The Antiphospholipid Syndrome

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ABSTRACT
The primary antiphospholipid syndrome is an autoimmune thrombotic disease. It is characterized by recurrent arterial or venous thrombosis, recurrent fetal loss or in-utero death and thrombocytopenia. The risk of cerebral vascular disease (CVD), pulmonary embolism (PE) or deep venous thrombosis (DVT). It has been described the detection of antibodies directed against diverse phospholipids, both anionic and neutral, such as cardiolipin phosphatidylserine, phosphatidylethanolamine and phosphatidic acid, also some seric co-factors like β2-Glycoprotein I, prothrombin, antithrombin III and annexin V. Several hypotheses have been proposed to explain the molecular and cellular mechanisms by which antiphospholipid antibodies (APLA) promote thrombosis. Treatment decisions are allotted into four main areas: prophylaxis, prevention of further thromboses of large vessels, treatment of acute thrombotic microangiophaty, and management of pregnancy in association with APS.

In 1965 Alarcon-Segovia and Osmundson described 11 patients with SLE who had peripheral vascular syndromes. Some of these cases were particularly interesting: one patient had chronic ulcers of the legs and livedo reticularis; clinical manifestations later found to be associated with aPL, and both circulating anticoagulant and false positive serologic tests for syphilis. Another had had 4 miscarriages, thrombophlebitis, livedo reticularis, thrombosis of the left ulnar artery, convulsions, and long-standing false positive tests for syphilis. A third patient had a history of false positive tests for syphilis, recurrent superficial thrombophlebitis, leg ulcers, intermittent claudication with evidence of popliteal artery occlusion, a vascular lesion of the brain system, and a terminal occlusion of a basilar artery. In 1980 Soulier and Boffa noted the occurrence of recurrent abortions, thromboses, and a circulating anticoagulant in patients not having a primary condition. In 1989, it was simultaneously described as Primary Antiphospholipid Syndrome (APS) by Hughes and Alarcon-Segovia. Nowadays the APS is a well known disease among rheumatologists, gynecologists and haematologists. It is considered a serious clinical problem, because of its association to multiple systems.

The disorder is characterized by recurrent arterial or venous thrombosis, recurrent fetal loss or in-utero death and thrombocytopenia. The risk of cerebral vascular disease (CVD), pulmonary embolism (PE) or deep venous thrombosis (DVT) in a patient under 50 years is about 7 to 8 times higher than in the normal people. There is also a relation to a positive test of lupus anticoagulant (LA) or positive results of anticardiolipin antibodies (ACA), which mainly attack phospholipid binding protein, β2-GP-I and prothrombin. The first antiphospholipid antibody was discovered in 1906 in patients with syphilis, and years later a mitochondrial phospholipid called cardiolipin was identified as the most important antigen.
Soon it was observed that many patients with Systemic Lupus Erythematosus (SLE) were positive to this test without any clinical or serological evidence of syphilis. This observation became the basis for the Venereal Disease Research laboratory (VDRL) test, which is currently still in use. A solid-phase immunoassay with greater sensitivity for anticardiolipin antibodies detection was developed in 1983, and soon investigators noted a strong correlation between the antibodies associated to lupus and thrombosis. Towards 1990, two independent groups discovered that certain anticardiolipin antibodies require the presence of a plasmatic protein named β₂-glycoprotein I, to bind the plasmatic phospholipids to the cardiolipin. In 1991, it was demonstrated that lupus anticoagulants may be also directed against prothrombin, another protein with affinity for phospholipids. The involvement of prothrombin as a co-factor for antiphospholipid antibodies has been established, reporting a prevalence between 50-90% of anti-prothrombin antibodies in patients with APS. The interaction between prothrombin and phospholipids results in the exposure of neo-epitopes with immunogenic capacity. The creation of neo-epitopes (epitope spreading) is one of the proposed mechanisms that tries to explain the transition from the initial stages of autoimmune diseases to their chronic phases. The demonstration that autoimmune anticardiolipin antibodies are directed against a phospholipid-binding protein rather than against a phospholipid led to the discover that some autoantibodies bind directly to β₂-glycoprotein I (β₂-GPI) in the absence of phospholipids.

**PATHOGENIC MECHANISMS**

Several hypotheses have been proposed to explain the molecular and cellular mechanisms by which antiphospholipid antibodies (APLA) promote thrombosis (Table 1). One of them deals with the activation of endothelial cells: binding of APLAs induces activation of endothelial cells, as assessed by up regulation of the expression of adhesion molecules, the secretion of cytokines and the metabolism of prostacyclins. The vascular endothelium is not just a passive non-thrombotic surface, which actively participates in the haemostatic regulation through the synthesis, expression and release of diverse components with pro- and anticoagulant properties. The vascular endothelium also interacts dynamically with different cells such as leucocytes, platelets, monocytes, and erythrocytes, among others. The most studied interaction is between endothelial cells and platelets. The oxygenation pathway of the arachidonic acid (AA) which leads to the eicosanoids and iso-eicosanoids synthesis has received special consideration within the physiopathogenic mechanisms of thrombosis. The released AA from phospholipid (PL) cellular membranes through the action of the Phospholipase A₂ are metabolized through different pathways: 1) the first pathway, which is enzymatic, generates eicosanoids. The TXA₂ has an important effect on the platelet aggregation and in vasoconstriction. Moreover, the PGI₁ is a powerful inhibitor of the platelet aggregation and has vasodilatation effects; it also maintains the antithrombotic properties of the endothelium. 2) The second pathway is non-enzymatic and gene-rates iso-eicosanoids. It was observed in egg cultures that the synthesis of PGI₁ did not increase in any significant way. The β₂-GPI is able to bind to the endothelial cells in a basal condition, and it has been demonstrated that it is a mediator between APL-binding the endothelium. Regarding in vitro studies on platelets, it was observed that there was an elevated production of metabolite TXA₂ (TXB₂) when the platelets stimulated with collagen or AA were incubated with the serum of patients with LES or APS. The immune antiphospholipids (APL) modify the function of the endothelium, and the binding of antibodies depends essentially on the presence of the β₂-GPI, which is adhered to the endothelial surface. It was observed that E-selectin, VCAM and ECAM induced the expression of adhesion molecules on its surface. This phenotype increases the adhesion and subsequently activates leucocytes and monocytes, which contributes the prothrombotic state of APS. Furthermore, it was determined that β₂-GPI induces the secretion of proinflammatory cytokines as Interleukines 6 and 1β in the endothelium. Some patients with APS were found to have higher plasmatic levels of Von Willebrand factor and tissue plasminogen activator (t-PA). Moreover, it was found that these endothelial activation markers were positive related with the increase of the 1+2 prothrombin frag-
ment, which indicates the activation of the coagulation system. Incidentally, a two-hit hypothesis has been suggested: (first hit) APL increases the risk of thrombotic events that take place in the presence of another thrombophilic condition and (second hit)23.

A second theory proposes that antiphospholipid antibodies modulate or interfere with the function of phospholipid-binding proteins involved in the haemostatic reactions and cells implicated in coagulation24. However, APLs alone are apparently unable to induce thrombotic manifestations independently. Meroni et al., propose that APLA increases the risk of thrombotic events that occur in the presence of another thrombophilic condition25. In line with this hypothesis are the experimental findings in murine models in which infusion of APLAs can increase clotting after mechanical injury to the vessel wall (Figure 1), which would not occur if the vessel wall were not injured26. Mechanisms by which antiphospholipid antibodies interfere with the regulatory functions of prothrombin, protein C, annexin V, and tissue factor have also been studied. These findings show that APLAs may interfere with protein C axis, a phospholipid-dependent major antithrombotic pathway, in multiple ways; among them the inhibition of thrombin formation and protein C activation via anti-thrombomodulin antibodies and achievement of activated protein C resistance and protein C/S deficit27,28. Annexin V has a high affinity for the anionic phospholipids, and one of its important properties is to act as an anticoagulant on the trophoblastic and endothelial surfaces29. There is evidence that β2-GPI adhesion to endothelial cells might take place in a different non-alternative mechanisms, spanning from an electrical charge interaction between the cation PL-binding site and anionic cell membrane structure (heparansulphate), to the binding annexin II29. Annexin II has been actually shown to behave as a high affinity endothelial β2-GPI receptor, though, the receptor is known as non-trans-membrane protein and it has been suggested to require a yet unknown “adaptor” protein to signal the cells30.

Antiphospholipid antibodies and β2-GPI also interfere with the fibrinolytic pathway through thrombomodulin by activating thrombin inducible fibrinolysis inhibitor and by increasing t-PA activity. These findings suggest that the impairment of fibrinolytic activity by APLAs might be one of the causes of thrombophilic diagnosis in APS31. More recent studies report the evidence of antibodies to factor XII in a significant number of patients with APS and suggest that this may lead to acquired factor XII deficiency. Coagulation factor XII, prekallicrein, and high molecular weight kininogen are known as plasma contact proteins, which participate in the intrinsic pathway of blood coagulation32. It is currently accepted that APLAs can react with endothelial cells, mainly through the binding β2–GPI expressed on cell membranes. Exogenous β2–GPI can bind to endothelial cells at the putative phospholipid-binding site located in the fifth domain of the molecule, or through annexin II, an endothelial cell receptor for tissue t-PA33.

Another theory but that it’s not as well describes as the others, focuses on the oxidant-mediated injury on the vascular endothelium. Most of the pathogenic mechanisms for APS are closely related to the events that collaborate in the formation of the atherosclerotic plaque, and in determining its stability and rupture33. Oxidized low-density lipoprotein (LDL), a major contributor to atherosclerosis, is taken up by macrophage activation, with subsequent damage to endothelial cells. Some studies on the clinical significance of the antibodies to oxidized LDL support the view that these antibodies are different from antibodies to β2–GPI. Clinical links of the antibodies to oxidized LDL are different from those of β2–GPI-dependent anticardiolipin antibodies34. The antibodies to the cardiolipin-β2–GPI complex are associated with arterial thrombosis in antiphospholipid syndrome. These results may indicate that antibodies to oxidized LDL do not interfere with blood coagulation as suggested for other types of antiphospholipid antibodies, but are more closely related to the disease process in the arterial vessel wall35. β2–GPI binds ox-LDL and the complexes with anti-β2–GPI antibodies are easily swallowed up by monocytes (foam cell formation). Anti-β2–GPI antibodies can activate endothelial cells inducing a pro-inflammatory and pro-coagulant phenotype. Antibodies to oxidized LDL appear regularly in patients with SLE and APS, and are related to arterial thrombosis in these patients36. Antibodies to oxidize LDL are heterogeneous in their specificity. Some of them show cross-reactivity with
Further activation
Surface-bound immune complexes

Minor vascular injury and mild platelet activation

Exposure of negatively charged phospholipids

Further activation

THROMBOSIS

β₂GPI

Surface-bound immune complexes

Figure 1. Putative pathogenic mechanism in the APS. As a consequence on the initial damage, anionic phospholipids are exposed on cells of the blood, on endothelium or on trophoblast. The potentially reactive phospholipids are covered up by phospholipid binding proteins such as β₂-GPI or prothrombin in the presence of antiphospholipid antibodies against these proteins. Immune complex deposits cause further cellular activation by unidentified signal transducing mechanisms in addition to the potential role of the platelet FcγRIIa; for platelets this would lead to release of granule contents and formation of multiple microvesicles, which would paradoxically provide a much larger negatively charged phospholipid surface, and therefore enhance rather than inhibit thrombin generation.
cardiolipin binding antibodies, most likely due to their binding to the common epitopes in oxidized lipids. It has been suggested that antiphospholipid antibodies recognize oxidized epitopes in phospholipids and thus are cross-reactive with several oxidized antigens, such as oxidized LDL. Antibodies to β2-GPI may show binding to oxidized LDL. Given the fact that antibodies to oxidized LDL bind to lipid-protein complexes and show cross-reactivity with anticardiolipin antibodies, they are considered antiphospholipid antibodies.

### CLINICAL FEATURES (TABLE 2)

There are no major differences in the clinical consequences of antiphospholipid antibodies between patients with primary antiphospholipid syndrome (Primary APS) and those with secondary antiphospholipid syndrome (secondary APS). The criteria of patients with primary APS are those who do not suffer of SLE or another autoimmune disease. There is some data that suggests primary APS has genetic bases, mostly in families with more than one case of primary APS. Primary APS prevails in young women; 54% of these have DVT, in which half of the cases lead to PE; 44% suffer of arterial occlusions in different sites; 34% have recurrent fetal loss and 20% suffer from minor manifestations. Secondary APS is related to the presence of SLE; its principal manifestations are recurrent venous thrombosis, autoimmune thrombocytopenia, autoimmune hemolytic anemia, recurrent fetal loss, leg ulcers and pulmonary hypertension (PHT).

### THROMBOSIS

Events are usually sporadic and appear unrelated to antibody levels. In most patients, recurrent events are confined to either the arterial or venous circulation, suggesting that factors that influence arterial and venous thrombosis may differ. DVT is the most frequent site affected in venous circulation, but thrombosis has also been described in the pulmonary vessels, inferior vena cava, renal, hepatic (Budd-Chiari syndrome), and sagittal veins. The most common presentations of arterial thrombosis are stroke or transient ischemic attacks; myocardial, adrenal, and gastrointestinal infarction, as well as gangrene of the extremities have also been described. Not all arterial episodes of ischemia or infraction are thrombotic in origin: emboli, especially from mitral or aortic valve vegetations, can also lead to cerebral events. Acute involvement at the capillaries, arterioles, or venules often results in a clinical picture indistinguishable from those of the hemorrhagic-uremic syndrome and thrombotic thrombocytopenic purpura as well as another thrombotic microangiopathies. Although renal manifestations are very common features of SLE, they were recently recognized as part of the antiphospholipid syndrome. Two mechanisms through which antiphospholipid antibodies may enhance thrombosis have been proposed: either a) they may interfere with phospholipid-dependent anticoagulant pathways; or b) they may bind to cell surfaces, induce cellular activation and by this means promote thrombosis. The term “catastrophic antiphospholipid syndrome” describes the development of thrombosis in multiple sites, either simultaneously or over a short period of time, often with life-threatening consequences.

### THROMBOCYTOPENIA

Sometimes patients with APS present thrombocytopenia alone. More frequently, however, mild to moderate thrombocytopenia (platelet counts in the range of 100,000–150,000/mm³) occurs in 40–50% of patients and accompanies other clinical complications. Investigators currently maintain that the true plaketary antigens would be membrane proteins, since they observed that 40% of the patients with APLAs had antibodies against glycoprotein IIb/IIIa and IbIX (as in thrombocytopenic idiopathic purpura), which would explain the low platelet count.

### MINOR FEATURES

These include cardiac valve vegetations (Libman-Sachs endocarditis), valvular insufficiency, livedo reticularis, leg ulcers, migraine headaches, and a variety of neurological complications, including chorea and transverse myelopathy.

### OBSTETRICAL MANIFESTATIONS

Women with APLAs have an unusually high proportion of pregnancy losses within the fetal period (10 or more weeks of gestation). In contrast, in unselected women with sporadic or recurrent miscarriage, pregnancy losses occur more commonly in the preembryonic period.
Table 2. Clinical manifestations of the APS

<table>
<thead>
<tr>
<th>Organ or system</th>
<th>Thromboembolism of large vessels</th>
<th>Primary Pathogenic process</th>
<th>Thrombotic microangiopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>Thrombosis of the aorta or axillary, hepatic, carotid, iliofemoral, mesenteric, pancreatic, popliteal, splenic or subclavian artery.</td>
<td>Myocardial infarction, myocardial microthrombi, myocarditis or valvular abnormalities.</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
</tr>
<tr>
<td>Cardiac</td>
<td>Angina, myocardial infarction, cardiac valve vegetations, valvular abnormalities, intracardiac thrombi, nonbacterial thrombotic (Libman-Sacks) endocarditis, peripheral embolization or atherosclerosis.</td>
<td>Myocardial infarction, myocardial microthrombi, myocarditis or valvular abnormalities.</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>Superficial thrombophlebitis, splinter hemorrhages, leg ulcers, distal cutaneous ischemia, skin infarcts, blue toe syndrome or acrocyanosis.</td>
<td>Myocardial infarction, myocardial microthrombi, myocarditis or valvular abnormalities.</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
</tr>
<tr>
<td>Endocrine or reproductive</td>
<td>Adrenal failure or infarction, testicular and/or prostate infarction, necrosis or failure of the pituitary gland.</td>
<td>Disseminated intravascular coagulation only in catastrophic antiphospholipid syndrome.</td>
<td>Microthrombi or microinfarctions.</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Budd-Chiari syndrome, hepatic, intestinal, splenic infarction, esophageal perforation, ischemic colitis, infarction of the gall bladder not attributable to gallstones, pancreatitis or ascites.</td>
<td>Intestinal, hepatic, pancreatic and splenic infarction or gangrene.</td>
<td>Microthrombi or microinfarctions.</td>
</tr>
<tr>
<td>Hematologic</td>
<td>Thrombocytopenia, hemolytic anemia or hemolytic uremic syndrome and thrombotic thrombocytopenia purpura.</td>
<td>Myocardial infarction, myocardial microthrombi, myocarditis or valvular abnormalities.</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Transient ischemic attack, cerebrovascular accident (thrombotic or embolic), choanal, seizures, multi-infarct dementia, transverse myelitis, encephalopathy, migraines, pseudotumor cerebri, cerebral venous thrombosis or mononeuritis.</td>
<td>Myocardial infarction, myocardial microthrombi, myocarditis or valvular abnormalities.</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Perforation of nasal septum or avascular necrosis of bone.</td>
<td>Myocardial infarction, myocardial microthrombi, myocarditis or valvular abnormalities.</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
</tr>
<tr>
<td>Obstetrical</td>
<td>Pregnancy loss, intrauterine growth retardation, HELLP syndrome (hemolysis, high liver enzymes and low platelet count associated with preeclampsia), oligohydramnios, uteroplacental insufficiency or preeclampsia.</td>
<td>Myocardial infarction, myocardial microthrombi, myocarditis or valvular abnormalities.</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
</tr>
<tr>
<td>Ophthalmological</td>
<td>Thrombosis of the retinal artery or vein, amaurosis fugax.</td>
<td>Retinitis</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Pulmonary emboli, pulmonary hypertension, pulmonary arterial thrombosis or alveolar hemorrhage.</td>
<td>Acute respiratory distress syndrome or alveolar hemorrhage.</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
</tr>
<tr>
<td>Renal</td>
<td>Thrombosis of the renal artery or vein, renal infarction, hypertension, acute renal failure, proteinuria, hematuria or nephritic syndrome.</td>
<td>Acute renal failure (often requiring dialysis), thrombotic microangiopathy or hypertension.</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
</tr>
<tr>
<td>Venous</td>
<td>Deep venous thrombosis of the legs or thrombosis of the adrenal, hepatic, mesenteric, portal or splenic vein or of the inferior vena cava.</td>
<td>Acute renal failure (often requiring dialysis), thrombotic microangiopathy or hypertension.</td>
<td>Livedo reticularis, superficial gangrene, purpura, ecchymoses or subcutaneous nodules.</td>
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</table>

six weeks of gestation. Women with positive antiphospholipid antibodies, who are pregnant, can also present complications such as premature delivery due to pregnancy-associated hypertensive disease and uteroplacental insufficiency. There is an adverse pregnancy outcome in women with APS who have poor placental perfusion due to localized thrombosis. APLAs may also impair trophoblastic invasion and hormone production, by this means promoting not only preembryonic and embryonic loss but also fetal loss and uteroplacental insufficiency.

**Catastrophic Antiphospholipid Syndrome**

A minority of patients with the APS present with an acute and devastating syndrome characterized by multiple simultaneous vascular occlusions throughout the body often resulting in death. It involves at least three different organ systems over a period of days or weeks with histopathological evidence of multiple occlusions of large or small vessels. The kidney is the most commonly affected organ (78% of patients), followed by the lungs (66%), the central nervous system (56%), heart (50%), and skin (50%). Disseminated intravascular coagulation, which does not occur in primary or secondary antiphospholipid syndrome, occurs in approximately 25% of patients with catastrophic antiphospholipid syndrome. Microvascular manifestations include renal thrombotic microangiopathy, adult respiratory distress syndrome, cerebral microthrombi and microinfarcts, and myocardial microthrombi. Most patients with renal involvement have hy-
pertension, often malignant, and approximately 25% require dialysis. The mortality rate is 50%; death is usually due to multiorgan failure.

**Antiphospholipid Antibodies in Normal Population**

Between 2-7% of seemingly healthy young people have positive APLAs — permanently or temporary — and in most of them are low titers. The possibility of these people to have related symptoms from these antibodies is not well established. The frequency of detection of these antibodies increases with the age. In a study in which it was related neurophysiologic parameters with cranial magnetic resonance (CMRI) in patients with and without APLAs, they found low positive titer in 15%, and medium and high titers in 8% of individuals with an average age of 60 years. This titers were not associated with apparently morphologic changes in the CMRI, although slight neurophysiological manifestations were found.

**Diagnosis and criteria for classification**

A patient with the APS must have at least one of two clinical criteria and at least one of two laboratory criteria (Table 3).

Preliminary classification criteria for the APS were formulated during a post-conference workshop held on October 10, 1998 in Sapporo, Japan, following the Eight International Symposium on Antiphospholipid antibodies. These classification criteria define the essential features of APS in order to facilitate studies of treatment and cause. The criteria include the clinical laboratory features that are more closely associated with APLAs in prospective studies and based on the strongest experimental evidence. Following the Ninth International APS Symposium in Tours, France, a discussion was organized on September 16, 2000 on the classification issues that were not addressed in the previous international classification workshop (in 1998). Dr. Lockshin and his group presented a study that evaluated the international Sapporo APS criteria. He noted that additional evaluation of the criteria was called for, especially in relation to group of subjects that were not previously evaluated; these groups include pregnancy outside of the context of overt connective tissue diseases, infectious diseases, and general neurologic disease and stroke. He also noted that, on the basis of their evaluation, the criteria seemed appropriate for clinical studies of APS. Moreover, Dr. Derkensen discussed the clinical problems of validating a diagnosis of APS, caused by inter-laboratory variation in ACA and LA. He noted that thrombosis in most clinical contexts is a multifactorial disease, and remarked on the need for more information on collateral risk factors for thrombosis in APS, along with the need for better imaging techniques to differentiate primary thrombosis from vasculitis, especially from CNS. Dr. W. Branch discussed pregnancy morbidity criteria for APS. He declared that continuing evaluation of the criteria is needed, especially for the number and types of pregnancy loss, and the relativity specificity of preeclampsia in relation to premature births caused by APS. Dr. R. Brey commented that further investigation is needed because APS may have several different physiopathologic mechanisms.

**Laboratory tests**

There are three subgroups detected of antiphospholipid antibodies: a) Lupus anticoagulant antibodies, b) anticardiolipin antibodies and c) β2-GPI. Division of these groups is based on the method of detection.

**Lupus Anticoagulant and Anticardiolipin Antibodies**

The occurrence of a circulating anticoagulant in the settings of SLE was first described in 1952. Afterward, a number of reports described an association between this inhibitor and clinical thrombosis (both arterial and venous) and recurrent fetal loss. The term Lupus Anticoagulant (LA) was given by a group of investigators in 1972. Lupus Anticoagulants are immunoglobulins (IgG, IgM, IgA mixtures) which interfere with one or more of the in-vitro phospholipids-dependent coagulation tests like activated partial thromboplastin time (APTT), kaolin clotting time (KCT), dilute Russell’s viper venom time, dilute prothrombin time and Teixetarin time (TT). Thromboembolic events are the most common clinical complications associated with LA. Thrombi may come out in the arterial, venous or microvascular circulation. Given the variety of
Table 3. Preliminary criteria for the classification of the APS.

<table>
<thead>
<tr>
<th>Clinical criteria&lt;sup&gt;15&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>1. Vascular thrombosis</td>
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<tr>
<td>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 histopathology, with exception of superficial venous thrombosis. For histopathological confirmation of thrombosis, should be present without significant evidence of inflammation in the vessel wall.</td>
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<tr>
<td>2. Pregnancy morbidity</td>
</tr>
<tr>
<td>One or more unexplained deaths of a morphologically normal fetus at or beyond the 10&lt;sup&gt;th&lt;/sup&gt; week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus; or One or more premature births of a morphologically normal neonate at or before the 34&lt;sup&gt;th&lt;/sup&gt; week of gestation because of severe preeclampsia or eclampsia, or severe placental insufficiency; or Three or are unexplained consecutive spontaneous abortions before the 10&lt;sup&gt;th&lt;/sup&gt; week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.</td>
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</tbody>
</table>

In studies of population who have more than 1 type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects to A, B or C above.

<table>
<thead>
<tr>
<th>Laboratory criteria</th>
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<tbody>
<tr>
<td>1. Anticardiolipin antibody of IgG and or IgM isotype in blood, present medium or high titer, on 2 or more occasions at least 6 weeks apart, measured by a standardized enzyme-linked immunosorbent for β2GPI dependent anticardiolipin antibodies.</td>
</tr>
<tr>
<td>2. Lupus anticoagulant present in plasma, on 2 or more occasions at least 6 weeks apart, detected according to the guidelines of the International society on Thrombosis and Haemostasis (Scientific Subcommitteee on Lupus Anticoagulants/Phospholipid-Dependent antibodies) in the following steps: Prolonged phospholipid-dependent coagulation demonstrated on a screening test, e.g. activated partial thromboplastin time, kaolin clotting time, dilute Russell’s viper venom time, dilute prothrombin time, Textarin time. Failure to correct the prolonged coagulation time on the screening test by mixing with normal platelet-poor plasma. Shortening or correction of the prolonged coagulation time on the screening test by the addition of excess phospholipid. Exclusion of other coagulopathies, e.g. factor VIII inhibitor or heparin, if appropriate.</td>
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</table>

Definite antiphospholipid antibody syndrome is considered to be present if at least 1 of the clinical criteria and 1 of the laboratory criteria are met.

In the case of LAs, several protein targets have been identified including prothrombin, β2-GPI, low and high molecular weight kininogens, and perhaps other vitamin K-dependent proteins such as protein C and protein S. Because of the frequent concordance of ACA and LA, both tests must be employed in evaluating patients with clinical findings suspicious for APS (Figure 2). LA is most frequently diagnosed in young adult-middle women, reflecting the greater incidence of autoimmune disease in this population. Between 30-40% of patients with SLE are found to have LA and/or ACA. The laboratory also plays an important role; it must have a coordinated system to evaluate plasma samples. The initial step is the preparation of the platelet poor plasma. Three or more screening tests are recommended, the labo-
ratory must have in mind other coagulopathies (such as factor VIII inhibitor) prior to definitive LA diagnosis.\(^{55}\)

**β₂-Glycoprotein I**

It was first reported in 1990 that the antigen for the antibodies in the ELISA system was not cardiolipin, but a plasma protein (β₂–GPI) captured on cardiolipin. This was based on the observation that following purification of APLAs by ion exchange chromatography or phospholipid affinity chromatography, these antibodies do not bind to phospholipids unless human plasma, human serum or bovine serum was also applied. The purified protein was separated from immunoglobulins sequenced and identified as β₂–GPI.\(^{57}\) The β₂–GPI has a molecular mass of 50 kDa; the plasma concentration is approximately 200 μg/ml, of which 40% is associated with lipoproteins of various classes and is also known as apolipoprotein H. β₂–GPI is known to bind to negatively charged substances such as phospholipids, heparin, lipoproteins and activated platelets, in addition, β₂–GPI inhibits the intrinsic blood coagulation pathway ADP-dependent platelet aggregation.\(^{47,58}\)

The β₂–GPI is the most common and best-characterized antigenic target. APLAs have a preference to bind β₂–GPI that has been immobilized on anionic phospholipid membranes or certain synthetic surfaces.\(^{59}\)

In a further study, it was observed that APLAs associated with viral infection bound cardiolipin (CL) in the modified CL-ELISA without the need for added β₂–GPI. On the contrary, APLAs from autoimmune patients only bind to CL in the modified CL-ELISA in the presence of β₂–GPI. It was the first time that APLAs had been related with the coagulation cascade, as β₂–GPI possesses numerous inhibitory functions in multiple coagulation pathways.\(^{58}\) Another group of investigators reported that APLAs bound directly to β₂–GPI coated oxidized irradiated wells but not untreated wells. APLAs binding to β₂–GPI adsorbed to these wells correlated well with β₂–GPI complexed to negatively charged phospholipids. Binding was observed to be inhibited by preincubation with beads coated with CL and β₂–GPI but not β₂–GPI, or CL-coated beads alone. These data holds up the theory that APLAs bind a cryptic epitope on β₂–GPI that is only exposed after interaction with a negatively charged phospholipid.\(^{47}\) It has been

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**Figure 2.** Screening and confirmation of lupus anticoagulant.\(^{35}\) Activated partial thromboplastin time (aPTT), dilute aPTT, dilute Russel viper venom time (dRVVT) Antiphospholipid Antibodies Detected in by Immunoassay.
suggested that the effect of autoantibodies to β₂-GPI on the interaction of β₂-GPI with phospholipid surfaces may contribute to the pathogenesis of the APS. It is now accepted that β₂-GPI is the target antigen towards which these autoantibodies are directed; this provided more specific clinical tests for identification of pathogenic antibodies. One of the most hopeful aspects of the discovery of β₂-GPI as a target antigen for AP-LAs is the possibility that AP-LAs interfere with the function of β₂-GPI in vivo, thus conferring a prothrombotic diathesis in the APS.

Mature human β₂-GPI protein is composed of 326 amino acids preceded by a putative leader sequence of 19 amino acids. The sequences of other mammalian species (rat, mouse, bovine) of β₂-GPI show that they are well conserved and are approximately 84% homologous to the amino acid sequence of human β₂-GPI. This protein has five N-glycosylation sites, abundant proline residues, and 11 internal disulphide bonds. β₂-GPI possesses five complement control protein (CCP) repeat domains, of which the fifth domain has an aberrant C-terminal tail. CCPs also called short consensus repeat (SCR) domains, have a common 60 amino acid sequence, each of which allows the formation of two internal disulphide bridges. Unlike other members of the CCP superfamily that are shown to be located in chromosome 1, the human β₂-GPI gene has been assigned to chromosome 17 (Figure 3). The fifth domain of β₂-GPI was reported to contain both phospholipids and ACA binding sites.

Figure 3. Amino acid sequence and location of disulfide bands in human β₂-GPI.
DIFFERENTIAL DIAGNOSIS

A differential diagnosis should be considered when there is unexplained arterial or venous thrombosis, thrombotic involvement of unusual sites like adrenal or renal veins, thrombosis in a patient younger than 50 years, recurrent thrombotic events, second or third trimester losses, and more than one APS event in the same individual. Confirmation should rely on an unequivocally positive tests for LA or medium to high titer of ACA. Other diagnosis that should be considered in these patients, include factor V Leiden (activated protein C resistance), Protein C, protein S, or antithrombin III deficiency; dysfibrinogenemias; abnormalities of fibrinolysis, nephrotic syndrome; polycythemia vera; paroxysmal nocturnal hemoglobinuria; and side effects of oral contraceptives. In patients with arterial occlusion, the possibility of hyperlipidemias, diabetes mellitus, hypertension, vasculitis, homocysteinuria and Buerger’s disease should also be taken into account. APS accounts for only a small fraction of pregnancy losses. Other possible causes that must be evaluated include chromosomal abnormalities, anatomic anomalies of the maternal reproductive tract, and maternal disorders such as infectious, endocrine, autoimmune and drug-induced disease

TREATMENT

Treatment decisions are allotted into four main areas: prophylaxis, prevention of further thromboses of large vessels, treatment of acute thrombotic microangiopathy, and management of pregnancy in association with APS. Only 10-15% of patients with APLAs develop complications, which makes the decision to treat more difficult. A low daily dose of aspirin (75-80 mg/day) is frequently used. Other studies examined the role of a higher dose of aspirin (325 mg/day) as a prophylactic agent. Aspirin did not offer protection against deep vein thrombosis and pulmonary embolism in male patients with ACA. In contrast, aspirin may provide protection against thrombosis in women with the APS and previous pregnancy loss. Hydroxichloroquine has been reported to decrease the titers of APLAs and may protect against future thrombosis in patients with SLE and secondary APS. According to the sixth American College of Chest Physicians (ACCP) guidelines for antithrombotic therapy for prevention and treatment of thrombosis, the basis of treatment of arterial or venous thrombosis in patients with established APS is low molecular weight heparins (LMWHs) followed by oral anticoagulants (warfarin) at an International Normalized Ratio (INR) of 2.5 for 12 months or longer. It is important to test the level of protein C before starting warfarin therapy; protein C deficiency has been implicated in thrombosis of the venules and capillaries of the skin. In cases where protein S and/or protein C are decreased, an alternative approach should be considered. The LMWHs have several advantages compared with unfractionated heparin. Perhaps the greatest advantage is the ability to move the acute management of thrombosis from the hospital to outpatient settings. Unfractionated heparin costs much less compared with LMWH, but this advantage is offset by the increased costs of hospitalization and laboratory testing associated with heparin therapy. Low-molecular weight is cleared by the kidneys, and thus any decrease in the glomerular filtration can prolong the plasma half-life, sometimes requiring dosing adjustments. If a patient decides to become pregnant while administering warfarin therapy, the drug should be discontinued because of its teratogenic effect. The patient may convert to therapeutic doses of either unfractionated heparin subcutaneously or LMWH. It was initially thought that there was a possibility that LMWH may cross the placenta. Several studies have shown that this does not occur in any trimester, concluding that LMWH is safe in pregnancy. However, it appears that the pharmacokinetics of enoxaparin during pregnancy is different. Antifactor Xa levels were measured in 40 pregnant women who received 40 mg/day of enoxaparin, the Antifactor Xa levels where lower during pregnancy than during the nonpregnant period, probably because of a greater clearance and larger volume of distribution of enoxaparin because of the pregnancy-associated increase in plasma volume and glomerular filtration rate. Therefore it is believed that when LMWH is used in pregnant patients, measurement of Antifactor Xa levels are necessary to ensure that an adequate dose is being used to reach the desired anticoagulation level.

In idiopathic venous thromboembolism, patients were traditionally treated for 3 months with
anticoagulation (warfarin). A randomized study was undertaken to evaluate whether an additional 2 years of anticoagulation would be superior versus placebo in patients with venous thromboembolism (VT). After a period of 10 months, a significantly greater rate of recurrent thrombosis was demonstrated in the placebo group (p<0.001). The longer duration of warfarin resulted in a 95% reduction in the risk of recurrent thrombosis. The conclusion was that anticoagulation might be used for more than 3 months. In contrast, it is accepted that long-term anticoagulation in patients with APS and thrombosis is necessary because of the increased risk for recurrent thrombosis. Recurrence rates for thrombosis among these patients range from 22-69%.

In general, warfarin, heparin and LMWH are well tolerated. One acute complication that can result from unfractionated heparin (less common with LMWH) is heparin-induced thrombocytopenia (HIT). This occurs after previous exposure to heparin in which antibodies recognize a complex of heparin and platelet factor 4. These immune complexes bind to platelet Fc receptors to mediate immune clearance, and an abrupt decrease in the platelet count occurs. If this situation develops, all forms of heparin should be discontinued, and patients should be treated with an alternative medication like lepirudin, a synthetic derivative of the anticoagulant secreted by the medicinal leech. Long term heparin use also results in progressive osteoporosis.

Bleeding is the most troublesome complication with chronic anticoagulation, which includes minor bleeding to hemorrhagic cerebral vascular accidents. In patients treated for APS, significant bleeding occurs when the level of anticoagulation becomes supratherapeutic. In women vaginal bleeding may be present, especially of anovulation or an anatomic reason such as an uterine leiomyoma.

**TREATMENT OF THE CATASTROPHIC ANTIPHOSPHOLIPID SYNDROME**

The catastrophic APS leads to rapid development of multiorgan failure because of the multiple vascular occlusions. Therefore, intravenous heparin should be started as soon as the diagnosis is made. Treatment with antithrombin III prevents the continuation of unchecked coagulation. The basic abnormality in these patients affects fibrinolytic system, with high levels of plasminogen activator inhibitor and a reduction in plasminogen activator. Another possible therapy is the use of tissue plasminogen activator (t-PA). Pentoxifylline is useful in improving erythrocyte flexibility and increases blood flow by decreasing blood viscosity. One of the most successful approaches is the use of plasmapheresis. Aggressive supportive therapy, including hemodialysis, inotropic support, intubation and mechanical ventilation, is often necessary. With resolution of the catastrophic APS, continuous replacement steroid therapy may be indicated if patients have adrenal hemorrhage during the acute disease. Lifelong anticoagulation therapy is indicated in these cases.

**REFERENCES**


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