Diagnostic value between IL-17 combined with IL-18 and CT angiography in carotid atherosclerosis was evaluated. A total of 158 patients with suspected carotid artery stenosis in People's Hospital of Dongying from March 2014 to April 2017 were selected as the subjects. One hundred and two patients with carotid atherosclerosis were selected as the atherosclerosis group, the other 56 patients with no obvious carotid artery abnormalities were selected as the disease control group. In addition, there were 100 healthy subjects selected as the healthy control group. The level of IL-17 and IL-18 in peripheral blood of all the subjects was detected by ELISA. The ROC curve was used to analyze the diagnostic value of IL-17 combined with IL-18 and CT angiography in atherosclerosis. The levels of IL-17 and IL-18 in the three groups were different (P<0.05). The level of IL-17 and IL-18 in the atherosclerosis and disease control groups was higher than that in the healthy control group, and the level of IL-17 and IL-18 in the atherosclerosis was higher than that in the disease control group. The sensitivity of IL-17 or IL-18 was less than the coincidence rate and sensitivity of IL-17 combined with IL-18 as diagnostic criteria, and AUC was also less than AUC of IL-17 combined with IL-18. The sensitivity and diagnostic accuracy of IL-17 combined with IL-18 in the diagnosis of carotid atherosclerosis were not significantly different from CT angiography (P>0.05), but the specificity was obviously lower than CT angiography (P<0.05). The diagnosis of atherosclerosis of IL-17 combined with IL-18 was more accurate, it can improve the diagnostic efficiency of atherosclerosis and be used as a routine method for screening the atherosclerosis.

Introduction
Carotid atherosclerosis is a very common disease in cardiovascular medicine, and it is the basis of cerebral vascular disease such as stroke. There are approximately sixty percent stroke patients related to it. It is one of the main causes of death worldwide (1,2). It is reported that the incidence of carotid atherosclerosis has declined in developed countries in recent years, while increased in developing countries, which may result from dietary patterns changing caused by economic development, because high-fat diet and low-cholesterol consumption play a very important role in the pathogenesis of carotid atherosclerosis (3,4).

Among non-invasive examination methods, CT angiography is a widely used method in the diagnosis of carotid and cervical atherosclerosis. It is a popular technique for diagnosis of carotid atherosclerosis as it is fast, convenient, repeatable and its temporal resolution is high (5,6). However, CT angiography is not suitable for all patients. It is closely related to the heart rate (7,8). In recent years, the diagnosis of serology and the diagnosis of molecular biology are very popular directions of study. IL-17 and IL-18 are two molecules associated with atherosclerosis. It is reported that the level of IL-17 protein and mRNA in the peripheral blood of patients with acute coronary syndrome is significantly higher than that of healthy subjects (9). IL-18 can induce the expression of γ interferon, a proinflammatory cytokine, which is reported to be involved in the rupture of atherosclerotic plaques (10). Therefore, we speculate that IL-17 and IL-18 have the diagnostic value of carotid atherosclerosis.

In this study, we compared and analyzed the diagnostic value of IL-17 combined with IL-18 and CT angiography in 158 patients with suspected carotid artery stenosis in order to provide a reference for the clinical diagnosis of carotid atherosclerosis.

Materials and methods

Clinical data. From March 2014 to April 2017 in People’s Hospital of Dongying (Dongying, China), 158 patients with
suspected carotid stenosis were selected as the subjects. One hundred and two patients with carotid atherosclerosis were selected as the atherosclerosis group. The other 56 patients with no obvious carotid artery abnormalities were selected as the disease control group, and there were 100 healthy subjects selected in the same period after health examination as the healthy control group. Carotid atherosclerosis conforms to the diagnostic standard of International Atherosclerosis Society (11), which included patients with suspected ischemic cerebrovascular disease with or without clinical symptoms, and excluded patients with carotid stenosis caused by arterial dissection, patients with stable vital signs, no obvious contraindication, and patients with coronary heart disease, patients with intermittent myocardial infarction, patients with acute myocardial infarction, patients with cerebral atherosclerosis, patients with infectious disease, patients with severe cardiac insufficiency, tumor patients, patients with grade III hypertension, patients with liver and kidney failure, patients with incomplete case information, and patients with mental or learning disability. The control group underwent physical examination to exclude organic diseases and Alzheimer’s disease. This study was approved by the Ethics Committee of People’s Hospital of Dongying, and the patients or their family members signed an informed consent.

ELISA detection of IL-17 and IL-18. The levels of IL-17 and IL-18 in peripheral blood of three groups of patients were detected, and the peripheral blood of patients in the morning under fasting condition was collected by nurses in People’s Hospital of Dongying. IL-17 test kit was purchased from R&D Systems, Inc. (Minneapolis, MN, USA) and IL-18 test kit was purchased from Beijing Keyingmei Technology (Beijing, China). ELISA was used to determine the level of IL-17 and IL-18 in the samples. First, the standard curve of IL-17 and IL-18 was established, then blank, standard and sample wells were set up, and standard sample was added to enzyme labeled well precoated with primary IL-17 or IL-18 monoclonal antibody (dilution, 1:300; cat. nos. HS750, D8000C; R&D Systems, Inc.) followed by incubation at 37℃ for 30 min. After washing, horseradish peroxidase labeled IL-17 or IL-18 antibodies were added. After incubation at 37℃ for 30 min, the unbound enzyme was removed, then the substrate was added to show color at room temperature without light. In 15 min, the absorbance value (OD value) of each well at 450 nm wavelength was read under the blank well, and the amount of OD value was positively correlated with the concentration of IL-17 or IL-18 in the sample. Three groups of repeated wells were set up, and the experiment was repeated 3 times.

CT angiography. CT angiography was performed in the atherosclerosis and disease control groups. The scanning equipment was GE Company Light Speed VCT (GE Healthcare, Milwaukee, WI, USA). The scan range from the arterial arch to the upper edge of the external auricle. The first sequence is plain scanning, and the second sequence is enhancement scanning with unchangeable parameters. Scanning parameters: voltage 120 KV, current 300-500 mA, detector width 128 mm x 0.625 mm, layer thickness 0.9 mm, layer spacing 0.5 mm, alignment 0.625 mm, FOV 180 mm, screw pitch 0.984, spherical tube rotation speed 0.5 sec/rot.

Table I. Comparison of the basic data.

| Factors                  | Atherosclerosis group | Disease control group | Healthy control group | F/χ² | P-value |
|--------------------------|-----------------------|-----------------------|-----------------------|------|---------|
| No. of patients          | 102                   | 56                    | 100                   |      |         |
| Male/female              | 72/30                 | 32/24                 | 52/48                 | 2.629| 0.302   |
| Age (years)              | 52.5±11.4             | 54.6±12.5             | 49.7±13.3             | 3.028| 0.050   |
| Hypertension (n, %)      | 82 (80.4)             | 10 (17.9)             | 58.125 <0.001         |      |         |
| Hyperlipidemia (n, %)    | 86 (84.3)             | 14 (25.0)             | 54,743 <0.001         |      |         |
| Diabetes (n, %)          | 32 (31.4)             | 5 (8.9)               | 10.154 0.001          |      |         |
| Weight (n, %)            | 63 (61.8)             | 32 (57.1)             | 59 (59.0)             | 0.353| 0.828   |
| ≥60 kg                   | 39 (38.2)             | 24 (42.9)             | 41 (41.0)             |      |         |
| <60 kg                   | 68 (66.7)             | 39 (69.6)             | 65 (65.0)             | 2.329| 0.312   |
| History of smoking (n, %)| 52 (51.0)             | 23 (41.1)             | 43 (43.0)             |      |         |
| Positive                 | 50 (49.0)             | 33 (58.9)             | 57 (57.0)             |      |         |
| Negative                 |                       |                       |                       | 2.097| 0.351   |
| Exercise habits (n, %)   |                       |                       |                       |      |         |
| Negative                 | 68 (66.7)             | 39 (69.6)             | 65 (65.0)             |      |         |
| Positive                 | 34 (33.3)             | 17 (30.4)             | 35 (35.0)             |      |         |

The nonionic contrast agent used for the VCT examination was omnipaque produced by GE Pharmaceutical Co., Ltd. (Shanghai, China). By injection of the anterior elbow vein, the rate of 4-4.4 ml/sec, the quantitative injection of 60-80 ml, and then the addition of the physiological saline 40 ml, image processing technology is used to display lesions on AM4.4 workstation. Two experienced radiologists used double blind methods to diagnose the images respectively.

Statistical analysis. SPSS19.0 [AsiaAnalytics (formerly SPSS China), Shanghai, China] was used. Enumeration data were expressed in (n, %), and χ² test was used for comparison of the rates. The measurement data were expressed as mean ± standard deviation. ANOVA was used for comparison among the multiple groups, and LSD test was used for comparison between the two groups. The receiver operating curve (ROC) was used to analyze the diagnostic value. P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical data. There were 158 cases of suspected carotid artery stenosis, among which 102 patients with carotid atherosclerosis in the atherosclerosis group, including 72 male and 30 female patients, with an average age of 52.5±11.4 years and 56 patients in the disease control group, including 32 male and 24 female patients, with an average age of 54.6±12.5 years. There were 100 healthy subjects in the healthy control group, including 52 males and 48 females, with an average age of 49.7±13.3 years. There was no significant difference among the three groups in basic data such as weight, age, sex, smoking history and exercise habits (P>0.05). The proportion
of patients with hypertension, hyperlipidemia and diabetes in the atherosclerosis group was significantly higher than that in the disease control group (P<0.05) (Table I).

Detection results of IL-17 and IL-18. The results of IL-17 and IL-18 test detected by ELISA in the three groups of patients showed that the levels of IL-17 and IL-18 in the three groups were all different. The levels of IL-17 and IL-18 in the atherosclerosis and disease control groups were higher than those in the healthy control group (P<0.05), and the level of IL-17 in the atherosclerosis group was higher than that in the disease control group (P<0.05) (Table II; Fig. 1).

Analysis of diagnostic value of CT angiography, IL-17 and IL-18 in 158 patients with suspected carotid atherosclerosis. The coincidence rate and the sensitivity of carotid atherosclerosis diagnosed by diagnostic criteria of IL-17 and IL-18 were less than those diagnosed by the diagnostic criteria of IL-17 combined with IL-18 (P<0.05), AUC was also less than AUC of IL-17 combined with IL-18 (P>0.05), and there was no significant difference among the three groups in specificity (P>0.05). The sensitivity and diagnostic accuracy of IL-17 combined with IL-18 in the diagnosis of carotid atherosclerosis were not significantly different from CT angiography (P>0.05), but the specificity was significantly lower than CT angiography (P<0.05) (Table III; Fig. 2).

Discussion

Carotid atherosclerosis poses a serious threat to human health. Approximately 60% of stroke patients are associated with carotid atherosclerosis, with a high incidence and mortality

| Factors                  | Atherosclerosis group | Disease control group | Healthy control group | F-value | P-value |
|--------------------------|-----------------------|-----------------------|-----------------------|---------|---------|
| No. of patients          | 102                   | 56                    | 100                   | 674.867 | <0.001  |
| IL-17 (ng/l)             | 30.35±5.12            | 21.12±3.33            | 10.32±2.45            |         |         |
| IL-18 (ng/ml)            | 336.5±132.5           | 214.7±58.9            | 114.9±30.6            |         |         |

*P<0.05, compared with the atherosclerosis group. **P<0.05, compared with the disease control group.

| Factors                  | IL-17                  | IL-18                  | IL-17 combined with IL-18 | CT angiography |
|--------------------------|------------------------|------------------------|---------------------------|----------------|
| AUC                      | 0.912                  | 0.750                  | 0.931                     |                |
| 95% confidence interval  | 0.870-0.954            | 0.674-0.823            | 0.893-0.968               |                |
| Diagnostic standard      | 13.45 ng/l             | 279.5 ng/ml            |                           |                |
| Sensitivity (%)          | 68.6 (70)              | 62.7 (64)              | 77.5 (79)                 | 73.5 (75)      |
| Specificity (%)          | 87.5 (49)              | 85.7 (48)              | 82.1 (46)                 | 94.6 (53)      |
| Diagnostic coincidence rate (%) | 75.3 (119) | 70.9 (112) | 81.6 (129) | 81.0 (128) |

*P<0.05, compared with IL-17 and IL-18 for each. **P<0.05, compared with IL-17 and IL-18 and IL-17 combined with IL-18 for each.

![Figure 1](image1.png)
A study was conducted to evaluate the diagnostic value of IL-17 and IL-18 in patients with carotid atherosclerosis. The coincidence rate and the sensitivity of carotid atherosclerosis diagnosed by diagnostic criteria of IL-17 and IL-18 were less than those diagnosed by the diagnostic criteria of IL-17 combined with IL-18 (P<0.05). AUC was also less than AUC of IL-17 combined with IL-18 (P>0.05), and there was no significant difference among the three groups in specificity (P>0.05). AUC (IL-17) = 0.912, AUC (IL-18) = 0.750, AUC (IL-17 and IL-18) = 0.931.

Figure 2. The ROC curves of IL-17 and IL-18 alone and IL-17 combined with IL-18 to diagnose carotid atherosclerosis. The coincidence rate and the sensitivity of carotid atherosclerosis diagnosed by diagnostic criteria of IL-17 and IL-18 were less than those diagnosed by the diagnostic criteria of IL-17 combined with IL-18 (P<0.05). AUC was also less than AUC of IL-17 combined with IL-18 (P>0.05), and there was no significant difference among the three groups in specificity (P>0.05). AUC (IL-17) = 0.912, AUC (IL-18) = 0.750, AUC (IL-17 and IL-18) = 0.931.

This study analyzed the level of IL-17 and IL-18 in peripheral blood of 102 patients with carotid atherosclerosis and few reports of IL-17 combined with IL-18 for the diagnosis of carotid atherosclerosis. It could be better to screen the carotid atherosclerosis, because its methodology is simple, and it has economic benefits with no adverse reactions, which is suitable for a wide range of individuals and will not create a physical or psychological burden to patients (22).

In conclusion, IL-17 combined with IL-18 has a high diagnostic value for atherosclerosis, and can be used as a routine method for screening and diagnosing atherosclerosis.
Ethics approval and consent to participate

The study was approved by the Ethics Committee of People’s Hospital of Dongying (Dongying, China). Patients who participated in this study, signed an informed consent and had complete clinical data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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