Antiphospholipid syndrome: an overview

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Abstract

ANTIPHOSPHOLIPID ANTIBODIES ARE A HETEROGENEOUS GROUP of autoantibodies that are detected by immunoassays and functional coagulation tests. The antigenic targets are negatively charged phospholipids and serum phospholipid-binding proteins. The latter antibodies are frequently associated with thrombosis, fetal loss and other clinical manifestations of the antiphospholipid syndrome. These antibodies are felt to be etiologically important in the syndrome, although the precise pathogenic mechanisms are still being determined. Proposed mechanisms include antibody-mediated interference with coagulation homeostasis, activation of platelets and endothelial cells and a T-cell immune response to serum phospholipid-binding proteins. The mainstay of therapy is anticoagulation, whereas immunosuppression is ineffective.

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Antiphospholipid syndrome is an autoimmune disease characterized by antiphospholipid antibodies and at least 1 clinical manifestation, the most common being venous or arterial thrombosis and recurrent fetal loss.[1–8] The syndrome occurs in isolation (primary antiphospholipid syndrome)[9–11] or in association with connective tissue diseases (secondary antiphospholipid syndrome), particularly systemic lupus erythematosus.[12] Antiphospholipid antibodies are heterogeneous and may be detected by immunoassays or functional coagulation assays. Current treatment strategies focus on anticoagulation,[13] whereas traditional forms of immunosuppression are unhelpful.[14]

Historical background

In 1906, Wassermann identified sera from patients with syphilis that reacted with extracts of syphilitic tissues.[15] The Wassermann reagin test was originally attributed to antibody reactivity against antigens derived from *Treponema pallidum*, until the use of normal human and animal tissues was found to give similar results.[16] It was not until 1941 that Pangborn isolated cardiolipin (diphosphatidylglycerol) from bovine heart, identifying it as the antigenic component of the reagin test.[17] Subsequently, the combination of cardiolipin, lecithin and cholesterol formed the basis of the flocculation test for syphilis referred to as the venereal disease research laboratory (VDRL) test.[16]

With the development of more specific tests for syphilis, such as the *T. pallidum* immobilization test, it became clear that infections other than syphilis could produce a positive Wassermann reagin or VDRL test. In 1952, Moore and Mohr identified 2 circumstances in which a biologic false-positive serologic test result for syphilis could occur.[18] Transient reactions followed acute viral infections and vaccination, whereas persistent (> 6 months) reactions were associated with autoimmune disorders such as systemic lupus erythematosus, Sjogren's syndrome and rheumatoid arthritis.

In 1952, Conley and Hartman reported the cases of 2 patients with hemorrhagic disorders who had prolongation of prothrombin time in addition to a biologic false-positive serologic test result for syphilis.[19] This was the initial description of the "lupus anticoagulant," detected by the prolongation of a phospholipid-dependent in-vitro coagulation test. Subsequent work confirmed that the lupus anticoagulant was attributable to the biologic false-positive serologic test result for syphilis[20,21] and, paradoxically, was associated with in-vivo thrombosis[22] rather than a bleeding diathesis.

In 1983, Harris and colleagues described a radioimmunoassay for anticardiolipin antibodies that was considerably more sensitive than previous binding assays or functional coagulation assays.[23] This development and the subsequent conversion to an enzyme-linked immunosorbent assay (ELISA)[24] greatly facilitated subsequent clinical and epidemiologic studies and the description of the antiphospholipid syndrome.

Antibody determination and antigenic specificity

Antiphospholipid antibodies are routinely detected by ELISA using plastic wells coated with negatively charged phospholipid (e.g., cardiolipin). Although this detects a heterogeneous group of antibodies, of interest are those most strongly associated with clinical manifestations. In such cases, the predominant reactivity is against serum phospholipid-binding proteins (initially called "cofactors") rather than reactivity against phospholipid per se (Fig. 1).[25–30] The most common of these proteins is β_2-glycoprotein I, which associates with negatively charged phospholipids through charge interactions. The physiologic role of β_2-glycoprotein I is unknown, but it has been suggested that it is a natural in-vivo anticoagulant in part because of its ability to bind to negatively charged phospholipids and thereby inhibit contact activation of the intrinsic coagulation pathway.[31–35] Although β_2-glycoprotein I is the predominant target of autoimmune "antiphospholipid" antibodies, other phospholipid-binding proteins have been described as playing a similar role. These include prothrombin, protein C, protein S and annexin V.[6]

In contrast to antibodies that target phospholipid-binding proteins, there are also antiphospholipid antibodies that bind directly to negatively charged phospholipids themselves (Fig. 1). These occur in patients with infections such as syphilis,[18,24] infectious mononucleosis[36,37] and AIDS,[38] and following exposure to certain medications.[39] These antibodies usually have no clinical sequelae. However, routine assays do not readily distinguish between these major antibody subsets.

The presence of antiphospholipid antibodies may also be inferred by the detection of a lupus anticoagulant (Fig. 2).[2,3,17] Internationally accepted criteria for the identification of lupus anticoagulant require the following: (1) prolongation of at least 1 phospholipid-dependent coagulation assay (e.g., dilute Russell viper venom test), (2) failure to correct this inhibition of in-vitro coagulation by the addition of normal plasma and (3) correction of inhibition of in-vitro coagulation by the addition of phospholipid.[40] The antigenic specificity of the autoantibodies responsible for the lupus anticoagulant includes prothrombin[41] and β_2-glycoprotein I.[42]

Classification criteria and diagnosis

Criteria for the classification of patients with definite antiphospholipid syndrome,[43] developed in 1998, provide a basis for including patients with the syndrome in research protocols rather than a guide to diagnosing the syndrome in individual patients. In order to fulfill the "Sapporo criteria" (Box 1), patients must have either vascular thrombosis or fetal loss and demonstrate evidence of antiphospholipid antibodies either by the detection of anticardiolipin antibodies or a positive lupus anticoagulant. Autoantibodies must be detected on at least 2 occasions 6 weeks apart in order to distinguish persistent autoimmune antibody responses from transient responses caused by infection or drug exposures. These classification criteria have been evaluated[45] and reported to have a sensitivity of 71% and a specificity of 98%, suggesting that the threshold for inclusion is high and that most cases have "definite" antiphospholipid syndrome. Thus, comparable to the situation encountered with the American College of Rheumatology (ACR) classification criteria for systemic lupus erythematosus,[46] whereby some patients with a diagnosis of systemic lupus erythematosus do not meet the ACR criteria, there are likely to be patients with the antiphospholipid syndrome who do not meet the Sapporo criteria because of the presence of unusual clinical manifestations of the syndrome. For example, clinical manifestations associated with the antiphospholipid syndrome that are not part of the classification criteria include livedo reticularis, cardiac valve disease and transient cerebral ischemia, and laboratory manifestations include hemolytic anemia and thrombocytopenia. Thus, in clinical practice a diagnostic workup for antiphospholipid antibodies should be considered in all patients with venous or arterial thrombosis and fetal loss for which there is no alternative explanation, particularly in the presence of recurrent manifestations. Likewise, unexplained thrombocytopenia, hemolytic anemia and prolongation of any phospholipid coagulation tests should lead to determination of antiphospholipid antibody status.

Pathogenic mechanisms

The in-vivo mechanisms responsible for thrombosis and fetal loss in patients with antiphospholipid syndrome remain unknown, although several potential pathogenic pathways

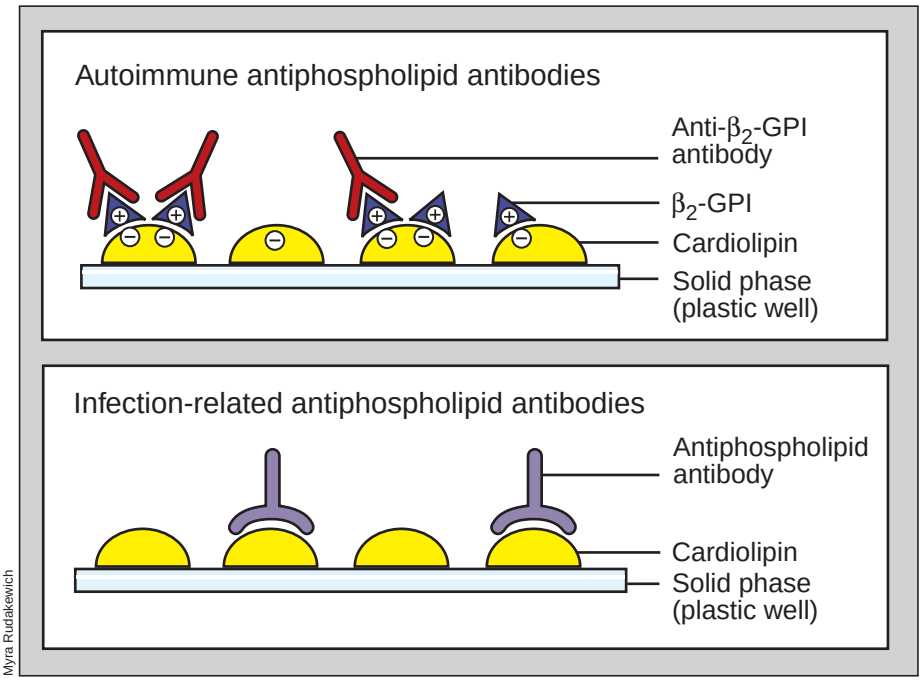

Fig. 1: Antiphospholipid antibody determination by enzyme-linked immunosorbent assay.

have been identified (Fig. 3). First, antiphospholipid antibodies may interfere with the function of the coagulation cascade leading to a procoagulant state.[47] Examples include inhibition of the activated protein C and antithrombin III pathways, inhibition of fibrinolysis and upregulation of tissue factor activity. As previously mentioned, β_2-glycoprotein I may function as an in-vivo anticoagulant[48] and, thus, antibodies that target the molecule may interfere with this role. Other proteins that are important in regulating coagulation, such as prothrombin, proteins C and S, and annexin V, may also be targeted by antiphospholipid antibodies.[47] Finally, there is evidence that the binding of annexin V to procoagulant surfaces may be inhibited by antiphospholipid antibodies.[49,50]

Several studies have examined the specific interaction between antibodies to β_2-glycoprotein I and in-vitro endothelial cell function. The direct binding of β_2-glycoprotein I to the endothelial cell surface is facilitated by the constitutive negative charge on the surface of endothelial cells, enhanced surface expression of negatively charged phosphatidylserine during apoptosis[51] and the fact that annexin II acts as a receptor for the binding of β_2-glycoprotein I to cultured endothelial cells.[52] Thus, antiphospholipid antibody binding to the endothelial cell surface in a β_2-glycoprotein-I–dependent manner leads to endothelial cell activation, which is manifested by upregulation of cell surface adhesion molecules and increased secretion of interleukin-6 and prostaglandins.[53–56] Because activated endothelial cells promote coagulation,[57] this may be a relevant pathogenic mechanism. There is also evidence that antiphospholipid antibodies promote the activation and aggregation of platelets.[47]

Data from experimental animals support a pathogenic role for antiphospholipid autoantibodies, particularly those with specificity for β_2-glycoprotein I, in both the generation of thrombosis[58,59] and the causation of fetal loss.[59,60] There is also evidence that viral[61] and bacterial peptides[62] may induce antiphospholipid antibody production in animals and promote thrombosis[61] and fetal loss.[62]

Recent work has suggested a direct role for cellular immune mechanisms in antiphospholipid syndrome. Peripheral blood mononuclear cells proliferate in response to native human β_2-glycoprotein I, and cell culture supernatants from peripheral blood mononuclear cells stimulated with β_2-glycoprotein I showed a predominance of interferon-γ production,[63] which has the potential to directly activate endothelial cells.

Finally, it is likely that other factors play a role in determining whether patients develop clinical manifestations of antiphospholipid syndrome. For example, a "second hit" may be required for thrombosis and fetal loss to occur. Although speculative, this may include traumatic injury to the vascular bed, nonimmunologic procoagulant factors or the presence of infection leading to cytokine production and endothelial cell activation. Recent data have also suggested that the presence of antibodies to nuclear lamin B1 nullifies

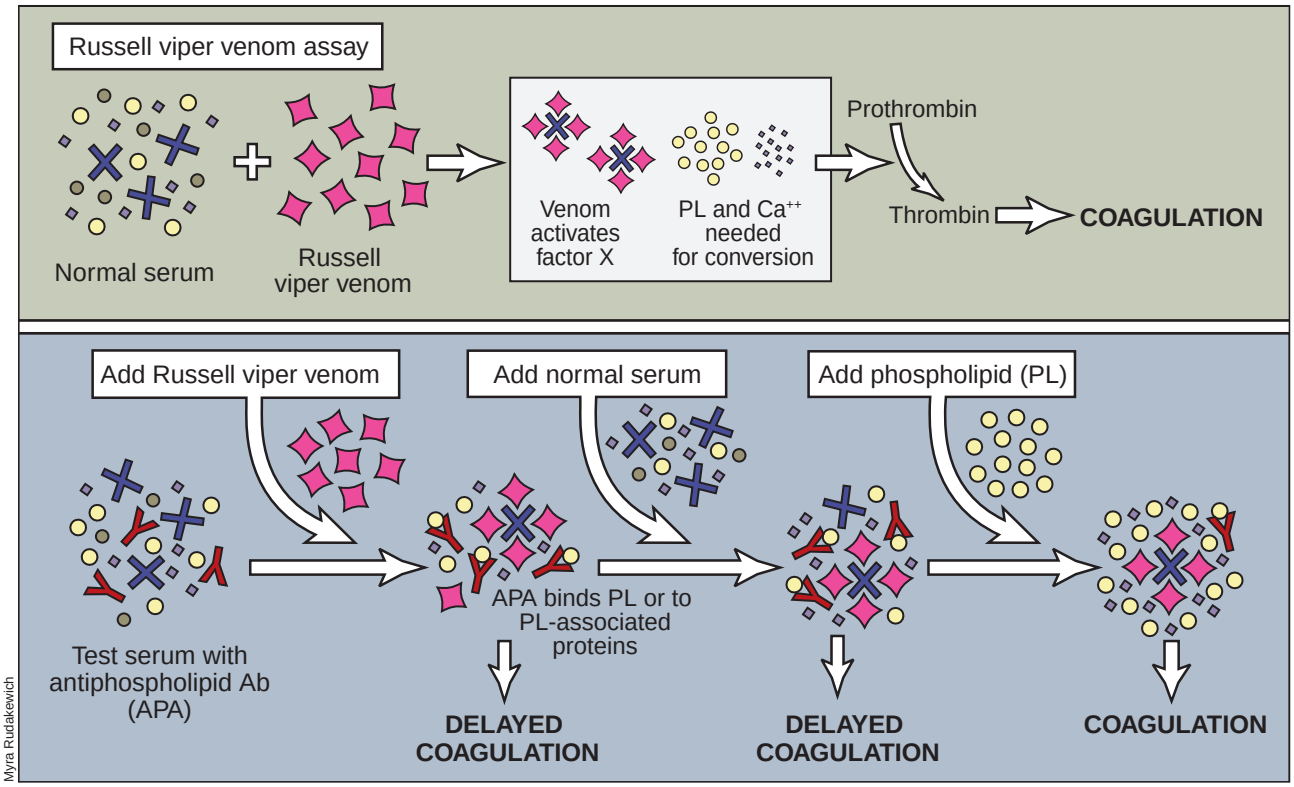

Fig. 2: Antiphospholipid antibody determination by lupus anticoagulant.

the prothrombotic risk associated with lupus anticoagulant in patients with systemic lupus erythematosus,[64] although the mechanism by which these autoantibodies confer this protective effect remains to be explained.

Risk and predictors

The risk of thrombosis associated with antiphospholipid antibodies has been studied most thoroughly in populations with systemic lupus erythematosus, of whom 12%–30% have anticardiolipin antibodies[65,66] and 15%–34% have lupus anticoagulant.[4,66] In patients with antiphospholipid antibodies, 38% have both anticardiolipin and lupus anticoagulant.[67] In general, about 50% of patients with systemic lupus erythematosus who have antiphospholipid antibodies have a history of either venous or arterial thrombosis.[9,68,69] In a prospective North American study, the incidence of thrombosis was found to be 2 per 100 person-years of follow-up.[70] A European study reported that up to 7% of patients with systemic lupus erythematosus develop a new thrombotic event over a 5-year period.[71] Recurrent episodes tend to mimic the original vascular event, with ve-

Box 1: Criteria for the classification of definite antiphospholipid syndrome*[43]

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.

Clinical criteria

1. Vascular thrombosis

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 the exception of superficial venous thrombosis. For histopathologic confirmation, thrombosis should be present without significant inflammation in the vessel wall.

2. Pregnancy morbidity

a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasonography or by direct examination of the fetus, **or**

b) One or more premature births of a morphologically normal neonate at or before the 34th week of gestation because of severe pre-eclampsia or eclampsia, or severe placental insufficiency, **or**

c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosal causes excluded.

In studies of populations of patients who have more than 1 type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects according to a, b or c above.

Laboratory criteria

1. Anticardiolipin antibody of IgG and/or IgM isotype in blood, present in medium or high titre, on 2 or more occasions, at least 6 weeks apart, measured by a standardized enzyme-linked immunosorbent assay for β_2-glycoprotein-I–dependent anticardiolipin antibodies.

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 of Thrombosis and Hemostasis (Scientific Subcommittee on Lupus Anticoagulants/Phospholipid-Dependent Antibodies) in the following steps:

a) 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.

b) Failure to correct the prolonged coagulation time on the screening test by mixing with normal platelet-poor plasma.

c) Shortening or correction of the prolonged coagulation time on the screening test by addition of excess phospholipid.

d) Exclusion of other coagulopathies, e.g., factor VIII inhibitor or heparin, as appropriate.

Note: Ig = immunoglobulin.
*No exclusions other than those contained within the above criteria are needed. However, because of the likelihood that thrombosis may be multifactorial in patients with the antiphospholipid antibody syndrome, the workshop participants recommend that (a) patient populations being studied should be assessed for other contributing causes of thrombosis, and (b) such populations should be stratified according to identifiable or probable risk factors, e.g., age or comorbidities. Specific limits were not placed on the interval between the clinical event and the positive laboratory findings. However, it was the view of many at the workshop that (a) information about such intervals should be assessed when relevant, and (b) the relatively strict definition of laboratory criteria (including the requirement that results again be positive on repeat tests performed at least 6 weeks after the initial test) would help to exclude antiphospholipid antibody positivity that represents an epiphenomenon to the clinical events. The pregnancy morbidity criteria were mainly developed by Branch and Silver.[44] Reproduced with permission from Wiley-Liss, Inc (*Arthritis Rheum* 1999;42[7]:1309-11).

nous thrombosis following venous occlusion and arterial thrombosis following arterial occlusion, although there are exceptions when patients occasionally develop both venous and arterial disease.[67]

Factors associated with a higher risk of thrombosis include a previous history of thrombosis[72,73] and the presence of lupus anticoagulant.[74] The higher the level of anticardiolipin antibodies, the greater the risk of thrombosis.[12,72,75,76] Traditional risk factors for thrombosis such as pregnancy and surgical procedures also increase the risk in patients with antiphospholipid antibodies.[77] Intuitively, one would anticipate that the presence of genetically determined procoagulant factors, such as Factor V Leiden mutation, would heighten the risk of thrombosis, but the data related to this are inconsistent.[67]

Thrombotic manifestations

The most common clinical manifestation of antiphospholipid syndrome is thrombosis, which can affect the vessels of any organ. Venous thrombosis, particularly of the lower limb, occurs in up to 55% of patients with the syndrome, half of whom also have pulmonary emboli.[9,68,69] Arterial thrombosis involves the brain in up to 50% of cases, causing transient ischemic attacks or strokes. Other anatomic sites for arterial thrombosis are the heart (25%), causing coronary occlusion, and the eye, kidney and peripheral arteries (25%).[9,68,69] Finally, vascular occlusion may occur through embolization from a central source such as vegetations on a mitral or aortic valve, which are reported in up to 4% of patients with antiphospholipid syndrome.[69] Some echocardiographic abnormalities may be detected in almost two-thirds of patients with the syndrome, although most of these abnormalities are of little clinical significance.[69]

Obstetric manifestations

The risk of pregnancy loss in women with antiphospholipid antibodies is greatest from the 10th week of gestation onward (fetal period).[78,79] This is in contrast to pregnancy loss in the general population, which is most frequent during the first 9 weeks of gestation. These facts are acknowledged in the Sapporo criteria for obstetric manifestations of the antiphospholipid syndrome,[43] in which the patient must have 1 or more otherwise unexplained losses in the fetal period up to 34 weeks of gestation, or 3 or more losses in the first 9 weeks of gestation. There is also evidence that women with antiphospholipid antibodies have an increased risk of giving birth to a premature infant because of pregnancy-associated hypertension and uteroplacental insufficiency.[80,81]

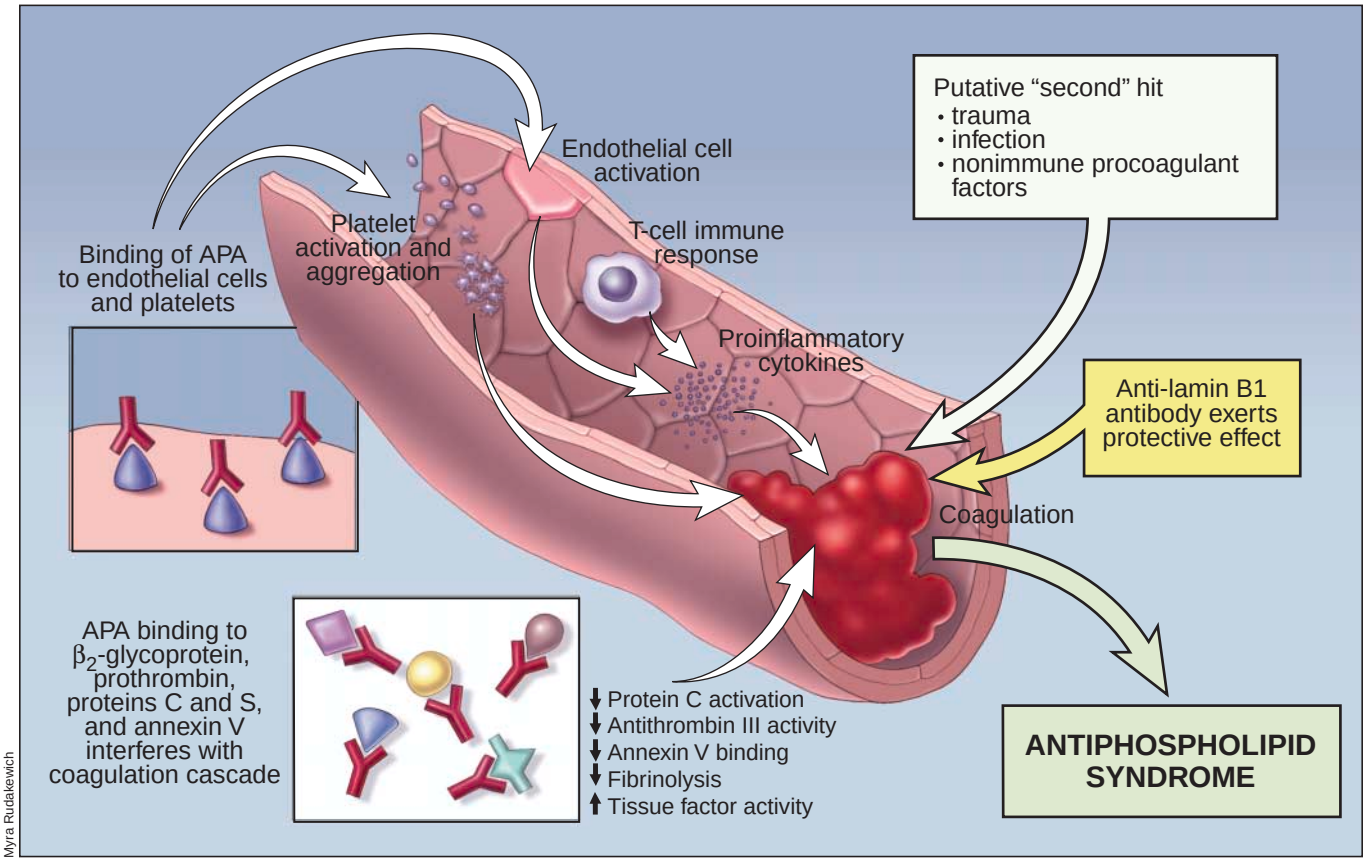

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Fig. 3: Pathogenic mechanisms in antiphospholipid syndrome.

Catastrophic antiphospholipid syndrome

Although most patients with antiphospholipid syndrome develop venous or arterial thrombosis at a single anatomical site, a minority present with multiorgan involvement of rapid onset that is associated with high mortality. In recognition of the dramatic nature of the clinical presentation, this has been called "catastrophic,"[82] which some have attributed to a "thrombotic storm."[83] Because of the relative infrequency of this presentation, estimated to be 0.8% in one large series,[84] clinical experience is based upon case series. Patients are arbitrarily required to have at least 3 different organ systems involved, with symptoms developing over a period of days to weeks. Although large-calibre vessels may be affected, typically there is an acute thrombotic microangiopathy affecting small-calibre blood vessels in multiple organs: 50% of cases included the kidneys, lungs, central nervous system, heart and skin. Although there are similarities to thrombotic thrombocytopenic purpura (TTP), there are several differences.[85] For example, TTP is more likely if there is a history of a viral prodrome, fever, profound rather than moderate thrombocytopenia, schistocytes on the peripheral blood smear, less severe renal impairment, purpura and histopathologic evidence of platelet thrombi. In addition, there is recurrence in 64% of cases with TTP but this is very rare in catastrophic antiphospholipid syndrome. An important difference in the management of these 2 conditions is that anticoagulation is essential for antiphospholipid syndrome but is inappropriate for TTP. Both conditions may be complicated by disseminated intravascular coagulation in about 25% of the cases. The disseminated intravascular coagulation may be clinically silent, cause thrombosis or bleeding, and is characterized by several laboratory abnormalities, including prolongation of the prothrombin time and the partial thromboplastin time, low levels of fibrinogen, antithrombin III and protein C, as well as elevated D-dimer concentrations.[82,86] Mortality is usually a consequence of multiorgan failure and is as high as 50%[82,86] in patients with catastrophic antiphospholipid syndrome.

Treatment

Full anticoagulation, the cornerstone of therapy in patients with antiphospholipid syndrome,[87] is not indicated in the absence of significant clinical manifestations of the syndrome, particularly thrombosis. Thus, when considering treatment options for prophylaxis in patients with antiphospholipid antibodies but without a history of thrombosis, there is only 1 study that suggests that ASA (325 mg/d) may lower the risk of thrombosis in women with a history of fetal loss.[88] In addition, there is evidence that hydroxychloroquine, which is frequently used in the treatment of patients with systemic lupus erythematosus, may also provide some protection from thrombosis in secondary antiphospholipid syndrome.[89] In patients known to have antiphospholipid antibodies, it is generally wise to avoid exposure to other procoagulant factors such as oral contraceptives and to minimize risk factors for atherosclerosis, which in itself can promote intravascular thrombosis.

The occurrence of even a single thrombotic event in a patient with antiphospholipid antibodies indicates lifelong anticoagulation, as the risk of recurrence varies between 20% and 70%.[13,90–92] Initial therapy is with heparin or low-molecular-weight heparin followed by warfarin. Whereas no randomized, placebo-controlled studies have been published that assess the role of anticoagulation in patients with antiphospholipid syndrome, data from several retrospective studies[13,90,91] have indicated that warfarin significantly reduces the recurrence rate of arterial and venous thrombosis provided the international normalized ratio is maintained above 2.0. It remains unclear if more aggressive anticoagulation with an international normalized ratio above 3.0 is more effective than a ratio of 2.0–2.9, an important point given the higher risk of hemorrhagic complications associated with this level of anticoagulation.[93] Low-dose ASA has not been shown to be effective in preventing recurrent thrombosis caused by antiphospholipid antibodies, but some experts have recommended adding it to warfarin therapy when there is evidence of ongoing ischemia.[94] Patients with catastrophic antiphospholipid syndrome are usually treated with full anticoagulation, and data from uncontrolled studies have suggested that plasmapheresis may improve survival.[82]

Evidence exists to support the use of anticoagulation in the prevention of obstetric complications of antiphospholipid syndrome. Most prospective studies have indicated that heparin plus low-dose ASA is more effective than ASA alone for preventing pregnancy loss in patients with antiphospholipid antibodies.[95–98] The dose of heparin is usually 5000 IU twice daily unless there is also a history of previous thromboembolic disease, in which case full anticoagulation is recommended. The recommended dose of heparin used to prevent pregnancy loss during the fetal period of gestation, as opposed to the first 9 weeks of gestation, is higher (7500–10 000 U twice daily)[80,81,99] because of the risk of maternal thromboembolism attributable to the pregnancy. It is generally agreed that regular heparin may be replaced by low-molecular-weight heparin, which has the advantage of the convenience of once-daily administration and decreased risks of heparin-induced thrombocytopenia and probably osteoporosis.[100] Intravenous immunoglobulin infusions[101] are not more effective than low-dose ASA and heparin in the prevention of pregnancy loss attributed to antiphospholipid antibodies, although some have advocated the use of intravenous immunoglobulin infusions (1–2 g/kg in divided doses over 2–5 days given monthly) in patients with antiphospholipid antibodies who continue to lose pregnancies despite receiving low-dose ASA and heparin.[102] High-dose prednisone does not prevent fetal loss[99] and is associated with increased maternal morbidity, including gestational diabetes, hypertension and sepsis. Warfarin should be avoided, particularly from weeks 6–12 of gestation, because of its teratogenic effects, and patients with previous thrombosis who are on warfarin should be

switched to heparin or low-molecular-weight heparin.[103]

In summary, antiphospholipid syndrome is characterized by venous and arterial thrombosis, fetal loss and an array of other clinical manifestations. Although etiologically linked to autoantibodies with specificity for serum phospholipid-binding proteins such as β_2-glycoprotein I, the precise pathogenic mechanisms underlying the syndrome remain unclear. Currently the mainstay of therapy is anticoagulation rather than immunosuppression, which is ineffective. Improvement in treatment awaits the further elucidation of pathogenic mechanisms.

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