Biomarkers of Plaque Instability in Acute Coronary Syndrome Patients

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SUMMARY

The aim of the study was to determine serum levels of matrix metalloproteinase 9 (MMP-9) and high sensitivity C reactive protein (hsCRP) in patients with diagnosis of acute coronary syndrome (ACS). The study included 150 patients divided in three groups: patients with significant coronary artery disease (CAD), patients without significant coronary artery disease and patients with acute myocardial infarction (MI). Method used for determination of coronary artery disease significance is coronary angiography, and CAD is determined as significant if level of stenosis is >50%. The group without significant CAD had lower MMP-9 serum concentrations than group with significant CAD, which has lower MMP-9 than group with acute MI. Difference in levels of MMP-9 serum concentration between groups with and without CAD is statistically significant. Level of serum hsCRP in group with MI is significantly higher than in other two groups. There is no significant difference in hsCRP serum level between group of patients with significant CAD and without significant CAD. Our results demonstrate the significance of MMP-9 and hsCRP level determination in assessment of acute coronary syndrome patients in the future as a biomarker of plaque instability.

Keywords: matrix metalloproteinase 9, high sensitivity C reactive protein, acute coronary syndrome, plaque instability

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1. INTRODUCTION

The diagnosis and management of acute coronary syndrome (ACS) are limited by the lack of a widely available and sensitive assay for use in assessment of coronary artery disease. The diagnosis of ACS in most hospitals is based on clinical grounds, invasive techniques such as coronary angiography. Furthermore, most hospitals do not have these specialized services and the assessment of urgency and difficultness of CAD remains largely based on clinical decisions. This suggests the need for an adjunctive biochemical test that could provide diagnostic information with the ability to correlate with CAD severity.

In particular, pro-inflammatory cytokines and acute phase reactants including C-reactive protein (CRP) have been used as biomarkers and predictors for acute coronary syndromes (ACS) (1). In parallel, pro-inflammatory cytokines such as interleukin-1 (IL-1) and tissue necrosis factor-α (TNF-α) have been shown to up-regulate matrix metalloproteinase-9 (MMP-9) (2). Matrix metalloproteinase (MMPs) are a large, tightly regulated family of zinc-endopeptidases that degrade different components of the extracellular matrix (ECM) under normal conditions, although they have also been reported in a wide range of pathological processes, including acute myocardial infarction (MI). Members of the MMP family are divided into classes based on their structure or substrate specificity, one of which is gelatinases MMP-9. Proinflammatory cytokines, released as a result of acute ischemic event, enhance the expression of adhesion molecules on endothelial cells. Adhesion molecules support the invasion of polymorphonuclear leukocytes and monocytes to the site of the lesion (3). Hemodynamic changes, injury, inflammation, and oxidative stress all appear to regulate MMP activity and induce MMP overexpression. MMPs are known to play an important role in the physiological and pathological remodeling of blood vessels (4). More importantly, they have been postulated to be involved in the destabilization and rupture of atherosclerotic lesions in unstable carotid plaques. Tissue destruction seems to be especially related to the overexpression of MMP-2 and MMP-9 (5).

Numerous prospective studies in different populations have shown that high levels of CRP predict future cardiovascular events, even in apparently healthy individuals (6). This has led to the recent position statement by the American Heart Association-Centers for Disease Control, recommending cutoff levels of CRP less than 0.0085, 0.0085–0.025, and more than 0.025 μM (<1.0, 1.0–3.0, and >3.0 mg/liter) for low, average, and high risks for subsequent acute cardiovascular event (7).

In addition to it being a risk marker, recent data from several laboratories strongly suggest that CRP is proatherogenic and prothrombotic (8).

Therefore, our aim was to study serum levels of MMP-9 and hsCRP in patients with significant CAD, patients with insignificant CAD and patients with acute MI and to determine possible correlation that will help in future risk assessment.

Risk factors	Group without CAD	Group with CAD	Group with IM	p
Smoking	24%	42%	36%	0,2907
Family history	48%	54%	16%	0,00496
Obesity	24%	24%	22%	0,9718
Hypertension	84%	80%	62%	0,4019
Hyperlipidemia	84%	86%	32%	0,0009
History of MI	10%	42%	22%	0,005
History of ICV			6%	

Table 1. Risk factors for ACS. Legend: CAD – Coronary Artery Disease, IM – Infarctus Miocardy, ICV- Cerebrovascular Insult.

2. MATERIALS AND METHODS

Patients were recruited during the period from August 2008 to January 2009. A total of 150 patients from which 100 are admitted to the Clinic for Cardiovascular disease University Clinical Centre Tuzla (UKC) with diagnosis of ACS and with aim to proceed coronary angiography, and 50 is admitted on Intensive Care Unit UKC Tuzla with diagnosis of acute MI.

Patients with history of chronic, malignant or acute infective disease were excludes from this study.

Demographic characteristics, as well as risk factors according to the World Health Association (WHO) i.e., smoking, hypertension and total blood cholesterol, were collected for each patient. CAD severity was assessed using the coronary angiography on group of patients admitted on Clinic for Cardiovascular disease (n=100) with criteria for significant coronary artery stenosis of stenosis >50%. Venous blood samples were drawn upon patient arrival at the hospital before any treatment. Laboratory methods of this study were conduct on Department for immunology, Policlinic for laboratory diagnostic University Clinical Centre Tuzla. Plasma MMP-9 concentrations were measured using a commercially available enzyme linked immunosorbent assay (R&D Systems ELISA). C-reactive protein was determined by a highly sensitive (hs), automatic nephelometric method («Dade Behring«). Significance of the results was determined based on their difference between known groups of patients.

Blood was drawn under standardized conditions before coronary angiography and stored at -80°C. From patients hospitalized because of acute myocardial infarction, blood sample was taken in period of 48 hours from beginning of first symptoms.

MMP-9 is determined as marker of plaque rupture and hsCRP, as indirect sign of chronic inflammatory process on coronary arteries.

We analyzed MMP-9 and hsCRP values in three determined group of patients and then compare results between group of patients with coronary artery disease verified by coronary angiography and group without CAD verified by same method. Same analyze was conduct with group of patients with IM.

Minimal detected dose of MMP-9 was 0,156 ng/ml. Values of hsCRP above 3 mg/dL point out inflammation, which together with patient's history and values of other cardiovascular markers can show inflammatory process at cardiovascular system.

Results of coronary angiography of our patients we get from Department for Interventional Cardiology Clinic for Cardiovascular Disease. Other laboratory tests were determined by routine diagnostic of acute myocardial infarction and angina pectoris during patient's hospitalization.

In our study we determined level of hsCRP for all patients from one blood sample taken before coronary angiography. We did just one determination of hsCRP because of good experience from our previous study on levels of hsCRP in patients with ACS. In that study we used good excluding criteria for ACS patients and results from two hsCRP levels determination two weeks apart, showed no significant difference. Excluding criteria are absence of maligned, chronic or acute inflammatory disease on basis of data from patient's clinical history.

2.1. Statistical analysis

In statistical analysis we use methods of descriptive statistic with help of «Medcalc» Computer program. For testing of statistical significance between groups we use parametric and nonparametric tests. The level of statistical significance was set at p=0.05, results are significant if p<0,05.

3. RESULTS

The group without CAD include 50 patients, mean age of 58,60 years, 27 (54%) of them male,. The group with CAD include 50 patients, mean age of 60,54, 31 (62%) of them male, and group with MI include 50 patients, mean age 63,82, 36 (72%) of them male,. Risk factor profiles are presented in Table 1.

There is no statistically significant difference in values of hsCRP between groups with and without CAD (p=0,0798). There is statistically significant difference in levels of MMP-9 between group with CAD and group without CAD (p=4,178E-08) (Table 2).

There is statistically significant difference in hsCRP between group with CAD and group with IM (p=1,84E-06). There is no statistically significant difference in MMP-9 values between group with CAD and group with IM (p=0,29) (Table 3).

There is statistically significant difference in hsCRP values between group without CAD and group with IM (p=8,94E-07). There is statistically significant difference in MMP-9 values between group without CAD and group with IM (p=2,17E-08) (Table 4).

In group without CAD highest percentage of patients have hsCRP values from 0 to 1 mg/l. In group with CAD highest percentage of patients have hsCRP values from 3 to 10 mg/l. In group with acute MI highest percentage of patients have hsCRP values higher than 10 mg/l (Table 5). In our study we also determine the number of coronary arteries with significant stenosis (>50%) and correlate it with MMP-9 and hsCRP values (Table 6). We analyzed relation between hsCRP and MMP-9 values in group of patients with acute myocardial infarction and conclude that there is weak positive correlation between hsCRP and MMP-9 values (Figure 1).

	Group with CAD	Group without CAD	P
hsCRP	3,09	2,23	0,0798
MMP-9	880,82	434,94	4,178E-08

Table 2. Values of laboratory markers between patients in group with and without CAD. Legend: CAD – Coronary Artery Disease, IM – Infarctus Miocardy, MMP-9 – Matrix Metalloproteinase 9, hsCRP – high sensitivity C Reactive Protein.

	Group with CAD	Group with IM	P
hsCRP	3,09	26,12	1,84E-06
MMP-9	880,82	986,12	0,29

Table 3. Values of laboratory markers between groups with CAD and IM Legend: CAD – Coronary Artery Disease, IM – Infarctus Miocardy, MMP-9 – Matrix Metalloproteinase 9, hsCRP – high sensitivity C Reactive Protein.

	Group without CAD	Group with IM	p
hsCRP (mg/L)	2,23	26,11	8,94E-07
MMP-9 (ng/mL)	434,94	986,12	2,17E-08

Table 4. Values of laboratory markers between patients in group without CAD and group with IM. Legend: CAD – Coronary Artery Disease, IM – Infarctus Miocardy, MMP-9 – Matrix Metalloproteinase 9, hsCRP – high sensitivity C Reactive Protein.

HsCRP (mg/L)	Group without CAD	Group with CAD	Group with IM
0-1	38%	14%	4%
1-2	26%	24%	14%
2-3	4%	22%	2%
3-10	26%	30%	18%
>10	6%	10%	62%

Table 5. Distribution of hsCRP values by AHA classification in tree study group of patients. Legend: CAD – Coronary Artery Disease, IM – Infarctus Miocardy, MMP-9 – Matrix Metalloproteinase 9, hsCRP – high sensitivity C Reactive Protein.

4. DISCUSSION

It is today widely accepted that inflammatory processes are involved in plaque rupture preceding acute coronary events (9). In daily practice we met with huge number of patients which beside coronary angiography analysis that shows that there is no CAD have one of the clinical manifestations of ACS. Often patients with CAD and stenosis higher than 50%, proven with coronary angiography, have no expected clinical manifestation. All patients in our study had diagnosis of ACS, which is working diagnosis for coronary angiography based on patient's clinical history, changes on ECG and values of biochemical parameters. In our study there is significant difference in average age of patients between group without CAD (58,60 years), in group with CAD (60,54) and acute IM (63,82 years) (p<0,03957). In all three analyzed groups there is higher number of man then women. In a group with IM 36%, in group with CAD 31%, and 27% of man in a group without CAD. Percentage of women is highest in group without CAD and the lowest in group with IM.

Number of stenosed coronary artery>50%	Percentage of patients in group with CAD
3	40%
2	28%
1	32%

Table 6. Number of stenosed coronary artery in CAD patients. Legend: CAD – Coronary Artery Disease.

Age and sex distribution of patients in our study is as expected by results of number of clinical studies conducted on patients with ACS or acute myocardial infarction (10). We notice a number of patients age 40 to 60 years with ACS or IM but it does not significantly affect results of our final statistical analysis.

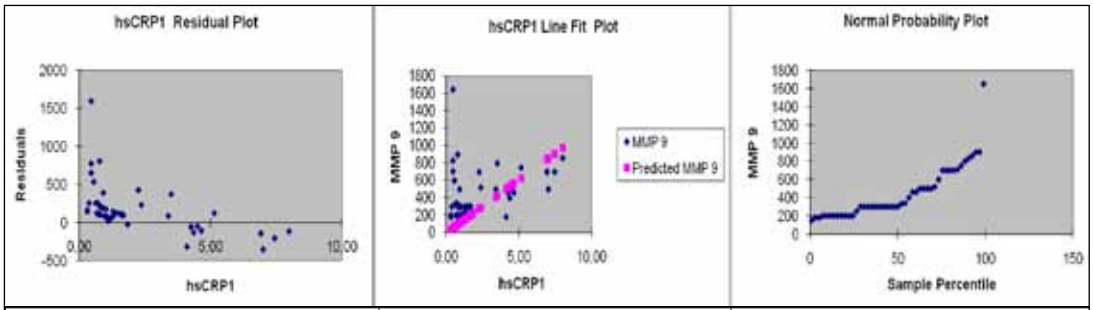
Many studies in last decade proved that certain markers of inflammation, systemic and locally, have very important role in development of atherosclerosis. So far, most studies exploring elevated levels of MMP-9 and its association to coronary artery disease (CAD) have been based on a experimental or clinical design, the latter showing elevated levels of plasma MMP-9 in patients with CAD (11). MMP-9 is considered to be a key determinant of extracellular matrix degradation, having collagen as the main substrate. Increased concentrations and activity of MMP-9 have been observed in human atherosclerotic vulnerable plaques with high inflammatory activity, suggesting a role in matrix degradation and plaque rupture (2). Increased expression and activity of MMPs have been identified in various pathological processes, such as general inflammation, tumor metastasis, respiratory diseases, myocardial injury, vascular aneurysms, and remodeling (4). Serum levels of MMP-9 have been reported to be elevated in patients with MI and unstable angina (5) as well as in stable angina (12). FINRISK retrospective study showed that MMP-9 is significantly high in persons with history of MI comparing with healthy individuals (13).

In January 2003 associate protocol from Centers For Disease Control and Prevention (CDC) and American Heart Association (AHA) point out hsCRP as marker of inflammation in estimation of risk for cardiovascular disease (7).

In our study levels of hsCRP are highest in group with IM as expected (26,11mg/l). In that group 62% of patients have levels of hsCRP higher than 10 mg/l.

Myocardial necrosis is reason for high hsCRP values in group of patients with IM, while mean hsCRP values in group with CAD is 3,09 mg/l which is above referent values (0-3 mg/l). Mean values of hsCRP in group without CAD is 2,23 mg/l. Values in group with and without CAD are not showing any statistically significant difference which point out the active plaque and active inflammatory process in both group of patients.

Based on AHA recommendation, patients in three study groups we can divide on subgroups based on hsCRP values and determine level of risk for acute cardiovascular event (IM, ICV, etc.) for those patients. Values of hsCRP in group with CAD are higher than in group without CAD but that difference is not statistically significant. In group of patients without CAD 38% of patients have hsCRP values 0-1 mg/l, which shows that there is highest percentage of patients with low risk for acute cardiovascular event. Values of hsCRP 3-10 mg/l, have 26% of patients which point out the fact that in this group are also highly risk patients. Group with CAD has 30% highly risk patients,



	hsCRP1	MMP 9
hsCRP1	1	
MMP 9	0,28437426	1

Figure 1. Correlation of hsCRP and MMP-9 values in group of patients with acute myocardial infarction

and 38% have hsCRP values lower than 2 mg/l. The fact is that more than one third of patients in group without CAD are highly risk patients and that more than one third of patients in group with CAD are low to middle risk for acute cardiovascular even, leads to conclusion that there is no significant difference in values of this marker between these two study group. Significant effect on hsCRP values probably had a patient's therapy. Consider high number of influence factors it is important that in group with CAD hsCRP values point out active plaque prone rupture and make risk estimation easier. Elevation of hsCRP after acute IM or unstable angina has positive correlation with consequence of disease (6). Coronary angiography determine level of coronary artery lumen stenosis but it is not adequate in determination of CAD severity because we know about plaque rupture and active inflammation even when plaque don't take more than 50% of vessel lumen (14).

Because MMP-9 has characteristic of plaque rupture marker and hsCRP as marker of active inflammation of atherosclerotic plaque it is interesting to follow these markers values in all three study group. Values of MMP-9 are the highest in group with IM, lower in group with CAD and the lowest in group without CAD. Values of hsCRP are also highest in group with IM and lowest in group without CAD. We can see that there is no statistically significant difference in hsCRP values between group with and without CAD, but difference in values of these markers is still there. Difference between these markers values between group with CAD and group with IM is statistically significant. We analyzed values of hsCRP and MMP-9 in group of patients with acute IM and conclude that there is weak positive correlation. The importance of determination both MMP-9 and hsCRP together is probably in the fact that mechanism of there synthesis is different. Our study shows that values of hsCRP are higher in patients with significant stenosis in three coronary artery than in patients with two, and in patients with two higher than in patients with one, but this difference is not statistically significant. Also, values of MMP-9 are higher in patients with significant stenosis in three coronary artery than in patients with two, and in patients with two are lower than in patients with significant stenosis on one coronary artery but these difference is not statistically significant. Austrian study also shows no significant correlation between hsCRP values and number of stenosed coronary artery (8).

HsCRP is highly sensitive but weekly specific marker so it is very important to combine it with patients clinical data but also with values of marker as MMP-9 who point out plaque rupture. here is a variety of factors influencing hsCRP and MMP-9 values, we exclude patients with acute or chronic inflammatory disease or malignancies. But is noted significant influence of therapy that CVD patients used on study markers. In our study there is no statistically significant correlation between hsCRP and MMP-9 levels and used therapy. In our study we take data from the patients about the drug therapy they use. Most of the patients use drugs from the group of ACE inhibitors, than beta blockers or Aspirin. Statin therapy is mostly used in group of patients with CAD same as therapy with ACE inhibitors. When we consider presence of list risk factors in all three group of patients than prescribe therapy get on importance. Data of therapy use we get from patients which not exclude possibility of incorrect and irregular use. Statins therapies can lover collagen degradation and MMP-9 activity. Clinical studies showed reduction in hsCRP level for 15-28% in period of 6 weeks statine usage, independently of LDL level reduction (10). Zaman in his study recommended combination of lipid reductors and antioxidants (to help plaque stabilization by lipid reduction, stabilization of fibrose cap and reduction of MMP-9 activity in plaque), ACE inhibitors (improvement of endotel dysfunction) and beta-blockers (reduction of blood stream influence on fibrose cap) (15).

5. CONCLUSION

Our study point out importance of hsCRP determination because it is marker of inflammation at atherosclerotic plaque and is important in assessment of plaque rupture risk in patients with ACS. MMP-9 is marker of plaque instability and point out at plaque rupture. The low but significant correlation between hsCRP and MMP-9 is of particular interest in our findings. This implies that hsCRP and MMP-9, at least to some extent, could be markers of different physiological pathways. It is important to correlate data about presence of other risk factors for CAD and correct data about used medical therapy with data of laboratory marker levels to get assessment of acute cardiovascular event, CAD and plaque rupture.

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