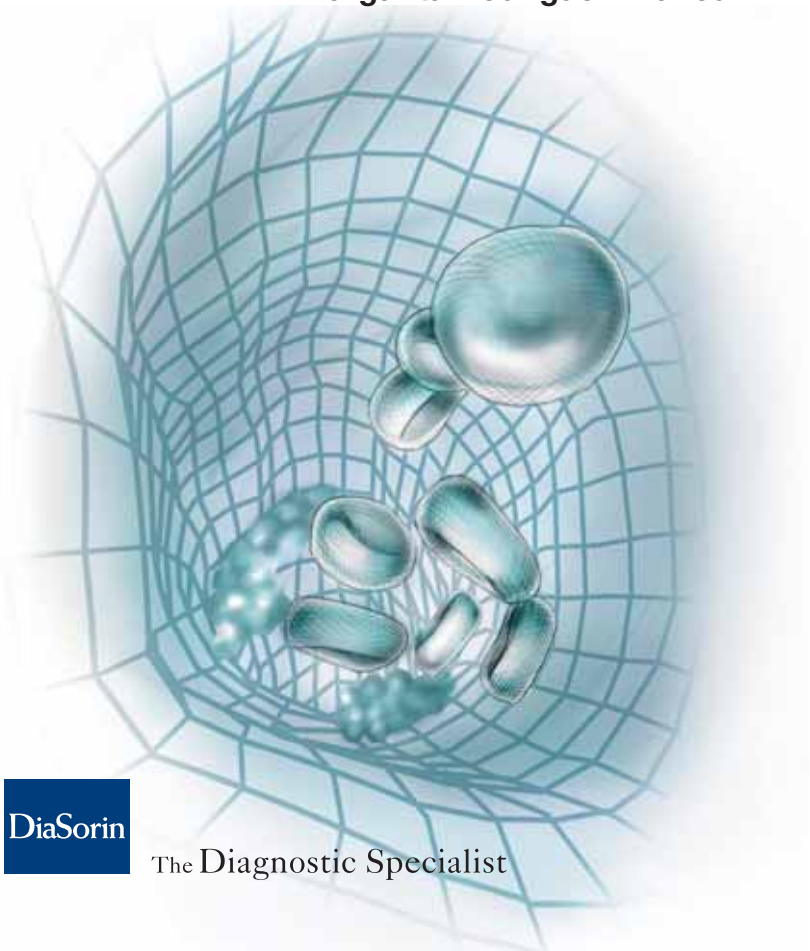
Antiphospholipid **Syndrome**

Ricard Cervera
Margarita Rodríguez-Mahou



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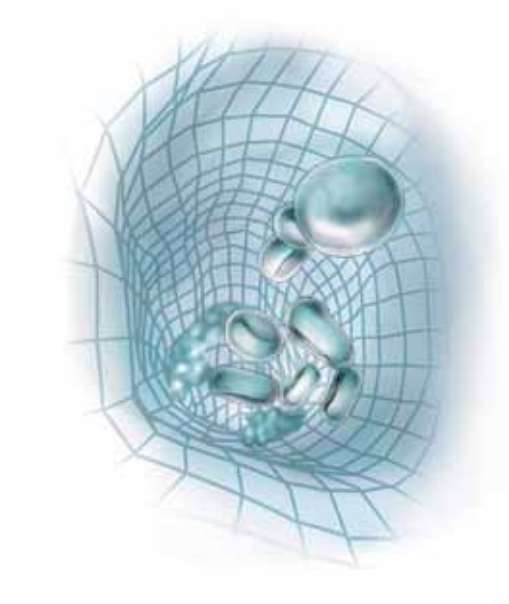

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Presentation

The antiphospholipid syndrome (APS), initially known as anticardiolipin syndrome, is characterized by the development of arterial and venous thrombotic phenomena, miscarriage and recurrent foetal loss, and thrombocytopoenia, in the presence of antiphospholipid antibodies (APA). It was first described by Graham R.V. Hughes in an editorial of the *British Medical Journal* in 1983. Now, almost 25 years after syndrome description, it seems to be opportune to recapitulate upon current knowledge and to comment on the direction to which present research is heading.

Undoubtedly, APS is widely recognized in current medical practice. One of its most outstanding characteristics is that although it was originally described in the field of Internal Medicine and Rheumatology (due to frequent association with systemic lupus erythematosus), its wide range of clinical manifestations has led to introduction into practically all medical, surgical and medical-surgical specialities.

In the 1950s, haematologists discovered the paradoxical phenomenon of increased coagulation time in lupic patients that did not present haemorrhages, which was termed as "lupic anticoagulant". Over the years, this phenomenon proved to be associated with thrombosis and also appeared in patients not affected by lupus, rendering the term doubly incorrect. It is now known that this phenomenon is due to the presence of APA

and is, together with anticardiolipin antibodies, one of the diagnostic markers of APS. For this reason, immunologists are now intensely engaged in research on the subject.

Miscarriage and recurrent foetal loss represent a core manifestation of this syndrome and as a result obstetricians, gynaecologists and perinatal paediatricians have become the main specialists in this pathology. APS is one of the main causes of cerebrovascular disorders in persons under the age of 50. This made neurologists develop interest in the syndrome together with occasional associations between APS and other neurological manifestations, such as vascular dementia, epilepsy, chorea, transverse myelopathy and even migraine. Pulmonary disorders, principally pulmonary embolism, but also pulmonary hypertension, alveolar haemorrhage or respiratory distress in adults challenged the interest of many pneumologists. Its involvement in ischaemic cardiopathy and, above all, in cardiac valve lesions means that it has become an interesting research subject for many cardiologists. Specialists in Intensive Care Medicine may encounter patients with manifestations of multisystemic thrombosis, often characterized by severe neurological disorders (coma, epileptic status), respiratory distress, cardiac or renal failure. Such condition has a mortality rate of over 50%, and is termed "catastrophic APS". Diagnosis of APS frequent when nephrologists observe renal microangiopathic thrombosis; ophthalmologists observe retinal venous thrombosis; endocrinologists observe adrenal insufficiency secondary to adrenal venous thrombosis; hepatologists observe hepatic regenerative nodular hyperplasia; otorhinolaryngologists diagnose hypoacusia due to microcirculation disorders of the ear; and even when surgeons

observe intestinal ischaemia due to mesenteric venous thrombosis in the course of urgent laparotomy, to name only a few representative examples.

Some estimations indicate that the incidence of APS reaches approximately five cases per 100,000 inhabitants per year and a prevalence of 40 cases per 100,000 inhabitants. However, many cases may remain undiagnosed, as it is the case for many deaths attributed to the so-called "tourist class syndrome" (pulmonary thromboembolism after long haul flights).

APS has irrupted into the XXI century and hundreds of researchers all over the world are trying to solve its multiple hidden enigmas. An indication of such interest is the fact that over 500 participants take part in the international monographic congresses held on the subject every two years. The experimental studies, on the one hand, as well as the forthcoming introduction of anticoagulant drugs or perhaps the use of other therapies on the other, will probably change our perception of this syndrome in coming years.

Summary

Antiphospholipid syndrome

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Introduction

The term "antiphospholipid syndrome" (APS) describes the association between antiphospholipid antibodies (APA) with the clinical manifestation of hypercoagulability, characterized by recurrent thrombosis and/or recurrent foetal loss, often accompanied by mild or moderate thrombocytopoenia. APA are a family of autoantibodies that recognize several combinations of phospholipids, proteins bound to phospholipids or both. Anticardiolipin antibody (ACA), lupic anticoagulant (LA) and anti-β2-glycoprotein I antibody are the most widely researched molecules.

Historic profile

Researchers first became aware of APA during the 1950s, after observing that a number of patients with systemic lupus erythematosus (SLE) presented false-positive luetic serology. In 1952, Conely and Hartman described the existence of a coagulation inhibitor in such patients, observing increased *in vitro* coagulation time, without greater tendency towards haemorrhage *in vivo*. In 1972, Feinstein and Rapaport introduced

the term LA to indicate this coagulation inhibitor. Subsequently, it was demonstrated that LA is associated with thrombotic and obstetric complications and thrombocytopoenia. Similarly, the LA phenomenon and false-positive luetic serology were found to be attributable to the existence of antibodies against anionic phospholipids. Then in 1983, ACA were detected for the first time by radioimmunoassay (RIA) and enzyme immunoassay (ELISA).

During the 1980s, the term APS was introduced with the observation that patients with a clinical record of thrombosis without SLE also present APS antibodies, leading to the term "primary APS". Subsequently, during the 1990s it was discovered that some ACA require the participation of a protein or cofactor (β2-glycoprotein I) for binding to phospholipids. In 1992, dependence on β2-glycoprotein I was observed in APS, but not in APA associated with syphilis or other infectious diseases. In 1993, anti-β2-glycoprotein I antibodies were described and in the following years other antibodies against several proteins (prothrombin, annexin V, proteins C and S, thrombomodulin, oxidized low-density lipoproteins) were added to the APA list. However, the clinical relevance of those antibodies has yet to be established.

Epidemiology

APS may appear as an isolated form, known as primary APS, or may be associated with other systemic autoimmune diseases. Table I shows the autoimmune diseases with which it is most frequently associated.

APA are also observed in other situations, such as infections, neoplasms or are associated with drug administration. More recently, a subgroup was described, in which development of multiple thromboses in small vessels of numerous organs is observed within short periods of time. Such situation is termed as catastrophic APS and is responsible for a mortality rate of up to 30%.

Just like other autoimmune diseases, APS is more frequent in women, with a ratio of 5:1. Approximately 30% of SLE patients present APA associated with thrombosis, spontaneous foetal loss and thrombocytopoenia at a percentage of 30-40%. The presence of APA is observed in 21% of young heart attack patients and its presence is equally detected in a high number of cerebrovascular disorders in persons under the age of 40.

Primary forms and those associated with other diseases present a practically identical clinical picture. However, in one study the SLE-associated form was more frequently observed in heart valve disease, haemolytic anaemia, neutropoenia, and lower concentrations of the complement C4 fraction.

Classification and diagnosis

APS classification criteria were established for the first time in 1998 at the International Symposium on APA in Sapporo, Japan. In 2004, such criteria were revised during the International Conference held in Sydney, Australia (Table II). Other manifestations that may be present in APS, such as thrombocytopoenia,

TABLE I. Diseases associated with APA

Autoimmune diseases

Systemic lupus erythematosus
Rheumatoid arthritis
Systemic sclerosis
Temporal arteritis
Sjögren's syndrome
Psoriatic arthropathy
Behçet's syndrome and dermatopolymyositis
Hashimoto's thyroiditis and mixed connective tissue disease

Infections

Viral diseases (HIV, chicken pox, hepatitis C)
Bacterial diseases (syphilis)
Parasitic diseases (malaria)

Lymphoproliferative diseases

Lymphomas
Paraproteinaemia
Drug-induced :
- Phenothiazines
- Quinidine
- Hydralazine
- Procainamide
- Phenytoin

Miscellaneous

Autoimmune thrombocytopoenia
Autoimmune haemolytic anaemia
Parenteral drug addiction
Guillain-Barré syndrome

haemolytic anaemia, transverse myelitis, valvulopathy, *livedo reticularis*, chorea or migraine were not considered classification criteria, but as associated manifestations (Table III).

TABLE II. Classification criteria for APS

Clinical criteria

1. Thrombotic phenomena

   One or more clinical episodes of arterial, venous or small vessel thrombosis in any organ or tissue. Such thromboses should be confirmed through Doppler or histological image studies, with the exception of superficial venous thrombosis. In histopathological confirmation, thrombosis should be present without evidence of vascular wall inflammation.

2. Obstetric manifestations

   a) One or more idiopathic deaths of a morphologically normal foetus after ten weeks of gestation, with normal foetal morphology confirmed through ultrasonography or direct examination.

   b) One or more premature births of a morphologically normal newborn child after 34 weeks of gestation, due to severe pre-eclampsia or eclampsia, or severe placental insufficiency.

   c) Three or more consecutive idiopathic miscarriages prior to ten weeks of gestation, after other causes, such as abnormalities in maternal anatomy, or where hormonal or parent chromosome alterations have been excluded.

cont'd

TABLE II. *Cont'd*

Laboratory criteria

1. ACA of IgG and/or IgM isotype in blood, present at a moderate or high titre, in two or more occasions, with a time lapse of at least 12 weeks, determined by ELISA for β2GPI-dependent ACA.
2. LA present in plasma, in two or more occasions, with a time lapse of at least 12 weeks, determined in accordance with directives established by the International Society of Thrombosis and Haemostasis:
   a) Increased phospholipid-dependent coagulation time demonstrated by screening assays, for example, activated partial thromboplastin time, kaolin time, Russell's time, diluted prothrombin or textarin time.
   b) Absence of increased coagulation time in screening assays on addition of platelet-poor plasma.
   c) Shortening or correction of increased coagulation time in screening tests on addition of phospholipids.
   d) Exclusion of other coagulation pathologies, such as factor VIII inhibitor, or heparin.
3. Anti-β2GPI antibodies of IgG and/or IgM isotype in blood, present at a moderate or high titre, on two or more occasions, with a time lapse of at least 12 weeks, determined by ELISA.

Definitive classification is established through both clinical and laboratory criteria.

Classification as APS should not be established if the time lapse between the clinical event and the positive determination of APA is less than 12 weeks or more than five years.

Such criteria are only classificatory and have not yet been prospectively validated. Consequently, other clinical (heart valve lesions, *livedo reticularis*...) and laboratory (thrombocytopoenia and/or haemolytic anaemia) criteria should also be taken into account in the diagnosis of APS.

TABLE III. Other clinical and laboratory manifestations associated with APA, not included within the classification criteria.

- Thrombocytopoenia
- Haemolytic anaemia
- Transverse myelopathy
- *Livedo reticularis*
- Heart valve lesions
- Chorea
- Migraine
- Nephropathy
- ACA IgA
- Anti-β2GPI antibodies of IgA isotype
- Anti-phosphatidylserine antibodies
- Anti-phosphatidylethanolamine antibodies
- Anti-prothrombin antibodies
- Anti-phosphatidylserine/prothrombin antibodies

In the diagnosis of catastrophic APS, clinical disorders of at least three different organs are required within a period of days or few weeks, together with anatomopathological evidence of multiple occlusions in large or small vessels. Some of the most recently established classification criteria are shown In Table IV.

Precipitating factors are usually present in syndrome development, which include infections, surgery, discontinuation of oral anticoagulation therapy and the use of oral contraceptives.

TABLE IV. Classification criteria for catastrophic APS

1. Clinical evidence of three or more affected organs, systems or tissues (a)
2. Development of manifestations occurring simultaneously or within one week
3. Anatomopathological confirmation of small vessel occlusion in at least one organ (b)
4. Analytical confirmation of the presence of APA (lupic anticoagulant or anticardiolipin antibodies) (c)

- **Definitive** catastrophic APS: The four aforementioned criteria
- **Probable** catastrophic APS:
  - The four aforementioned criteria, except where only two organs, systems or tissues are affected
  - The four aforementioned criteria, except confirmation of the presence of APA in a second determination at least six weeks later, due to premature patient's death
  - Criteria 1, 2, and 4
  - Criteria 1, 3 and 4 and the development of the third thrombosis after the first week but before one month, despite anticoagulation.

a) In general, clinical evidence of thrombosis, confirmed by imaging techniques where appropriate. Renal failure is defined as an increase of 50% in serum creatinine, acute high blood pressure (>180/100 mmHg) and/or proteinuria (> 500 mg/24 hours).

b) In anatomopathological confirmation signs of thrombosis should be present. However, occasionally vasculitis may concurrently be present.

c) If the patient is not previously diagnosed with APS, analytical confirmation requires that APA are detected on two or more occasions, with a time lapse of at least six weeks (not necessarily at the time when thrombosis is detected), according to preliminary criteria for the classification of definitive APS.

Clinical manifestations

Recurrent thrombosis

Thrombosis is the main complication in APS and may affect vessels of any size, either arterial or venous. The main characteristic of thrombosis is recurrence. Over 50% of patients usually present recurrent thromboembolism, generally in the same vessels as the previous episode.

Venous thrombosis is described in any vessels, although the most frequently affected area is the deep venous circulation in lower extremities, which may also be accompanied by pulmonary thromboembolism. On rare occasions, recurrent thromboembolic episodes may lead to development of chronic pulmonary hypertension. Superficial venous thrombosis may also appear in the jugular (Fig. 1), subclavian, brachial, and other veins.

The most frequent arterial thrombosis is occlusion of intercranial arteries, manifested as transitory ischaemic or cerebrovascular disorders. Cerebral thrombosis is occasionally associated with *livedo reticularis* and arterial hypertension (Sneddon's syndrome). Recurrent of thrombosis may lead to multi-infarct dementia (Fig. 2). Other less frequent neurological manifestations associated with the presence of APA are chorea, epilepsy, migraine and transverse myelitis. In addition to cerebral dysfunction, arterial thrombosis may also occur in any other organ of the body.

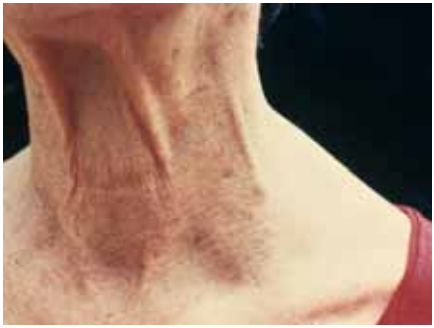

Figure 1.
Jugular venous thrombosis

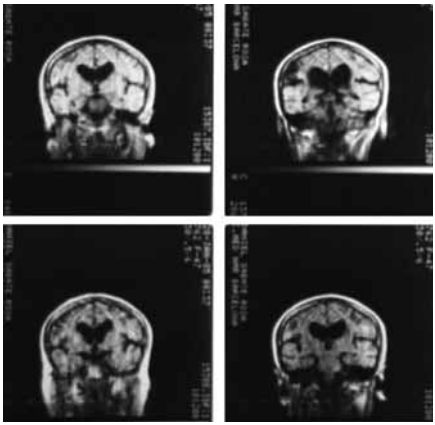

Figure 2.
Multi-infarct dementia

There are several cardiac manifestations of APS including myocardial alteration secondary to thrombotic occlusion of intramyocardial arteries (Fig. 3), formation of intracardial thrombosis (Fig. 4), coronary heart disease and valvulopathy. The most frequently occurring pathology is valvular alterations,

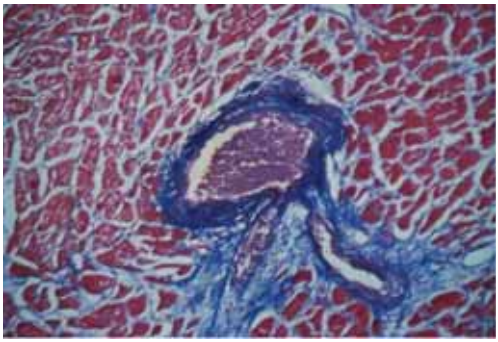

Figure 3.
Intramyocardial arterial thrombosis

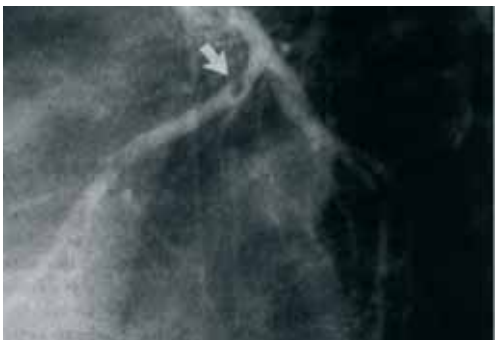

Figure 4.
Coronary thrombosis

with a prevalence of over 30%. The mitral valve is the most commonly affected, followed by the aortic valve. Such condition usually causes valvular insufficiency and the lesions that occur vary from thickening of the valves to formation of "warts" or protrusions (non-bacterial thrombotic endocarditis or Libman-

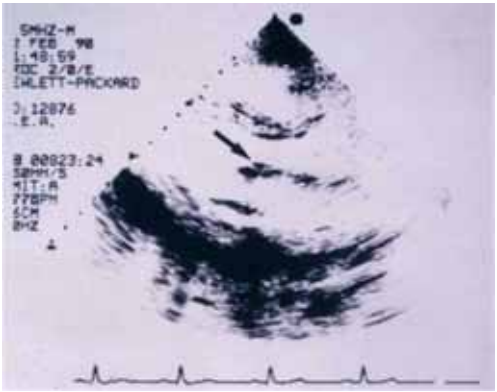

Figure 5.
Non-bacterial thrombotic endocarditis on the mitral valve

Sacks endocarditis) (Fig. 5). Such valvular disorders may associate secondary cerebral ischaemia with cardioembolic phenomena.

Renal manifestations are also mainly of thrombotic nature. The clinical picture includes lesions of the renal artery or one of its branches, renal infarcts, thrombosis of the renal veins or intrarenal vascular lesions, characterized by renal thrombotic microangiopathy (Fig. 6).

Digestive manifestations are infrequent. Episodes of intestinal ischaemia, liver (Fig. 7) and spleen infarction, splenoportal thrombosis and even thrombosis of the suprahepatic veins are described. APS is currently considered as one of the most frequent causes of the Budd-Chiari syndrome and nodular regenerative liver hyperplasia (Fig. 8).

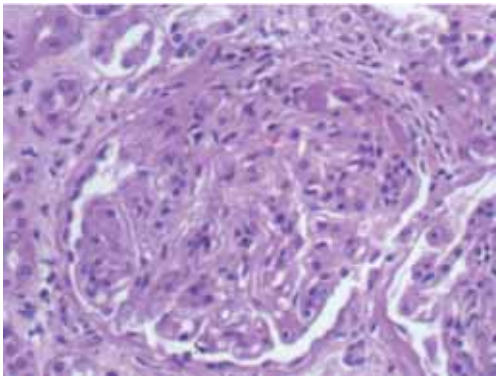

Figure 6.
Renal thrombotic microangiopathy

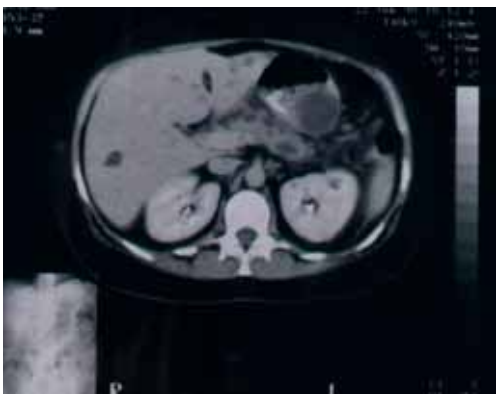

Figure 7.
Hepatic infarction

Ocular lesions include retinal vessel occlusion, central retinal venous or arterial thrombosis and optic neuropathy. Vessel occlusive disease may cause both arterial ischaemia and retinal venous thrombosis.

A wide range of cutaneous manifestations are described,

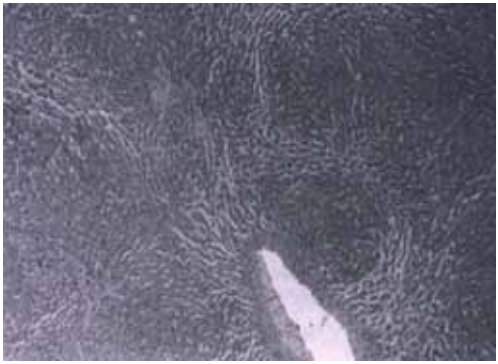

Figure 8.
Nodular regenerative liver hyperplasia

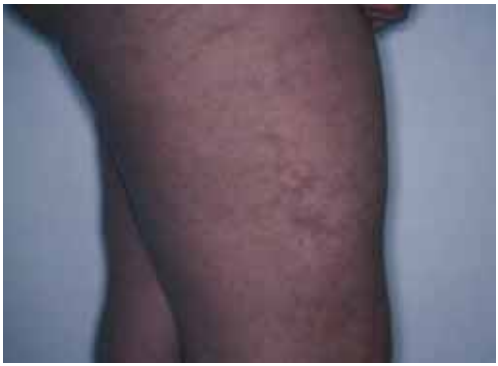

Figure 9.
Livedo reticularis

including *livedo reticularis* (Fig. 9), necrotizing vasculitis, ulcers (Fig. 10) and cutaneous nodules, thrombophlebitis, necrosis and cutaneous gangrene (Fig. 11), subungueal splinter haemorrhages (Fig. 12), ecchymosis and purpura. Of all such conditions, the most frequent one is *livedo reticularis*.

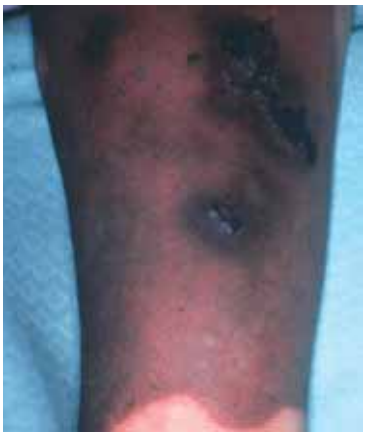

Figure 10.
Cutaneous ulcer

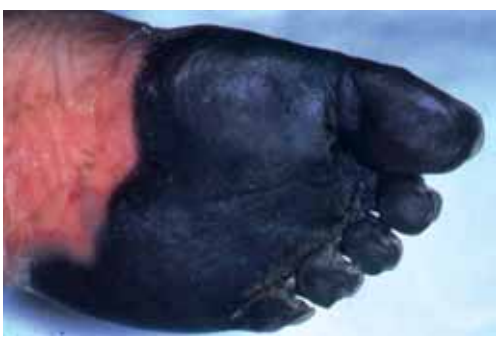

Figure 11.
Cutaneous necrosis

Haematological alterations

The main haematological alterations associated with APA are thrombocytopoenia and autoimmune haemolytic anaemia.

The prevalence of thrombocytopoenia is over 20%, but is usually mild to moderate and generally no treatment is required.

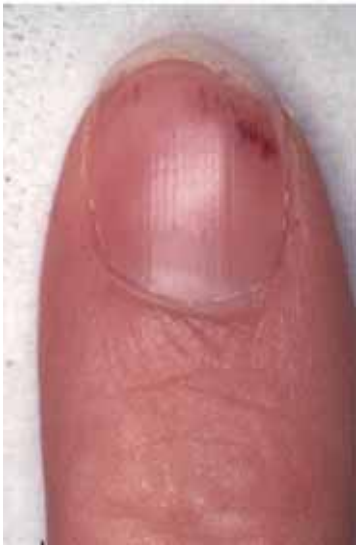

Figure 12.
Subungueal splinter haemorrhage

Occasionally, the condition may be the initial manifestation of the syndrome.

Some patients with APA may develop haemolytic anaemia with positive direct Coombs' test, microangiopathic haemolytic anaemia with the presence of schistocytes in peripheral blood or occasionally, disseminated intravascular coagulation.

Obstetric complications

APS is closely associated with obstetric complications. In fact, with a prevalence of approximately 10%, the presence of APA is currently considered as one of the main known causes

of recurrent miscarriage among unselected women with a history of previous miscarriages. In one study, the presence of APA was studied in embryofoetal loss in women with a history of previous miscarriages. Comparison of the presence or absence of APA and incidence of foetal loss revealed that recurrent miscarriage in APA-positive women is 90%, while in APA-negative cases is 34%. In most cases the percent term pregnancies are less than 20% before diagnosis of APS, and consequently, no prophylactic treatment is given. Loss may occur at any time during pregnancy. Although it is more frequent in the first three months, foetal death seems to be specifically APS-related. Other associated obstetric complications are intrauterine growth retardation, preeclampsia, prematurity and foetal distress.

Treatment

Controversy exists about optimal APS treatment and the subject is under constant revision. As a consequence of the low number of patients suffering from this condition, it is difficult to carry out prospective studies, to enable definitive conclusions to be drawn. Although seems to exist a clear association between APA and thrombosis, treatment should not be zeroed in to elimination or reduction of those antibodies through immunosuppressive therapy (plasma refills, cyclophosphamide or intravenous gammaglobulins), given that no clear correlation exists between APA concentrations and thrombotic episodes. Consequently, treatment of those patients should be based on anti-platelet or anticoagulant drugs.

1. Elimination of risk factors: In APA patients, additional vascular risk factors, such as arterial hypertension, hypercholesterolaemia, smoking or the use of oral contraceptives containing oestrogens should be curbed or eliminated. Special care is also required in postoperative or bedridden patients, to whom appropriate prophylaxis of venous thrombosis should be carried out with subcutaneous heparin.

2. Prophylaxis in asymptomatic patients: Prophylactic treatment with low doses of acetylsalicylic acid (ASA) (100 mg/day) is recommended, although it is controversial in patients with persistently positive LA or high-titre ACA of IgG isotype with no background of thrombosis.

3. Treatment of thrombosis: Treatment is the same used for thromboembolism in the general population. Initial treatment starts with intravenously administered heparin or low-molecular weight heparin, and subsequently, anticoagulation is maintained through oral administration of coumarins.

   However, due to the high frequency of recurrent thrombosis in APS, anticoagulation should be maintained over long periods of time or even for life. Although anticoagulant dose is a matter of controversy, International Normalized Ratio (INR) should be maintained within the range of 2-3, given that there is a greater risk of haemorrhage with an INR above 3. Treatment with ASA at low doses does not avoid the risk of recurrent thrombosis.

4. Treatment of catastrophic APS: Due to the high mortality rate, anticoagulation should be started immediately with heparin, and APA levels should be reduced with plasma refills or intravenous gammaglobulins. The use of plasma refills is derived from its proven effectiveness in thrombotic thrombocytopoenic purpura and in haemolytic uraemic syndrome. Similarly, high doses of glucocorticoids should be administered to treat systemic inflammatory response syndrome, which generally accompanies the catastrophic syndrome. Additionally, it should be taken into account that treatment with antibiotics should be rapidly started when infection is suspected or a necrosed organ is to be removed. Extreme care should be taken with APS patients that require surgery or other invasive techniques.

5. Prophylaxis of miscarriage and foetal loss: Pregnant women with APA should be considered at high risk and should be kept under close observation. The growing foetus and the umbilical artery flow should be monitored, so as to enable early detection of abnormalities in uteroplacental circulation. The percent miscarriages or foetal losses in patients not undergoing prophylactic treatment are high, over 80%. Percent term pregnancies are notably increased with the administration of prophylactic treatment, with a success rate of up to 70%. However, a high incidence of foetal and obstetric complications still remains. Low-dose ASA treatment (75-100 mg/day), started at conception and lasting until delivery was demonstrated to be a safe and effective prophylactic treatment. Concurrent treatment

with ASA and subcutaneous low-molecular weight heparin may also be administered, especially in cases where ASA treatment only is not effective. Prednisone was demonstrated to be much less effective and its administration at high doses is associated with high maternal morbidity rate and should be administered for non-obstetric reasons, such as intense thrombocytopoenia. When its administration is considered necessary, it should be used preferably at low doses (< 30 mg/day), in order to avoid other complications. In cases where the woman suffered previous thromboembolism and coumarin is used as anticoagulant, treatment should be suspended, due to potential teratogenicity, and substituted with subcutaneous low-molecular weight heparin at therapeutic levels. When these measures fail, gammaglobulins may be administered intravenously (0.4 g/kg/day for five days or 1 g/kg at single doses repeated monthly) on an individual basis.

6. Treatment of thrombocytopoenia: Thrombocytopoenia associated with the presence of APA is usually moderate (the number of platelets is usually above 50,000/mL) and does not require treatment. In cases of intense thrombocytopoenia, prednisone is usually effective. Other alternatives consist of intravenous administration of gammaglobulins, administration of danazole, or even splenectomy. The use of rituximab has recently been demonstrated to be effective in refractory cases.

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Antiphospholipid antibodies as diagnostic markers of antiphospholipid syndrome

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Antiphospholipid antibodies (APA) are a group of heterogeneous IgG, IgA and IgM autoantibodies that bind with anionic phospholipids, such as cardiolipin, or with plasma proteins, which have affinity for phospholipid surfaces, such as β2-glycoprotein I (β2GPI), prothrombin, annexin V, protein C, protein S and the quininogens (Table 1). Phospholipids are polar lipids that form part of the cell membrane. Phosphatidylserine, phosphatidylinositol, phosphatidic acid and cardiolipin are negatively charged, while phosphatidylcholine is neutral and phosphatidylethanolamine is dipolar. Phospholipids are involved in the blood coagulation cascade. The appearance of clinical signs, mainly vascular thrombosis and recurrent miscarriage that are related to the presence of APA, is proof of antiphospholipid syndrome (APS) or Hughes syndrome (in honour of the English rheumatologist, who first described APS in 1983).

Current recommended classification criteria for APS involve the use of enzyme immunoassay (ELISA), which measures β2GPI-dependent anticardiolipin antibodies (ACA) IgG and IgM and/or

TABLE I. Different specificities of antiphospholipid antibodies

Reagin
Antibodies against anionic phospholipids
Cardiolipin
Phosphatidylserine
Phosphatidic acid
Phosphatidylinositol
Antibodies against neutral phospholipids
Phosphatidylcholine
Dipolar antiphospholipid antibodies
Phosphatidylethanolamine
Antibodies against proteins that bind to phospholipids
β2-glycoprotein I
Prothrombin
Annexin V
Protein C
Protein S
High- and low-molecular weight quininogens

lupic anticoagulant (LA), in accordance with the International Society of Thrombosis and Haemostasis Subcommittee on lupic anticoagulant/antiphospholipid antibodies. However, a reduced number of patients exists, who present all the typical manifestations of APS, but persistently give negative results in APA detection tests. This has lead to the concept of "seronegative APS". Although it is well known that routine ACA and/or LA tests may sometimes score negative, repeated tests and rigorous

differential diagnoses are mandatory, before estabilishing a "seronegative APS" diagnosis.

The two main aims in the search for APA are: To diagnose APS in the light of suggestive clinical evidence and to assess the risk of thromboembolism in a patient.

Anticardiolipin antibodies

Autoantigen

Cardiolipin is an anionic phospholipid, historically known as an antigen, used in the reagin test for diagnosis of syphilis. It is part of the antigen used in the *Venereal Disease Research Laboratory* test (VDRL), together with lecithin and cholesterol for syphilis screening.

In 1990, three independent research groups observed that purified ACA IgG in APS patients bind to cardiolipin only in the presence of a plasma protein, which is the true antigen. The protein was identified as β2-glycoprotein I (β2GPI) or apolipoprotein H. Cardiolipin may play an important *in vivo* role in facilitating binding between antibody and plasma protein that binds to phospholipid.

Biological function

Phospholipids are responsible for maintaining plasma membrane structure in a dynamic state, interleaving with the function of proteins present on the cell surface. They also play a fundamental role in the coagulation cascade (Fig. 1). Their

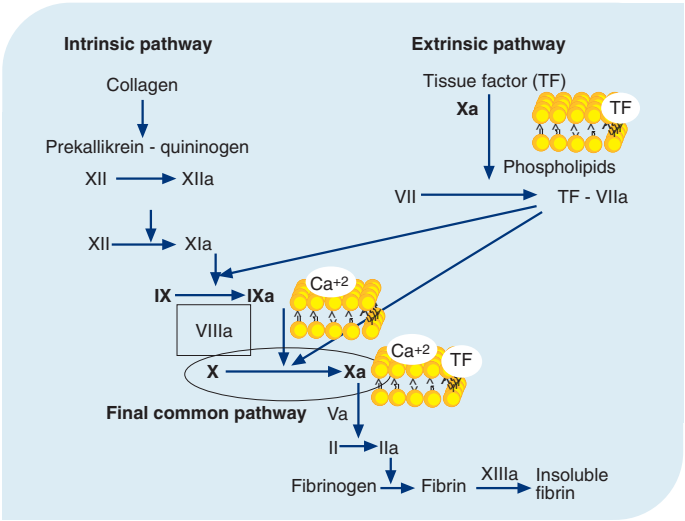

Figure 1. Coagulation cascade

presence is critical in several steps along the extrinsic, intrinsic and final common pathways. The activation of factors IX and X (intrinsic pathway), factor X (extrinsic pathway) and factor II (common pathway) requires the presence of phospholipids (Fig. 1). Although no direct evidence indicates what physiopathological role APA play, autoantibodies against proteins that bind to phospholipids were hypothesized to contribute directly to thrombotic diathesis, by interfering with coagulation cascade reactions, taking place on the membranes of anionic phospholipids *in vivo*.

ACA autoantibodies

Antibodies bind to negatively charged phosphodiester groups of cardiolipin. However, glyceride is an essential group in such

binding reaction. In fact, when it is substituted with benzyl rings, the molecule antigenicity is lost.

Phospholipids may simulate the conformation of the phosphate-sugar groups of polynucleotides. Monoclonal murine and human antibodies to single-stranded DNA cross-react with cardiolipin, while monoclonal ACA react with nucleic acids, with single-stranded and double-stranded DNA and with nucleosomes. The counter-argument against this hypothesis is that no correlation is found between ACA and anti-DNA concentrations, and that thrombosis or miscarriage is not associated with the presence of anti-DNA antibodies.

Cofactor-dependent ACA may be of IgG, IgM, or IgA isotype in APS. ACA of IgG isotype are more prevalent than ACA IgM in APS, and IgA is occasionally associated with clinical manifestations of APS.

ACA IgM may also be induced by drugs, may appear after infections, or may be synthesized as acute-phase reaction to non-specific stimuli. The latter effects are transitory and may disappear with time, with no link to thrombosis.

The clinical relevance of ACA IgA is uncertain. Recent studies seem to suggest that ACA IgA is similar to ACA of IgG isotype in patients with SLE, as far as thrombogenicity and binding to cofactor β2GPI are concerned. The injection of IgA from APS patients causes thrombosis in murine models designed to study clot formation. The prevalence of these

autoantibodies in SLE patients varies between 1 and 44%. Given the low prevalence of isolated ACA IgA antibodies in APS patients, these antibodies should be tested, when ACA IgG or IgM is negative.

Pathogenic role

β2GPI plays an essential role in ACA reaction in primary APS patients and in other autoimmune patients. ACA in patients with thrombosis or miscarriage may even be linked to β2GPI in the absence of phospholipids or anti-β2GPI antibodies. ACA antibodies present in determined infections bind to cardiolipin in the absence of β2GPI. These differences may explain why ACA present in autoimmune diseases are associated with thrombosis or foetal loss, while those that are detected in infectious diseases are not. ACA recognize a neoepitope that appears in β2GPI on binding with cardiolipin, given that the binding of cardiolipin to β2GPI induces conformational changes in the latter, which is coated to the well of the ELISA polystyrene plate. Both contact with an oxidized surface (after gamma-irradiation of the plate) and presence of anionic phospholipids induce a conformational change in β2GPI, causing exposure of a cryptic epitope, recognized by ACA.

Genetics

The haplotypes HLA-DR7 and DR5 are more frequent in patients with ACA antibodies. Familial susceptibility to APA was demonstrated to be high in APS patients, indicating that genetic factors may play a role.

TABLE II. Sensitivity and specificity of APA in the diagnosis of APS

Test	Sensitivity (%)	Specificity (%)
LA	94	79
ACA/β2GPI	54	86
aPT/PS	57	92
ACA/β2GPI or aPT/PS (Combination of ELISAs)	77	92

LA: Lupic anticoagulant;
ACA/β2GPI: β2-glycoprotein-dependent anticardiolipin antibodies;
aPT/PS: phosphatidylserine-dependent antiprothrombin antibodies.

Detection methods

The first ACA tests were carried out using radioimmunoassay (RIA), with successive modifications to solve basic problems. The introduction of foetal or adult bovine serum increases the antibody binding to cardiolipin. Such an effect is attributable to β2GPI.

ACA may be detected either by RIA or the more currently used Enzyme Immunoassay (ELISA), using solid-phase cardiolipin as antigen. Detection is carried out in serum or plasma. ACA of IgG, IgM and/or IgA isotype are expressed in GPL, MPL and/or APL units per mL respectively, where one unit is the binding activity of 1 mg/mL of affinity-purified ACA. In spite of recent international efforts, inter-laboratory consensus has still to be reached on ACA measurement. However, the use of semi-

quantitative positivity grading (low positive, medium positive or high positive) seems to be more appropriate clinically and is less susceptible to errors.

The ACA test has a sensitivity of 94% (Table II). However, the main problem arises from the specificity of cofactor-independent ACA, given that they may be positive in a great variety of diseases (syphilis, viral and parasitic diseases, rheumatoid arthritis and other autoimmune diseases).

Due to fluctuations in serum levels of APA, repeated quantification after a time lapse of 12 weeks is recommended.

Standardization of ACA detection test

Numerous meetings and international forums were organized in an attempt to reach consensus on the standardization of ACA tests. At a first workshop meeting, ACA detection methods were determined. Measurement units were also established and the use of six calibrators was introduced, so as to facilitate development of ACA antibody tests worldwide. A second workshop demonstrated that semi-quantitative measurements of ACA levels permit maximum inter-laboratory concordance. The third and fourth workshops attempted to solve the most controversial aspects of ACA specificity, together with analysis of commercial kits that have recently become available on the market.

ACA calibrators

Since the initial development of ACA detection tests, patients with SLE and other autoimmune diseases without a history of

previous thrombotic episode(s) or foetal loss(es) were observed to present high ACA levels. Many of these-false positive cases occur in patients with low-titre ACA, indicating that patients with signs of APS tend to present high-titre ACA. Consequently, it became evident that ACA grading as positive or negative is not sufficient, but titre determination is mandatory to establish a more specific diagnosis of APS. A series of ACA IgG calibrators was established with pools of sera with high concentrations of defined ACA IgG and normal serum mixed in variable concentrations. The concentration of every calibrator was assessed according to the proportions of IgG-positive serum and normal serum in the pool. The use of these calibrators allowed validation of all ACA detection techniques to be carried out. In fact results obtained by ELISA or any other method have to correlate with the calculated calibrator concentration of ACA IgG, in order that the technique is valid. It additionally permitted the calculation of ACA levels in unknown samples, using the calibration curve, independently of the technique used. Furthermore, secondary calibrators derived from the IgG calibration curve were prepared starting from the original calibrators. Similar procedures were used for IgM, and subsequently for IgA.

More recently, monoclonal ACA calibrators were introduced, in which preparations of monoclonal antibodies are serially diluted and correlated with previously prepared calibrators. Monoclonal calibrators offer the advantage of providing a single-specificity reference sample which may be used perpetually. Conversely, original polyclonal calibrators offer the advantage of originating from patients with APA poly-specificities.

Variations in ACA detection techniques

Low-, medium- and high-positive and negative titres are defined on the basis of the calibration curve. Numerous retrospective studies demonstrated that the diagnosis of APS is more probable when detecting medium and high levels of ACA, constituting APS diagnostic criteria. Nevertheless, in our experience, low titres of ACA and anti-β2GPI may also be associated with manifestations of APS. In the presence of suggestive clinical evidence in a determined patient, the titre of antibodies should be considered on an individual basis.

Anti-β2-glycoprotein I autoantibodies

Autoantigen

β2-glycoprotein I (β2GPI) is a protein that binds to phospholipids with a molecular weight of 50 kD and circulates in plasma at a concentration of approximately 200 µg/mL. It is composed of 326 amino acids and contains five homologous domains. Each of the first four domains (I-IV) is composed of 60 amino acids (aa) with highly conserved tryptophan, proline, and cysteine. The fifth domain is of particular importance because it contains the KNKEKK sequence that is responsible for binding to the phospholipid.

Biological function

In vitro, β2GPI binds to negatively charged molecules, such as phospholipids (cardiolipin and phosphatidylserine), heparin and some lipoproteins, as well as cell membranes of activated platelets and endothelial cells.

β2GPI is considered as a natural anticoagulant. It was demonstrated to affect coagulation and platelet function, inhibiting contact activation in the intrinsic coagulation pathway, prothrombinase platelet activity and ADP-dependent platelet aggregation. More recently, β2GPI has been shown to be involved in the elimination of apoptotic cells through binding to phosphatidylserine. This appears to affect metabolism of lipoproteins, enhancing clearance of oxidized products such as oxidized low-density lipoproteins (LDL). The functions of β2GPI are less well known *in vivo*.

Anti-β2GPI antibody

Free or monomeric β2GPI has a relatively low affinity for negatively charged phospholipids. Anti-β2GPI antibodies may cross-react with two β2GPI-phospholipid complexes, increasing binding affinity by over 100-fold. A possible hypothesis to explain how cross-reactivity on the cell surface may challenge prothrombotic mechanisms is platelet activation. Anti-β2GPI antibodies bind to two β2GPI molecules, inducing conformational changes. This interaction increases the affinity of the β2GPI dimer for phospholipids on the cell surface and the binding sites to the protein. β2GPI may interact with a receptor on the cell surface belonging to the low-density lipoprotein receptor family: apolipoprotein E receptor 2 (ApoER2). This interaction induces phosphorylation of ApoER2 followed by phosphorylation of p38MAP kinase and synthesis of thromboxane A2. These events may alter the haemostatic balance towards a prothrombotic state, thus increasing the risk of developing thrombosis in patients with anti-β2GPI antibodies.

Detection methods

As previously mentioned, Galli et al. and Virad et al. demonstrated that ACA from patients presenting SLE and APS are directed against β2GPI coated on polystyrene plate wells. Koike and Matsuura concluded that β2GPI was the main ACA antigen and that phospholipids acted as a binder of β2GPI to the solid phase.

Anti-β2GPI antibodies may be detected by ELISA, in accordance with Arvieux et al. methodology. Purified bovine or human β2GPI antigen from normal plasma is used and coated onto polystyrene plate wells in the absence of phospholipids.

Koike and Matsuura observed that a high-density negative charge is necessary to induce a structural change in the native β2GPI molecule, in order to make it immunogenic. This structural change may be induced in several ways: by binding β2GPI to the main (head) phosphate region of phospholipids or by previously gamma-irradiating the polystyrene plate, causing oxidation of the well surface and thus favouring protein binding. This structural change was confirmed by demonstrating that β2GPI-dependent ACA did not bind to β2GPI on the plates that are commonly used, but did so when the well was coated with β2GPI on oxygenated plates. The possible explanations for this phenomenon are the following:

1. When the well is coated with β2GPI on oxygenated plates, the C-O and C=O bonds may induce conformational alterations in β2GPI that cause exposure of cryptic epitopes.

2. As a result of low anti-β2GPI avidity, the bond of these autoantibodies to solid-phase β2GPI may require a high-density antigen. This system eliminates most false-positive or non-specific reactions, that are often observed on surfaces coated with cardiolipin.

Standardization tests of anti-β2GPI, together with the availability of a calibrator, allow results to be quantified and compared in inter-laboratory studies.

Just as for ACA, three different isotypes of anti-β2GPI antibodies are detected: IgG, IgM and IgA. Anti-β2GPI IgG antibodies are considered as being more specific than IgM. Recently several authors reported that anti-β2GPI IgA antibodies may also be associated with thrombosis and APS. In general, there is a good correlation between the presence of anti-β2GPI antibodies and the presence of ACA. However, in a small subgroup of patients presenting clinical manifestations of APS, only anti-β2GPI antibodies are detected, while results for ACA and LA are negative.

Lupic anticoagulant

Lupic anticoagulant (LA), a term coined by Feinstein and Rapaport in 1972, is an acquired coagulation inhibitor that alters prothrombinase activation to prothrombin (PT). It prolongs coagulation times through *in vitro* interference with phospholipids, without specifically affecting the activity of coagulation factors. LA presence is rarely associated with bleeding. LA was first detected in plasma of SLE patients.

LA is currently considered as the most important acquired risk factor in thrombosis and foetal loss.

Autoantigens

Two proteins, β2GPI and prothrombin (PT), are mostly involved in LA. However, annexin V was also found to be involved in the LA phenomenon as an autoantigen.

LA antibody

LA has been associated with the presence of autoantibodies of IgG, IgM and/or IgA isotype directed against β2GPI and/or PT, or annexin V.

LA increases phospholipid-dependent coagulation time (particularly in Russel's viper venom test, RVVT, see below). However, the activity of these antibodies is inhibited by phospholipid excess. Molecular mimicry between infectious agents and the β2GPI molecule is a proposed mechanism that may generate anti-β2GPI antibodies. In fact, there is an important homology between β2GPI-related peptides (target epitopes for anti-β2GPI antibodies) and several common pathogens. Furthermore, the polymorphism of β2GPI, especially allele Val247, was recently associated with high frequency of anti-β2GPI antibodies and greater reactivity than the Leu247 allele of β2GPI. There is a possibility that this polymorphism may favour the appearance of molecular mimicry.

The exact mechanism through which these immunoglobulins determine thromboembolism and foetal loss is unknown, but

several theories were proposed. One suggests that the antibodies responsible for *in vitro* LA also interfere with the *in vivo* inhibition pathways of phospholipid-dependent coagulation. In fact, phospholipid-dependent inactivation of factors Va/VIIIa through the thrombomodulin-protein C-protein-S system or through the phospholipid-dependent inhibition of tissue factor (TF) may be altered in the presence of LA.

Alternatively, β2GPI (annexin V or other potential LA auto-antigens) may exert an *in vivo* anticoagulant effect, while anti-β2GPI antibodies (or anti-annexin V) may alter it. In such a way, the formation of thromboses may occur on the surface of activated monocytes, platelets or endothelial cells. Conversely, the fact that congenital deficiency of β2GPI is not a risk factor for thrombosis seems to contradict this hypothesis.

Detection of lupic anticoagulant

LA is detected using coagulometric techniques that reveal the presence of antibodies directed against the phospholipid fraction of the prothrombin activation complex. Confirmation of the presence of LA should comply with the following four conditions (Fig. 2):

1. **Increase of at least one of phospholipid-dependent coagulation times in patient plasma under diagnosis.** Several tests may be used to assess intrinsic pathway coagulation (Fig. 1) [activated partial thromboplastin time (aPTT), diluted activated partial thromboplastin time (dAPTT), coagulation time with kaolin],

extrinsic pathway coagulation (diluted prothrombin time), or final common pathway coagulation [diluted Russell's viper venom time (dRVVT), textarin and ecarin time, taipan snake venom time].

1.1. The most widely used test is the aPTT test (>10 sec with respect to control). A normal coagulation time does not exclude the existence of LA when a low-sensitivity reagent to APA is used.

1.2. Inhibition test of thromboplastin diluted from 1/100 to 1/1000 (TIT). Prothrombin time is performed at low phospholipid concentrations, therefore coagulation time will be increased in the presence of LA. In this process, thromboplastin of diluted prothrombin time is used. The study is carried out with venous blood samples added with 3.8% trisodium citrate diluted 1/10. The results of TIT on samples diluted 1/100 and 1/1000 are expressed as patient TIT to healthy control TIT ratio.

1.3. Diluted Russell's viper venom test (dRVVT). Russell's viper venom is a snake venom, which converts factor X into its activated form, Xa in the presence of calcium ions. Factor Xa, together with phospholipids and factor V, convert prothrombin into thrombin.
The dRVVT is a modification of prothrombin time, specific for LA. The results of RVVT are expressed as patient dRVVT to healthy control dRVVT ratio.

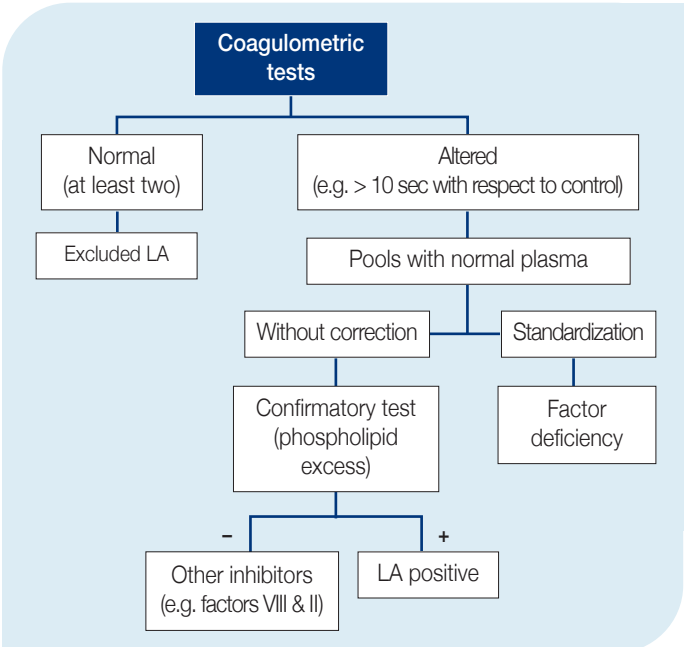

Figure 2. Lupic anticoagulant detection algorithm

2. Shortening or correction of increased coagulation time, after addition of an excess of phospholipids, either synthetic or obtained from platelet lysate (confirmatory test).

   The confirmatory Russell's viper venom test (dRVVT) is used to confirm the presence of LA in plasma in case the dRVVT result is positive. Confirmatory dRVVT is a coagulation test with Russell's viper venom and a phospholipid excess. The results may be

expressed as patient confirmatory dRVVT to healthy control confirmatory dRVVT ratio. It is also of interest to express the result as patient dRVVT to patient confirmatory dRVVT ratio.

3. Decision to correct increased coagulation time, after mixing patient plasma with normal plasma (1:1 ratio). This excludes deficiencies in coagulation factors contained in normal plasma.

   Exner test has fallen into disuse. However, it is a technique applied to patients with increased aPTT that cannot be corrected with control plasma and in cases when previous tests are negative. The results are expressed graphically, with the coagulation time in seconds plotted on the ordinate (y axis) and the data from the five control tubes and patient plasma pools on the abscissa (x axis). Positive result: the existence of a lupic inhibitor occurs when the region of the curve near the ordinate (y axis) is convex; that is to say that patient coagulation is not corrected with 50% or 20% control plasma. Negative result: patient coagulation time is corrected with control plasma.

4. Other coagulopathies may be excluded if the confirmatory test is negative (e.g., inhibitors of factor VIII or II). Paradoxically, LA increases *in vivo* coagulation times, but enhances clot formation *in vivo*.

Although there is high concordance between LA and ACA, antibodies are not identical. LA is generally more specific than ACA for APA, but less sensitive. Given that in 35% of patients ACA and LA do not coexist (are mutually exclusive), both tests should be carried out in order to rule out the existence of APA with absolute certainty. LA may prove positive in 10 to 20% of ACA-negative patients. Consequently, both tests should be performed in order to establish a more accurate diagnosis. In contrast with the case of ACA, the LA test cannot be assessed in patients undergoing anticoagulant treatment. A time lapse of at least two weeks must pass after suspension of anticoagulant treatment. On the other hand, in some subjects LA may also slightly alter prothrombin time, which would affect INR values (parameter assessed during dicoumarin treatment). The presence of anti-prothrombin antibodies may cause a functional deficit of this protein, as it is generally observed in association with LA. When such situation leads to significant bleeding, this rare condition is known as LA-hypoprothrombinaemia.

Given that anti-β2GPI and aPT antibodies have an LA effect, specific ELISA tests for each antibody may offer an advantage over coagulometric studies, which only provide qualitative estimation of an *in vitro* phenomenon, or at least they may be related with the clinical course. However, two recent systematic revisions do not support the substitution of coagulation tests with ELISA tests and the subject remains a matter of debate.

False-positive syphilis serology

False-positive syphilis serology may be encountered in APA patients to a lesser extent. This means that the reagin screen test is positive, given that VDRL or RPR test use a mixture of phospholipids as substrate, rendering the specific treponemal test negative. The standard non-treponemal serology test for the detection of syphilis (VDRL) consists of suspended flocculation, using a phospholipid compound made up of cardiolipin, cholesterol, lecithin as an antigen substrate. Since 1941, cardiolipin has been recognized as a phospholipid obtained from bovine heart that can be used as an antigen substrate in the detection of such antibodies. A positive VDRL test result with negative treponemal tests is an indirect marker of the presence of APA. This false-positive result is always at low titre (<1/8), while it is detected at high titre in syphilis (presence of luetic reagin). However, it is considered as a low-sensitivity technique for APA detection.

Anti-prothrombin antibodies

Autoantigen

Prothrombin is thrombin precursor, the final factor of the coagulation cascade that leads to fibrin formation. Prothrombin is a key enzyme in the balance between procoagulation and anticoagulation. It enhances coagulation through positive feedback, as well as anti-coagulation through protein C pathway activation.

aPT autoantibody

Antiprothrombin antibodies (aPT) were first identified by Loeliger in 1959. During the ensuing 15 years, numerous SLE patients, with haemorrhage complications associated with the presence of LA and acquired hypoprothrombinaemia were reported. Hypoprothrombinaemia was postulated as the result of the rapid clearance of complexes formed by PT and aPT antibodies in the blood stream. Subsequently, the presence of aPT antibodies was reported in patients with LA with no apparent strong hypoprothrombinaemia.

In 1995, Arvieux et al. demonstrated that aPT antibodies may be detected by ELISA, using purified human prothrombin as antigen, coated directly onto irradiated polystyrene plates or onto plates coated with anionic phospholipids (phosphatidylserine) in the presence of calcium. Such a procedure was used, given that antibodies cannot be detected when prothrombin is coated onto non-irradiated plates, because bonding requires an appropriate anionic surface.

Calli et al. observed that prothrombin is recognized more effectively when it is bound to ELISA plates coated with phosphatidylserine in the presence calcium ions. In this way, aPT antibodies may be detected through the bond to phospholipids. Consequently, the antibodies would be directed against cryptic or neo-epitopes that are exposed when prothrombin binds to anionic phospholipids and/or may act as low-affinity antibodies that bivalently bind to coated prothrombin. A high percentage of aPT are species-specific for human proteins and a minority reacts with the bovine protein.

The epitopes that are recognized by aPT have not been totally defined. Numerous studies suggest that antibodies bind to prothrombin, to prethrombin 1 (the carboxy-terminal region of prothrombin), to DIP-α thrombin (the carboxy-terminal region of prethrombin 1) and to fragment 1. Reactivity with coated thrombin is not observed. These findings suggest that most aPT are either polyclonal or oligoclonal.

Given that the amino-terminal region of prothrombin shares homology with other vitamin K-dependent proteins, it was suggested that aPT recognize a common epitope within this region of prothrombin, as is also the case with protein C and protein S. Results of studies of Rao et al. are however against this hypothesis.

aPT antibodies may inhibit the formation of the thrombokinase complex. This complex is formed by the binding of prothrombin to phospholipids on the damaged cell membrane. Prothrombin is subsequently cleaved enzymatically into thrombin. Antibodies directed against coagulation factors, such as prothrombin, are circulating pathogenic antibodies. They inhibit coagulation factors directly and therefore increase coagulation time.

The presence of these autoantibodies is associated with thrombosis in SLE and APS patients. aPT were identified as the antibodies responsible for LA activity, measured in plasma of 15% of APS patients. High titres of aPT imply the risk of suffering from deep venous thrombosis, pulmonary thromboembolism and myocardial infarction.

Anti-annexin V antibodies

Autoantigen

Annexin V is a 36-kD protein with large tissue distribution, which is mainly encountered in the placenta and vascular endothelium. It is a placental anti-coagulant protein (PAP), which not only belongs to a family of proteins that bind to calcium-dependent phospholipids, but is also a powerful vascular anticoagulant protein.

Annexin V shows great anticoagulant activity, due to its inhibitory effect on prothrombin activation and its capacity to prevent the formation of arterial and venous clots in normal blood stream.

This protein shows affinity for phosphatidylserine (PS), which is mainly found between plasma membrane layers and on the cell surface. In this way, once PS is exposed on the cell surface, annexin V may bind to it and inhibit its procoagulant and inflammatory activity.

Annexin V is necessary to maintain placental integrity, where it may exert a regulatory effect on clotting, as it was demonstrated in murine models.

Annexin V autoantibody

Annexin V autoantibodies were detected for the first time in 1995 in SLE patients. They are associated with thrombotic events and recurrent miscarriage in APS, as well as in patients with systemic sclerosis with digital ischaemia.

Mechanisms of synthesis of anti-annexin V antibodies have not yet been clarified. In the frame of apoptosis, it has been proposed that annexin V expression increases in the extracellular membrane, which may constitute an antigenic stimulus for specific production of antibodies. Annexin V antibodies have been thought to interfere with annexin V function and to induce thrombosis and/or vascular occlusion.

Nakamura et al. reported that LA could induce apoptosis in endothelial cells through binding with annexin V, which plays a role in the prevention of foetal loss, and arterial and venous thrombosis in physiological conditions. Those authors demonstrated that anti-annexin V IgG increase activated partial promboplastin time and has a high affinity for phospholipids. They argued that a potential overlap between anti-annexin V and ACA may exist.

It has been suggested that anti-annexin V antibodies interfere with the protection that annexin V affords to placental villi, leading to the exposure of procoagulant anionic phospholipids, which trigger blood coagulation in the placental vessels. Such process may be an important factor inducing thrombosis and recurrent miscarriage in APS.

Anti-annexin V antibodies are highly APS-specific and are also encountered at high levels in SLE patients. Patients that present these autoantibodies have a high incidence of arterial and venous thrombosis. It has been suggested that anti-annexin

V may affect the clinical course of the disease. They have also been identified as a risk factor for recurrent miscarriage in SLE.

Anti-phosphatidylserine antibodies

Autoantigen

Phosphatidylserine (PS) is a negatively charged phospholipid that is a member of the antigen group, to which APA are directed. Anti-PS are less frequently identified under laboratory conditions, given that they may be classified as "minor phospholipid antigens", together with other types, such as phosphatidic acid, phosphatidylglycerol and phosphatidylinositol.

From a physiological point of view, PS is a more relevant antigen than CL in the detection of APA, given that CL is a component of the plasma membrane internal surface, while PS is found on the external surface of platelet and endothelial cell plasma membrane. Additionally, PS also participates in the coagulation cascade and plays a role in clot formation.

aPS autoantibody

aPS antibodies present a stronger correlation with LA than with ACA and are detected in SLE patients with negative ACA. PS is detected in serum of APS patients but not of patients with syphilis. On the other hand, CL is recognized by antibodies from both patient groups.

However, the usefulness of aPS in the diagnosis of APS is a matter of controversy. The determination of aPS is of diagnostic relevance only in the case of suspected APS, when

low-positive or cut-off values of ACA, LA and anti-β2GPI are detected (Fig. 3).

Anti-phosphatidylethanolamine antibodies

Anti-phosphatidylethanolamine (aPE) antibodies are detected by ELISA using this neutral phospholipid, and are of a different nature from LA and ACA. Part of these antibodies depends on protein cofactors, such as quininogens.

They are often associated with LA and ACA in autoimmune diseases, but may also be detected alone in patients with clinical manifestations of APS. For aPE antibodies, IgM is the most frequently detected isotype, either alone or in association with IgG.

Anti-protein C and protein S antibodies

Autoantigens

Protein C and protein S are natural inhibitors of blood coagulation. Protein C is a vitamin K-dependent proenzyme anticoagulant, synthesized in the liver and circulating in blood. It is activated by thrombin in the presence of an endothelial cell cofactor called thrombomodulin and becomes an active enzyme known as activated protein C (APC). APC acts as an anticoagulant by proteolytic cleavage of coagulation factors V and VIII (Va and VIIIa), preventing fibrin formation.

Protein S is a vitamin K-dependent glycoprotein, synthesized mainly in the liver, but also in endothelial cells. It is also present

in platelets. Protein S belongs to coagulation regulatory mechanisms, and acts as a mandatory cofactor for APC in the proteolytic cleavage of procoagulant factors Va and VIIIa. Approximately 60% of total plasma protein S antigen circulates bound to C4b binding protein, while the remainder circulates freely. Only the free form has anticoagulant activity.

aPC and aPS autoantibodies

Quantitative and qualitative protein C and S deficiencies are venous thrombosis risk factors, and represent the physiopathological association between anti-protein C and S antibodies and venous thrombosis. Other authors did not find similar associations between anti-protein C and S antibodies and thrombosis, in spite of the strong correlation between both autoantibodies.

Other autoantibodies

During recent years, numerous studies were carried out to determine the importance of other autoantibodies and their possible relationship with thrombosis and foetal loss in APA patients. The following antibodies are worthy of mention: antithrombomodulin, anti-factor XII, high- and low- molecular weight anti-quininogen, anti-oxidized LDL (with respect to atheroma plaque formation) and antiplatelet antibodies. These autoantibodies are generally not used in clinical practice and their relevance is therefore still under study. Given that all these proteins are involved in the initiation and control of coagulation, it is conceivable that the antibodies that curb their availability or block their function may affect the procoagulant and anticoagulant balance.

Laboratory tests in seronegative patients

There are cases of patients with manifestations of APS that do not present ACA, anti-β2GPI, or LA antibodies (Fig. 3). Seronegative APS is the term that was proposed to define those patients. However, before considering those patients as seronegative, the following points should be taken into consideration:

- Concordance between LA and ACA is not absolute.
- LA may be undetectable or very weak positive, if the plasma sample is not platelet-free.
- A small proportion of patients negative for ACA IgG and IgM is positive for ACA IgA.
- A small proportion of negative ACA and LA patients may occasionally present antibodies against membrane phospholipids, such as aPS, aPI, aPE, phosphatidic acid, phosphatidylcholine or phosphatidylethanolamine.
- Decreased APA are observed in patients affected by nephrotic syndrome. This is due to the renal loss of APA of IgG isotype, reduced antibody synthesis or increased APA catabolism.
- APA, especially in the case of LA, may decrease during corticoid treatment.

- APA may disappear temporarily during the course of thrombotic events, probably as a result of their consumption. Determinations immediately after a thrombotic episode are not therefore considered as definitive.

Differential diagnosis of APS

The algorithm for the diagnosis of APS requires repetition of tests and careful exclusion in each patient of other possible causes for the presence of APA (Fig. 3), such as infections, old age, drug administration like beta-blockers, quinin and neuroleptic drugs, etc. It should also be remembered that the first manifestation of APS usually appears in young subjects (< 40 years of age). Drug-induced APA is not usually associated with thrombotic complications. It generally disappears 6 to 12 months after drug suspension in only 50% of cases.

Thrombosis risk assessment using conventional tests

Meta-analysis carried out by Wahl et al. demonstrated that in SLE patients, the presence of LA was the best predictive marker of thrombotic risk, with a relative risk of a first episode of venous thrombosis of 5.61 and of a recurrence of 11.6, while the presence of ACA implied a relative risk of only 2.5 and 3.91, respectively. These claims were confirmed through meta-analysis carried out by Galli, who demonstrated that the relative risk associated with the presence of LA was between

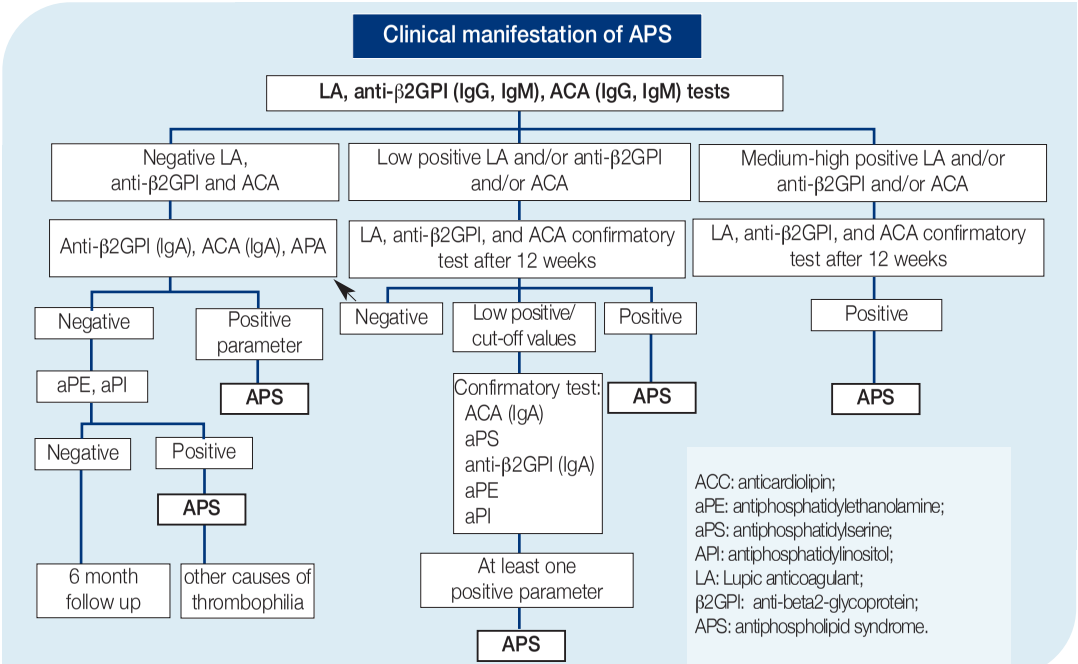

Figure 3. Diagnostic algorithm of antiphospholipid syndrome

5 and 16, independently of the coagulometric test used for detection. In this work, a significant association between thrombosis and the presence of ACA was only demonstrated in the case of IgG isotype, for values above 33 or 49 GPL U/mL.

Biological classification of APS

The criticism on ACA ELISA, and its weak predictive value in thrombotic risk prompted some authors to propose to the ISTH Subcommittee of Standardization for APA that APS should be given a biological classification, thus rejecting in principle the use of ACA titres (Table III).

In order to validate such classification, a British team of researchers analyzed the serological profile of patients presenting persistent APA and studied their distribution, according to different biological types, taking into account that patients presenting ACA only would be classified as type 4.

This study clearly shows that the titre of ACA must be taken into consideration, when high diagnostic sensitivity for APS should be obtained. In effect, patients that have both LA and

TABLE III. Proposed biological classification of antiphospholipid syndrome

Type 1	LA + Anti-β2GPI
Type 2	LA
Type 3	Anti-β2GPI
Type 4	Others: aPT, aPS, ACA, aPI, etc.

anti-β2GPI (type 1), present higher ACA titres than those presenting LA (type 2) or anti-β2GPI (type 3) independently. Those presenting lower ACA titres were patients with ACA only (type 4), with an average value of 11.5 GPL U/mL.

However, in our experience, a subgroup of patients with APS manifestations exists, in which titres of anti-β2GPI antibodies are low and ACA is negative and vice versa.

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Notes

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