The biochemical markers associated with TCD to evaluate the curative effect after Carotid Artery Stenting

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Research Article

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Abstract

Introduction This study associated the expression of GFAP and S100B in serum with the imaging tools (TCD and DWI) to confirm these cerebral biochemical markers as surrogate outcome methods for evaluating the curative effect after CAS.

Methods and materials 72 patients with unilateral carotid stenosis who underwent CAS were enrolled in the operation group. The blood samples of the operation patients were collected on four different time points: T1: the day before operation; T2: 1 day (24 hours) after operation; T3: 3 days (72 hours) after operation; T4: 30 days after operation. The operation patients were performed on the MRI after CAS to evaluate the post-operative lesion and received the TCD to monitor the changes of hemodynamics. 47 patients who were excluded for carotid artery stenosis by DSA were selected as the control group. The blood samples of patients in control group were collected at D1 (before DSA) and D2 (24 hours after DSA). The concentrations of GFAP and S100B in serum were measured with ELISA.

Results (1) The MFV (pre-operation, post-operation, 30 days follow-up: 47.65±17.24, 62.37±18.25, 70.29±16.89; $P<0.05$) and PI (pre-operation, post-operation, 30 days follow-up: 0.78±0.21, 0.98±0.19, 1.02±0.20; $P<0.05$) increased significantly in the ipsilateral MCA after CAS. And at 30 days follow-up, the CVR improved significantly (post-operation, 30 days follow-up: 27.47±12.13, 31.92±10.94; $P<0.05$). Patients with different degrees of stenosis, the more severe stenosis in carotid artery, the more obvious improvement of CVR at the 30 days follow-up. (2) After CAS, the serum concentrations of GFAP and S100B increased to the peak at 24 hour after operation (T2), and then decrease gradually (T2>T3>T4; $P < 0.05$). Furthermore, the serum concentrations of GFAP ($r=0.71$, $P<0.05$) and S100B ($r=0.78$, $P<0.05$) correlated positively with CVR at 30 days after CAS. (3) 29/72 patients (40.28%) were shown the emerging hyperintense in DWI after CAS.

Conclusion Our finding proven that the trend of GFAP and S100B in serum after CAS had a positive correlation to the improved hemodynamics which was verified by TCD. We recommend the biochemical markers (GFAP and S100B) associated with TCD to evaluate the curative effect after CAS.

Introduction

Carotid Artery Stenting (CAS) is a minimally invasive endovascular method advocated in the recent 20 years. Among patients with symptomatic or asymptomatic carotid stenosis, CAS and Carotid Endarterectomy (CEA) are preventive measures for ischemic cerebrovascular disease. The risk of the composite primary outcome of stroke, myocardial infarction, and death do not significantly differ in patient groups undergoing CAS or undergoing CEA [1]. Studies have confirmed that the rate of symptomatic internal carotid artery stenosis is >50%, the rate of asymptomatic internal carotid artery stenosis is >80%, and at least one patient with high risk factors for CEA treatment, the efficacy of CAS is not inferior to CEA [2]. Three large international multicenter randomized controlled trials also failed to demonstrate that CAS was less effective than CEA [3-4]. But in terms of health-related quality of life, CAS

is easy to accept for patients because of its minimally invasive operation and short postoperative hospitalization period [5].

In clinical work, Transcranial Doppler (TCD) is used to detect the stenosis of intracranial artery before CAS [6], monitor the embolus during CAS [7] and analyze the cerebral hemodynamic changes after CAS [8-9]. Some researchers pointed out that the ischemic brain lesion after CAS could increase the risk of recurrent cerebrovascular events [10]. So they recommended the application of Diffusion Weighted Imaging (DWI) as a surrogate outcome measure for procedural stroke in carotid revascularisation procedure [11]. But the two above imaging tools in some extent have the disadvantages of poor repeatability, high costs and susceptible to the operators.

The expression of glial fibrillary acidic protein (GFAP) and S100B (S100B protein) in cerebrospinal fluid and/or serum are parallel to the size of infarction, the deficit of neurological function, the prognosis of disability after cerebral stroke and the degree of brain injury [12-15]. But the application of biochemical markers after CAS in clinical work reported rarely. Therefore, this study associated the expression of GFAP and S100B in serum with the imaging tools (TCD and DWI) to confirm these cerebral biochemical markers as surrogate outcome methods for evaluating the curative effect after CAS.

Materials And Methods

Objects: From December 2017 to November 2019, Patients who diagnose unilateral carotid stenosis according to the results of Digital Subtraction Angiography (DSA), then underwent CAS were assigned as the operation group. The inclusion criteria of the operation group are: (1) symptomatic stenosis was ≥ 50% and the clinical symptoms were consistent with the vascular area of stenosis; (2) asymptomatic stenosis was ≥ 70%; (3) age was > 18 years. Exclusion criteria of the operation group: (1) patients with nervous system related tumor diseases (such as meningioma, gliocytoma, primary or secondary malignant tumor of the brain, etc.); (2) patients with infectious diseases of nervous system (such as encephalitis, meningitis, myelitis, etc.); (3) patients with demyelination and degenerative diseases of nervous system (such as multiple sclerosis, optic neuromyelitis, Parkinson's disease, Alzheimer's disease, etc.); (4) patients with the severe heart, liver, kidney and lung disease could not tolerate surgery. At the same time, these patients who were excluded from intra- and extracranial vascular stenosis by DSA in neurology department were selected as the control group.

Operative procedures: The patients in the operation group were given dual antiplatelet therapy (aspirin 100mg and clopidogrel 75mg) seven days before CAS. The operation was performed by two experienced neuroscientists and all of the operation patients used the cerebral protection devices. Under the guidance of the guiding wire, the guiding catheter was placed near the opening of internal carotid artery in the affected side, and the protective umbrella was placed at the distal of the stenosis artery. Then the stent was placed along the guide wire in the stenosis area. The stent was implanted successfully after precise positioning. Then the angiography showed that the carotid artery was unobstructed, the stent was well

attached to the wall, no obvious residual stenosis was found, and the anterior blood flow was normal (TICI Level 3) [16]. Aspirin was taken for life after operation, combined with clopidogrel at least 6 months.

The standard of carotid artery stenosis: The degree of carotid artery stenosis was determined by two neurologists independently, the decision was reached by consensus. The criteria were calculated abide by the standard of European Carotid Surgery Study (ECST) [17] and North American symptomatic carotid endarterectomy (NASCET) [18]. And the degree of carotid artery stenosis was separated to three grades according to the results of DSA: Grade 1: 50-70%, Grade 2: 70-90%, Grade 3: >90%.

The measurement of serum samples: 5ml of venous blood was taken from the operation patients in the morning before operation (T1), 24 hours after CAS (T2), 72 hours after CAS (T3) and 30 days (T4) after CAS. Similarly, the serum was collected from the control patients in the morning before DSA (D1) and 24 hours after DSA (D2). These blood samples were centrifuged and stored at - 80°C.

The ELISA for detecting the concentrations of GFAP and S100B in serum was as follows: anti-GFAP (Human GFAP ELISA KIT, ZC-34594) and anti-S100B (Human S100B ELISA KIT, ZC-32056) antibodies were coated in 96 well microporous plates to make solid-phase carriers, and standards or samples are added to the micropores respectively. The GFAP and S100B are attached to their specific antibody binding on the solid-phase carriers, then after thorough washing, GFAP and S100B antibodies are added. After the unconjugated biotin antibodies are cleaned and the HRP labeled avidin is added, then they are washed thoroughly again. TMB substrate was added and converted to blue under the catalysis of peroxidase, and finally to yellow under the action of acid. The color depth was positively correlated with concentration of either GFAP or S100B protein. The absorbance (OD value) was measured at 450 nm wave length with the microplate reader, and the sample concentrations were calculated.

The evaluation of imaging tools: The examination was measured using a 2-MHz TCD machine. For the operation patients, the middle flow velocity (MFV) in middle cerebral artery (MCA), pulsatility index (PI systolic velocity minus diastolic velocity divided by MFV), cerebral vascular reactivity (CVR) on the ipsilateral side were measured before CAS, 24 hours after CAS and 30 days after CAS. The measurement of CVR was performed using the breath holding test (BHT). We performed the technique as breath holding for a minimum of 15 seconds after normal inspiration. And a percentage increase of MFV inferior to 20% was considered impaired CVR.

The Magnetic Resonance Imaging (MRI) was performed before CAS and 24 hours after CAS. Also, the scan sequences including T1-weighted, T2-weighted, fluid attenuated inversion recovery (FLAIR) and DWI. Hyperintense which is presented on the post-operation but is not presented on the pre-operation DWI sequences was considered as the occurrence of new ischemic lesion after CAS.

Statistical analysis: SPSS 23.0 was used for statistical analysis. The continuous variables were expressed as mean ± standard deviation ($\chi \pm s$), T test and one-way repeated measures analysis of variance (ANOVA) were used for the comparisons between groups and subgroups. The categorical

variables were expressed as frequency and percentage, and chi-square test was used for comparison. Correlation analysis used the method of Spearman. Statistical significance was defined as $P < 0.05$.

Results

Demographic data: Overall, 72 patients (age: 69.34±7.56 year, male: 47) were enrolled in the operation group and 47 patients (age: 64.25±10.83 year, male:27) were enrolled in the control group respectively. And there was no statistical difference between the operation group and the control group in demographic data (age, sex, diabetes mellitus, hypertension, heart disease, current smoker, HbA1c, uric acid, creatinine, obesity). The demographic data in the control and operation groups are shown in Table 1.

CAS: Among the operation patients, 19 patients were diagnosed the carotid artery stenosis in Grade 1 (50-70%), 38 patients in Grade 2 (70-90%) and 15 patients in Grade 3 (>90%) respectively. The changes in carotid artery stenosis before and after CAS are shown in Figure 1.

28 of 72 patients (38.89%) who had occurred the neurological symptom (stroke, transient ischemic attack and amaurosis fugax) before CAS. Besides, 5 of 72 (6.94%) patients occurred the complications (2 TIA, 2 stroke and 1 death) after CAS. And there were no myocardial infarction and hemorrhage at the 30 days follow-up visits after CAS.

TCD: After CAS, the MFV (pre-operation, post-operation, 30 days follow-up: 47.65±17.24, 62.37±18.25, 70.29±16.89; $P<0.05$) and PI (pre-operation, post-operation, 30 days follow-up: 0.78±0.21, 0.98±0.19, 1.02±0.20; $P<0.05$) increased significantly in the ipsilateral MCA. And the CVR improved significantly (post-operation, 30 days follow-up: 27.47±12.13, 31.92±10.94; $P<0.05$) at 30 days follow-up. For patients with different degrees of carotid artery stenosis, the more severe stenosis in carotid artery, the more obvious improvement of CVR at the 30 days follow-up after CAS. But the CVR had no statistical change at 24 hours after CAS (pre-operation, post-operation: 23.39±10.21, 27.47±12.13; $P>0.05$). The hemodynamic data in the operative patients is shown in Table 2.

DWI: Although some operative patients had occurred the neurological symptom before CAS, we operated the treatment after the stage of acute cerebral infarction for safety reasons. So the symptomatic patients who performed on MRI before CAS had no hyperintense in DWI images. However, there were 29 patients showed the emerging hyperintense in DWI after CAS. Among the 29 patients with hyperintense in DWI, 5 patients (2 TIA, 2 stroke and 1 death) occurred the ischemic events, and the remaining 24 patients showed the DWI+ rather than neurological deficits.

Biochemical markers: (1) The serum concentrations of GFAP and S100B in the control patients had no significant change after DSA ($P > 0.05$). (2) After CAS, the serum concentrations of GFAP and S100B increased to the peak at 24 hour after operation (T2), and then decrease gradually. There were the lowest values at the 30 days after CAS ($T2>T3>T4$; $P < 0.05$). Furthermore, the serum concentrations of GFAP ($r=0.71$, $P<0.05$) and S100B ($r=0.78$, $P<0.05$) correlated positively with CVR at 30 days after CAS. The

serum concentrations of GFAP and S100B in the patients before and after operation are shown in Table 3 and Figure 2. (3) Among the 5 patients with post-operative symptoms, the serum values of GFAP and S100B in 2 TIA increased temporarily at 24 hours after operation (T2), and then came back to the basal at 30 days after operation (T4). But for the 2 stroke and 1 death patients, the serum GFAP and S100B increased continuously even at the 30 days after CAS. And the fatal patient maintained a higher level than the stroke at any time points during the 1 month follow-up. The details for the 5 patients with post-operative complications are shown in Figure 3.

Table 1 Demographic data in the control and operation patients

	operation group (n=72)	control group (n=47)	T or χ2	P value
Mean±SD age (year)	69.34±7.56	64.25±10.83	-0.359	0.78
Sex (male)	47 (65.28)	27 (57.45)	1.002	0.09
Diabetes	33 (45.83)	10 (21.28)	0.358	0.07
Hypertension	51 (70.83)	21 (70.00)	0.694	0.63
Heart disease	12 (16.67)	9 (19.15)	0.454	0.32
Current smoker	13 (18.06)	8(17.02)	1.369	0.35
HbA1c (%)	5.88±0.40	6.04±0.76	-2.000	0.11
Uric Acid	304.23±47.30	325.44±50.68	1.267	0.27
Creatinine	67.35±12.69	59.39±10.96	2.598	0.22
Obesity	18 (25.00)	9 (19.15)	0.546	0.51

CAS: Carotid Artery Stenting; DSA: Digital Subtraction Angiography; HbA1c: glycosylated hemoglobin; Hypertension: systolic pressure≥140 mmHg and/ or diastolic pressure≥90 mmHg; Obesity: body mass index≥30 kg/m^2.

Table 2 The hemodynamic data in the operation patients

TCD values	pre-operation	post-operation	30 days follow-up
MFV（cm/s）	47.65±17.24	62.37±18.25*	70.29±16.89*
CVR（cm/s）	23.39±10.21	27.47±12.13&	31.92±10.94*
PI	0.78±0.21	0.98±0.19*	1.02±0.20*

MFV: middle flow velocity; PI: pulsatility index; CVR: cerebral vascular reactivity; MCA: middle cerebral artery.

With the release of carotid artery stenosis and the reconstruction of cerebral blood flow after CAS, the MFV and PI increased significantly in the ipsilateral MCA,(*P<0.05); the CVR improved significantly at 30 days follow-up (*P<0.05); but the CVR had no statistical change at 24 hours after CAS (&P>0.05).

Table 3 The serum concentrations of GFAP and S100B in the operation patients

Group	GFAP（pg/ml）	S100B（ng/ml）
D1 (before DSA)	20.059±10.219	0.853±0.162
D2 (24h after DSA)	21.392±09.022	0.909±0.127
T1 (before CAS)	25.392±11.022	1.500±0.804
T2 (24h after CAS)	30.877±14.979	2.074±1.082
T3 (72h after CAS)	27.038±12.294	1.786±0.975
T4 (30d after CAS)	21.038±13.458	0.886±0.315

Discussion

Carotid artery stenosis is an independent risk factor for cerebral ischemic disease [19]. The stenosis can reduce the blood flow velocity in the distal artery and weaken the cerebral vascular reactivity. The disordered hemodynamics caused by stenosis lead to the occurrence of hypoperfusion cerebral infarction. Besides, the embolus detached from the carotid atherosclerotic plaques can induce the cerebral embolism. CAS can not only dilate the stenosis in the carotid artery, but also eliminate the source of embolus, it is the optimal and minimally invasive endovascular prevention method for cerebral ischemic disease.

There are three pivotal compensatory mechanisms for regulating cerebral blood flow [20]. Firstly, the collateral circulation engages in the early stage of chronic hypoperfusion. When the collateral circulation cannot maintain the perfusion, then the CVR begins to participate in the regulation. But when the cerebral artery dilation still cannot meet the demands, that is, the CVR fails. The oxygen extraction fraction (OEF) has to increase and the brain metabolic reserve begins to work. If the above three mechanisms cannot meet the blood and oxygen supply of brain tissue activities, finally stroke will occur. This study utilized the TCD to evaluate the hemodynamic changes before and after CAS. With the release of carotid artery stenosis, the MFV and PI in the ipsilateral MCA increased significantly after the operation, and the CVR improved significantly at the 30 days after CAS. Furthermore, for patients with different degrees of

stenosis, the more severe stenosis in carotid artery, the more obvious improvement in CVR at the 30 days follow-up. But a long-term change is require further follow-up.

Astrocytes are the main glial cells in brain tissue, they have the effect on adjusting neurotransmitters, promoting immune responses, regulating intracranial blood flow, resisting oxidants and so on [21]. GFAP and S100B are the major components and signature proteins of astrocytes, and their presence is essential to the maintenance of astrocytes morphological structures and normal functions [22-23]. The increase of GFAP and S100B in cerebrospinal fluid (CSF) [24-25] and/ or blood [26] reflects the formation of astrocyte filaments in the central nervous system. A rapidly rising in GFAP and S100B suggests the acute injury of brain tissue; whereas a moderate increase suggests the astrocytes proliferation, the scars formation, and the delayed ischemic tolerance [27]. The moderate increase in GFAP and S100B plays an important role in promoting neuronal survival and repairing tissue after brain injury [28]. In addition, Herrmann et al [29] found that the release of GFAP and S100B were significantly correlated with the incidence of cerebral infarction. While for patients with lacunar or mild stroke, GFAP was found to be a more sensitive cerebral biochemical marker.

The results in this study showed that the serum concentrations of GFAP and S100B increased temporarily at 24h after CAS. Meanwhile, the MRI which performed on the post-operation patients contemporaneously revealed the emerging hyperintense signals in DWI sequences. So the DWI (+) shown in the operative patients maybe ascribe to the particulate embolism after CAS [11]. Because astrocytes are very sensitive to cerebral ischemia and hypoxia, the embolism could cause the astrocytes damaged and produced excessive GFAP and S100B into CSF. These proteins as biochemical markers can be in turn released into the peripheral blood through impaired blood-brain barrier, so the overexpression of GFAP and S100B could be detected in blood serum. Besides, these patients who are shown the DWI (-) and also had the increase of both markers in the serum simultaneously, this results are considered as the cause of reperfusion after CAS [30]. It has been confirmed that the moderate increase of GFAP and S100B in serum is concerned with repair after cerebral injury [28]. Therefore, the concentrations of GFAP and S100B at 72 hours after CAS were still keep the higher level than pre-operation, suggesting the brain tissue is under the injury repairing caused by embolism. In addition, it is reported that the reactive gliocytosis has dual effects [22]. When it cannot be solved in the stage of acute injury, the reactive gliosis have negative impact on the injured tissue. If the intervention measures are adjusted correctly in the best time window, new methods of treating cerebal injury may be developed. However, with the release of carotid artery stenosis and the reconstruction of cerebral blood flow after CAS, the injured tissue caused by hypoperfusion was recovered gradually, which was reflected in the decrease of both GFAP and S100B at 30 days after CAS. Also, the downtrend of GFAP and S100B after CAS had the positive correlation to the improved hemodynamics which was verified by TCD. The data in our current study is consistent with the results observed by Wunderlich MT [31].

For patients with symptomatic (2 TIA, 2 stroke and 1 death) after CAS, the increased values of GFAP and S100B in serum are more significant than patients without complications. Furthermore, for these patients

who remained the neurological deficits (2 stroke and 1 death), the serum GFAP and S100B still cannot return to the baseline even at 30 days follow-up. And the fatal patient maintained a higher level than the stroke at any time points during the 1 month follow-up, which is different from the positive curative patients.

Limitations

There are also some limitations in our study. Firstly, we only collected the serum and monitored the hemodynamics before and after operation, so the data during operation is missed. Secondly, we demonstrated that the DWI (+) was related to embolism, but the reperfusion was not confirmed by image tools in this study.

Conclusion

Our finding proven that the trend of GFAP and S100B in serum after CAS had a positive correlation to the improved hemodynamics which was verified by TCD. We recommend the biochemical markers (GFAP and S100B) associated with TCD to evaluate the curative effect after CAS. The detection of GFAP and S100B in serum has the advantages of simplicity, low price and repeatability. In addition, the correct intervention in serum GFAP and S100B at the best time window after CAS may lead to the development of new treatments for postoperative cerebral lesion, and this requires further studies.

Declarations

Ethics approval and consents to participate: This study was reviewed by the ethics committee of Sichuan Provincial People's Hospital, and all the experimental protocols for involving human data in the study were in accordance with the declaration of Helsinki. All of the patients and representatives/guardian/next of kin of the patients signed the informed consents legally.

Competing interests: The author(s) confirm that this article content has no conflict of interest.

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Author's contributions: Xiaofan Yuan and Jianhong Wang designed the study; Shu Yang and Fuqiang Guo performed the operation; Xiaofan Yuan and Lei Guo carried out the study; Duozi Wang analyzed the results; XiaofanYuan and FuqiangGuo wrote the manuscript.

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Figures

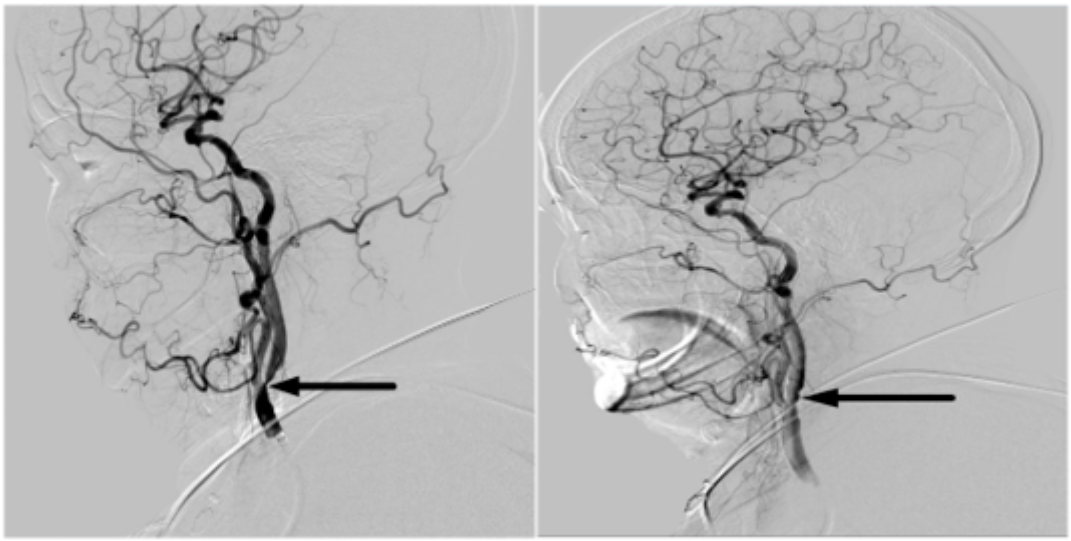
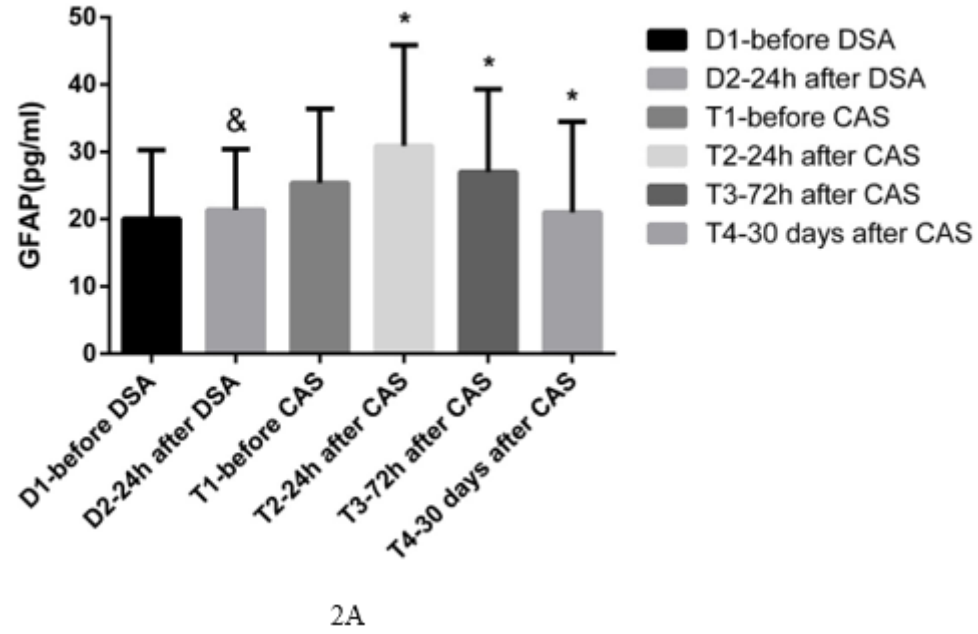
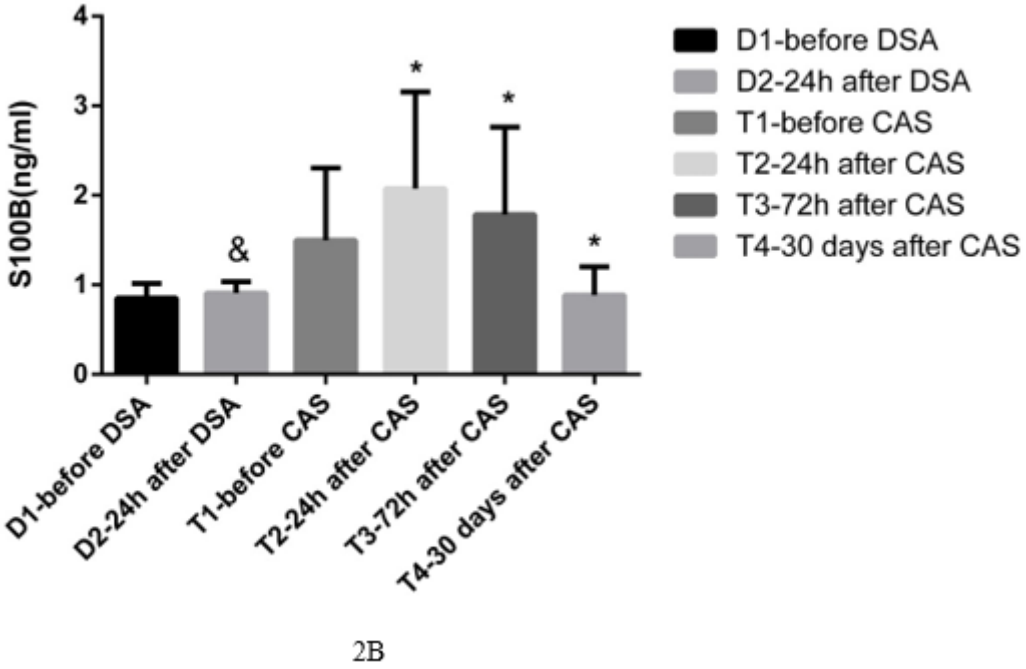


Figure 1

1A: The DSA showed the carotid artery stenosis in Grade 3 (>90%) before carotid artery stenting. 1B:The stenosis in carotid artery sinus was improved obviously and the reperfusion was restored (TICI 3) after carotid artery stenting.



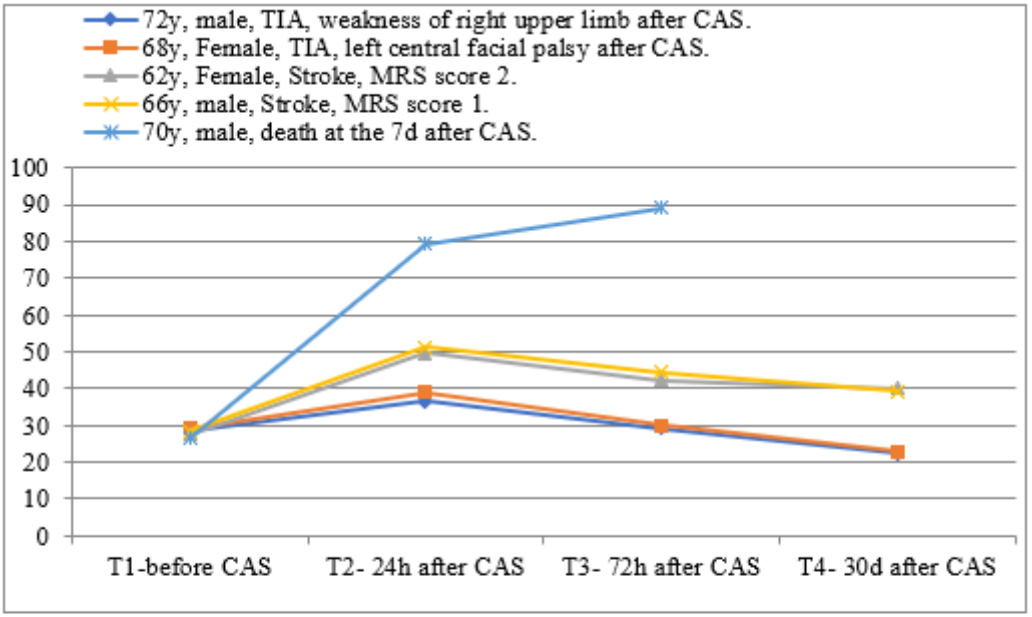
2A



2B

Figure 2

The serum concentrations of GFAP (2A) and S100B (2B) did not change significantly after DSA (&P>0.05). After CAS, the serum concentrations of GFAP (2A) and S100B (2B) increased to the peak at 24 hour after operation (T2), and then decrease gradually (T2>T3>T4; *P < 0.05).



3A

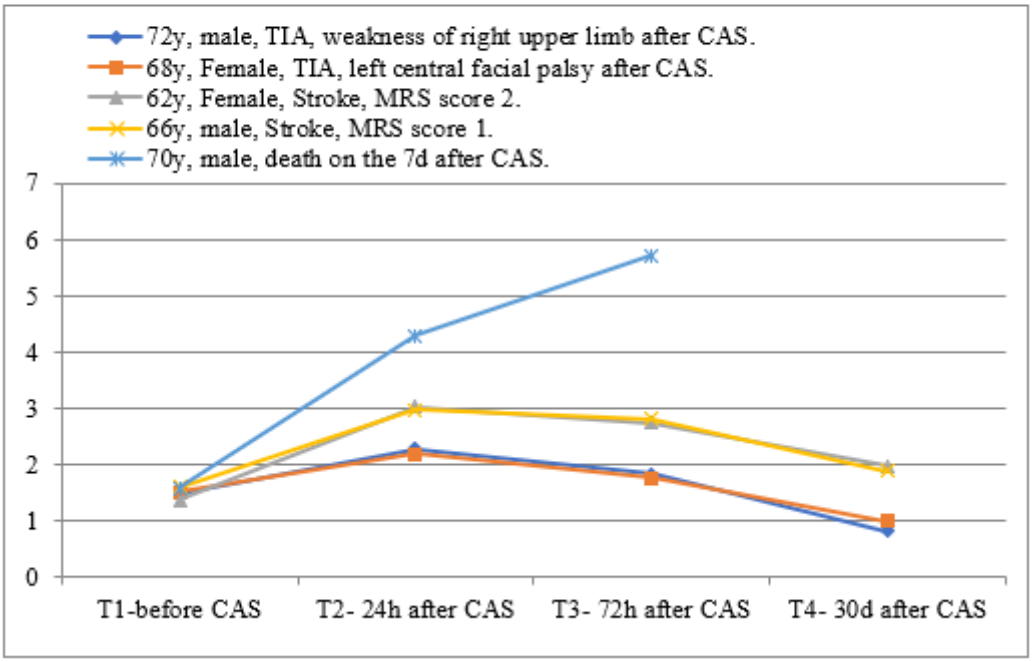


Figure 3

The serum values of GFAP (3A) and S100B (3B) in 2 TIA increased temporarily at 24 hours after operation (T2), and then came back to the basal at 30 days after operation (T4). For the 2 stroke and 1 death, the serum GFAP and S100B increased permanently, and the fatal patient maintained a higher level than the stroke at any time points after operation.