

## ANTI-OXLDL ANTIBODIES, HOMOCYSTEINE AND APOLIPOPROTEINS IN PRIMARY ANTIPHOSPHOLIPID SYNDROME

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**Summary:** The aim of this study was to investigate the influence of: autoantibodies (anticardiolipin, anti-oxLDL, anti-β<sub>2</sub>gpl, lupus anticoagulant), apolipoproteins (apo) AI and apoB, and homocysteine on the clinical features of patients with primary antiphospholipid syndrome, and to compare analyzed parameters with those from control subjects. Patients who were positive for anti-oxLDL antibodies had venous thrombosis associated with lower levels of apolipoprotein AI ( $p < 0.05$ ). Patients with a history of thrombosis of peripheral arterial blood vessels had higher concentrations of apo B ( $p < 0.05$ ) and this finding was also present in patients with a history of cerebrovascular insults ( $p < 0.05$ ). Patients with myocardial infarction had significantly higher concentrations of homocysteine ( $p < 0.05$ ). In patients with PAPS, testing the above-mentioned parameters is justified.

**Key words:** primary antiphospholipid syndrome, atherothrombosis, anti-oxLDL antibodies, homocysteine, apolipoproteins

### Introduction

The antiphospholipid syndrome (APS) may be associated with another autoimmune disease (secondary APS = SAPS), or unrelated to an underlying disease (primary APS = PAPS). The antiphospholipid syndrome is an acquired thrombotic disorder characterized by venous and arterial thromboses and/or miscarriages and repeated detection of antiphospholipid antibodies (1).

The term »antiphospholipid antibodies« traditionally includes anticardiolipin antibodies (aCL) and lupus anticoagulant (LA). The modern meaning of antiphospholipid antibodies implies large and various groups of autoantibodies which bind to different antigens (oxidized LDL, apolipoprotein AI, etc.) (2).

Antibodies against oxidized low density lipoproteins (anti-oxLDL antibodies) are involved in the de-

velopment of atherosclerosis in animal models. In humans, it is still not clear whether *in vivo* immune response to oxLDL is proatherogen or antiatherogen (3).

Lipids may through their effects on the coagulation and fibrinolytic system contribute to the development of venous thrombosis, and this association has been investigated in a few studies with conflicting results (4). Hyperlipidemia represents a fundamental risk factor for coronary heart disease. However, nearly half of all myocardial infarctions occur in individuals with no evidence of increased low density lipoprotein (LDL) cholesterol. Thus, clinical research has focused on the development of novel biomarkers for coronary heart disease that might detect patients prone to thrombosis. Some of them are apolipoproteins AI, B and homocysteine (5). Decreased apolipoprotein AI (apo AI) levels and increased apolipoprotein B (apo B) levels are risk factors for the development of atherosclerosis.

Elevated homocysteine (Hcy) concentrations are associated with thrombosis and with an increased risk of atherothrombotic vascular diseases, but this association has not yet been proven to be causal in randomized controlled trials (6).

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Since the thrombotic tendency of antiphospholipid syndrome shares several pathways with atherosclerosis (7), the aim of this study was to investigate the association of anti-oxLDL antibodies, Hcy, apo AI, apo B with clinical and serological features of patients with primary antiphospholipid syndrome. Furthermore, we compared analyzed parameters in patients and in control subjects.

## Patients and Methods

### Analyzed subjects

All investigations in this study were routinely conducted in the laboratories of the Clinical Center of Serbia. This study included 33 consecutive patients (24 women and 9 men) with PAPS, according to Sapporo criteria (8). The mean age of analyzed patients was  $41 \pm 14$ , and ages ranged from 21 to 78 years. Also, the study included 28 blood donors (21 women and 7 men) which were predominantly laboratory personnel (including the authors of this article). Exclusion criteria for control subjects were the presence of acute or chronic diseases and taking medications which could in any way affect the analyzed parameters. The mean age of control subjects was  $37 \pm 12$  and ages ranged from 18 to 72 years.

### Methods

After overnight fasting (12 hours) and after 24 hours without intensive physical activity, patients were resting immediately prior to venepuncture. The use of tourniquet was never longer than three minutes. Blood samples for serum and plasma were collected from the antecubital vein, and then centrifuged for 10 minutes at 3 000 rotations per minute. For the purpose of avoiding falsely positive higher levels of homocysteine, serum was separated from coagulum in 45 minutes.

Homocysteine concentrations were determined by HPLC (high pressure liquid chromatography) method, using commercial reagents from BIORAD, Munich, Germany, and fluorescent detector Hewlett-Packard 1046A.

Antibody levels were estimated by ELISA in patient sera using commercial reagents from Imtec Immunodiagnostika, Berlin, Germany, for the detection of anti-oxLDL antibodies (synchronous detection of the IgG and the IgM isotypes), and for the detection of anti- $\beta$ 2gpl antibodies of the IgG isotype and the IgM isotype. Anticardiolipin antibodies of the IgG isotype and the IgM isotype were estimated by ELISA using commercial kits from Varelisa Pharmacia Diagnostics, Freiburg, Germany.

The presence of lupus anticoagulant was detected according to the recommendations from the

Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society of Thrombosis and Haemostasis (9).

Apolipoproteins AI and B were estimated by immunonephelometry using the commercial kits of DADE Behring with the Behring Nephelometer Analyzer II, Behring Diagnostics GmbH, Germany.

### Statistical analysis

Mean and standard deviation (SD) were calculated for all analyzed parameters (except for lupus anticoagulant). Comparison between analyzed patients and control subjects was done with Student's t-test or, when appropriate, Mann-Whitney test. Categorical variables were compared by  $\chi^2$  test. In all above-mentioned tests,  $p < 0.05$  was considered statistically significant. Statistical analysis was done using the SPSS 10 program (SPSS Inc, Chicago, IL, USA).

## Results

### Clinical features of analyzed patients

Arterial thrombosis was present in 45.45% (15/33) of patients, and venous thrombosis in 39.39% (13/33) of investigated patients. The most frequent clinical findings were cerebrovascular insults, present in 48.48% (16/33) of patients. Twenty five percent (6/24) of women had recurrent miscarriages, while 21.21% (7/33) of patients had pulmonary embolism. Myocardial infarctions and thromboses of small blood vessels were equally present in 18.18% (6/33) of investigated patients.

### Serological features of investigated subjects

Table 1 shows mean values and standard deviations of analyzed parameters in the investigated subjects.

Lupus anticoagulant was present in 89.3% (25/28) of patients, while anti-oxLDL antibodies were present in 75.8% (25/33) of investigated patients. Anticardiolipin antibodies of the IgG isotype were present in 57.6% (19/33) of patients, while IgM isotype was present in 60.6% (20/33) of analyzed patients. Anti- $\beta$ 2gpl antibodies of the IgG isotype were present in 51.5% (17/33) of patients, while IgM isotype was present in 33.3% (11/33) of analyzed patients.

### Association of investigated parameters with clinical and serological features of analyzed patients

Lower apolipoprotein AI levels and positive finding of anti-oxLDL antibodies were present in patients with a history of venous thrombosis ( $\chi^2 = 3.86$ ,  $p < 0.05$ ), which is shown in Figure 1.

Table I Mean values and standard deviations (SD) for investigated parameters in analyzed subjects

Investigated parameters	Patients (mean ± SD)	Control subjects (mean ± SD)
ApoA1 (g/L)	1.51 ± 0.21	1.46 ± 0.32
ApoB (g/L)	0.94 ± 0.28	0.83 ± 0.22
Homocysteine (μmol/L)	10.86 ± 3.69	9.39 ± 2.01
Anti-oxLDL (U/mL)	57.34 ± 44.15***	19.63 ± 5.69
aCL IgG (GPLU)	40.23 ± 36.14***	3.12 ± 1.90
aCL IgM (MPLU)	41.99 ± 43.39***	2.55 ± 1.52
Anti-β2gpI IgG (U/mL)	32.57 ± 37.70***	3.13 ± 1.82
Anti-β2gpI IgM (U/mL)	20.63 ± 35.41***	1.97 ± 1.19

GPLU (cardiolipin binding activity of 1 μg/mL an affinity-purified IgG anticardiolipin antibody preparation)  
MPLU (cardiolipin binding activity of 1 μg/mL an affinity-purified IgM anticardiolipin antibody preparation)  
\*(p < 0.05), \*\*\*(p < 0.01), \*\*\* (p < 0.001)

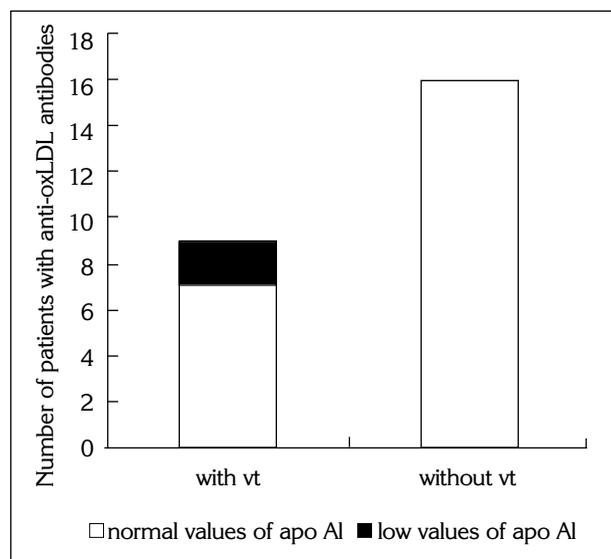


Figure 1 The presence of decreased apo AI values and positive finding of anti-oxLDL antibodies in patients with history of venous thrombosis (vt) ( $\chi^2 = 3.86, p < 0.05$ ).

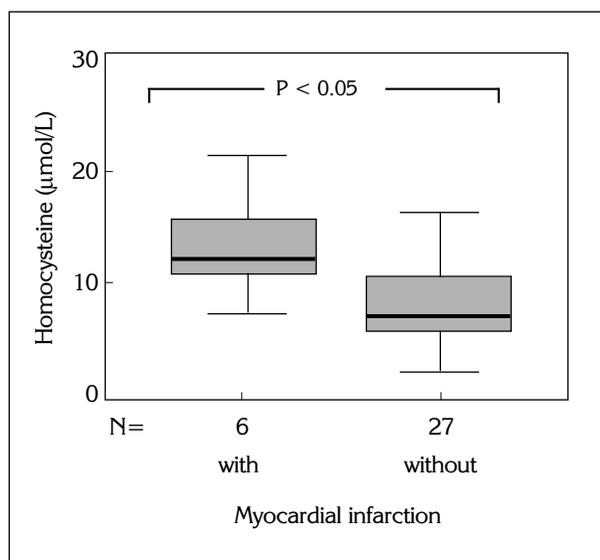


Figure 2 The association between increased homocysteine concentrations and a history of myocardial infarction in analyzed patients (t-test,  $p < 0.05$ ).

Elevated homocysteine levels and positive findings of anti-oxLDL antibodies were present in patients with pulmonary emboli ( $\chi^2 = 12.35, p < 0.01$ ).

In patients older than 40 years, elevated homocysteine levels were associated with a history of venous thrombosis ( $\chi^2 = 4.61, p < 0.05$ ). Homocysteine concentrations were increased in patients with a history of myocardial infarction, in comparison to patients with no history of myocardial infarction (t-test,  $p < 0.05$ ), which is shown in *Figure 2*.

Elevated apo B levels were present in patients with a history of thrombosis of peripheral arterial

blood vessels in, comparison to patients without the above-mentioned clinical finding (t-test,  $p < 0.05$ ).

Elevated apo B concentrations were present in patients with a history of cerebrovascular insults, in comparison to patients without this clinical finding (t-test,  $p < 0.05$ ).

In patients with positive findings of anti-oxLDL antibodies, the presence of thrombosis of small blood vessels was associated with the presence of anti-β2gpI antibodies of the IgM isotype ( $\chi^2 = 5.25, p < 0.05$ ). Concentrations of anticardiolipin antibodies of the IgM isotype were associated with a history of recurrent miscarriages ( $p < 0.05$ ).

The presence of lupus anticoagulant was associated with a history of arterial thrombosis ( $\chi^2 = 4.48$ ,  $p < 0.05$ ) and with cerebrovascular insults ( $\chi^2 = 3.87$ ,  $p < 0.05$ ). Positive findings of lupus anticoagulant and anticardiolipin antibodies of the IgM isotype were associated with arterial thrombosis ( $\chi^2 = 4.17$ ,  $p < 0.05$ ).

## Discussion

Patients with antiphospholipid syndrome have increased risk for atherosclerosis and cardiovascular complications (10). The way in which antiphospholipid antibodies participate in the pathogenesis of endocardiac lesion is not clear yet (1). Some studies indicated that antibodies against cardiolipin and oxLDL are in positive correlation with the rate of progression of atherosclerotic plaques in patients with atherosclerosis of the carotid artery, that above-mentioned antibodies are independent risk factors for subsequent myocardial infarction (11) and that anti-oxLDL antibodies could be the marker for arterial thrombosis in the antiphospholipid syndrome (12). Stauffenberger et al. (13) used an enzyme immunoassay for the measurement of homocysteine, and they found significant correlation between homocysteine levels and the rate of stenosis of coronary arteries in women. In our study, homocysteine concentrations were determined by HPLC method, and we found that myocardial infarctions were associated with hyperhomocysteinemia, but no association between the presence of anti-oxLDL antibodies and the history of myocardial infarctions was shown.

Around one third of venous thromboses are complicated by pulmonary embolism (1). Avivi et al. (14) concluded that hyperhomocysteinemia was associated with thromboembolic complications in patients with PAPS. We found that hyperhomocysteinemia was associated with the presence of venous thrombosis in patients older than 40 years. Also, hyperhomocysteinemia was associated with a history

of pulmonary embolism in patients with positive finding of anti-oxLDL antibodies. Interestingly, we found that decreased apo AI levels and positive finding of anti-oxLDL antibodies were present in patients with a history of venous thrombosis.

Fialová et al. (15) showed that pregnant women with a history of recurrent miscarriages had increased anti-oxLDL antibodies levels. Some studies indicated that hyperhomocysteinemia is a risk factor for recurrent miscarriages and thromboses, but according to Lee et al. (16) abnormal metabolism of homocysteine does not play a significant role in the pathogenesis of APS because no difference regarding concentrations of homocysteine between female patients with APS and control subjects was found. In our study, elevated levels of anticardiolipin antibodies of the IgM isotype were present in patients with recurrent miscarriages, but no association with the presence of anti-oxLDL antibodies was discovered. Also, we did not find any difference in mean concentrations of homocysteine between female patients and control subjects.

Eighty percent of patients with APS have either recurrent venous thrombosis or recurrent arterial thrombosis, whereas only 20% have both arterial and venous thrombosis. The period between the initial occurrence and the recurrence may last several days or several years (1). Considering the fact that thrombosis is the main complication in atherosclerosis the process known as atherothrombosis whose main clinical manifestations are coronary heart disease (myocardial infarctions and angina), peripheral arterial disease, cerebral ischemia (17) according to our results, in patients with PAPS the testing of anti-oxLDL antibodies, homocysteine, apolipoproteins AI and B (18) is justified.

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## ANTI-OXLDL ANTITELA, HOMOCISTEIN I APOLIPOPROTEINI U PRIMARNOM ANTIFOSFOLIPIDNOM SINDROMU

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*Kratak sadržaj:* Cilj ove studije je bio istraživanje uticaja: autoantitela (antikardiolipinska, anti-oxLDL, anti-β2gpl, lupus antikoagulans), apolipoproteina (apo) AI i apoB i homocisteina na kliničke odlike pacijenata sa primarnim antifosfolipidnim sindromom, kao i poređenje sa analiziranim parametrima u kontrolnih subjekata. Pacijenti koji su imali pozitivan nalaz anti-oxLDL antitela, imali su venske tromboze povezane sa sniženim nivoom apo AI ( $p < 0,05$ ). Pacijenti sa istorijom tromboza perifernih arterijskih krvnih sudova su imali povišene koncentracije apoB ( $p < 0,05$ ) i ovaj nalaz je bio prisutan i u pacijenata sa cerebrovaskularnim insultima ( $p < 0,05$ ). Pacijenti sa infarktima miokarda su imali značajno povišene koncentracije homocisteina ( $p < 0,05$ ). U pacijenata sa PAPS je opravdano testirati navedene parametre.

*Ključne reči:* primarni antifosfolipidni sindrom, aterotromboza, anti-oxLDL antitela, homocistein, apolipoproteini

### References

- Gromnica-Ihle E, Schösl W. Antiphospholipid syndrome. *Int Arch Allergy Immunol* 2000; 123: 67–76.
- Mackworth-Young CG. Antiphospholipid syndrome: multiple mechanisms. *Clin Exp Immunol* 2004; 136: 393.
- Opes-Virella M. Lipoprotein autoantibodies: measurement and significance. *Clin Diagn Lab Immunol* 2003; 10 (4) 499–505.
- Doggen CJM, Smith NL, Lemaitre RN, Heckbert SR, Rosendal FR, Psaty BM. Serum lipid levels and the risk of venous thrombosis. *Arterioscler Thromb Vasc Biol* 2004; 24 (10): 1970–5.
- Ridker PM. Beyond cholesterol: C-reactive protein and homocysteine as predictors of cardiovascular risk. In: *Handbook of lipoprotein testing*. Rifai N, Warnick GR, Dominiczak MH (ed). AACCC Press 2002; 1–29.
- Hankey GJ, Eikelboom JW, Ho WK, Van Bockxmeer FM. Clinical usefulness of plasma homocysteine in vascular disease. *MJA* 2004; 181: 314–8.
- Ames PRJ. Medical Perspective. Antiphospholipid antibodies, thrombosis and atherosclerosis in systemic lupus erythematosus. A unifying »membrane stress syndrome« hypothesis. *Lupus* 1994; 3: 371–7.
- Wilson WA, Gharavi AE, Koike T, et al. International statement on preliminary classification criteria for definite antiphospholipid syndrome. Report of an International Workshop. *Arthritis Rheum* 1999; 42 (7): 1309–11.
- Brandt JT, Triplett DA, Alving B, Scharer J. Criteria for the Diagnosis of Lupus Anticoagulans: An Update on behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society of Thrombosis and Haemostasis. *Thrombosis Haemostasis* 1995; 74 (4): 1185–90.
- Nicolo D, Monestier M. Antiphospholipid antibodies and atherosclerosis. *Clin Immunol* 2004; 112: 183–9.
- Lefvert AK. Heterogeneity of autoantibodies against cardiolipin and oxidatively modified low density lipoproteins revealed by human monoclonal antibodies. *J Intern Med* 2000; 247: 385–90.
- Bećarević M, Andrejević S, Bonači-Nikolić B, Obradović I, Miljić P, Majkić-Singh N. Anti-oxLDL antibodies – marker for arterial thrombosis in antiphospholipid syndrome? *Clin Lab* 2005; 51 (5+6): 279–83.
- Stauffenberger MT, Lange RA, Hillis LD, Cigarroa J, Hsu RM, Devaraj S, Jialal I. Hyperhomocysteinemia measured by immunoassay: a valid measure of coronary artery atherosclerosis. *Arch Pathol Lab Med* 2004; 128: 1236–6.
- Avivi J, Lanir N, Hoffman R, Brenner B. Hyperhomocysteinemia is common in patients with antiphospholipid syndrome and contributes to the expression of major thrombotic events. *Blood Coagul Fibrinolysis* 2002; 13 (2): 169–72.
- Fialová L, Mikulíková L, Malbohan J, Benešová O, Štípek S, Zima T, Zwinger A. Antibodies against oxidized low density lipoproteins in pregnant women. *Physiol Res* 2002; 51: 355–61.
- Lee RM, Brown MA, Ward K, Nelson L, Branch DW, Silver RM. Homocysteine levels in women with antiphospholipid syndrome and normal fertile controls. *J Reprod Immunol* 2004; 63 (1): 23–30.

17. Leys D. Atherothrombosis: a major health burden. *Cerebrovasc Dis* 2001; 1 Suppl 2: 1–4.
18. Bećarević M. Clinical significance of the determination of potential biomarkers for atherothrombosis in primary

antiphospholipid syndrome. Thesis. Belgrade, Serbia and Montenegro: Faculty of Pharmacy, University of Belgrade 2005.

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