ORIGINAL RESEARCH

Cong-Lin Liu PhD^{1,2}

De-Liang Shen PhD¹

Kui-Zhu MD¹

Jun-Nan Tang MD¹

Qi-Min Hai MD¹

Jin-Ying Zhang PhD¹

Levels of interleukin-33 and interleukin-6 in patients with acute coronary syndrome or stable angina

¹Department of Cardiology, the First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan 450052, China.

²Institute of Clinical Medicine, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China.

Abstract

Purpose: The purpose of this study was to investigate the levels of interleukin-33 (IL-33) and interleukin-6 (IL-6) in patients with acute coronary syndrome or stable angina.

Methods: Serum IL-33 and IL-6 were measured with Enzyme Linked Immunosorbent Assay (ELISA) in patients with acute coronary syndrome (ACS, n = 40), and stable angina pectoris (SAP, n = 43). IL-33 and IL-6 were also determined in 30 healthy subjects (control group).

Results: The serum level of IL-33 in the ACS group (78.60 ± 44.84 ng/L) was lower than in the SAP (102.58 ± 37.21 ng/L, $P < 0.01$) or control groups (130.24 ± 10.17 ng/L, $P < 0.01$). The serum level of IL-6 in the ACS group (39.90 ± 12.64 ng/L) was higher than in the SAP (18.68 ± 11.89 ng/L, $P < 0.05$) or control groups (6.28 ± 17.72 ng/L, $P < 0.05$). There were no differences in serum levels of IL-33 and IL-6 among the single-, double- and triple-vessel lesion groups. IL-33 and IL-6 levels were negatively correlated with each other in the ACS ($r = -0.871$, $P < 0.01$) and SAP groups ($r = -0.788$, $P < 0.01$).

Conclusion: The serum level of IL-33 was lower in patients with ACS or SAP and was negatively correlated with the serum level of IL-6. Thus, IL-33 and IL-6 may be used as biomarkers for evaluating inflammatory response and severity of coronary heart disease in patients with ACS or SAP.

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Correspondence to:

Prof. Jinying Zhang,
Department of Cardiology,
the First Affiliated Hospital, Zhengzhou University,
Zhengzhou, Henan 450052, PR China.
E-mail: jy Zhang@zzu.edu.cn

Acute coronary syndrome (ACS) is one of the most critical clinical syndromes and rupture of vulnerable plaque in the coronary artery is considered to be responsible for the pathogenesis of acute coronary syndrome [1]. Studies have shown the process of inflammation affects the stability of the atherosclerotic plaque and may be the initiating link of the early stages of atherosclerosis [2]. Recent studies suggest that the pro-inflammatory cytokine interleukin family plays an important role in the pathogenesis of atherosclerosis [3]. Interleukin-33 (IL-33), a new member of the IL-1 superfamily of cytokines [4], is essential in the regulation of the inflammatory response as well as in congenital and acquired autoimmune diseases [5]. It has been found that expression of IL-33 in human atherosclerotic plaques is associated with plaque instability [6]. Interleukin-6 (IL-6) is a pro-inflammatory cytokine that is triggered by vulnerable plaque or necrotic myocardium and correlates with the severity of coronary artery disease (CAD) [7].

This study measured the serum levels of IL-33 and IL-6 in patients with ACS or SAP. At the same time, coronary angiography was performed in patients with CAD to investigate the relationship among IL-33, IL-6 and the severity of coronary lesions.

Methods

Subjects

The study was approved by the Institution Review Board of the First Affiliated Hospital of Zhengzhou University. Informed consent was obtained from all participants before the study.

From 1 August 2012 to 28 February 2013, 83 consecutive patients (54 men, mean age 56.50 ± 12.54 years, range 40-78) with coronary artery disease, confirmed by coronary angiography, were recruited from the Department of Cardiology in the First Affiliated Hospital of Zhengzhou University. All the enrolled patients were classified into two groups: a group with ACS and a group with SAP. The ACS group consisted of 16 patients with ST elevation acute myocardial infarction (STEMI) and 24 patients with unstable angina pectoris (UAP). The SAP group consisted of 43 patients with stable angina pectoris. Percutaneous Coronary Intervention (PCI) was performed in 83 patients with ACS and SAP.

Thirty healthy subjects (17 men, mean age 51.50 ± 12.81 years, range 42-76) with no signs of ischemic heart disease, ECG criteria suggestive of acute coronary syndrome or past history of cardiovascular disease or diabetes were also recruited for the measurement of serum levels of IL-33 and IL-6.

Diagnostic criteria of STEMI include detection of rise of cardiac biomarker values (troponin I (TnI), troponin T (TnT), or CK-MB) with at least one value above the 99th percentile of the upper reference limit and with at least one of the following: symptoms of ischemia; new or presumably new significant ST-T changes or new LBBB; development of pathological Q waves in the ECG; imaging evidence of new loss of viable myocardium, or new regional wall motion abnormality; and, identification of an intracoronary thrombus by angiography [8]. UAP was defined as chest pain at rest with either a ST segment depression of at least 0.1 mV or a T-wave inversion in two or more continuous electrocardiographic leads. No biomarker of myocardial necrosis has been released (based on two or more samples collected at least 6 h apart, with a reference limit of the 99th percentile of the normal population) [9]. SAP was diagnosed as chest pain of at least six months' duration accompanied by evidence of severe coronary artery disease on coronary angiography and by the absence of clinically evident ischemic episodes during the week preceding arteriography [10].

Those who had a previous history of cardiomyopathy, valvular heart disease, tumor, inflammatory diseases, liver and renal dysfunction, cerebral vessel diseases or peripheral angiopathy were excluded from the study, as were those who were pregnant.

Both STEMI and UAP patients were managed with intravenous heparin before PCI. Other drugs used in all three groups of patients included β -blockers, aspirin, nitrates, statins and angiotensin converting enzyme inhibitors.

Detailed self-reported questionnaires containing information on the history of hypertension, smoking status, alcohol consumption and so on were collected at baseline and used for analysis. Clinical findings, such as blood pressure and heart rate, were assessed upon hospital admission by the internal medicine doctor.

Blood samples to be used for assessment of serum IL-33 and IL-6 levels were drawn after admission within 60 minutes, into tubes containing EDTA and aprotinin immediately centrifuged for 10min at $1000 \times g$ at 4°C and stored at -80°C until assayed in a blinded fashion in a single batch. Samples were thawed only once. Blood to be used for assessments of serum high-density lipoproteins, low-density lipoproteins, total cholesterol, triglyceride, NT-proBNP, CK-MB and high-sensitivity C-reactive protein (hs-CRP) levels was drawn into tubes containing anticoagulant and the tubes were then sent to the clinical laboratory for determination.

TABLE 1. Comparison of baseline demographic and clinical characteristics of patients with ACS or stable angina and the control group.

Clinical variables	ACS group	SAP group	Control group	P value
Number of patients	40	43	30	
Age (years)	57.89 ± 12.74	55.24 ± 8.39	51.50 ± 12.81	0.259
Male (n; %)	24 (60%)	30 (69.77%)	17 (56.67%)	0.725
Medical history				
Hyperlipidemia (n; %)	16 (40%) ^Δ	25 (58.14%)	0	0.011
Type 2 diabetes (n; %)	10 (25%)	15 (34.88%)	0	0.168
Hypertension (n; %)	28 (70%) ^{Δ*}	16 (37.21%)*	6 (20%)	0.040
Family history of CAD (n; %)	2 (5%)	0	0	
Smoking history (n; %)	7 (17.5%)	5 (11.63%)	2 (6.67%)	0.691
Diagnostic tests				
SBP (mmHg)	139.75 ± 19.04	128.10 ± 17.74	118.10 ± 22.62	0.288
DBP (mmHg)	87.61 ± 11.58	81.29 ± 12.49	80.00 ± 4.08	0.154
Heart rate (/min)	77.93 ± 11.36	70.57 ± 10.50	75.71 ± 10.12	0.127
HDL (mmol/L)	0.99 ± 0.28	0.93 ± 0.22	1.15 ± 0.24	0.109
LDL (mmol/L)	2.96 ± 2.09	2.87 ± 0.86	2.71 ± 0.57	0.900
TC (mmol/L)	4.56 ± 2.17	4.54 ± 0.97	4.35 ± 0.81	0.934
TG (mmol/L)	1.71 ± 0.83	2.02 ± 0.98	1.47 ± 0.57	0.312
Single-vessel disease (n; %)	12 (30%)	18 (41.86%)		0.137
Double-vessel disease (n; %)	10 (25%)	6 (13.95%)		0.245
Triple-vessel disease (n; %)	18 (45%)	9 (20.93%)		0.101

Data are presented as mean ± SD

Abbreviations: ACS: acute coronary syndromes; SAP: stable angina; CAD: coronary artery disease; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL: high-density lipoproteins; LDL: low-density lipoproteins; TC: total cholesterol; TG: triglyceride.

^ΔP < 0.05, ^{ΔΔ}P < 0.01 in comparison with SAP group,

*P < 0.05, **P < 0.01 in comparison with control group

Measurements of IL-33 and IL-6

Levels of serum IL-33 and IL-6 were determined using ELISA kits (Nanjing Jiancheng Technology Industry Co. Ltd, Nanjing, China). The antibodies were coated onto microlite ELISA plates using 10 μL per well in 100 μL of phosphate-buffered saline. Plates were then blocked in bovine serum albumin and wells were filled with 40 μL IL-33 or IL-6 plasma samples or recombinant standards. After incubation overnight, the plates were washed and then incubated with anti-IL-6 or anti-IL-33 in an assay buffer, per 100 μL in each well. After 2 hours, plates were washed, and detection was with methyl acridinium ester-labeled streptavidin. These assay kits had a lower limit of detection of 5 ng/L for IL-33 and 2 ng/L for IL-6. Experiments were performed in triplicate.

Echocardiographic study

Two-dimensional echocardiography was carried out using a digital imaging system (Vivid-7; GE Medical System). The left ventricular end-systolic volume (LVESV) and the left ventricular end-diastolic volume (LVEDV) were measured using Simpson's method, and the left ventricular ejection fraction (LVEF, %) was calculated by (LVEDV - LVESV)/LVEDV × 100%.

Coronary angiography

Coronary angiography was performed using a approach of the radial artery with six Fr diagnostic catheters. Images were recorded in multiple projections for left and right coronary arteries on 35 mm cine film. All coronary stenoses were assessed by two experienced cardiologists who had knowledge of neither the levels of IL-33 and IL-6 nor the identity and clinical characteristics of the patients.

Statistical Analysis

Statistical analysis was performed with SPSS[®] version 17.0 (SPSS, Chicago, IL, USA). Quantitative data were expressed as mean values $\pm$ standard deviation (SD) and categorical data as percentages. Data between groups were compared by Student's *t* test. Categorical data was compared with chi-squared test. Comparison among the three groups was performed by analysis of variance of univariate (ANOVA) and Fisher's PLSD test. The relationship between serum levels of IL-33 and IL-6 were explored using Pearson's correlation technique was analyzed by univariate linear relation. Correlation coefficient was tested by *t*-test. $P < 0.05$ was considered as statistically significant.

Results

General characteristics of the study subjects

Demographic and clinical characteristics of the study participants are shown in Table 1. Among the three groups, there was no significant difference in sex, age, smoking history, family history of coronary artery disease or diabetes, blood pressure, heart rates and blood cholesterol levels. A greater number of patients with ACS had hypertension compared with those in the SAP group ($P < 0.05$); meanwhile, a greater number of patients with SAP had hyperlipidemia compared with ACS group ($P < 0.05$).

Comparisons of the mean levels of serum IL-33 and IL-6

Comparisons of serum levels of IL-33 and IL-6 and other biochemical features and left ventricular function among patients with ACS or SAP and healthy controls are shown in Table 2. The level of IL-33 in ACS group was lower than the SAP and control group ($P < 0.01$), and the level of IL-6 in the ACS group was higher than the SAP and control group ($P < 0.01$). There was no difference in serum IL-6 level between the SAP and control groups and there was no difference in hs-CRP, NT-proBNP, CK-MB or echocardiographic parameters among the three groups. The Gensini score, an indicator of the degree of coronary atherosclerosis, was higher in the ACS group than in the SAP group, but the difference was not significant ($P = 0.178$).

Relationship between serum levels of IL-33 and IL-6 and severity of vascular lesions

The serum levels of IL-33 and IL-6 in single-, double- and triple-vessel disease groups were higher than that in the control group ($P < 0.05$); however, there was no difference in the serum levels of IL-33 and IL-6 among the three patient groups (Table 3).

Correlations of IL-33 and IL-6 in ACS and SAP group

In patients with ACS, the level of IL-33 showed a negative correlation with the level of serum IL-6 ($r = -0.871$, $P < 0.01$; Figure 1). Similarly, in the SAP group, the level of IL-33 was also

TABLE 2. Comparison of biochemical features and left ventricular function in patients with ACS or stable angina and the control group

	ACS group	SAP group	Control group	<i>P</i> value
Number of patients	40	43	30	
IL-33 (ng/ L)	78.60 $\pm$ 44.84 ^{**Δ}	102.58 $\pm$ 37.21 [*]	130.24 $\pm$ 10.17	0.012
IL-6 (ng/ L)	39.90 $\pm$ 12.64 ^{*Δ}	18.68 $\pm$ 11.89	6.28 $\pm$ 17.72	0.014
hs-CRP (mg/L)	5.32 $\pm$ 10.59	2.01 $\pm$ 2.43	3.92 $\pm$ 11.44	0.759
NT-proBNP (pg/ml)	550.08 $\pm$ 110.78	151.43 $\pm$ 58.17	260.96 $\pm$ 368.11	0.435
CK-MB (U/ L)	36.25 $\pm$ 61.97	10.00 $\pm$ 4.78	12.43 $\pm$ 7.31	0.197
LVEDV (ml)	119.8 $\pm$ 52.26	92.67 $\pm$ 27.97	107.80 $\pm$ 16.16	0.510
LVESV (ml)	48.60 $\pm$ 34.28	34.33 $\pm$ 15.50	40.40 $\pm$ 8.92	0.599
LVEF (%)	51.60 $\pm$ 10.06	64.00 $\pm$ 6.93	62.10 $\pm$ 8.06	0.124
Gensini Score	31.65 $\pm$ 32.99	17.35 $\pm$ 25.75		0.178

Data are presented as mean $\pm$ SD

Abbreviations: IL-33: interleukin-33; IL-6: interleukin-6; hs-CRP: high-sensitivity C-reactive protein; EDV: end diastolic volume; ESV: end systolic volume; LVEF: left ventricular ejection fraction.

^{Δ} $P < 0.05$, ^{$\Delta\Delta$} $P < 0.01$ in comparison with SAP group

^{*} $P < 0.05$, ^{**} $P < 0.01$ in comparison with control group

TABLE 3. Comparisons of serum IL-33 and IL-6 in single, double and triple-vessel disease.

	n	IL-33 (ng/ L)	IL-6 (ng/ L)
Single vessel lesion group	30	85.60 ± 46.41*	20.49 ± 12.53*
Double vessel lesion group	16	81.00 ± 50.01*	33.12 ± 28.46*
Triple-vessel lesion group	27	77.86 ± 51.10*	43.05 ± 17.63*
Control group	30	130.24 ± 10.17	6.28 ± 17.72
P value		0.063	0.076

Data are presented as mean ± SD

*P<0.01 compared with control group.

negatively correlated with the level of serum IL-6 ($r = -0.788$, $P < 0.01$; Figure 2).

Discussion

The inflammatory response plays an important role in the development of ACS. Based on the consensus that the inflammatory response is the cause of instability of coronary plaque, resulting in the pathogenesis of ACS, researchers have conducted extensive studies on a variety of inflammatory markers, including IL-1, IL-6 and hs-CRP, and found that they all play a role in the pathogenesis of ACS. Studies of AMI suggest that cytokines are preferentially produced by inflammatory cells in the pre-infarct zone and that persistent elevation of cytokines results from an increased infiltration of inflammatory cells [11]; however, the nature of the relationships among various cytokines in the inflammatory reaction are still unclear.

This is the first study assessing the correlation of IL-33 and IL-6 levels in patients with ACS or SAP. The major findings of the this study are as follows: 1) the serum level of IL-33 in the ACS group was lower than either the SAP group or the health subjects and the serum levels of IL-6 showed the opposite tendency; 2) the serum levels of IL-6 were similar between patients with SAP and health subjects; 3) there were no differences in serum levels of IL-33 or IL-6 among patients with single-, double- or triple-vessel lesions; and, 4) the IL-33 levels were negatively correlated with IL-6 levels in the ACS and SAP groups.

IL-33, a recently described member of the IL-1 family, has been reported to be the natural ligand for membrane-bound ST2. IL-33 is broadly expressed in many tissues, including the heart, and Sanada *et al.* [12] found it to be a biomechanically-induced protein predominantly synthesized by cardiac fibroblasts. It was recognized as one of the key cytokines enhancing the Th2-type immune responses and had been shown to promote the production of Th2 cytokines, such as IL-4, IL-5 and IL-13, by innate-type lymphoid cells, basophils, mast cells, and Th2 cells [13,14]. A study by Milovanovic *et al.* [15] suggested

that ST2 alters the Th1/Th2 balance and leads to enhanced Th1/Th17 immune response; in addition, the fact that strong pro-inflammatory type of ST2^{-/-} antigen-presenting cells is accompanied by higher production of IL-33 indicates that IL-33 exhibits an intracrine inflammatory role in APC and may therefore promote inflammation. Furthermore, McLaren [16] revealed that IL-33 significantly reduced the mRNA expression of key genes implicated in the uptake and efflux of cholesterol (like SR-A, CD36 and SR-B1) in THP-1 macrophages and primary human monocyte-derived macrophages (HMDMs) and simultaneously increases gene expression involved in cholesterol efflux, including Apolipoprotein E, therefore decreased AcLDL/OxLDL uptake and enhances cholesterol efflux. In summary, IL-33 plays an important role in the cardiovascular system and IL-33/ST2 signaling was revealed as a crucial cardioprotective fibroblast-cardiomyocyte paracrine system. Miller [17] also found, in mice deficient in apolipoprotein E, that administration of recombinant IL-33 induced Th2 cytokines and protective ox-LDL antibodies, which significantly reduced atherosclerotic plaque development and exerted cardioprotective effects. These results demonstrate a novel role for IL-33 in the control of Th1/Th2 balance and indicate that IL-33 may be used as a new biomarker for the severity of coronary heart disease and may also be a potential therapeutic target for patients with ACS or SAP. The results of this study demonstrate that IL-33 levels decreased as clinical severity increased from SAP to ACS, suggesting that IL-33 might reflect the severity of CAD. Further studies should be conducted to investigate the correlation between level of IL-33 and stability of atherosclerotic plaques.

In the current study, a higher serum concentration of the pro-inflammatory cytokine IL-6 was found in the ACS group in comparison with the SAP and control groups, which is consistent with the results of earlier studies [18,19]. IL-6 is a proximal mediator produced under different stimuli, such as IL-1 β , in various tissues [20]. During tissue injury or infection, IL-6 is mainly produced by hepatocytes as the major initiators

FIGURE 1. Correlation between levels of IL-33 and IL-6 in patients with ACS ($r = -0.871$, $P < 0.01$).

FIGURE 2. Correlation between levels of IL-33 and IL-6 in patients with stable angina ($r = -0.788$, $P < 0.01$).

of the acute-phase reaction [21]. In patients with CAD, elevated circulating IL-6 concentrations may be the result of a variety of stimuli, including various clinical risk factors that lead to the release of IL-6 from numerous cell types, including smooth muscle cells and macrophage/foam cells found in atheromatous plaques [22]. Anderson *et al.* [18] reported that IL-6 and the soluble IL-6 receptor (sIL-6R) were elevated and IL-6R mRNA was decreased in patients with AMI, in comparison with patients with CAD and controls, supporting the hypothesis that blocking IL-6 receptor binding may be pathogenic in AMI. Several lines of evidence suggest that IL-6 may be used as a biomarker for evaluating the severity and prognosis of coronary heart disease. López-Cuenca *et al.* [19] demonstrated that IL-6 concentrations decreased after the acute phase of CAD and that obtaining serial measurements of IL-6 concentration improved the prognostic risk stratification of patients with CAD. A study by Ammirati [23] suggested that IL-6+ STEMI patients were at an increased risk of death and that determination of the combined cytokine index may improve the prediction of fatal outcomes. Considerable evidence has been obtained for the diagnostic and prognostic role of IL-6 in patients with CAD [24].

This study also detected a negative correlation between levels of IL-33 and IL-6 in patients with ACS and SAP although it is unclear how the two cytokines affect each other. Previous studies, in primary blood-derived human basophils, have demonstrated that IL-33 induces modest production of multiple cytokines, including IL-4, IL-5, IL-6, IL-8, IL-13 and GM-CSF [25,26]. Basophils are functionally related to mast cells and are targets of IL-33. Following IL-33 stimulation, basophils produce several cytokines, particularly IL-4, IL-6 and IL-13, which enhance basophile adhesion, integrin expression, chemotaxis, degranulation and survival [25,27]. IL-33 can also activate dendritic cells (DCs) to produce IL-5, IL-6 and IL-13 by CD4+ T cells [28], suggesting an additional indirect role of IL-33 in the production of IL-6; therefore, IL-33 may increase the availability of IL-6 in the process of inflammation. Increased level of IL-6 may also exert a negative effect on the level of IL-33, but the mechanism of this process needs to be further investigated.

This is the first study that investigates the correlation between levels of IL-33 and IL-6 in patients with ACS and SAP and the results suggested that IL-33 and IL-6 are both involved in the pathogenesis of ACS and may have mutual effects with each other. Thus, both IL-33 and IL-6 show promise as new biomarkers for the evaluation of severity of CAD and may be associated with stability of vulnerable plaques.

In conclusion, our study demonstrates that, in patients with ACS, there is a decreased serum level of IL-33 and an increased serum level of IL-6. A negative correlation was found between IL-33 and IL-6 levels in the ACS and SAP groups. IL-33 and IL-6 show promise for use as biomarkers for evaluating inflammatory response and severity of CAD in patients with ACS or SAP. The interaction among different cytokines remains to be investigated.

This study has several limitations. First, it was conducted at a single center and the number of cases was relatively small. Second, it did not assess long-term effects on atherosclerotic plaque progression and the scale of in-stent restenosis. Third, it did not compare levels of IL-33 and IL-6 at different time points. Thus, further studies should involve a larger number of cases at different time points and a longer follow-up on all patients in the study.

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