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Clinical Investigations

Peripheral Levels of Matrix Metalloproteinase-9, Interleukin-6, and C-Reactive Protein Are Elevated in Patients with Acute Coronary Syndromes: Correlations with Serum Troponin I

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Summary

Background: Acute coronary syndromes (ACS) are characterized by activation of systemic and local inflammatory mediators. The interrelation between these soluble inflammatory markers and their association with markers of myocardial necrosis have not been extensively studied.

Hypothesis: The study was undertaken to evaluate the association of the systemic levels of matrix metalloproteinase-9 (MMP-9) and the tissue inhibitor of metalloproteinase-1 (TIMP-1), with C-reactive protein (CRP), interleukin-6 (IL-6), and serum troponin-I in patients admitted with ACS.

Methods: Analysis of serum concentrations of the above inflammatory markers was performed in 53 patients with unstable angina (UA) and in 15 with non-ST-segment elevation myocardial infarction (NSTEMI) within 48 h of admission, and 34 patients with stable coronary artery disease.

Results: Compared with patients with stable angina, those with ACS had elevated admission levels of MMP-9 ($p = 0.04$), CRP ($p < 0.001$), and IL-6 ($p = 0.001$), but not TIMP-1 ($p = 0.55$). Compared with patients with UA, those with NSTEMI also had higher levels of IL-6 ($p < 0.001$), CRP ($p = 0.002$), and MMP-9 ($p = 0.05$).

Conclusions: In patients with ACS, the admission levels of inflammatory mediators, including MMP-9, CRP, and IL-6 are significantly elevated, specifically in association with serum troponin I. Systemic and local markers of inflammatory activity may be directly associated with myocardial injury.

Key words: matrix metalloproteinase-9, acute coronary syndrome, troponin, inflammation

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Introduction

Matrix metalloproteinases (MMPs) are a family of endopeptidases, secreted by a variety of inflammatory or tumor cells as zymogens (pro-MMPs), subsequently activated by proteinases.[1] Expression of pro-MMPs is strictly regulated,[2] and their action is inhibited by specific tissue inhibitors of metalloproteinases (TIMPs).[3] The system of MMPs is considered important in acute coronary syndromes (ACS), because it may affect the endurance of the vascular matrix and fibrous cap, rendering it vulnerable to rupture.[4,5]

The aim of the present study was to investigate differences of MMP-9 and TIMP-1 in the peripheral blood of patients admitted with ACS and stable angina, in correlation with the widely accepted markers of inflammatory activity, interleukin-6 (IL-6), and C-reactive protein (CRP) and serum troponin I (TnI) as a marker of myocardial necrosis.

Methods

Study Patients

This study analyzed the demographic, clinical, and biochemical data of 68 consecutive patients who were admitted to the coronary care unit with ACS. The definitions proposed by the American College of Cardiology/American Heart Associ-

ation Committee on management of patients with ACS were used.[6] Unstable angina was defined according to Braunwald's classification IIIb,[7] with chest pain at rest associated with electrocardiographic changes within the preceding 48 h. Of the 68 patients, 53 had unstable angina and 15 had maximum TnI levels that exceeded the predefined value of 1.5 ng/ml; thus, they were classified as having non-ST-segment elevation myocardial infarction (NSTEMI). During the same period, 34 consecutive patients (28 men, age 61 ± 8 years, and 6 women, age 60 ± 8 years), admitted to the hospital for elective diagnostic workup because of stable angina, were included in the study. In this group all patients had either a previous diagnosis of stable coronary artery disease, or coronary angiography during the index admission documenting stable coronary obstructive lesions > 50% in diameter. Patients with concurrent inflammatory conditions, neoplastic disease, or if they were on steroidal or nonsteroidal anti-inflammatory medications were excluded from the study. The protocol was approved by the Institution's Review Board and informed consent was obtained from all participating patients.

Blood Collection and Laboratory Methods

In the ACS group, MMP-9, TIMP-1, IL-6, and CRP were measured on admission from peripheral blood within 48 h from the last episode of resting ischemia. In addition, TnI was measured every 6 h for 48 h from admission, and the maximum value, before cardiac catheterization, was captured. In the group with stable coronary disease, blood samples for the four inflammatory markers were taken from the peripheral blood, before catheterization or any percutaneous intervention, and subsequently analyzed.

Matrix metalloproteinase-9, TIMP-1, and high-sensitivity IL-6 were assessed in the patient's sera by use of commercially available ELISA kits (R&D Systems, Minneapolis, Minn., USA). C-reactive protein was assessed by use of a high-sensitivity nephelometric method (The Minineph human C-reactive protein kit, the Binding Site, Birmingham, U.K.). Serum TnI was measured by a one-step enzyme immunoassay based on the "sandwich" principle, using the commercially available flex reagent cartridge of dimension clinical chemistry system (Dade Behring, Ltd., Walton Manor, Walton, U.K.). The diagnostic cut-off point for myocardial infarction was 1.5 ng/ml.

Statistical Analysis

Associations between categorical variables were tested by use of contingency tables and the calculation of Pearson's chi-square test. Comparison among groups of the study and the inflammatory markers were tested by calculations of the nonparametric Kruskal-Wallis criterion due to the skewed distribution of the markers. Correlations were evaluated by the calculation of Spearman's rho-coefficient. Normality was assessed using the Kolmogorov-Smirnov criterion. All reported p values are based on two-sided tests and compared with a significance level of 5%.

Results

Demographic and Catheterization Data

Age and gender in all three groups were well matched (p for age > 0.05 and p for gender differences > 0.8) (Table I).

TABLE I Baseline patient characteristics

	Stable angina (n = 34)	Unstable angina (n = 53)	NSTEMI (n = 15)	p Value
Age	61 ± 8	62 ± 9	65 ± 10	0.06
Male sex (%)	82	85	87	0.92
Current smoking (%)	24	42	40	0.05
Hypertension (%)	53	62	67	0.59
Dyslipidemia (%)	88	83	77	0.35
Diabetes (%)	29	28	13	0.49
Family history CAD (%)	38	54	33	0.19
Number of diseased vessels (stenosis ≥ 50%)				0.08
None (%)	3	0	7	
One (%)	39	36	14	
Two (%)	24	36	14	
Three (%)	33	28	64	
LVEF (%)				0.014
> 50%	82	76	47	
40–50%	15	16	33	
< 40%	3	8	20	

Abbreviations: CAD = coronary artery disease, LVEF = left ventricular ejection fraction, NSTEMI = non-ST-segment elevation myocardial infarction.

Cardiac catheterization was performed during the index hospitalization in all but one patient (97.1%) in the stable angina group; one patient had already received a diagnosis of coronary artery disease from a recent angiogram. Similarly, 66 of 68 (97.1%) patients with ACS underwent cardiac catheterization, which revealed similar coronary anatomical severity in all groups (Table I).

Levels of Inflammatory Markers

The differences in peripheral blood levels of the inflammatory markers analyzed in the three clinical groups are presented in Table II. An incremental relation that reached statistical significance for MMP-9, CRP, and IL-6 levels was evident. In regard to serum concentrations of TIMP-1, a gradual step-up was seen that did not reach statistical significance. Since MMP-9 and its natural inhibitor, TIMP-1, are metabolically linked, we also analyzed the product MMP-9 $\times$ TIMP-1 in the three clinical groups and observed a significant increase across the clinical groups (Table II).

Intercorrelations of Inflammatory Markers

We examined the relationship of the systemic blood levels of the four inflammatory markers in our total patient population. Significant correlations were observed between IL-6 and CRP ($r = 0.43$, $p < 0.001$), Il-6 and TIMP-1 ($r = 0.45$, $p = 0.001$), and Il-6 and MMP-9 ($r = 0.28$, $p = 0.031$). No other significant intercorrelations of inflammatory markers were observed.

Then we focused only on the group with unstable angina (UA), observing similar relationships; IL-6 levels correlated positively with CRP ($r = 0.46$, $p = 0.001$) and TIMP-1 ($r = 0.54$, $p < 0.001$); in addition, CRP correlated positively with TIMP-1 ($r = 0.43$, $p = 0.007$).

Levels of Serum Troponin-I and Correlations with Inflammatory Markers

To identify the relationship between systemic inflammation and markers of myocardial necrosis, the levels of TnI were analyzed in all patients and correlated with the levels of the four inflammatory markers, yielding significant correlations between maximum TnI and IL-6 ($r = 0.26$, $p = 0.04$) as well as with MMP-9 ($r = 0.29$, $p = 0.04$). No changes were observed in the significance of the aforementioned relationships when we stratified our analysis by group of study. Patients with NSTEMI had higher blood levels of IL-6 than those with UA (17.2 ± 15.6 pg/ml vs. 5.3 ± 9.2 pg/ml, $p < 0.001$). Similarly, compared with those with UA, patients with NSTEMI had higher blood levels of CRP (17.8 ± 20.5 mg/dl vs. 7.6 ± 7.5 mg/dl, $p = 0.002$) and MMP-9 (869 ± 306 ng/ml, vs. 705 ± 359 ng/ml, $p = 0.05$).

Discussion

The main finding of our study was the documentation, from peripheral blood samples, of increased concentrations of inflammatory markers in patients with UA and NSTEMI. Activation of the inflammatory system was more pronounced in patients with cardiac enzyme elevation. Furthermore, the contributory confirmation of the role of MMP-9 and TIMP-1 in ACS, with or without evidence of myocardial necrosis, is proposed by their association with IL-6 and CRP.

Previous Studies

In a previous study, Kai *et al.*, in smaller groups of patients, reported for the first time that MMP-2 and MMP-9 were elevated in the peripheral blood of patient with ACS.[8] Levels of TIMPs or other inflammatory markers, however, were not measured. Our results are in concordance with this study regarding MMP-9 levels. In our study, admission levels of MMP-9 in the ACS group were almost twice as high as those in patients with stable angina. In addition, approximately a 40% increase in the peripheral plasma levels of TIMP-1 was documented.

More recently, Inocubo *et al.* analyzed the differences of MMP-9 and TIMP-1 in peripheral and great cardiac vein blood, obtained from stable patients and those with ACS and culprit lesions in the left anterior descending artery.[9] The

TABLE II Levels of inflammatory markers across the groups of the study presented as mean ± standard deviation

	Stable angina (n = 34)	UA (n = 53)	NSTEMI (n = 15)	p Value for trend
Matrix metalloproteinase-9 (ng/ml)	439 ± 222	705 ± 359	869 ± 306 [a]	0.04
Tissue inhibitor of metalloproteinase-1 (ng/ml)	186 ± 59	256 ± 105	271 ± 87	0.55
Matrix metalloproteinase-9 X tissue inhibitor of metalloproteinase-1 / 100	677.1 ± 957.5	1635.6 ± 946.1	2536 ± 1340.1 [a]	0.02
C-reactive protein (mg/dl)	2.1 ± 1.7	7.6 ± 7.5 [b]	17.8 ± 20.5 [b]	< 0.001
Interleukin-6 (pg/ml)	4.7 ± 9.8	5.9 ± 9.3 [b]	17.2 ± 15.6 [b]	0.001

[a] Bonferroni corrected p value < 0.05 for the comparison between UA or NSTEMI group with stable angina group.

[b] Bonferroni corrected p value < 0.01 for the comparison between STE UA or NSTEMI group with stable angina group.

Abbreviations: UA = unstable angina, STE = ST-segment elevation, NSTEMI = non-ST-segment elevation myocardial infarction.

MMP-9 and TIMP-1 concentrations did not differ between the groups in the systemic blood samples, yet significant differences were observed in the great cardiac vein samples. As previously suggested by detailed histologic examinations of atherectomy specimens, the site of enhanced MMP and TIMP activity is probably the active atherosclerotic coronary plaque.[10] Our findings, consistent with more recent reports,[11, 12] differ from the study by Inocubo *et al.* in the concentrations of MMP-9 and TIMP-1 in the systemic blood specimens. This discrepancy may be due to the smaller number of patients compared with those in our study. Additional factors may include temporal differences in blood specimen collection in relation to the last ischemic episode and differences in clinical baseline characteristics or in the sensitivity of the laboratory measurement kit used.

Correlations between Inflammatory Mediators

Another interesting finding in the present study is the association between the plasma levels of MMP-9, TIMP-1, and IL-6 with CRP. The latter two inflammatory markers have been associated with culprit lesion complexity[13] and are currently widely accepted as having important clinical and prognostic implications. These associations are in agreement with previous studies supporting a pathophysiologic link between proinflammatory cytokines and the metalloproteinase system.[12, 14, 15] Similarly, the close correlation between CRP and IL-6 in the ACS group confirms previous observations by Biasucci *et al.*[16]

Inflammatory Mediators and Troponin-I

Our finding of higher IL-6, CRP, and MMP-9 in patients with NSTEMI, compared with those with UA, also suggests that soluble and local markers of inflammatory activity may be directly associated to myocardial injury.[17] Very recently, in a detailed study, Cusack *et al.* suggested that, in patients with UA, the enhanced inflammatory response lay within the "downstream myocardium" rather than at the unstable atherosclerotic plaque.[18] In that study, IL-6 concentrations in the aortic root were not different between TnT positive and TnT negative patients with ACS, nor was a direct correlation found between IL-6 and TnT levels. Recently, Mazzone *et al.* also reported on the correlation of serum troponin T and inflammatory mediators in patients with ACS.[19] Although patients with UA had higher values of IL-6 than those with stable angina, the difference was not statistically significant. Furthermore, no correlation was found between IL-6 levels and troponin T values in patients with UA. Our data are different from those provided in that study, possibly because of the larger patient cohort and the higher percentage of patients with resting ischemia within the previous 48 h.

Study Limitations

This study documents the concomitant elevation of systemic inflammatory mediators early in the course of ACS, using a single blood level drawn shortly after admission. Although significant correlations were observed from these relatively small patient groups, it is likely that serially determined blood concentrations would have yielded more information regarding changes of these markers over time. The statistical power of the present data does not permit prospective evaluation of the MMP-9 levels regarding future cardiac events, and large future studies are necessary to establish this association in patients with ACS.

Conclusions

Our study provides data that may elucidate the complex inflammatory mechanisms among markers involved as primary instigators for the development of acute coronary syndromes. The simultaneous increase in plasma levels of both MMP-9 and its inhibitor TIMP-1 is of great interest. It can be speculated that the balance of production versus inhibition of MMPs is shifted toward enhanced proteolytic activity in patients with ACS. Furthermore, since the activity of MMPs depends on their rate of synthesis and activation, but also on the balance between active enzyme and its inhibitors, the increased expression of TIMPs represents a local negative feed-back mechanism, aiming at limiting the degradation of extracellular matrix proteins, with potentially important therapeutic implications.

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