The expression of interleukin-25 increases in human coronary artery disease and is associated with the severity of coronary stenosis

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ABSTRACT

Objective: Interleukin (IL) 25, also known as IL-17E, is an inflammatory cytokine and has been demonstrated to be closely related to cardiovascular diseases by regulating immunity and inflammation, including atherosclerosis. This study was aimed to evaluate the expression of IL-25 in patients with coronary artery disease (CAD).

Methods: In this study, the expression of IL-25 in normal (n=6) and atherosclerotic (n=10) human coronary arteries was detected by immunofluorescent staining. In addition, the serum IL-25, IL-6, and tumor necrosis factor (TNF) α concentrations in stable angina pectoris (SAP, n=44), unstable angina pectoris (UAP, n=46), acute myocardial infarction (AMI, n=34), and non-CAD (control, n=36) were measured using enzyme-linked immunosorbent assay (ELISA) kits.

Results: IL-25 was significantly increased in coronary arteries of CAD patients when compared with normal coronary arteries, with macrophages and T lymphocytes being the sources of IL-25, especially macrophages. Moreover, the serum concentrations of IL-25 were markedly elevated in CAD patients and gradually increased in SAP, UAP, and AMI groups. In addition, IL-25 levels were positively correlated with the IL-6 and TNF-α levels, and Gensini score in CAD patients. Logistic regression analysis showed that IL-25 was independently positively correlated with the occurrence of acute coronary syndrome (ACS). A receiver operator characteristic curve suggested that IL-25 presented a significant diagnosis value in ACS.

Conclusion: IL-25 is increased in the coronary arteries and serum of CAD patients and is associated with the severity of coronary stenosis and the occurrence of ACS, suggesting that IL-25 may be one of the biomarkers of ACS. *(Anatol J Cardiol 2020; 23: 151-9)*

Keywords: interleukin 25, coronary artery disease, inflammation, Gensini score

Introduction

Coronary artery disease (CAD) is one of the most fatal cardiovascular diseases with a high hospitalization rate and mortality (1). Inflammation has been identified to participate in CAD partly by acting on atherosclerosis (2). Based on the critical role of various inflammatory factors and mediators in the pathophysiological processes of atherosclerosis, there are increasing numbers of investigations focused on novel and efficient therapies to ameliorate the immune and inflammatory response of atherosclerosis (3-6).

The interleukin (IL) 17 cytokine family, including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25), and IL-17F, was found to be involved in inflammatory responses (7, 8). Growing evidence showed that the IL-17 cytokine family played important roles in cardiovascular diseases. For instance, IL-17A was increased in the plasma of unstable angina and acute myocardial infarction patients, and no significance was noted between patients with complex and simple lesions (9, 10). Animal studies also showed that IL-17A is increased in the left ventricle infarcted region of rats after myocardial infarction. Moreover, endothelial cells in the infarcted region were observed to stain positively for IL-17A, which suggested that IL-17A might be involved in cardiac repair and inflammation (11). Liao et al. (12) reported that IL-17A might have the capacity to regulate cardiomyocyte apoptosis and neutrophil infiltration to participate in myocardial ischemia/reperfusion injury (13).

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IL-25 is also a member of the IL-17 cytokine family, which possesses unique structures and has the capacity to regulate immune and inflammatory responses (14) and angiogenesis (15). Further evidence showed that IL-25 promotes cell proliferation, inhibits apoptosis (16), and affects the development of fibrosis and hypoxic ischemic injury (17), tumor metastasis (18) and metabolism processes (19, 20). Experiments using IL-25 for the treatment of atherosclerosis-prone apolipoprotein (apo) E-deficient mice showed its ability to protect against atherosclerosis without affecting the adaptive Th2 responses (21). However, the IL-25 levels in patients with CAD are still unclear. In this study, we detected the serum and coronary artery tissue expression of IL-25 in CAD and attempted to explore the possible clinical associations between IL-25 and CAD.

Methods

Collection of coronary artery tissues

This study protocol was approved by the Medical Ethics Committee of the Renmin Hospital of Wuhan University. All donors or their families signed an informed consent form before tissue harvesting. Human coronary artery tissue with atherosclerotic plaques (n=10) was obtained from CAD patients undergoing heart transplantation, whereas control arteries (n=6) were obtained from normal donors who failed to match for a transplant recipient. All donors in whom other diseases were reported to affect the expression of IL-25 including allergic diseases, autoimmune diseases, and tumor were ruled out. The tissues were fixed in formalin at the end of the surgery and then embedded in paraffin for immunofluorescence.

Immunofluorescence

After the tissue samples were fixed in formalin, coronary artery tissues embedded in paraffin were cut into 5 µm slices. After washing in PBS, the sections were subsequently sealed with 8% sheep serum for 1 h and incubated at 4°C overnight with primary antibodies. Immunofluorescent staining was used for IL-25 expression by anti-IL-25 antibodies (R&D Systems, USA). Double immunofluorescent staining was used to detect the source of IL-25 by anti-CD4 antibodies (Biolegend, USA), anti-CD68 antibodies (Abcam, UK), and anti-α- smooth-muscle actin (SMA) antibodies (Abcam, UK). Then, the sections were washed with PBS followed by a secondary antibody incubation at 37°C for 1 h. The nuclei were stained with 4′,6-diamidino-2-phenylindole (R&D Systems, USA). Finally, the sections were visualized by fluorescence microscopy.

Collection and conservancy of human blood samples

Patients who suffered from chest pain and had undergone coronary angiography were recruited from January 2018 to March 2018 in the Renmin Hospital of Wuhan University. The study protocol was approved by the Medical Ethics Committee of the Renmin Hospital of Wuhan University. The exclusion crite-

ria for this study included patients who were too young or too old (<20 or >80 years old) (n=5) and those with severe liver damage, severe renal dysfunction, and a prior history of drug assumption (n=2) (22). Meanwhile, all donors in whom other diseases were reported to affect the expression of IL-25 including allergic diseases, autoimmune diseases, and tumor were ruled out.

According to the diagnoses, 37 patients were assigned to the non-CAD group (control group), 44 patients to the stable angina pectoris (SAP) group, 48 patients to the unstable angina pectoris (UAP) group, and 34 patients to the acute myocardial infarction (AMI) group. The CAD patients group included the SAP, UAP, and AMI groups. Vacutainers coated with sodium heparin were used to collect blood samples upon admission. Thirty-four AMI patients underwent reperfusion therapy after blood collection. Serum was isolated after centrifugation for 20 min at 4000 g within 1 h of collection, aliquoted, and stored at −80°C until analysis. In addition, patients with blood sample hemolysis (n=1) in the control group and lipaemia (n=2) in the UAP group were also excluded from this study. All of the donors or their families have provided an informed consent.

Measurement of serum IL-25, IL-6, and TNF-α levels

Serum concentrations of IL-25 (R&D Systems, USA), IL-6, and TNF-α (both from eBioscience, USA) were measured using the enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

Gensini score

The Gensini score was used to estimate the severity of coronary stenosis according to the results of a coronary angiography as previously reported (22). The coronary angiography was conducted by the attending practicing physicians. Then, we recorded the subjects' Gensini scores according to the results of the coronary angiography.

Echocardiography measurement

The patients' left ventricular ejection fraction (LVEF) was measured using a VIVID E9 (GE Healthcare, USA) ultrasonography machine as previously reported (22). The LVEF values were recorded for all subjects.

Statistical analysis

The statistical software package SPSS 19.0 was used for data analysis. All data were tested for normality using the Kolmogorov–Smirnov test. Clinical characteristics with normal distribution were presented as mean±standard deviation. Student's t-tests were performed when two normal distribution groups were compared and one-way analysis of variance (ANOVA) was used to test the difference between multiple groups. Data without normal distribution were presented as the median (minimum-maximum) and compared by means of the Mann–Whitney U test. The correlations in continuous variables with normal distribution were evaluated by Pearson correlation analysis. Spearman's correlation analysis

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was used in continuous variables without normal distribution. Simple linear regression analyzes and subsequent binary logistic regression analyzes were used to identify whether IL-25 was an independent predictor of ACS. A receiver operator characteristic (ROC) curve was used to evaluate the diagnosis value of cytokines in ACS. P<0.05 was considered statistically significant.

Results

Basic clinical characteristics of patients who provided coronary artery tissues

From the basic clinical characteristics of the patients who provided coronary artery tissues, several significant differences were found between control and CAD patients, including higher levels of TC and LDL-C, but lower LVEF in CAD patients. However, no difference was observed in other clinical characteristics, including gender, age, smoking, hypertension, BMI, SBP, DBP, Glu, TG, HDL-C, eGFR, CRP, and time. These clinical characteristics of patients are shown in Table 1.

Expression of IL-25 in human atherosclerotic plaques

To determine the expression of IL-25 in human atherosclerotic plaques, immunofluorescent staining was performed. We found that IL-25 was highly expressed in coronary arteries with atherosclerotic plaques but lowly expressed in normal arteries (Fig. 1a). Specific antibodies, such as human anti-CD4 antibodies, anti-CD68 antibodies, and anti-α-SMA antibodies, were used to show markers for T-helper cells, macrophages, and smooth muscle cells, respectively. In addition, immunofluorescent double staining displayed that IL-25 was almost not expressed by smooth muscle cells in atherosclerotic arteries but was expressed by macrophages and T lymphocytes, especially the macrophages (Fig. 1b).

Basic clinical characteristics of patients who provided blood samples

Serum levels of Glu, LDL-C, and cardiac troponin I (TnI), as well as the percent of smoking and obesity, SBP, DBP, CRP, and the Gensini score were significantly higher in CAD groups when compared to those of the control group. However, there were no significant differences between the control and the CAD groups in terms of other clinical parameters including diabetes and hyperlipidemia, age, HbAlc, TG, TC, HDL-L, eGFR, ALT, LVEF, NT-pro-BNP, and oral hypoglycemia. The basic clinical characteristics of each group are presented in Table 2.

Serum IL-25, IL-6, and TNF-α levels in the four groups

According to the results of the ELISA, IL-25 levels were significantly higher in the CAD patients compared to those in the control group (Table 3) and gradually increased in the SAP, UAP, and AMI groups (150.7 pg/mL vs. 379.8 pg/mL vs. 484.7 ng/mL vs. 634.3 ng/mL, p<0.05) (Fig. 2a). Homoplastic changes were found in IL-6 and TNF-α levels (Fig. 2b and 2c); however, not all of the differences noted were statistically significant. The serum levels of the various cytokines in the four groups of patients are listed in Table 3.

The correlation between serum cytokine levels and the Gensini score

Pearson correlation analysis or Spearman's correlation analysis were performed in 124 CAD patients to evaluate the relationship among IL-25, IL-6, and TNF-α and the Gensini score. The results showed that serum levels of IL-25 were markedly positively correlated with IL-6 (r=0.304, p<0.001) and TNF-α (r=0.403, p<0.001). The concentrations of IL-6 were also identified to be positively correlated with TNF-α (r=0.201, p=0.026). Additionally, the concentrations of serum IL-25 (r=0.495, p<0.001), IL-6 (r=0.440, p<0.001), and TNF-α (r=0.507, p<0.001) were positively correlated with the Gensini score (Fig. 3a–3c).

The association between serum IL-25 and ACS

Simple linear regression analysis and binary logistic regression analysis were performed to confirm whether IL-25 is a possible predictor for ACS. The results of the simple linear regression analysis suggested that IL-25, IL-6, TNF-α, cTnI, CRP, Glu, LDL, and SBP levels showed a potential (p<0.05) correlation

Table 1. Clinical characteristics of the patients who provided tissues

Characteristics	Control	CAD
Gender (M/F)	3/3	6/4
Age (years)	62.0 (46.0-76.0)	62.5 (48.0-73.0)
Smoking (%)	50	50
Hypertension (%)	50	70
BMI (kg/m²)	20.6 (17.9-23.6)	21.1 (18.8-24.5)
SBP (mm Hg)	125.5 (118.0-136.0)	118.0 (102.0-137.0)
DBP (mm Hg)	78.5 (66.0-87.0)	88.5 (68.0-99.0)
Glu (mmol/L)	5.6 (3.8-6.5)	5.95 (4.9-7.1)
TG (mmol/L)	1.4 (0.7-1.9)	1.9 (0.9-2.3)
TC (mmol/L)	3.4 (3.0-4.6)	4.0 (3.4-6.0)*
HDL-C (mmol/L)	1.40 (0.88-1.90)	1.4 (0.9-1.8)
LDL-C (mmol/L)	2.2 (1.9-3.3)	3.5 (2.0-4.5)*
eGFR (mL/min)	92.0 (80.0-100.0)	87.5 (78.0-104.0)
CRP (mg/L)	3.0 (0.44-4.5)	3.9 (2.0-4.5)
LVEF (%)	56.0 (50.0-60.0)	31.0 (24.0-36.0)*
Time (h)	3.4 (2.9-4.0)	3.1 (2.5-4.2)

*P<0.05 vs. control. BMI - body mass index; CRP - C-reactive protein; DBP - diastolic blood pressure; eGFR - estimated glomerular filtration rate; Glu - fasting glucose; HDL-C - high-density lipoprotein cholesterol; LDL-C - low-density lipoprotein cholesterol; LVEF - left ventricular ejection fraction; SBP - systolic blood pressure; TC - total cholesterol; TG - total triglycerides; Time - time intervals between the beginning of surgery and collection of tissue sampling; CAD - coronary artery disease; M - male; F - female
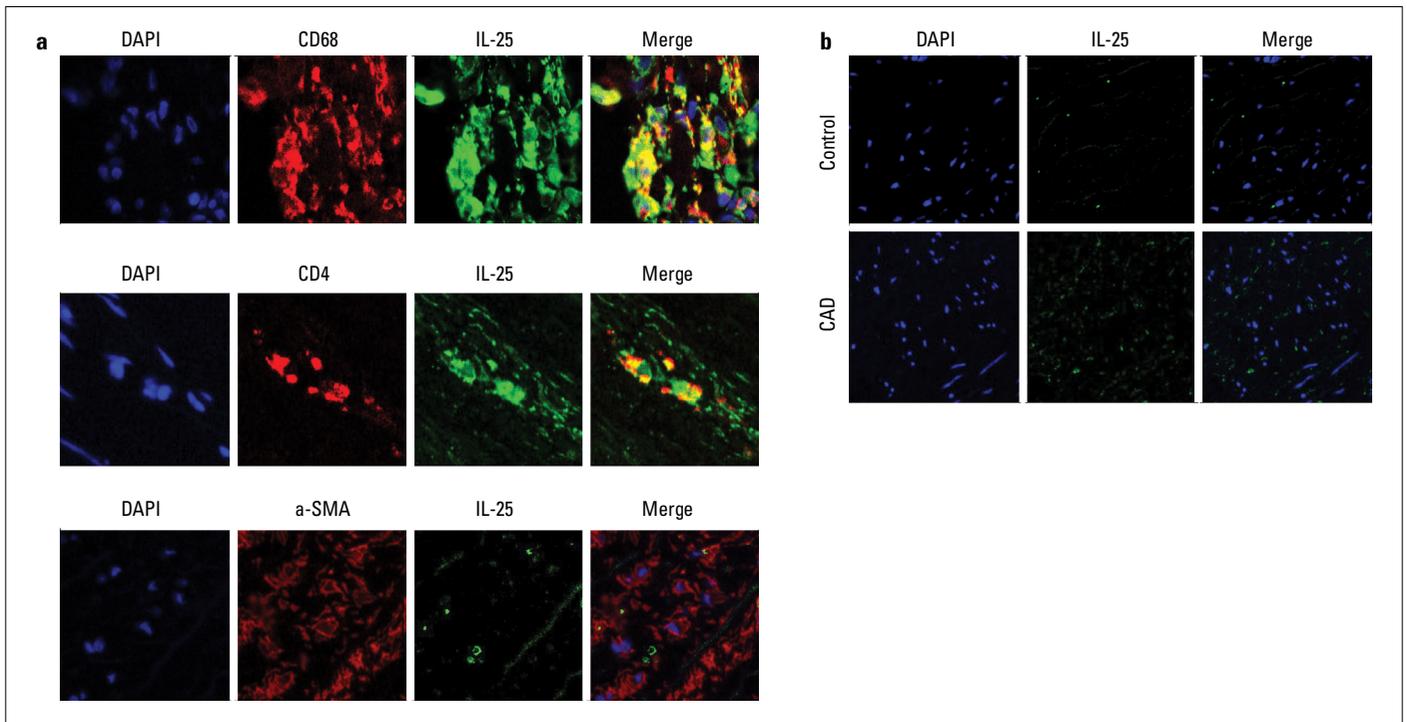
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Figure 1. IL-25 expression in human atherosclerotic plaques. (a) IL-25 levels in normal and CAD coronary artery tissues were measured by immunofluorescent staining (200×). (b) Double staining for IL-25 expression, Th cells, macrophages, and smooth muscle cells in human atherosclerotic plaques (400×)
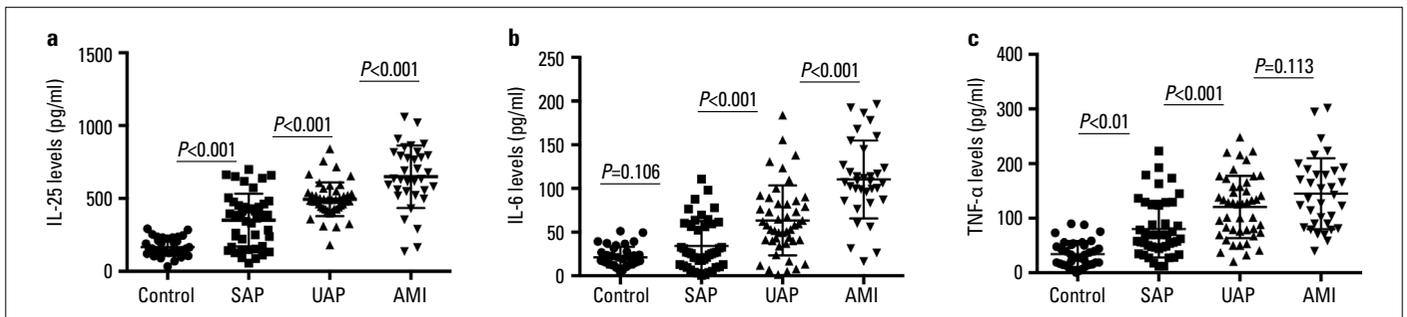
CAD - coronary artery disease

Figure 2. Serum IL-25, IL-6, and TNF-α levels in the four groups. (a) Serum IL-25 levels in patients with SAP, UAP, AMI, and control groups were measured. (b) Serum IL-6 levels were measured in the four groups. (c) Serum TNF-α levels were measured in each group
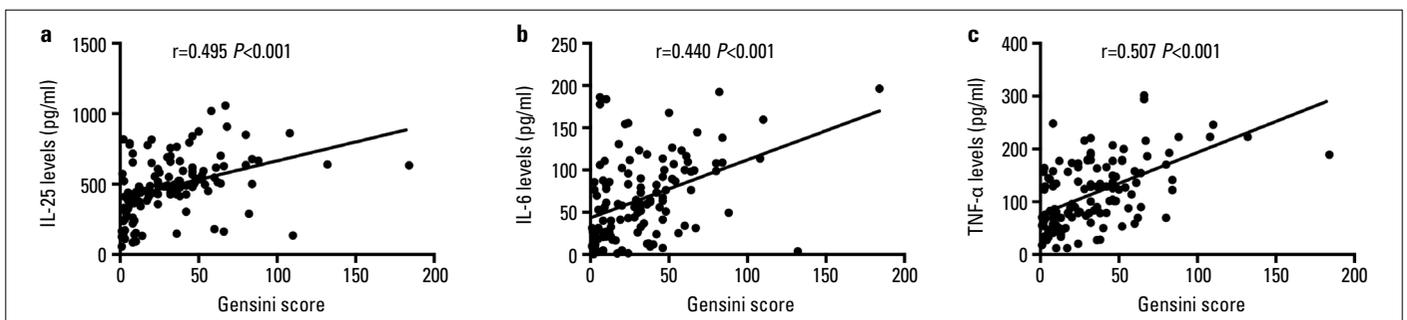
Figure 3. Spearman's correlation of circulating cytokines and Gensini score. Correlation among (a) IL-25 levels, (b) IL-6 levels, (c) TNF-α levels, and Gensini score

with the onset of ACS whereas smoking, DBP, BMI, HbAlc, TC, age, and gender showed no obvious trends (p>0.05) with the onset of ACS. Furthermore, IL-25, IL-6, TNF-α, cTnI, CRP, Glu, LDL, and SBP levels were used to perform binary logistic regression analysis. The further results showed that serum levels of IL-25 (OR 1.006, 95% CI 1.002 to 1.011; p=0.004), IL-6 (OR 1.036, 95% CI

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Table 2. Information of clinical characteristics of patients who provided blood samples

Characteristics	Control	SAP	P value	UAP	P value	AMI	P value
Elderly (n, %)	16 (44.4%)	24 (54.5%)	0.372	20 (43.5%)	0.931	16 (47.1%)	0.828
Gender (M/F)	20/16	28/16	0.466	31/15	0.276	21/13	0.601
Smoking (n, %)	7 (19.4%)	27 (61.4%)*	<0.001	26 (56.5%)*	0.001	20 (58.8%)*	0.001
Obesity (n, %)	16 (44.4%)	38 (86.4%)*	<0.001	36 (78.3%)*	0.002	24 (70.6%)*	0.028
Diabetes (n, %)	9 (25.0%)	13 (29.5%)	0.653	16 (34.8%)	0.343	11 (32.4%)	0.499
Hyperlipidemia (n, %)	14 (38.9%)	12 (27.3%)	0.273	15 (32.6%)	0.557	16 (47.1%)	0.493
Hypertension (n, %)	25 (69.4%)	38 (86.4%)	0.067	33 (71.7%)	0.822	24 (70.6%)	0.917
Age (years)	60±16	64±10	0.280	63 (43-80)	0.691	62±10	0.787
BMI	23.7 (21.2-27.6)	25.4 (21.5-29.1)*	<0.001	25.7 (20.9-28.5)*	0.003	25.6 (20.9-28.3)*	0.007
Glu (mmol/L)	5.4±1.1	6.8±2.1*	<0.001	6.5 (4.6-11.8)*	<0.001	6.8 (4.3-17.5)*	0.001
HbAlc (%)	5.9 (5.3-6.9)	6.1 (5.3-7.6)	0.515	6.2±0.6	0.593	6.2±1.0	0.592
TG (mmol/L)	1.7±0.9	1.6±1.0	0.488	1.5 (0.6-3.5)	0.866	2.0 (0.7-4.8)	0.274
TC (mmol/L)	4.2 (2.4-7.0)	4.0±1.1	0.073	4.4 (2.8-7.7)	0.463	4.1 (2.5-6.7)	0.477
HDL-C (mmol/L)	1.1±0.5	1.1 (0.6-1.9)	0.957	1.1±0.3	0.799	1.0±0.3	0.110
LDL-C (mmol/L)	2.3±0.7	3.0 (1.5-4.5)*	<0.001	3.1 (1.7-4.6)*	<0.001	3.2 (1.1-4.5)*	0.001
SBP (mm Hg)	155±21	147 (110-210)*	0.027	143 (97-180)*	0.001	145 (100-221)	0.142
DBP (mm Hg)	86 (69-119)	80 (64-112)*	0.027	82 (58-108)*	0.016	85 (58-126)	0.353
eGFR (mL/min)	92±6	90 (76-109)	0.927	94 (70-110)	0.143	91 (75-109)	0.995
ALT (U/L)	36.5 (23.0-49.0)	34.0 (20.0-67.0)	0.663	39.2±9.4	0.387	35.5 (18.0-74.0)	0.787
AST (U/L)	32.0 (20.0-45.0)	30.0±5.3	0.573	30.4±5.8	0.959	29.7±7.4	0.444
CRP (mg/L)	6.4±16.0	28.6±27.9*	<0.001	33.7 (0.2-135.2)*	<0.001	55.8 (3.6-183.0) *	<0.001
Gensini score	0 (0-0)	18.3±23.7*	<0.001	32.0 (3.0-88.0)*	<0.001	52.5 (2.0-184.0) *	<0.001
LVEF (%)	54.0 (44.0-67.0)	54.5 (45.0-67.0)	0.745	55.0 (45.0-67.0)	0.536	54.0 (43.0-67.0)	0.568
NT-pro-BNP (pg/mL)	190±67	186±61	0.923	191±62	0.918	199±84	0.934
TnI (10⁻³ ng/L)	13.2 (4.3-34.6)	23.6 (4.2-45.8)*	<0.001	17.7 (8.9-43.2)*	0.007	2689.5 (1438.0-5429.0)*	<0.001
Medications, n (%)							
Oral hypoglycemics	6 (16.7%)	8 (18.2%)	0.860	9 (19.7%)	0.738	10 (29.4%)	0.208
Insulin	1 (2.8%)	5 (11.4%)	0.149	5 (10.9%)	0.165	3 (8.8%)	0.280
Aspirin	17 (47.2%)	24 (54.5%)	0.517	25 (54.3%)	0.524	19 (55.9%)	0.472
Statin	8 (22.2%)	16 (36.4%)	0.172	19 (41.3%)	0.070	19 (55.9%)*	0.004
ACEI/ARB	11 (30.6%)	15 (34.1%)	0.739	18 (39.3%)	0.423	15 (44.1%)	0.244
Beta-blockers	4 (11.1%)	7 (15.9%)	0.538	10 (21.7%)	0.207	7 (20.6%)	0.280
CCB	8 (22.2%)	18 (40.9%)	0.078	16 (34.8%)	0.218	14 (41.2%)	0.090
Diuretic	2 (5.6%)	3 (6.8%)	0.818	3 (6.5%)	0.857	1 (2.9%)	0.592

*P<0.05 vs. control. ACEI - angiotensin-converting enzyme inhibitor; ALT - alanine aminotransferase; ARB - angiotensin receptor blocker; AST - aspartate transaminase; AMI - acute myocardial infarction; BMI - body mass index; CCB - calcium channel blocker; CRP - C-reactive protein; cTnI - cardiac troponin I; DBP - diastolic blood pressure; eGFR - estimated glomerular filtration rate; Glu - fasting glucose; HbA1c - hemoglobin A1c; HDL-C - high-density lipoprotein cholesterol; LDL-C - low-density lipoprotein cholesterol; LVEF - left ventricular ejection fraction; NT-pro-BNP - N-terminal pro-brain-type natriuretic peptide; SBP - systolic blood pressure; SAP - stable angina pectoris; UAP - unstable angina pectoris; TG - total triglycerides; TC - total cholesterol

1.017 to 1.055; p<0.001), and Glu (OR 1.471, 95% CI 1.040 to 2.081; p=0.029) seemed to be independent predictors for ACS (Table 4). In this study, part of variables including TNF-α, cTnI, CRP, LDL, and SBP levels were not the independent predictors for the occurrence of ACS.

The diagnostic value of cytokines and cTnI in ACS

We further applied ROC analysis on diagnostic values of serum cTnI, IL-25, IL-6, and TNF-α concentrations in ACS. As shown in Figure 4a and 4b, each of these cytokines and cTnI seem to have particular diagnostic value for ACS, whereas the

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Table 3. Serum IL-25, IL-6, and TNF-α levels in each group

	Control	SAP	P value	UAP	P value	AMI	P value
IL-25 (pg/mL)	150.7 (33.2-292.4)	379.8 (57.3-699.3)*	<0.001	484.7 (180.9-841.0)*	<0.001	634.3 (135.7-1058.0)*	<0.001
IL-6 (pg/mL)	21.4±11.8	34.4±28.3	0.106	57.8 (1.5-183.9)*	<0.001	107.5 (16.4-196.4)*	<0.001
TNF-α (pg/mL)	30.4 (0.6-90.0)	80.0±51.9*	<0.001	127.3 (20.4-248.1)*	<0.001	139.1 (39.5-301.5)*	<0.001

*$P<0.05$ vs. control. Values are median, unless otherwise stated. Values in parentheses are minimum and maximum. Mann–Whitney U test was used to compare the concentrations of IL-25, IL-6, and TNF-α in CAD serum. SAP - stable angina pectoris; UAP - unstable angina pectoris; AMI - acute myocardial infarction; TNF-α - tumor necrosis factor-α

Table 4. Association between cytokines, clinical characteristics, and the presence of acute coronary syndrome assessed by simple linear regression analysis and further multiple linear regression analysis

Variables	Simple linear			Binary logistic		
	β	95% CI	P value	OR	95% CI	P value
IL-25	0.644	0.524 to 0.764	<0.001	1.006	1.002 to 1.011	0.004
IL-6	0.591	0.465 to 0.718	<0.001	1.036	1.017 to 1.055	<0.001
TNF-α	0.548	0.417 to 0.680	<0.001	1.011	1.000. to 1.021	0.052
cTnI	0.485	0.348 to 0.623	<0.001	1.002	0.997 to 1.007	0.470
CRP	0.409	0.265 to 0.552	<0.001	1.016	0.998 to 1.035	0.079
Glu	0.239	0.086 to 0.391	0.002	1.471	1.040 to 2.081	0.029
LDL-C	0.225	0.072 to 0.378	0.004	1.359	0.574 to 3.218	0.486
SBP	-0.156	-0.311 to -0.001	0.049	0.981	0.954 to 1.009	0.176
DBP	-0.081	-0.237 to 0.076	0.310			
TC	0.121	-0.035 to 0.277	0.128			
Age	0.017	-0.014 to 0.174	0.829			
Gender	0.052	-0.105 to 0.209	0.517			
BMI	0.092	-0.064 to 0.249	0.246			
Smoking	0.150	-0.005 to 0.305	0.058			
HbA1c	0.077	-0.080 to 0.233	0.335			

BMI - body mass index; CRP - C-reactive protein; cTnI - cardiac troponin I; DBP - diastolic blood pressure; Glu - fasting glucose; HbA1c - hemoglobin A1c; LDL-C - low-density lipoprotein cholesterol; SBP - systolic blood pressure; TC - total cholesterol; TNF-α - tumor necrosis factor-α

combination of three cytokines presented a bit more preferable diagnostic value than three cytokines individually did in ACS.

Discussion

In the present study, we found for the first time that IL-25 was increased in human atherosclerotic arteries and that T lymphocytes and macrophages, especially the macrophages, rather than smooth muscle cells, were the source of IL-25 in atherosclerotic plaques. We also found that IL-25 levels were increased in the serum of CAD patients and positively correlated with the severity of coronary stenosis. Binary logistic regression analysis suggested that IL-25 might independently correlate with the onset of ACS.

Previous studies had demonstrated that IL-25 was associated with the progression of various diseases of the immune system, such as asthma (23), rheumatoid arthritis (24), multiple sclerosis (25) and inflammatory bowel disease (26). However, IL-25 expression in various diseases involving other systems is controversial. Growing evidence has shown that IL-25 is upregulated in serum of patients with allergic diseases such as respiratory diseases exacerbated by aspirin (27) but without significant changes in patients with severe asthma (28) or uncontrolled asthma (29). In addition, Aalberse et al. (30) showed that IL-25 was also highly enhanced in children's plasma response to peanuts. In autoimmune diseases, IL-25 was observed to be elevated in the plasma of rheumatoid arthritis patients and serum of Churg-Strauss syndrome patients compared to healthy individuals (31, 32). In our study, the results showed that serum concentrations and atherosclerotic plaques expression of IL-25 in CAD patients were significantly increased and that IL-25 was also positively correlated with the Gensini score, further con-
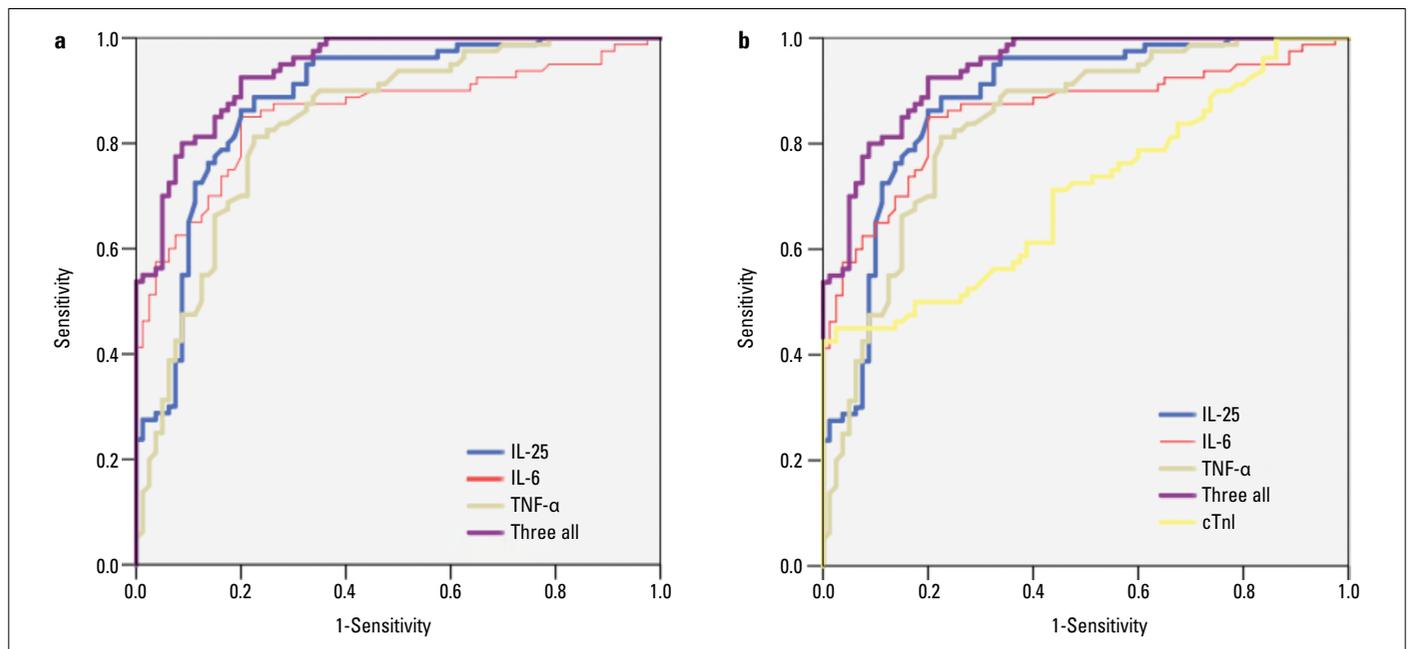
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Figure 4. Diagnostic value of cytokines and cTnI in ACS. (a) ROC curve of IL-25, IL-6, TNF-α, and combining the three cytokines for ACS. (b) ROC curve of IL-25, IL-6, and TNF-α, combining the three cytokines and cTnI for ACS

firming that IL-25 levels may be closely related with the severity of coronary stenosis. These results suggested that IL-25 might play an important role in the progression of atherosclerosis.

After it was first found to be expressed by Th2-polarized CD4+ T cells, IL-25 has been identified to be extensively produced and to locally or generally participate in inflammatory, infectious, or autoimmune diseases (33-35). IL-25 has been identified to be produced by several different cells, such as bone marrow-derived mast cells (36), alveolar macrophages (37), eosinophils, basophils (38), epithelial, (15) and endothelial cells (39). Our results of immunofluorescence double staining were consistent with the above conclusions, and for the first time, we suggest that macrophages and T lymphocyte are important sources of IL-25 in human atherosclerotic plaques. This suggestion is a significant addition to the sources of IL-25 and indicates that IL-25 may influence the development of atherosclerosis via regulation of the inflammatory response.

Atherosclerosis is well known to be an underlying pathophysiological mechanism of CAD and various inflammatory cells and factors have been identified to play important roles in the development of atherosclerosis (40). Clinical, experimental, and animal studies have supported the hypothesis that inflammatory cells are recruited to the intima during the beginning of atherosclerosis and monocytes further differentiate into tissue macrophages after translocation to the arteries and subsequently become foam cells. In response to the development of an atheroma, one type of macrophages named M1 macrophages have the function of secreting the proinflammatory cytokines such as IL-1β and TNF-α, which are involved in the stimulation of IL-6 production (41). Kampits et al. (42) reported that CAD patients who experienced major events had higher concentrations of

TNF-α and IL-6. Min et al. (43) found that serum levels of TNF-α and IL-6 were significantly more elevated in ACS patients than those in controls and that these levels were associated with high Gensini scores. In our present study, we found that the concentrations of serum TNF-α and IL-6 are not only increased in CAD patients but also associated with IL-25 levels and Gensini scores, which reflect the severity of coronary stenosis. Our results are consistent with previous findings and further suggested the predictive role of TNF-α and IL-6 in CAD as shown in the Spearman correlation analysis and logistic regression analysis. Therefore, we surmised that IL-25 could affect levels of TNF-α and IL-6 in CAD patients.

As important members of inflammatory cytokines, IL-6, and TNF-α have been identified to be synthesized by macrophages, B cells, CD4 effector T-helper cells, and some other immune cells (44). As a Th2 cytokine, IL-25 has been reported to regulate several inflammatory and immune cells in the progression of several diseases. For instance, Kleinschek et al. (45) suggested that experimental autoimmune encephalomyelitis IL-25 knockout mice presented increased levels of IL-23 in the periphery and enhanced T cells in the central nervous system, which produced IL-17, IFN-γ, and TNF. IL-25 also enhanced the functions of Th2 memory cells through the sustained expression of GATA-3, c-MAF, and JunB independent of IL-4 (38). In contact hypersensitivity induced by fluorescein isothiocyanate, IL-25 secreted by mast cells and nonimmune cells can stimulate dermal dendritic cells to produce IL-1β and contributes to the activation of Th17 instead of Th2 cells (46). Furthermore, with exogenous IL-25 treatment of apoE/Rag-1 deficient mice, splenic innate lymphoid type 2 cells were extensively enhanced and plasma IL-5 was also increased. In addition, apoE-

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deficient mice presented reduced aortic atherosclerosis after injection of recombinant IL-25, which suggests that IL-25 may have a protective role in atherosclerosis (21). IL-25 deficient mice were also used and showed an aggravation of the aortic atherosclerotic plaques with elevated Th1/Th17 proinflammatory response, suggesting a role for IL-25 in the maintenance of the T cells in patients suffering from hyperlipidemias (47). Our studies first found that high serum IL-25 levels were associated with an increased risk of suffering ACS. Data in ROC analysis suggested that IL-25 might have the function of reflecting the stability of plaque. The small sample size of our study might contribute to these results, although further large amount of subjects will be more convincing. These results indicated that inflammatory cytokine IL-25 would be tightly related to atherosclerosis progress, especially unstable plaque, and shows potential to be a biomarker for ACS patients.

Study limitations

However, there are some limitations in the present study. For instance, oxidative stress, which also plays critical roles in the development of atherosclerosis, might be another mechanism for IL-25 involving in ACS. Therefore, further investigations should concentrate on the relationship between IL-25 and oxidative stress in atherosclerosis. Another limitation of the study is that the number of the patients was too small and more subjects should be included to reduce any margins of error.

Conclusion

The results of our study identified that IL-25 is highly expressed in atherosclerotic arteries of CAD patients and is mainly produced by macrophages and T lymphocyte cells. Additionally, IL-25 levels in serum were first significantly increased in CAD patients especially in AMI patients and positively correlated with the severity of coronary atherosclerosis. Further analysis identified that high serum IL-25 levels were associated with an increased risk of suffering plaque instability and high predictive value of IL-25 in ACS.

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