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## Markers of inflammation and antioxidant enzyme activities in restenosis following percutaneous coronary intervention

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**Abstract:** The efficacy of percutaneous coronary intervention (PCI) is often compromised by the need for repeat revascularization because of restenosis development. Numerous studies have tried to establish the predictive value of different biochemical markers of restenosis, with conflicting results. The aim of this study was to assess the prognostic significance of inflammatory and lipid markers, and major antioxidant enzyme activity for the development of in-stent restenosis (ISR) following PCI. Serum high sensitive C-reactive protein (hs-CRP), soluble intercellular cell adhesion molecule-1 (sICAM-1), transforming growth factor-beta (TGF- $\beta$ ), lipoprotein(a) (LP(a)) and oxidized low-density lipoprotein (oxLDL) levels, as well as serum extracellular superoxide dismutase (EC-SOD) and catalase (CAT) activities were determined in 44 patients before the stent implantation procedure, and after 6-month follow-up. The results after follow-up revealed that in patients that developed angiographically confirmed ISR, the increase in serum hs-CRP levels was significantly higher compared to those without stenosis. Stent implantation induced compensatory increases in the activities of serum antioxidant enzymes at follow-up, with significantly lower CAT activity in patients with ISR, possibly contributing to stenosis development. No significant changes in the circulating levels of ICAM-1, TGF- $\beta$ , oxLDL and Lp(a) were observed between the groups. In conclusion, serum hs-CRP level and CAT activity may be considered as useful biochemical markers for monitoring patients during follow-up after stent implantation.

**Keywords:** coronary restenosis; hs-CRP; EC-SOD; catalase; oxLDL; Lp(a).

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## INTRODUCTION

Percutaneous coronary intervention (PCI) with stent implantation has revolutionized the management of coronary artery disease (CAD), enabling greater efficacy in the prevention of acute occlusions. The efficacy of PCI is limited because of the development of in-stent restenosis (ISR), a long-term complication that occurs in 20–30 % of patients 6 months after the procedure.<sup>1</sup> Great efforts have been made in attempts to elucidate the molecular mechanism(s) leading to ISR, and to search for potential markers that would help identify patients at higher risk for ISR.

Experimental studies showed that ISR is a complex and multifactorial process strongly influenced by the inflammatory response of the arterial wall to stent deployment. Previous studies also reported that percutaneous coronary interventions induce the production of vascular reactive oxygen species (ROS). The increase in ROS after stent implantation was shown to induce a chain reaction resulting in oxidative stress, which could lead to endothelial dysfunction, neointimal formation, stent thrombosis and eventual restenosis.<sup>2</sup> Stent implantation, in particular, can precipitate arterial intimal cellular proliferation and extracellular matrix synthesis, which is mediated largely by inflammatory processes.<sup>3</sup> There is evidence that inflammatory response further enhances oxidative stress in the growing lesions, while, on the other hand, oxidative stress, which has pro-inflammatory properties, could cause inflammation.<sup>4</sup> Several studies suggested that restenosis after PCI is associated with increased circulating levels of inflammatory markers, such as pro-inflammatory cytokines and C-reactive protein (CRP) before or after the procedure.<sup>5</sup> Several studies demonstrated that CRP could induce oxidative stress *in vitro*. It was also published that cardiomyocytes from patients with CAD produce CRP locally and that human recombinant CRP induces significant increase in the production of reactive species in endothelial cells, leading to a concentration-dependent induction of apoptotic cell death, which can be attenuated in the presence of antioxidants.<sup>6</sup>

Similar relations were shown with serum levels of other inflammatory markers, such as adhesion molecules (ICAM-1 or VCAM-1). It was noted that arterial endothelial expression and raised serum concentrations of the soluble form of intercellular adhesion molecule-1 (sICAM) are implicated in the development of CAD after coronary artery bypass surgery.<sup>7</sup> A significant correlation between raised concentrations of CRP and soluble ICAM1 concentrations after transplantation was also reported.<sup>7</sup> In addition, immunohistochemical analyses of human and animal samples showed that TGF- $\beta$  expression is upregulated following balloon- or stent-induced vascular injury.<sup>8</sup> Furthermore, upregulation of TGF- $\beta$  was shown to increase intimal thickening, whereas blockade of TGF- $\beta$  attenuated this process.<sup>9</sup>

The characteristic histological finding of restenotic tissue is intimal hyperplasia, with a variable lipid component. This histological appearance suggests that similar cellular processes may play important roles in both atherogenesis and restenosis. Many previous studies revealed that serum levels of low- and high-density lipoprotein cholesterol (LDL-C and HDL-C, respectively) are the major predictors of atherosclerotic coronary artery disease, and an inverse relation between HDL levels and the restenosis rate in patients following PCI was demonstrated,<sup>10</sup> possibly attributed to the scavenger function of HDL cholesterol. In addition, it was shown that hypercholesterolemia is associated with increased level of oxidative stress, contributing to an increased oxidation of LDL.<sup>11</sup> The accumulation of oxidized low-density lipoproteins (oxLDLs) in atherosclerotic lesions from coronary and carotid arteries was demonstrated,<sup>12</sup> as well as an elevation of circulating levels of oxLDLs in patients with unstable angina or acute myocardial infarction.<sup>13</sup> Lipoprotein(a) [Lp(a)] is a low-density lipoprotein (LDL)-like particle in which apolipoprotein (apo)B-100 is bound by a disulfide bridge to apo(a).<sup>14</sup> Lp(a) was identified in the atherosclerotic plaque and it was shown that Lp(a) plaque levels correlate with its concentration in plasma.<sup>15</sup> In numerous reports, associations between elevated serum concentrations of Lp(a) and CAD, myocardial infarction, cerebrovascular disease and stenosis of coronary artery bypass vein grafts were established.<sup>13,15</sup> However, to the best of our knowledge, data on the association between the level of circulating oxLDL and Lp(a) with clinical outcome after coronary stenting are limited, even though some studies suggested that elevated plasma concentration of lipoprotein(a) could be a risk factor for restenosis after PCI.<sup>15</sup>

Bearing in mind the role of inflammatory and lipid markers, as well as the serum antioxidative status, it was hypothesized that several inflammation (hs-CRP, sICAM-1, TGF- $\beta$ ) and lipid markers (Lp(a), oxLDL), as well as the activity of major plasma antioxidant enzymes (EC-SOD and catalase) could be regarded as prognostic markers for the development of in-stent restenosis following PCI.

## EXPERIMENTAL

### *Study group*

The study group consisted of 81 consecutive patients with CAD who were successfully treated with either balloon coronary angioplasty or stent implantation in the Clinical Center of Montenegro during 2010 and 2011. Out of this cohort, 44 patients who had successful coronary stent implantation underwent follow-up angiography and a series of biochemical analyses were performed prior to PCI (percutaneous coronary intervention) (baseline) and at follow-up, 6 months after the procedure. All patients fulfilled the following criteria: confirmed myocardial ischemia, coronary vessel constriction (defined reduction > 50 % vessel lumen diameter), no contraindications to the administration of antiplatelet agents, and written agreement to undergo follow-up angiography. Conventional clinical risk factors for CAD were recorded such as age, gender, current smoking habit (>5 cigarettes/day), hypertension (systolic

blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg or antihypertensive medication), and hyperlipidemia (total cholesterol > 5.17 mmol L<sup>-1</sup>, LDL-cholesterol > 2 mmol L<sup>-1</sup> or cholesterol-lowering medications). The patients received a single heparin injection (5000 I.U.) after obtaining blood sample and prior to PCI, followed by post-operative administration of treatment oral antiplatelet agents (aspirin, 100 mg and clopidogrel, 75 mg) that was continued throughout follow-up period.

The clinical and angiographic exclusion criteria were fasting glucose levels > 6.5 mmol L<sup>-1</sup>, obesity (*BMI* > 25 kg m<sup>-2</sup>), acute or chronic inflammatory conditions, liver or kidney dysfunction, treatment with steroids, pregnancy and presence of mental disorders. The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethical Committee of the Clinical Center of Montenegro and written informed consent was obtained from all patients for routine control angiographic examination after 6 months.

#### *Angiographic assessment*

Coronary intervention and angiographic assessment were performed with a digital angiographic system (Siemens Axiom interventional cardiology systems), and quantitative coronary analysis (QCA) was performed on a digital angiographic workstation (Axiom Syngo artis zee family of C-arm system). Restenosis was defined as a percent diameter stenosis *DS* > 50 % at the site of the treated lesion in at least 2 orthogonal views.

#### *Laboratory tests*

Blood samples were obtained two hours before PCI (baseline), and at follow-up, 6 months after the procedure, after fasting for at least 10 h. All the analyses were performed at the Center for Clinical Laboratory Diagnostics of the Clinical Center of Montenegro, unless stated otherwise. Peripheral venous blood (10 mL) was collected in the morning, after fasting for a minimum of 10 h. Serum was separated at 3000 rpm at 4 °C for 15 min. The supernatant was collected, and aliquots were stored at -80 °C until analysis. The presence of hemolysis was followed by measurement of plasma hemoglobin and all patients with hemoglobin concentration > 50 mg L<sup>-1</sup> were excluded from the study. Serum levels of glucose, triacylglycerol, cholesterol, VLDL and LDL were determined using standard commercial kits (Abbott Laboratories), whereas the concentration of hs-CRP was determined using laser nephelometry (BNII Siemens). The concentrations of ICAM-1, TGF- $\beta$ , Lp(a) and oxLDL were determined employing a ChroMate 4300 microplate reader (Awareness Technology Inc., Palm City, FL, USA) using enzyme-linked immunosorbent assays (DRG International, Springfield, NJ, USA) at the Laboratory for Research at the Faculty of Medicine, University of Montenegro. Measurement of the serum activity of antioxidative enzymes was performed at the Institute of Medical and Clinical Biochemistry, Faculty of Medicine, Belgrade, Serbia. The superoxide dismutase (SOD) activity in serum was measured spectrophotometrically, using the method of Misra and Fridovich, based on the ability of SOD to inhibit auto-oxidation of epinephrine at alkaline pH (pH 10.2).<sup>16</sup> Catalase activity was determined spectrophotometrically by measuring the degradation of H<sub>2</sub>O<sub>2</sub> using ammonium molybdate.<sup>17</sup>

#### *Statistical analysis*

Data are expressed as mean  $\pm$  *SD* or median, as indicated in the text or Figure legends. The student's *t*-test was used to compare differences between continuous variables. The significance of differences between the frequencies of incidence of risk factors was tested using the  $\chi^2$ -test. *P* values < 0.05 were considered significant. All statistical calculations were realized using a trial version of IBM 17 SPSS for Windows.

## RESULTS AND DISCUSSION

The results of role of the investigated inflammatory (hs-CRP, sICAM-1 and TGF- $\beta$ ) and lipid (Lp(a) and oxLDL) markers, as well as the serum antioxidative status and their prognostic significance for the development of ISR following PCI are as follows.

*Baseline characteristics*

Of the 44 patients who underwent stent implantation, 31 (70.5 %) were male and 13 (29.5 %) were female and all were between the ages of 38 and 75 ( $56.0 \pm 5.5$  years). In-stent restenosis (ISR) occurred within 6 months of stent implantation in 8 patients (18.2 %). In univariate analysis, age and gender did not differ between the groups with and without restenosis ( $p > 0.05$ ). No significant association between restenosis and hypercholesterolemia, hypertension or smoking was observed (Table I). However, there were significant differences in positive family history for CAD and previous angina.

TABLE I. Baseline characteristics of the patients stratified by stent implantation with restenosis or without restenosis. CAD - coronary artery disease; Values are mean  $\pm$  SD, \* $p < 0.05$

| Risk factor               | No restenosis (n = 36) | Restenosis (n = 8) |
|---------------------------|------------------------|--------------------|
| Age, years                | 60.2 $\pm$ 8.1         | 61.2 $\pm$ 7.3     |
| Male, %                   | 69.4                   | 75.0               |
| Smoking, %                | 44.4                   | 33.3               |
| Hypercholesterolemia, %   | 55.6                   | 33.3               |
| Hypertension, %           | 75.0                   | 66.7               |
| Family history for CAD, % | 33.3                   | 55.6 *             |
| Previous angina, %        | 36.1                   | 50.0 *             |

This study focused on the association between the biochemical risk factors (inflammatory biomarkers, level of anti-oxidant protection and lipid markers) and ISR as an adverse event following coronary angioplasty and stent implantation. Moreover, the standard panel of biochemical analyses (serum concentration of glucose, triacylglycerol, cholesterol, VLDL, LDL, HDL, troponin I, AST, CK-MB and cystatin C) was also performed at the same time points, in order to assess the patients glycemic and lipid profile (Table II). No significant difference was observed in the biochemical profile neither at the pre-procedural time point nor after 6-month follow-up between the patients without restenosis and those developing ISR.

*Inflammatory biomarkers in restenosis*

Even though it is well established that multiple factors can contribute to ISR, the underlying mechanisms remain elusive. Numerous studies in animal models and on human arterial segments *in vitro* have reported the critical role of inflammation in the restenotic process, either because of pre-existing inflammatory lesion,

TABLE II. Biochemical parameters determined in serum of those that underwent stent implantation prior to PCI (baseline) and after 6-month follow-up; values are mean  $\pm$  SD;  $p > 0.05$

| Parameter                             | No stenosis      |                   | In-stent restenosis |                   |
|---------------------------------------|------------------|-------------------|---------------------|-------------------|
|                                       | Baseline         | Follow-up         | Baseline            | Follow-up         |
| Glucose, mmol L <sup>-1</sup>         | 5.05 $\pm$ 0.75  | 4.85 $\pm$ 0.69   | 5.17 $\pm$ 0.64     | 4.92 $\pm$ 0.45   |
| Triacylglycerol, mmol L <sup>-1</sup> | 2.08 $\pm$ 0.85  | 2.07 $\pm$ 0.78   | 1.79 $\pm$ 1.07     | 1.63 $\pm$ 0.87   |
| Cholesterol, mmol L <sup>-1</sup>     | 5.63 $\pm$ 1.03  | 5.44 $\pm$ 0.99   | 4.60 $\pm$ 0.9      | 5.43 $\pm$ 1.83   |
| VLDL / mmol L <sup>-1</sup>           | 1.47 $\pm$ 1.08  | 1.54 $\pm$ 1.18   | 2.28 $\pm$ 2.04     | 2.04 $\pm$ 1.73   |
| LDL / mmol L <sup>-1</sup>            | 3.83 $\pm$ 0.74  | 3.70 $\pm$ 0.54   | 3.81 $\pm$ 1.32     | 3.69 $\pm$ 1.08   |
| HDL / mmol L <sup>-1</sup>            | 1.06 $\pm$ 0.26  | 1.05 $\pm$ 0.25   | 1.14 $\pm$ 0.16     | 1.13 $\pm$ 0.16   |
| AST / U L <sup>-1</sup>               | 21.25 $\pm$ 7.18 | 24.74 $\pm$ 14.25 | 18.75 $\pm$ 5.52    | 21.68 $\pm$ 10.21 |
| CK-MB / U L <sup>-1</sup>             | 16.0 $\pm$ 0.32  | 16.97 $\pm$ 6.64  | 15.6 $\pm$ 3.73     | 19.48 $\pm$ 5.06  |
| Troponin I, ng L <sup>-1</sup>        | 0.05 $\pm$ 0.04  | 0.17 $\pm$ 0.44   | 0.02 $\pm$ 0.01     | 0.08 $\pm$ 0.16   |
| Cystatin C, ng L <sup>-1</sup>        | 0.88 $\pm$ 0.22  | 0.90 $\pm$ 0.20   | 0.8 $\pm$ 0.17      | 0.79 $\pm$ 0.17   |

or an inflammatory response developing after the procedure. However, the data regarding the relationship between inflammatory marker levels prior to PCI and after the follow-up period in human subjects have been somewhat controversial. Some reports revealed association between the hs-CRP concentration before the intervention and restenosis during the following 6–12 months,<sup>5</sup> whereas other studies failed to establish a relationship between ISR and preprocedural hs-CRP levels.<sup>18</sup> Hence, the aim of the present study was to investigate the serum hs-CRP level both before and 6 months after PCI, and to assess if an increase in the hs-CRP level during the follow-up period was more pronounced in patients with angiographically confirmed restenosis. The obtained results showed that the preprocedural hs-CRP level was not significantly different between patients with no stenosis and those developing ISR ( $p = 0.8117$ , Fig. 1). However, the increase in serum hs-CRP concentrations after 6-month follow-up was more pronounced ( $p = 0.029$ ) in patients who developed restenosis (from 9.08 $\pm$ 6.53 to 13.91 $\pm$ 5.53 mg L<sup>-1</sup>), compared to those without ISR (10.29 $\pm$ 0.29 to 12.27 $\pm$ 6.48 mg L<sup>-1</sup>, Fig. 1). It was previously demonstrated that stent deployment itself is associated with an increase in the hs-CRP level up to 48 h after the procedure, resulting in an increased ISR rate.<sup>19</sup> Elevated hs-CRP levels were also associated with increased rate of death or myocardial infarction 30 days after stenting, most likely due to the pro-thrombotic action of CRP.<sup>19</sup> The present results confirmed the previously published data that the post-intervention rise in inflammatory markers reflected the inflammatory response,<sup>18</sup> and indicated towards a link between raised post-intervention CRP levels and development of ISR. This rise in the hs-CRP level may be an indicator of the extent of inflammation in atheromatous lesions, and such plaques could be prone to the development of ISR. Moreover, CRP appeared to be involved in foam cell formation, promotion of monocyte chemotaxis and facilitation of LDL (low-density lipoprotein) uptake by macrophages *in*

*vitro*.<sup>20</sup> These findings suggest that CRP may play a causal role in vascular disease and therefore could be considered as a possible target of therapy.

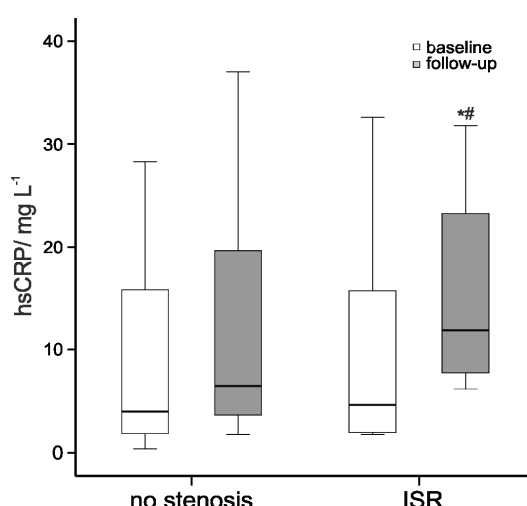


Fig. 1. The concentration of high-sensitivity CRP (hs-CRP) in the serum of patients that underwent stent implantation prior to PCI (baseline) and after 6-month follow-up. \* –  $p < 0.05$  compared to baseline enzyme activity, # –  $p < 0.05$ , compared to patients without stenosis at the same time point.

One of the events contributing the inflammatory response following PCI is leukocyte activation and infiltration (*e.g.*, monocyte, T-lymphocyte and granulocyte). Vascular injury induced by PCI may upregulate local cytokine expression leading to the release of pro-inflammatory factors. The cytokines released from activated macrophages, smooth muscle cells, lymphocytes and cells forming the vascular wall may stimulate neointimal formation.<sup>15</sup> Animal models showed that various cell components of the vascular wall express adhesion molecules, such as ICAM-1 or VCAM-1, for up to 30 days after balloon injury, which facilitates leukocyte interaction with the arterial wall.<sup>21</sup> Furthermore, inflammatory cytokines, such as tumor necrosis factor- $\alpha$  or interleukin-1, stimulate adhesion molecule expression, and these cytokines were shown to be upregulated at the arterial site after angioplasty.<sup>22</sup> However, results of studies that investigated the use of sICAM-1 as a biomarker of prognosis for cardiovascular disease are contradictory.<sup>23</sup> In this study, it was evaluated whether the levels of circulating adhesion molecule ICAM increased following the development of ISR. The results failed to confirm changes in sICAM-1 in the group of patients with ISR, compared to patients without stenosis (Table II). These results are in accordance with a previously published study that suggested a more dominant role for sVCAM-1, but not sICAM-1, in development of restenosis following PCI.<sup>24</sup>

The role of the transforming growth factor-beta (TGF- $\beta$ ) in restenosis has been studied for over two decades. TGF- $\beta$  is a family of cytokines with a variety of functions, including fibrosis, growth, differentiation and apoptosis. Both human and animal studies demonstrated that TGF- $\beta$  is upregulated at sites of vas-



cular injury. However, emerging data indicated that the role of TGF- $\beta$  in restenosis is complex, and could be attributed not only to TGF- $\beta$ -mediated vascular fibrosis, but to intimal thickening and arterial remodeling as important events in restenosis development.<sup>9</sup> In animal studies, systemic suppression of TGF- $\beta$  activity accelerated the development of atherosclerosis, but in humans, the plasma TGF- $\beta$  concentration was found to be negatively correlated with atherosclerosis.<sup>25</sup> In the present study, the plasma TGF- $\beta$  concentration did not differ in patients with ISR, in comparison with patients without stenosis, neither before the procedure nor after 6-month follow-up (Table II). Even though most studies are based on the rationale that TGF- $\beta$  promotes intimal hyperplasia, it appears that some components of the TGF- $\beta$  signaling cascade may favor outward or adaptive remodeling.<sup>9</sup> Therefore further studies are required to help delineate the multifunctional role and mechanisms of TGF- $\beta$  signaling in different restenotic lesions.

#### *Antioxidative enzyme activity in restenosis*

Previous studies on the mechanisms of restenosis post-balloon angioplasty showed increased oxidative stress and impaired redox processes as possible factors contributing to restenosis development.<sup>26</sup> Although the mechanisms participating in redox imbalance are not fully elucidated, it is certain that the increased oxidative stress is in close association with the inflammatory response that is observed following stent implantation. It was shown that the oxidative stress in the vascular wall developed immediately after the procedure, and persisted during all stages of ISR, including the stage of neointimal hyperplasia (proliferation and migration of smooth muscle cells and synthesis of an extracellular matrix), which is the leading mechanism of ISR development.<sup>26</sup> In addition, active forms of oxygen are known to modify the aggregation functions of platelets, thus also contributing to restenosis development.<sup>27</sup>

In contrast to the role of ROS in immune defense against microbial agents, ROS in vascular cells could be regarded as signaling molecules,<sup>4</sup> playing a role in activation and expression of pro-atherogenic genes.<sup>28</sup> Signal transmission *via* ROS occurs at free sulfhydryl groups of cysteine residues, which are present in many enzymes, including kinases, phosphatases, phospholipases, *etc.*, as well as transcriptional factors (*e.g.*, NF- $\kappa$ B). For instance, reactive oxygen species (ROS) are known to stimulate TGF- $\beta$ -induced gene expression *via* Smad-dependent and Smad-independent pathways.<sup>9</sup>

Bearing in mind the important role of oxidative stress in ISR development, the protective scavenging function of plasma enzymes seems crucial to prevent the deleterious action of ROS. The most important plasma enzymes participating in scavenging activity are extracellular superoxide dismutase (EC-SOD), a major component of antioxidative defense in blood vessels, and catalase.<sup>29</sup> EC-SOD



represents a major defense system against superoxide, being a target for oxidative damage as well. It also inhibits the reaction between superoxide and NO, maintaining endothelium-dependent vasodilatation.<sup>30</sup> Numerous studies provided evidence that decreased activity of antioxidant enzymes contributed to development of atherosclerotic lesions, but the data on EC-SOD activity in heart diseases were not consistent. It was shown that the levels of EC-SOD protein appearing in the plasma was reduced in subjects with coronary artery disease,<sup>31</sup> or history of myocardial infarction.<sup>32</sup> In addition, the activities of EC-SOD and catalase in blood of patients with myocardial infarction after reperfusion were shown to be significantly decreased compared to the controls.<sup>33</sup> It was also reported that local gene therapy with EC-SOD in atherosclerotic hyperlipidemic rabbits could inhibit ISR.<sup>29</sup> However, other studies failed to establish a difference in activities of EC-SOD and catalase between patients that developed in-stent restenosis, compared to those with CAD, but no stenotic lesions.<sup>33</sup>

In the present study, the serum EC-SOD activity was increased after 6-month follow up in patients without stenosis (from  $67.3 \pm 9.49$  to  $108.92 \pm 12.87$  U mL<sup>-1</sup>,  $p < 0.01$ ), as well as in patients with ISR ( $73.4 \pm 11.98$  to  $117.28 \pm 11.96$  U mL<sup>-1</sup>,  $p < 0.05$ ). However, no significant change in the rate of increase in EC-SOD activity was observed after 6-month follow-up between the two groups, as shown in Fig. 2 ( $p > 0.05$ ). Given the role of SOD in dismutation of superoxide anions, it is possible that the observed increase in its activity after follow-up was an adaptive phenomenon in response to increased O<sub>2</sub><sup>•-</sup> concentration that inevitably follows stent implantation. It is important to note that, in this study, only EC-SOD released into the plasma was determined, whereas the majority of enzyme remained attached to endothelial cell membrane glycoproteins. It is possible that, although there was increased EC-SOD release into plasma after follow-up in both

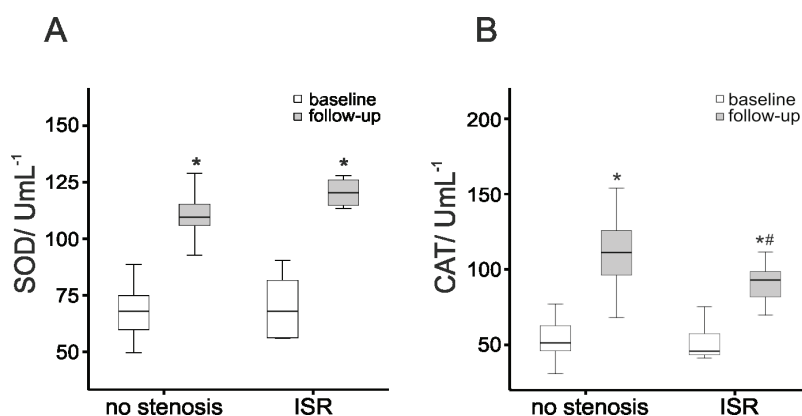


Fig. 2. SOD (A) and catalase (B) activities in the serum of patients that underwent stent implantation prior to PCI (baseline) and after 6-month follow-up. \* –  $p < 0.05$  compared to baseline enzyme activity, # – compared to patients without stenosis at the same time point.

groups, there will be a difference in overall amount of available enzyme between patients with ISR and patients without stenosis, as suggested previously.<sup>29</sup>

SOD dismutates  $O_2^{\bullet-}$  into  $H_2O_2$ , which, in turn, is scavenged by catalase. It was shown that CRP can directly induce ROS formation in endothelial progenitor cells (EPC) by inducing expression of MnSOD and down regulating glutathione peroxidase expression, leading to excessive production of  $H_2O_2$ , resulting in  $H_2O_2$ -induced EPC death.<sup>31</sup> In addition, increased  $H_2O_2$  production in patients with restenosis was reported.<sup>34</sup> The protective role of catalase against hydrogen peroxide-induced endothelial cell injury was documented *in vitro* and in animal models. Catalase overexpression protected the endothelium of human aorta against apoptosis caused by the oxidized forms of low-density lipoproteins (oxLDL),<sup>35</sup> whereas a 3-day catalase treatment *in vivo* decreased the blood pressure of spontaneously hypertensive wild-type mice and ameliorated the *ex vivo* function of the aorta endothelium.<sup>36</sup> In the present study, the increased EC-SOD activity during follow-up resulted in increased availability of the catalase substrate ( $H_2O_2$ ). The present results showed that the increase in catalase activity ( $X \pm SD$ ) was significantly lower in patients with ISR after 6-month follow-up – from  $51.61 \pm 12.84$  U L<sup>-1</sup> at the baseline to  $87.06 \pm 12.90$  U L<sup>-1</sup> after follow-up ( $p = 0.0036$ ). In patients without stenosis the changes were from  $54.07 \pm 12.66$  to  $119.25 \pm 29.86$  U L<sup>-1</sup> ( $p = 0.0001$ ) compared to subjects without stenosis, as shown in Fig. 2. The difference between the groups at follow-up was statistically significant ( $p = 0.0142$ ), despite the difference in the sample size. The data showed, for the first time, that a decrease in catalase protective activity might be one of the factors contributing to the development of ISR.

#### *Lp(a) and oxLDL in restenosis*

The role of lipoproteins as risk factors for the development of coronary restenosis after PCI was investigated in several studies. Lp(a) was independently associated with the presence of coronary atherosclerosis on angiography in the general population.<sup>13</sup> However, there are fewer data on the role of Lp(a) in patients with established CAD, implicating that Lp(a) might be less predictive of vascular risk in patients with established vascular disease.<sup>37</sup> Since it was shown that Lp(a) has a high degree of homology to plasminogen, thus inhibiting plasminogen activation,<sup>14</sup> the hypothesis was that patients with elevated Lp(a) concentrations in blood may have a greater tendency to thrombosis, after platelet activation initiated by arterial injury during stent implantation, leading to increased growth factor release and a propensity to coronary restenosis.

In the present study, the preprocedural level of Lp(a) was higher in patients that had developed ISR, but due to small sample number of patients included in the study, it did not reach the level of significance (Table III). Further studies are

needed to help clarify how relevant the Lp(a) concentration might be as a predictive factor for ISR.

TABLE III. Concentration of soluble intercellular cell adhesion molecule-1 (sICAM-1), and transforming growth factor-beta (TGF- $\beta$ ) in serum of patients that underwent stent implantation prior to PCI (baseline) and after 6-month follow-up; values are mean  $\pm$  SD

| Species                            | No stenosis        |                    | In-stent restenosis |                    |
|------------------------------------|--------------------|--------------------|---------------------|--------------------|
|                                    | Baseline           | Follow-up          | Baseline            | Follow-up          |
| sICAM-1 / ng mL <sup>-1</sup>      | 62.68 $\pm$ 12.49  | 62.02 $\pm$ 17.84  | 60.55 $\pm$ 18.54   | 59.91 $\pm$ 17.25  |
| TGF- $\beta$ / pg mL <sup>-1</sup> | 248.61 $\pm$ 24.39 | 244.47 $\pm$ 26.32 | 228.38 $\pm$ 52.26  | 246.13 $\pm$ 48.78 |

It was shown that oxLDL impairs endothelium relaxation by inhibition of the expression of eNOS,<sup>37</sup> reduces the responsiveness of smooth muscle cell to NO,<sup>71</sup> induces the expression of adhesion and inflammatory molecules.<sup>38</sup> All of the above contribute directly to dysfunction of the endothelium. OxLDL levels, auto-antibodies against epitopes of oxLDL and oxLDL:LDL ratio<sup>39</sup> were independently associated with increased risk for coronary atheromatosis and ischemic heart disease, with increased levels of oxLDL and MDA-LDL being related to plaque instability. However, other studies failed to find a significant association between oxLDL and clinical presentation of coronary artery disease or rate of complications in the first year after coronary stenting.<sup>40</sup> In the present study, the plasma concentration of oxLDL was increased after 6-month follow-up, with a trend of more pronounced increase in the group with ISR, but further study including larger number of patients with restenosis is required to assess whether this finding is relevant (Table IV).

TABLE IV. Concentration of oxLDL and Lp(a) in serum of patients that underwent stent implantation prior to PCI (baseline) and after 6-month follow-up; values are mean  $\pm$  SD

| Species                   | No stenosis        |                    | In-stent restenosis |                    |
|---------------------------|--------------------|--------------------|---------------------|--------------------|
|                           | Baseline           | Follow-up          | Baseline            | Follow-up          |
| oxLDL / U L <sup>-1</sup> | 169.10 $\pm$ 20.23 | 182.47 $\pm$ 17.02 | 166.19 $\pm$ 15.36  | 188.02 $\pm$ 14.22 |
| Lp(a) / U L <sup>-1</sup> | 194.28 $\pm$ 68.71 | 195.11 $\pm$ 52.64 | 209.55 $\pm$ 480.90 | 191.35 $\pm$ 33.13 |

Further investigation is required to determine the appropriate biochemical markers and the algorithm of their changes during follow-up will ameliorate diagnostic and prognostic assessment of patients undergoing PCI. Taking into account the fact that PCI has replaced more invasive surgical procedures for dealing with coronary stenosis, and that it has become the golden standard for treatment of acute myocardial infarction, follow-up of these patients and early detection of complications of myocardial ischemia using serum/plasma markers could contribute to better selection of patients for which the invasive procedure of follow-up angiography is indicated.

## CONCLUSIONS

Based on the results of this and other studies, it may be concluded that inflammatory processes and oxidative stress play important roles in the formation of in-stent restenosis after coronary stent implantation. It is proposed that the serum hs-CRP level and catalase activity may be useful for monitoring and planning management of patients during follow-up after stent implantation, but warrants confirmation by larger, well-designed prospective and randomized studies.

## STUDY LIMITATIONS

The main limitation of this study is the relatively small number of patients, as only patients that fulfilled the inclusion criteria and who were subjected to follow-up angiography were included in the study.

## LIST OF ABBREVIATIONS

PCI – percutaneous coronary intervention  
ISR – in-stent restenosis  
CAD – coronary artery disease  
hs-CRP – high sensitive C-reactive protein  
sICAM-1 – soluble intercellular cell adhesion molecule-1  
sVCAM-1 – soluble vascular cell adhesion molecule-1  
TGF- $\beta$  – transforming growth factor-beta  
Lp(a) – lipoprotein(a)  
oxLDL – oxidized low-density lipoprotein  
SOD – superoxide dismutase  
CAT – catalase  
ROS – reactive oxygen species  
EPC – endothelial progenitor cells  
LDL-C – low-density lipoprotein cholesterol  
HDL-C – high-density lipoprotein cholesterol  
VLDL – very low-density lipoprotein  
LDL – low-density lipoprotein  
HDL – high-density lipoprotein  
AST – aspartate transaminase  
NF- $\kappa$ B – nuclear factor kappa  
CK-MB – creatine kinase-MB  
eNOS – endothelial NO synthase  
MDA – malondialdehyde

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## ИЗВОД

## ПОКАЗАТЕЉИ ЗАПАЉЕЊА И АКТИВНОСТ ЕНЗИМА АНТИОКСИДАТИВНЕ ЗАШТИТЕ КОД РЕСТЕНОЗЕ НАКОН ПЕРКУТАНЕ КОРОНАРНЕ ИНТЕРВЕНЦИЈЕ

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Успешност перкутане коронарне интервенције је често угрожена потребом да се понови ревакуларизација услед појаве стенозе. Иако је, с тим у вези, у бројним истраживањима процењиван предиктивни значај различитих биохемијских показатеља, нису добијени конзистентни резултати. Циљ овог истраживања је био да се процени прогностички значај појединих маркера запаљења, као и активност ензима антиоксидативне заштите, у развоју рестенозе након уградње стента. Концентрације С-реактивног протеина (hs-CRP), солубилног међућелијског адхезивног молекула-1 (sICAM-1), фактора трансформације раста бета (TGF- $\beta$ ), липопротеина(а) (Lp(a)) и оксидованог липопротеина мале густине (oxLDL), као и активност супероксид-дисмутазе (SOD) и каталазе (CAT) у серуму одређиване су код 44 пацијента пре уградње стента, као и након шестомесечног периода праћења. Резултати након периода праћења указали су на значајан пораст концентрације hs-CRP-а у серуму код пацијената са ангиографски потврђеном поновном стенозом, у односу на пацијенте без стенозе. Уградња стента је довела до пораста активности SOD и CAT у серуму, али је након шестомесечног праћења активност CAT била значајно нижа код пацијената са стенозом, што би могао бити један од чинилаца који доприноси њеном развоју. Није уочена разлика у концентрацији sICAM-1, TGF- $\beta$ , oxLDL и Lp(a) између испитиваних група. У закључку, концентрација hs-CRP и активност CAT у серуму би се могли у будућности размотрати као корисни показатељи праћења пацијената након уградње стента у циљу благовременог уочавања претеће стенозе.

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