Serum lipids and anti-oxidized LDL antibodies in primary antiphospholipid syndrome

M. Bećarević[1], S. Andrejević[2], P. Miljić[3], B. Bonači-Nikolić[2], N. Majkić-Singh[1]

[1]Institute for Medical Biochemistry, [2]Institute for Allergology and Clinical Immunology, [3]Institute for Haematology, Clinical Centre of Serbia, Belgrade, Serbia.

Abstract

Objective

The link between specific antibodies and atherogenesis in primary antiphospholipid syndrome (PAPS) is less strong than for thrombosis, although clearly the two processes are related and thrombosis is the main complication of atherosclerosis, a process known as atherothrombosis. The aim of this study was to investigate the influence of serum lipid levels and anti-oxidized LDL (oxLDL) antibodies on the clinical features of 42 patients with PAPS (mean age 40.45 ± 13.37; 32 women and 10 men), and to compare them with 47 control subjects (mean age 39.68 ± 13.93; 33 women and 14 men).

Methods

Total cholesterol, HDL and triglyceride concentrations were determined by enzymatic methods. LDL was calculated according to the Friedwald formula. Anticardiolipin, anti-oxidized LDL and anti-β2glycoprotein I antibodies were detected by ELISA.

Results

*A significant association was found between arterial events and triglyceride, LDL and cholesterol concentrations, but multivariate analysis showed that cholesterol concentrations were the most important predictor of arterial events (*p = *0.012). Cerebrovascular insults were the most significantly associated with cholesterol concentrations (*p = *0.011). Myocardial infarctions were more frequently present in patients more than 40 years of age (*p = *0.032). No significant association of the investigated parameters with venous thromboses was found. Recurrent abortions were not associated with the presence or concentrations of the investigated parameters. Although patients had increased concentrations of anti-oxLDL antibodies, no significant association was found between the titres of anti-oxLDL antibodies and clinical features of APS.*

Conclusions

In patients with PAPS, lipid concentrations are a better predictor for arterial events than anti-oxLDL antibodies.

Key words

Anti-oxLDL antibodies, atherothrombosis, cholesterol, primary antiphospholipid syndrome, triglyceride.

Mirjana Bećarević, MSc; Slađana Andrejević, PhD; Predrag Miljić, MSc; Branka Bonači-Nikolić, PhD; Nada Majkić-Singh, PhD.

Please address correspondence to: Mirjana Bećarević, MSc, Institute for Medical Biochemistry, Clinical Center of Serbia, Višegradska 26, 11000 Belgrade, Serbia.
E-mail: bmyrjana@yahoo.co.uk

Reprints will not be available from the authors.

Received on August 30, 2006; accepted in revised form on December 15, 2006.

Abbreviations:
aβ2gpI: anti-β2-glycoprotein I antibodies
aCL: anticardiolipin antibodies
aoxLDL: anti-oxidized LDL antibodies
APS: antiphospholipid syndrome
HDL: high- density lipoproteins
LDL: low-density lipoproteins
PAPS: primary antiphospholipid syndrome

Competing interests: none declared.

Introduction

Antiphospholipid syndrome (APS) may be primary (PAPS) or secondary to another autoimmune disease or malignancy (1) and it is characterized by arterial and/or venous thromboses and recurrent abortions, accompanied by elevated titers of antiphospholipid antibodies: anticardiolipin (aCL), anti-β2-glycoprotein I (β2gpI) and/or lupus anticoagulant (2-4). The other target antigens for the antiphospholipid antibodies include prothrombin (5), oxidized LDL (oxLDL) (6), etc.

On the one hand, experimental data indicate that anti-oxLDL antibodies may be protective (7-9), while on the other hand, a correlation was found between the existence and titers of anti-oxLDL antibodies and the extent of atherosclerosis, thrombosis and other cardiovascular or autoimmune diseases (10-13). George *et al*. (14) indicated that oxLDL aggravates the clinical manifestations of APS and suggested that autoantibodies cross-reactive with oxLDL may provide a pathogenic mechanism for accelerated atherosclerosis in APS.

During atherogenesis, LDL becomes trapped in the subendothelial space and is subsequently oxidized, and oxidized LDL is the major antigen implicated in atherosclerosis (15).

However, despite the fact that premature atherosclerosis has been reported in autoimmune diseases, the presence of underlying atherosclerosis has not been widely analysed in patients with PAPS. Studies in humans with PAPS have involved a relatively small number of patients. In addition, the role of classical risk factors for atherosclerosis (serum lipid levels) in PAPS patients is unclear because most clinical studies include patients with primary and secondary antiphospholipid syndrome and patients with systemic lupus erythematosus. Moreover, the link between antiphospholipid antibodies and atherogenesis in PAPS is less strong than for thrombosis, although clearly the two processes are related (16) and thrombosis is the main complication of atherosclerosis, a process known as atherothrombosis (17). In addition, the occurrence of placental infarctions and vascular thrombosis in women with recurrent abortions (18) suggests that this complication may be a part of a generalized thrombotic disorder.

Therefore, the aim of this study was to investigate the influence of serum lipid levels and anti-oxLDL antibodies on the clinical features of patients with PAPS. For comparison we also studied the same parameters in control subjects.

Patients and methods

Patients

Our study was approved by the local ethical committee and all participants gave their written informed consent. This study included 42 consecutive patients (32 women and 10 men) with PAPS, diagnosed using the updated Sapporo criteria (4, 19). The mean age of the analysed patients was 40.45 ± 13.37, and the ages ranged from 19 to 78. Our patients were not on any medications that might have affected the analysed parameters.

This study also included 47 blood donors (33 women and 14 men) predominately drawn from laboratory personnel. Exclusion criteria for the control subjects were the presence of acute or chronic diseases and the taking of medications that in anyway might have affected the analyzed parameters. The mean age of the control subjects was 39.68 ± 13.93 (range 18 to 73) yrs.

The body mass index (BMI) was calculated as the weight (kg)/height^2 (m^2). Individuals with BMI ≥ 30 kg/m^2 were considered as obese. Persons with BMI 25 - 30 kg/m^2 were considered to have an elevated BMI, while persons with BMI ≤ 25 kg/m^2 had a normal BMI.

Methods

After overnight fasting (12h) and 24h without intensive physical activity, patients were placed in a resting position immediately prior to venepuncture. The application of a tourniquet was never for longer than three minutes. The blood samples were collected for serum and plasma evaluation from the antecubital vein, and centrifuged for 10 minutes at 3000 rotations per minute.

Antibody levels were estimated by ELISA in patient sera using commercial reagents of Imtec Immunodiagnos-

tika, GmbH, Germany for the detection of anti-oxLDL antibodies (synchronous detection of the IgG and IgM isotypes). The test is based on the simultaneous incubation of serum samples with both oxidized LDL and the native LDL, with the subsequent determination of anti-oxLDL antibodies. The cut-off value for anti-oxLDL antibodies was 30 U/mL.

Commercial reagents of Imtec Immunodiagnostika, GmbH, Germany were used to detect anti-β2gpI antibodies (IgG and IgM isotypes) and anticardiolipin antibodies (IgG and IgM isotypes). The cut-off value for IgG aCL antibodies was 48 U/mL, while 44 U/mL was the cut-off value for IgM aCL antibodies, and 7 U/mL was the cut-off value for IgG and IgM anti-β2gpI antibodies.

The presence of lupus anticoagulant was detected according to the recommendations of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society of Thrombosis and Haemostasis (20).

Serum total cholesterol, HDL and triglyceride concentrations were measured by enzymatic methods using commercial reagents from Randox Laboratories Ltd., United Kingdom. LDL-cholesterol was calculated according to the Friedwald formula (21).

The cut-off value for total cholesterol was < 5.20 mmol/L, for HDL ≥ 1.60 mmol/L, for LDL < 3.40 mmol/L, and for triglyceride the cut-off was < 1.70 mmol/L.

Statistical analysis

Statistical analysis was conducted in three steps. In the first step, descriptive statistics were used to summarize the patients' characteristics. Continous variables were expressed as the mean ± SD. Secondly, the association between clinical risk factors for an APS-related event was examined using contingency table analysis, the t-test or the Mann-Whitney test, where appropriate. Comparisons between patients and control subjects were carried out by the Mann-Whitney test, t-test and χ^2- test, as appropriate. In the third step, logistic regression was performed with the APS-related event as the dependent variable and those clinical risk factors whose association with the event was statistically significant in the preeceding analysis as the independent variables. In all of the above-mentioned tests, p <0.05 was considered as statistically significant.

Analyses were conducted using SPSS 10 (SPSS Inc, Chicago IL, USA).

Results

The comparison of the parameters investigated between PAPS patients and healthy control subjects is shown in Table I.

In the patient group, 23.8% (10/42) had hypertension, which was more frequent in comparison with the control subjects who were all normotensive (χ^2 = 12.607, p = 0.000). Obesity was more frequent among patients than in control subjects but the difference was not significant (4/42, 9.5% *vs* 2/47, 4.3%; χ^2 = 0.979, p =not significant = ns). There were more smokers among patients in control subjects, but the difference was not significant (14/42, 33.33% *vs* 9/47, 19.10%; χ^2 = 2.329, p = ns). No association was found between the levels of the investigated parameters and smoking in the patients.

Patients were then divided in two groups based on the presence/absence of at least one arterial event, which is shown in Table II.

Among the patients, 54.76% (23/42) had at least one arterial event and Table III shows the association of the investigated parameters with arterial events. Arterial events were more frequent in patients older than 40 years of age (χ^2 = 10.380, p = 0.001), while no significant association with BMI, smoking or hypertension was found between patients with and without arterial events. A significant association was found between arterial events and triglyceride, LDL and cholesterol concentrations, but multivariate analysis showed that cholesterol concentrations were the most important predictor of arterial events (OR = 2.388, p = 0.012, B = 0.870, 95% CI for OR = 1.209 to 4.717).

Table IV shows the number of patients with the clinical features of PAPS and the autoantibodies evaluated in this study. Patients with arterial events were divided as follows: patients with thromboses of the peripherial arterial blood vessels, patients with cerebrovascular events, and patients with myocardial infarctions. Cholesterol concentrations were the most important predictor of cerebrovascular insults (OR = 3.200, p = 0.011, B = 1.163, 95% CI for OR = 1.310 to 7.814). Multivariate analysis showed that patients older than 40 years had more frequent myocardial infarctions (OR = 11.500, p = 0.032, B = 2.442, 95% CI for OR = 1.238 to 106.850). No significant association of the investigated parameters with ve-

Table I. Comparison of the parameters investigated in PAPS patients and control subjects.

Investigated parameters	PAPS (n = 42)	Control subjects (n = 47)	*P* value
Age, years (mean ± SD)	40.45 ± 13.37	39.68 ± 13.93	0.690
Cholesterol (mmol/l), mean ± SD	5.17 ± 1.18	4.87 ± 0.96	0.180
HDL (mmol/l), mean ± SD	1.18 ± 0.23	1.54 ± 0.27	0.000
LDL (mmol/l), mean ± SD	3.22 ± 1.04	2.79 ± 0.92	0.062
Triglyceride (mmol/l), mean ± SD	1.72 ± 0.78	1.18 ± 0.46	0.000
aCl IgG (U/ml), mean ± SD	195.07 ± 176.06	20.4 ± 30.34	0.000
aCl IgM (U/ml), mean ± SD	160.47 ± 154.70	11.22 ± 6.68	0.000
aβ2gpI IgG (U/ml), mean ± SD	27.53 ± 35.31	3.13 ± 1.82	0.000
aβ2gpI IgM (U/ml), mean ± SD	20.46 ± 34.15	1.98 ± 1.19	0.012
aoxLDL (U/ml), mean ± SD	62.22 ± 46.90	25.24 ± 13.14	0.000
*Lupus anticoagulant (%)	28/32 (87.50%)	0/47 (0%)	0.000

aCL IgG: anticardiolipin antibodies of the IgG isotype; aCL IgM: anticardiolipin antibodies of the IgM isotype; aβ2gpI IgG: anti- β2glycoprotein I antibodies of the IgG isptype; aβ2gpI IgM: anti-β2glycoprotein I antibodies of the IgM isptype; aoxLDL: anti-oxLDL antibodies.
Comparisons between groups were carried out by the Mann-Whitney test.
*Comparisons between groups were carried out by the χ^2- test.

Table II. Clinical features of the patients investigated.

	Clinical events	No. of pts. (%)
Arterial events (n = 23; 17 women)	1. Cerebrovascular insults	9/23 (39.13)
	2. Thromboses of peripherial arterial blood vessels	7/23 (30.43)
	3. Myocardial infarctions	7/23 (30.43)
Non-arterial events (n = 19; 15 women)	1. Recurrent abortions	6/15 (40.00)
	2. Venous thromboses	13/19 (68.42)

Table III. Comparison of the analysed parameters between patients with and without arterial events.

Investigated parameters	Arterial events (n = 23) (mean ± SD)	Non-arterial events (n = 19) (mean ± SD)	*P* value
Cholesterol (mmol/l), mean ± SD	5.61 ± 1.08	4.63 ± 1.08	0.011
HDL (mmol/l)), mean ± SD	1.17 ± 0.25	1.19 ± 0.22	0.909
LDL (mmol/l), mean ± SD	3.56 ± 0.97	2.80 ± 0.98	0.044
Triglyceride (mmol/l), mean ± SD	1.96 ± 0.89	1.42 ± 0.49	0.023
aoxLDL (U/ml), mean ± SD	62.89 ± 50.53	61.37 ± 43.25	0.834
aCl IgG (U/ml), mean ± SD	192.62 ± 171.64	198.10 ± 185.91	0.704
aCl IgM (U/ml), mean ± SD	184.05 ± 175.82	130.28 ± 120.91	0.674
aβ2gpI IgG (U/ml). mean ± SD	26.19 ± 32.68	29.24 ± 39.31	0.683
aβ2gpI IgM (U/ml), mean ± SD	21.69 ± 36.67	18.89 ± 31.61	0.176
*Lupus anticoagulant, (%)	14/17 (82.35%)	14/15 (93.33%)	0.603

aCL IgG: anticardiolipin antibodies of the IgG isotype; aCL IgM: anticardiolipin antibodies of the IgM isotype; aβ2gpI IgG: anti- β2glycoprotein I antibodies of the IgG isptype; aβ2gpI IgM: anti-β2glycoprotein I antibodies of the IgM isptype; aoxLDL: anti-oxLDL antibodies.
Groups were compared bythe Mann-Whitney test.
*Comparisons between groups were carried out by the χ^2- test.

Table IV. The number of patients with the clinical features of PAPS and the autoantibodies analyzed in this study.

Clinical features	LA (n= 32)	aCL (n = 42) Any	aCL (n = 42) IgG	aCL (n = 42) IgM	aβ2gpI (n = 41) Any	aβ2gpI (n = 41) IgG	aβ2gpI (n = 41) IgM	aoxLDL (n = 41)
				Arterial events (n= 23)				
CVI (n = 9)	6	7	5	6	6	5	2	6
MI (n = 7)	4	5	4	2	3	2	1	5
PAT (n = 7)	4	7	4	5	6	3	5	6
				Non-arterial events (n = 19)				
VT (n= 13)	9	10	6	8	7	6	5	11
RA (n = 6)	5	5	5	4	5	4	3	5
Sum (%)	28/32 (87.50)	34/42 (80.95)	24/42 (57.14)	25/42 (60.97)	27/42 (65.85)	20/41 (48.78)	16/41 (39.02)	33/41 (80.49)

aCL IgG: anticardiolipin antibodies of the IgG isotype; aCL IgM: anticardiolipin antibodies of the IgM isotype; aβ2gpI IgG: anti-β2glycoprotein I antibodies of the IgG isptype; aβ2gpI IgM: anti-β2glycoprotein I antibodies of the IgM isotype; aoxLDL: anti-oxLDL antibodies; CVI: cerebrovascular insults; IM: myocardial infarctions; LA: lupus anticoagulant; PAT: thromboses of peripherial arterial blood vessels; RA: recurrent abortions; VT: venous thromboses.

nous thromboses was found. Recurrent abortions were not associated with the presence or concentrations of the investigated parameters.

Patients with PAPS had significantly elevated anti-oxLDL antibodies in comparison with control subjects, but the presence and titers of anti-oxLDL antibodies did not predict any of the clinical manifestations of APS. Investigation of the presence of multiple antibodies with clinical features of PAPS showed no significant association, except in the case of the simultaneous presence of anti-oxLDL and anti-β2gpI antibodies, which was associated with thromboses of the peripherial arterial blood vessels (χ^2 =7.00, p = 0.008). Rigidly selected patients with very high positivity for antiphospholipid antibodies (80.95%, 87.50%, 65.85% for anticardiolipin antibodies, lupus anticoagulant, anti-β2gpI antibodies, respectively, Table IV), and the relatively small number of patients included in our study (n = 42) are reasons for the lack of a statistically significant association between antiphospholipid antibodies and the clinical features of PAPS.

Discussion

The main clinical manifestations of atherothrombosis are coronary heart disease (CHD), peripherial vascular disease, and stroke (17). The relationship between the triglyceride concentration and the risk of CHD has not been firmly established because most studies have reported increased triglyceride concentrations to be positively correlated with an increased risk of CHD in univariate analysis, but when other risk factors for CHD were included in the multivariate model, triglyceride often lost its significance (22). However, there is evidence that increased triglyceride concentrations may be an independent risk factor for CHD, especially in women (23). A subgroup of patients with a high prevalence of anticardiolipin antibodies and premature atherosclerotic peripherial vascular disease had a low occurrence of dyslipidaemia (24). Ames *et al*. (25) reported no significant changes in total cholesterol, HDL or LDL levels in patients with antiphospholipid antibodies, suggesting that other factors might be important in the development of atherosclerosis. Similarly to Ames *et al*. (25), we did not find significant changes in total cholesterol and LDL concentrations, but we did find that HDL concentrations were significantly lower in patients with PAPS in comparison to healthy control subjects (Table I). In addition, we found that triglyceride and LDL concentrations were significantly associated with the arterial events in patients with PAPS (Table III), but multivariate analysis showed that cholesterol concentrations were

the most important predictor for this clinical finding.

Hyperlipidaemia and hypertension increase the risk of thrombosis in patients with antiphospholipid antibodies (26). It has been reported that cerebrovascular events with a high titer of anticardiolipin antibodies of the IgG isotype were associated with smoking and hyperlipidaemia and that recurrent events were significantly more common among smokers and hyperlipdaemics (27, 28). Our findings were similar because we demonstrated that cerebrovascular insults in patients with PAPS were associated with elevated cholesterol concentrations, but we did not find any association with hypertension and smoking.

An independent risk factor for myocardial infarction and sudden cardiac death are high titers of antiphospholipid antibodies. The risk is even higher in patients who also have antibodies against oxLDL (12). Autoantibodies against oxLDL and phospholipids occur both in healthy individuals and in patients with cardiovascular diseases (9). Karvonen *et al*. (7) reported that anti-oxLDL antibodies were inversely associated with the risk of cardiovascular diseases. In our study, no significant association between anti-oxLDL antibodies and myocardial infarctions was found, and multivariate analysis showed that only ageing appears to be a strong risk factor for myocardial infarctions in PAPS.

Previously it was reported that hypertension and smoking were associated with arterial events, but not with venous events in APS patients (29). Doggen *et al*. (30) reported that elevated triglyceride levels were associated with a doubling of the risk of venous thrombosis in women, whereas elevated HDL cholesterol levels were associated with a decreased risk and total cholesterol levels were not associated with venous thrombosis. We did not find a significant association of any of our parameters with venous thromboses.

Although previously mentioned studies (10-12) have reported that anti-oxLDL antibodies are pathogenic, some studies (8) provide evidence for a protective role of anti-oxLDL antibodies in atherosclerosis and CHD. Antibodies may neutralize immunogens, and this is the reason why humoral immunity to oxLDL can reduce the incidence of atherosclerosis (31). Although in our study 80.49% of the analysed patients had elevated titres of anti-oxLDL antibodies (with higher concentrations in patients with arterial events, p = ns), those antibodies did not discriminate between arterial or venous events, but confirmed the tendency towards vascular events. In this sense, anti-oxLDL antibodies should have the same significance as anticardiolipin antibodies.

Although patients with PAPS had high titers of anti-oxLDL antibodies, our results demonstrate that a better predictor for arterial events in PAPS are elevated serum lipid levels. Therefore, our recommendation would be that testing for serum lipids should be mandatory in patients whose first clinical manifestation of APS are at least one arterial event, especially in those who are more than 40 years old. Our results have very important implications for the new treatment strategies. Statins are to be strongly recommended for the prevention of accelerated atherothrombosis in PAPS patients, similarly to patients with systemic lupus erythematosus (32). This should help physicians to introduce additional therapy (lipid lowering drugs, such as statins) to minimize the probability of recurrent thrombotic episodes and prevent the clinical manifestations of atherothrombosis in patients with PAPS.

References

1. MARAI J, ZANDMAN-GODDARD G, SHOENFELD Y: The systemic nature of the antiphospholipid syndrome. *Scand J Rheumatol* 2004; 33: 365-72.
2. HUGHES GRV: Thrombosis, abortion, cerebral disease and the lupus anticoagulant. *Br Med J* 1983; 287: 1088-9.
3. SHOENFELD Y: Systemic antiphospholipid syndrome. *Lupus* 2003; 12: 497-8.
4. MIYAKIS S, LOCKSHIN M, ATSUMI T *et al*.: International Consensus Statement on an update of the classification criteria for the definite antiphospholipid syndrome. *J Throm Haemostasis* 2006; 4: 295-306.
5. NOJIMA J, KURATSUNE H, SUEHISA H *et al*.: Anti-prothrombin antibodies combined with lupus anticoagulant activity is an essential risk factor for venous thromboembolism in patients with systemic lupus erythematosus. *Br J Haematol* 2001; 114: 647-54.
6. MACKWORTH-YOUNG CG: Antiphospholipid syndrome: multiple mechanisms. *Clin Exp Immunol* 2004; 136: 393-401.
7. KARVONEN J, PAIVANSALO M, KESANIEMI YA, HORKKO S: Immunoglobulin M type of autoantibodies to oxidized low-density lipoprotein has an inverse relation to carotid atherosclerosis. *Circulation* 2003; 108: 2107-12.
8. HULTHE J, WIKLUND O, HURT-CAMEJO E, BONDJERS G: Antibodies to oxidized LDL in relation to carotid atherosclerosis, cell adhesion molecules, and phospholipase A2. *Arterioscler Thromb Vasc Biol* 2001: 21; 269-74.
9. LEFVERT AK: Heterogeneity of autoantibodies against cardiolipin and oxidatively modified LDLs revealed by human monoclonal antibodies. *J Intern Med* 2000; 247:
10. BEĆAREVIĆ M, ANDREJEVIĆ S, BONAČI-NIKOLIĆ B, OBRADOVIĆ I, MILJIĆ P, MAJKIĆ-SINGH N: Anti-oxLDL antibodies - marker for arterial thromboses in antiphospholipid syndrome? *Clin Lab* 2005; 51: 279-83.
11. BEĆAREVIĆ M, MIRKOVIĆ D, MILJIĆ P *et al*.: Anti-oxLDL antibodies, homocysteine and apolipoproteins in primary antiphospholipid syndrome. *Jugoslov Med Biohem* 2006; 25: 167-72.
12. GROMNICA-IHLE E, SHOSSLER W: Antiphospholipid syndrome. *Int Arch Allergy Immunol* 2000; 123: 67-76.
13. WADA Y, KURODA T, MURASAWA A, TANABE N, NAKANO M, GEJYO F: Autoantibodies against oxidized low-density lipoprotein (LDL) and carotid atherosclerosis in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2005; 23: 482-6.
14. GEORGE J, BLANK M, HOJNIK M *et al*.: Oxidized low-density lipoprotein (oxLDL) but not LDL aggravates the manifestations of experimental antiphospholipid syndrome (APS). *Clin Exp Immunol* 1997; 108: 227-33.
15. NICOLLO D, MONESTIER M: Antiphospholipid antibodies and atherosclerosis. *Clin Immunol* 2004; 112: 183-9.
16. SHERER Y, SHOENFELD Y: Antiphospholipid antibodies: are they proatherogenic or an epiphenomenon of atherosclerosis? *Immunobiology* 2003; 207: 13-6.
17. LEYS D: Atherothrombosis: a major health burden. *Cerebrovasc Dis* 2001; 11: 1-4.
18. OUT HJ, KOOIJMAN CD, BRUINSE HW, DERKSEN RHWM: Histopathological findings in placenta from patients with intrauterine fetal death and antiphospholipid antibodies. *Eur J Obst Gynecol Reprod Biol* 1991; 41: 179.
19. WILSON WA, GHARAVI AE, KOIKE T *et al*.: International statement on preliminary classification criteria for definite antiphospholipid symdrome. Report of an international workshop. *Arthritis Rheum* 1999; 42: 1309-11.
20. BRANDT JT, TRIPLETT DA, ALVING B, SCHARRER J: Criteria for the diagnosis of lupus anticoagulans: An update on behalf of the Subcommittee on Lupus Anticoagulant/ Antiphospholipd Antibody of the Scientific and Standardisation Committee of the International Society of Thrombosis and Haemostasis. *Thrombosis Haemostasis* 1995; 74: 1185-90.

21. FRIEDWALD WT, LEVY RI, FREDRICKSON DS: Estimation of the concentration of low-density lipoprotein cholesterol without the use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499.
22. COLE TG, KLOTZSCH SG, MCNAMARA JR: Measurement of triglyceride concentration. *In* RIFAI N, WARNICK GR and DOMINICZAK MH (Eds.): *Handbook of Lipoprotein Testing*. Washington DC, AACC Press, 2000; 207-20.
23. SCHAEFER EJ, MCNAMARA JR: Overview of the diagnosis and treatment of lipid disorders. *In* RIFAI N, WARNICK GR and DOMINICZAK MH (Eds.): *Handbook of Lipoprotein Testing*. Washington DC, AACC Press, 2000; 77-102.
24. NITYANAND S, BERGMARK C, DE FAIRE U, SWEDENBORGJ, HOLM G, LEFVERT K: Antibodies against endothelial cells and cardiolipin in young patients with peripherial atherosclerotic disease. *J Intern Med* 1995; 238: 437-43.
25. AMES PRJ, TOMMASINO C, ALVES J *et al*.: Antioxidant susceptibility of pathogenic pathways in subjects with antiphospholipid antibodies: a pilot study. *Lupus* 2000; 9: 688-95.
26. PETRI M: Thrombosis and systemic lupus erythematosus: The Hopkins Lupus Cohort perspective. *Scand J Rheumatol* 1996; 25: 191-3.
27. VERRO P, LEVINE SR, TIETJEN GE: Cerebrovascular ischemic events with high positive anticardiolipin antibodies. *Stroke* 1998; 29: 2245-53.
28. LEVINE SR, DEEGAN MJ, FUTRELL N, WELCH KM: Cerebrovascular and neurological disease associated with antiphospholipid antibodies: 48 cases. *Neurology* 1990; 40: 1181-9.
29. ERKAN D, YAZICI Y, PETERSON MG, SAMMARITANO L, LOCKSHIN MD: A cross-sectional study of clinical thrombotic risk factors and preventive treatments in antiphospholipid syndrome. *Rheumatology* 2002; 41: 924-9.
30. DOGGEN CJM; SMITH NL, LEMAITRE *et al*.: Serum lipid levels and the risk of venous thrombosis. *Arterioscler Thromb Vasc Biol* 2004; 24: 1970-5.
31. SHOENFELD Y, WU R, DEARING L, MATSURA E: Are anti-oxidized Low-Density Lipoprotein antibodies pathogenic or protective? *Circulation* 2004; 110: 2552-8.
32. SCHATTNER A, NAPARSTEK Y: The future treatment of systemic lupus erythematosus. *Clin Exp Rheumatol* 2005; 23: 254-60.