Review

Antiphospholipid syndrome: a comprehensive review of a complex and multisystemic disease

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ABSTRACT

The antiphospholipid syndrome (APS) is an acquired thombophilia, which is characterized by one or more thrombotic episodes and obstetric complications in the presence of antiphospholipid (aPL) antibodies (Abs). APL Abs are detected by laboratory tests such as lupus anticoagulant (LAC), anticardiolipin (aCL) and anti-β_2-glycoprotein I (β_2GPI) Abs. This article reviews the most current aspects of APS with emphasis on the pathophysiology of the disease, clinical manifestations, laboratory tests, and current modalities of treatment.

Introduction

The term thrombophilia was used for first time by Egeberg in 1965, when a family with predisposition to thromboses showed antithrombin III deficiency (1). Later, this term was extended to explain a propensity state which leads to thrombosis. Three kinds of thrombophilias can be distinguished: inherited, acquired and mixed (2, 3). The main acquired thrombophilia is the APS which is characterized by the presence of aPL Abs, recurrent venous and/or arterial thrombosis and/or fetal loss and sometimes autoimmune thrombocytopenia (2, 3). APS can appear as primary (idiopathic) or secondary - in association with systemic lupus erythematosus (SLE) (3). Other variants of APS have also been reported, including the "seronegative" APS, the "Catastrophic" APS or "CAPS", other microangiopathic syndromes such as Thrombotic thrombocytopenic purpura-Hemolytic uremic syndrome (TTP-HUS), HELLP syndrome, disseminated intravascular coagulation or the rare but still possible "lupus anticoagulant-hypoprothrombinemia syndrome (4).

Antiphospholipid antibodies: Epidemiology

APL Abs are present in APS and are autoantibodies with specificity against proteins that bind to anionic phospholipids (2). APL Abs are also found in other conditions such as infections, cancer, and can be induced by some medications (5-8). The prevalence of aPL Abs in asymptomatic individuals is 1–5% and the incidence may increase with age and with coexisting chronic diseases (9). An association of certain HLA II alleles has been reported in patients with APS (10).

In patients with SLE, the prevalence of aPL Abs has been between 12–30% for IgG and IgM anticardiolipin (aCL) Abs, and 15–34% for LAC (11). Palomo *et al*. found 44.4% of aCL Abs in patients with SLE and 3.3% in healthy individuals, in the regional hospital of Talca, Chile (12). When other types of aPL Abs were considered, the prevalence in SLE patients reached 60% (12).

There is no sufficient published information that determines the percentage of asymptomatic individuals (aPL positive) that will develop clinical manifestations ofAPS in the future. On the other hand, the possibility that a patient with SLE and aPL Abs will develop APS in the following 20 years has been reported between 50–70% (13).

Antigens recognized by aPL Abs

APL Abs owe their name to the fact that initially these Abs were believed to recognize anionic phospholipids. Nowadays, it is known that aPL Abs have specificity against some proteins with affinity for these phospholipids. Several target antigens have been described as being recognized by these Abs including: $\beta_2$glycoprotein I (β_2GPI), prothrombin (PT), several components of the protein C system, annexin A5, tissue factor pathway inhibitor (TFPI); proteins of the fibrinolytic system and other proteins of the coagulation cascade, such as: Factor XII, XI, VII. Of these antigens, the most studied are β_2GPI and PT (14-22).

β_2GPI is a 50 kDa glycoprotein, synthesized mainly in the liver, which has a noticeable affinity for negatively charged molecules, such as anionic phospholipids, heparin, lipoproteins, and activated platelets (23). Potential antithrombotic properties of β_2GPI have been identified. Hulstein *et al*. found that β_2GPI inhibits von Willebrand factor (VWF)-induced platelet aggregation. β_2GPI binds to the A1 domain of VWF but preferably when the A1 domain is in its active glycoprotein Ibalpha-binding conformation (24). This mode of action could contribute to the thrombosis and consumptive thrombocytopenia observed in patients with anti-β_2GPI Abs.

Pathogenesis

APL Abs have prothrombotic activity and several mechanisms seem to be responsible for effects on the fibrinolitic system, cellular effects (platelets, monocytes and endothelial cells) and activation of complement (summarized in Table I) (25-27).

a) Alterations of the coagulation and fibrinolysis systems

β_2GPI is a cell surface-binding plasma protein. The affinity of β_2GPI for the cellular surfaces appears to be low. However, in the presence of Abs against β_2GPI, that affinity increases significantly. The increase in the affinity of β_2GPI for the plasmatic membrane can modify its function and affect the coagulation/fibrinolysis rate on the cellular surface when interfering with other proteins that bind to phospholipids such as coagulation factors and protein C (28).

Yang *et al*. have shown that 28% of APS patients have Abs that react with plasmin that interfere with the plasmin-mediated lysis of fibrin clots, suggesting that plasmin may be an important driving Ag for some aPL specific B cells in APS patients (26). Then, the induced anti-plasmin Ab may act either directly, by binding to plasmin and inhibiting its fibrinolytic activity, or indirectly, by cross-reacting with other homologous proteins in the coagulation cascade to promote thrombosis (29).

Recently, Chen *et al*. have found that five out of seven patient-derived IgG monoclonal aCL Abs react with thrombin, activated protein C, and plasmin (30). All three protein are trypsin-like serine proteases (SP), and are highly homologous in their catalytic domains. Importantly, among these SP autoantigens, the reactive aCL Abs bind to plasmin with the highest affinity, and thus plasmin serve as a major driving autoantigen for some aCL Abs in approximately 30% of APS patients who are positive for IgG anti-plasmin Ab (30). Lu *et al*. studied plasmin-reactive aCL and have shown that these antibodies may bind to tissue plasminogen activator (tPA) and that some of the tPA-reactive aCL may inhibit tPA activity and, thus, may be prothrombotic in the host (31).

The Abs against PT may either have an anticoagulant or a procoagulant activity, based in their ability to interfere with the action of the prothombinase complex, and to act at level of the lipid surface (32). The procoagulant activity of these Abs is based on: (i) increase of the binding of prothrombin to anionic phospholipids, which favors the formation of thrombin and (ii) interference on the action of the antithrombin III (natural anticoagulant) (16).

In a significant percentage of the patients with APS, Abs against TFPI activity have been found (18,33). IgG fractions of these Abs interfere with the TFPI favoring the generation of thrombin.

In addition, impaired fibrinolysis has been reported in patients with APS. Lower activity of intrinsic fibrinolysis in euglobulin fractions from APS patients has been demonstrated (34). β_2GPI is proteolytically cleaved by plasmin in domain V (nicked β_2GPI) and becomes unable to bind to phospholipids, reducing antigenicity against aPL Abs. Nicked β_2GPI binds to plasminogen and suppresses plasmin generation in the presence of fibrin, plasminogen, and tPA, thus, nicked β_2GPI plays a role in the extrinsic fibrinolysis (35).

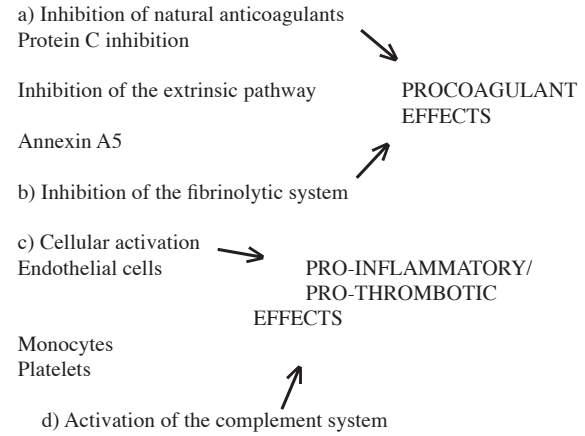
b) Effects of aPL Abs on target cells

APL Abs can also exert its prothrombotic effect by activating endothelial cells (EC), platelets and monocytes.

i) Endothelial cells and monocytes: The activation of the EC by anti-β_2GPI Abs involves an increase in the expression of cellular adhesion molecules (ICAM-1, VCAM-1 and E-selectin), favoring the adhesion of leukocytes and the activation of the coagulation (36). Studies have shown that EC expressed significantly higher amounts of cellular adhesion molecules (ICAM-1, VCAM-1 and E-selectin), IL-6 production, and alteration in prostaglandin metabolism when incubated with aPL Abs and β_2GPI *in vitro* (37, 38) (Fig. 1).

In addition, one of the mechanisms contributing to thrombosis in APS patients might be the increased expression of tissue factor (TF) - the major initiator of the coagulation *in vivo*, by EC and monocytes (39). There is a close relationship between TF and vascular permeability factor/vascular endothelial growth factor (VPF/VEGF). VEGF may stimulate monocyte TF expression through its receptor, the tyrosine

Table I. Pathogenic mechanisms triggered by antiphospholipid antibodies.



kinase Flt-1. Cuadrado *et al*. showed *in vivo* that purified monocytes from APS patients have higher levels of VEGF and flt-1 than healthy people, and this correlated with IgG aCL titers and TF expression in monocytes. Furthermore, studies indicated that monocyte VEGF and Flt-1 levels are significantly higher in patients with aPL Abs and thrombosis compared to patients without previous thrombosis. Thus, VEGF might act as a regulatory factor in aPL-mediated monocyte activation and TF expression, thereby contributing to the proinflammatory-prothrombotic phenotype of APS patients (40).

The intracellular events triggered by aPL Abs on EC and other target cells are not completely understood. To address this question Vega-Ostertag *et al*. examined the effects of aPL Abs on transcription, expression and function of TF on EC and on phosphorylation of p38 mitogen activated protein kinase (MAPK) (41). They showed that IgG aPL Abs increased the production of IL-6 and IL-8 by EC significantly when compared to cell treated with control IgG. TF expression was determined by ELISA and aPL Abs increased the expression of TF on EC significantly and in a dose-dependent fashion. Incubation of EC with aPL Abs increased transcription of TF mRNA. These effects were blocked when cells were pre-treated with an specific p38MAPK inhibitor (SB203580) *in vitro* and *in vivo* (42). Other studies have shown activation of nuclear factor-kappa B (NF-κB) in monocytes and in ECs after incubation with aPL Abs *in vitro* (41, 42). Furthermore there pro-inflammatory effect scan be abrogated by fluvastatin (43-45).

In patients with primary APS stimulation of monocytes has been observed. Expression of TF on the surface of monocytes is increased, which may contribute to thrombosis in these patients. APL induces TF expression in monocytes from APS patients by activating, simultaneously and independently, the phosphorylation of MEK-1/ERK proteins, and the p38 MAP kinase-dependent nuclear translocation and activation of NF-κB/Rel proteins (46). It has been found that anti-β_2GPI Abs, purified from the plasma of patients with APS, increase the expression of TF and the procoagulant activity in normal monocytes (47-49). Procoagulant activity of monocytes was reported to be increased in patients with SLE, although correlation with positive LAC was not found (50).

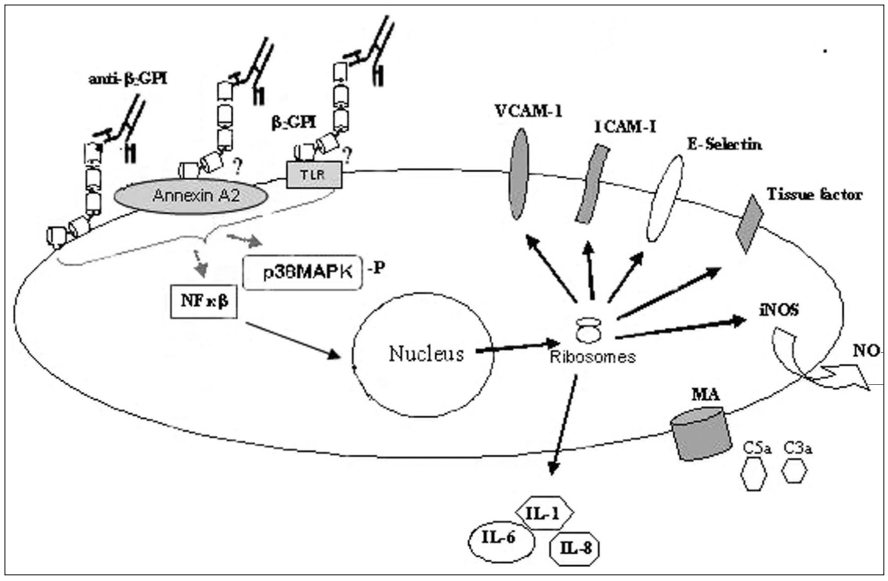


Fig. 1. Diagrammatic representation of events triggered by aPL Abs on EC. APL Abs bind to $\beta_2$glycoprotein I (β_2GPI) that in turns bind to endothelial cell (EC) membrane receptors [*i.e*. toll-like receptors (TLR), annexin A2] and induce phosphorylation of p38 mitogen activated protein kinase (p38MAPK), nuclear factor-kappa B (NF-κB) activation and translocation, induction of nitric oxide synthase (iNOS), leading to a pro-inflammatory and pro-thrombotic effect (*i.e*. expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), E-selectin, tissue factor (TF), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8). aPL Abs may also enhance effects on EC by activating complement and by releasing complement split products (C3a, C5a).

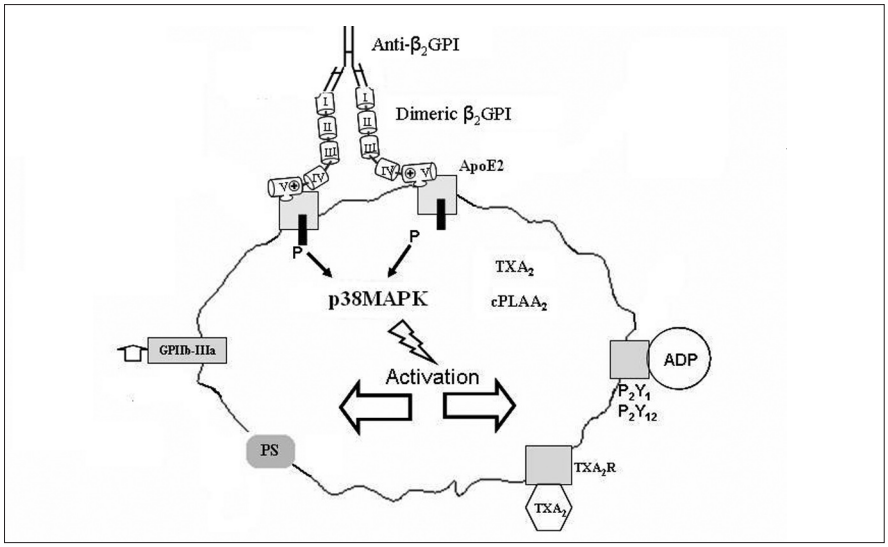


Fig. 2. Diagrammatic representation of events triggered by aPL Abs on platelets. APL Abs bind to dimerized $\beta_2$glycoprotein I (β_2GPI) that in turns bind to platelets receptor(s) [*i.e*. apolipoprotein ER2' (apoER2')], and induce cell activation/aggregation through phosphorylation of p38 mitogen activated protein kinase (p38MAPK), phospholipase A2 (cPLA2, and release of thromboxane A2 (TXA2) and expression of GPIIbIIIa.

Such procoagulant activities and TF expression in normal monocytes were induced by purified IgG from APS patients (51). In another study and in order to address the question how the binding of aPL/ β_2GPI to these cell surfaces causes production of pro-co-

agulant molecules Bohgaki *et al*. investigated aPL-inducible genes in peripheral blood mononuclear cells using a cDNA array system (52). Two hours after exposure to EY2C9, a monoclonal IgM aCL Abs established from an APS patient, mRNAs related to the mitogen-activated protein kinase (MAPK) pathway, such as p38-regulated/activated protein kinase (PRAK), SP-1, TNF receptor-associated factor 6 (TRAF6) and SAPK4 (p38δ), were increased more than two-fold. TF and inflammatory cytokines such as TNF-α and IL-1 expression were also confirmed using real-time PCR. Using monocyte cell line RAW264.7, phosphorylation of p38 MAPK, translocation of NF-κB to the nuclear fraction, and expression of TF mRNA were demonstrated after treatment with monoclonal aCL Abs. These phenomena were observed only in the presence of β_2GPI. Moreover, a specific p38 MAPK inhibitor SB203580 decreased aCL/ β_2GPI-induced TF mRNA expression in mononuclear cells (52).

ii) Platelets: APL Abs harness the platelet activation. The platelets of patients with APS display greater expression of CD63 and they release larger amounts of P-selectin to the plasma than the platelets of normal individuals. Also aPL Abs increase the expression of the GPIIb-IIIa, stimulate the platelet aggregation in the presence of subaggregating concentrations of platelets agonists and increase the synthesis of thromboxane A2 *in vitro* (53-57) (Figure 2). Vega-Ostertag *et al*. showed that theses effects of aPL Abs on platelets are also mediated by p38MAPK (58). In order to study intracellular pathways activated by aPL Abs, Vega-Ostertag *et al*. examined their effects on: phosphorylation of p38MAPK, ERK1/ERK2 and cytosolic phospholipase A_2 ($cPLA_2$); intracellular Ca^{2+} mobilization; and TXA2 production (58). The effects of the specific inhibitor for SB203580 on aPL-mediated enhancement of platelet aggregation and on TXB2 production were also determined. Treatment of the platelets with IgG aPL Abs or with their $F(ab`)_2$ fragments resulted in a significant increase in phosphorylation of p38MAPK. Neither IgG aPL nor their $F(ab`)_2$ significantly increased the phosphorylation of ERK1/ERK2. Furthermore, pretreatment of the platelets with SB 203580 completely abrogated aPL-mediated enhanced platelet aggregation. Platelets treated with $F(ab)_2$ derived from aPL produced significantly larger amounts of TXB2 when compared to controls, and this effect was completely abrogated by treatment with SB 203580. cPLA2 was also significantly phosphorylated in platelets treated with thrombin and $F(ab`)_2$ derived from aPL Abs (58). The data strongly indicates that aPL Abs induce TXB2 production mainly through the activation of p38MAPK and subsequent phosphorylation of cPLA2, and that the ERK1/ERK2 pathway does not seem to be involved, at least in early stages of aPL-mediated platelet activation.

c) Activation of the complement system

Some studies have recently suggested the involvement of the complement system in APS. APL Abs may activate the complement system and may favor the generation of C5a, a molecule that attracts and activates neutrophils and monocytes and that leads to the release of inflammatory mediators and others molecules (59, 60). Using specific complement inhibitors or mice deficient in several complement components, Girardi *et al*. has shown that C4, C3, C5 and C5a-C5aR are required to induce fetal injury by aPL Abs (57). Furthermore, Pierangeli *et al*. showed that mice deficient in complement C3 and C5 are resistant to the enhanced thrombosis and EC activation that is induced by aPL Abs (60).

A proposed mechanism for APL-induced fetal damage is that when these Abs act on the placenta they may generate C5a, which attracts and activates neutrophils and monocytes that in turn stimulate the release of inflammatory mediators and other molecules, such as proteolytic enzymes, chemokines, cytokines, C3 and properdin. Neutrophils have been implicated in pregnancy loss in an antibody-independent form, and C5a could enhance this effect in APS (59). Furthermore, Fischetti *et al*. showed that in C6-deficient rats and in animals treated with an anti-C5 mini-antibody and aPL Abs. the number of intravascular platelet-leukocyte aggregates and thrombotic occlusions is markedly reduced, suggesting the contribution of the terminal complement complex to the aPL antibody-mediated intravascular thrombosis (61).

Given the participation of the complement system in thrombosis and fetal loss, it is tempting to speculate that the inhibition of complement activation may be beneficial for the treatment of thrombosis and pregnancy complications in women with APS. Further studies in humans are needed to confirm these postulated mechanisms.

Receptor (s) for β_2GPI on target cells

Studies have shown that the binding of aPL Abs to target cells involve β_2GPI (62). Recent publications have focused on the identification of receptor(s) proteins that may be involved in binding to β_2GPI/aPL Abs complexes and in transducing signaling to target cells. At least three receptors have been suggested, a toll-like receptor and annexin A2 on EC and monocytes, and apolipoprotein ER`2 (apoER2`) on platelets (62-64).

i) Toll-like receptors (TLR): The current hypothesis is that anti-β_2GPI Abs activate EC and this process involves Toll-like receptor (TLR-4), a receptor for bacterial lipopolysaccharide (LPS). The relationship between TLR-4 and β_2GPI is apparently supported by the molecular mimicry shared by β_2GPI and different microbial pathogens. Raschi *et al*. proposed that β_2GPI might interact with TLR-4 and that anti-β_2GPI Abs might cross-link the complex eventually triggering the inflammatory cascade (65). In order to investigate the *in vivo* pathogenic role of TLR- 4 in APS, Pierangeli *et al*. looked at the thrombogenic activity of aPL Abs in lipopolysaccharide (LPS) non-responsive ($LPS^{-/-}$) mice. For this purpose IgGs from two APS patients, one aPL- negative SLE patient and one IgG control (from healthy donor) were evaluated for thrombosis in LPS ($^{-/-}$) mice and in the LPS ($^{+/+}$) wild type animals. The two IgG-APS induced significantly smaller thrombus size and lower number of leukocytes adhering to EC adhering in LPS $^{-/-}$ compared to $LPS^{+/+}$

mice (66). The same researchers studied the association between TLR-4 gene polymorphisms and APS. Two polymorphisms of TLR-4 gene, Asp299Gly and Thr399Ile, were evaluated in APS patients with arterial/venous thrombosis and in controls of the same ethnic origin. Both polymorphisms prevalence was significantly lower in patients than in controls (66) The findings suggest that TLR-4 is involved in *in vivo* aPL interaction with EC. Others have shown involvement of TLR-2 (67).

ii) Annexin A2: Some studies have shown that aPL/anti-β_2GPI Abs bind β_2GPI that, in turn, binds annexin A2 on the surface of EC and then intracellular signaling is induced (63, 68). Because annexin A2 does not span the cell membrane, this interaction may require an adaptor protein able to transduce intracellular signaling. It has been suggested that β_2GPI adhesion to negatively charged cell membrane structures may facilitate the binding to the putative receptors and that aPL/β_2GPI Abs may, in turn, increase the binding affinity to the receptors (69).

The possibility that anti-β_2GPI Abs activate EC by the interaction with some molecules, and then producing an inflammatory response, may explain the apparent paradox raised in annexin A2 studies (63, 68). Both anti-β_2GPI and anti-anexin A2 bivalent Abs can induce activation of EC. Since annexin A2 is not a transmembrane protein, it is likely that more than one receptor participate in cellular activation, being annexin A2 able to act as an obligatory primary protein.

In summary, the presence of thrombophilic vascular processes in patients with APS suggests a putative reactivity of aPL Abs with EC and monocytes. Such reactivity seems to be secondary to the binding of anti-β_2GPI Abs to β_2GPI present on the target cell membrane (25).

iii) Apolipoprotein E receptor 2` (ApoER2`): de Groot *et al*. have proposed the concept that after binding to the cellular surface, probably via heparin sulphates or a protein-binding site, β_2GPI interacts with apoER2` (70). Experiments with deletion mutants have shown that the domain V of β_2GPI contains the binding site for the members of the LDLR-family (71). That interaction of β_2GPI with ApoER2' is not sufficient to trigger the signaling: the presence of the aPL Abs is required. The interaction of β_2GPI with apoER2' results in phosphorylation of apoER2' followed by phosphorylation of p38MAP kinase and synthesis of thromboxane A2 (TXA2) in platelets. TXA2 is the mayor eicosanoid produced in platelets and has a potent aggregatory and vasoconstrictor activity. Patients with APS have shown increased levels of thromboxane breakdown products in their urine, an important indication that activation of platelets occurs in this group of patients (71-73).

Clinical features

Since first reported as a syndrome in the 1980s, APS has become a systemic conditions. Almost any organ/tissue may be involved in the disease, such as the brain, the kidney, the heart, the placenta, the blood and the endocrine system. Prof. Shoenfeld recently suggested to add to the syndrome the word 'systemic' in analogy to its sister disease Systemic Lupus Erythematosus (74).

i) Thromboses: Patients with APS can present spontaneous venous or arterial thromboembolism that can compromise any location or organ. These thrombotic events are observed in approximately 30% of the patients with aPL Abs (3). Clinical differences do not exist between primary and secondary APS. The symptomatology is related to the nature and size of the compromised vessel. The size of the affected vessel determines the organ compromise, which will may have two possible origins thrombotic microangiopathy or ischemic, secondary to thromboembolism (3).

Venous thrombosis is the most frequent manifestation of the APS (2/3 of the cases), specially of the inferior extremities. In a follow-up of six years this complication has been observed in 29-55% of the patients, of whom more than half present pulmonary emboli. Thrombosis can appear spontaneously, or associated with predisposing or triggering factors such as bed rest, trauma, surgery, infections, use of oral contraceptives, etc. (3).

Arterial thrombosis is less frequent (1/3 of the cases) and can present as ischemia or infarct. Brain is the most compromised organ (50%) and thrombosis may present in the form of vascular accident or transient ischemic crisis. The rest of arterial thrombotic events are divided into coronary (23%) and others (27%); *(i.e*. subclavia, kidney or retinal arteries). Also arterial emboli phenomenon can be observed secondary to vegetations of the mitral or aortic valve (75).

Clinical manifestations of the capillaries, arterioles or venules in APS are often indistinguishable from the hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), or others thrombotic microangiopathies (76).

ii) Neurologic manifestations: Transient ischemic attacks or cerebral vascular accidents (CVA) are often seen in young people with APS with no traditional risk factors for brain vascular disease. The infarcts of the central nervous system (CNS) are generally small without evidence of vasculitis in the biopsy. Others neurological manifestations include: migraine, Sneddon syndrome (CVA, arterial hypertension and livedo reticularis), chorea, convulsions, transverse myelitis, encephalopaty, cerebral venous thrombosis, pseudotumor cerebri, Guillan-Barre syndrome, neurosensorial deafness, psychotic disorders, motor neuron disease, transient global amnesia, myasthenia gravis, transient global amnesia, multiple mononeuritis or amaurosis fugax (77-80). Shoenfeld *et al*. found, in a large study of patients with primary and secondary APS, that epilepsy is common in those patients and most of the risk seems to be linked to vascular disease, as manifested by extensive CNS involvement, valvulopathy and livedo reticularis and also the presence of SLE (81).

iii) Cardiac manifestations: APS is associated with coronary disease in young people. APS should be suspected when classic risk factors of cardiovascular disease do not exist or there is evidence of coronary thrombotic occlusion or emboli without angiographic evidence of atheresclerotic disease (82). The mitral and/or aortic valves compromise including thickening, vegetations, re-

gurgitations and stenosis (not infectious endocarditis) may be present in APS patients, detected with the examination of transesophagic ecography (83). Hojnik *et al*. indicated in a publication, that aPL Abs may promote the formation of valve thrombi (84).

iv) Obstetric manifestations: Repeated spontaneous miscarriages are a characteristic manifestation of APS. The exact mechanism is unknown. During differentiation to syncytium, trophoblasts express cell membrane anionic phospholipids that can bind β_2GPI. Adhered β_2GPI can be recognized by the aPL Abs that, once bound, interfere with trophoblast cell maturation, resulting in defective placentation (85). The action of the complement system has been postulated as an inductive mechanism of the fetal damage possible due to the action of complement mediators, among them C5a (59). Complement activation may explain the pathogenic effect in experimental animal models. Salmon *et al*. used a mouse model of APS induced by passive transfer of human aPL Abs, demonstrating that complement activation plays an essential and causative role in pregnancy loss and fetal growth restriction, and that blocking activation of the complement cascade rescues pregnancies (86). These studies underscore the importance of inflammation in fetal injury associated with aPL Abs and raise the importance of developing and testing targeted complement inhibitory therapy for patients with APS, providing that these effects in animal models are confirmed in human studies. Other complications of the pregnancy associated to aPL Abs are: intrauterine growth retardation, HELLP syndrome (hemolytic anemia, elevated liver enzymes, low platelet count associated to pre-eclampsia), uteroplacental insufficiency and preeclampsia (85).

v) Renal manifestations: Renal manifestations in APS depend on the type of vessel affected. For example, if large vessels are involved thrombosis of the vein or renal artery, renal infarct, hypertension, acute or chronic renal insufficiency, proteinuria, haematuria or nephrotic syndrome, thrombotic microangiopathy resempling hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) or hypertension may occur (87-89).

vi) Pulmonary manifestations: In APS, pulmonary manifestations may appear as spontaneous thrombosis of the pulmonary vessels, or demonstrated as pulmonary hypertension, pulmonary embolism, alveolar haemorrhage or as an acute respiratory distress syndrome (90).

vii) Ophtalmological manifestations: Common ophtalmological manifestations of APS in the eye include: thrombosis of the central vein of the retina and of the retinal artery, amaurosis fugax or retinitis (91).

viii) Gastrointestinal manifestations: Mesenteric and porta vein thrombosis, Budd-Chiari syndrome, hepatic infarction, intestinal or splenic, esophagic perforation, ischemic colitis, infarction of the alithiasic biliary bladder, pancreatitis or ascites can be observed in patients with APS (92-94).

ix) Endocrine manifestations: Among the endocrine complications associated with APS suprarenal glands infarction and necrosis of pituitary gland have been described (95).

x) Hematological manifestations: Frequently observed hematological manifestations in APS include: haemolytic anemia, hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and disseminated intravascular coagulation in cases of catastrophic APS. When present, thrombocytopenia appear as moderated in 20-40% of the patients (96).

xi) Cutaneous manifestations: Livedo reticularis may be observed in 11-22% of the patients with APS even in the absence of SLE. Capillary thrombosis can be demonstrated in addition to haemorrhage, necrotizing purpura or peripheral gangrene (97). Toubi and Shoenfeld suggested the inclusion of livedo reticularis as "minor" criterion for APS (98).

xii) Catastrophic APS (CAPS): CAPS is defined as a clinical manifestation that compromises at least three organ systems in a brief period of time and include multiple occlusions of big or small vessels. About 75% of the cases are women at a mean age of 40 years. Most of them are primary APS and few cases have also SLE or another autoimmune disease. It is characterized by a "thrombotic storm" with massive venous thromboembolism, with respiratory insufficiency, cerebrovascular accident (CVA), increased hepatic enzymes, renal damage, suprarrenal insufficiency and skin infarction. The organ compromised with major frequency is the kidney and secondly the lungs followed by CNS, heart and skin. Mortality rate is high due to multiorgan failure, if this condition is not promptly identified and treated (99-103).

Classification criteria for APS

In 1999, the first classification criteria for APS were published after an international meeting in Sapporo, Japan, and updated recently after another international meeting and workshop in Sydney, Australia (104,105). The clinical current and laboratory criteria are as follows:

1) Clinical criteria

a) Vascular thrombosis

One or more clinical events of arterial, venous or little vessels thrombosis in any tissue or organ. Thrombosis must be confirmed by imaging or doppler or histopathology, with exception of superficial venous thrombosis. The histopathology study does not have to demonstrate significant evidence of inflammation of the blood vessel.

b) Pregnancy morbidity

i. One or more unexplained deaths of morphologically normal fetuses, at the 10 or more weeks of gestation, with a normal fetal morphology confirmed by ultrasound or direct examination of the fetus.

ii. One or more newborn premature losses of morphologically normal to the 34 weeks of gestation or before, due to:
   - Severe preeclampsia or eclampsia defined according to Standard definitions , or
   - Recognized placental insufficiency.

iii. Three or more consecutive spontaneous abortions without explanation before the 10 weeks of gestation, excluding hormonal or anatomical alterations from the mother or chromosomic alterations of both parents.

2) Laboratory criteria

The presence of**,** at least one of these tests:

a) LAC in the plasma, in two or more separate occasions in a period of 12 weeks, detected according to the guidelines of the International Society of Thrombosis and Haemostasis (Scientific Subcommittee on lupus anticoagulant/phospholipids dependent antibodies).

b) IgG or IgM or aCL Abs in plasma or serum, in medium-high titers (>40 GPL or MPL units, respectively), in two or more separate occasions in a period of 12 weeks, measured by standardized ELISA.

c) IgG or IgM or anti-β_2GPI antibodies present in the serum or plasma (in titer above the 99th percentile), present in two or more separate occasions in a period of 12 weeks, measured by standardized ELISA, according to recommended procedures.

Patients can be classified as having APS if one clinical criteria and at least one laboratory criteria are present.

Treatment of thrombosis in APS

The current therapeutic recommendations for APS are based mainly on observational studies of the association between aPL Abs and thrombosis, particularly recurrent thrombosis (106-109). Treatment is centered on the use of anticoagulants. Initially heparin is used, and then followed by oral anticoagulant with international normalized ratio (INR) between 2 and 3 (110, 111). In patients with recurrent thrombosis, besides the regime previously mentioned, the addition of low dose aspirin is advised. It also becomes necessary to investigate other possible reasons for thrombophilia (*i.e*. factor V Leiden or prothrombin G20210A mutations, protein C, protein S deficiencies, etc.). It is also recommended to inform the patients with APS of other prothrombotic risks, such as: tobacco, hormone replacement therapy or the use of illicit drugs such as cocaine.

In the case of CAPS plasma exchange can be indicated, although real benefits have not been found in controlled studies (112). Recently, the use of rituximab, a monoclonal antibody that diminishes selectively B lymphocytes (CD20+) has shown good results in a small number of CAPS patients that were the resistant to other treatments (113-115).

In case of APS in pregnant women, the use of unfractionated heparin and aspirin has resulted in 80% of live births, in comparison with 40% when using only with aspirin (116). The prophylactic treatment with heparin has improved the outcome of pregnancies in patients with APS (116).

Hydroxychloroquine should likely be an integral component of the treatment in all cases. This drug is gaining increased attention as therapeutic agent in APS. There are studies that demonstrate that hydroxycloroquine significantly diminished thrombus size and time of thrombus persistence in mice injected with aPL Abs and reverted aPL-mediated platelet activation (56, 111, 117). Hydroxychloroquine may be of benefit in APS patients who are unable to tolerate high levels of oral anticoagulation due to hemorrhagic side effects, or in those who continue to experience thrombotic events despite oral anticoagulation. Besides it could be useful in patients which significant titer of aCL antibodies or a positive LAC test, who have not had any previous thromboembolic events. However, clinical trials to establish its real efficacy are needed.

Some studies have suggested that statins could have effects in some aPL-mediated pathogenic effects. For example, Meroni *et al*. showed that fluvastatin prevented the expression of adhesion molecules and IL-6 in endothelial cells treated with aPL Abs (40) Furthermore, other study showed that the thrombogenic and proinflammatory effects of aPL Abs *in vivo* could be abrogated in mice fed witch fluvastatin (43, 44). In addition fluvastatin showed to reduce induction of TF on endothelial cells *in vitro* (45). The data presented provide a rationale for using statins as a therapeutic tool in treatment of thrombosis in APS.

Summary

APS is an acquired thombophilia, which is characterized by recurrent thrombotic events and obstetric complications in the presence of aPL Abs. The diagnostic of APS is based in the discovery of one clinical criteria and one laboratory criteria at least. The treatment of APS is based fundamentally in the use of orals anticoagulants with or without aspirin.

Pathogenic mechanisms include effects on the coagulation cascade, cellular activation and complement activation. Lately much has been advanced in the knowledge of cellular receptors that participate in signaling transduction. Further studies are needed to clarify how aPL Abs affect cell surface molecules and how signal transduction events occur. Understanding intracellular events in aPL-mediated EC, platelet and monocyte activation may help in designing new targeted therapies for thrombosis in APS. Understanding molecular events triggered by aPL Abs may help to device new modalities of treatment for clinical manifestations of APS (*i.e*. use of specific inhibitors, antibodies, etc.). *In vivo* studies in animal models followed by clinical trials in humans will need to be performed to determine the safety and effectiveness of specific inhibitors to be used in the treatment of complications of APS.

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