Correlation of carotid contrast-enhanced ultrasonography parameters with nerve damage and plaque properties in patients with atherosclerosis cerebral infarction

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ABSTRACT

Objective: To explore the correlation of carotid contrast-enhanced ultrasonography parameters with nerve damage and plaque properties in patients with atherosclerosis cerebral infarction.

Methods: A total of 176 patients with atherosclerosis cerebral infarction who were sent to this hospital for medical treatment between August 2014 and February 2018 were enrolled in cerebral infarction group, and 100 healthy elderly subjects who received physical examination in this hospital during the same period were enrolled in normal control group. Carotid CEUS parameter levels as well as serum contents of indexes related to nerve injury and plaque property were compared between the two groups, and Pearson test was used to evaluate the correlation of CEUS parameters levels with nerve damage and plaque properties in patients with cerebral infarction.

Results: CEUS parameter Tp level in cerebral infarction group was lower than that in normal control group whereas P and AUC levels were higher than those in normal control group; serum nerve damage-related indexes SAA, NT-proBNP, Hcy, NSE and copeptin contents were higher than those in normal control group; serum plaque property-related indexes Lp-PLA2, MMP-9, Cat S and CD62P contents were higher than those of normal control group while APN and Cys C contents were lower than those of normal control group. Correlation analysis confirmed that carotid CEUS parameter levels in patients with cerebral infarction were correlated with the contents of indexes related to nerve damage and plaque property.

Conclusion: Carotid CEUS parameters are obviously abnormal in patients with atherosclerosis cerebral infarction, they are directly correlated with the specific nerve damage and plaque properties, and they can be used as the reliable indexes to forecast the risk of cerebral infarction and evaluate its severity.

1. Introduction

Carotid atherosclerosis is the clinical main cause of cerebral infarction. How to early predict the risk of cerebral infarction, accurately assess the severity of cerebral infarction and determine the risk of cerebral infarction recurrence after cerebral infarction are all focuses of current clinical research[1,2]. Conventional carotid artery ultrasound can determine the existence of plaque, the intima thickness and so on, but cannot provide deeper information such as plaque stability. Carotid contrast-enhanced ultrasonography can sensitively detect the neovascularization in plaques to clarify the stability of plaques, which is currently a highly recommended gold standard for the examination of carotid lesions[3,4]. In this study, the differences in carotid CEUS parameters were compared between patients with atherosclerotic cerebral infarction and healthy elderly people, and the internal correlation of CEUS parameter levels with nerve damage and plaque property of cerebral infarction was further determined to lay a foundation for the subsequent application of the technology in predicting the risk of cerebral infarction and judging its condition.
2. Information and methods

2.1 Inclusion and exclusion criteria

Inclusion criteria: (1) the patients who were diagnosed with cerebral infarction by head CT, which was caused by carotid atherosclerotic plaque shedding; (2) the subjects of the study were 60-79 years old; (3) the patients had cerebral infarction attack for the first time and received no other treatment before sent to this hospital; (4) those who were without history of cerebral hemorrhage or brain trauma; (5) those whose family members signed the informed consent.

Exclusion criteria: (1) the patients who died within a short period of time after being admitted to the hospital due to cerebral infarction; (2) the patients combined with Alzheimer’s disease or Parkinson’s disease; (3) the patients combined with intracranial infection; (4) the patients who dropped out of the study group, which resulted in incomplete data collection.

2.2 Case information and grouping

According to the above inclusion and exclusion criteria, 176 patients with atherosclerotic cerebral infarction treated in this hospital between August 2014 and February 2018 were included in the cerebral infarction group, and 100 healthy elderly subjects who had physical examination in this hospital during the same period were included in the normal control group. Cerebral infarction group included 96 males and 80 females, and they were 61-78 years old; normal control group included 54 males and 46 females, and they were 60-79 years old. The distribution of these basic data of the two groups was similar, and the follow-up study plan was approved by all members of the hospital ethics committee.

2.3 Carotid CEUS

Both groups of subjects received carotid CEUS after enrollment, and the specific steps were as follows: Sonovye microbubble contrast agent and 5mL of normal saline were configured into suspension. The subjects first received two-dimensional ultrasonography and then 2 mL of contrast agent was injected via median cubital vein. At this time, contrast mode was activated to collect images. Image analysis was conducted by QLAB imaging analysis software and the region of interest was tracked dynamically. The imaging time-intensity curve was drawn, and the imaging parameters time to peak (Tp), peak intensity ratio (P) and area under the curve (AUC) were obtained.

2.4 Nerve damage–related and plaque property–related index content detection

After the patients were enrolled, peripheral blood samples of the two groups (at the same time point) were collected at the first time, and the serum was isolated and stored in cryogenic environment. Enzyme-linked immunosorbent assay was adopted to detect serum contents of nerve damage-related indexes and plaque property-related indexes, nerve damage-related indexes included serum amyloid protein A (SAA), N-terminal pro-B-type natriuretic peptide (NT-proBNP), homocysteine (Hcy), neuron-specific enolase (NSE) and copeptin, and plaque property-related indexes included lipoprotein-associated phospholipase A2 (Lp-PLA2), matrix metalloproteinase-9 (MMP-9), adiponectin (APN), cathepsin S (Cat S), cystatin C (Cys C) and P-selectin (CD62P).

2.5 Statistical processing

Carotid CEUS parameters, nerve damage-related indexes and plaque property-related indexes all belonged to measurement data and were recorded into software SPSS23.0, and the statistical value P was calculated and used to judge whether there was statistical significance in differences between groups (P<0.05 was the standard for statistical significance in difference in this paper).

3. Results

3.1 Carotid CEUS parameters

Comparison of carotid CEUS parameters Tp (s), P (dB) and AUC (dB·s) levels between the two groups was as follows: Tp level in cerebral infarction group was lower than that in normal control group whereas P and AUC levels were higher than those in normal control group. The differences in carotid CEUS parameters Tp, P and AUC levels were statistically significant between the two groups (P<0.05), specifically shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Tp</th>
<th>P</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>100</td>
<td>30.65±4.09</td>
<td>27.84±3.09</td>
<td>1 450.83±165.27</td>
</tr>
<tr>
<td>Cerebral infarction group</td>
<td>176</td>
<td>20.74±2.81</td>
<td>56.19±6.72</td>
<td>2 156.33±249.71</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Nerve damage–related indexes

Comparison of serum nerve damage-related indexes SAA (mg/L), NT-proBNP (pmol/L), Hcy (μmol/L), NSE (μg/L) and Copeptin (pmol/L) contents between the two groups was as follows: serum...
SAA, NT-proBNP, Hcy, NSE and copeptin contents of cerebral infarction group were higher than those of normal control group. The differences in serum nerve damage-related indexes SAA, NT-proBNP, Hcy, NSE and copeptin contents were statistically significant between the two groups ($P<0.05$), specifically shown in Table 2.

### 3.3 Plaque property-related indexes

Comparison of serum plaque property-related indexes Lp-PLA2 (ng/mL), MMP-9 (ng/mL), APN (mg/L), Cat S (pg/mL), Cys C pg/mL and CD62P (pg/mL) contents between the two groups was as follows: serum Lp-PLA2, MMP-9, Cat S and CD62P contents of cerebral infarction group were higher than those of normal control group while APN and Cys C contents were lower than those of normal control group. The differences in serum plaque property-related indexes Lp-PLA2, MMP-9, APN, Cat S, Cys C and CD62P contents were statistically significant between the two groups ($P<0.05$), specifically shown in Table 3.

### 3.4 Correlation analysis

Pearson test showed that carotid CEUS parameter Tp level in patients with cerebral infarction was negatively correlated with nerve damage-related indexes SAA, NT-proBNP, Hcy, NSE and copeptin contents; it was negatively correlated with plaque property-related indexes Lp-PLA2, MMP-9, Cat S and CD62P contents, and positively correlated with APN and Cys contents. $P$ and AUC levels were positively correlated with nerve damage-related indexes SAA, NT-proBNP, Hcy, NSE and copeptin contents; they were positively correlated with plaque property-related indexes Lp-PLA2, MMP-9, Cat S and CD62P contents, and negatively correlated with APN and Cys contents ($P<0.05$).

### 4. Discussion

The patients with cerebral infarction are often accompanied by carotid plaque, which is the main cause of current cerebral infarction and long-term secondary cerebral infarction. Early identifying the severity of carotid plaque is a necessary step to guarantee the patients’ life safety. Two-dimensional ultrasound is the main method for clinical screening of carotid plaque, and can provide information such as plaque diameter and intima thickness. Recent studies have shown that angiogenesis in plaques is one of the important factors leading to decreased stability and rupture of plaques, and the measurement of blood flow in plaques can more accurately determine the risk of carotid plaques. Contrast-enhanced ultrasonography uses the microvesicles generated by contrast agent as reflectors and scatterers to significantly enhance the images of the target organ tissue, and can sensitively detect the neovascularization in plaques. Therefore, it is recommended as a necessary examination method for clinical high-risk patients with cerebral infarction [5,6]. In this study, the differences in carotid CEUS parameter levels were first compared between patients with atherosclerotic cerebral infarction and healthy elderly subjects, and the results showed that compared with those in normal control group, the CEUS parameter Tp level in cerebral infarction group was lower while $P$ and AUC levels were higher. The new blood vessels in the plaque are not completely developed, and the contrast agent can leak through the wall, so the transit time is shortened; the presence of neovascularization increases the content of contrast agent in the plaque, so the contrast intensity in the local plaque finally increases. The above results are consistent with the conclusions of previous studies on carotid plaque, indicating that there are indeed plaques rich in new blood vessels in the carotid artery of patients with atherosclerotic cerebral infarction. The correlation of plaque CEUS parameters with specific nerve damage and plaque properties in patients with cerebral infarction is
elaborated below.

Local cerebral artery occlusion in patients with cerebral infarction leads to hypoxic-ischemic injury in the local blood-supplying nerve tissue, which is also the fundamental reason for the abnormality of patients’ body function, intelligence and so on[7,8]. The nerve damage of cerebral infarction can lead to the fluctuation of serum contents of a series of factors, which is closely related to the degree of specific nerve damage. The detection of their contents is helpful to quantitatively determine the nerve damage in patients with cerebral infarction. SAA is synthesized by macrophages and fibroblasts in the liver, it can be combined with plasma high-density lipoprotein cholesterol, restrain lecithin cholesterol acyltransferase activity and increase the lipid deposit in arterial hardening area, and more studies have shown that SAA is the bridge between inflammation and lipid metabolism, and that its content in serum of patients with acute cerebral infarction is significantly higher than that of normal people and obviously correlated with the specific area of cerebral infarction[9]. NT-proBNP is a new biological indicator for cerebrovascular diseases, its content is relatively high in the brain, and the specific content is positively correlated with the severity of cerebral infarction[10]; Hcy, as an intermediate metabolite of methionine, can affect platelet adhesion and activate coagulation factors, and directly participates in the occurrence of vascular obstructive lesions[11]; NSE is specifically highly expressed in brain tissue and accounts for 40%~65% of enolase. When neurons are damaged, it is released from neurons into cerebrospinal fluid and peripheral blood, which can be detected within a short time after cerebral infarction[12]; copeptin has been previously used for the diagnosis of diabetes insipidus, and current studies have shown that the abnormal increase of its serum content is highly correlated with the adverse prognosis of cerebrovascular diseases[13]. In this paper, the contents of the above nerve damage-related indexes in serum of patients with cerebral infarction were all abnormally higher than those of normal control group, indicating that these indexes indeed have neurospecificity. Further correlation analysis showed that the CEUS parameter Tp level in patients with cerebral infarction was negatively correlated with the contents of above nerve damage-related indicators while P and AUC levels were positively correlated with them, confirming that the CEUS parameter levels could objectively reflect the degree of nerve damage in patients with cerebral infarction.

The nature of carotid atherosclerotic plaques is not only directly related to the risk of cerebral infarction, and the patients with current cerebral infarction should be on the alert against the risk of secondary cerebral infarction caused by plaque detachment. There are many factors closely related to the plaque properties in serum, which are involved in the occurrence and evolution of plaques, and can be used as indirect indicators to evaluate the stability of plaques. Lp-PLA2 can hydrolyze the acyl on glyceryl phosphatide Sn-2 sites, more than 80% of the Lp-PLA2 in circulating blood is combined with low density lipoprotein cholesterol by the apolipoprotein B, it is the initial link causing atherosclerotic plaque formation, and the hydrolysis of low density lipoprotein by Lp-PLA2 can further stimulate macrophages to devour lipoprotein to form foam cells, accelerate the atheromatous plaque formation and reduce its stability[14,15]; the macrophages and foam cells aggregated during the formation of atherosclerotic plaques can secrete MMP-9, which leads to accelerated decomposition of extracellular matrix and rapid accumulation of inflammatory cells caused by vascular endothelium damage. As a result, the volume of atherosclerotic plaques keeps increasing. At the same time, MMP-9 can decompose and thin the fibrous cap on the surface of the plaque, so it easily ruptures when blood flow changes[16,17]; APN is a typical adipokine, which can inhibit the secretion of various interleukins and TNF-, and reduce the exacerbation of atherosclerosis caused by inflammatory reactions[18,19]; Cat S can degrade collagen and elastin in vivo. Studies have confirmed that the expression level of Cat S in the unstable region of atheromatous plaque is higher than that in the stable region[20]; Cys C can regulate the activity of cysteine cathepsins and stabilize the release/degradation balance of extracellular matrix. Cys C is less expressed in those with acute cerebral infarction than in healthy people; CD62P is a marker that recognizes activated platelets. After inflammatory mediators activate platelets, the expression of CD62P in the serum increases, which can mediate the mutual adhesion between neutrophils and monocytes, and thus exacerbate the degree of microcirculation disorder. The results of the study showed that compared with those of normal control group, the serum levels of Lp-PLA2, MMP-9, Cat S and CD62P were higher in the cerebral infarction group while the levels of APN and Cys C were lower. Correlation analysis showed that the levels of CEUS parameters Tp, P and AUC in patients with cerebral infarction were directly correlated with the contents of the above plaque property-related indicators, confirming that the CEUS parameter levels could objectively reflect the properties of plaques in patients with cerebral infarction.

Compared with those in healthy people, carotid CEUS parameter levels are significantly abnormal in patients with atherosclerotic cerebral infarction, their levels are directly correlated with the nerve damage degree and plaque properties, and they can be used as the reliable indexes to assess the severity of cerebral infarction and predict the risk of long-term recurrence of infarction.
References


