ORIGINAL ARTICLE

UDC: 616.379-008.64:616.12-037
DOI: 10.2298/VSP140204055P

Matrix metalloproteinase-9 index as a possible parameter for predicting acute coronary syndrome in diabetics

Indeks matriks metaloproteinaze-9 kao mogući parametar predviđanja akutnog koronarnog sindroma kod dijabetičara

Srdjan Popović*†, Fadil Canović*, Miroljub Ilić*, Sašo Rafajlovski‡, Vesna Dimitrijević-Srećković*†, Dragana Matanović§, Svetlana Vujović*‡, Predrag Djordjević†, Draško Gostiljac*

***Clinic for Endocrinology, Diabetes and Metabolic Diseases; §Clinic for Physical Medicine and Rehabilitation, Clinical Center of Serbia, Belgrade, Serbia; †Faculty of Medicine, University of Belgrade, Belgrade, Serbia; ‡Military Medical Academy, Belgrade, Serbia**

Abstract

Background/Aim. Matrix metalloproteinase-9 (MMP-9) index is the ratio of active MMP-9 and total MMP-9 levels. It reflects the importance of MMP-9 in acute coronary syndrome (ACS). **Methods.** The study included 3 groups of patients (n = 87): the group 1 – non-diabetic subjects without ACS (control); the group 2 – diabetic patients with ACS [subgroups with unstable angina pectoris (UAP), myocardial infarction (MI) or reinfarction]; and the group 3 non-diabetics patients with ACS. Total and active MMP-9 were measured and used to create MMP-9 index. **Results**. MMP-9 index, as a marker showed good sensitivity and specificity, of ACS in diabetics, with a cut-off value over 58.2. MMP-9 was higher in the study groups than in the control one. MMP-9 correlated with ACS occurrence and type of cardiovascular event. A statistically significant difference was found among the groups according to active MMP-9 ($p < 0.001$). The same was found with active MMP-9 between the control and the group with MI ($p < 0.001$). The control was highly statistically significantly different from the group of patients with UAP ($p < 0.01$). Statically significant differences in MMP-9 index was found between the control and the diabetics with ACS ($p < 0.001$). Statistically significant difference of MMP-9 index was also found in the controls compared to the value in non-diabetic patients with ACS ($p < 0.01$). **Conclusion.** MMP-9 index may be a possible marker of atheromatous plaque rupture in diabetics.

Key words:
diabetes mellitus; acute coronary syndrome; matrix metalloproteinase 9; prognosis.

Apstrakt

Uvod/Cilj. Indeks matriks metaloproteinaza-9 (MMP-9) predstavlja odnos nivoa aktivne i ukupne MMP-9. On odražava značaj MMP-9 u akutnom koronarnom sindromu (ACS). **Metode.** Ova studija obuhvatila je tri grupe bolesnika (n = 87): grupa 1 – nedijabetičari bez ACS (kontrola); grupa 2 – dijabetičari sa ACS [podgrupa sa nestabilnom anginom pektoris (UAP), miokardijalnim infarktom (MI) ili reinfarktom]; grupa 3 – nedijabetičari sa ACS. Određivan je nivo ukupne i aktivne MMP-9 da bi se dobio MMP-9 indeks. **Rezultati.** Index MMP-9, kao marker za ACS, pokazao je dobru senzitivnost i specifičnost za *cut off* (granične) vrednosti od preko 58.2. Kod dijabetičara MMP-9 bio je viši u ispitivanim grupama nego u kontroli. Index MMP-9 korelisao je sa pojavom ACS i tipom koronarnog događaja. Statistički značajna razlika dobijena je između grupa prema aktivnom MMP-9 ($p < 0,001$). Isto je nađeno sa aktivnim MMP-9 između kontrolne grupe i grupe sa MI ($p < 0,001$). Kontrola se visoko statistički značajno razlikovala od grupe bolesnika sa UAP ($p < 0,01$). Statistički značajne razlike u indeksu MMP-9 nađene su između kontrole i grupe dijabetičara sa ACS ($p < 0,001$). Statistički značajna razlika nađena je kod MMP-9 indeksa između kontrole u poređenju sa vrednostima kod nedijabetičara sa ACS ($p < 0,01$). **Zaključak.** Indeks MMP-9 može biti potencijalni marker za rupturu ateromatoznog plaka kod dijabetičara.

Ključne reči:
dijabetes melitus; akutni koronarni sindrom; matriks metaloproteinaza 9; prognoza.

Correspondence to: Draško Gostiljac, Clinic for Endocrinology, Diabetes and Metabolic Diseases, Belgrade, Serbia.
E-mail: doctor@med.bg.ac.rs

Introduction

Unstable atherosclerotic plaque is the pathophysiological substrate of acute coronary syndrom (ACS) [1]. Enzymes matrix metalloproteinases (MMPs), especially MMP-9 secreted by inflammatory cells of atheromatous plaque (macrophages), smooth muscle cells and endothelial cells belong to a large family of zinc-binding, calcium-dependent endopeptidases that are involved in the degradation and remodeling of extracellular matrix [2, 3]. They are a growing group of proteolytic enzymes involved in numerous processes including embryogenesis, interstitial metabolism, angiogenesis, etc. [4]. Matrix activity was observed in carcinogenesis, in some degenerative processes, and inflammatory conditions including atherogenesis. MMPs primarily MMP-9 has clinical significance in the serum of diabetic patients in inflammatory processes involving in plaque rupture and leading to acute coronary event with elevated levels [5–13]. MMP-9 is localized on the shoulders of plaque, a thin area which is suspected for rupture. The potential role of MMP-9 as a marker for risk stratification in patients with ACS was examined in a study on a larger number of patients showing that the values of MMP-9 were associated with future lethal cardiovascular events [6]. In patients with unstable angina pectoris (UAP) MMP-9 showed the increase of 70%, indicating active synthesis, compared with patients with stable angina. Although there is no conclusive data to show correlation of MMP with atheromatous plaques and localization of MMPs in the shoulder region of vulnerable lesions, a direct connection to the real rupture of unstable plaque has been described.

The aim of the study was to find out if the enzyme MMP-9 in the serum of diabetics and MMP-9 index could be early and safe markers of atheromatous plaque rupture and ACS.

Methods

We investigated patients admitted due to ACS to the Coronary Care Unit, Clinical Center of Serbia, Belgrade, during the period February, 2012 to February, 2013. Ethical principles were respected and all patients gave their consent to participate. A total of 87 patients of both sexes (57 male, 30 female) were examined. The patients were 40–80 years old (61.1 ± 10.3 year). The main criterion for inclusion was the presence of ACS (acute myocardial infarction or unstable angina pectoris). The two groups were formed depending on the diagnosis of diabetes mellitus (DM): diabetics with ACS (DM + ACS) and the group of non-diabetic patients with ACS. The diagnosis of ACS was based on clinical, electrocardiographic findings, biochemical analysis and diagnosis of DM based on the current American Diabetes Association criteria [14]. The patients with damaged hepatic function, severe anemia, neoplastic illness, infectious or autoimmune disease were excluded. DM + ACS was divided into two subgroups: UAP and the group with myocardial infarction or reinfarction (MI). The control consisted of healthy subjects (age 55.3 ± 8.9 years). MMP-9 activity was measured using detection enzymes in their pro-forms activated by binding to the active MMP-9 in the single-level enzymatic process by the method of Verheijen et al. [15]. The active form of MMP-9 linked to the detection enzyme was determined by binding to specific chromogenic peptide substrate complex which absorbs light of the wavelength of 405 nm. The concentration of active MMP-9 in the sample was determined by interpolation from the standard curve. Biotrak of MMP-9 activity assay system is equivalent to ELISA determination. The measuring range of the method is: the total MMP-9 1.0–32 ng/mL, and active MMP-9 0.5–16 ng/mL. MMP-9 index is defined as the relative ratio of the active and total form of MMP-9 multiplied by 100 [(MMP-9 active/MMP-9 total) ×100].

The results were reported as the mean value ± standard deviation and percentage. The differences between the groups were assessed by two-way analysis of variance (ANOVA-with Bonferroni post hoc analysis) for continuous variables and one-way non-parametric analysis of variance (Kruskal-Wallis) for category variables. Potential cutoffs for MMP-9 index were evaluated using receiver operating characteristic (ROC) curve analysis. The correlations between parameters were analyzed with Pearson's and Spearman's test. The differences were considered statistically significant at $p < 0.05$. SPSS 12.0 software was used for statistical analysis.

Results

Table 1 shows the values of total MMP-9, active MMP-9 and MMP-9 index in the examined groups and the groups formed on coronary events.

Total MMP-9 in the examined groups

The values of total MMP-9 show a statistically significant difference among the three examined groups ($p < 0.01$). There was a statistically significant difference of total MMP-9 between the control and DM + ACS group ($p < 0.001$). Also, there is a statistically significant difference of total MMP-9 between the groups of diabetics and non-diabetics with ACS ($p < 0.05$). There was no statistically significant difference of total MMP-9 between the control and the group of non-diabetics with ACS.

Active MMP-9 in the examined groups

The values of active MMP-9 showed a statistically significant difference among the three examined groups ($p < 0.001$). The value of active MMP-9 was significantly different between the control and both groups with ACS, diabetics ($p < 0.001$) and non-diabetics ($p < 0.001$). The groups of diabetics and non-diabetics with ACS were statistically significantly different ($p < 0.001$).

MMP-9 index in the examined groups

A significant difference in the values of MMP-9 index among the three examined groups was found ($p < 0.001$), as well as between the control and DM + ACS group ($p < 0.001$). There was a significant difference in the value of MMP-9 index in the control compared to the value in the non-diabetics ($p < 0.0$).

Table 1

The values of matrix metallproteinaze-9 (MMP-9)

Marker	Examined groups ($\bar{x} \pm SD$)			*p*
	DM + ACS	ACS	Control	
Total MMP-9 level (ng/mL)	51.7 ± 13.4	42.2 ± 11.1	37.1 ± 12.4	< 0.01
Active MMP-9 level (ng/mL)	36.8 ± 11.2	28.4 ± 7.3	16.2 ± 7.9	< 0.001
MMP-9 index	70.6 ± 16.8	68.8 ± 15.6	42.9 ± 10.1	< 0.001
	Coronary events groups ($\bar{x} \pm SD$)			
	UAP	MI	Control	
Total MMP-9 level (ng/mL)	44.4 ± 15.5	46.7 ± 12.3	37.1 ± 12.4	< 0.05
Active MMP-9 level (ng/mL)	28.9 ± 10.4	32.7 ± 9.9	16.2 ± 7.9	< 0.001
MMP-9 index	66.7 ± 16.5	71.4 ± 16.0	42.9 ± 10.1	< 0.01

x – mean value; SD – standard deviation; DM – patients with diabetes mellitus; ACS – patients with acute coronary syndrome; UAP – patients with unstable angina pectoris; MI – patients with myocardial infarction or reinfarction.

Total MMP-9 in the coronary events groups

The values of total MMP-9 showed a statistically significant difference among the three groups formed on coronary events ($p < 0.05$). There was a statistically significant difference in total MMP-9 between the control group and the MI group ($p < 0.01$).

Active MMP-9 in the coronary events groups

The values of active MMP-9 show a statistically significant difference among the three groups formed on coronary events ($p < 0.001$). The value of active MMP-9 was significantly different between the control and both groups with ACS, UAP ($p < 0.001$) and MI ($p < 0.001$). The groups of UAP and MI were not significantly different in values of active MMP-9 ($p > 0.05$).

MMP-9 index in the examined groups

The values of MMP-9 index showed statistically significant difference among the three groups formed on coronary events ($p < 0.01$). The value of active MMP-9 was significantly different between the control and both coronary events groups, UAP ($p < 0.001$) and MI ($p < 0.001$). The value of MMP-9 index in the UAP group was not significantly different than the value in the MI group ($p < 0.05$).

Statistical analysis of the data presented in Figure 1 showed a significant difference in the percentage of patients with elevated total MMP-9 between the control group and the DM + ACS group ($p < 0.01$).

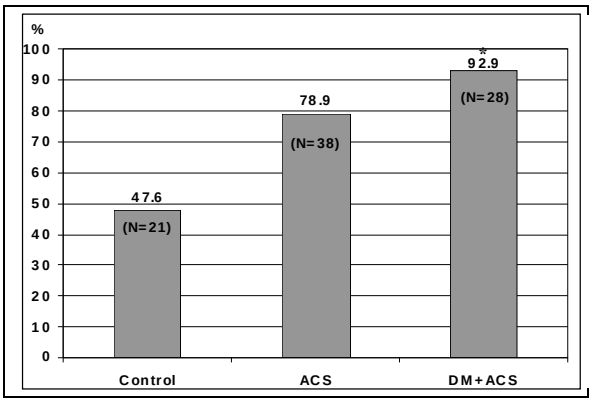

Fig. 1 – Distribution of elevated total matrix metalloproteinase-9 (MMP-9) levels.

*** *p* < 0.01 compared to control; N – number of patients; ACS – patient with acute coronary syndrome; DM – patient with diabetes mellitus.**

Statistical analysis of data presented in Figure 2 showed a significant difference in the percentage of patients with elevated total MMP-9 between the control group and the group of patients with myocardial infarction or reinfarction ($p < 0.01$).

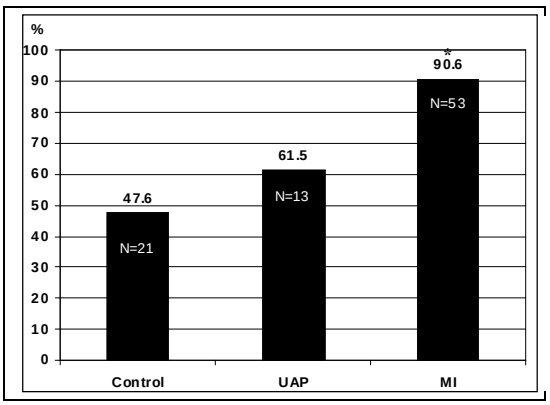

Fig. 2 – Distribution of elevated total matrix metalloproteinase-9 (MMP-9) levels in the coronary event groups.

****p* < 0.01 compared to control ; N – number of patients; UAP – patients with unstable angine pectoris; MI – patients with myocardial infarction or reinfarction.**

Data presented in Figure 3 show a significant difference between the control group and both groups with ACS (diabetics and non-diabetics) ($p < 0.001$). The groups of diabetics and non-diabetics with ACS did not differ significantly ($p > 0.05$).

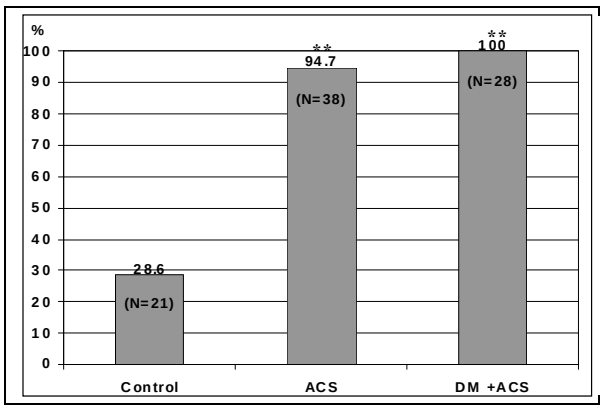

Fig. 3 – Distribution of elevated active matrix metalloproteinase-9 (MMP-9) levels

**** p < 0.001 compared to control; N – number of patients; ACS – patient with acute coronary syndrome; DM – patient with diabetes mellitus.**

Data presented in Figure 4 show a significant difference between the control and MI group ($p < 0.001$). The control group was significantly different from the UAP group ($p < 0.01$), too.

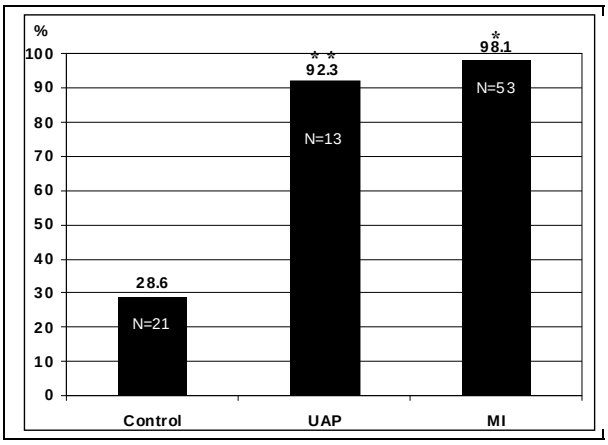

Fig. 4 – Distribution of elevated active matrix metalloproteinase-9 (MMP-9) levels in coronary event groups

***p < 0.01 compared to control; **p < 0.001 compared to control; N – number of patients; UAP – patients with unstable angine pectoris; MI – patients with myocardial infarction or reinfarction.**

Table 2 shows the significant correlations of total MMP-9 levels and other ACS parameters. In the DM + ACS group, elevated total MMP-9 level showed the strongest correlation with the elevated Homeostatic Model Assessment of Insulin Resistance (HOMA IR) score. Elevated cholesterol levels significantly correlated with elevated total MMP-9 level in the DM + ACS group. The presence of hypertension and smoking showed a statistically significant correlation between DM + ACS with total MMP-9.

The presence of hypertension and smoking in the DM + ACS group significantly correlated with serum levels of active MMP-9.

There was a good correlation between MMP-9 index and hyperinsulinemia and pain duration in the group of diabetics with ACS.

The largest area under the ROC curve (AUC) was of active MMP-9 level, the lowest AUC was of total MMP-9 level, and AUC of MMP-9 index was between active and total MMP-9 level. The AUC of total MMP-9 level showed a significant difference ($p = 0.0041$) compared to the area of 0.5. The AUC of active MMP-9 level and MMP-9 index showed a significant difference ($p = 0.0001$) compared to the area of 0.5. AUC of total MMP-9 is significantly different from the AUC of active MMP-9 ($p < 0.05$). The AUC for MMP-9 index was not significantly different compared to the AUC of total or active MMP-9 level. The AUC of MMP-9 index had the lowest standard error (SE). The resulting cut-off value for MMP-9 index was over 58.2.

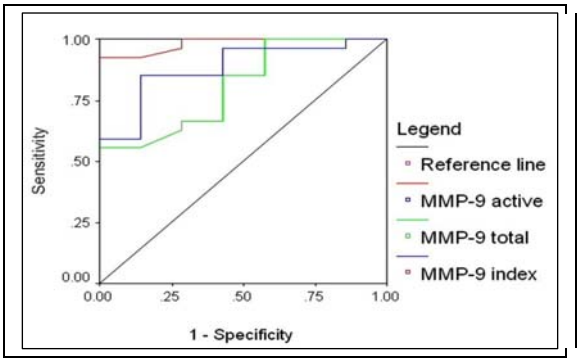

Fig. 5 – Receiver operating characteristic (ROC) curves of total matrix metalloproteinase-9 (MMP-9) level, active MMP-9 level and MMP-9 index in diabetics with acute coronary syndrom (ACS)

Table 3 shows the values of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of MMP-9 level as predictor of ACS in diabetics. Total MMP-9 level had good sensitivity, but poor specificity with a high PPV and NPV. Active MMP-9 had good sensitivity and specificity, with a high PPV and NPV. MMP-9 index showed good sensitivity, excellent specificity and PPV, and acceptable NPV.

Table 2

Correlations of matrix metalloproteinase-9 (MMP-9) with other important parameters of acute coronary syndrome (ACS)

Parameter	MMP-9	Group	*r*	*p*
Age	Total	All	0.353	< 0.01
Smoking	Total	DM + ACS	0.387	< 0.05
Hypertension	Total	DM + ACS	0.469	< 0.05
HOMAIR (high values)	Total	DM + ACS	0.557	< 0.05
Cholesterol (elevated levels)	Total	DM + ACS	0.422	< 0.05
Smoking	Active	DM +ACS	0.426	< 0.05
Hypertension	Active	DM + ACS	0.395	< 0.05
Insulinemia (high values)	Index	DM + ACS	0.542	< 0.05
Pain duration	Index	DM + ACS	0.382	< 0.05

***r* – coefficient of correlation; DM – diabetes mellitus.**

Table 3

Matrix metalloproteinase-9 (MMP-9) parameters as predictors of acute coronary syndrome (ACS) in diabetics

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV(%)
MMP-9 total level	100	44.4	84.4	100
MMP-9 active level	92.6	88.9	96.2	80
MMP-9 index	77.8	100	100	60

PPV – positive predictive value of the test; NPV – negative predictive value of the test.

Discussion

The levels of MMP-9 and tissue inhibitor metalloproteinases-1 (TIMP-1) are significantly increased in coronary arteries with unstable plaques [16]. As TIMP-1 is a potential inhibitor of MMP-9, its increase during the acute phase of MI may indicate production of MMP-9 [17–19]. Diabetes and/or hyperglycemia have a big influence on the structure and function of the heart and vascular tissue [20], inducing proinflammatory changes, including increased MMP and other factors potentially relevant to plaque rupture and thrombosis [21]. There are studies showing no changes of MMP-9 in diabetics [22]. Diabetes is also associated with plaque instability and carries a high risk for an acute coronary event. Sampling of MMP-9 values is related to the occurrence of acute coronary event and the diagnosis of ACS. Our results showed that the values of total MMP-9 level were higher in diabetics with ACS, than in the non-diabetics and the control. Elevated total MMP-9 level was highest in the patients with MI, then in the UAP and the control group. The mean value of total MMP-9 level in the control group was slightly above the upper limit of the reference values (37.1 ng/mL) and there was a statistically significant difference compared to the mean value obtained in the group of diabetics with ACS. The total MMP-9 level, active form, reflects the presence of ACS more precisely. The control group had statistically significantly lower values of active MMP-9 level compared to the groups of diabetics and non-diabetics with ACS. Elevated active MMP-9 level was highest in the patients with MI, then in the UAP group and the control group. The number of patients with highly active MMP-9 level in the control group was highly statistically significantly different compared to the groups with MI and UAP. The mean values of active MMP-9 level between the groups of diabetics and non-diabetics with ACS were highly significantly different. In the group of diabetics with ACS, a total MMP-9 level showed the strongest correlation with the values of HOMA IR index. There was a correlation of elevated cholesterol with the elevated total MMP-9 level in diabetics with ACS. Hypertension and smoking showed a correlation with the values of total MMP-9 level in the group of diabetics. Insulin resistance is closely related to the progression of atherosclerosis and total MMP-9 level may reflect the degree of existing atherosclerosis. In the group of diabetics there was correlation between active MMP-9 level with hypertension and smoking and without HOMA IR. IR is a chronic condition, and active MMP-9 level is a parameter of acute events. A correlation was found with hypertension, also a chronic condition, but may be a sudden increase of pressure in the form of hypertensive crisis, which can trigger an acute coronary event. A correlation with smoking can be the result of the influence of carbon monoxide on the development of coronary vasoconstriction of blood vessels, which in the ground of unstable plaque and hypertension increases the turbulent flow of blood which in turn contributes to plaque rupture. This study examined the active MMP-9 level in diabetics with ACS who are particularly exposed to numerous risk factors including IR [23]. Dysfunction in autonomic nervous system may result in pain absence during coronary event. It is necessary to predict coronary events in diabetics. The level of active MMP-9 level is associated with both plaque rupture and the massiveness of the rupture. There is no adequate information about genetic polymorphisms of MMPs. They can be partially explained by changes in the distribution of MMP-9 at the individual level. If the levels of total and active MMP-9 are directly related to the genetic polymorphism that may result in higher levels of both forms of MMP-9 without ACS. MMP-9 index reflects the impact of both forms of MMP-9. Therefore, we examined the relative ratio of active and total MMP-9, which we define as MMP-9 index. The mean value of MMP-9 index in the group of diabetics was highest, slightly lower in the non-diabetics with ACS, and lowest in the control group. MMP-9 index was very highly statistically significantly different among the groups. Our results showed that the mean MMP-9 index was slightly higher in the patients with MI than in the group with UAP but with no statistically significant differences. These results are expected because level of active MMP-9 is a marker of plaque rupture, which occurs in UAP and MI. MMP-9 index showed a statistically significant correlation with hyperinsulinemia and duration of pain in the group of diabetics with ACS. In order to prove the validity of MMP-9 index we determined sensitivity and specificity for each parameter individually (total, active MMP-9 and MMP-9 level index). Analyzing the ability of total MMP-9 level as a marker of ACS in diabetics we found an important AUC (amounted to 0.774) and the cut-off value for acute coronary events was over 27.6 ng/mL, while level of active MMP-9 in the same group the AUC was 0.936 and the cut-off value over 23.8 ng/mL. The AUC of active and total MMP-9 level differed significantly compared to the ACU of MMP-9 index. In diabetics with ACS, the AUC of MMP-9 index was 0.914 and highly significantly different compared to the AUC of 0.5. MMP-9 index cut-off value was 58.2. The AUC of MMP-9 index had the lowest values of SE.

The sensitivity of total MMP-9 level as a test for ACS in diabetics was 100%, but its specificity was low (only 44.4%). Active MMP-9 level had a good sensitivity and specificity. MMP-9 index showed a very good sensitivity and excellent specificity satisfying the requirements for screening test of ACS in diabetics. MMP-9 index is a relative number which eliminate several possible influences of the variability of active and total MMP-9 level values.

Analysing the available literature does not reveal similar results in terms of active MMP-9 level. MMP-9 index has been first postulated in this study. Total MMP-9 and active MMP-9 levels can be determined relatively quickly and easily. Their commercialization further reduces the costs and allows determination of the proposed MMP-9 index. This index could be a good marker for triage in population of diabetics and selection of candidates for elective coronary angiography. We believe that the use of this index in the secondary prevention of acute coronary events in diabetics may significantly improve cardiovascular outcomes. Without any doubt, it

is necessary to set a prospective randomized study on a larger number of patients, which may eventually correct the cut-off value of MMP-9 index determined by our study.

Conclusion

MMP-9 index could be a good marker for prediction of acute coronary syndrome in diabetic patients and for triage for elective coronary angiography.

Acknowledgements

The authors would like to thank the patients, cardiologists, laboratory staff of the Emergency Unit, and the Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia, Belgrade, Serbia.

Disclosures

None of the authors have any competing interests.

R E F E R E N C E S

1. *Cola C, Clementi E, Biondi-Zoccai G, Sangiorgi G.* From carotid plaque biology to serologic markers of vulnerability to predict the risk of cerebrovascular events. Acta Chir Belg 2007; 107(2): 129−42.
2. *Fingleton B.* Matrix metalloproteinases as valid clinical targets. Curr Pharm Des 2007; 13(3): 333−46.
3. *Page-McCaw A, Ewald AJ, Werb Z.* Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol 2007; 8(3): 221−33.
4. *Dabek J, Kulach A, Gasior Z.* The role of matrix metalloproteinases in acute coronary syndromes. Eur J Int Med 2007; 18(6): 463−6.
5. *Hansson GK.* Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005; 352(16): 1685−95.
6. *Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G,* et al. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation 2003; 107(12): 1579−85.
7. *Newby AC.* Do metalloproteinases destabilize vulnerable atherosclerotic plaques. Curr Opin Lipidol 2006; 17(5): 556−61.
8. *Tayebjee MH, Lip GY, MacFadyen RJ.* Matrix metalloproteinases in coronary artery disease: clinical and therapeutic implications and pathological significance. Curr Med Chem 2005; 12(8): 917−25.
9. *Kameda K, Matsunaga T, Abe N, Fujiwara T, Hanada H, Fukui K,* et al. Increased pericardial fluid level of matrix metalloproteinase-9 activity in patients with acute myocardial infarction: possible role in the development of cardiac rupture. Circ J 2006; 70(6): 673−8.
10. *Wagner DR, Delagardelle C, Ernens I, Rouy D, Vaillant M, Beissel J.* Matrix metalloproteinase-9 is a marker of heart failure after acute myocardial infarction. J Card Fail 2006; 12(1): 66−72.
11. *Jones CB, Sane DC, Herrington DM.* Matrix metalloproteinases: a review of their structure and role in acute coronary syndrome. Cardiovasc Res 2003; 59(4): 812−23.
12. *Tsuzuki M, Morishima I, Yoshida T, Hayashi Y, Miura M, Hirai T,* et al. Inverse correlation between soluble CD40 ligand and soluble CD40 is absent in patients with unstable angina. Heart Vessels 2005; 20(6): 245−50.
13. *Fiotti N, Altamura N, Orlando C, Simi L, Reimers B, Pascotto P,* et al Metalloproteinases-2, -9 and TIMP-1 expression in stable and unstable coronary plaques undergoing PCI. Int J Cardiol 2008; 127(3): 350−7.
14. *American Diabetes Association.* Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; 33(Suppl 1): S62−9.
15. *Verheijen JH, Nieuwenbroek NM, Beekman B, Hanemaaijer R, Verspaget HW, Ronday HK,* et al. Modified proenzymes as artificial substrates for proteolytic enzymes: colorimetric assay of bacterial collagenase and matrix metalloproteinase activity using modified pro-urokinase. Biochem J 1997; 323(3): 603−9.
16. *Zeng B, Prasan A, Fung KC, Solanki V, Bruce D, Freedman SB,* et al. Elevated circulating levels of matrix metalloproteinase-9 and -2 in patients with symptomatic coronary artery disease. Intern Med J 2005; 35(6): 331−5.
17. *Fukuda D, Shimada K, Tanaka A, Kusuyama T, Yamashita H, Ehara S,* et al. Comparison of levels of serum matrix metalloproteinase-9 in patients with acute myocardial infarction versus unstable angina pectoris versus stable angina pectoris. Am J Cardiol 2006; 97(2): 175−80.
18. *Lindsey ML.* MMP induction and inhibition in myocardial infarction. Heart Fail Rev 2004; 9(1): 7−19.
19. *Vanhoutte D, Schellings M, Pinto Y, Heymans S.* Relevance of matrix metalloproteinases and their inhibitors after myocardial infarction: a temporal and spatial window. Cardiovasc Res 2006; 69(3): 604−13.
20. *Maekawa K, Tsujino T, Saito K, Kim JI, Ikeda Y, Emoto N,* et al. Inhibitory effect of insulin on vasopressin-induced intracellular calcium response is blunted in hyperinsulinemic hypertensive patients: role of membrane fatty acid composition. Heart Vessels 2006; 21(4): 205−12.
21. *Aljada A, Ghanim H, Mohanty P, Syed T, Bandyopadhyay A, Dandona P.* Glucose intake induces an increase in activator protein 1 and early growth response 1 binding activities, in the expression of tissue factor and matrix metalloproteinase in mononuclear cells, and in plasma tissue factor and matrix metalloproteinase concentrations. Am J Clin Nutr 2004; 80(1): 51−7.
22. *Baugh MD, Gavrilovic J, Davies IR, Hughes DA, Sampson MJ.* Monocyte matrix metalloproteinase production in Type 2 diabetes and controls - a cross sectional study. Cardiovasc Diabetol 2003; 2: 3.
23. *Gostiljac D, Dorđević PB, Djurić D, Peruničić J, Lasica R, Colak E,* et al. The importance of defining serum MMP-9 concentration in diabetics as an early marker of the rupture of atheromatous plaque in acute coronary syndrome. Acta Physiol Hung 2011; 98(1): 91−7.

Received on February 4, 2014.
Revised on April 17, 2014.
Accepted on May 12, 2014.
Online First August, 2014.