Clinical Implications of Elevated Serum Interleukin-6, Soluble CD40 Ligand, Metalloproteinase-9, and Tissue Inhibitor of Metalloproteinase-1 in Patients with Acute ST-segment Elevation Myocardial Infarction

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ABSTRACT

Background: Atherosclerosis is widely accepted as a chronic inflammatory disease. Research paid much attention to sensitive specific serum biomarkers for vulnerable plaques. The markers not only serve as diagnostic tools for the identification of patients with acute coronary syndrome (ACS), but also help us to identify high-risk patients. However, the existing data are limited and have been conflicting.

Hypothesis: Circulating interleukin-6 (IL-6), soluble CD40 ligand (sCD40L), metalloproteinase-9 (MMP-9), and tissue inhibitor of metalloproteinase-1 (TIMP-1) might correlate with the onset and the cardiac mortality of patients with ST-segment elevation myocardial infarction (STEMI).

Methods: Serum levels of IL-6, sCD40L, MMP-9, and TIMP-1 were measured by sandwich enzyme-linked immunosorbent assay (ELISA) in 263 patients with STEMI and 262 age- and gender-matched control subjects without coronary artery disease (CAD). The patients with STEMI were then followed prospectively for 24 mo for the occurrence of cardiac mortality.

Results: Compared with the control subjects, patients with STEMI exhibited higher levels of IL-6 ($p<0.001$), sCD40L ($p<0.001$), MMP-9 ($p<0.001$), TIMP-1 ($p = 0.045$), and MMP-9/TIMP-1 ratio ($p = 0.007$). Significant and positive correlations between MMP-9 and TIMP-1 ($r = 0.610$, $p\leq0.001$), IL-6 and creatine kinase (CK) ($r = 0.159$, $p = 0.022$), and IL-6 and Troponin-I (TnI) ($r = 0.141$, $p = 0.042$) were observed by Spearman's correlations analysis. Logistic regression analysis revealed that IL-6 significantly and independently correlated with the occurrence of STEMI, and IL-6 was an independent predictor for cardiac mortality during a 24-mo follow-up in patients with STEMI.

Conclusion: The present study indicates that elevated admission level of IL-6, but not of sCD40L, MMP-9, or TIMP-1, might indicate the onset of STEMI, and could provide prognostic value for future cardiac mortality within 2 y in patients with STEMI.

Key words: myocardial infarction, cytokine, inflammation

Introduction

Atherosclerosis is widely accepted as a chronic inflammatory disease initiated by different vascular and extravascular sources.[1,2] An unstable, and subsequently ruptured, atherosclerotic coronary plaque with superimposed thrombosis constitutes the most common, general, and pathological background of the acute coronary syndrome (ACS).[3] Previous studies implicate that ACS is triggered by the following stages: proinflammatory cytokines, such as interleukin-6 (IL-6) and chemoattractants; induce leukocyte chemoattraction to the endothelium, and together with the co-stimulatory pair CD40 ligand (CD40L), activate plaque macrophages; the activated macrophages then produce matrix metalloproteinases (MMPs) that disintegrate extracellular plaque matrix; thus causing plaque instability. To elucidate the role of inflammation in the pathogenesis of ACS, many studies have focused on the sensitive specific biomarkers for vulnerable plaques, especially serum biomarkers.[4] The new markers not only serve as diagnostic tools for the identification of patients with ACS, but also help us to identify high-risk patients. However, the existing data are limited and have been conflicting. In this study, we evaluated the circulating levels of serum IL-6, soluble CD40 ligand (sCD40L), metalloproteinase-9 (MMP-9), and tissue inhibitor of metalloproteinase-1 (TIMP-1), and attempted to determine their clinical implication in Chinese patients with ST-segment elevation myocardial infarction (STEMI).

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Methods

Subjects

We enrolled 263 consecutive patients (203 men and 60 women) with STEMI who were admitted to our institute within 6 h of symptoms onset, and fulfilled all of the following criteria:[5] (1) typical, prolonged chest pain at rest (>30 min); (2) ST-segment elevation ≥0.2 mV at the J point in 2 or more contiguous, precordial leads, or ≥0.2 mV in 2 or more adjacent limb leads on the standard 12-lead electrocardiogram (ECG); and (3) presentation in the first 6 h since the onset of chest pain. Diagnosis of acute myocardial infarction (AMI) was confirmed by increased serial serum markers of myocardial damage (>2-fold increase over the upper normal range required for creatine kinase [CK] and troponin-I [TnI]). Patients with equivocal or uninterpretable ECGs (i.e., left bundle branch block, paced rhythm, or persistent ST-segment elevation after a previous MI) were not included in the study. In this study, 262 age- and gender-matched subjects who had normal coronary arteries confirmed by medical records, physical examination, ECG, and angiography were selected as controls.

Furthermore, this study did not include patients with a history of hematological, neoplastic, renal, liver, or thyroid disease, or patients receiving treatment with anti-inflammatory drugs. Patients with acute or chronic infections and autoimmune disease were also excluded from the study. The study protocol was approved by the ethics committee of our institution, and written informed consent was obtained from all participating subjects.

Clinical Data Collection

A special questionnaire was used to collect information on lifestyle, environmental factors, and medical history of the study population. Men who reported smoking at least 1 cigarette per day for at least 1 y were defined as current smokers, and ex-smokers were defined as abstainers for at least 1 y. The subjects were asked to indicate in a 1–10 visual-spatial scale their level of psychological distress in the 2 wk before hospital admission. They were also asked to indicate their negative life events which occurred in the previous 6 mo (i.e., serious illness or death of a family member, divorce or separation, forced to change job, feelings of insecurity at work, serious financial trouble, been legally prosecuted, etc.). Diabetes mellitus was defined as a previous diagnosis, use of diet or antidiabetic medicines, or fasting venous blood glucose level ≥126 mg/dL on 2 occasions in previously untreated patients. Patients who received medications for hypertension, or those with seated systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg on at least 3 separate clinic visits were also identified. Patients who used cholesterol-lowering medicines or had a total serum cholesterol level ≥200 mg/dL were classified as having hypercholesterolemia. Height, weight, and waist circumference were measured, and body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

Patients with STEMI were followed-up for up to 24 mo after admission using a standardized protocol that included outpatient visits, telephone contacts, and the recording of recurrent cardiac events. A death was classified as cardiac if the predominant and immediate cause was related to myocardial infarction (MI) or ischemia, arrhythmia, refractory congestive heart failure, or sudden death.

Blood Collection and Assays

After admission, in respect of every subject, peripheral venous blood was drawn. After clotting, the samples were centrifuged at 2500 rpm for 10 min, and the serum was frozen and stored at −70°C until analyzed. Sandwich enzyme-linked immunosorbent assay (ELISA) was performed for measuring concentrations of serum IL-6, MMP-9, and TIMP-1, using Quantikine commercial kits (R&D Systems Europe, Ltd., Abingdon, UK), and of serum sCD40L using Bender Medsystems commercial kits (Bender Medsystems Inc., Burlingame, Calif., USA). The lower detection limits were 0.7pg/mL for IL-6, 0.156ng/mL for MMP-9, 0.08ng/mL for TIMP-1, and 0.095ng/mL for sCD40L. All other biochemistry measurements were carried out by our biochemistry department using standard methods.

Statistical Analysis

Data are expressed as means±standard deviation (SD) for normally distributed variables, and serum biomarkers were expressed as median and interquartile ranges, as these values were non-normally distributed. Qualitative data are presented as numbers (percentages). The comparison of the serum biomarkers levels between the control and STEMI patients were performed using the nonparametric Mann-Whitney U test, and the differences of normally distributed variables between the 2 groups were evaluated by t test. For categorical variables, chi-square tests were performed. Correlations between serum biomarkers of inflammation and myocardial necrosis were assessed using Spearman's rank correlations test for these continuous variables with non-normal distribution, and logistic regression was used to analyze the relationship between these biomarkers and the onset and clinical outcome of STEMI. A value of $p<0.05$ was considered statistically significant. All calculations were performed using SPSS statistical software for windows V11.5 (SPSS, Inc., Chicago, Ill., USA).

Results

Clinical Characteristics

Compared with the control subjects, the patients with STEMI tended to have a higher rate of diabetes mellitus ($p<0.001$), smoking ($p<0.001$), and more psychosocial stress ($p<0.001$). No significant differences in the other

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baseline clinical data were observed between the STEMI and the control groups (Table 1).

Levels of Inflammatory Markers

The concentrations of IL-6 ($p<0.001$), sCD40L ($p<0.001$), MMP-9 ($p<0.001$), TIMP-1 ($p = 0.045$), and MMP-9/TIMP-1 ratio ($p = 0.007$) were significantly higher in the STEMI group than those in the control group (Figure 1).

Correlations Between Markers of Inflammation and Myocardial Necrosis

Spearman's correlations analysis showed that MMP-9 significantly and positively correlated with TIMP-1 ($r = 0.610$, $p\leq0.001$). No other significant intercorrelations of inflammatory markers were observed. To identify the relationship between systemic inflammation and markers of myocardial necrosis, the levels of peak CK, CK-myocardial

TABLE 1: Baseline characteristics of the study population

Variable	STEMI (n = 263)	Control (n = 262)	p-value
Gender (male)	203 (77.2%)	202 (77.1%)	0.981
Age(y)	61.10±12.43	60.21±11.99	0.409
BMI (Kg/m²)	25.26±3.24	25.47±3.33	0.474
Waist circumference (cm)	90.44±11.31	89.92±9.48	0.567
Family history of CAD	21 (8.0%)	15 (5.7%)	0.306
Hypertension	118 (44.9%)	138 (52.7%)	0.074
Hypercholesterolemia	50 (19.0%)	68 (26.0%)	0.057
Diabetes mellitus	59 (22.4%)	26 (9.9%)	<0.001
Cigarette smoking (current and ex-smoker)	190 (72.2%)	142 (54.2%)	<0.001
Sedentary lifestyle	119 (45.2%)	135 (51.5%)	0.150
Psychosocial stress score (≥5)	170 (64.6%)	122 (46.6%)	<0.001
Negative life events (occurred in the previous 6 mo)	75 (28.5%)	58 (22.1%)	0.093
Medication	—	—	—
Beta-blocker	207 (78.7%)	—	—
ACE inhibitor	235 (89.4%)	—	—
Statins	236 (89.7%)	—	—
Antiplatelet agents	250 (95.1%)	—	—
Reperfusion therapy	184 (70%)	—	—
Peak CK (unit/l)	1337.0 (493.5–2619.8)	—	—
Peak CK-MB (unit/l)	106.0 (46.3–192.8)	—	—
Peak TnI (ng/mL)	29.5 (10.5–99.7)	—	—
LVEF <50%	27 (10.3%)	—	—
Killip's class ≥2	72 (27.4%)	—	—
Number of diseased vessels ≥2 (stenosis ≥50%)	183 (69.6%)	—	—

Age, BMI, and waist circumference are presented as mean±SD; peak CK, CK-MB, and TnI are presented as medians (25th–75th percentile); categorical variables are presented as numbers (%). *Abbreviations:* ACE = angiotensin-converting enzyme; BMI = body mass index; CAD = coronary artery disease; CK = creatine kinase; CK-MB = creatine kinase-myocardial fraction; LVEF = left ventricular ejection fraction; STEMI = ST-segment elevation myocardial infarction; TnI = Troponin-I.

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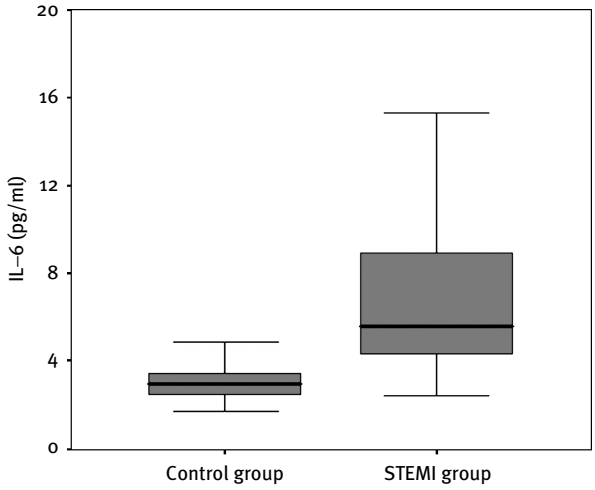

Figure 1: Patients with STEMI had higher levels of inflammatory markers such as IL-6, sCD40L, MMP-9, TIMP-1, and MMP-9/TIMP-1 ratio.

fraction (CK-MB), and TnI were analyzed in STEMI patients, and significant and positive correlations between IL-6 and CK ($r = 0.159$, $p = 0.022$), and IL-6 and TnI ($r = 0.141$, $p = 0.042$) were observed (Table 2).

Logistic Regression Analysis of Inflammatory Markers to STEMI

In order to determine the relationship between the biomarkers and conventional risk factors mentioned above and STEMI, the significant different variables between the 2 groups were selected and analyzed using logistic regression analysis. The variables remaining in the equation were type 2 diabetes mellitus, cigarette smoking, psychosocial stress score, and IL-6 (Table 3).

Association of Inflammatory Markers with Cardiac Mortality at 24 mo

Follow-up information was available for 231 patients (87.8%) from the 263 STEMI patients at 24 mo. There were a total of 27 deaths classified as cardiac in etiology. Interleukin-6 was the only biomarker found to be an independent predictor of cardiac mortality based on logistic regression analysis (Table 4).

Discussion

The present study showed that patients with STEMI had significantly higher concentrations of IL-6, sCD40L, MMP-9, TIMP-1, and MMP-9/TIMP-1 ratio than control subjects. Furthermore, serum IL-6 significantly and positively correlated with TnI and CK. Logistic regression analysis revealed that IL-6 had independent correlation with the onset of STEMI, and was an independent predictor for cardiac mortality during a 24-mo follow-up in patients with STEMI. The results suggest that measuring serum IL-6 level may provide valuable information for long-term risk stratification after MI.

Among these biomarkers, proinflammatory cytokines IL-6 has been the most widely studied and accepted as a valuable inflammatory marker to identify those at high risk of a cardiac event. Interleukin-6 is one of the main triggers of C-reactive protein (CRP) release, thus the stronger correlation between IL-6 and CRP has been well established, and IL-6 can provide additional predictive value, over that provided by CRP, for the risk stratification and incidence of future cardiovascular events.[6] In apparently healthy men, elevated levels of IL-6 are associated with increased risk of future MI, which support a role for cytokine-mediated inflammation in the early stages of atherogenesis.[7] In patients with unstable angina pectoris, IL-6 is a strong predictor of the risk of serious coronary events.[8] Rallidis LS et al. reported that circulating IL-6 levels correlated closely with left ventricular geometric changes during the remodeling process in patients with reperfused MI.[9] In the present study, IL-6 significantly and independently correlated with the onset of STEMI and cardiac mortality during 24-mo follow-up, suggesting that IL-6 might play a key role in the development of CAD.

Structurally, the CD40-CD40L is a pair of transmembrane glycoproteins belonging to the tumor necrosis factor receptor family. In recent years, accumulating evidence supported the involvement of the CD40-CD40L receptor-ligand pair in atherosclerosis, thrombosis, and inflammation. Previous studies have demonstrated that CD40-CD40L interaction

TABLE 2: Spearman's correlations analysis between assessed biomarkers (n = 263)

	CD40L	MMP-9	TIMP-1	Peak CK	CK-MB	Peak TnI
IL-6	0.071	0.004	0.119	0.159*	0.065	0.141*
CD40L	—	0.088	0.054	0.034	0.055	0.020
MMP-9	—	—	0.610**	0.102	0.066	0.035

**p<0.001; *p = 0.05; figures are Spearman's correlation coefficients R. *Abbreviations:* CK = creatine kinase; CK-MB = creatine kinase-myocardial fraction; IL-6 = interleukin-6; MMP-9 = metalloproteinase-9; sCD40L = soluble CD40 ligand; TIMP-1 = tissue inhibitor of metalloproteinase-1; TnI = Troponin-I.

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TABLE 3: Logistic regression analysis to study risk factors related to STEMI

	B	Wald	P	EXP(B)	95% CI
Diabetes mellitus	0.935	9.633	0.002	2.546	1.411–4.595
Cigarette smoking	0.786	12.305	<0.001	2.195	1.415–3.405
Psychosocial stress score (≥5)	0.631	8.752	0.003	1.879	1.237–2.854
IL-6	0.017	6.151	0.013	1.017	1.004–1.030

Abbreviations: CI = confidence interval; IL-6 = interleukin-6; STEMI = ST-segment elevation myocardial infarction.

TABLE 4: Logistic regression analysis for cardiac mortality at 24 mo in the entire cohort

	B	Wald	P	EXP(B)	95% CI
Killip's class ≥2	2.741	25.482	<0.001	15.506	5.349–44.953
IL-6	0.007	4.975	0.026	1.007	1.001–1.013

Abbreviations: CI = confidence interval; IL-6 = interleukin-6.

may be involved in the diverse pathogenic process of ACS, which is characterized by plaque rupture and superimposed thrombosis.[10] The CD40L on T-cells binds to the CD40 receptor on macrophages and this activates macrophages.[11] At this crucial stage, activated macrophages then synthesize and secrete MMPs, which degrade vascular extracellular collagen matrix, thereby weakening the cap of the coronary plaque.[12] The CD40-CD40L system could also induce functional expression of endothelial adhesion molecules. Furthermore, CD40-CD40L interaction induces the expression of procoagulant tissue factor on monocytes, endothelial cells, and smooth muscle cells, probably promoting the thrombosis following the plaque rupture.[11] It has been shown that elevated sCD40L not only plays a central role in the pathogenesis of atherosclerosis and CAD, but also identifies patients with ACS that are at high-risk for ischemic events.[13,14] Our findings also support the notion that sCD40L is involved in multiple stages of atherosclerosis and ACS.

The MMPs superfamily includes the collagenases, the gelatinases, and the stromelysins. An important mechanism for the regulation of the activity of the MMPs is via binding to a family of naturally occurring tissue inhibitors (TIMPs). Under normal circumstances, the TIMPs are in delicate balance with the MMPs, and matrix is digested in a highly regulated fashion. However, during certain disease states, including atherosclerosis, there is an imbalance between the activities of these 2 families of proteins leading to tissue destruction. Enhanced synthesis of MMPs has been reported in unstable coronary atherosclerotic plaques, suggesting a pathogenic role of these molecules in the development of ACS, and recent investigations have shown that patients with vulnerable atherosclerotic plaques and ACS exhibited significantly higher levels of MMPs than patients with stable angina and normal control subjects.[15] Our finding that serum MMP-9 and TIMP-1 were both elevated in patients with STEMI is in accord with the literature, and is consistent with the growing evidence implicating macrophages and matrix degradation in the etiology of plaque rupture.[12] Thus, we speculate that TIMP-1, following the activation of MMP-9, plays a protective role during the early phase of STEMI. However, the MMP-9/TIMP-1 ratio was also remarkably increased in STEMI patients, which indicates that an imbalance between MMP-9 and TIMP-1 in the microenvironment of the vulnerability atherosclerotic plaques may be responsible, at least in part, for plaque disruption leading to occurrence of cardiovascular events eventually.

The exact inflammatory mechanisms in the development and progression of atherosclerosis remain unclear. Previous studies suggest that the interaction of various inflammatory factors might play an additional role on instability and rupture of plaque. In the present study, we did not find any significant correlations between proinflammatory cytokines and the metalloproteinase system. Thus, we speculate that the role of IL-6 in the evolution of the pathological process is primary and independent of MMP-9, TIMP-1, and sCD40L. Our finding of close correlation between IL-6 and CK, TnI confirms a previous study by Manginas A et al. suggesting that systemic markers of inflammatory activity may be directly associated with myocardial injury.[16]

Our study had several limitations. First, the differences were observed in some of the baseline characteristics in the STEMI and control groups. We attempted to minimize the effect of these differences by multivariate analysis. The second control group consisted of patients with stable CAD and healthy subjects, so as to elucidate the role of inflammation in different phases of CAD. Third, in our study, there was a lack of tissue samples to directly link

circulating and tissue concentrations of the inflammatory marker. Serum levels of the inflammatory marker cannot be directly related to tissue concentration; therefore, elevations of these biomarkers do not necessarily reflect instability of the plaque rupture that gave rise to the AMI. Ideally, correlations should have been established between local and systemic inflammatory markers.

Conclusion

In Patients with STEMI, the admission levels of IL-6, sCD40L, MMP-9, TIMP-1, and MMP-9/TIMP-1 ratio were significantly elevated, and admission level of elevated IL-6, but not of sCD40L, MMP-9, or TIMP-1, might indicate the onset of STEMI, and could provide prognostic value for future cardiac mortality within 2 y in patients with STEMI. This suggests that measuring serum IL-6 level may provide valuable information for long-term risk stratification after MI.

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