REVIEW ARTICLE

Antiphospholipid syndrome

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The antiphospholipid syndrome (APS) is characterized by thrombosis, recurrent fetal death and the presence of circulating antiphospholipid (aPL) antibodies[1]. Antiphospholipid antibodies are directed against anionic phospholipids or protein-phospholipid complexes, and the two that are the most clinically characterized and relevant are the lupus anticoagulant and anticardiolipin antibodies. In this paper we offer a concise review of the vast body of published work, highlight the clinical features of the syndrome and the diagnostic difficulties associated with testing for lupus anticoagulant and anticardiolipin antibodies, outline the proposed mechanisms of thrombosis and fetal loss in APS and present evidence for various treatment modalities.

CLINICAL FEATURES

Antiphospholipid syndrome can be divided into two types—a primary form, with no associated systemic disease, and a secondary form, in which systemic lupus erythematosus (SLE) or a related connective tissue disease is present. The major clinical consequence of APS is a tendency to both venous and arterial thrombosis. It is noteworthy, however, that thrombotic events in a particular patient with APS tend to segregate into venous or arterial—e.g. a venous thrombosis is more likely to be followed by another venous thrombosis than by an arterial thrombosis[2]. Almost every vascular bed can be involved by thrombosis in APS. The most common site for venous thrombosis is the deep venous system of the lower limb[3]. Other sites include the retinal, renal and hepatic veins, thrombosis of the last causing Budd–Chiari syndrome. The most frequent manifestation of arterial thrombosis is ischaemic stroke or transient ischaemic attack (TIA)[4]. Other manifestations include retinal artery occlusion, myocardial infarction and peripheral arterial occlusion.

Recurrent pregnancy failure, thought to be in large part due to placental insufficiency secondary to thrombosis, is associated with APS[5]. First-trimester miscarriages are quite frequent in the normal (aPL-antibody-negative) population but late pregnancy loss is more specific. Livedo reticularis may be seen in APS—a purple, lace-like rash most prominent on the limbs, probably due to dermal microvascular thrombosis. Other cutaneous features of APS are superficial thrombophlebitis, splinter haemorrhages and skin infarcts. Thrombocytopenia is often seen in APS and is associated with a thrombotic rather than a bleeding tendency, much as happens in heparin-induced thrombocytopenia, to which APS has some similarities[6]. The echocardiogram is often abnormal in patients with APS, 4% of patients having aortic or mitral valve sterile vegetations, akin to those seen in verrucous or Libman–Sacks endocarditis[7].

The frequency of migraine in aPL-antibody-positive individuals with stroke or TIA is said to be twice that of the general population[8], but whether the association is causal is uncertain. Tietjen et al.[9], in a large prospective study, found that the frequency of anticardiolipin antibodies was no higher in migraine sufferers, under 60 years old, with or without aura, than in controls. Other purported neurological manifestations of APS include chorea, transverse myelitis and a vascular dementia. An uncommon and severe variant of APS is termed catastrophic antiphospholipid syndrome, characterized by rapidly progressive microvascular thrombosis leading to multiorgan failure. In a review of 50 such patients, the mortality rate was 50%, major causes of death being cardiac disorders and respiratory failure from adult respiratory distress syndrome[10].

ASSAYS FOR ANTIPHOSPHOLIPID ANTIBODIES

The conventional tests for antiphospholipid antibodies are coagulation assays for lupus anticoagulant and an enzyme-linked immunosorbent assay (ELISA) for anticardiolipin antibodies[11]. There is partial concordance between these two methods—80% of patients with lupus anticoagulant have anticardiolipin antibodies, though $<50\%$ of patients with anticardiolipin antibodies have lupus anticoagulant.

The lupus anticoagulant was first described by Conley and Hartmann at Johns Hopkins in 1952[12]; the term is a double misnomer, since most patients with lupus

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anticoagulant do not have lupus, and in vivo it is a procoagulant rather than an anticoagulant. The presence of lupus anticoagulant is reckoned to confer a 30% lifetime risk of a thrombotic event[13]. Lupus anticoagulant inhibits phospholipid-dependent coagulation, so there may be prolongation of APTT (activated partial thromboplastin time) and dilute RVVT (Russell viper venom time). These can be used as screening tests, with the latter more sensitive than the former[14], but two further steps are needed to confirm the presence of lupus anticoagulant—a mixing study to demonstrate that the APTT does not normalize when the patient's plasma is mixed with normal plasma (i.e. there is an inhibitor present rather than a factor deficient); and a test to demonstrate phospholipid-dependence (e.g. a platelet neutralization procedure)[15]. Lupus anticoagulant assays have limitations, since they are confounded by heparin and warfarin and there is no current method to quantify titre.

Cardiolipin is the phospholipid antigen conventionally used in testing for antiphospholipid syndrome. This phospholipid is mainly found intracellularly in the mitochondrial membrane, and antibodies to it are responsible for the false-positive VDRL (Venereal Disease Reference Laboratory) test for syphilis that one often sees in patients with APS. The first quantitative anticardiolipin antibody test was established by Harris et al. in 1983 at the Hammersmith Hospital[16]; this solid-phase radioimmunoassay was later refined to the safer, easier to perform, ELISA. The anticardiolipin antibody ELISA is much less specific for patients at risk of thromboembolism than the lupus anticoagulant assays[17]. This is because anticardiolipin antibodies can also be found in various non-thrombotic contexts—for example, in patients taking phenothiazines, hydralazine, phenytoin, or valproate and in those with infections such as syphilis, Lyme disease, hepatitis C or HIV[18]. These antibodies can also be detected in a proportion of the normal population, perhaps in response to common viral illnesses[19]. The anticardiolipin antibodies found in these groups generally carry little risk of thrombosis (although there are a few case reports of thrombosis in patients with drug-induced antibodies), and are likely to be in low titre. Evidence has been accumulating that these 'benign' anticardiolipin antibodies can be immunologically distinguished from the 'pathogenic' anticardiolipin antibodies associated with thrombosis and APS.

B_2-glycoprotein I (β2-GPI) is a 50 kd phospholipid-binding plasma glycoprotein that is a member of the complement control protein family. 'Pathogenic' anticardiolipin antibodies are dependent on β2-GPI for binding[20,21]. Indeed, the anticardiolipin antibodies detected by ELISA in the sera of APS patients do not actually recognize cardiolipin but rather bind to epitopes on β2-GPI. In contrast, 'benign' anticardiolipin antibodies bind to cardiolipin directly and are not dependent on β2-GPI for binding. Anti-β2-GPI antibodies can help to differentiate between these two groups[22].

The exact roles of β2-GPI and phospholipid in antibody binding are disputed[23]. One hypothesis is that β2-GPI bound to a surface (e.g. cell-membrane phospholipid or the plastic of an assay plate) undergoes a conformational change, and certain antiphospholipid antibodies bind to exposed neoepitopes[24]. A more likely possibility is that, although anti-β2GPI antibodies have low intrinsic affinity, bivalent/multivalent attachment results from immobilization of the concentrated antigen on a membrane, allowing high avidity binding, which is detectable in ELISAs[25].

The anticardiolipin antibody isotype that is mainly implicated in thrombosis is IgG—specifically IgG_2[26], whereas infection-related 'benign' aCLs are typically IgG_1 and IgG_3[27]. Recent data suggest that quantification of antiphospholipid antibody titre is clinically important, since titre correlates with risk of thrombo-occlusive events[28,29]. In a study by Levine et al. of a group of patients who presented with focal cerebral ischaemia, those with an anticardiolipin IgG titre >40 had a six-fold greater risk of subsequent TIAs than the lower-titre group. It is important to retest for persistence of anticardiolipin antibodies after at least two months, to exclude transient antibodies that may have no clinical significance[30].

Anti-prothrombin antibodies are found in 50–90% of patients with APS, particularly in those with the lupus anticoagulant[31]. Whether these antibodies are a risk factor for thromboembolic events has yet to be established.

PRELIMINARY CLASSIFICATION CRITERIA FOR 'DEFINITE' APS

To facilitate studies of treatment and causation, an international consensus statement on preliminary classification criteria for 'definite' APS has lately been published (Sapporo Workshop Criteria)[32]. The clinical criteria used were vascular thrombosis (arterial, venous, or small vessel) and pregnancy morbidity (including fetal death and three or more unexplained consecutive spontaneous miscarriages before the tenth week of gestation). The laboratory criteria included the presence, on two or more occasions at least six weeks apart, of medium or high titre IgG and/or IgM anticardiolipin antibodies in blood, and of lupus anticoagulant in plasma. Definite APS was considered present in a particular patient if at least one of the clinical criteria and one of the laboratory criteria were met. Other features such as thrombocytopenia, haemolytic anaemia, transient cerebral ischaemia, transverse myelopathy, livedo reticularis,

cardiac valve disease, chorea and migraine were not included as criteria for definite APS.

A HETEROGENEOUS GROUP OF AUTOANTIBODIES

A multitude of protein targets for 'antiphospholipid' antibodies have been described including β2-GPI, prothrombin, protein C, protein S, thrombomodulin, annexin V, kininogens, C4-binding protein (a complement protein that regulates free protein S levels), and vascular heparan sulphate proteoglycan[33]. Furthermore, there is extensive cross-reactivity between anticardiolipin antibodies and other negatively charged phospholipids, such as phosphatidylserine and phosphatidylinositol. Thus, antiphospholipid antibodies are a very heterogeneous family of autoantibodies, and most patients with antiphospholipid syndrome have a mixture of autoantibodies reacting with various phospholipids and plasma proteins, some of which are involved in the coagulation and anticoagulation cascades.

In a paper that generated much discussion Toschi et al.[34] reported a very high prevalence (44%) of antibodies to one or more of seven different phospholipids (which it should be noted were all β2-GPI dependent) in a population of 77 non-SLE patients aged 50 years or less with cryptogenic stroke or TIA. Furthermore, nearly one-quarter of the patients who lacked anticardiolipin antibodies showed immunoreactivity to one of the other phospholipids. Among the antiphospholipid antibodies studied, those with specificity for phosphatidylinositol had the highest prevalence. Toschi et al. concluded that, if one assesses only anticardiolipin antibodies and lupus anticoagulant in young stroke patients, one may be underestimating the true prevalence of antiphospholipid antibodies. However, when Branch et al.[35] evaluated antibodies to several phospholipids they concluded that, if lupus anticoagulant and anticardiolipin are absent, testing for individual aPL antibodies is not worth while.

MECHANISM OF THROMBOSIS

Histopathologically and characteristically, the vascular occlusions in antiphospholipid syndrome are non-inflammatory: the thrombus is fibrin-platelet and there is no evidence of vasculitis. There are many theories but no consensus as to why pathological clotting occurs in antiphospholipid syndrome. As already mentioned, antiphospholipid antibodies can bind to various plasma proteins involved in anticoagulation—such as protein C, protein S and thrombomodulin—and by altering their function may create a permissive thrombotic environment[33]. Similarly, autoantibody activity against β2-GPI, which is thought to have anticoagulant properties and antiplatelet activity by the inhibition of ADP-mediated platelet aggregation, may have a prothrombotic effect. However, it should be noted that individuals with inherited deficiencies of β2-GPI do not seem to have an increased risk for thrombosis[36]. Studies of the recently produced β2-GPI knockout mice may shed further light on this[37]. Some antiphospholipid antibodies bind vascular endothelial cells, and in vitro studies have shown that adhesion molecule expression is increased on endothelial cells in the presence of antiphospholipid antibodies, facilitating platelet adherence[38]. Furthermore, there is evidence that patients with APS have increased levels of antibodies to oxidized LDL, which is associated with progression of atherosclerosis and risk of thrombo-occlusive events[39,40].

Rand et al. have proposed another hypothesis for the mechanism of thrombosis in APS—antibody-mediated disruption of 'the annexin V antithrombotic shield'[41]. Annexin V is thought to form a protective carpet shielding anionic phospholipids from participating in coagulation reactions; these phospholipids would otherwise serve as efficient cofactors for the assembly of coagulation factor complexes. The clustering of annexin V on the surface is disrupted by high-affinity antiphospholipid antibodies, resulting in a prothrombotic state. Another group has suggested that inhibition of annexin V binding to procoagulant phospholipid surfaces is dependent upon anti-β2-GPI antibodies[42].

Another possibility is dysfunction of factors important in maintaining lipid symmetry of the membrane bilayer—such as aminophospholipid translocase, 'floppase' and lipid scramblase—resulting in exposure of normally secluded negatively charged phospholipids to the cell surface. This loss of membrane phospholipid symmetry has been shown to occur in apoptosis—indeed, Ranch et al. hypothesized that apoptotic cells not only serve as targets of aPL antibodies but also participate in the induction of aPL antibodies[43].

PREGNANCY LOSS

aPL antibodies are found in less than 2% of normal pregnant women and in up to 20% of women with recurrent pregnancy loss[44]. These antibodies are associated with adverse pregnancy outcome at all gestational ages—from first-trimester miscarriage, through second-trimester pregnancy loss, pre-eclampsia and intrauterine growth retardation to preterm labour[45,46]. These complications are thought to be caused largely by uteroplacental insufficiency from multiple placental thromboses, infarcts and a spiral artery vasculopathy in decidual vessels. Some workers have proposed alternative mechanisms for pregnancy loss, since

thrombosis is neither a universal nor a specific feature of aPL-associated miscarriage[47]. These include abnormal eicosanoid metabolism induced in gestational tissues by aPL antibodies, leading to impaired trophoblast invasion and expansion[48].

STROKE

In the Antiphospholipid Antibodies in Stroke Study (APASS) in 1993, 255 consecutive first ischaemic stroke patients were compared with age and sex matched non-stroke controls. The frequency of anticardiolipin antibodies was substantially higher in those with ischaemic stroke (10% *v* 4%) and furthermore their presence seemed to be an independent risk factor for stroke in these patients[49]. In the two-year follow-up APASS study anticardiolipin positivity did not carry an increased risk of subsequent thrombo-occlusive events or death[50]; however, the cut-off titre for positivity was only 10 GPL units and, since low titres of anticardiolipin antibodies often represent a transient non-specific phenomenon, the effect of high titres was considerably diluted—furthermore, the median follow-up of 2 years may have been too short for detection of a small but clinically important difference.

Stroke associated with anticardiolipin antibodies affects a younger population and proportionately more females than typical atherothrombotic stroke[51]. In a recent prospective study of patients with high titres of anticardiolipin antibodies (>100), 26 of the 27 patients had recurrent cerebrovascular ischaemic events despite treatment (most were on aspirin and coumadin) over the 3 years of follow-up[52]. These events were more likely to be TIAs (mean rate 25% per year) than strokes (5% per year). A high titre of anticardiolipin antibodies was associated with other stroke risk factors, including cigarette smoking and hyperlipidaemia—an observation made also by others. One hypothesis is that endothelial injury caused by the presence of conventional stroke risk factors leads to exposure of antigens that are normally secluded within the phospholipid bilayer, thus stimulating an antiphospholipid antibody response. Young patients with persistently high anticardiolipin IgG levels (>100 units) and stroke or TIA were almost invariably cigarette smokers.

TREATMENT OF ANTIPHOSPHOLIPID SYNDROME

Modification of cardiovascular risk factors is important: the patient should avoid the contraceptive pill, refrain from smoking, exercise regularly and maintain ideal weight; hypercholesterolaemia and hypertension should be treated, and diabetes closely controlled if present. With regard to antithrombotic therapy, two retrospective studies have been helpful. One was a review[2] of 70 patients with aPL-associated thrombosis, on various empiric treatment regimens, with a five-year follow-up. Warfarin with an intermediate to high intensity of anticoagulation (international normalized ratio [INR] >2.6) was effective in preventing further thrombotic events. In contrast, lower intensity warfarin and aspirin (dose 80–325 mg per day) were ineffective. In another study[53], the efficacy of high-intensity warfarin (INR >3), low intensity warfarin (INR <3) with and without low-dose aspirin, and low-dose aspirin (75 mg per day) alone was assessed in the secondary prevention of thrombosis in 147 patients with APS. Median follow-up was 6 years and 69% had recurrent thrombotic events. Recurrence rates per year were 0.01 for high intensity warfarin, 0.23 for low intensity warfarin, 0.18 for aspirin alone and 0.29 for the untreated group. Aspirin conferred no additional benefit when added to warfarin. The conclusion was that an INR >3 is effective in preventing thrombotic events, and that there is no benefit from low-dose aspirin or an INR of 2–3. However, before these data are extrapolated to a particular patient with APS, we do well to remember that the trials were not prospective or randomized, and that high-intensity anticoagulation carries a risk of serious haemorrhage[54].

In contrast, the obstetric management of APS is now clearly delineated by two prospective randomized studies. In the trial reported by Rai *et al.*[55], pregnant women with recurrent miscarriage associated with aPL antibodies were randomized to low-dose aspirin with or without a low-dose of unfractionated heparin (5000 U) twice daily, at the first detection of fetal cardiac activity and continued until 34 weeks' gestation. The rate of live births was significantly higher in the combination group than in the group receiving low-dose aspirin alone. In the study conducted by Kutteh *et al.*[56], pregnant women with a history of three or more pregnancy losses and aPL antibody levels of >27 IgG or >23 IgM phospholipid units were randomized to receive low-dose aspirin with or without adjusted doses of heparin to maintain a mid-interval APTT of 1.5 fold. Treatment was begun when fetal cardiac activity was first detected and continued until term. This study likewise showed that the live-birth rate was significantly greater for the treatment combination group than for those who received low-dose aspirin alone (80% *vs* 44%). With aspirin and heparin treatment the live-birth rate of women with APS approaches that of normal aPL-antibody-negative women.

Another treatment approach is immunotherapy. There is, however, no conclusive evidence supporting this approach, most of the reported studies being small and non-controlled. Corticosteroids and other immunosuppressant therapies, such as azathioprine, cyclophosphamide and methotrexate, have been reported in some studies to decrease titres of lupus anticoagulant and anticardiolipin antibodies, but do not seem to decrease thrombotic

risk[57,58]. The most promising immunotherapies, which still need to be comprehensively studied in a controlled fashion (in view of their success in other antibody-mediated autoimmune diseases such as myasthenia gravis and idiopathic thrombocytopenic purpura) are intravenous immunoglobulin (IVIG) and plasma exchange[59,60]. However, a prospective, placebo-controlled, randomized pilot study of pregnant women with APS showed no benefit from IVIG over and above that conferred by aspirin and heparin therapy[61]; furthermore, no significant clinical benefit was shown in a recent meta-analysis of IVIG therapy for women with unexplained recurrent miscarriage[62].

CONCLUSIONS

Antiphospholipid syndrome is an important cause of hypercoagulability, predisposing to both venous and arterial thromboses and recurrent fetal death due to placental insufficiency. Despite the name, the antibodies associated with APS are predominantly directed against phospholipid-binding plasma proteins, such as β2-GPI and prothrombin, rather than phospholipids themselves. When APS is suspected, confirmatory laboratory tests include coagulation assays for lupus anticoagulant and ELISA detection of anticardiolipin antibodies, the former being more specific and the latter more sensitive. Interpretation of the pathological significance of anticardiolipin antibodies can be problematic since these antibodies are found in various non-thrombotic contexts (certain infections, drug therapy) and even in apparently healthy people. The associated presence of lupus anticoagulant, anticardiolipin antibody in high titre (>40 GPL units for IgG isotype), persistence of anticardiolipin antibody for at least 6 weeks, and its dependence on the plasma glycoprotein β2-GPI for binding suggests pathogenicity. Pending formal assay standardization, testing for antibodies to β2-GPI and to non-cardiolipin phospholipids is indicated in patients who are strongly suspected to have APS but who have negative tests for anticardiolipin antibody and lupus anticoagulant. For prevention of recurrent thrombosis in APS the present recommendation is to maintain an INR >3. However, this is based on retrospective non-controlled evidence, and high-intensity anticoagulation carries an important risk of haemorrhage. Until definitive data from prospective trials are available, the intensity of anticoagulation will need to be individualized for patients with APS, the risks of haemorrhagic complications being weighed against the benefits of preventing re-thrombosis. Prospective randomized trials have shown the efficacy of aspirin and heparin treatment in the prevention of pregnancy loss in APS. Evidence for the use of other treatment strategies, such as immunotherapy, remains unpersuasive.

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