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REVIEW ARTICLE

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Antibody specificity and related clinical features in antiphospholipid syndrome

Abstract The concept of antiphospholipid syndrome (APS) has been widely accepted. Antiphospholipid antibodies originally included anticardiolipin antibodies and lupus anticoagulants. However, recent advances have shown that most pathogenic antiphospholipid antibodies are directed to phospholipid binding proteins such as β_2-glycoprotein I (β_2-GPI) and prothrombin. Other candidates for antigens of so-called antiphospholipid antibodies are protein C, protein S, factor V, factor X, annexin V, high and low molecular weight kininogens, and complex with β_2-GPI and oxidized low-density lipoprotein (LDL). These autoantibodies directed to different phospholipid binding proteins are present in the blood stream, and contribute to triggering procoagulant effects on endothelial cells and platelets, leading to thrombosis. The heterogeneity of these antiphospholipid antibodies appears to explain various clinical manifestations in patients with APS. The preliminary classification criteria for definite APS and a general management policy have been proposed, although successful treatment of patients with antiphospholipid antibodies have only been shown by retrospective studies. Further prospective investigations are required to confirm the diagnostic and therapeutic criteria for patients with APS.

Key words Anti-β_2-GPI antibodies · Anticardiolipin antibodies · Fetal loss · Lupus anticoagulants · Thrombosis

Introduction

The clinical significance of antiphospholipid antibodies and the concept of antiphospholipid syndrome (APS) are well known.[1–8] This syndrome is also called "Hughes' syndrome",[9] after the scientist who first reported the clinical significance of lupus anticoagulants (LA) and APS.[1–3,10,11] Antiphospholipid antibodies are significantly associated with thrombosis and fetal loss. At the present time, antiphospholipid antibodies are considered to be an acquired risk factor for thrombosis, especially in patients under 40 years old.[12]

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Specificity of antiphospholipid antibodies

Published research on antiphospholipid antibodies can conveniently be separated in to the periods before and after 1990.

Historical review of classical antiphospholipid antibodies

Investigations into antiphospholipid antibodies began with the Wassermann test in 1907, which was reviewed by Matsuura.[13] Then in 1941, Pangborn[14] identified cardiolipin as the essential antigenic component of tissue extracts used in this test. Persistent false-positive results for more than 6 months, i.e., chronic BFP-STS (biologically false-positive test for syphilis), was adopted in the revised criteria for the classification of systemic lupus erythematosus (SLE).[15]

In the early 1950s, acquired coagulation inhibitors were observed in patients with SLE, who showed prolonged phospholipid-dependent coagulation tests in vitro.[16] These inhibitors were subsequently termed lupus anticoagulants (LA).[17,18] However, in 1963, Bowie et al.[19] reported the increased incidence of thrombosis in vivo in SLE patients who had circulating anticoagulants or LA. In 1983, Hughes et al.[10,11] reported the significant association between LA and clinical features such as thrombosis.

Later, solid-phase immunoassays for detecting anticardiolipin antibodies were developed.[20,21] Then, clinical investigations into antiphospholipid antibodies were revolutionized by the enzyme-linked immunosorbent assay

(ELISA). As a consequence, the new concept of anticardiolipin syndrome was proposed in 1986.[1] However, as antibodies directed to other negatively charged phospholipids such as phosphatidylserine were found, the term, "antiphospholipid syndrome" (APS) was advocated by Hughes and Harris.[2,3] Arterial or venous thrombosis, fetal loss, and thrombocytopenia were listed as clinical features of APS. This clinical significance of antiphospholipid antibodies was confirmed by the excellent review of Love and Santoro in 1990,[22] although only anticardiolipin antibodies and LA were measured.

Heterogenous specificities of antiphospholipid antibodies

After 1990, a series of publications reported that the major group of autoantibodies detected in anticardiolipin assays were directed not only to phospholipids, but also to phospholipid binding plasma proteins and/or complexes of these proteins with phospholipids in patients with APS.

β_2-Glycoprotein I (β_2-GPI)

In 1990, three groups of investigators reported that so-called anticardiolipin antibodies did not bind to solid-phase cardiolipin or cardiolipin liposomes, but did bind to serum/plasma cofactor, β_2-glycoprotein I (β_2-GPI), or complexes of β_2-GPI and cardiolipin.[23–26] β_2-GPI, a 50-kd glycoprotein, consists of 326 amino acids, and is a noncomplement member of the complement control protein family, which has five consensus repeats, or so-called sushi domains.[27] The fifth domain contains a major phospholipid binding region.[27,28] Cryptic epitope is expressed by the conformational change when β_2-GPI interacts with negatively charged phospholipids or an oxygen-substituted solid-phase surface by irradiation.[29] Major epitopes occur in the fourth domain, but they are reported to exist in the fifth and first domains also.[27,30,31] It was also shown that anti-β_2-GPI antibodies had low intrinsic affinity, and high-avidity binding to β_2-GPI was dependent on a multivalent attachment to immobilized antigen.[32]

The plasma level of β_2-GPI is approximately 200 μg/ml in healthy people, and is under genetic control.[33] There is an interesting paper which reports that valine/leucine polymorphism at position 247 of β_2-GPI affects the reactivity of anti-β_2-GPI antibodies.[34] Autoimmune anti-β_2-GPI antibodies in SLE patients have a stronger reactivity to valine^{247} β_2-GPI than to leucine^{247} β_2-GPI. It is supposed that the substitution of amino acid at the 247 position alters the interaction between the fourth and fifth domains, leading to the reactivity of autoantibodies against β_2-GPI, as this position is located at the beginning of the fifth domain.

Characterization of the T cell response induced by β_2-GPI in vitro revealed that the response was associated with the presence of the DR53-associated alleles, the responding T cells were $CD4^+$ and were restricted by HLA class II, and antigenic peptides were located in the fourth and/or fifth domains.[35] Moreover, it was recently reported that the major repertoire of β_2-GPI reactive $CD4^+$ T cells represented restricted antigen recognition and a cytokine profile such as IL-6,[36] and that T cell receptor β-chains of β_2-GPI-reactive T cells were highly restricted in patients with APS.[37]

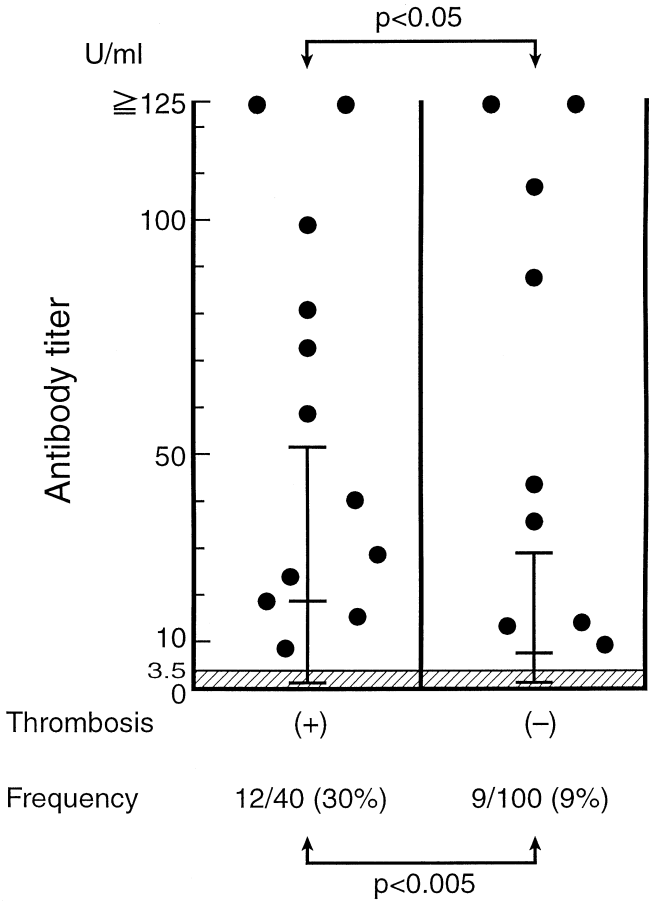

Fig. 1. Thrombosis and IgG β_2-GPI-dependent anticardiolipin antibodies in patients with systemic lupus erythematosus (SLE). One hundred and forty patients with SLE were divided into two groups: 40 patients with thrombosis and 100 without thrombosis. The frequency of IgG β_2-GPI-dependent anticardiolipin antibodies was siginificantly higher in patients with thrombosis than in those without thrombosis. Moreover, the titer of these antibodies in patients with thrombosis was 18.3 ± 34.1 U/ml, which was significantly higher than 7.2 ± 22.2 U/ml in those without thrombosis

An ELISA kit to detect serum IgG anti-β_2-GPI antibodies is commercially available. In this kit, antigen is immobilized on plates by adding β_2-GPI to cardiolipin-coated plates, and the antibodies detected are β_2-GPI-dependent anticardiolipin antibodies, or anti-β_2-GPI-cardiolipin complex antibodies (phospholipid-dependent anti-β_2-GPI antibodies) in Japan.[38–42]

Reports on the clinical significance of β_2-GPI-dependent anticardiolipin antibodies have been published.[38–41] These antibodies are significantly associated with thrombosis, fetal loss, and other clinical features of APS. Figure 1 shows the significant association between IgG β_2-GPI-dependent anticardiolipin antibodies and thrombosis in 140 patients with SLE. Recently, another ELISA kit has been developed using β_2-GPI coated on irradiated plates as the antigen.

The usefulness of this kit should be evaluated in the near future, but it has been reported that anti-β_2-GPI antibodies examined by irradiated plates were also associated with thrombosis.[43]

Prothrombin

It was suggested that antibodies directed to prothrombin were associated with hypoprothrombinemia and bleeding complications in patients with SLE.[44] Several ELISAs to detect antiprothrombin antibodies were later developed, although the standardized assays are not yet commercially available. Prothrombin is coated on plates in two different ways: prothrombin coated on irradiated or highly activated plates,[45] and prothrombin with phosphatidylserine coated on plates.[46] Antiprothrombin antibodies may be of intrinsically low affinity and/or recognize conformational epitopes formed when prothrombin binds to anionic phospholipids.

A review of retrospective studies revealed that IgG and/or IgM antiprothrombin antibodies were associated with thrombosis, but the sensitivity against arterial thrombosis was relatively low.[47] It is necessary to investigate which isotype of antiprothrombin antibodies and which prothrombin coated on plates by irradiation or complex with phosphatidylserine as antigen are more useful in the diagnosis of APS.

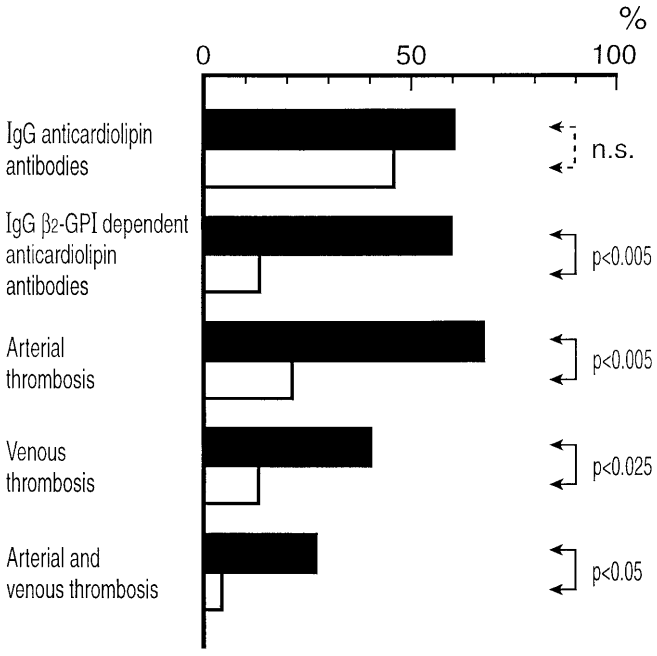

Fig. 2. Clinical siginificance of lupus anticoagulants (LA) in patients with SLE. LA were examined in 67 patients with SLE. LA were not associated with IgG anticardiolipin antibodies, but were significantly associated with IgG β_2-GPI-dependent anticardiolipin antibodies and thrombosis. *Solid bars*, LA positive (n = 15); *open bars*, LA negative (n = 52)

Lupus anticoagulants

Lupus anticoagulants (LA) were measured by various methods, and it was found that the frequency of LA ranged from 6% to 52% in patients with SLE.[6] However, the Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis published their criteria for the diagnosis of LA in 1995.[48] These criteria are listed below.

1. Demonstration of an abnormal phospholipid-dependent screening test for hemostasis such as prolonged activated partial thromboplastin time (APTT), diluted Russell viper venom time (dRVVT), and kaolin clotting time (KCT).
2. Failure to correct the prolonged screening coagulation test upon mixing with normal platelet-poor plasma.
3. Shortening or correction of the prolonged screening test value on the addition of excess phospholipids or hexagonal-phase phospholipids.
4. Other coagulopathies such as factor VIII inhibitors and the use of heparin have been ruled out.

LA are considered to be heterogenous in terms of antigen recognition.[30,49–51] Some studies showed that subpopulations of anti-β_2-GPI antibodies as well as antiprothrombin antibodies had lupus anticoagulant activity in assays such as APTT and dRVVT. It was reported that antibodies to the third or fourth domain of β_2-GPI demonstrated LA activity, but antibodies to the fifith domain of β_2-GPI did not.[30] A recent study revealed that dRVVT is more sensitive in detecting the LA activity of anti-β_2-GPI antibodies than KCT or tissue thromboplastin inhibition (TTI).[52] Seven test kits for dRVVT are commercially available in the United States.[53] One of them, LA screen (Gradipore), is also available in Japan. The frequency of LA was 29% in our 67 patients with SLE when examined by APTT, and cross-mixing tests using diluted APTT and the LA screen kit (Gradipore). LA are significantly associated with IgG β_2-GPI-dependent anticardiolipin antibodies and thrombotic events in these patients (Fig. 2).

Other phospholipid-binding proteins

Many investigators have reported that so-called antiphospholipid antibodies are actually directed to other phospholipid binding proteins. Thus, the term "antiphospholipid–protein antibodies" was advocated.[54] It was shown that a group of these antibodies was directed to a protein C pathway such as protein C and protein S.[55] Antibodies to factor V were also detected.[56]

Annexin V has been reported to be placental anticoagulant I.[57] Annexin V has a high calcium-dependent binding affinity for negatively charged phospholipids and blood plateletes, and shows in vitro anticoagulant effects. Anti-annexin V antibodies examined by ELISA showed a siginificant association with thrombosis, fetal loss, and prolonged APTT in patients with SLE.[58–60]

It was shown that anti-phosphatidylethanolamine antibodies were also predictive for recurrent fetal losses.[61] High and low molecular weight kininogens are necessary for the

binding sites of pathogenic anti-phosphatidylethanolamine antibodies.[62]

Epitopes of anti-β_2-GPI antibodies occur on the β_2-GPI molecule when $CuSO_4$-oxidized low-density lipoprotein (oxLDL) binds to β_2-GPI,[63,64] whereas it was reported that antiphospholipid antibodies could bind to oxidized low-density lipoprotein.[65] These studies revealed that immune responses against β_2-GPI could induce atherogenic effect in in vitro oxLDL uptake by macrophages, and that this phenomenon might lead to atherosclerosis in patients.

Antibodies to malondialdehyde-modified lipoprotein (a) were also reported in patients with APS, and were associated with the atherosclerosis.[66]

There remains a possibility that inhibitors or antibodies to von Willebrand factor-cleaving protease play a role in the mechanism of thrombosis in some patients with catastrophic APS.[67]

Antiendothelial antibodies

Some groups of autoantibodies were shown to bind to endothelial cells,[68] and those were reviewed by Meroni et al.[69]

Thrombomodulin is an endothelial cell receptor for thrombin, and plays a critical role in protein C activation. Vascular heparan sulfate proteoglycan, which is expressed on endothelial cells, plays an important role in vascular structure and function such as hemostasis. It has been demonstrated that both antibodies to thrombomodulin and antibodies to heparan sulfate or heparin were detected in sera from patients with SLE.[70–72] Moreover, a recent study revealed that β_2-GPI could adhere to human endothelial cell membranes and offer a suitable epitope for circulating anti-β_2-GPI antibodies in the blood.[69] It is believed that anti-β_2-GPI antibodies have the potential to induce endothelial cell activation when they bind to the adhered β_2-GPI. This phenomenon was observed by increased adhesion molecule expression and the upregulation of cytokine secretion and arachidonic acid metabolism. Therefore, it is postulated that antiphospholipid antibodies such as anti-β_2-GPI induce proinflammatory and procoagulant endothelial phenotypes.

Diagnosis

The concept of APS has been already established.[2–9] It was shown that APS occurred in a small group of unselected patients with stroke or transient ishemic attacks, irrespective of SLE.[73] Recurrent idiopathic fetal losses in apparently healthy women were reported to be attributable to APS in approximately 5%–15% of cases.[22] These patients, who had no underlying diseases, were diagnosed as having primary APS. The majority of patients with secondary APS have SLE as an underlying disease. Anticaridiolipin antibodies are positive in approximately 40% of patients with SLE, and LA are detected in approximately 30% of these patients. Harris and Hughes[2,3] proposed the original diagnostic criteria for APS. They adopted arterial or venous thrombosis, recurrent fetal losses, and thrombocytopenia as the clinical features, and mentioned the positive results of IgG anticardiolipin antibodies and LA in the serological features.

During the last 10 years, it has been reported that IgM anticardiolipin antibodies predict clinical feature of APS such as thrombosis,[4,74] and that thrombocytopenia in patients with APS can be caused by antiplatelet membrane glycoprotein antibodies.[75]

The new preliminary classification criteria for definite APS, the "Sapporo criteria," were proposed in 1999.[76] Vascular thrombosis and pregnancy morbidity were listed as clinical criteria. Vascular thrombosis means one or more clinical episodes of arterial, venous, or small-vessel thrombosis in any tissue or organ. Thrombosis must be confirmed by imaging or Doppler studies, or by histopathology. Pregnancy morbidity includes (a) one or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or (b) one or more premature births of a morphologically normal neonate at or before the 34th week of gestation because of severe preeclampsia or eclampsia, or severe placental insufficiency, or (c) three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, in which maternal anatomic or hormonal abnormalities, and paternal and maternal chromsomal causes were excluded. β_2-GPI-dependent anticardiolipin antibodies of IgG and/or IgM isotype and lupus anticoagulants were listed as laboratory criteria. Positive results on two or more occasions were required, at least 6 weeks apart. Definite APS is considered to be present if at least one of the clinical criteria and one of the laboratory criteria are met. The validity of the Sapporo criteria was confirmed elsewhere.[77] However, it is necessary to investigate whether other clinical features such as cardiac valvular diseases and livedo reticularis, and other immunological features such as IgA anticardiolipin antibodies and antibodies directed to phospholipid-binding proteins other than β_2-GPI, should be included in the criteria or not in the future.

Mechanism

Thrombosis

A "two-hit phenomenon" was proposed as the mechanism of thrombosis in patients with APS.[78–80] Autoantibodies to phospholipids or phospholipid-binding proteins are continually present in the circulating blood in the first hit, and then a local trigger against coagulation and/or fibrinolysis is required at the sites of endothelial cells and platelets in the second hit. In this model, high-affinity neutralizing antibodies directed to phospholipid-binding proteins may directly inhibit the antigen's function, e.g., the prothrombinase activity of β_2-GPI, and decrease the plasma antigen levels through the clearance of antigen–antibody complexes. This hypothesis has been demonstrated in animal models of APS which were developed by the passive trans-

Table 1. Mechanisms of thrombosis and related so-called antiphospholipid antibodies and other antibodies reported in antiphospholipid syndrome

Mechanisms	Antibodies involved
1. Inhibition of the protein C pathway	
Protein C activation	Anti-protein C, anti-thrombomodulin
Inactivation of factors Va and VIIIa	Anti-protein C, anti-protein S, anti-β_2-GPI
2. Inhibition of antithrombin III	Anti-heparan sulfate, anti-heparin, anti-β_2-GPI
3. Impaired fibrinolysis	Anti-β_2-GPI, anti-PE/HMWK
4. Increase monocyte tissue factor expression	Anti-β_2-GPI
5. Increased expression of adhesion molecules on endothelial cells	Anti-β_2-GPI
6. Enhanced platelet activation	Anti-β_2-GPI, anti-CD36
7. Increased platelet thromboxane A_2	Anti-β_2-GPI
8. Inhibition of β_2-GPI	Anti-β_2-GPI
9. Displacement of annexin V	Anti-β_2-GPI, anti-annexin V

PE/HMWK, phosphatidylethanolamine/high molecular weight kininogen

fer of immunoglobulins from patients with APS or monoclonal anticardiolipin antibodies.[81,82] On the other hand, intrinsically low-affinity antibodies to phospholipid-binding proteins may bind with high avidity, via bivalent or multivalent attachments, to antigens on the cell membrane. Alternatively, when the antigens are bound to receptor proteins on the cell surface, cross linking by these antibodies to phospholipid-binding proteins may trigger signal transductions and cellular responses. For example, as mentioned before, it was observed that anti-β_2-GPI antibodies stimulated the upregulation of adhesion molecules on endothelial cells.[69]

There are many reports on the mechanism of thrombosis. Abnormal findings in coagulation and fibrinolysis were reviewed in patients with APS.[8,78–80] These different factors may be due to the various specificities of antibodies directed to phospholipid-binding proteins, leading to different clinical manifestations in patients with APS (Table 1). It has been suggested that antibodies which alter the prostacyclin/thromboxane balance are associated with arterial thrombosis, whereas antibodies which inhibit the protein C pathway are associated with venous thrombosis.

Fetal loss

Antiphosholipid antibodies are primarily associated with fetal death occuring in the second or third trimester.[4] Fetal loss is caused by hypoxia due to insufficient uteroplacental blood flow.[83] Placental infarction is the most important pathological finding in these patients,[84] although other findings include chorionic villitis and atherosis, and abnormal placental human chorionic gonadotropin (hCG) secretion may be related to fetal loss.[85] It is suggested that placental infarction results from decreased amounts of annexin V on the surface of placental villi in APS patients with recurrent fetal losses.[86]

Treatment

Thrombosis

The management of thrombosis in patients with APS has been reviewed in the literature,[87–89] although most of the results were obtained by retrospective studies.

The treatments for thrombosis in patients with APS are divided into three periods.

In the first period, treatment is considered for people with antiphospholipid antibodies in their sera, but who do not show any clinical features such as thrombosis or fetal loss. When they have any risk factors for atherosclerosis, e.g., hypertension, hyperlipidemia, or diabetes mellitus, these diseases should be treated. The use of oral contraceptives is forbidden in women. A patient with SLE who had high titers of IgG anticardiolipin antibodies, LA, and IgG β_2-GPI-dependent anticardiolipin antibodies at her first visit to the hospital was reported to have developed deep vein thrombosis in a leg after 5 years during which she received no antiplatelet or anticoagulation therapy.[12] A low dose of aspirin should be administered to patients with only serological features after their consent has been obtained.

In the second period, various treatments, including the use of heparin, are given to each clinical manifestation of thrombosis in different organs.

The third period is important in terms of the prophylaxis, as approximately 53% of patients have recurrent thrombotic events in spite of aspirin or warfarin therapy.[90] An antiplatelet agent such as aspirin and/or anticoagulation therapy by warfarin are administered. The dose of warfarin is controversial. Strict warfarin anticoagulation therapy at the high level of prothrombin time international normalized ratio (INR) over 3.0 was reported to be effective, but serious adverse side effects were found, such as intracranial hemorrhage or a tendency to bleed.[91] Warfarin anticoagulation therapy at the relatively high level which maintains prothrombin time INR between 2.6 and 3.0 is required to prevent recurrent thrombosis. Lifelong anti-

coagulation therapy is necessary, and endothelial cell damage markers such as plasma levels of von Willebrand factor (vWF) and APC-PCI (activated protein C-protein C inhibitor) complex may be useful to monitor thrombotic events.[92,93]

Plasmapheresis as well as immunosuppressive agents are required for the treatment of catastrophic APS.[94,95]

Fetal loss

Patients who have their first pregnancy with antiphospholipid antibodies in their sera may be treated by aspirin only. However, patients with antiphospholipid antibodies who have a history of fetal loss or thrombosis should be fully managed. Warfarin should not be used because of its teratogenic effect. A typical regimen includes both subcutaneous heparin, 5000–10000 units every 12 h, and low doses (for example, a daily dosage of 81 mg) of aspirin.[4,88,89,96] Treatment is begun as soon as a viable pregnancy is diagnosed, usually at 5–6 weeks gestation. Heparin treatment is maintained at the time of delivery, although the dosage may be reduced to 5000 units every 12 h. Heparin is reinstituted for 4–6 weeks after delivery to prevent thrombosis in the postpartum period. Low molecular weight heparin and intravenous immunoglobulin therapy are another treatment choice.[88,96] However, prednisone is not used nowadays because it shows a higher frequency of adverse side effects, including infection, preeclampsia, gestational diabetes, and osteonecrosis.

Conclusion

The spectrum of antigenic specificities appears to explain the heterogeneity of so-called antiphospholipid antibodies, and may ultimately lead to the various clinical manifestations in patients with APS. It is necessary to investigate other clinical and serological effects in order to establish definite diagnostic criteria, and to suggest a satisfactory management policy.

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