Heart Vessels (2007) 22:371–375
DOI 10.1007/s00380-007-0991-z

ORIGINAL ARTICLE

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Interleukin-18: a strong predictor of the extent of coronary artery disease in patients with unstable angina

Received: October 30, 2006 / Accepted: April 20, 2007

Abstract The aim of this study was to confirm that plasma interleukin (IL)-18 level is associated with the extent of coronary artery disease in unstable angina patients. Previous studies have shown that patients with unstable angina have significantly higher plasma IL-18 levels than healthy volunteers. However, the association between IL-18 and the extent of coronary artery atherosclerosis in patients with unstable angina remains unclear. Plasma concentrations of IL-18 and high-sensitivity C-reactive protein (hs-CRP) were measured in 166 consecutive patients admitted for coronary arteriography. One hundred and eighteen patients with unstable angina had coronary artery disease (coronary artery disease group; severity score: 2.32 ± 1.47; Gensini score: 31.3 ± 25.9), and 48 patients with coronary risk factors and without coronary artery lesions served as the risk control group. Plasma levels of IL-18 were higher in the coronary artery disease group than in the risk control group ($P = 0.062$). Additionally, plasma levels of IL-18 were significantly higher in 77 coronary artery disease patients with severity score ≥2 than in the risk control group (242.3 ± 110.6 vs 209.8 ± 120.3 pg/ml, $P = 0.016$). By univariate analysis, log-transformed plasma IL-18 concentration was positively correlated with coronary artery disease severity score ($r = 0.244$, $P = 0.009$). By multiple regression analyses, the association between coronary artery disease severity score and IL-18 remained significant ($\beta = 0.733$, $P = 0.017$) when controlling for age, diabetes mellitus and left ventricular ejection fraction. Additionally, coronary artery disease severity score was greater in the highest tertile (>246 pg/ml) of plasma IL-18 levels than in the middle (176–246 pg/ml) and the lowest (<176 pg/ml) tertiles (2.79 ± 1.52 vs 2.05 ± 1.08 vs 2.13 ± 1.66, $P = 0.028$). Of note, plasma hs-CRP level had no significant correlation with coronary artery severity. Plasma IL-18 level is associated with the extent of coronary artery disease in unstable angina patients, suggesting the link between IL-18 and coronary artery atherosclerosis in these patients.

Key words Interleukin-18 · Coronary artery disease · C-reactive protein

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Introduction

Interleukin (IL)-18 is a proinflammatory cytokine produced mainly by monocytes and macrophages.[1] Interleukin-18, originally identified as an interferon-γ-inducing factor in Kupffer cells and macrophages, plays a central role in orchestrating the cytokine cascade and accelerates atherosclerotic plaque growth and plaque vulnerability in experimental and animal models.[2–6] Interferon-γ appears to play a key role in both plaque development and stability.[5] Plasma IL-18 level has been identified as an independent predictor of coronary heart disease in healthy, middle-aged men and men with risk factors for atherosclerosis and coronary artery disease.[7] Recently, increased IL-18 expression has been reported in human unstable atherosclerotic plaque,[6] suggesting that IL-18 plays an important role in atherosclerotic plaque destabilization causing acute ischemic syndrome.[8,9] Indeed, plasma IL-18 level has been shown to be significantly increased in 11 patients with unstable angina compared with that in 11 healthy volunteers.[9] It is possible that IL-18 is predictive for coronary events either through a correlation with coronary artery disease extent (a disease marker) or as an indicator of inflammation that leads to atherothrombotic events such as plaque rupture (a process marker), leading to acute coronary syndrome. We hypothesized that IL-18 is associated with the extent of coronary artery atherosclerosis in patients with unstable angina. Accordingly, this study investigated plasma IL-18 levels in

unstable angina patients with coronary artery disease of different severities as measured by coronary angiography.

Materials and methods

Study population

This study comprised 166 consecutive patients admitted to our hospital for coronary angiography owing to unstable angina ($n = 118$) or because of a clinical suspicious of coronary artery disease in patients with multiple coronary risk factors ($n = 48$). Unstable angina was defined as the presence of typical chest pain syndrome at rest and ST segment modifications indicative of myocardial ischemia, crescendo angina, or new-onset angina within 2 months prior to coronary angiography. Exclusion criteria were as follows: myocardial infarction within 2 months preceding the study, evidence of hemodynamically significant valvular heart diseases, congenital heart disease, surgery or trauma during the month preceding the study, known cardiomyopathy, known malignant diseases, febrile conditions, acute or chronic inflammatory disease on study entry, overt congestive heart failure, renal insufficiency (creatinine >2.0 mg/dl), abnormal liver function, adjunctive platelet glycoprotein IIb/IIIa inhibitor prior to coronary angiography, or oral anticoagulant therapy within 4 weeks preceding the study. Informed consent was obtained from all subjects, and the study protocol was approved by the Institutional Review Committee on Human Research at our institution.

Measurement of angiographic variables

Coronary angiographic morphology of stenotic lesions was classified by at least the two best angiogram projections. Using a contrast-filled guiding catheter used as the calibration standard, quantitative angiographic analysis of the percentage of minimal lumen diameter stenosis was determined using a digital edge-detection algorithm (DUKE System) and by selecting the end-diastolic frames demonstrating the stenosis in its most severe and non-foreshortened projection.

Coronary angiograms

Coronary angiograms were scored using two techniques. (a) *Severity score*. In this score, a value of 1 was given to any of 15 arterial segments (as defined by the American Heart Association)[10] that contained greater or equal to 70% stenosis, giving a potential score range from 0 to 15. This score gives a more complete measure of disease severity than simply the number of major arteries involved.[11] (b) *Gensini score*. This scoring system assigns a different severity score depending on geometrically increasing severity of lesion, the cumulative effects of multiple obstructions and the significance of their locations.[12,13]

Blood sampling and measurement of IL-18 and hs-CRP

Blood samples from all study subjects were obtained immediately after vascular puncture prior to coronary angiography in the fasting, non-sedative state on study entry. Plasma concentrations of high-sensitivity C-reactive protein (hs-CRP) were measured by immunonephelometry using the BN system (Dade Behring, Newark, DE, USA). Blood samples were also drawn into an evacuated tube containing K_3 ethylenediamine tetraacetic acid. Blood samples with gross hemolysis were discarded. Mixtures of blood and K_3 ethylenediamine tetra acetic acid were immediately centrifuged (model 5400; Kubota Corp; Tokyo, Japan) at 3000 rpm for 10 min at room temperature. The plasma was immediately separated and frozen at –80°C until the assay. The soluble IL-18 concentrations of human plasma samples were quantified using a commercially available enzyme-linked immunosorbent assay (MBL, Naka-ku, Nagoya, Japan). The samples were processed according to the manufacturer's instructions. All standards and samples were tested in duplicate. The mean intra-assay coefficient of variances of IL-18 was 8.6%. The mean inter-assay coefficient of variances of IL-18 of 19 samples was 7.8%.

Statistical analysis

Continuous variables were described as the mean ± SD. Because distributions of inflammatory marker levels appeared to be skewed, they were normalized by log-transformation. Categorical variables were compared using the Chi-Square test (2-tailed). Continuous variables between the two groups were compared using the Wilcoxon rank sum test. The associations between IL-18 levels and atherosclerotic risk factors were examined by Pearson correlation analysis for continuous variables, and by unpaired *t*-test for categorical variables. Also, relationships between coronary severity scores and other continuous variables were examined by Spearman rank correlation analysis. For categorical variables, severity score was compared using Wilcoxon rank sum test. Subsequently, a multiple regression model with stepwise selection was used to examine associations between coronary severity scores and other parameters with a value of $P < 0.1$ in univariate analysis. Finally, coronary severity score was compared across the IL-18 tertiles by Kruskal–Wallis test. Statistical analysis was conducted using a statistical software program (SAS for Windows, version 8.02; SAS Institute, Cary, NC, USA). The level of significance was set at $P < 0.05$.

Results

Baseline characteristics of the studied patients

One hundred and eighteen patients with unstable angina had coronary artery disease (coronary artery disease group; severity score: 2.32 ± 1.47; Gensini score: 31.3 ± 25.9), and 48 patients with multiple coronary risk factors and without

Table 1. Baseline characteristics and plasma levels of markers of inflammation of patients studied

Variables	Coronary artery disease group (n = 118)	Risk control group (n = 48)	P value
Age, years	61.7 ± 9.6	60.3 ± 10.3	0.311
Men, n (%)	86 (72.9)	26 (54.2)	0.020
Body mass index (kg/m^2)	26.2 ± 3.1	26.7 ± 3.2	0.567
Hypertension, n (%)	78 (66.1)	31 (64.6)	0.852
Systolic blood pressure (mmHg)	145.4 ± 23.2	146.0 ± 29.6	0.832
Diastolic blood pressure (mmHg)	76.9 ± 9.1	76.2 ± 11.7	0.392
Diabetes, n (%)	48 (40.7)	12 (25.0)	0.057
Fasting blood sugar (mg/dl)	129.8 ± 62.2	126.0 ± 65.3	0.983
Dyslipidemia, n (%)	87 (73.7)	30 (62.5)	0.005
Cholesterol (mg/dl)	206.6 ± 44.0	185.2 ± 32.8	0.002
Triglyceride (mg/dl)	197.4 ± 141.7	168.6 ± 94.8	0.291
LDL-cholesterol (mg/dl)	143.5 ± 43.1	117.8 ± 27.7	0.031
HDL-cholesterol (mg/dl)	37.0 ± 8.7	36.6 ± 9.7	0.628
Smokers, n (%)	40 (33.9)	11 (22.9)	0.164
Homocysteine (μmol/l)	10.9 ± 5.1	11.1 ± 4.2	0.310
Left ventricular ejection fraction (%)	57.3 ± 7.8	61.4 ± 5.6	0.005
Medications			
β-blocker, n (%)	94 (79.7)	25 (52.1)	0.0003
Ca-channel blocker, n (%)	48 (40.7)	26 (54.2)	0.113
ACEI or ARB use, n (%)	47 (39.8)	14 (29.2)	0.193
Aspirin, n (%)	98 (83.1)	39 (81.3)	0.782
Clopidogrel, n (%)	38 (32.2)	12 (25.0)	0.359
Statins, n (%)	36 (30.5)	6 (12.5)	0.018
Fibrates, n (%)	23 (19.5)	2 (4.2)	0.012
Inflammatory markers			
hs-CRP (mg/l)	7.35 ± 15.76	2.97 ± 5.33	0.009
Interleukin-18 (pg/ml)	226.5 ± 100.6	209.8 ± 120.3	0.062

Data are expressed as mean ± SD or number (%) of patients
ACEI, angiotensin converting enzyme inhibitor; ARB, type 1 angiotensin receptor blockers; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein

coronary artery lesions served as the risk control group. All of the patients in the risk control group had at least one risk factor for atherosclerosis and coronary artery disease, including current smoker (22.9%), diabetes mellitus (25.0%), hypertension (64.6%), dyslipidemia (62.5%), family history of documented coronary artery disease, documented peripheral vascular disease, post-menopausal female, and male older than 40 years. Table 1 summarizes the baseline characteristics for each group. There was no difference between the two groups in terms of age, body mass index, systolic and diastolic blood pressure, fasting plasma triglyceride, blood sugar, homocysteine and creatinine levels, and blood leukocyte counts. However, there were more male patients in the coronary artery disease group than in the risk control group, and plasma levels of cholesterol were significantly higher in the coronary artery disease group than in the risk control group. Additionally, the percentages of patients taking β-blocker, statins, and fibrates were significantly higher in the coronary artery disease group than in the risk control group. Furthermore, the left ventricular ejection fraction was significantly lower in the coronary artery disease group than in the risk control group. Plasma levels of IL-18 were higher in the coronary artery disease group than in the risk control group, although the difference did not reach statistical significance (P = 0.062). However, plasma levels of IL-18 were significantly higher in 77 coronary artery disease patients with severity score ≥2 than in the risk control group (242.3 ± 110.6 vs 209.8 ± 120.3 pg/ml, P = 0.016). Additionally, plasma levels of IL-18 were significantly higher in 75 coronary artery disease patients with Gensini score ≥9 than in the risk control group (240.6 ± 111.1 vs 209.8 ± 120.3 pg/ml, P = 0.022). Plasma levels of hs-CRP were significantly higher in the coronary artery disease group than in the risk control group.

Associations between IL-18 levels and atherosclerotic risk factors

Associations between IL-18 levels and atherosclerotic risk factors are shown in Table 2. Levels of plasma IL-18 were higher in men than in women. Plasma IL-18 levels were similar between patients with hypertension, diabetes mellitus, dyslipidemia, or smoking, and those without them.

Associations between extent of coronary artery disease and IL-18 levels

To clarify the link between the extent of coronary atherosclerosis and IL-18, associations of the coronary artery severity scores with plasma IL-18 levels were examined. Of note, by univariate analysis, log-transformed plasma IL-18 concentration was positively correlated with the extent of coronary artery disease (Table 2). By multiple regression analyses (Table 3), the association between coronary artery disease severity score and IL-18 remained significant (β =

Table 2. Associations of plasma interleukin-18 levels with atherosclerotic risk factors and coronary artery disease severity score in the coronary artery disease group

	r or Mean ± SD	*P* value
Age, years	–0.11	0.237
Sex, men/women	241.5 ± 105.7/186.4 ± 72.6	0.005
Body mass index, kg/m^2	0.14	0.132
Hypertension, yes/no	221.9 ± 81.8/235.7 ± 130.5	0.802
Systolic blood pressure	0.05	0.597
Diastolic blood pressure	0.01	0.973
Diabetes mellitus, yes/no	235.4 ± 115.2/220.4 ± 89.7	0.495
Fasting blood glucose	0.10	0.300
Dyslipidemia, yes/no	224.0 ± 103.4/229.8 ± 97.9	0.760
Total cholesterol	–0.01	0.921
Triglyceride	0.12	0.212
Smoking, yes/no	239.8 ± 93.6/219.7 ± 104.0	0.204
hs-CRP, mg/dl	0.13	0.154
Severity score	0.244	0.009
Gensini score	0.19	0.043
LVEF	–0.11	0.250

hs-CRP, high-sensitivity C-reactive protein; LVEF, left ventricular ejection fraction

Table 3. Multiple regression analyses of coronary artery severity score in the coronary artery disease group

	β	*P*
IL-18, pg/ml	0.733	0.017
Diabetes mellitus, yes/no	0.55	0.036
LVEF	–0.043	0.002

Levels of inflammatory markers were analyzed as log-transformed values. IL-18, interleukin-18; LVEF, left ventricular ejection fraction

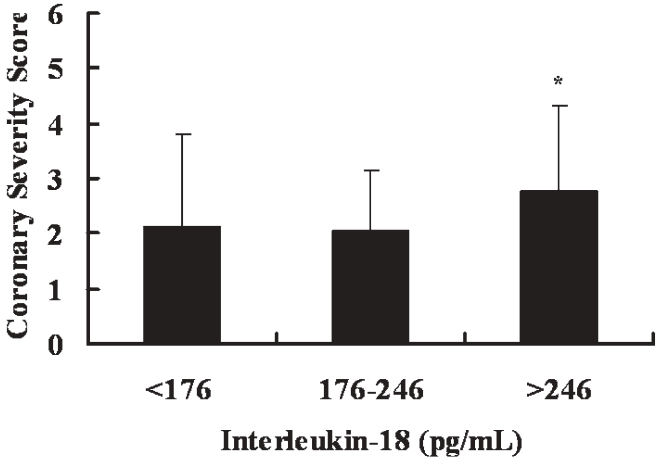

Fig. 1. Coronary artery disease severity score according to tertiles of plasma interleukin-18 levels. *P = 0.028

0.733, P = 0.017) when controlling for age, diabetes mellitus, and left ventricular ejection fraction. Given the association between IL-18 and the extent of coronary artery disease, the coronary artery disease severity score was compared across the tertiles of plasma IL-18 levels. Coronary artery disease severity score was greater in the highest tertile (>246 pg/ml) of plasma IL-18 levels than in the middle (176–246 pg/ml) and the lowest (<176 pg/ml) tertiles (2.79 ± 1.52 vs 2.05 ± 1.08 vs 2.13 ± 1.66, P = 0.028) (Fig. 1). Furthermore, plasma levels of IL-18 were significantly higher in the 39 patients with high coronary artery disease severity scores and plasma IL-18 levels >246 pg/ml than in the risk control group (P < 0.0001). Of note, plasma hs-CRP level had no significant correlation with coronary artery disease severity score (β = –0.006, P = 0.950).

Discussion

In this study, we found that plasma IL-18 level was associated with the extent of coronary artery disease in unstable angina patients with coronary artery disease. Also, the association was independent of traditional atherosclerotic risk factors, and hs-CRP level.

In this study, IL-18 levels had no correlations with traditional atherosclerotic risk factors (Table 2). These findings are approximately in line with those of Blankenberg et al.,[7] who showed no associations of plasma IL-18 levels with such risk factors.

Mallat et al. has shown that plasma IL-18 levels are higher in patients with acute coronary syndrome compared with those in healthy volunteers.[9] Moreover, serum IL-18 level has been identified as a strong independent predictor of the development of coronary heart disease in healthy men, and as a strong independent predictor of cardiovascular death in patients with stable and unstable angina.[7,8] However, the association of elevated IL-18 level and the extent of coronary artery disease remains to be examined. In this study, our findings indicate that plasma IL-18 level is independently associated with the extent of coronary artery disease, suggesting its link with coronary artery atherosclerosis. Atherosclerosis is a progressive inflammatory disease.[14] Pathological studies have provided evidence that not all disruptions of atherosclerotic ruptures cause clinically apparent events.[15,16] Such subclinical episodes of plaque disruption with local thrombin activation and subsequent healing may indicate a pathway for atherosclerotic lesion progression due to continuous inflammatory process. Therefore, it was not surprising to observe significantly elevated plasma IL-18 levels only in patients with extensive coronary artery disease (higher coronary artery severity score) compared to risk control group. Therefore, our findings strongly support experimental evidence of IL-18-mediated inflammation leading to acceleration of atherosclerotic plaque growth,[2,3,4,6] and it could be that elevated IL-18 may be a stronger marker of the degree of atherosclerosis than of thrombotic risk. On the other hand, this study showed that CRP was not associated with coronary artery severity. This finding is approximately in line with that of Hunt et al.,[17] who showed no association of serum hs-CRP level with the extent of coronary artery disease as assessed by electron beam computed tomography. Therefore, our findings indicate that CRP is not strongly associated with coronary artery atherosclerosis. Additionally, this study showed that plasma levels of hs-CRP were significantly higher in unstable angina patients than in the risk control group. Because CRP has been associated with clinical events,[18–20] it could be

that elevated CRP may be a stronger marker of thrombotic risk than of the degree of atherosclerosis.

Increased IL-18 expressions have been observed in human unstable atherosclerotic plaque, predominantly colocalized with macrophages.[6] Interleukin-18 is a proinflammatory cytokine produced mainly by monocytes and macrophages. Interleukin-18 acts in synergy with IL-12 to induce interferon-γ. Interferon-γ plays a key role in both plaque development and stability.[5] These findings are in accordance with the hypothesis that IL-18 plays an important role in atherogenesis, supporting the link between IL-18 and coronary artery atherosclerosis.

Study limitations

Several limitations of this study deserve consideration. First, IL-18 was measured using samples that were stored at –80°C. Consequently, the possibility of protein degradation cannot be eliminated. However, in the laboratory, the mean interassay coefficient of variances was 7.8% for IL-18 in 19 samples measured separately for a similar amount of frozen time. Therefore, the possibility of protein degradation was not a significant factor in this study. Second, there were more male patients in the coronary artery disease group than in the risk group, and the percentages of patients taking β-blockers, statins, and fibrates were higher in the coronary artery disease group than in the risk control group. However, multiple regression analysis showed that the association between IL-18 and coronary artery disease severity score remained significant when controlling for these variables. Finally, the association of red blood cell 5-methyltetrahydrofolate and severity of coronary artery disease was not assessed in this study. Golbahar et al. found that low red blood cell 5-methyltetrahydrofolate was associated with the severity of coronary artery disease independent from plasma homocysteine and other cardiovascular risk factors.[21] In conclusion, plasma IL-18 level is associated with the extent of coronary artery disease in unstable angina patients, suggesting the link between IL-18 and coronary artery atherosclerosis in these patients.

Acknowledgment The authors would like to thank the National Science Council, Taiwan, Republic of China, for financially supporting this research under the Contract number NSC 92-2314-B-182A-199.

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