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Original Article

Increased circulating macrophage-colony stimulating factor and monocyte chemoattractant protein-1 are predictors of in-hospital events in Chinese patients with unstable angina pectoris

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Abstract: Background: Macrophage colony stimulating factor (MCSF) and monocyte chemoattractant protein-1 (MCP-1) play an important role in the activation of monocyte resulting in the onset and progression of atherosclerosis. However, it remains unclear whether MCSF and MCP-1 have a predictive value to in-hospital events in Chinese patients with unstable angina pectoris. Methods: 110 subjects were divided into unstable angina pectoris (UAP) group (n = 60), stable angina pectoris (SAP) group (n = 30), and controls (n = 20). Blood samples were collected to measure the levels of MCSF and MCP-1 by ELISA. The severity of coronary stenosis was evaluated using coronary angiography and Gensini score method. The cardiac events of patients with UAP were recorded during the hospital. Results: The levels of MCSF and MCP-1 in UAP group were significantly higher than SAP and control group. MCSF and MCP-1 of patients with type II lesions were significantly higher than type I and type III, but MCSF and MCP-1 were not correlated with Gensini score. The incidence of cardiac events in UAP patients with high levels of MCSF and MCP-1 were significantly increased than that with non-high levels. The levels of MCSF and MCP-1 in UAP patients with cardiac events were significantly higher than that without cardiac events. Furthermore, multivariable logistic regression analysis revealed that MCSF and MCP-1 were all risk factors for in-hospital cardiac events. Conclusion: MCSF and MCP-1 are increased in unstable angina pectoris and may be biomarkers to evaluate the stability of plaque and prognosis of in-hospital cardiac events in Chinese patients with unstable angina pectoris.

Keywords: Macrophage colony stimulating factor, monocyte chemoattractant protein-1, unstable angina pectoris, short-term prognosis

Introduction

Increasing evidences suggest that inflammation plays a pivotal role in atherosclerotic plaque instability and rupture resulting in acute coronary syndrome [1, 2]. Activated monocytes/macrophages is implicated in this process through migrating to the fibrous cap of atherosclerotic plaque and producing proinflammatory cytokines, metalloproteinases cytokines, chemokines and tissue factor, thus leads to a progression of plaque destabilization [3, 4]. The monocyte chemotactic protein (MCP-1) and monocyte colony-stimulating factor (MCSF) are two important cytokines involved in monocytes activation and atherogenesis [5-7].

Previous studies found that increased MCSF beyond the acute phase was strongly predictive of long-term outcome in patients with severe unstable angina [8] and circulating MCP-1 played an important role in the pathogenesis development of acute coronary syndrome (ACS) [9]. Shyy et al. [5] also found that human MCSF stimulated the gene expression of MCP-1 and increased the adhesion of monocytes to endothelial monolayers. However, it remains unclear whether circulating MCP-1 and MCSF are changed and have a predictive value to in-hospital

Table 1. Patient characteristics in three groups of subjects

	UAP[a] (n = 60)	SAP (n = 28)	Control (n = 20)
Age, year	61.65±9.70	63.71±11.19	58.07±9.71
F/M sex, n	40/20	18/10	12/8
SBP, mmHg	135.65±12.24	134.29±11.41	132.27±13.11
DBP, mmHg	86.75±9.15	87.79±8.68	86.67±7.93
Glucose, mmol/l	6.15±1.68	6.01±1.57	5.93±1.02
TC, mmol/l	5.44±1.01	5.99±1.88	5.34±0.84
LDL-C, mmol/l	3.49±0.88	3.97±1.60	3.34±0.74
HDL-C, mmol/l	1.07±0.21[b,c]	1.22±0.20	1.28±0.32
TG, mmol/l	1.88±0.99	1.77±1.00	1.61±0.75
LVEF, %	64.97±5.44	64.50±8.63	67.73±5.55
MCSF, pg/ml	698.91±181.93[b,c]	481.59±89.75	397.35±82.71
MCP-1, pg/ml	125.12±62.92[b,c]	78.50±19.89	68.89±18.21

[a]UAP, unstable angina pectoris; SAP, stable angina pectoris; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, high density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; LVEF, left ventricular ejection fraction; MCSF, macrophage colony stimulating factor; MCP-1, monocyte chemoattractant protein-1. [b]$P<0.05$ compared with control. [c]$P<0.05$ compared with SAP.

events in Chinese patients with unstable angina.

Materials and methods

Patients

Sixty patients with UAP were recruited from the Department of Cardiology, Qilu Hospital, Shandong University from December 2012 to June 2014. All patients with UAP had anginal episodes at rest or angina during a mild degree of effort within the preceding 48 hours with no evidence of myocardial necrosis by enzymatic criteria. For comparison, 30 sex- and age-matched patients were randomly selected as the SAP group who had typical effort angina or positive treadmill exercise testing with no episode of angina at rest. 20 healthy subjects without a history of cardiovascular disease and having normal findings on physical examination, chest roentgenography and electrocardiography echocardiography served as control group. The exclusion criteria in the study included a myocardial infarction within the previous month, the presence of any ECG abnormalities invalidating ST-segment analyses, elevated serum levels of cardiac-related enzymes, thrombolytic therapy, or body temperature >38.0°C. Furthermore, all patients with inflammatory diseases (e.g., infections, autoimmune diseases), malignancies, pulmonary disease, liver or kidney disease were also excluded. Informed consent was obtained from all subjects based on a protocol approved by the Ethics Committee of Qilu Hospital, Shandong University.

Coronary angiography

Coronary angiography was performed by standard technique and the diagnosis of coronary artery disease was confirmed by least 75% reduction in the internal diameter of the right, left anterior descending, or left circumflex coronary arteries and their branches, or >50% reduction in the internal diameter of the left main trunk. The plaques were divided into type I, type II and type III using the method of Ambrose Ja et al. [10]. Gensini score was calculated for each patient according to coronary angiography results as previously described [11, 12].

Laboratory measurements

A fasting morning blood sample was drawn from each patient at inclusion in the study. Aliquots of plasma were stored at -80°C and analysis was performed within a year. Plasma MCSF and MCP-1 were determined by enzyme-linked immunoassay (R&D system, USA) according to the manufacturer's instructions. The intra-assay coefficients of variation were 5% for both tests.

In-hospital follow-up

All patients were given standard therapy according to physician preference. During intensive hospitalization, we noted any occurrence of in-hospital events, defined as cardiac death, non-fatal acute myocardial infarction, and recurrence of angina pectoris after conventional treatment. In-hospital events were evaluated by cardiologists who treated the patients without knowing the circulating MCSF and MCP-1 levels.

Statistics

Statistical analysis was performed with SPSS13.0 for Windows. Continuous data were presented as mean ± SD and were compared by

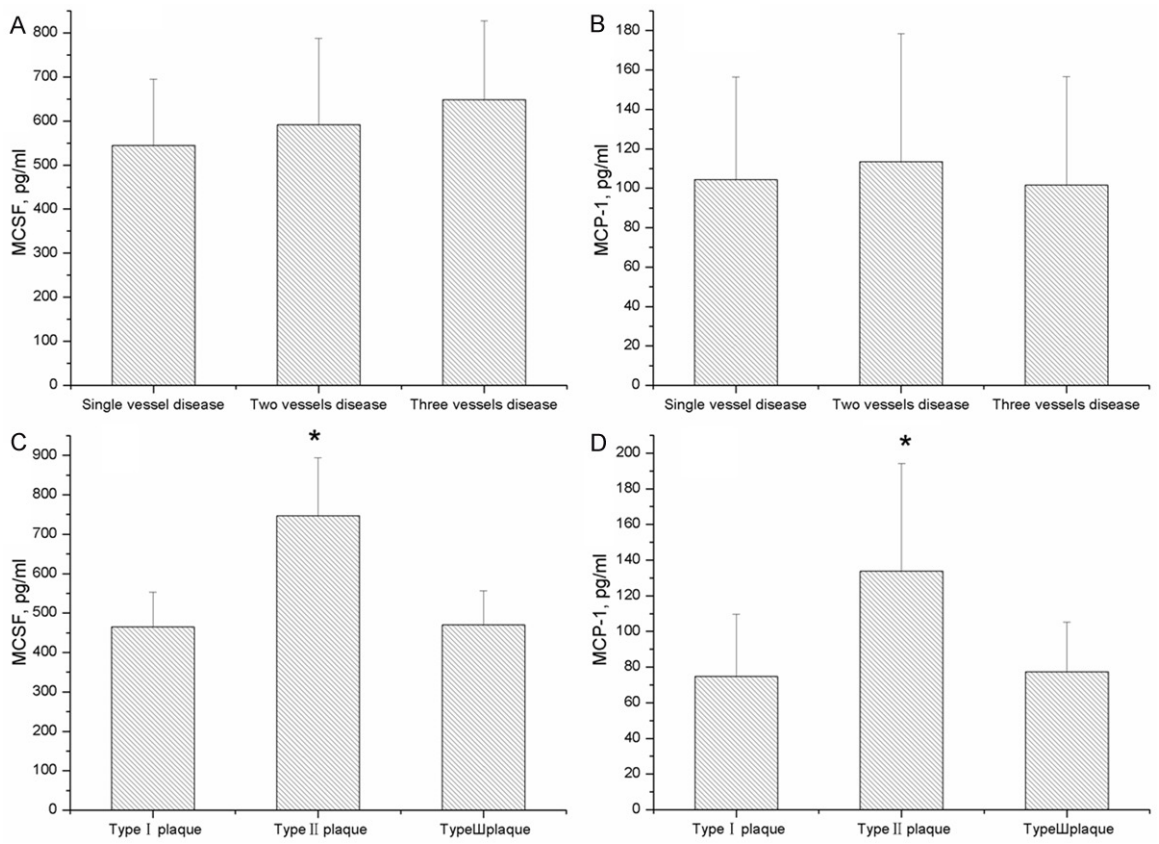

Figure 1. Comparison of MCSF and MCP-1 concentrations between patients with different vessel disease or different plaque types in UAP group. MCSF and MCP-1 of patients with type II lesions were significantly higher than that with type I and type III (C and D), but the levels of MCSF and MCP-1 were not different among the patients with single vessel disease, two vessel disease, multiple vessel disease (A and B). *P<0.05, compared with patient of type I and type III plaque.

means of one-way analysis of variance with Scheffe posteriori comparisons and student's t test as appropriate. Categorical data were presented as proportion and Chi square test was used for comparison. Multivariat analysis with multiple logistic regression method was used to further analyze covariables associated with in-hospital events. P<0.05 was considered statistically significant.

Results

Patient characteristics

The three groups were well matched in terms of age and gender. Compared with SAP and controls, high-density lipoprotein-cholesterol (HDL-C) was lower in UAP groups, with no difference between SAP groups and controls (**Table 1**). With respect to other cardiovascular risk factors, there was no difference between UAP and SAP groups.

Comparison of MCSF and MCP-1 and in-hospital events

The levels of MCSF and MCP-1 in UAP group were significantly higher than SAP group and control group (P<0.05, **Table 1**). MCSF and MCP-1 of patients with type II lesions were significantly higher than that with type I and type III (P<0.05 or P<0.01, **Figure 1**), but the levels of MCSF and MCP-1 were not different among the patients with single vessel disease, two vessel disease, multiple vessel disease (P> 0.05, **Figure 1**). The levels of MCSF and MCP-1 were not correlated with Gensini score (P>0.05).

Linear correlation analysis revealed that MCP-1 was positively associated with age, TC and GLu (r = 0.330, P<0.01; r = 0.406, P<0.001; r = 0.434, P<0.001), but MCSF was not associated with risk factors of CHD (P>0.05). And MCP-1 was positively correlated with MCSF (r = 0.630, P<0.01).

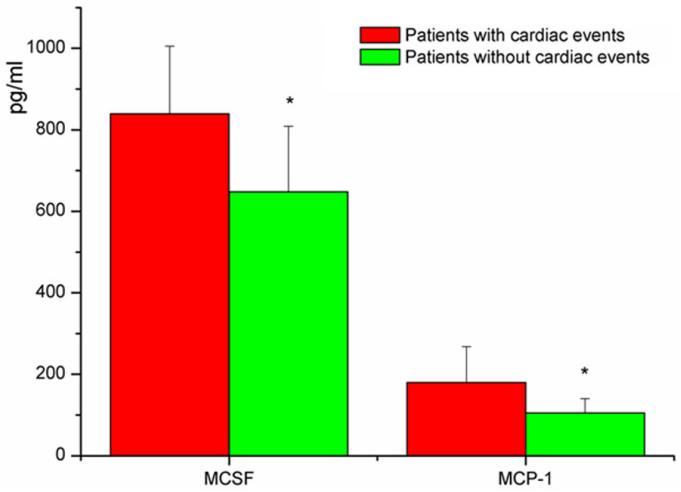

Figure 2. Comparison of MCSF and MCP-1 concentrations between patients with and without cardiac events in UAP group. The levels of MCSF and MCP-1 in UAP patients with cardiac events were significantly higher than that without cardiac events. *P<0.01 compared with patients without cardiac events.

Table 2. Logistic regression analysis of cardiac events in patients with unstable angina pectoris

	Partial regression coefficient	Standardized partial regression coefficient	P	OR
MCSF	1.021	185.751	0.004	2.776
MCP-1	0.674	42.408	0.045	1.962

MCSF, Macrophage colony stimulating factor; MCP-1, monocyte chemoattractant protein-1; OR, odds ratio.

We defined the 95% credibility interval of the MCSF (228.04-543.36 pg/ml) and MCP-1 (29.73-96.65 pg/ml) concentrations in control group as the normal reference range. The levels of MCSF and MCP-1 were considered high beyond the upper limit of normal reference range. The incidence of cardiac events in UAP patients with high levels of MCSF and MCP-1 were significantly higher than the non-high level ones (36.84% *vs.* 9.09%, 36.11% *vs.* 12.50%, respectively; all P<0.05). According to the in-hospital cardiac events, UAP patients were divided into the groups with cardiac events and without cardiac events. The levels of MCSF and MCP-1 in the former group were significantly higher than the latter (P<0.05 or P<0.01, **Figure 2**). Multivariable logistic regression analysis revealed that MCSF and MCP-1 were all risk factors for cardiac events (Partial regression coefficient were 1.021 and 0.674 respectively; OR were 2.776 and 1.962 respectively; P = 0.004 and 0.045 respectively; **Table 2**).

Discussion

The present study demonstrated that circulating MCSF and MCP-1 were increased in Chinese patients with unstable angina pectoris. Increased MCSF and MCP-1 were correlated with atherosclerotic plaque type and were all risk factors for in-hospital cardiac events.

It has become increasingly recognized that atherosclerosis is a chronic inflammatory process, in which activated monocytes/macrophages play a very important role [13, 14]. The monocyte chemotactic protein (MCP-1) and monocyte colony-stimulating factor (MCSF) are two important cytokines involved in monocytes activating [15, 16]. Both MCP-1 and MCSF have been located in atherosclerotic lesions of humans and animal models [17, 18]. Previous studies have shown that MCSF and MCP-1 concentrations were higher in patients with unstable than with stable angina [19, 20]. In the present study, MCSF and MCP-1 were also increased in Chinese patients with UAP compared with SAP and control group. Furthermore we found that MCSF and MCP-1 in UAP patients with type II lesions were significantly higher than that with type I and type III. These data suggest that high levels of MCSF and MCP-1 may reflect the unstable plaque leading to acute coronary syndrome.

Previous studies also found that increased MCSF beyond the acute phase were strongly predictive of long term outcome in patients with severe unstable angina [8] and circulating MCP-1 played an important role in the pathogenesis development of ACS [9]. However it is unclear whether circulating MCP-1 and MCSF have a predictive value to in-hospital events in Chinese patients with unstable angina. Here, we found that MCP-1 was positively associated with age, TC and GLu, but MCSF was not associated with risk factors of CHD. According to the in-hospital cardiac events, UAP patients were divided into the groups with cardiac events and without cardiac events. The levels of MCSF and MCP-1 in the former group were significantly higher than the latter. Multivariable logistic

regression analysis revealed that MCSF and MCP-1 were all risk factors for in-hospital cardiac events. The mechanism may be that MCSF causes monocyte/macrophage activation and MCP-1 serves as a chemoattractant for monocytes, resulting in monocyte migrating to fibrous cap of an atherosclerotic plaque, producing metalloproteinases and causing plaque destabilization [3, 4].

The major limitation of the study is the relatively small size of the sample. Larger and long-term studies are necessary to investigate whether the prognostic value of MCSF and MCP-1 is independent of or additive to other inflammatory marker.

Conclusion

MCSF and MCP-1 are increased in unstable angina pectoris and may be biomarkers to evaluate the stability of plaque and prognosis of in-hospital cardiac events in Chinese patients with unstable angina pectoris.

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Disclosure of conflict of interest

None.

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