

# ANTI-OXIDIZED LDL AUTOANTIBODY ASSESSMENT AS MARKERS OF THE IMMUNE RESPONSE IN ARTERIOSCLEROSIS

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## REZUMAT

**Obiective:** Acest studiu a avut ca scop verificarea ipotezei potrivit căreia marker-ul imun al oxidării lipoproteinelor cu densitate mică (oxLDL) - autoanticorpilor anti-oxLDL (oLAB) reflectă sindroamele coronariene acute.

**Material și metode:** Probele au fost recoltate de la pacienți cu infarct miocardic acut (IMA), angină instabilă (AIS), angină stabilă (AS) și de la subiecți sănătoși. Pentru măsurarea nivelurilor serice ale autoanticorpilor anti-oxLDL a fost utilizată tehnica ELISA, iar pentru profilul lipidic teste enzimatic colorimetrice.

**Rezultate:** Concentrația de oLAB a fost crescută la grupul IMA ( $p < 0,05$ ) și AIS ( $p < 0,05$ ) comparativ cu lotul martor.

**Concluzii:** Acest studiu sugerează faptul că sistemul imun joacă un important rol în ateroscleroză și contribuie la disfuncția endotelială și la destabilizarea plăcii aterosclerotice.

**Cuvinte cheie:** ateroscleroza, oxLDL, autoanticorpi

## ABSTRACT

**Objectives:** This study was conducted to test the hypothesis that the immune marker of oxidized low-density lipoprotein (oxLDL) - anti-oxidized LDL autoantibody (oLAB) reflects acute coronary syndromes

**Material and Methods:** Serial samples were obtained from patients with acute myocardial infarction (AMI), unstable angina (UA), stable angina (SA) and from healthy subject. ELISA assay was used to quantitate serum levels of autoantibodies against oxLDL (oLAB) and enzymatic colorimetric tests for lipid profile.

**Results:** Titers of oLAB were higher in the AMI group ( $p < 0.05$ ) and UA group ( $p < 0.05$ ).

**Conclusion:** This study suggests that the immune system plays an important role in atherosclerosis and contributes to endothelial dysfunction and plaque destabilization.

**Key Words:** atherosclerosis, oxLDL, autoantibodies

## INTRODUCTION

Cardiovascular diseases constitute the primary cause of mortality in the modern society and are due to an extensive atheromatosis of the coronary vessels.<sup>1-3</sup>

The pathogenesis of the arteriosclerosis includes complex interactions among the arterial wall cells (endothelial cells, macrophages, smooth muscle cells) and plasma lipoproteins, mainly the low-density lipoproteins (LDL) and high-density lipoproteins (HDL).<sup>4,5</sup> According to the actual predominant data, the formation of atheroma

commences with the adhesion of blood monocytes to the vessel endothelium, which is induced by oxidized forms of LDL (oxLDL) and their penetration into the subendothelial space.<sup>4-6</sup> At this level the monocytes differentiate in macrophages, take up large quantities of cholesterol and are transformed into foam cells.<sup>7,8</sup>

Foam cells present receptors, which differ from the standard LDL receptors and recognize and bind modified forms of LDL and mainly oxLDL.<sup>3,5,9,10</sup>

LDL oxidation is a complex procedure. During this process both the lipid and the protein content of LDL are subject to chemical modifications, which depend on the locally produced free radicals.<sup>1,8,11,12</sup>

One of the most important changes noted during LDL oxidation is the peroxidation of polyunsaturated fatty acids. This peroxidation results in the formation of oxidized phospholipids.<sup>13-15,18</sup> oxLDL represents an antigen with high immunogenicity and thus the immune system produces anti-oxLDL autoantibodies (oLAB).<sup>11,12,16-18</sup>

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Received for publication: Nov. 11, 2003. Revised: Jul. 14, 2004

oLAB were detected and described in various diseases<sup>1,6,11</sup> and although their clinical significance was demonstrated only in carotidian and coronary arteriosclerosis<sup>1,18,20</sup>, it was suggested that they should represent a biological marker of the lipidic peroxidation.<sup>1,16,18-20</sup>

The purpose of the present study consists in realizing a lipid profile and the seric oLAB level of patients with cardiovascular diseases, acute myocardial infarction (AMI), unstable angina (UA), stable angina (SA), and to demonstrate the role of the immune system in the progression of arteriosclerosis.

## MATERIAL AND METHODS

The study represents the interdisciplinary collaboration between the Department of Biochemistry, Immunology and Cardiology I and was performed between October 2002-June 2003.

### Patient selection

The study was performed on two groups.

- The first group, formed of 40 patients (32 males and 8 females) with an average age of 65 years and 68.9 years, respectively. The patients were diagnosed in the Cardiology Centre and also in the Coronary Unit of the County Clinical Hospital no.1 Timisoara: 24 patients (60%) with AMI, 6 patients (15%) with UA and 10 patients (25%) with SA.

Patients with history of inflammatory and autoimmune organic or systemic disease were excluded from this study.

- The second group was formed of 17 healthy subjects with an average age of 60.4 years, who did not suffer acute or chronic diseases and did not present a history of cardiovascular diseases.

### Collection and preserving the blood samples

Every subject from the two groups gave samples of venous blood. The serum samples obtained were used for the assessment of the lipid profile, using the enzymatic colorimetric test and also to determine the oLAB titer from the immunoenzymatic ELISA method.

The sera were kept in the freezers at -20°C until testing, using duplicate samples.

### Immunoenzymatic determination (ELISA)

For measuring the oLAB concentration in the serum of the subjects within the study were utilized immunoenzymatic sandwich techniques with Cu<sup>2+</sup> oxLDL as antigen. The ELISA BIOMEDICA AUSTRIA kit was used to determine oLAB (type Ig G specific) with a variation coefficient (VC) = 4.3 .

### Colorimetric enzymatic determination

For the determination of the lipid profile in the serum of the studied subjects the enzymatic colorimetric methods were utilized, using the BIOCON GERMANY kits for the determination of total serum cholesterol, triglyceride and the cholesterol in fractions HDL and LDL.

### Statistic data processing

The values obtained (including titer oLAB) were expressed in the form of medium  $\pm$  standard deviation values. The groups were compared using the t-Student test considering the minimum of the statistic significance 0.05 (p<0.05).

## RESULTS

Tables 1a,b,c show the lipid profile of the study groups and the statistic interpretation obtained by comparing the groups of patients with the witness group.

**Table 1a.** Lipid profile for AMI group compared to the control group

	Total Cholesterol (mg%)	Triglycerides (mg%)	HDL-Cholesterol (mg%)	LDL-Cholesterol (mg%)
Healthy subjects group (M)	160.20 $\pm$ 18.40	88.80 $\pm$ 28.40	70.52 $\pm$ 16.50	79.51 $\pm$ 18.60
AMI	227.30 $\pm$ 97.17	198.78 $\pm$ 61.24	27.43 $\pm$ 14.16	165.40 $\pm$ 64.55
Statistical significance	p > 0.05 (nonsignificant)	p < 0.0001 (very significant)	p < 0.0001 (very significant)	p < 0.001 (very significant)

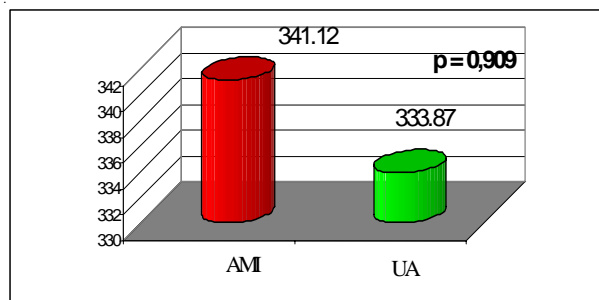
**Table 1b.** Lipid profile for UA lot compared to the control group

	Total Cholesterol (mg%)	Triglycerides (mg%)	HDL-Cholesterol (mg%)	LDL-Cholesterol (mg%)
Healthy subjects group (M)	160.20 $\pm$ 18.40	88.80 $\pm$ 28.40	70.52 $\pm$ 16.50	79.51 $\pm$ 18.60
UA	205.85 $\pm$ 85.85	231.33 $\pm$ 149.68	33.16 $\pm$ 13.47	148.97 $\pm$ 35.86
Statistical significance	p > 0.05 (nonsignificant)	p < 0.05 (significant)	p < 0.001 (very significant)	p < 0.05 (significant)

**Table 1c.** Lipidic profile for SA lot compared to the control group

	Total Cholesterol (mg%)	Triglycerides (mg%)	HDL-Cholesterol (mg%)	LDL-Cholesterol (mg%)
Healthy subjects group (M)	160.20±18.40	88.80±28.40	70.52±16.50	79.51±18.60
SA	196.19±74.73	247.29±126.32	22.8±9.02	125.61±23.24
Statistical significance	p > 0.05 (nonsignificant)	p < 0.01 (very significant)	p < 0.001 (very significant)	p < 0.01 (very significant)

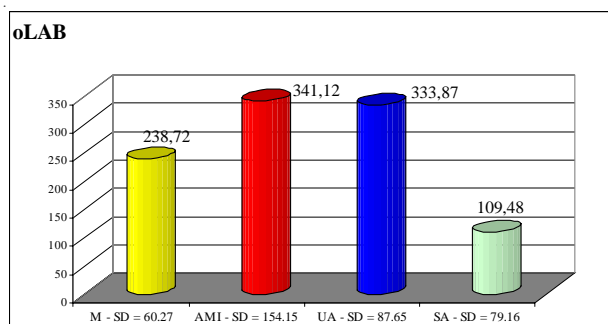
Concentration of oLAB in the group with AMI is 341.12±154.15, in the group with UA is 333.87±87.65 and in the group with SA 109.48±79.16 compared with the oLAB values of the healthy subjects group 238.72±60.27. Table 2 displays the levels of oLAB for all the four groups studied, together with the statistical significance, while Figure 1 shows the titers of oLAB in the group of patients compared to the healthy subjects group, expressed in mU/ml.



**Figure 3.** Titers of oLAB in AMI and UA groups

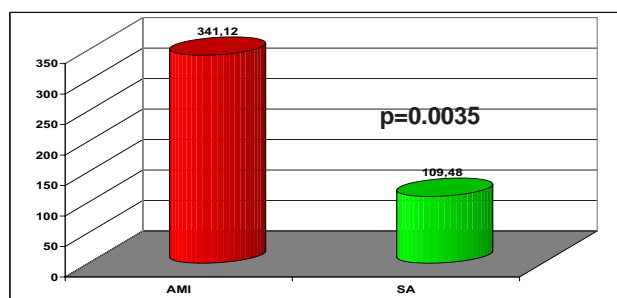
**Table 2.** oLAB titers of the studied groups

	M	AMI	UA	SA
oLAB (mU/ml)	238.72±60.27	341.12±154.15	333.87±87.65	109.48±79.16
Statistical significance		p < 0.05 (significant)	p < 0.05 (significant)	p < 0.05 (significant)



**Figure 1.** Titers of oLAB in the studied groups, expressed in medium values in mU/ml

Using as reference the group of patients with AMI and compared to the group with UA and SA we observe significantly increased values of the titers of oLAB in the group with AMI compared to the group with SA (p<0.01) (Fig.2) and the difference non significantly compared to the group UA (p>0.05) (Fig.3).



**Figure 2.** Titers of oLAB in AMI and SA groups

## DISCUSSIONS

Analyzing the results of the lipid profile and the oLAB we found that patients present higher serum triglycerides (p<0.05) and LDL fractions (p<0.05), compared to the healthy subjects group. The total serum cholesterol values did not differ significantly between the the studied groups. The HDL fractions were lower in the group of patients (p<0.05) compared to the healthy subjects.

Titers of autoantibodies against oxLDL were present in both patients and healthy subjects group. The highest values of the oLAB serum concentration belong to the patients with AMI and UA (p<0.05), between the two not existing a significant statistical difference. The lowest values of the oLAB serum concentration belong to the patients with SA, and a significant statistical difference was noted between the patients with AMI (p<0.01) and the healthy subjects group (p<0.05).

Several recent studies have shown the presence of autoantibodies to oxLDL in patients with coronary artery disease (AMI, UA, SA). In these studies, patients have shown higher autoantibody titers to oxLDL compared to normal subjects (confirming partially the results of our study), a finding that indicates that these titers may have a diagnostic and/or prognostic value.<sup>1,6,7,11,14-16</sup> However, there are studies according to

which no differences have been observed in the titers of the autoantibodies, between patients and healthy controls.<sup>17,20</sup> The causes for this inconsistency in the results of various clinical studies are not known.<sup>16</sup>

In this study, the titer of the autoantibodies against oxLDL can differentiate the patients with AMI and UA from the patients with SA and from the healthy witness subjects.

## **CONCLUSIONS**

The results of our study demonstrated the elevated oLAB serum concentration of the patients with AMI and UA compared to the patients with SA and the healthy subjects in correlation with the lipidic profile.

The reason for this increase would be due to the fact that the immune system is stimulated by the increased concentration of oxLDL and oxidized epitopes specifically released from the broken atherosclerotic plaque, while in the cases of AMI a secondary stimulation pathway is added, by the release of apoptotic myocytes which contain oxidized phospholipids.

The implication of lipid peroxides in the atherosclerotic pathogenesis is generally accepted. Numerous publications show pathologic implications of oLAB in the cardio-vascular diseases,<sup>1,6-8,10,11,16,20</sup> finding high levels of oLAB predictive for the progressing arteriosclerosis, but still it is not demonstrated if oLAB can serve as an useful instrument in the prediction of cardiac risk.

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