

Prevalence and Clinical Associations of Lupus Anticoagulant, Anticardiolipin Antibodies, and Anti-beta2-glycoprotein I Antibodies in Patients with Systemic Lupus Erythematosus

Kwang-Sook Woo¹, Kyung-Eun Kim¹, Jeong Man Kim¹, Jin-Yeong Han¹, Won-Tae Chung², and Kyeong-Hee Kim¹

Departments of Laboratory Medicine¹ and Internal Medicine², Dong-A University College of Medicine, Busan, Korea

Background : The presence of antiphospholipid antibodies (aPLs) is associated with the clinical features of antiphospholipid syndrome (APS), which comprises venous and arterial thrombosis and pregnancy loss, and systemic lupus erythematosus (SLE). The prevalence of aPLs has been reported to be different in patient populations affected by either of these conditions. We performed a retrospective study to evaluate the prevalence and clinical associations of aPLs, including lupus anticoagulant (LAC), anticardiolipin (aCL), and anti-beta2-glycoprotein I antibodies (anti-beta2-GPI) in a cohort of Korean patients with SLE.

Methods : This study included samples from 88 SLE patients for whom aPL testing had been advised between June 2006 and July 2009 at the Dong-A University Hospital. Serum and plasma samples were tested for LAC, aCL (IgG, IgM), and anti-beta2-GPI (IgG, IgM) antibodies. Clinical data from patients were obtained from a review of medical records.

Results : LAC was the most common (34.1% of total patients, 30/88) antibody, followed by IgM aCL (31.8%, 28/88), IgG aCL (18.2%, 16/88), and IgM and IgG anti-beta2-GPI (both 5.7%, 5/88 each). Positivity for LAC was strongly associated with venous/arterial thrombosis ($P=0.002$).

Conclusions : LAC was the most common antibody detected in Korean SLE patients and is shown to have a significant association with the presence of venous/arterial thrombosis. The measurement of LAC may be clinically useful in identifying patients with SLE who are at a high risk for venous/arterial thrombosis. (*Korean J Lab Med 2010;30:38-44*)

Key Words : *Systemic lupus erythematosus, Antiphospholipid antibodies, Antiphospholipid syndrome*

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by diverse clinical manifestations including the excessive production of autoantibodies. Antiphospholipid antibodies (aPLs) are found in a large percent-

age of patients with SLE; these antibodies are also associated with the antiphospholipid syndrome (APS), which comprises venous and arterial thrombosis and pregnancy loss [1, 2]. Further, other clinical conditions such as thrombocytopenia have been associated with these antibodies [3]. APS is diagnosed when there is laboratory evidence of the presence of aPLs and characteristic clinical manifestations [4].

Several studies in SLE patients have suggested that lupus anticoagulant (LAC) is probably more frequently associated with thrombotic events and recurrent pregnancy losses (RPLs) than anticardiolipin (aCL) antibodies [5-7]. These studies have also shown that the detection of anti-beta2-glycoprotein I antibodies (anti-beta2-GPI) are correlated

Received : February 12, 2009 Manuscript No : KJLM09-030
Revision received : December 21, 2009
Accepted : January 13, 2010
Corresponding author : Kyeong-Hee Kim, M.D.
Department of Laboratory Medicine, Dong-A University
College of Medicine, 1 Dongdaesin-dong 3-ga, Seo-gu,
Busan 602-715, Korea
Tel : +82-51-240-2850 Fax : +82-51-255-9366
E-mail : progreen@dau.ac.kr

*This study was supported by research funds from Dong-A University.

with the manifestations of APS [8, 9].

Detection of aPLs in patients with a history of thrombosis or pregnancy-related complications is an essential step in both the diagnosis and management of APS. Examination of aPL in SLE patients of the old or reproductive age groups is also essential for the evaluation of the risk of thrombosis and for developing strategies to improve the pregnancy outcome in these patients. Therefore, the development of adequate laboratory tests for the detection of APS is clinically relevant.

Differential prevalence of aPLs has been reported in different populations of SLE patients [10]. Few comprehensive studies on aPLs expressed in SLE patients have been performed in a Korean cohort [11]. Different types of aPLs such as LAC, IgM, and IgG aCL and IgM and IgG anti-beta2-GPI in SLE patients are rarely evaluated.

In this study, to evaluate the prevalence of aPLs, including LAC, aCL, and anti-beta2-GPI in Korean patients, we performed a retrospective study of the laboratory findings in patients with SLE during the course of the disease. Furthermore, we investigated the relationship between APS manifestations and the presence of aPLs.

MATERIALS AND METHODS

1. Patients

We included SLE patients for whom aPL testing, including tests for LAC, IgM and IgG aCL and IgM and IgG anti-beta2-GPI, had been performed at the same time during treatment at Dong-A University Hospital between June 2006 and July 2009. Eighty-eight SLE patients who fulfilled the American College of Rheumatology classification criteria [12] were included in a single-center, retrospective cohort study. We reviewed all patient charts for sex, age, medications, and clinical manifestations of APS, including venous/arterial thrombosis and pregnancy loss. Venous thrombosis was confirmed by venography or ultrasonography and arterial thrombosis by computerized tomography, magnetic resonance imaging, or arteriography. Thrombocytopenia (platelet count: $<100,000/\mu\text{L}$) was also observed

[4]. Informed consent was obtained from all patients, and blood samples were collected. Fresh plasma and serum samples were obtained during treatment and tested for LAC, IgM, and IgG aCL and IgM and IgG anti-beta2-GPI.

2. Detection of lupus anticoagulant

Blood samples were collected in vacuum tubes containing sodium citrate (0.109 M). Platelet-free plasma was prepared by centrifugation at $3,000\times g$ for 15 min at room temperature. LAC was screened using a LAC-sensitive activated partial thromboplastin time (APTT) reagent, PTT-LA (Diagnostica Stago, Asnières, France). Tests were performed according to the manufacturer's recommendations. PTT-LA is a reagent containing cephalin prepared from rabbit cerebral tissue with a siliceous activator.

Results were presented as the clotting times of the tested plasma samples. The prolongation of PTT-LA clotting time was determined as positive results: evidence of inhibition was demonstrated by mixing studies, and the lack of a specific inhibitor for any specific coagulation factor must be ruled out with further testing [12]. Confirmation tests were not performed.

3. Detection of aCL antibodies

Blood samples were collected from patients, and sera were separated. Analysis of IgM and IgG anticardiolipin antibodies was performed using the QUANTA Lite™ ACA, IgM, and IgG ELISA kit (Inova Diagnostics, San Diego, CA, USA) for semiquantitative detection in human sera. The assay was performed with polystyrene plates coated with purified cardiolipin antigen and both bovine and human beta2-GPI. Patient sera were added to each well. Each specimen was incubated at room temperature for 30 min. Unbound sample was washed away, and enzyme-labeled anti-human IgM or IgG antibodies were added to each well. After a second incubation at room temperature for 30 min and subsequent washing, the activity of the remaining enzyme was measured by adding a chromogenic substrate and measuring the intensity of the developed color. Optical absorbance

was measured at 450 nm by using a microplate reader (Model 680 Microplate Reader; Bio-Rad Laboratories, Hercules, CA, USA). The anticardiolipin antibody levels were evaluated by comparing the development of color intensity in wells with that for a five-point calibration curve. The values of anticardiolipin antibodies were expressed in standard IgM anticardiolipin units (MPL) or IgG anticardiolipin units (GPL). In accordance with the manufacturer's instructions, 15 GPL units/mL for IgG and 12.5 MPL units/mL for IgM were considered positive values for this study.

4. Detection of anti-beta2-GPI antibodies

For the measurement of anti-beta2-GPI antibodies, a semiquantitative ELISA assay was performed with the commercially available QUANTA Lite™ beta2 GPI ELISA kit (Inova Diagnostics, San Diego, CA, USA). Sera from patients were collected, and tests were performed following the same procedure described above. Polystyrene plates coated with purified beta2-GPI antigen were used. The values of anti-beta2-GPI antibodies were expressed in standard IgM anti-beta2-GPI units (SMU) or IgG anti-beta2-GPI units (SGU). In accordance with the manufacturer's instructions, the cut-off value for the positive results was set at >20 SMU and >20 SGU.

Table 1. Demographic data from patients with systemic lupus erythematosus (N=88)

Variable	No APS (N=82)	APS (N=6)
Sex (Female/male)	74/8	5/1
Median age (range)	32 (17-62)	30.5 (27-35)
Clinical manifestation*		
Arterial/venous thrombosis	0 (0.0)	6 (100.0)
Pregnancy loss [†]	2 (2.7)	1 (20.0)
Thrombocytopenia	9 (11.0)	2 (33.3)
Medication*		
Corticosteroids	82 (100.0)	6 (100.0)
Hydroxychloroquine	43 (52.4)	4 (66.7)
Azathioprine	15 (18.3)	0 (0.0)
NSAIDs	32 (37.8)	2 (33.3)
Mycophenolate mofetil	9 (11.0)	1 (16.7)
Methotrexate	10 (12.2)	0 (0.0)

*Values are expressed as numbers (%); [†]Women only are evaluated. Abbreviations: NSAIDs, nonsteroidal antiinflammatory drugs; APS, antiphospholipid syndrome.

5. Statistical methods

Statistical analysis was performed using MedCalc version 9.3 (MedCalc Software, Mariakerke, Belgium). Distributions of IgM and IgG aCL, IgM and IgG anti-beta2-GPI antibodies, and LAC in patients were analyzed. The results for each test (for IgM and IgG aCL, LAC, and IgM and IgG anti-beta2-GPI antibodies) were analyzed by the chi-square test to determine the associations between each of these antibodies and specific clinical events of APS, including thrombocytopenia. A *P* value less than 0.05 was considered significant.

RESULTS

Eighty-eight SLE patients were recruited into the study. The demographic data of the patients are shown in Table 1. Among the 88 SLE patients (79 females and 9 males), 6 (6.8%) presented with at least one of the diagnostic criteria for APS, such as thromboembolism or pregnancy loss, whereas 82 (93.2%) did not. Eleven patients had thrombocytopenia; 2 of them were included in the APS group. The median age of patients was 32 yr (age range, 17–62 yr) in patients without APS and 30.5 yr (age range, 27–35 yr) in patients with APS. During the course of their disease, the majority of patients was treated with prednisone.

General distributions of aCL and anti-beta2-GPI anti-

Table 2. Distribution of lupus anticoagulant, anticardiolipin antibodies, and anti-beta2-glycoprotein I antibodies in patients with systemic lupus erythematosus

LAC	aCL*	anti-beta2-GPI*	N (%)		
			No APS (N=82)	APS (N=6)	Total (N=88)
+	+	+	4 (4.9)	0	4 (4.5)
+	+	-	11 (13.4)	1 (16.7)	12 (13.6)
+	-	+	0	0	0
+	-	-	9 (11.0)	5 (83.3)	14 (15.9)
-	+	+	3 (3.7)	0	3 (3.4)
-	+	-	15 (18.3)	0	15 (17.0)
-	-	+	1 (1.2)	0	1 (1.1)
-	-	-	39 (47.6)	0	39 (44.3)

*A positive IgG and/or IgM isotype was regarded as a positive result. Abbreviations: LAC, lupus anticoagulants; aCL, anticardiolipin antibodies; anti-beta2-GPI, anti-beta2-glycoprotein I antibodies; Refer Table 1.

Table 3. Prevalence of lupus anticoagulant, anticardiolipin antibodies, and anti-beta2-glycoprotein I antibodies in 88 patients with systemic lupus erythematosus

	Prevalence (%)		
	No APS (N=82)	APS (N=6)	Total (N=88)
LAC	24 (29.3)	6 (100.0)	30 (34.1)
IgG aCL	15 (18.3)	1 (16.7)	16 (18.2)
IgM aCL	27 (32.9)	1 (16.7)	28 (31.8)
IgG anti beta2-GPI	5 (6.1)	0 (0.0)	5 (5.7)
IgM anti beta2-GPI	5 (6.1)	0 (0.0)	5 (5.7)

Abbreviations: Refer Tables 1, 2.

bodies in patients with and without APS are shown in Table 2. The distributions of LAC, aCL, and anti-beta2-GPI antibodies in patients with SLE indicated that at least 1 type of aPL could be detected in 49 (55.7%) patients. Only 4 patients (4.5%) had positive results for all the aPL tests. One patient was positive only for anti-beta2-GPI, but negative for aCL and LAC. Among the 6 patients with APS, all were positive for LAC, 1 was also positive for IgG aCL, and the other 5 were negative for both aCL and anti-beta2-GPI.

Table 3 shows the prevalence of LAC, IgM/IgG aCL, and IgM/IgG anti-beta2-GPI in SLE patients. LAC was the most common (34.1%) antibody detected in the population group, followed by IgM aCL (31.8%), and IgG aCL (18.2%). Five patients each (5.7% of the total) were positive for IgM and IgG anti-beta2-GPI, and none of these patients had APS manifestations.

The relationship between specific clinical events and laboratory findings and aPL isotypes in SLE patients is shown in Table 4. Positivity for LAC was strongly associated with venous/arterial thrombosis ($P=0.002$).

DISCUSSION

Clinical manifestations and abnormal laboratory values associated with APS are relatively common among patients with SLE [13]. According to the International Consensus Document, which provides updated classification criteria for APS [4], a definite diagnosis for APS requires the following criteria to be fulfilled: vascular thrombosis and/or pregnancy morbidity and the presence of at least 1 of the

Table 4. Relationship between individual antiphospholipid tests and clinical features of antiphospholipid syndrome in 88 patients with systemic lupus erythematosus

	Arterial/venous thrombosis		<i>P</i>	Pregnancy loss*			Thrombocytopenia		
	Yes (N=6)	No (N=82)		Yes (N=3)	No (N=76)	<i>P</i>	Yes (N=11)	No (N=77)	<i>P</i>
LAC+ (N=30)	6	24	0.002	1	23	0.67	7	23	0.12
IgG aCL+ (N=16)	1	15	0.65	0	14	0.94	4	12	0.21
IgM aCL+ (N=28)	0	28	0.20	0	23	0.56	5	23	0.48
IgG anti beta2-GPI+ (N=5)	0	5	0.77	0	5	0.40	1	4	0.86
IgM anti beta2-GPI+ (N=5)	0	5	0.77	0	5	0.40	1	4	0.86

*Only female patients are evaluated.

Abbreviations: Refer Table 2.

following antibodies—LAC, IgM/IgG aCL, and IgM/IgG anti-beta2-GPI.

Simultaneous tests for aPLs such as LAC, IgM, and IgG aCL and IgM and IgG anti-beta2-GPI are rarely performed as part of routine clinical investigation for SLE patients in Korea. We evaluated the frequencies as well as isotype distribution of LAC, aCL, and anti-beta2-GPI. Results from a few previous studies performed in different populations of SLE patients have estimated the prevalence of aPLs to range approximately between 10% and 80% [2, 9, 14, 15]. In this study, the overall prevalence of aPLs was 53.7%. The prevalence of LAC was 34.1%; IgM aCL, 31.8%; IgG aCL, 18.2%; IgM anti-beta2-GPI, 5.7%; and IgG anti-beta2-GPI was 5.7% of the total patients. The frequencies of these aPLs were similar to those reported in previous studies, except for that of anti-beta2-GPI; the prevalence of anti-beta2-GPI in the present study was lower than that reported in studies performed in Western countries [9, 14, 15]. Findings similar to ours have been reported in previous studies: the prevalence of IgG anti-beta2-GPI in 272 Chinese SLE patients was reported to be low, at 7.7% and of 61 Korean SLE patients, 2.6% were positive for IgG anti-beta2-GPI and 0%, for IgM anti-beta2-GPI [16, 17]. How-

ever, the prevalence of anti-beta2 GPI in Korean patients with SLE reported by Lee et al. [11] was higher than that observed in our study. The discrepancy in the results pertaining to the prevalence of anti-beta2 GPI in Korean SLE patients may be attributed to the difference in the assays used in the studies (home-based or commercial assays) and relatively small study population. Further studies will be required to determine the clinical roles and racial disparity in the prevalence of anti-beta2 GPI in Korean SLE patients.

The significant association between the presence of LAC and aCL and the clinical manifestations of APS and thrombocytopenia is a well-known phenomenon. Recent studies indicate that the risk of thrombotic events and RPLs is more strongly associated with LAC than with aCL. Our results indicated a strong association of LAC with venous/arterial thrombosis; these findings were consistent with those of previous studies [5, 7]. These results suggest that LAC has a greater predictive value for thrombosis in SLE patients than other aPLs. The detection of LAC appeared to be the preferred choice of assay for the examination of functional aPL antibodies.

Previous studies have revealed a specific association between aCL antibodies and clinical manifestations of APS [14, 18]. A scientific committee recently recommended the replacement of the aCL test with anti-beta2 GPI and LAC tests [19]. However, available evidence indicates that anti-beta2 GPI cannot yet be confirmed as a substitute for aCL [20, 21]. In the updated classification criteria for APS, the aCL test continues to be a laboratory criterion for the diagnosis of APS [4]. A study on the persistence of aCL failed to establish that the risk of thrombosis increases in SLE patients with negative LAC and transiently-positive aCL [22]. Our study found no significant association between aCL and the clinical manifestations of APS; this finding may be explained by the lack of follow-up of aCL in our patients.

The test for anti-beta2 GPI has 2 methodological advantages over the aCL test. The former is less affected by the presence of infectious antibodies against cardiolipin and it does not require cofactors [23]. Several reports have shown that anti-beta2-GPI may be the only test that yields pos-

itive results in APS patients [24, 25]. In contrast to previous studies, no case was observed with a negative result for aCL and LAC and a positive result for anti-beta2-GPI. In this study, the anti-beta2-GPI test did not provide additional information for the diagnosis of APS in SLE patients. We performed the aCL and anti-beta2-GPI tests using the same manufacturer's reagent, which may be 1 of the causes for different findings.

aPLs are frequently observed in patients initially diagnosed with idiopathic thrombocytopenic purpura; thrombocytopenia is more common in patients with APS and SLE than in patients with APS alone [26, 27]. The International Consensus Committee recommends closer follow up in thrombocytopenic patients with persistent aPL, despite the absence of clinical manifestations of APS because of increased risk for thrombosis in such patients. In our study, thrombocytopenia was more frequent in the SLE patients with APS than in those without APS; however, the results of the aPL tests were not significantly associated with thrombocytopenia.

The incidence of APS in SLE patients is high, ranging from 20% to 30% [2, 18]. However, in our study, the incidence of APS in patients with SLE was as low as 6.8%. This finding was consistent with those of a study reporting that the incidence of venous thromboembolism in Korean patients was lower than that in Western populations [28].

In conclusion, we found that 56% of SLE patients had detectable levels of at least 1 type of aPL; LAC was the most common aPL and was shown to have a significant association with the presence of venous/arterial thrombosis. Therefore, the measurement of LAC may be clinically useful in the identification of Korean SLE patients who may be at a high risk for venous/arterial thrombosis.

REFERENCES

1. Sammaritano LR, Gharavi AE, Lockshin MD. Antiphospholipid antibody syndrome: immunologic and clinical aspects. *Semin Arthritis Rheum* 1990;20:81-96.
2. Love PE and Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythemato-

- sus (SLE) and in non-SLE disorders. Prevalence and clinical significance. *Ann Intern Med* 1990;112:682-98.
3. Uthman I, Godeau B, Taher A, Khamashta M. The hematologic manifestations of the antiphospholipid syndrome. *Blood Rev* 2008;22:187-94.
 4. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006;4:295-306.
 5. Galli M, Luciani D, Bertolini G, Barbui T. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. *Blood* 2003;101:1827-32.
 6. Galli M and Barbui T. Antiphospholipid antibodies and pregnancy. *Best Pract Res Clin Haematol* 2003;16:211-25.
 7. Somers E, Magder LS, Petri M. Antiphospholipid antibodies and incidence of venous thrombosis in a cohort of patients with systemic lupus erythematosus. *J Rheumatol* 2002;29:2531-6.
 8. Tsutsumi A, Matsuura E, Ichikawa K, Fujisaku A, Mukai M, Kobayashi S, et al. Antibodies to beta 2-glycoprotein and clinical manifestations in patients with systemic lupus erythematosus. *Arthritis Rheum* 1996;39:1466-74.
 9. Sahin M, Duzgun N, Tunc SE, Tutkac H. Antibodies to beta2-glycoprotein-I: relation of anticardiolipin antibodies with clinical and laboratory parameters in patients with systemic lupus erythematosus. *Clin Biochem* 2007;40:526-30.
 10. Mok CC, Tang SS, To CH, Petri M. Incidence and risk factors of thromboembolism in systemic lupus erythematosus: a comparison of three ethnic groups. *Arthritis Rheum* 2005;52:2774-82.
 11. Lee SS, Cho ML, Joo YS, Kim WU, Hong YS, Min JK, et al. Isotypes of anti-beta2-glycoprotein I antibodies: association with thrombosis in patients with systemic lupus erythematosus. *J Rheumatol* 2001;28:520-4.
 12. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
 13. Ghirardello A, Doria A, Ruffatti A, Rigoli AM, Vesco P, Calligaris A, et al. Antiphospholipid antibodies (aPL) in systemic lupus erythematosus. Are they specific tools for the diagnosis of aPL syndrome? *Ann Rheum Dis* 1994;53:140-2.
 14. Petri M. Epidemiology of the antiphospholipid antibody syndrome. *J Autoimmun* 2000;15:145-51.
 15. Danowski A, Kickler TS, Petri M. Anti-beta2-glycoprotein I: prevalence, clinical correlations, and importance of persistent positivity in patients with antiphospholipid syndrome and systemic lupus erythematosus. *J Rheumatol* 2006;33:1775-9.
 16. Mok MY, Chan EY, Fong DY, Leung KF, Wong WS, Lau CS. Antiphospholipid antibody profiles and their clinical associations in Chinese patients with systemic lupus erythematosus. *J Rheumatol* 2005;32:622-8.
 17. Lee SG, Cha HS, Yang YS. The association of HLA-DRB1 and DQB1 alleles and a study of anticardiolipin antibody and anti-beta2 glycoprotein I antibody in Korean SLE patients. *Immune Netw* 2002;2:227-32.
 18. Swadźba J, Iwaniec T, Szczeklik A, Musiał J. Revised classification criteria for antiphospholipid syndrome and the thrombotic risk in patients with autoimmune diseases. *J Thromb Haemost* 2007;5:1883-9.
 19. Scientific and Standardization Committee. Lupus AntiCoagulant/Phospholipid-dependent antibodies. Annual Report. Boston: International Society for Thrombosis and Haemostasis, 2002.
 20. Obermoser G, Bitterlich W, Kunz F, Sepp NT. Clinical significance of anticardiolipin and anti-β2-glycoprotein I antibodies. *Int Arch Allergy Immunol* 2004;135:148-53.
 21. Nash MJ, Camilleri RS, Kunka S, Mackie IJ, Machin SJ, Cohen H. The anticardiolipin assay is required for sensitive screening for antiphospholipid antibodies. *J Thromb Haemost* 2004;2:1077-81.
 22. Martinez-Berriotxo A, Ruiz-Irastorza G, Egurbide MV, Garmendia M, Gabriel Erdozain J, Villar I, et al. Transiently positive anticardiolipin antibodies and risk of thrombosis in patients with systemic lupus erythematosus. *Lupus* 2007;16:810-6.
 23. Arvieux J, Roussel B, Jacob MC, Colomb MG. Measurement of antiphospholipid antibodies by ELISA using β2-glycoprotein I as an antigen. *J Immunol Methods* 1991;143: 223-9.
 24. Lee EY, Lee CK, Lee TH, Chung SM, Kim SH, Cho YS, et al. Does the anti-beta2-glycoprotein I antibody provide additional information in patients with thrombosis? *Thromb Res* 2003;111:29-32.
 25. Ebeling F, Pettersson T, Muukkonen L, Vahtera E, Rasi V. Beta-2-glycoprotein I antibodies in patients with thrombosis. *Scand J Clin Lab Invest* 2003;63:111-8.
 26. Stasi R, Stipa E, Masi M, Oliva F, Sciarra A, Perrotti A, et al. Prevalence and clinical significance of elevated antiphospholipid antibodies in patients with idiopathic thrombocytopenic purpura. *Blood*

1994;84:4203-8.

27. Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps MT, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1000 patients. *Arthritis Rheum* 2002;46:1019-27.
28. Kim TM, Kim JS, Han SW, Hong YS, Kim I, Ha J, et al. Clinical predictors of recurrent venous thromboembolism: a single institute experience in Korea. *Thromb Res* 2009;123:436-43.