Phospholipid-Dependent Anti- β_2 -Glycoprotein I (β_2 -GPI) Antibodies and Antiphospholipid Syndrome

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A portion of anticardiolipin antibodies is defined as phospholipid-dependent anti- β_2 -glycoprotein I (β_2 -GPI) antibodies and recognizes the conformationally altered β_2 -GPI which interacts with anionic phospholipids. We studied the clinical significance of IgG phospholipid-dependent anti- β_2 -GPI antibodies in patients with antiphospholipid syndrome (APS). The subjects consisted of 60 APS patients. IgG phospholipid-dependent anti- β_2 -GPI antibodies were detected by ELISA in 32 of the 60 patients (53%). Significantly higher incidences of prolonged APTT and lupus anticoagulants were found in patients with these anti- β_2 -GPI antibodies. Moreover, significantly lower incidences of malar rash, serositis, LE cell preparation and anti-Sm antibodies were found in patients with these anti- β_2 -GPI antibodies. It was found that 88% of the patients with these anti- β_2 -GPI antibodies satisfied less than five of the revised criteria items for the classification of SLE. These findings indicate the clinical characteristics of APS patients with IgG phospholipid-dependent anti- β_2 -GPI antibodies.

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Introduction

The paradoxical phenomenon of lupus anticoagulants (LA) in systemic lupus erythematosus (SLE) patients with thrombosis has generated intense interest (1). An association has been found between the presence of antiphospholipid antibodies such as anticardiolipin antibodies (aCL) and LA, and clinical features such as thrombosis, intrauterine fetal loss, and thrombocytopenia. Thus, the concept of antiphospholipid syndrome (APS) has been proposed (2–6). It is well-known that IgG is the predominant isotype related to these clinical manifestations (7–9).

Recent studies have shown that patients with APS can be classified into two subsets: patients with primary APS who do not have any underlying collagen diseases, and patients with secondary APS based on a definite diagnosis of a collagen disease (10–13). However, the differences or overlapping features in patients with primary APS and secondary APS based on SLE have not been clarified in terms of their clinical aspects. On the other hand, the serological heterogeneity of aCL has been documented (14–16). β_2 -glycoprotein I (β_2 -GPI)-dependent

aCL have been defined as a portion of aCL which requires β_2 -GPI as a cofactor for binding to cardiolipin in patients with SLE (16, 17). These β_2 -GPI-dependent aCL react with the complex of β_2 -GPI and cardiolipin, and recognize an epitope appearing on conformationally altered β_2 -GPI (17, 18), or clustered β_2 -GPI at high density (19). The important roles of β_2 -GPI as an antigen have been reviewed by Roubey (20). The controversy regarding whether so-called aCL bind to β_2 -GPI has been eliminated by the subsequent reports (21–23). We, therefore, use the term “phospholipid-dependent anti- β_2 -GPI antibodies” in this study. It is possible that phospholipid-dependent anti- β_2 -GPI antibodies inhibit the function of β_2 -GPI such as the inhibition of prothrombinase activity (24), and that this phenomenon leads to thrombotic events.

We previously reported that IgG phospholipid-dependent anti- β_2 -GPI antibodies are found in sera from patients with SLE as well as from patients with primary APS (25), and that these phospholipid-dependent anti- β_2 -GPI antibodies are associated with thrombosis in patients with SLE and lupus-like disease (26). The clinical significance of these anti- β_2 -GPI antibodies has been elucidated in patients with SLE (27). However, the

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frequency of IgG phospholipid-dependent anti- β_2 -GPI antibodies was 15% in patients with SLE, whereas the frequency of IgG aCL by conventional enzyme-linked immunosorbent assay (ELISA) was 44% in the same patients (27). Thus, some patients with APS have phospholipid-dependent anti- β_2 -GPI antibodies, and others do not.

In this study, we extended our investigation to clarify the clinical significance of IgG phospholipid-dependent anti- β_2 -GPI antibodies in patients with APS.

Patients and Methods

Patients

The subjects consisted of 60 Japanese patients with APS (49 females and 11 males; mean age 35.8 ± 11.7 years). They were diagnosed as having APS according to the previously published criteria (6). Thrombosis, intrauterine fetal loss and thrombocytopenia were found in 28, 18 and 38 of these patients, respectively. IgG aCL measured by conventional ELISA were positive in sera from all patients.

Forty-two patients satisfied the revised criteria for the classification of SLE established by the American Rheumatism Association (ARA: American College of Rheumatology) (28). Patients with SLE were considered clinically active according to the criteria of Schur and Sandson (29).

Medical records were retrospectively reviewed. Serum samples from each patient were kept at -20°C until use.

Enzyme-linked immunosorbent assay

Sera were examined for IgG phospholipid-dependent anti- β_2 -GPI antibodies by the previously described ELISA (16, 18, 25). Human β_2 -GPI was purified by combined column chromatography (17), and was used as a cofactor in this assay. Purity was confirmed by analytical electrophoresis on SDS-polyacrylamide gel (16). The activity of these antibodies was expressed as units relative to the standard serum. When antibody activity was enhanced by the addition of β_2 -GPI at 3.5 unit/ml or more, the serum was determined to be positive for IgG phospholipid-dependent anti- β_2 -GPI antibodies, as in the previously published article (16). This 3.5 unit/ml represented the mean value plus six times the standard deviation in sera from 283 healthy controls.

The reliability of this ELISA was tested by intraassay, interassay and recovery tests. In the intraassay, the antibody activity was measured in five different lots of the same sample. In the interassay, the antibody activity in the same sample was measured five different times. In the recovery test, different sera of various known titers were used: sample A was added to an equal volume of sample B, and whole antibody activity was measured. Results (%) were calculated as follows:

$$\text{whole antibody activity} - \frac{\text{antibody activity of sample A}}{\text{antibody activity of sample B}} \times 100$$

IgG aCL were screened by conventional ELISA according

to the previously described method (30). The activity of IgG aCL was expressed as units relative to the standard serum. The cutoff point was 17 unit/ml, which was the mean value plus six times the standard deviation in sera from healthy controls.

Statistical analysis

The relationship between serum antibodies and clinical manifestations was determined by Fisher's exact test. A probability value of $p < 0.05$ was considered statistically significant.

Results

Reliability of ELISA for phospholipid-dependent anti- β_2 -GPI antibodies

The results for the two samples in the intraassay were as follows: means $\pm$ SD, 34.4 ± 3.1 unit/ml and 15.8 ± 1.4 unit/ml; CV, 8.9% and 8.7%. The results for the two samples in the interassay were as follows: means $\pm$ SD, 30.0 ± 1.5 unit/ml and 15.2 ± 1.0 unit/ml; CV, 5.0% and 6.3%. In the recovery test, the results ranged from 86.4% to 110.0%, with a mean rate of 98.6%.

Frequency of IgG phospholipid-dependent anti- β_2 -GPI antibodies

IgG phospholipid-dependent anti- β_2 -GPI antibodies were detected in 32 of the 60 patients (53%) with APS. Sera from all patients with IgG phospholipid-dependent anti- β_2 -GPI antibodies reacted positively for IgG aCL by conventional ELISA.

Clinical features of APS patients with phospholipid-dependent anti- β_2 -GPI antibodies

These 60 APS patients were divided into two groups: 32 patients with IgG phospholipid-dependent anti- β_2 -GPI antibodies and 28 patients without anti- β_2 -GPI antibodies. There were no significant differences in age, female to male ratio and dosage of prednisolone in the patients with and without phospholipid-dependent anti- β_2 -GPI antibodies (Table 1).

The incidences of thrombosis, intrauterine fetal loss and thrombocytopenia were not significantly different. However, significantly lower incidences of malar rash and serositis were found in patients with phospholipid-dependent anti- β_2 -GPI antibodies than in those without anti- β_2 -GPI antibodies. The incidence of renal disorder was lower in patients with anti- β_2 -GPI antibodies, but the difference was not significant.

Eighteen (56%) of the 32 patients positive for phospholipid-dependent anti- β_2 -GPI antibodies and 24 (86%) of the 28 patients negative for anti- β_2 -GPI antibodies were diagnosed as having SLE according to the revised criteria for the classification by the ARA (28). This observation was confirmed by the cumulative incidences of the revised criteria items (Fig. 1). It was found that 88% of the patients with phospholipid-dependent anti- β_2 -GPI antibodies satisfied less than 5 revised criteria items, and that the cumulative incidences for less than 3, 4 and 5 of the revised criteria items were significantly different in patients with and without phospholipid-dependent anti- β_2 -GPI antibodies.

We found nine patients with APS based on SLE who fulfilled

Table 1. Clinical Characteristics of Antiphospholipid Syndrome (APS) Patients with IgG Phospholipid-dependent Anti- β_2 -glycoprotein I (β_2 -GPI) Antibodies

Clinical manifestations	Positive for phospholipid-dependent anti- β_2 -GPI (n=32)	Negative for phospholipid-dependent anti- β_2 -GPI (n=28)	p value
Age (years)*	34.6 $\pm$ 10.6	37.2 $\pm$ 12.8	n.s.
Female to male ratio	29 : 3	20 : 8	n.s.
Dosage of prednisolone (mg/day)*	18.2 $\pm$ 17.1	16.5 $\pm$ 15.3	n.s.
Thrombosis	14 (44%)	14 (50%)	n.s.
Intrauterine fetal loss**	14/24 (58%)	4/14 (29%)	n.s.
Thrombocytopenia	20 (63%)	18 (64%)	n.s.
Malar rash	8 (25%)	17 (61%)	<0.01
Serositis	1 (3%)	8 (29%)	<0.05
Renal disorder	12 (38%)	17 (61%)	<0.10
Prolonged APTT	24 (75%)	7 (25%)	<0.01
BFP-STs	18 (56%)	9 (32%)	<0.10
LE cell preparation	9 (28%)	15 (54%)	<0.05
Anti-Sm antibodies	1 (3%)	6 (21%)	<0.05

*Results are expressed as mean value $\pm$ standard deviation. **The incidence of intrauterine fetal loss was determined in pregnant patients.

four of the revised criteria items for the classification of SLE by the ARA (28), and had IgG phospholipid-dependent anti- β_2 -GPI antibodies (Table 2). All patients had a hematological disorder, and sera from eight patients revealed an immunological disorder and positive reactions for antinuclear antibodies (ANA). Six of these patients fulfilled a combination of revised criteria items such as hematological disorder, immunological disorder, ANA, and arthritis.

Serological features of APS patients with phospholipid-dependent anti- β_2 -GPI antibodies

All patients reacted positively for IgG aCL measured by conventional ELISA. The mean titer of IgG aCL measured by

Figure 1. Cumulative incidences of revised criteria items for the classification of SLE. Cumulative incidences of revised criteria items for the classification of SLE in patients with IgG phospholipid-dependent anti- β_2 -glycoprotein I (β_2 -GPI) antibodies (●—●) and in those without anti- β_2 -GPI antibodies (○---○) are shown. The incidence of fulfillment of less than three, four and five criteria items was significantly higher (*: $p < 0.05$, **: $p < 0.01$) in patients with IgG phospholipid-dependent anti- β_2 -GPI antibodies than in those without these anti- β_2 -GPI antibodies.

conventional ELISA was 50.4 $\pm$ 31.4 unit/ml in patients with phospholipid-dependent anti- β_2 -GPI antibodies and 26.0 $\pm$ 9.2 unit/ml in those without these anti- β_2 -GPI antibodies. This difference was significant ($p < 0.01$) (Fig. 2).

A significantly higher incidence of prolonged APTT was found in patients with phospholipid-dependent anti- β_2 -GPI antibodies than in those without these anti- β_2 -GPI antibodies (Table 1). LA, which were examined by the cross mixing test of diluted APTT and the Staclot-LA test (Diagnostica Stago, France), were detected in 17 (53%) of the 32 patients with phospholipid-dependent anti- β_2 -GPI antibodies and in 4 (14%) of the 28 patients without these anti- β_2 -GPI antibodies. This difference was significant ($p < 0.025$). The frequency of biologically false positive serological tests for syphilis (BFP-STs) was higher in patients with phospholipid-dependent anti- β_2 -GPI antibodies, but the difference was not significant.

However, the frequencies of LE cell preparation and anti-Sm antibodies were significantly lower in patients with phospholipid-dependent anti- β_2 -GPI antibodies than in those without these anti- β_2 -GPI antibodies.

Discussion

The original report noted that the majority of patients with anticardiolipin syndrome, that is APS, had "atypical" lupus (3).

Table 2. Revised Criteria Items for the Classification of SLE in Patients with Antiphospholipid Syndrome (APS) Who Satisfied Four Criteria Items and had IgG Phospholipid-dependent Anti- β_2 -glycoprotein I (β_2 -GPI) Antibodies

Number of patients	Hematological disorder	Immunological disorder	ANA	Arthritis	Photo-sensitivity	Renal disorder	Malar rash
6	+	+	+	+	(-)	(-)	(-)
1	+	+	+	(-)	+	(-)	(-)
1	+	+	+	(-)	(-)	+	(-)
1	+	(-)	(-)	+	(-)	+	+

In hematological disorders, hemolytic anemia, leucopenia and thrombocytopenia were found in 2, 5 and 5 patients, respectively. In immunological disorders, positive reactions for LE cell preparation, anti-nDNA antibodies and BFP-STs were detected in 2, 8 and 4 patients, respectively. Anti-Sm antibodies were negative in all patients. The titers of ANA in patients with positive results were greater than a 128 serum dilution by the indirect immunofluorescent method using HEp-2 cells as substrate.

Figure 2. Titers of IgG anticardiolipin antibodies (aCL) in patients with antiphospholipid syndrome (APS). Dots represent individual patients, and the horizontal lines indicate the mean value plus standard deviation of IgG aCL. The titers of IgG aCL were significantly higher in patients with IgG phospholipid-dependent anti- β_2 -glycoprotein I (β_2 -GPI) antibodies than in those without these anti- β_2 -GPI antibodies.

Recent studies have shown that patients with APS are divided into two subsets: those with primary APS who do not have any underlying collagen diseases, and those with secondary APS based on a definite diagnosis such as SLE (10–13). However, differences or overlapping features in patients with primary APS and secondary APS based on SLE have been a source of controversy (31–37). Some investigators have argued that it is difficult to distinguish primary APS from secondary APS based on SLE on the basis of the revised criteria for the classification of SLE by the ARA alone (31, 34, 36, 37). One reason for this problem is that thrombotic events in patients with APS cause serositis, renal disorder, and neurological disorder, which are listed as the revised criteria items. Hematological disorders such as hemolytic anemia and thrombocytopenia, and immunological disorders such as BFP-STs, and low titer of ANA are also found in patients with APS.

On the other hand, it was reported that the 1982 revised criteria for the classification of SLE by the ARA were 96% sensitive and 96% specific for SLE (28). However, as the disease controls were rheumatoid arthritis, scleroderma, polymyositis and dermatomyositis in this study, APS may be distributed in both SLE patients and non-SLE patients according to these revised criteria. Thus, another serological marker should be studied to distinguish APS patients from definite SLE patients.

aCL represent one of the serological features for the diagnosis of APS (2–6). IgG is the most frequent isotype of aCL in these patients (7–9). Recent studies have revealed heterogeneous characteristics of the aCL: a portion of aCL does not recognize the cardiolipin structure directly, but binds to the complex of cardiolipin and β_2 -GPI, specifically the conformationally altered β_2 -GPI, as a cofactor, especially in patients with SLE (17, 18). These antibodies also recognize clustered β_2 -GPI at high density on anionic phospholipids (19). These aCL have

been defined as β_2 -GPI-dependent aCL (17), and are referred to as phospholipid-dependent anti- β_2 -GPI antibodies in this study. We have already reported that these antibodies are found in sera from patients with SLE and primary APS (25). The clinical significance of these anti- β_2 -GPI antibodies has been studied in patients with SLE (25–27). Sustained positive reactions for IgG phospholipid-dependent anti- β_2 -GPI antibodies were observed over periods ranging from 4 to 98 months (27). However, the frequency of IgG phospholipid-dependent anti- β_2 -GPI antibodies was lower than that of IgG aCL by conventional ELISA (27). On the basis of these results, we investigated the clinical characteristics of patients with APS who had positive serological reactions for IgG phospholipid-dependent anti- β_2 -GPI antibodies.

Significantly higher incidences of prolonged APTT, LA and percentage of patients fulfilling less than five of the revised criteria items, and significantly lower incidences of malar rash, serositis, LE cell preparation and anti-Sm antibodies were observed in patients with IgG phospholipid-dependent anti- β_2 -GPI antibodies. The revised criteria items in these APS patients who satisfied four items suggested that they had an atypical or mild form of SLE.

The titers of IgG aCL by conventional ELISA were also higher in patients with IgG phospholipid-dependent anti- β_2 -GPI antibodies than in those without these anti- β_2 -GPI antibodies. This result suggests that the association between high titers of IgG aCL and thrombosis reported in previous articles (7, 9) may be due to the β_2 -GPI dependency of so-called aCL.

Thus, the results of the present study suggest that patients with APS who have IgG phospholipid-dependent anti- β_2 -GPI antibodies have unique clinical features, and represent the group of patients with APS, who were described in the original article (3). Recent studies have demonstrated that antiphospholipid antibodies require other cofactors such as prothrombin, protein C, and protein S (38, 39). The clinical significance of IgG anti-annexin V antibodies has been also studied in SLE patients with thrombosis (40). There is a possibility that so-called aCL which do not recognize conformationally altered β_2 -GPI may bind to these other cofactors. Further studies are therefore necessary to determine whether patients with APS can be classified into subsets on the basis of the cofactor dependency of antiphospholipid antibodies.

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References

- 1) Bowie EJW, Thompson JH Jr, Pascuzzi CA, Owen CA Jr. Thrombosis in systemic lupus erythematosus despite circulating anticoagulants. *J Lab Clin Med* **62**: 416, 1963.
- 2) Harris EN, Gharavi AE, Hughes GRV. Anti-phospholipid antibodies. *Clin Rheum Dis* **11**: 591, 1985.
- 3) Hughes GRV, Harris EN, Gharavi AE. The anticardiolipin syndrome. *J Rheumatol* **13**: 486, 1986.
- 4) Alarcón-Segovia D, Delezé M, Oria CV, et al. Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. *Medicine* **68**: 353, 1989.
- 5) Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. *Ann Intern Med* **112**: 682, 1990.
- 6) Harris EN. Antiphospholipid antibodies. *Br J Haematol* **74**: 1, 1990.
- 7) Harris EN, Chan JKH, Asherson RA, Aber VR, Gharavi AE, Hughes GRV. Thrombosis, recurrent fetal loss, and thrombocytopenia. Predictive value of the anticardiolipin antibody test. *Arch Intern Med* **146**: 2153, 1986.
- 8) Gharavi AE, Harris EN, Asherson RA, Hughes GRV. Anticardiolipin antibodies: isotype distribution and phospholipid specificity. *Ann Rheum Dis* **46**: 1, 1987.
- 9) Ishii Y, Nagasawa K, Mayumi T, Niho Y. Clinical importance of persistence of anticardiolipin antibodies in systemic lupus erythematosus. *Ann Rheum Dis* **49**: 387, 1990.
- 10) Asherson RA. A "primary" antiphospholipid syndrome? *J Rheumatol* **15**: 1742, 1988.
- 11) Asherson RA, Khamashta MA, Ordi-Ros J, et al. The "primary" antiphospholipid syndrome: major clinical and serological features. *Medicine* **68**: 366, 1989.
- 12) Alarcón-Segovia D, Sanchez-Guerrero J. Primary antiphospholipid syndrome. *J Rheumatol* **16**: 482, 1989.
- 13) Mackworth-Young CG, Loizou S, Walport MJ. Primary antiphospholipid syndrome: features of patients with raised anticardiolipin antibodies and no other disorder. *Ann Rheum Dis* **48**: 362, 1989.
- 14) McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. Antiphospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: β_2 -glycoprotein I (apolipoprotein H). *Proc Natl Acad Sci USA* **87**: 4120, 1990.
- 15) Galli M, Comfurius P, Maassen C, et al. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. *Lancet* **335**: 1544, 1990.
- 16) Matsuura E, Igarashi Y, Fujimoto M, Ichikawa K, Koike T. Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease. *Lancet* **336**: 177, 1990.
- 17) Matsuura E, Igarashi Y, Fujimoto M, et al. Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. *J Immunol* **148**: 3885, 1992.
- 18) Matsuura E, Igarashi Y, Yasuda T, Triplett DA, Koike T. Anticardiolipin antibodies recognize β_2 -glycoprotein I structure altered by interacting with an oxygen modified solid phase surface. *J Exp Med* **179**: 457, 1994.
- 19) Roubey RAS, Eisenberg RA, Harper MF, Winfield JB. "Anticardiolipin" autoantibodies recognize β_2 -glycoprotein I in the absence of phospholipid. Importance of Ag density and bivalent binding. *J Immunol* **154**: 954, 1995.
- 20) Roubey RAS. Autoantibodies to phospholipid-binding plasma proteins: A new view of lupus anticoagulants and other "antiphospholipid" autoantibodies. *Blood* **84**: 2854, 1994.
- 21) Harris EN, Goldsmith G, Pierangeli S, Gharavi AE, Branch W. Phospholipid binding antibodies warrant continued investigation. *Blood* **85**: 2276, 1995.
- 22) Koike T, Tsutsumi A, Ichikawa K, Matsuura E. Antigenic specificity of the "anticardiolipin" antibodies. *Blood* **85**: 2277, 1995.
- 23) Roubey RAS. Response to Harris et al and Koike et al. *Blood* **85**: 2278, 1995.
- 24) Nimf J, Bevers EM, Bomans PHH, et al. Prothrombinase activity of human platelets is inhibited by β_2 -glycoprotein-I. *Biochim Biophys Acta* **884**: 142, 1986.
- 25) Kaburaki J, Kuwana M, Yamamoto M, Kawai S, Matsuura E, Ikeda Y. Disease distribution of β_2 -glycoprotein I-dependent anticardiolipin antibodies in rheumatic diseases. *Lupus* **4**: S27, 1995.
- 26) Kaburaki J, Kuwana M, Ikeda Y, Yamamoto M, Kawai S, Matsuura E. Cofactor (β_2 -glycoprotein I)-dependent anticardiolipin antibodies and thrombosis. *J Rheumatol* **21**: 1371, 1994.

- 27) Kaburaki J, Kuwana M, Yamamoto M, Kawai S, Matsuura E, Ikeda Y. Clinical significance of phospholipid-dependent anti- β_2 -glycoprotein I (β_2 -GPI) antibodies in systemic lupus erythematosus. *Lupus* **4**: 472, 1995.
 - 28) Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* **25**: 1271, 1982.
 - 29) Schur PH, Sandson J. Immunologic factors and clinical activity in systemic lupus erythematosus. *N Engl J Med* **278**: 533, 1968.
 - 30) Taki M, Ikeda Y, Handa M, et al. Role of antiphospholipid antibodies in the pathogenesis of thrombosis in antiphospholipid syndrome. *Acta Haematol Jpn* **53**: 1620, 1990.
 - 31) Piette J-C, Wechsler B, Frances C, Godeau P. Systemic lupus erythematosus and the antiphospholipid syndrome: reflections about the relevance of ARA criteria. *J Rheumatol* **19**: 1835, 1992.
 - 32) Ehrenstein MR, Swana M, Keeling D, Asherson RA, Hughes GRV, Isenberg DA. Anti-DNA antibodies in the primary antiphospholipid syndrome (PAPS). *Br J Rheumatol* **32**: 362, 1993.
 - 33) Asherson RA. SLE and the antiphospholipid antibody syndrome. *J Rheumatol* **20**: 1801, 1993.
 - 34) Piette J-C, Wechsler B, Frances C, Papo T, Godeau P. Exclusion criteria for primary antiphospholipid syndrome. *J Rheumatol* **20**: 1802, 1993.
 - 35) Vianna JL, Khamashta MA, Ordi-Ros J, et al. Comparison of the primary and secondary antiphospholipid syndrome: a European multicenter study of 114 patients. *Am J Med* **96**: 3, 1994.
 - 36) Piette J-C. Primary antiphospholipid syndrome (APS) or systemic lupus erythematosus related APS: from theory to practice. *J Rheumatol* **21**: 1368, 1994.
 - 37) Asherson RA, Cervera R. "Primary", "Secondary" and other variants of the antiphospholipid syndrome. *Lupus* **3**: 293, 1994.
 - 38) Bevers EM, Galli M, Barbui T, Comfurius P, Zwaal RFA. Lupus anticoagulant IgG's (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. *Thromb Haemost* **66**: 629, 1991.
 - 39) Oosting JD, Derksen RHW, Bobbink IWG, Hackeng TM, Bouma BN, de Groot PG. Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C, or protein S: an explanation for their pathogenic mechanism? *Blood* **81**: 2618, 1993.
 - 40) Kaburaki J, Ikeda Y. Thrombosis in patients with SLE and antiphospholipid syndrome. *Rinsho Ketsueki (Jpn J Clin Haematol)* **36**: 320, 1995 (Abstract in English).
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