAMA 1993;269:2386-91.
34. Bornstein RA, Benoit BG, Trites RL. Neuropsychological changes following carotid endarterectomy. Can J Neurol Sci 1981;8:127-32.
35. Antonelli Incalzi R, Gemma A, Landi F, Pagano F, Capparella O, Snider F, et al. Neuropsychologic effects of carotid endarterectomy. J Clin Exp Neuropsychol 1997;19:785-94.
36. Rollason WN, Robertson GS, Cordiner CM, Hall DJ. A comparison of mental function in relation to hypotensive and normotensive anaesthesia in the elderly. Br J Anaesth 1971;43:561-6.
37. Owens M, Pressman M, Edwards AE, Tourtellotte W, Rose JG, Stern D, et al. The effect of small infarcts and carotid endarterectomy on postoperative psychologic test performance. J Surg Res 1980;28: 209-16.
38. Jordan WD Jr, Voellinger DC, Doblar DD, Plyushcheva NP, Fisher WS, McDowell HA. Microemboli detected by transcranial Doppler monitoring in patients during carotid angioplasty versus carotid endarterectomy. Cardiovasc Surg 1999;7:33-8.
39. Kilminster S, Treasure T, McMillan T, Holt DW. Neuropsychological change and S-100 protein release in 130 unselected patients undergoing cardiac surgery. Stroke 1999;30:1869-74.
40. Rasmussen LS, Christiansen M, Johnson J, Gronholdt ML, Moller JT. Subtle brain damage cannot be detected by measuring neuron-specific enolase and S-100B protein after carotid endarterectomy. J Cardiothorac Vasc Anesth 2000;14:166-70.
41. Brightwell RE, Sherwood RA, Athanasiou T, Hamady M, Cheshire NJW. The neurological morbidity of carotid revascularization: using markers of cellular brain injury to campare CEA and CAS. Eur J Vasc Endovasc Surg 2007;34:552-60.

Submitted May 31, 2009; accepted Oct 4, 2009.

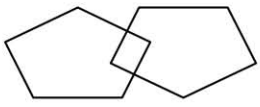
Additional material for this article may be found online at www.jvascsurg.org.

APPENDIX (online only).

Mini-Mental State Examination (MMSE)

Patient's Name: _____ Date: _____

***Instructions:* Ask the questions in the order listed. Score one point for each correct response within each question or activity.**

Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day of the week? Month?"
5		"Where are we now: State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible. Number of trials: _____
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, …) Stop after five answers. Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.) 
30		TOTAL

(Adapted from Rovner & Folstein, 1987)

Instructions for administration and scoring of the MMSE

Orientation (10 points):

- Ask for the date. Then specifically ask for parts omitted (e.g., "Can you also tell me what season it is?"). One point for each correct answer.
- Ask in turn, "Can you tell me the name of this hospital (town, county, etc.)?" One point for each correct answer.

Registration (3 points):

- Say the names of three unrelated objects clearly and slowly, allowing approximately one second for each. After you have said all three, ask the patient to repeat them. The number of objects the patient names correctly upon the first repetition determines the score (0-3). If the patient does not repeat all three objects the first time, continue saying the names until the patient is able to repeat all three items, up to six trials. Record the number of trials it takes for the patient to learn the words. If the patient does not eventually learn all three, recall cannot be meaningfully tested.
- After completing this task, tell the patient, "Try to remember the words, as I will ask for them in a little while."

Attention and Calculation (5 points):

- Ask the patient to begin with 100 and count backward by sevens. Stop after five subtractions (93, 86, 79, 72, 65). Score the total number of correct answers.
- If the patient cannot or will not perform the subtraction task, ask the patient to spell the word "world" backwards. The score is the number of letters in correct order (e.g., dlrow=5, dlorw=3).

Recall (3 points):

- Ask the patient if he or she can recall the three words you previously asked him or her to remember. Score the total number of correct answers (0-3).

Language and Praxis (9 points):

- Naming: Show the patient a wrist watch and ask the patient what it is. Repeat with a pencil. Score one point for each correct naming (0-2).
- Repetition: Ask the patient to repeat the sentence after you ("No ifs, ands, or buts."). Allow only one trial. Score 0 or 1.
- 3-Stage Command: Give the patient a piece of blank paper and say, "Take this paper in your right hand, fold it in half, and put it on the floor." Score one point for each part of the command correctly executed.
- Reading: On a blank piece of paper print the sentence, "Close your eyes," in letters large enough for the patient to see clearly. Ask the patient to read the sentence and do what it says. Score one point only if the patient actually closes his or her eyes. This is not a test of memory, so you may prompt the patient to "do what it says" after the patient reads the sentence.
- Writing: Give the patient a blank piece of paper and ask him or her to write a sentence for you. Do not dictate a sentence; it should be written spontaneously. The sentence must contain a subject and a verb and make sense. Correct grammar and punctuation are not necessary.
- Copying: Show the patient the picture of two intersecting pentagons and ask the patient to copy the figure exactly as it is. All ten angles must be present and two must intersect to score one point. Ignore tremor and rotation.

(Folstein, Folstein & McHugh, 1975)

Interpretation of the MMSE

Method	Score	Interpretation
Single Cutoff	<24	Abnormal
Range	<21	Increased odds of dementia
	>25	Decreased odds of dementia
Education	21	Abnormal for 8th grade education
	<23	Abnormal for high school education
	<24	Abnormal for college education
Severity	24-30	No cognitive impairment
	18-23	Mild cognitive impairment
	0-17	Severe cognitive impairment

Sources:

- Crum RM, Anthony JC, Bassett SS, Folstein MF. Population-based norms for the mini-mental state examination by age and educational level. JAMA 1993;269:2386-91.
- Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-98.
- Rovner BW, Folstein MF. Mini-mental state exam in clinical practice. Hosp Pract 1987;22:99, 103, 106, 110.
- Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. J Am Geriatr Soc 1992;40:922-35.

Source: www.medicine.uiowa.edu/igec/tools/cognitive/MMSE.pdf Provided by NHCQF, 0106-410