The correlation between vulnerable carotid plaque and inflammation—an exploratory research based on Histopathology

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Research Article

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Abstract

Background

Vulnerable plaques with ruptured fibrous cap were prone to produce emboli and cause distal arterial embolism. The identification of vulnerable plaque in humans before it becomes symptomatic has been elusive to date. Inflammation related ratio of leukocytes and their subtypes had been proved that they can predict cardiovascular diseases, while we aimed to explore the correlation between those and vulnerable carotid plaque.

Methods

Ischemic stroke patients admitted to the Department of Neurology were analyzed as study group (neurology group) from January 2019 and December 2020. Besides, patients who underwent carotid endarterectomy (CEA) during the same period were collected as control group (neurosurgery group) for compare. All patients were categorized into stable and vulnerable plaque groups based on the characteristics of plaque assessed by carotid doppler ultrasonography (CDU). The H&E staining characteristics of carotid plaque after CEA were analyzed to test the feasibility of ultrasound grouping in the study group. The inflammation-related ratio (PLR: platelet-to-lymphocyte ratio, NLR: neutrophil-to-lymphocyte ratio, MLR: monocyte-to-lymphocyte ratio) were collected to analyze. Spearman linear correlation analysis and logistic regression analysis were used to evaluate the correlation between factors and plaque vulnerability, and multivariate analysis was used to exclude confounding factors.

Results

In study group, comparisons of hs-CRP and NLR among the vulnerable plaque group and stable plaque group showed a statistically significant difference \( p < 0.05 \). Multivariate logistic analysis revealed that elevated levels of NLR were independent risk factors for carotid plaque vulnerability in the study group \( (2.399; 1.468–3.921; p < 0.001) \). Incorporating the control group’s data (neurosurgery group), patients with vulnerable carotid plaques present with higher hs-CRP and lower HDL-C. Low level HDL-C would lose its protective effect on vulnerable carotid plaque \( (0.15; 0.023–0.958; p = 0.045) \). The ultrasonic and pathological characteristics of carotid plaques in the control group had strong consistency.

Conclusions

The inflammation reflected by the high level of hs-CRP plays a vital role in forming vulnerable carotid plaques. NLR is expected to effectively predict vulnerable carotid plaque for stroke patients at the first visit and is easier to obtain in clinical.
Background

Stroke represents a worrying public health problem [1] and the main cause of death and disability in China. Statistics have shown that stroke became the top leading cause of years of life lost in 2017 [2]. Atherosclerosis is the main cause of stroke. Its pathological mechanism includes gradual accumulation of lipids and inflammatory cells [3], resulting in the thickening of intima-media. Plaques with ruptured fibrous cap are prone to produce emboli and cause distal arterial embolism. The atherosclerotic plaque prone to such clinical events is termed high-risk or vulnerable plaque. In recent years, a number of studies have shown that patients with vulnerable carotid plaques are more prone to ischemic stroke, regardless of the degree of vascular stenosis [4–5].

A variety of detection methods can be used to investigate the characteristics and vulnerability of carotid plaque, including Computed Tomography (CT) [6], Magnetic Resonance (MR) [7], and Carotid Doppler Ultrasound (CDU) [8]. CDU is recognized to be a preferably tool for diagnosis and evaluation of atherosclerosis at present due to the advantages of noninvasive, convenient, and real-time dynamics, which can be used to assess the vulnerability of atherosclerotic plaques based on the detection of echoes and morphology of the plaques and the filling of color blood flow signals [8].

Inflammatory mechanisms are considered to plays an important role in in the formation of vulnerable plaques. Hansson et al. reported that the accumulation of macrophages, activated T cells, dendritic cells and mast cells increase the possibility of plaque rupture [9]. Some previous studies have shown that leukocytes and their subtypes, neutrophils and lymphocytes also play a significant role. Correlation ratio such as PLR (platelet-to-lymphocyte ratio), NLR (neutrophil-to-lymphocyte ratio), MLR (monocyte-to-lymphocyte ratio) can be used as a predictor of cardiovascular disease [10–12]. However, researches about the relationship between these ratios and the evolution of carotid atherosclerotic plaque in patients with stroke are rare. This study investigates whether these inflammation-related ratios are associated with vulnerable carotid plaque.

Methods

Study Population

Ischemic stroke patients admitted to the Department of Neurology were analyzed as study group (the neurology group) from January 2019 and December 2020. All patients underwent routine examination of carotid doppler ultrasonography (CDU), computed tomography angiography (CTA), computed tomography perfusion imaging (CTP) within 48 hours of admission. The inclusion criteria in the neurology group included: (1) first onset and no relevant medical treatment before onset; (2) diagnosed as moderate-severe (50%-99%) carotid stenosis by multimodal imaging examination based on CDU. Exclusion criteria were as follows: (1) patients who have severe infection within a month such as pulmonary infection, etc.; (2) patients with history of malignant tumor radiotherapy and chemotherapy;
(3) patients with missing data; (4) patients with non-atherosclerotic carotid artery stenosis, such as carotid artery dissection, Takayasu arteritis, etc.

In addition, we collected consecutive patients with carotid atherosclerosis who underwent carotid endarterectomy (CEA) as control group (neurosurgery group). The inclusion criteria included: patients meet the North American Symptomatic Carotid Endarterectomy Trial [13] (NASCET): (1) symptomatic stroke patients with more than 50% carotid stenosis; (2) asymptomatic stroke patients with more than 70% carotid stenosis. The exclusion criteria in the neurosurgery control group were similar to those mentioned above. Healthy subjects without carotid plaque and previous history of cerebrovascular disease were regarded as control group (healthy group).

The study was approved by the local Ethics Committee (No.196) (see supplementary file), and informed consent was obtained from all patients.

**Data Collection**

From each patient, the following clinical data were obtained within 1 hour of admission: age, sex, height, weight, blood pressure. Hypertension was described as systolic blood pressure (SBP) $\geq 140$ mmHg and/or diastolic blood pressure (DBP) $\geq 90$ mmHg, or a history of taking hypertension drugs [14]. Besides, patient's neurological manifestations and signs were recorded, and the National Institutes of Health Stroke Scale (NIHSS) score was completed.

Venous blood samples were collected after 8 hours fasting on the morning after admission in order to conduct routine blood tests and biochemistry tests assessing total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), high sensitivity C-reactive protein (hs-CRP), fasting blood glucose, etc. The diagnosis of diabetes depended on previous history of diabetes, or fasting blood glucose (FBG) $\geq 7.00$ mmol/L and/or 2-hour postprandial blood glucose $\geq 11.10$ mmol/L [15]. The diagnosis of dyslipidemia was defined as total cholesterol (TC) $\geq 5.20$ mmol/L and/or triglyceride (TG) $\geq 1.70$ mmol/L, and/or low-density lipoprotein cholesterol (LDL-C) $\geq 3.40$ mmol/L, and/or high-density lipoprotein cholesterol (HDL-C) $\leq 1.00$ mmol/L [16]. The laboratory indicators above were completed in the department of clinical laboratory.

**Pathological Procedure**

After carotid endarterectomy, we macroscopic observed its characteristics such as lipid necrosis core, calcification, fresh bleeding, and old hemorrhage blood. Tissues were immediately fixed in 10% paraformaldehyde for 24 hours, embedded in paraffin after dehydration and wax dipping. Approximately 3 mm above and below the plaque lesion area was retained as a carotid atherosclerotic plaque specimen for each patient and. Further transverse sections of 5 µm thickness at a 1 mm interval were obtained. Hematoxylin and eosin staining was used to accurately observe the infiltration of inflammatory cells.
Assessment Of Carotid Arteries

All carotid doppler ultrasonography examinations were performed using a CX-50 scanner (Phillips Medical System, Netherlands) equipped with a linear array L12-3 probe and a convex array C5-1 probe. We dynamically observed the two-dimensional morphology of carotid, the filling of color blood flow signals, the hemodynamic changes of spectrum doppler, the plaque morphology of multi-slice, multi-angle, and recorded the carotid plaque characteristics including size, morphology, and property. According to the morphological and characteristics of the plaques on CDU, plaque echogenicity was mainly classified as follows: (1) uniformly hypoechoic; (2) predominantly hypoechoic: hypoechoic with small < 25% hyperechoic regions; (3) predominantly hyperechoic: hyperechoic with small < 25% hypoechoic regions; (4) uniformly hyperechoic [17]. Among them, predominantly hyperechoic plaque and uniformly hyperechoic plaque were confirmed to have a higher percent calcium and fibrous content which were the characteristics of stable plaques. Uniformly hypoechoic plaque and predominantly hypoechoic plaque were vulnerable carotid plaques which were associated with larger lipid cores and prone to have ruptured fibrous cap [18]. The ultrasonic and pathological characteristics of carotid plaques in the control group (neurosurgery group) had strong consistency (Supplementary Table 1). Therefore, we continued to use this ultrasonic examination standard to evaluate the patients in the study group. Those with stable plaques were classified as stable plaque group. Those with vulnerable plaques or both stable plaques and vulnerable plaques were classified as vulnerable plaque group. Besides, carotid artery stenosis degree was quantitatively evaluated using the NASCET system. The ratio of the luminal diameter at the greatest narrow section to the normal luminal diameter at the far section was measured.

All data of the study were collected and interpreted by 2 experienced ultrasound doctors, and results were entered into an institutional database. In case of persistent disagreement, a third expert ultrasound doctor was consulted, and a final decision was reached by consensus (Supplementary Table 2).

Statistical analysis

Continuous variables with normal distribution were expressed as mean ± SD; continuous variables without normal distribution were expressed as [median (1st quartile,3rd quartile)]; categorical variables were expressed as percentages (proportions). T test, Chi-Square test, the nonparametric Mann-Whitney U test was used to analyze baseline data between groups, while Fisher exact test and Continuity Adj. Chi-Square test was used if necessary. The correlation between variables and carotid plaque vulnerability was tested by Spearman correlation analysis. Multivariate logistic regression analysis was then applied to individuate the variables independently associated with the vulnerable carotid plaque, and the odds ratios (OR) and 95% confidence intervals (CI) were calculated.

All statistical analyses were performed using SPSS for windows (version 26.0, IBM Corporation, New York, USA). A two-tailed p value of < 0.05 was considered as statistically significant.
Results

In the study group (neurology group), patients with vulnerable plaque accounted for 85.2% (123/147). Patients with vulnerable carotid plaques were present with higher level of Leukocyte, Neutrophil, hs-CRP while the level of Lymphocyte was lower. Inflammation-related biomarker characteristics such as PLR, NLR, MLR, and hs-CRP were statistically significant between vulnerable plaque group and stable plaque group. In terms of blood lipids, no statistical difference was found in the study group. After incorporating the data of the control group, we found that patients in the study group exhibited higher level of Leukocyte, Neutrophil and NLR than patients in the control group (neurosurgery group), meanwhile both significantly higher than those in the control group (healthy group). Focusing on the neurosurgery group, we found that patients with vulnerable carotid plaques were present with a higher level of hs-CRP (3.78 mg/L vs 1.74 mg/L, respectively; \( p < 0.05 \)), while no statistical difference was found in the remaining inflammation-related biomarker characteristics. In terms of blood lipids, patients with vulnerable carotid plaques tend to have a lower level of HDL-C than patients with stable plaques (0.95 mmol/L vs 1.08 mmol/L, respectively; \( p < 0.05 \)). The remaining patient characteristics were listed in Table 1.

As illustrated in Figs. 1 to 2 of the control group data (neurosurgery group), inflammatory cell infiltration could be seen around the ruptured fibrous cap of vulnerable carotid plaques after HE staining. The blood inflammation biomarker characteristics of patients in Fig. 1 (PLR 66.85; NLR 0.94; MLR 0.25) and Fig. 2 (PLR 126.76; NLR 3.00; MLR 0.23) were similar to the degree of infiltration in the HE staining. This also meant that the blood inflammation biomarker characteristics of patients in the study group can reflect actual inflammatory cell infiltration in vulnerable plaque to some extent.

Table 2 Correlation analysis between influence factors and vulnerable carotid plaque

<table>
<thead>
<tr>
<th>Influence factor</th>
<th>Study group (Neurology group)</th>
<th>Control group (Neurosurgery group)</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>0.043</td>
<td>Diabetes</td>
<td>0.557</td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>0.028</td>
<td>SBP</td>
<td>0.931</td>
<td></td>
</tr>
<tr>
<td>Leukocyte</td>
<td>0.011</td>
<td>Leukocyte</td>
<td>0.317</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>0.004</td>
<td>Lymphocyte</td>
<td>0.644</td>
<td></td>
</tr>
<tr>
<td>Neutrophil</td>
<td>&lt; 0.001</td>
<td>Neutrophil</td>
<td>0.358</td>
<td></td>
</tr>
<tr>
<td>PLR</td>
<td>0.003</td>
<td>PLR</td>
<td>0.990</td>
<td></td>
</tr>
<tr>
<td>NLR</td>
<td>&lt; 0.001</td>
<td>NLR</td>
<td>0.975</td>
<td></td>
</tr>
<tr>
<td>MLR</td>
<td>0.004</td>
<td>MLR</td>
<td>0.995</td>
<td></td>
</tr>
<tr>
<td>hs-CRP</td>
<td>0.003</td>
<td>hs-CRP</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.831</td>
<td>HDL-C</td>
<td>0.039</td>
<td></td>
</tr>
</tbody>
</table>
In the Spearman correlation analysis, vulnerable carotid plaque had correlation with diabetes, SBP, Leukocyte, lymphocyte, Neutrophil, PLR, NLR, MLR, hs-CRP in the study group, while hs-CRP and HDL-C in the control group (neurosurgery group) (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate OR [95% CI]</th>
<th>p</th>
<th>Multivariate OR [95% CI]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte</td>
<td>1.366 [1.055–1.767]</td>
<td>0.018</td>
<td>1.186 [0.849–1.657]</td>
<td>0.316</td>
</tr>
<tr>
<td>PLR</td>
<td>1.017 [1.005–1.029]</td>
<td>0.005</td>
<td>1.006 [0.989–1.022]</td>
<td>0.505</td>
</tr>
<tr>
<td>NLR</td>
<td>2.399[1.468–3.921]</td>
<td>0.001</td>
<td>2.409 [1.075–5.395]</td>
<td>0.033</td>
</tr>
<tr>
<td>MLR</td>
<td>108.642[2.074-5691.383]</td>
<td>0.02</td>
<td>0.004[0.001–14.684]</td>
<td>0.286</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>1.112[1.025–1.207]</td>
<td>0.011</td>
<td>1.048[0.954–1.15]</td>
<td>0.33</td>
</tr>
</tbody>
</table>

To determine the independent predictors of vulnerable carotid plaque, the variables found to be statistically significant in the Spearman correlation analysis were included in the univariate logistic regression analysis. Elevated levels of NLR were independent risk factors for carotid plaque vulnerability in the study group. Although there were significant differences in Leukocyte, PLR, MLR, and hs-CRP between the stable plaque group and vulnerable plaque group, they were not considered as independent risk factors (Table 3). In addition, hs-CRP and HDL-C were the related factors of plaque vulnerability in the control group (neurosurgery group) and high level of HDL-C was independent protect factor for the occurrence of vulnerable carotid plaques (Table 4).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate OR [95% CI]</th>
<th>p</th>
<th>Multivariate OR [95% CI]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP</td>
<td>1.148 [1.009–1.307]</td>
<td>0.036</td>
<td>1.124 [0.995–1.27]</td>
<td>0.062</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.119 [0.020–0.692]</td>
<td>0.018</td>
<td>0.15[0.023–0.958]</td>
<td>0.045</td>
</tr>
</tbody>
</table>

**Discussion**

In this study, we found that inflammation was involved in the formation of vulnerable carotid plaque. After statistical analysis, it could be found that hs-CRP was both correlated with plaque vulnerability in
study group and control group (neurosurgery group). The study of Lombardo et al. had showed that high levels of hs-CRP not only caused complex coronary plaque, but also increased the risk of carotid plaque vulnerability [19]. Many previous studies believed that hs-CRP was an independent risk factor for cardiovascular disease [20–21]. However, the influence of inflammation on plaque vulnerability was often disturbed by many other factors. According to the results of multivariate logistic binary regression analysis, hs-CRP was not an independent risk factor of plaque vulnerability, suggesting that the relationship between plaque vulnerability and hs-CRP was complex.

Based on the data of the study group, we found that the level of Leukocyte, Lymphocyte, Neutrophil and NLR in the study group was statistically significant than that in the control group (healthy group) whether patients with vulnerable carotid plaques or not. Inflammation played a part in the formation of carotid plaque. Some scholars established an animal model of atherosclerosis and finally found that neutrophil respectively have a strong correlation and precise predictability for carotid intimal and media thickness of atherosclerosis [22]. In order to minimize uncontrollable confounding factors in human clinical research, we included ischemic stroke patients who had not been treated with medical treatment currently as study group. Their various test ratios in blood were closer to the initial level of atherosclerotic plaque. Núñez et al. found that lymphocyte will gradually apoptosis in the process of the development and destabilization of the atherosclerotic plaque [23]. In our study, the level of lymphocyte, Neutrophil and NLR were all correlated with the vulnerability of carotid plaque. Patients with vulnerable carotid plaque had significantly higher level of NLR than those with stable plaque. It could also be found that inflammatory cells were more likely to appear at the rupture of fibrous cap from Fig. 1–2. After the formation of carotid plaque, it was still eroded by long-term inflammatory cells, and the fiber cap on the surface tended to be broken and damaged. Thus, the vulnerable carotid plaque was formed finally. This might be one of the reasons why the inflammatory levels of patients with vulnerable carotid plaques in the study group were higher than that of patients with stable plaques. More Than This, elevated level of NLR was independent risk factor for occurrence of carotid plaque vulnerability in the study group. This phenomenon more confirmed that inflammation was correlated with the formation and destabilization of carotid plaque.

Lymphocytes gradually decreased in the process of inflammatory reaction, and neutrophils gradually increased with the thickening of the inner and middle membrane——the balance between lymphocytes and neutrophils was broken. Other relevant studies on NLR have similar conclusions. Imtiaz et al. discovered that NLR level has association with systemic inflammation and individuals with chronic conditions tend to have higher level of NLR [24]. A study, which consisted of 588 consecutive AIS patients, showed the independently association between NLR and the carotid plaque vulnerability detected by carotid ultrasonography in Chinese patients [25]. In several other studies, monocytes, PLR, MLR and other values were also associated with the course of atherosclerosis [26–28]. However, in our study, we found those inflammation-related ratios could distinguish stable plaque from vulnerable carotid plaque, but they were not independent risk factors of plaque vulnerability, which has not been reported in previous studies.

After comparing the data of the study group and the control group (neurosurgery group), we found that the overall inflammation-related biomarker characteristics in the neurosurgery group were lower than that
in the study group, especially leukocyte, neutrophil, NLR, and hs-CRP. In the neurosurgery group, NLR was substantially higher than that in the healthy control group regardless of having vulnerable carotid plaque or not, while NLR in patients with vulnerable carotid plaques was only slightly higher than that in patients with stable plaques, with no significant differences were found. Carotid atherosclerotic plaque formation involved vascular inflammation, lipid accumulation, intima thickening and fibrosis, arterial stiffness, remodeling, and plaque rupture or erosion. The prophase medical drug treatment might partially alleviate the imbalance between neutrophils and lymphocytes, but the formed intima-media thickening, carotid plaque, and even carotid stenosis are irreversible. Thus, this was the reason for the relatively high level of NLR in the neurosurgery group. It was noteworthy that high level of HDL-C was a protect factor of vulnerable carotid plaque in the neurosurgery group. Patients with lower levels of HDL-C were more likely to lose its protective effect on vulnerable carotid plaque. Touboul et al. had similar discoveries that low level of HDL-C may be a risk factor in intima-media thickness of common carotid artery [29]. In addition, the existing carotid plaques and carotid stenosis make partial patients still have clinical symptoms, such as unilateral limb hemp, speech slurred, headache, dizziness, memory loss, or more, pushing patients hospitalized again for CEA. This also indicated that we should give standardized medical treatment to patients in the early stage of carotid plaque formation, which may have a specific impact on the prognosis of patients.

There are still several limitations that should be taken into consideration that may affect proper causal inferences. First, in order to horizontally compare patients in the study group (neurology group) and control group (neurosurgery group), we did not include patients with mild carotid stenosis and patients whose plaques did not cause carotid stenosis. This creates a bias: fewer patients with stable plaques in cases of carotid stenosis. Secondly, this is a single-center study, and the sample size is not large enough, which means we need to conduct a multi-center study on the population of carotid stenosis in the next step. Furthermore, we did not statistics the prognosis of stroke patients, and we are unable to judge whether inflammation and inflammation ratio are related to the prognosis of stroke patients. In this regard, we will carry out the next research and further analyze the plasma and plaque tissue of CEA patients.

**Conclusions**

Inflammation response plays an important role in the formation of vulnerable carotid plaques, although this correlation may be concealed by clinical drug treatment. NLR, an inflammation ratio, is expected to be an effective predictor of carotid plaque vulnerability for stroke patients at the first visit and is easier to obtain in clinical.

**Abbreviations**

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol;
PLR: platelet to lymphocyte ratio; NLR: neutrophil to lymphocyte ratio; MLR: monocyte to lymphocyte ratio.

References


Table 1

Table 1 are available in the Supplementary Files section.
Figure 1

73-year-old asymptomatic male with severe stenosis of the left internal carotid artery (ICA). **A:** The vertical section of two-dimensional carotid doppler ultrasonography (CDU) showed that a plaque extended from the anterior wall of the carotid bulb to the ICA and the length of it was about 23.4mm. The echo of the plaque presented as uniformly hypoechoic, classifying it as vulnerable plaque. The arrow represented the rupture of fiber cap. **B:** The cross-sectional color doppler flow imaging of CDU showed no color blood flow signal at the plaque filling, and the red blood flow area in the lumen was the residual lumen after stenosis. The asterisk symbol indicates carotid plaque. **C:** The vertical section of spectrum doppler: PSV:221cm/s, EDV:69.3cm/s, RI:0.686. After comprehensive evaluation, the stenosis rate was 72.1%. **D-E:** The lower right corner was the cross-sectional and reconstructed images of computed tomography angiography (CTA) and the arrow in the enlarged view indicated the stenosis of ICA. **F:** The cross-sectional images of computed tomography perfusion imaging (CTP) showed that no obvious abnormal ischemia-reperfusion was found in the TTP values of bilateral regions of interest. **G:** The plaque length of gross specimen after eversion carotid endarterectomy (CEA) was about 23mm, which was similar to that
measured by preoperative ultrasound. 

**H:** The lower right corner was the image of H&E staining (×2). The square area of it represented a large number of lipid cells in plaque (×10) and the circular area of it showed the rupture of fiber cap. 

**I:** Enlarged view around fiber cap rupture in H&E staining (×40) and the arrow represented few inflammatory cells infiltration around the broken fiber cap.

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**Figure 2**

68-year-old symptomatic male with severe stenosis of the left internal carotid artery (ICA). 

**A:** The vertical section of two-dimensional carotid doppler ultrasonography (CDU) showed that a plaque extended from the anterior wall of the carotid bulb to the ICA and the length of it was about 23.7mm. The echo of the plaque presented as hypoechoic with small <25% hyperechoic regions, classifying it as vulnerable plaque. The hypoechoic region indicated by the arrow represents intra-plaque hemorrhage. 

**B:** The cross-sectional color doppler flow imaging of CDU showed no color blood flow signal at the plaque filling, and the red blood flow area in the lumen was the residual lumen after stenosis. The asterisk symbol indicates carotid plaque. 

**C:** The vertical section of spectrum doppler: PSV:618cm/s, EDV:298cm/s, RI:0.517. After comprehensive evaluation, the stenosis rate was 90.9%. 

**D-E:** The lower right corner was the cross-sectional and reconstructed images of computed tomography angiography (CTA) and the arrow in the enlarged view indicated the stenosis of ICA. 

**F:** The cross-sectional images of computed tomography
perfusion imaging (CTP) showed that obvious abnormal ischemia-reperfusion was found in the TTP values of left regions of interest. \textbf{G:} The plaque length of gross specimen after eversion carotid endarterectomy (CEA) was about 23mm, which was similar to that measured by preoperative ultrasound. \textbf{H:} The lower right corner was the image of H&E staining (×2). The square area of it represented a large number of thrombus and intra-plaque hemorrhage in plaque (×10) and the circular area of it showed the rupture of fiber cap. \textbf{I:} Enlarged view around fiber cap rupture in H&E staining (×40) and the arrow represented large degree of inflammatory cell infiltration around the broken fiber cap.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation12.6.docx](#)
- [supplementarytable.docx](#)
- [Table1.docx](#)