Perioperative Embolization Load and S-100β Do Not Predict Cognitive Outcome after Carotid Revascularization

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Background: Cognitive changes after carotid revascularization have been reported in 10–20% of patients. The etiology of cognitive impairments remains largely unknown. This study evaluates the predictive value of S-100β serum values and perioperative microembolization on cognition after carotid revascularization.

Methods: Forty-six patients with significant carotid stenosis underwent carotid endarterectomy (CEA, n = 26), transfemoral carotid artery stenting with distal protection (CASdp, n = 10), or transcervical carotid stenting with dynamic flow reversal (CASfr, n = 10). Twenty-six matched vascular patients without carotid stenosis were recruited as controls. All patients underwent comprehensive cognitive testing on the day before and 1 month after carotid revascularization. S-100β analysis was performed in 31 cases pre-, peri-, and 2, 6, and 24 hr after carotid surgery, and in 25 patients transcranial Doppler monitoring was done during surgery.

Results: In the 3 treatment groups similar transient increases in S-100β values were observed. CASdp was associated with a higher embolic load than CEA and CASfr, while CEA was also associated with less microembolization than CASfr. Cognitive improvement or deterioration could not be predicted by S-100β or perioperative embolic load for any of the investigated cognitive domains.

Conclusions: Cognitive deterioration could not be predicted using perioperative embolic load and S-100β changes. A similar inverted u-curve of the S-100β levels was observed in the 3 groups and may be caused by impairment in the blood–brain barrier during intervention, and not due to cerebral infarction. Distal protection CAS is associated with a higher embolic load than transcervical CAS using dynamic flow reversal and CEA, but the long-term impact of this higher embolic load is yet unknown. Perfusion-related measures seem promising in their ability to predict cognitive decline.

INTRODUCTION

To reduce the stroke risk in patients with significant carotid artery stenosis, carotid endarterectomy (CEA) and carotid artery stenting (CAS) are performed.1,2 Many studies have shown that CAS with distal protection filters (CASdp) is associated with higher stroke rates and incidence of postoperative lesions on diffusion-weighted magnetic resonance imaging (DW MRI).3 In an effort to reduce these higher strokes and new DW MRI lesion rates, proximal protection is increasingly used.4,5 Transcervical CAS with dynamic flow reversal (CASfr) has been shown to be safe with a low stroke, death, and myocardial infarction rate.6,7 Furthermore, it is associated with

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a reduced number of new DW MRI lesions compared
with transfemoral CAS with distal protection devices
(CASdp).6,7 Manipulation in the aortic arch and
origin of the common carotid is avoided, and ideally
during flow reversal emboli should theoretically not
be able to damage the brain.7

Besides a focus on stroke and other primary
outcome measures, as Siddiqui and Hopkins8 stated,
it is important to assess the cognitive effects of carotid
revascularization, as even asymptomatic patients
may sometimes benefit from revascularization.
Indeed, recent studies9–13 reported improvements
for some patients, while other patients showed cogni-
tive declines. On the one hand, carotid revasculariza-
tion may improve the blood flow to the brain and
hence result in cognitive improvement. On the other
hand, perioperative microembolization and hypo-
perfusion, and postoperative hyperperfusion may
cause cognitive decline.11,12 To date, it is unclear
how these factors interact. In this study, we will focus
on possible factors predicting negative cognitive
outcome, such as perioperative embolization load
and indicators of ischemic brain damage.

To study perioperative embolization during ca-
rotid revascularization, transcranial Doppler ultra-
sonography (TCD) is an effective tool.13 Larger
embolic loads for CASdp have been observed in
comparison with CEA.14–16 Although Ribo et al.17
revealed that CASfr is able to reduce emboli showers
typically observed during stent deployment, direct
comparisons of transcervical CASfr with CASdp
and CEA have not been published. It is important
to examine perioperative embolization as it has
shown to be linked with new DW MRI lesions post
intervention.18

A sensitive marker of cerebral injury and blood–
brain barrier dysfunction19–21 is the neuroprotein
S-100β. S-100β rises in patients with focal brain dam-
age after ischemic territorial middle cerebral artery
(MCA) infarction,19 which makes this biomarker
especially relevant to study cerebral damage after ca-
rotid revascularization because the MCA arises from
the internal carotid. Studies connecting S-100β to
perioperative embolization assessed by TCD reported
conflicting results.22,23 S-100β shows maximum
levels most often within 24 hr after cardiac surgery,24
has a biological half-life of approximately 25 min,
and is rapidly excreted by the kidney.25

The factors that may lead to cognitive deterio-
ration after carotid treatment have not been clearly
identified. This study will directly compare periop-
ervative embolic load and S-100β serum level
changes on cognitive outcome will be investigated.

**MATERIALS AND METHODS**

**Patients and Controls**

Between February 2011 and January 2014, 46 pa-
tients with significant internal carotid artery stenosis
without ostial common carotid artery lesions or tan-
dem lesions (≥80% for asymptomatic and ≥60% for
symptomatic lesions on duplex ultrasound) were
included, of which 26 underwent CEA, 10 CASdp,
and 10 CASfr. Exclusion criteria were history of pre-
vious carotid interventions, coronary artery bypass
grafting or stroke within 2 years, age >80 years, psy-
chiatric or neurological disorders, alcohol abuse, and
a Mini Mental State Examination (MMSE) score
lower than 24.

To avoid any influence of recent brain damage on
the possible cognitive changes evoked by revascular-
ization, symptomatic patients were also excluded if
they suffered from a recent acute stroke.9 So only
symptomatic patients who experienced amaurosis
fugax and/or transient ischemic attack were included.
Twenty-six patients with peripheral arterial disease
were selected from the vascular outpatient’s clinic as
a matched control group using the same exclusion
criteria. Controls were matched for sex, age, and so-
cioeconomic status (SES). The Hollingshead’s in-
dex,26 a computed score based on education and
occupation level, was used as a measurement of
SES. All controls had <50% carotid stenosis on
duplex ultrasound. All 46 carotid patients participated
in the cognitive study. Of these, only 31 patients had
S-100β evaluation, and 25 patients received TCD
monitoring due to logistical reasons or a poor trans-
temporal insonation window for TCD in some pa-
tients.27 Twenty-one carotid patients had S-100β,
TCD monitoring, as well as cognitive assessment.
The control group solely underwent cognitive testing.
The Ghent University Hospital’s Ethical Committee
approved this prospective study and all participants
gave written informed consent.

**Carotid Revascularization Procedures**

The choice between CEA and CAS was based on the
individual and anatomical characteristics, comor-
bidities of the patient, and the patient’s preference.
The decision was made by a multidisciplinary team
taking into account the international guidelines.28
CEA was routinely carried out under general anes-
thesia using selective shunting and Dacron patch
plasty. CAS was carried out under local anesthesia
with selective predilation, mandatory stenting, and
selective postdilation. All CAS patients received
dual antiplatelet therapy (aspirin and clopidogrel).
In transfemoral CAS, distal filter embolic protection
was always used, while in transcervical CAS dynamic flow reversal was created between the common carotid artery and contralateral common femoral vein using the ENROUTE™ Neuroprotection System (Silk Road Medical, Sunnyvale, CA).

**Neuropsychological Assessment**

Besides the MMSE as a dementia-screening test, a neuropsychological test battery consisting of 13 tests was used, of which 18 variables were derived. These variables were allocated to their respective cognitive domain: attention, long-term memory, executive functioning, fine motor abilities, or visuospatial functioning (see Table II in Plessers et al. for more information about the specific neuropsychological tests). Neuropsychological examinations were performed by M.P. 1 day before and 1 month after surgery and took approximately 90–120 min to complete. Identical time intervals were used for the control group.

**Transcranial Doppler**

Perioperative TCD monitoring was performed unilaterally using a commercially available TCD system (DWL Doppler Box™; Compumedics Germany GmbH, Germany). A 2-MHz transducer was placed on the ipsilateral temporal skull window before the start of the carotid surgery and recordings of the intervention were made from incision until closure.

Emboli were counted manually according to consensus statements. Only unidirectional high-intensity transient signals (<300 ms) at least 7 dB higher than that of the background signal with a distinctive “chirp,” “snap,” or “moan” sound were recorded as emboli. Because fluid-filled syringes always contain small air bubbles, even after thorough desufflation and these small air bubbles are of low clinical value, embolic signals directly related to the injection of contrast fluid were discarded. A global TCD score with 1 s of emboli showers or curtains counting as 10 separate emboli was computed as done previously by Brightwell et al. As such, we obtained a mean global embolic score that is comparable for the 3 types of surgery.

**Serum S-100β Biomarker**

Blood samples were collected immediately before carotid surgery, after declamping or retrieval of the embolic protection device, and 2, 6, and 24 hr post intervention. Blood samples were centrifuged at 1500 rpm for 15 min at 20°C. The resulting serum was stored in multiple aliquots at −25°C. Serum levels of S-100β were determined using an automatic electrochemiluminescence assay (S100 Cobas®) with a measuring range of 0.005–39 μg/L. The median value and 95th percentile for healthy adults is 0.046 and 0.105 μg/L, respectively. The biochemist responsible for carrying out these analyses was blinded to the revascularization group and TCD data.

**Neurological Evaluation**

All patients received a preoperative (1 day before surgery) and postoperative (after 1 month) clinical neurological evaluation by one of the authors (D.H.).

**Statistical Analyses**

Raw cognitive scores were rescaled so that higher scores represent better cognitive results. Next, difference scores were calculated for every subject: difference score = test score after 1 month – preoperative test score. Thereafter, z-scores were calculated using the difference scores of the control group as a test–retest measurement error by the following formula: “z-score = (difference score patient – mean difference score control group)/standard deviation difference score control group.” This way, the z-scores represent deviations from the expected test–retest effects. The larger the absolute z-score, the more the subject deviates from what is expected as a normal test–retest difference. Thus, the control group data were used to estimate the practice effect. Domain z-scores were the calculated mean of the relevant variables and represent the mean change of a cognitive domain when compared with the mean change of the control group.

Linear regression analysis was used to assess the predictive value of S-100β and TCD perioperative embolization on the 5 cognitive domain scores for all carotid patients. Chi-squared tests were performed to compare categorical variables such as clinical symptoms in cross tables and one-way analysis of variance (ANOVA) was used to compare continuous variables, such as embolic load. Changes over time and between patient groups for S-100β were examined with repeated measures ANOVA with Huynh-Feldt correction. Residuals for every variable showed a normal distribution.

**RESULTS**

Demographic characteristics are displayed in Table I. All revascularization groups and the control group had similar preoperative MMSE scores (F(3,71) = 0.80, P = 0.50). Most patients experienced no neurological symptoms post intervention except for 1 CEA patient who had a cranial nerve injury and 1 CASdp patient who suffered from a minor stroke in
hospital. No death or myocardial infarct occurred and no patients were lost to follow-up.

Because S-100β levels showed the highest mean peak value 2 hr after surgery, this value was used in further analysis. None of the cognitive changes in long-term memory, attention, executive functioning, fine motor abilities, and visuospatial functioning could be predicted using S-100β or the TCD microembolic load for the whole group of carotid patients (Table II).

However, a significant difference between the treatment groups for perioperative embolization was observed ($F(2,24) = 55.91, P < 0.001$). CASdp ($M = 584$) was associated with a significant higher embolic load than CEA ($M = 62, P < 0.001$) and CASfr ($M = 184, P < 0.001$) and CEA was also associated with fewer emboli than CASfr ($P = 0.02$; Fig. 1).

Because the last measurement of S-100β (24 h postsurgery) was missing in some patients ($n = 5$) and the acute effect of the surgery on S-100β at 24 hr had already dissolved, we decided to perform the repeated measures test only on the 4 first measurements to avoid listwise deletion of cases and a subsequently lower statistical power. There was an expected transient increase in the S-100β level in every group ($F(2.45,56.43) = 30.97, P < 0.001$), but no significant group differences ($F(2.23) = 0.69, P = 0.51$) or interactions ($F(4.91,56.43) = 1.55, P = 0.19$) could be detected (Fig. 2). There was no correlation between the amount of perioperative emboli and rise in S-100β ($r = -0.18, P = 0.44$).

### Table I. Demographic characteristics

<table>
<thead>
<tr>
<th>Demographic and clinical characteristics</th>
<th>CEA ($n = 26$)</th>
<th>CASdp ($n = 10$)</th>
<th>CASfr ($n = 10$)</th>
<th>Control ($n = 26$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>68.2 (6.7)</td>
<td>64.8 (9.2)</td>
<td>70.5 (7.0)</td>
<td>67.3 (7.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>15 (58%)</td>
<td>7 (70%)</td>
<td>6 (60%)</td>
<td>17 (65%)</td>
<td>0.89</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>29.5 (14.6)</td>
<td>33.1 (13.2)</td>
<td>27.8 (11.3)</td>
<td>29.6 (12.7)</td>
<td>0.85</td>
</tr>
<tr>
<td>Symptomatic lesion</td>
<td>12 (46%)</td>
<td>4 (40%)</td>
<td>2 (20%)</td>
<td>–</td>
<td>0.35</td>
</tr>
<tr>
<td>No. of days between symptoms and surgery</td>
<td>40.7 (39.7)</td>
<td>19.3 (15.2)</td>
<td>60.0 (42.4)</td>
<td>–</td>
<td>0.42</td>
</tr>
<tr>
<td>Contralateral carotid artery stenosis (&gt;$50%$)</td>
<td>10 (38%)</td>
<td>6 (60%)</td>
<td>5 (50%)</td>
<td>–</td>
<td>0.49</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8 (31%)</td>
<td>4 (40%)</td>
<td>4 (40%)</td>
<td>6 (23%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>19 (73%)</td>
<td>3 (30%)</td>
<td>5 (50%)</td>
<td>17 (65%)</td>
<td>0.095</td>
</tr>
<tr>
<td>Statins</td>
<td>24 (92%)</td>
<td>7 (70%)</td>
<td>9 (90%)</td>
<td>23 (88%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Familial vascular risk factors</td>
<td>20 (77%)</td>
<td>7 (70%)</td>
<td>8 (80%)</td>
<td>16 (62%)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Values are in mean (standard deviation) or $n$ (%).

### Table II. Linear regression analysis of the 5 cognitive domains with S-100β and embolic load as predictors

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>$F$ value</th>
<th>df1, df2</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-term memory</td>
<td>2.31</td>
<td>3, 19</td>
<td>0.12</td>
</tr>
<tr>
<td>Attention</td>
<td>0.62</td>
<td>3, 19</td>
<td>0.61</td>
</tr>
<tr>
<td>Executive functioning</td>
<td>0.82</td>
<td>3, 19</td>
<td>0.50</td>
</tr>
<tr>
<td>Fine motor abilities</td>
<td>0.54</td>
<td>3, 19</td>
<td>0.66</td>
</tr>
<tr>
<td>Visuospatial functioning</td>
<td>1.04</td>
<td>3, 19</td>
<td>0.40</td>
</tr>
</tbody>
</table>

### DISCUSSION

CEA, CASdp, and CASfr resulted in a similar cognitive evolution, around 10–20% of patients show either cognitive improvement or deterioration after revascularization (see Plessers et al.). None of the changes in the 5 cognitive domains could be predicted using S-100β serum levels and perioperative embolic load. It appears to be difficult to predict which patients will show postoperative cognitive decline. Many studies trying to find a relationship between S-100β and cognition after carotid revascularization failed to find an association and it was concluded that the predictive value of S-100β on cognition is inconclusive. The transient increase in S-100β early after CASdp and especially CEA has
been noted in several studies, and is most often regarded as a consequence of an impaired blood–brain barrier caused by balloon dilation or clamping rather than the consequence of brain damage.\textsuperscript{33–37} Indeed, the fact that a correlation between S-100\textsubscript{b} and embolic load could not be demonstrated in this study seems to implicate that S-100\textsubscript{b} may not be an ideal measure for cerebral infarction after carotid revascularization, but actually represents changes in the blood–brain barrier.\textsuperscript{33–37} We did not find a group difference between CEA, CASdp, and CASfr on S-100\textsubscript{b}, which confirms the findings of Brightwell et al.\textsuperscript{22} who compared CEA with CASdp.

In contrast, TCD analysis unveils significant differences in perioperative embolic load between the treatment modalities. Previous studies\textsuperscript{14–16} have shown that CASdp is associated with a higher embolic burden than CEA. Furthermore, this study shows that CASfr causes less embolization than CASdp. It appears that direct carotid access and dynamic flow reversal protect the brain better against microembolization than transfemoral carotid stenting with distal protection. This study confirms that distal filters do not always result in a reduction of perioperative microembolization.\textsuperscript{38} Direct carotid access avoids any manipulation in the aortic arch while flow reversal is possibly more effective because protection is in place before crossing the lesion.\textsuperscript{7} The low embolization rate during CEA can however not yet be achieved with CASfr.

The detected embolic load did not predict cognitive decline after surgery, as also found by other studies.\textsuperscript{15,39,40} Probably other factors such as embolic size and type (gaseous versus particulate) are more important than the mere number of emboli, that is, larger and particulate emboli are expected to have a worse outcome than small and gaseous emboli.\textsuperscript{9} Up until now, current technology allows no valid differentiation between gaseous and particulate emboli.\textsuperscript{41,42} Technological advances may have the potential to further improve the clinical relevance of TCD monitoring.

This exploratory study illustrates that it is difficult to predict postoperative cognitive deterioration, even when combining different datasets such as perioperative embolization and S-100\textsubscript{b}. It appears that cognitive evolution after revascularization is unpredictable.\textsuperscript{39} Indeed, recent studies and systematic reviews point out that the vast majority of studies that have attempted to correlate cognitive changes with the amount and size of new DW MRI ischemic lesions after revascularization have failed to find this association.\textsuperscript{9,43} Most of these lesions appear silent. In contrast, research has described that restoring a preoperative low blood flow in MCA is associated with improved cognition following intervention.\textsuperscript{44–47} While postoperative hyperperfusion is linked with cognitive decline.\textsuperscript{48–50} It seems that embolization, DW MRI, and S-100\textsubscript{b} remain unable to predict cognitive changes so far, while measures focusing on perfusion may be the key to successfully detect patients at risk for cognitive decline or patients who are likely to benefit from carotid revascularization.\textsuperscript{7}

Although our study is limited due to a small sample size and the lack of brain MRI data, we can conclude that if there is an effect of perioperative embolization and S-100\textsubscript{b} on cognition, this effect is not very robust. Other studies have often failed to find associations between these measures and cognition in CEA and CASdp\textsuperscript{15,30,31,39,40} and the combination of these measures does not also appear to predict cognitive deterioration as shown in this study. The strength of this study is that differences in S-100\textsubscript{b} serum levels and perioperative embolization between CEA, CASdp, and CASfr have been studied including its effect on cognitive alterations using a comprehensive neuropsychological test battery. Our study did not rely on short screening instruments such as the MMSE, which are considered insufficient.\textsuperscript{9,52} Finally, we had no drop-outs at 1 month, preventing possible subgroup confounders.

**CONCLUSION**

In conclusion, this study shows no clear influence of S-100\textsubscript{b} serum levels and perioperative embolization...
on cognitive changes after carotid revascularization. CEA, CASdp, and CASfr show a similar inverted u-curve in S-100β values. CASdp is associated with a higher embolization rate in comparison with CEA and CASfr, while CEA is associated with fewer emboli than CASfr. Further research remains warranted.

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