56 Antiphospholipid Antibodies and Variants

Robin L Brey and Christian L Stallworth

Overview

The antiphospholipid syndrome (APS) was first described by Harris, Hughes and colleagues in 1983.[1] The major clinical features consist of arterial[2–6] and venous[7] thrombosis leading to tissue ischemia or recurrent fetal loss,[8,9] and thrombocytopenia in the presence of antiphospholipid antibodies (aPLs).[10] A variety of neurological manifestations have also been described. Some of these are related to thrombosis, but others are not (see Table 56.1). Prior to the recognition of all the major facets of the clinical syndrome, thrombosis, and less commonly bleeding, were associated with the presence of a prolongation of phospholipid-dependent coagulation tests. This was called the Lupus anticoagulant (LA) effect because the coagulation test was prolonged as it might be in a patient on an anticoagulant medication, and it occurred most commonly in patients with systemic lupus erythematosus (SLE). A similar thrombotic tendency was seen in patients with antibodies to cardiolipin as detected by solid-phase radioimmunoassay and later enzyme-linked immunosorbent assay (ELISA) techniques.[11] There was some overlap between patients with the LA and immunoreactivity to cardiolipin using solid-phase testing, thus researchers initially thought that the same prothrombotic antibody must be detected by both techniques. Cardiolipin is found primarily in the plasma membranes of mitochondria,[12] and is not readily accessible to the immune system. However, cross-reactivity to other negatively-charged phospholipids was soon recognized. Phosphatidylserine is found in abundance in the plasma membranes of all cells and is exposed during cell activation and membrane re-modeling. In addition, phosphatidylserine immunoreactivity is more strongly associated with fetal loss[9,13] and thrombosis[14,15] than is cardiolipin immunoreactivity. In 1990, three groups independently identified the need for a co-operative phospholipid-binding protein in order to detect most, but not all, aPLs.[16–19] This protein was sequenced and identified as beta-2-glycoprotein-1 (β_2GP-1). Considering the substantial immunogenic quality of proteins versus the low immunogenicity of lipids, the involvement of a phospholipid-binding protein in the aPL immune response made sense biologically. Proteins such as prothrombin, annexin V, protein C, protein S, low molecular weight kininogens and factor XI[20] have also been shown to bind phospholipids, but β_2GP-1 is by far the most common and well characterized protein with this ability.[12,14] For some LAs, β_2GP-1 probably also plays a co-factor role; however, for most, prothrombin is probably more important.[21]

Epidemiology

Case–control studies of aPL-associated stroke in young people have been uniformly positive.[4,22,23] Some[24–27] but not all[28,29] case–control studies among older adults have found aPLs to be associated with ischemic stroke. Case–control studies have been criticized because of the difficulty of establishing the temporal relationship. However, studies that obtained blood within 7 days of the event,[27] or even within 6 hours of onset,[30] have had positive findings. While these time periods may be too short to allow the development of measurable IgG levels due to a primary or amnestic immune response,[31] they do not preclude the possibility of antibodies induced by a recent prior febrile illness. Indeed, infection-associated cerebral infarction is not only quite common, but is also associated with higher levels of anticardiolipin antibodies of the IgG isotype.[32]

Three prospective studies of antiphospholipid antibodies and stroke have been performed. Two of these were negative.[33,34] However, both were limited in statistical power and suffered from technical limitations. The first study to show a prospective association between anticardiolipin antibodies and ischemic stroke evaluated patients enrolled in the Honolulu Heart Program.[35] The overall risk factor-adjusted odds ratio (OR) for aCLs (anticardiolipin antibodies) of the IgG class was 1.5 (95% confidence interval (CI)

Table 56.1 Neurological syndromes associated with antiphospholipid antibodies

1. Cerebrovascular ischemia
   - Stroke
   - Transient ischemic attack
   - Cerebral venous sinus thrombosis
2. Ocular ischemia
3. Dementia
   - Acute ischemic encephalopathy
     - with Sneddon's syndrome
     - without Sneddon's syndrome
4. Atypical migrainous-like events
5. Seizures
6. Chorea
7. Transverse myelopathy
8. Guillain–Barré syndrome
9. Diabetic peripheral neuropathy
10. Sensorineural hearing loss
    - Sudden onset
    - Progressive
11. Transient global amnesia
12. Psychiatric disorders
13. Orthostatic hypotension

1.0–2.3), which was increased for the age group 56–70 years at baseline (OR 2.1, 95% CI 1.3–3.4). When the association with stroke over the 20-year follow-up period was examined by 5-year intervals, the odds ratio for the last 5-year interval was significantly lower than for each of the first three 5-year intervals. This suggests that the antibodies may be changing over time or that they may be a reflection of a similarly changing physiological state.

Etiology

Stroke mechanisms in patients with aPL

Cerebral ischemic events can occur in any vascular territory.[36] Cerebral angiography typically demonstrates intracranial branch or trunk occlusion, or is normal in about one-third of patients so studied.[2] Data from in-vitro studies, from experimental animal models, and from clinical studies showing an association of aPLs with placental infarction, peripheral and cerebral venous thrombosis, strongly suggest that these antibodies cause stroke through induction of a prothrombotic state. A higher than expected frequency of coronary artery[37] and peripheral arterial[38] graft occlusion has been noted in patients with aPL as well. These clinical observations, coupled with recent findings of endothelial cell activation by aPLs,[39,40] support the hypothesis that aPLs may act in concert with other vascular risk factors that damage endothelial cells.

A variety of cardiac valvular lesions have been associated with aPLs, making cardiac emboli a possible stroke mechanism in some patients. Two-dimensional transthoracic echocardiography is abnormal in one-third of patients, typically demonstrating nonspecific left-sided valvular (predominantly mitral) lesions characterized by valve thickening.[41,42] These may represent a potential cardiac source of stroke.[41–44] In a large consecutive autopsy series, a higher incidence of cardiac valvular abnormalities and "bland" (non-vasculitic) thromboembolic lesions were found in patients with aPLs.[45] Classic Libman–Sacks verrucous valvular lesions may also be attributable to phenomena associated with APS. In 1924, Libman and Sacks originally described a form of valvular and mural endocarditis,[46] which would later be attributed to SLE.[47,48] These valvular lesions are typically clinically silent and are frequently associated with thickened, functionally impaired cardiac valves that are prone to hemodynamic deterioration. The causative mechanisms for this pathology have yet to be defined.[48]

Origin of antiphospholipid antibodies

There are no data to suggest that the severity of the thromboembolic event, including stroke, influences aCL titer. aCLs do not appear to be a result of the thrombotic event in the brain[4,5] or elsewhere.[49]

Some aPLs may arise due to infection by common viruses and bacteria.[50] Gharavi and colleagues demonstrated that pathogenic aPL and anti-β_2GP-1 antibody production can be induced following immunization with mutant forms of β_2GP-1 containing the aPL binding site alone.[51] A subsequent Genebank screen yielded seven proteins with sequence homology to the mutant proteins. Four of the peptides were identified as part of viruses or bacteria to which humans are commonly exposed (human cytomegalovirus and *Bacillus subtilus*). Interestingly, these exhibited a greater ability to bind PL than the mutants they generated. Normal mice immunized with these viral protein fragments developed aPLs and suffered intrauterine fetal death, spinal cord infarction and thrombosis,[52] suggesting that infection may well be the trigger for pathogenic aPL production.[50] A recent report describing APS associated with cytomegalovirus infection illustrates that infection-induced aPL may occasionally be associated with thrombosis.[53] In support of this proposal, Vermylen provides corroborative evidence for infective origins of aPL. Varicella-associated neutralizing antibodies against protein S present clinical resemblance to purpura fulminans,[54] a congenital homozygous protein S deficiency that may exhibit widespread cutaneous thrombosis and necrosis shortly following birth.[55] Similar symptoms may arise from IgG and IgM antibodies to protein S in children recovering from varicella infection. Ultimately, common bacterial or viral infection might incite similar mechanisms in humans and yield aPL production in patients that leads to APS.

Pathophysiology and pathogenesis

The range of clinical manifestations in APS might be explained by the breadth of autoantibodies and their characteristics (specificity, affinity/avidity, valency, titer). Consequently, mechanisms may be widely characterized by those involving antibody interference with hemostatic reactions, and those involving cell-mediated events.[56] It is important to remember, however, that not all patients with aPLs suffer thrombosis or recurrent fetal loss. aPLs associated with some infections such as syphilis or certain medications are usually transient, contain a more restricted range of phospholipid immunoreactivity, and are not associated with clinical symptoms.[57] In addition, aPLs may be found in otherwise normal people. A prospective blood-bank study found that approximately 6.5% of normal subjects had ELISA detected aPL IgG.[58] Many aPL levels normalized with time, though, and no thrombotic events occurred in aPL+ patients over a 12-month period. Krnic-Barrie and colleagues[59] described recurrent thrombosis after many years of follow-up, therefore 12 months of follow-up may not be sufficient to assess aPL-associated thrombosis risk.

One of the most promising aspects of the discovery of β_2GP-1 as a target antigen for aPLs is the possibility that aPLs interfere with the function of β_2GP-1 in vivo, thereby conferring a prothrombotic diathesis in APS.[60] β_2GP-1, a minor natural anticoagulant, competes in vitro for available phospholipid surface area needed for assembly of the prothrombinase complex, thereby inhibiting prothrombinase activity.[61] This

inhibitory capacity is weak under normal physiological conditions. However, aPL may cross-link membrane-bound β_2GP-1 and increase β_2GP-1's inhibitory abilities.[20,62] Also, β_2GP-1's mobility remains high on phospholipid surfaces due to ionic binding interactions. Once complexed with aPL, however, there could be a higher affinity for phospholipids that would impede coagulation by competing with coagulation factors for the available catalytic surface. Therefore, bivalency would be essential for cross-linking two β_2GP-1 molecules and inducing a correct spatial orientation of their phospholipid binding domains, thereby markedly increasing their affinity for the phospholipid surface.[20] aPLs can also interfere with the protein C pathway by inhibiting thrombin formation, interfering with thrombomodulin expression, and inhibiting the degradation of factor Va by activated protein C (activated protein C resistance).[12] Interestingly, aPL-related activated protein C resistance does not appear to be associated with a mutation in the coagulation factor V gene.[63,64]

A variety of effects on platelets, coagulation proteins and endothelial cells have been ascribed to aPLs, making them not only serological markers for APS, but also direct contributors to the development of thrombosis and other clinical manifestations. β_2GP-1 binding to phosphatidylserine may serve in the identification of apoptotic cells, since cells undergoing apoptosis are known to redistribute PL to expose phosphatidylserine on the extracellular surface.[65] In vitro opsonization of apoptotic cells by β_2GP-1 antibodies enhances scavenger macrophages binding. With respect to platelet–antibody interactions, Arnout has drawn attention to the similarities between the pathogenic mechanism for heparin-induced thrombocytopenia and a potential mechanism for aPL-induced thrombosis.[66] Heparin-induced thrombocytopenia involves antibody binding of a protein (mainly platelet factor 4) that is bound to heparin, which then interacts with the cellular Fc-gamma receptor to induce activation of a prothrombotic process. There is emerging evidence that aPL binding to phospholipid complexes on various cells, including platelets and vascular endothelium, also results in their activation through the Fc-gamma receptor.[19] Campbell has demonstrated the induction of a dose-dependent increase in the activation and aggregation of human platelets using aPL from patients with APS.[67] This effect appears to be mediated through binding to phosphatidylserine[68] or β_2GP-1.[14,69] The feasibility of β_2GP-1 binding to endothelial cells is more controversial since they infrequently express large amounts of negatively-charged structures at the cell surface. Recent evidence suggests that β_2GP-1 may be involved in lipid metabolism and serve as an endothelial growth factor. Meroni and colleagues have demonstrated that β_2GP-1 probably binds to endothelial cells through heparin-sulphate (HS) proteoglycan, which plays a role in the function of vascular endothelial growth factors.[19]

Both passive and active immunization of normal laboratory mice with either aPL or β_2GP-1 results in the induction of an experimental APS, including thrombocytopenia, placental infarction and fetal loss, myocardial infarction and neurological dysfunction.[70] Brey and colleagues were able to accelerate neurological dysfunction in an autoimmune mouse strain by immunization with β_2GP-1.[71] Immunization with β_2GP-1 in both normal and autoimmune mouse strains leads to the development of antibodies to both β_2GP-1 and phospholipids.[70]

Many investigators favor a "two hit" hypothesis to explain how aPLs might lead to thrombosis; (1) continually circulating aPLs and phospholipid-binding proteins require (2) a local trigger (i.e. infection, injury, EC activation) to induce site-specific thrombosis or amplify the thrombotic process.[72] This hypothesis proposes aPL induction of a prothrombotic state in which thrombosis is triggered by an otherwise insufficient local trigger.[56] Thrombosis would require a second triggering factor, so explaining why patients with persistent serum autoantibodies display clotting events only occasionally, and in the absence of detectable immunoglobulin deposits.[19] The origins of atherosclerosis comprise a multifaceted histopathological process. It has been proposed that the oxidative modification of LDL in vivo may be a central contributor to atherogenesis. Therefore, aPLs may play a role in atherosclerosis because of a cross-reactivity with oxidized aPLs.[73,74]

Although there is some evidence that aPLs may actually lead to accelerated atherosclerosis,[73–75] the majority of evidence favors a prothrombotic mechanism that amplifies thrombosis in certain settings. Pierangeli and Harris demonstrated larger clot size with a longer time to dissolution in mice treated with human aPLs compared to control IgG using a pinch clamp injury model.[76] Taken together, these studies provide important evidence that antibodies to phospholipids and phospholipid-binding proteins like β_2GP-1 can cause thrombosis and other antibody-mediated clinical manifestations.

Genetics

The development of most autoimmune diseases, including the aPL response, is highly likely to be influenced by multiple genes. Deficiencies in two complement protein genes, C2 and C4, located within the MHC genetic region,[77] have been associated with aPLs, primarily in African Americans.[78] Cytokine genes are located within this region as well, such as tumor necrosis factor (TNF). TNF is a potent pro-inflammatory cytokine that is important in coagulation and also in the aPL response. Thus it is possible that aPL/APS may be associated with one or more polymorphic genes in strong linkage disequilibrium with specific HLA class II alleles. Other non-MHC disease susceptibility gene abnormalities may be important factors that influence whether thrombosis is seen in association with aPLs such as prothrombin, factor V Leiden, tissue factor pathway inhibitor, fibrinogen, thrombomodulin, and platelet collagen receptor genes.[79,80]

Many clinical observations suggest that aPLs (with

or without associated thrombosis) can be familial. Both siblings and consecutive generations of the same family have been shown to express aPL immunoreactivity.[81–83] While the precise basis for the familial clusterings of aPLs are unclear, a common genetic background is considered to be a likely causal connection.[83] Some family studies have evaluated the association between aPLs and HLA alleles, particularly DR4 and DR7.[81,82,84] However, in one project where seven families were studied using strictly applied criteria for APS, evidence for a dominant inheritance was seen but there was no association with HLA, fas, or other candidate genes.[83] It is possible that genetic heterogeneity is present, and some of these candidate genes may be important in a subset of families.

Most but not all population-based studies also demonstrate that HLA genes have a role in conferring susceptibility to developing APS in patients with or without SLE. The vast majority of these studies have been performed in patients with SLE, with fewer performed in patients without SLE.

Similar associations of aPLs/APS with HLA alleles are seen in patients with APS but without SLE as in family studies described above.[84] From these studies it appears that HLA-DR4 may be more important in Anglo Saxon populations, whereas HLA-DR5 and -DR7 may be more important in populations of Latin origin. All of these studies evaluated relatively small numbers of patients, often with different clinical manifestations of APS. These same HLA class II alleles are also associated with aCLs, LA or both in populations with SLE, and with similar ethnic differences. A large study specifically addressing ethnic differences in the association of HLA class II alleles with a type of aPL, anti-β_2GP-1, contained some patients with and without SLE.[85] In this study, the HLA-DR4 allele was increased in anti-β_2GP-1 positive SLE patients as compared to anti-β_2GP-1 negative SLE patients and normal controls, especially in Mexican Americans and, to a lesser extent, whites. When SLE and non-SLE patients were combined, the HLA-DR6 was increased in African Americans. HLA-DR7 was not increased in any ethnic group, and HLA-DR53 was increased in Mexican Americans only. Thus, genetic variability due to ethnic differences needs to be carefully considered in future studies of the association between HLA class II alleles and APS, including ethnic stratification of subjects.

Clinical features

Thrombotic episodes in patients with APS are primarily venous, but if arterial thrombosis occurs, the brain is affected most often.[10] The average age of onset of aPL-associated cerebral ischemia is several decades younger than the typical cerebral ischemia population.[5] Regardless of age, patients with cerebral ischemia often have other risk factors for cerebrovascular disease.[4,5,27,86]

The data regarding the risk of an initial thrombotic event associated with aPLs have been well studied; however, the risk of recurrent stroke associated with aPLs is less clear. Recurrent stroke and thromboembolic events in patients with aPLs have been reported to occur both early (within the first year of an index episode of cerebral ischemic)[5,87] and late (5–10 years).[59,88] The initial type of the thromboembolic event (i.e. arterial, venous, miscarriage) appears to be the most likely type of event to recur in a given patient in some[3] but not all studies.[59,89] Shah and colleagues studied APS in patients with and without SLE over a 10-year period and found recurrent thromboembolic events to be common in both groups.[88] In their series of 52 patients with aPLs, 9/31 (29%) patients with APS developed recurrent thrombotic episodes and 11/21 (52%) patients with aPLs but without clinical manifestations developed them over the follow-up period. Krnic-Barrie and colleagues[59] retrospectively evaluated 61 patients with APS in patients with and without SLE for an average of 6.4 years to identify risk factors for the development of recurrent thrombosis. There was no difference between patients with APS with or without SLE regarding recurrent arterial or venous events (arterial: 55% versus 38%; venous: 47% versus 50%). In patients with primary and secondary APS, recurrent arterial events were associated with Caucasian race and venous events with the puerperium or oral contraceptive use. High titers of aCL have been associated with recurrent events in patients without SLE[5,87,90] and with SLE.[49,91] In a study of 141 patients with APS related to SLE, the presence of both aCLs and LA were associated with thrombotic events.[6] In this study 84% of patients with an abnormal aCL IgG level and LA had a thrombotic event as compared to 16% with an abnormal aCL IgG only, 9.1% with LA only, and 3.8% with neither. All patients with high levels of aCL and LA and none of the patients without LA had an arterial thrombosis. The correlation between a high level of aCL and LA has been previously reported; however, Verro and colleagues did not find a similar relationship between very high titer and risk for recurrent thrombotic events.[92] Cerebrovascular and other thrombotic events have been reported in patients with isolated IgM aCL; however, in our experience, it is very unusual to have an isolated aCL IgM level without an aCL IgG level being present also. In addition, the diagnostic criteria for APS (described below) accept an isolated aCL IgM level if moderately positive (40–60 MPL units) if it remains positive over a 2-month period and the appropriate clinical features are also present.[93]

The Warfarin Aspirin Recurrent Stroke Study/Antiphospholipid Antibody in Stroke (WARSS/APASS) Collaboration is the largest study to date that is evaluating the risk for recurrent stroke in patients with standardized clinical data collection and treatment protocols in nearly 2000 patients. Preliminary analysis of these data suggest that there is no difference in recurrent thrombotic events between aPL-positive and aPL-negative stroke patients in this study (unpublished data), but final results examining the importance of titer, isotype and aCL versus LA positivity are pending.

Classification

APS is classified as secondary if it occurs in an individual with SLE or another collagen disease, and primary in the absence of SLE. However, as described above, primary and secondary APS are indistinguishable[59,88] with regard to the types of thromboses experienced and the risk of recurrent thrombotic events.

Diagnosis

The diagnosis of APS can be made if the patient has one or more arterial or venous thrombotic events, or two or more otherwise unexplained episodes of fetal loss or thrombocytopenia in the presence of antiphospholipid antibodies that persist over a period of at least 2 months (see Table 56.2).[93] The presence of antiphospholipid antibodies can be detected using one of the following two methodologies. First, aPLs can be detected with solid-phase testing using cardiolipin or other negatively-charged phospholipids as the detecting antigen. The presence of β_2GP-1 alone is not considered sufficient evidence for diagnosis of APS because of the lack of data in the literature confirming the association of anti-β_2GP-1 with thrombosis and recurrent fetal loss. The second method of aPL detection is the prolongation of phospholipid-dependent coagulation tests, also called the LA. The criteria for the diagnosis of an LA is as follows: demonstration of an abnormality in an in vitro phospholipid-dependent coagulation test; proof that the abnormality does not correct with the addition of an equal volume of normal plasma; and proof that the abnormality does correct with the addition of phospholipid.[93]

Clinical variants

Sneddon's syndrome and other aPL-associated dementia Recurrent stroke in patients with livedo reticularis (Sneddon's syndrome) has been associated

Table 56.2 Research criteria for antiphospholipid antibody syndrome diagnosis

Diagnosis

One or more clinical criteria and one or more laboratory criteria described below are required for the diagnosis of the antiphospholipid antibody syndrome.

Clinical criteria

One or more episodes of vascular thrombosis or pregnancy morbidity as described below are required for the diagnosis of antiphospholipid antibody syndrome. While other clinical features may be associated with aPL in some studies, there is insufficient evidence to consider them to be diagnostic features at this time.

Vascular thrombosis

I One or more clinical episodes of arterial, venous or small vessel thrombosis in any tissue or organ are required for the diagnosis of Antiphospholipid Antibody Syndrome.
Thrombosis must be confirmed via imaging, Doppler studies or histopathology, with the exception of superficial venous thrombosis.

Pregnancy morbidity

I One or more unexplained deaths of a morphologically normal fetus at or beyond the tenth week of gestation with normal fetal morphology documented by ultrasound or exam are required for the diagnosis of antiphospholipid antibody syndrome.
or
I One or more premature births or a morphologically normal neonate at or before the 34th week of gestation because of pre-eclampsia, or severe placental insufficiency are required for the diagnosis of antiphospholipid antibody syndrome.
or
II Three or more unexplained consecutive spontaneous abortions before the tenth week of gestation with maternal anatomical or hormonal abnormalities and exclusion of maternal and paternal chromosomal causes are required for the diagnosis of antiphospholipid antibody syndrome.

Laboratory criteria

The presence of either anticardiolipin antibodies (aCL) or Lupus anticoagulant as described below is required for the diagnosis of antiphospholipid antibody syndrome.

I Anticardiolipin antibody (aCL) of IgG and/or IgM isotype in blood, present in medium or high titer, on two or more occasions, 8 weeks or more apart, and measured by a standardized ELISA for β_2GP-1-dependent aCL.

II Lupus anticoagulant (LA) present in plasma on two or more occasions 6 weeks or more apart and detected according to the guidelines of the International Society of Thrombosis and Hemostasis, in the following steps:
i) Demonstration of a prolonged phospholipid-dependent coagulation screening test, e.g. APTT, KCT, dRV VT, dPT, Textarin time
ii) Failure to correct the prolonged screening test by mixing with normal platelet-poor plasma.
iii) Shortening or correction of the prolonged screening test by the addition of excess phospholipid.
iv) Exclusion of other coagulopathies as appropriate, e.g. factor VIII inhibitor, heparin.

(Adopted from the International Consensus Statement On Preliminary Classification Criteria for Definite Antiphospholipid Syndrome: Report of an International Workshop[93])

with aPLs.[94] The frequency of aPLs in patients with Sneddon's syndrome has ranged from zero to 85%.[94–96] This syndrome is also frequently accompanied by dementia, most likely on the basis of multiple infarctions. Sneddon's original patients all had focal neurological deficits, which he considered to be "limited and benign," leaving little residual disability.[97] Subsequent descriptions of the syndrome have revealed a spectrum of clinical neurological manifestations. Zelger and colleagues described three stages of neurological involvement: "prodromal" symptoms such as dizziness or headaches preceding focal neurological deficits by years; recurrent focal neurological deficits due to recurrent cerebral ischemia, also lasting years; and progressive cognitive impairment leading to severe dementia.[95] Tourbah and colleagues correlated the magnetic imaging (MRI) abnormalities found in 26 patients with Sneddon's syndrome with disability, presence of cardiovascular risk factors, cardiac valvular abnormalities on ECHO and titer of aPLs.[96] Disability (defined by memory disturbance or inability to perform activities of daily living) was found in 50% of the patients. Severe disability, which was consistent with dementia, was present in over half of the patients with disability. Systemic hypertension was present in 65%, cardiac valvular abnormalities in 61% and aPLs in 42% of patients, with no correlation found between any of these and MRI abnormalities. The presence of disability was correlated with increasing severity of MRI lesions.

An aPL-associated dementia without the other features of Sneddon's syndrome has also been described Although there is experimental evidence of aPL binding to neurons[98,99]). In many patients this appears to be due to multiple cerebral infarctions.[100] In addition, the catastrophic APS can present with an acute organic brain syndrome characterized by fulminant encephalopathy.[101] Asherson reported that in a group sample of 50 patients with catastrophic APS, over 50% had CNS involvement.[102]

Progressive sensorineural hearing loss Toubi and colleagues studied the association between aCLs and sudden or progressive sensorineural hearing loss in 30 patients and matched normal controls. None of the control group had aCLs, whereas 27% of the patient group had aCLs in low–moderate titers.[103] Of the patients with aCLs, five of eight had sudden deafness. In addition, two of five patients with sudden deafness and aCLs relapsed as compared with none of six patients without them. Naarendorp and Spiera reported six patients with SLE or a lupus-like syndrome with sudden sensorineural hearing loss, all of which had aCLs or LA.[104] The authors suggest that sudden sensorineural hearing loss may be a previously unrecognized manifestation of APS and that the mechanism is likely to be vascular, and speculate that the appropriate treatment for these patients may be anticoagulant therapy.

Venous sinus thrombosis A review by Carhaupoma and colleagues suggests that aPLs may be an important factor contributing to cerebral venous sinus (CVT) thrombosis even in the presence of other potential risk factors for thrombosis,[105] including the syndrome of activated protein C resistance due to factor V leiden mutation.[64] The onset of CVT in patients with aPLs occurs at a younger age and has more extensive superficial and deep cerebral venous system involvement than CVT without aPLs. Headache, papilloedema, seizures, focal deficits, coma and death contribute to the clinical classification of CVT, along with pathological identification of hemorrhagic infarction.[106] Patients who present with CVT typically exhibit headache (85%), long tract signs (35%), cognitive disturbances (25%) and visual dysfunction (40%). In addition, a higher rate of post-CVT migraine and more infarctions on brain imaging studies are seen in patients with aPLs than in those without them.[105]

Transient global amnesia Transient global amnesia (TGA), a syndrome of sudden, unexplained memory loss, has been associated with aPL immunoreactivity.[107] The etiology of transient global amnesia in patients without aPLs is controversial and is thought to be related to ischemia or epileptiform activity in bilateral hippocampal areas. As both cerebral ischemia and epilepsy have been associated with aPLs, either could play a role in aPL-associated TGA.

Ocular manifestations Many reports of stroke and transient ischemic attack associated with aPLs include some patients with ocular ischemia as well.[108,109] The ophthalmological ischemic manifestations commonly associated with aPLs include anterior ischemic optic neuropathy, branch and central retinal artery occlusions, cilioretinal artery occlusions, combined artery and vein occlusions and amaurosis fugax.[109] These manifestations are found in patients with both primary and secondary APS.

Laboratory testing

As mentioned above, both phospholipid-dependent coagulation tests and immunoreactivity to negatively-charged phospholipids in solid-phase testing are used to detect aPLs. Laboratory testing to detect LA must be performed using platelet-poor plasma, or a high false-negative rate may result. Sometimes patients with an LA have a prolongation of the prothrombin time (PT) as well. This can cause some difficulty in accurately monitoring the PT when using warfarin therapy in these patients. Use of the activated factor X assay has been suggested as a more accurate monitoring test in this instance.[110]

In addition to these tests for the diagnosis of APS, other laboratory tests and the presence of certain clinical features may also be helpful in suggesting the diagnosis in certain patients (see Table 56.3). Some patients with APS who do not fulfill American College of Rheumatology criteria for the diagnosis of SLE[111] may have a positive antinuclear antibody (ANA) or erythrocyte sedimentation rate test. Thrombocytopenia is a clinical manifestation of APS, and therefore all patients with APS should have a platelet count performed.

Table 56.3 Factors warranting evaluation of aPL presence in stroke

- Patient < 40 years of age
- Stroke is recurrent
- Thrombocytopenia, fetal loss or venous thrombosis have been documented
- Lupus or other connective disease is present

When bleeding is seen in patients with APS it is usually in the setting of severe thrombocytopenia: thus it is important to know what the patient's platelet count is before initiating therapy to treat thrombotic disease. The VDRL antigen contains cardiolipin, and can be "falsely" positive in patients with aPLs.

Imaging

Brain magnetic resonance imaging studies in patients with APS (primary or secondary) have revealed small foci of high signal in subcortical white matter scattered throughout the brain.[112–114] This type of pattern is seen in many other disease processes, and is, as such, non-specific. The correlation between MRI lesions in patients with aPLs and clinical nervous system symptoms is reported to be high by some investigators[112–115] and not by others.[116,117] Figure 56.1 illustrates multiple cerebral infarction on brain imaging in a 23-year-old woman with aPLs and SLE.

Toubi and colleagues[114] found aPL immunoreactivity in 53/96 (55%) SLE patients with CNS manifestations as compared to 20/100 (20%) of SLE patients without them. In this study, 53 patients with CNS manifestations underwent MRI imaging and 33 showed high-density lesions that were interpreted as "suggestive of vasculopathy." MRI abnormalities were seen more frequently in patients with aPL immunoreactivity as compared to those without. Some of these patients with MRI abnormalities had seizures or psychiatric disturbances but not stroke. This suggests that in some cases aPL-associated neurological manifestations may be due to an aPL–brain phospholipid interaction, whereas in others the underlying pathogenic feature may be thrombotic.

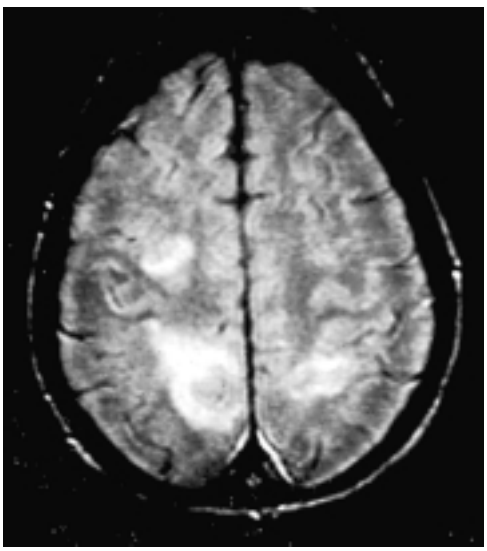

Figure 56.1 23-year-old female with history of SLE, antiphospholipid antibodies and stroke. Image obtained on 1.5 Tesla MRI Scanner (TR = 2000, TE = 30). Note two lesions in the patient's left parietal region and one small lesion in the right parietal region.

Sailer and colleagues[117] studied 35 SLE patients with inactive SLE using brain MRI and PET imaging, neuropsychological testing, a neurological examination and serum testing for aPLs and antineuronal antibodies. Twenty patients had neurological deficits, three had psychiatric symptoms and ten had cognitive impairment. No differences in global glucose utilization by PET imaging were seen between SLE patients as compared to those without neurological or cognitive abnormalities. On MRI imaging, the number and size of the white matter lesions correlated with the presence of neurological deficit, but were unrelated to the severity of cognitive impairment. Large lesions (8 mm or greater) were associated with high aCL IgG levels. Tietjen also found an association between MRI lesions and aCL levels in young patients with migraine-associated transient focal neurologic events.[115] In a study evaluating the association between neuropsychological abnormalities and aPLs in an elderly population, no association between aCLs and MRI lesions was found, supporting Sailer's findings in the group with cognitive impairment only.[116] Hachulla and colleagues performed brain MRI in patients with primary and secondary APS.[118] Both cerebral atrophy and white matter lesions were more common in both groups with respect to control subjects. The number and volume of white matter lesions were increased in patients with primary and secondary APS who also had neurological symptoms. Only a weak correlation was found between the presence of a LA and cerebral atrophy.

In addition to MRI, Specker and colleagues[119] report the use of transcranial Doppler technique for the assessment of stroke risk in patients with aPL. Transcranial Doppler has been used to identify microembolic signals, which have been detected in the intracranial circulation of patients with carotid artery disease,[120–122] artificial heart valves[123] and coagulopathy,[120,122] but are infrequently associated with small vessel disease.[122] They are thought to arise from vascular air bubbles, formed elements, and experimentally induced emboli,[124–126] and are detected based on the site, degree, extent and surface of stenosis.[127] Two of their patients with the highest event rates were aPL-positive, and the rate of microembolic signals correlated with the titer of IgG-aCL, arguing for a pathophysiological association between aPLs and microembolic signals. The use of transcranial Doppler to detect microembolic signals in APS is an inexpensive, non-invasive method. It may offer a new approach in risk stratification and possibly therapy monitoring in patients with aPLs.[128]

Differential diagnosis

Occasionally patients with aPLs will present with a clinical picture that is similar to multiple sclerosis (MS). Patients with definite MS and no thrombotic disease do not appear to have an increase in the frequency of aPL.[129] Sometimes it is difficult to distinguish MS from SLE or Sjogren's syndrome, both conditions that have an increased frequency of aPLs. Many patients with one autoimmune disease will have several others as well. Thus some, although probably a minority of patients, have both APS and MS.

Antiphospholipid antibodies are also common in patients with HIV.[130] Strokes and other thrombotic events are common in HIV-infected patients as well. It is not yet clear whether the magnitude of thrombosis risk in an HIV-infected patient with aPLs is increased.

Treatment

General treatment issues

Treatment of APS can be directed at thrombo-occlusive events using antithrombotic medications, or at modulating the immune response with immunotherapy. In the case of thrombotic manifestations, both approaches have been used (see Table 56.4).[36,59,88,131–135] Because the mechanism by which aPLs lead to thrombosis is probably heterogeneous, it is likely that the most appropriate therapeutic choice for a given patient may depend on which of these the patient's thrombo-occlusive episode was due to. For platelet or prostaglandin abnormalities, antiplatelet drugs may be expected to be beneficial; whereas for thrombomodulin, Protein C, or Protein S abnormalities, anticoagulation might be needed. This may provide a partial explanation for the discrepant findings in the aPL treatment literature on thromboembolic manifestations.

Therapies aimed at modulating the immune response in preventing both thrombotic and non-thrombotic neurological manifestations of APS also have variable success.[131,133,134] As with aPL-associated thrombosis, a more precise definition of the nature of the aPL-target tissue interaction would help guide more rational therapeutic decision making.

Table 56.4 Treatment for thrombosis associated with antiphospholipid antibodies

Anti-thrombotic therapy
Aspirin
Dipyridamole
Warfarin
Low molecular weight heparin
Immune system modulation
Intravenous immunoglobulin therapy
Plasmaphoresis
Corticosteroids
Cyclophosphamide
Azathiaprine

Treatment of aPL-associated cerebrovascular ischemia

Primary prevention The strong association between aPLs and incident ischemic stroke suggests that primary prevention strategies should be sought; however, it is unclear what, if any, treatment should be offered when aPL is discovered in a patient with no prior thrombotic episodes. Many patients with aPL-associated stroke also have other cardiovascular disease risk factors such as smoking and hyperlipidemia.[86,87,92] It is possible that primary preventative strategies may be more important in asymptomatic patients who also have other cardiovascular disease risk factors. Conventional risk factor reduction strategies should not be neglected in this group, however. Many patients with the various manifestations of APS tend to have repeated episodes of the same manifestations.[5] It is unclear, for example, whether young women with recurrent fetal loss due to aPLs require primary preventative therapy for stroke or other arterial or venous thrombotic manifestations. The prevalence of aPL is highest in patients with SLE than in any other population,[136] and aPL increases the risk of thrombosis from two- to nine-fold in patients with SLE. This suggests that a primary prevention strategy may be more important in this group than any other.[91,136]

Secondary prevention Prevention of recurrent disease in a patient found to have aPLs at the time of a thrombo-occlusive event is the source of great concern and anxiety for both patients and physicians. The same factors for determining the risk of a first aPL-associated thrombo-occlusive event described above are important in determining the risk for recurrence, e.g. antibody characteristics and the presence of other cardiovascular disease risk factors. A variety of treatment modalities have been suggested to prevent recurrent events in patients with aPL-associated thrombosis.[36,59,88,131–135] These are based on case reports and selected or retrospective series, and are therefore all limited in their clinical applicability and generalizability. These studies provide guidance in an area where no prospective data exist and are very important. However, there are limitations to utility of these data that should not be over-stepped. Standardized treatment recommendations for stroke associated with aPLs cannot be made nor extrapolated at this time.

In their long-term follow-up study of 61 patients with aPL, Krnic-Barrie and colleagues found that there was no difference in the number of recurrent thrombotic events between patients who were taking aspirin and those who were taking warfarin.[59] Both of these groups had fewer events than patient groups who were on no therapy or who were taking corticosteroid therapy. Patients taking corticosteroid therapy in general had SLE and were likely to have other medical problems that could have predisposed them to developing thrombosis. Nonetheless, these data underscore the notion that corticosteroid therapy is not effective in preventing thrombosis in patients with aPLs.

One important problem that is difficult to overcome is that "natural history" data regarding the risk for recurrent thrombosis, either with no medication or with standardized treatment, is unavailable for aPL-associated stroke. Controlled epidemiological studies are needed that assess the risk of recurrent thrombo-occlusive events in an ischemic stroke population. In addition, more basic work is needed to identify unique characteristics of pathogenic aPLs. Assays need to be developed that consistently allow us to differentiate pathogenic from non-pathogenic aPLs, and allow us to discriminate among the many pathogenic mechanisms for thrombosis. This knowledge would permit separation of aPL-positive stroke patients into groups that need (1) no treatment; (2) antiplatelet therapy; (3) warfarin; or (4) other immune-modulating therapy. The WARSS/APASS already mentioned above will provide much needed information on aPL-associated ischemic stroke. Half of the WARSS/APASS patients were randomized to warfarin and half to aspirin. Although there is not sufficient power to detect a definitive treatment difference between the two groups, preliminary results from the study suggest no treatment difference between warfarin and aspirin and provide information on the natural history of aPL-associated recurrent thrombotic events, as both data collection and treatment are controlled. Because both aCLs and LA are tested in all participating patients, some information on the risk differences associated with each will be available, as well.

References

1. Hughes GRV. Thrombosis, abortion, cerebral disease and lupus anticoagulant. Br Med J 1983;187:1088–1089
2. Antiphospholipid Antibodies in Stroke Study (APASS) Group. Clinical and laboratory findings in patients with antiphospholipid antibodies and cerebral ischemia. Stroke 1990;21:1268–1273
3. Rosove MH, Brewer PMC. Antiphospholipid thrombosis: clinical course after the first thrombotic event in 70 patients. Ann Intern Med 1992;117:303–308
4. Brey RL, Hart RG, Sherman DG, Tegeler CT. Antiphospholipid antibodies and cerebral ischemia in young people. Neurology 1990;40:1190–1196
5. Levine SR, Brey RL, Sawaya KL, et al. Recurrent stroke and thrombo-occlusive events in the antiphospholipid syndrome. Ann Neurol 1995;38:119–124
6. Nojima J, Suehisa E, Akita N, et al. Risk of arterial thrombosis in patients with anticardiolipin antibodies and lupus anticoagulant. Br J Haematol 1997;96: 447–450
7. Wahl DG, Guillemin F, de Maistre E, et al. Meta-analysis of the risk of venous thrombosis in individuals with antiphospholipid antibodies without underlying autoimmune disease or previous thrombosis. Lupus 1998;7:15–22
8. Rand JH, Wu XX, Andree HA, et al. Pregnancy loss in the antiphospholipid antibody syndrome—a possible thrombogenic mechanism. N Engl J Med 1997;337(3): 154–160
9. Levy RA, Avvad E, Olivera J, Porto LC. Placental pathology in antiphospholipid syndrome. Lupus 1998;7:S81–S85
10. Hughes, GRV. Thrombosis, abortion, cerebral disease and lupus anticoagulant. Br Med J 1983;187:1088–1091
11. Loizou S, McCrea JD, Rudge AC, et al. Measurement of anticardiolipin antibodies by an enzyme linked immunosorbent assay (ELISA): standardization and quantitation of results. Clin Exp Immunol 1985;62: 738–745
12. McNeil HP, Chesterman CN, Krilis SA. Immunology and clinical importance of antiphospholipid antibodies. Adv Immunol 1991;49:193–280
13. Rote NS, Dostal-Johnson D, Branch DW. Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin. Am J Obstet Gynecol 1990;163:575–584
14. Inanc M, Radway-Bright EL, Isenberg DA. Beta-2-glycoprotein 1 and anti-beta-2-glycoprotein 1 antibodies: where are we now? Br J Rheumatol 1997;36:1247–1257
15. Tuhrim S, Rand JH, Godbold JH, et al. Elevated antiphosphatidylserine antibodies are a risk factor for ischemic stroke. Neurology 1998;50:A246
16. McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. Antiphospholipid antibodies are directed to a complex antigen that includes a lipid-binding inhibitor of coagulation: (β2-glycoprotein 1 (apolipoprotein H). Proc Natl Acad Sci USA 1990;87:4120–4124
17. Galli M, Comfurius P, Maassen C, et al. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. Lancet 1990;335: 1544–1547
18. Matsuura E, Igarashi Y, Fujimoto M, et al. Anticardiolipin cofactors and the differential diagnosis of autoimmune disease. Lancet 1990;336(8708):177–178
19. Meroni PL, Del Papa N, Raschi E, et al. β2-glycoprotein 1 as a co-factor for antiphospholipid reactivity with endothelial cells. Lupus 1998;7(Suppl 2):S44–S47
20. Amout J, Wittevrongel C, Vanrusselt M, et al. β_2GP-1 dependent lupus anticoagulants form stable bivalent antibody-β_2GP-1 complexes on phospholipid surfaces. Thromb Haemost 1998;79:79–86
21. Galli M, Barbui T. Prothrombin as a co-factor for antiphospholipids. Lupus 1998;7:537–540
22. Nencini P, Baruffi MC, Abbate R, et al. Lupus anticoagulant and anticardiolipin antibodies in young adults with cerebral ischemia. Stroke 1992;23(2):189–193
23. Angelini L, Ravelli A, Caporali R, et al. High prevalence of antiphospholipid antibodies in children with idiopathic cerebral ischemia. Pediatrics 1994;94(4 Pt 1):500–503
24. Kushner MJ. Prospective study of anticardiolipin antibodies in stroke. Stroke 1990;21(2):295–298
25. Chakravarty KK, Byron MA, Webley M, et al. Antibodies to cardiolipin in stroke: association with mortality and functional recovery in patients without systemic lupus erythematosus. Q J Med 1991;79: 397–405
26. Hess DC, Krauss J, Adams RJ, et al. Anticardiolipin antibodies: a study of frequency in TIA and stroke. Neurology 1991;41:525–528
27. Antiphospholipid Antibodies in Stroke Study Group (APASS). Anticardiolipin antibodies are an independent risk factor for first ischemic stroke. Neurology 1993;43:2069–2073
28. Muir KW, Squire IB, Alwan W, Lees KR. Anticardiolipin antibodies in an unselected stroke population. Lancet 1994;344:452–456
29. Metz LM, Edworthy S, Mydlarski R, Fritzler MJ. The frequency of phospholipid antibodies in an unselected stroke population. Can J Neurol Sci 1998;25:64–69

30. Camerlingo M, Casto L, Censori B, et al. Anticardiolipin antibodies in acute non-hemorrhagic stroke seen within six hours after onset. Acta Neurol Scand 1995;92:60–71
31. Barret JT. Natural resistance and acquired immunity. *In:* Harshberger SE, ed. Textbook of Immunology. St Louis: CV Mosby, 1983:203–222
32. Ameriso SF, Wong VL, Quismorio FP Jr, Fisher M. Immunohematologic characteristics of infection-associated cerebral infarction. Stroke 1991;22:1004–1009
33. Ginsburg KS, Liang MH, Newcomer L, et al. Anticardiolipin antibodies and the risk for ischemic stroke and venous thrombosis. Ann Intern Med 1992;117: 997–1002
34. Sletnes KE, Smith P, Abdolnoor M, et al. Antiphospholipid antibodies after myocardial infarction and their relation to mortality, reinfarction, and non-haemorrhagic stroke. Lancet 1992;339:451–453
35. Brey RL, Abbott RD, Sharp DS, et al. Beta-2-glycoprotein 1-dependent (β2GP1-dep) anticardiolipin antibodies are an independent risk factor for ischemic stroke in the Honolulu Heart Cohort. Stroke 1999;39:252 (Abst)
36. Coull BM, Levine SR, Brey RL. The role of antiphospholipid antibodies and stroke. Neurol Clin 1992;10:125–143
37. Klemp P, Cooper RC, Strauss FJ, et al. Anticardiolipin antibodies in ischemic heart disease. Clin Exp Immunol 1988;74(2):254–257
38. Ciocca RG, Choi J, Graham AM. Antiphospholipid antibodies lead to increased risk in cardiovascular surgery. Am J Surg 1995;170:198–200
39. Del Papa N, Raschi ER, Catelli L, et al. Endothelial cells as a target for antiphospholipid antibodies: role of anti-beta-2-glycoprotein 1 antibodies. Am J Repr Immunol 1997;38:212–217
40. Del Papa N, Guidali L, Sala A, et al. Endothelial cells as target for antiphospholipid antibodies. Arthritis Rheumatol 1997;40:551–561
41. Ford SE, Lillicrap DM, Brunet D, Ford PM. Thrombotic endocarditis and lupus anticoagulant, a pathogenetic possibility for idiopathic rheumatic type valvular heart disease. Arch Pathol Lab Med 1989;113:350–353
42. Khamashta MA, Cervera R, Asherson RA, et al. Association of antibodies against phospholipids with valvular heart disease in patients with systemic lupus erythematosus. Lancet 1990;335(8705):1541–1544
43. Badui E, Solorio S, Martinez E, et al. The heart in the primary antiphospholipid syndrome. Arch Med Res 1995;26:115–120
44. Nesher G, Ilany J, Rosenmann D, Abraham AS. Valvular dysfunction in antiphospholipid syndrome: prevalence, clinical features and treatment. Semin Arthritis Rheum 1997;27:27–35
45. Ford SE, Kennedy LA, Ford PM. Clinico-pathological correlations of antiphospholipid antibodies. Arch Path Lab Med 1994;118:491–495
46. Libman E, Sacks B. A hitherto undescribed form of valvular and mural endocarditis. Arch Intern Med 1924;33:701–737
47. Gross, L. The cardiac lesion in Libman–Sacks disease with a consideration of its relationship to acute diffuse lupus erythematosus. Am J Pathol 1940;16:375–408
48. Ziporen, L, Goldberg I, Arad M, et al. Libman–Sacks endocarditis in the antiphospholipid syndrome: immunopathologic findings in deformed heart valves. Lupus 1996;5:196–205
49. Alarcon-Segovia D, Deleze M, Oria CV, et al. Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus: a prospective analysis of 500 consecutive patients. Medicine 1989;68(6):353–365
50. Gharavi AE, Peirangeli SS. Origin of antiphospholipid antibodies: induction of aPL by viral peptides. Lupus 1998;7:S52–S54
51. Gharavi AE, Tang H, Gharavi EE, et al. Induction of aPL by immunization with a 15 amino acid peptide. Arthritis Rheum 1995;38:5296 [Abstr]
52. Gharavi AE, Tang H, Gharavi EE, et al. Induction of antiphospholipid antibodies by immunization with a viral peptide. Arthritis Rheum 1996;39:S319 [Abstr]
53. Labarca JA, Rabaggliati RM, Radrigan FJ, et al. Antiphospholipid syndrome associated with cytomegalovirus infection: case report and review. Clin Infec Dis 1997;24:197–200
54. Vermylen J, Van Geet C, Arnout J. Antibody-mediated thrombosis: relation to the antiphospholipid syndrome. Lupus 1998;7:S63–S66
55. Marlar RA, Neumann A. Neonatal purpura fulminans due to homozygous protein C or protein S deficiencies. Semin Thromb Hemost 1990;16(4):299–309
56. Roubey RAS. Mechanisms of autoantibody-mediated thrombosis. Lupus 1998;7(Suppl 2):S114–S119
57. Drouvalakis KA, Buchanan TTC. Phospholipid specificity of autoimmune and drug-induced lupus anticoagulants; association of phosphatidylethanolamine reactivity with thrombosis in autoimmune disease. J Rheumatol 1998;25:290–295
58. Vila P, Hernandez MC, Lopez-Fernandez MF, Batlle J. Prevalence, follow-up and clinical significance of the anticardiolipin antibodies in normal subjects. Thromb Haemostat 1994;72:209–213
59. Krnic-Barrie S, Reister O'Connor C, Looney SW, et al. A retrospective review of 61 patients with antiphospholipid syndrome. Arch Intern Med 1997;157: 2101–2108
60. Sheng Y, Kandiah DA, Krilis SA. Beta-2-glycoprotein-1: target antigen for antiphospholipid antibodies. Immunological and molecular aspects. Lupus 1998a; 7:S5–S9
61. Roubey RAS, Pratt CW, Buyon JP, Winfield JB. Lupus anticoagulant activity of autoimmune antiphospholipid antibodies is dependent upon β2GP-1. J Clin Invest 1992;90:1100–1104
62. Willems GM, Janssen MP, Pelsers MM, et al. Role of divalency in the high-affinity binding of anticardiolipin antibody-β2GP-1 complexes to lipid membranes. Biochemistry 1996;35(43):13833–13842
63. Bokarewa MI, Bremme K, Falk G, et al. Studies on antiphospholipid antibodies, APC-resistance and associated mutation in the coagulation factor V gene. Thromb Res 1995;78:193–200
64. Deschiens M-A, Conard J, Horellou MH, et al. Coagulations studies, factor V Leiden, and anticardiolipin antibodies in 40 cases of cerebral venous sinus thrombosis. Stroke 1996;27:1724–1730
65. Casciola-Rosen L, Rosen A, Petri M, Schlissel M. Surface blebs on apoptotic cells are sites of enhanced procoagulant activity implications for coagulation events and antigenic spread in systemic lupus erythematosus. Proc Natl Acad Sci USA 1996;93:1624–1629
66. Arnout J. The pathogenesis of antiphospholipid antibody syndrome: hypothesis based on parallelisms with heparin-induced thrombocytopenia. Thromb Haemost 1996;75:536–541
67. Campbell AL, Pierangeli SS, Wellhausen S, Harris EN.

Comparison of the effects of anticardiolipin antibodies from patients with the antiphospholipid antibody syndrome and with syphilis on platelet activation and aggregation. Thromb Haemostat 1995;73:529–534
68. Vasquez-Mellado J, Llorente L, Richaud-Patin Y, Alarcon-Segovia D. Exposure of anionic phospholipids upon platelet activation permits binding of beta-2-glycoprotein 1 and through it that of IgG antiphospholipid antibodies. J Autoimmunity 1994;7:335–348
69. Duhaut P, Berruyer M, Pinede L, et al. Anticardiolipin antibodies and giant cell arteritis: a prospective, multicenter case–control study. Arthritis Rheumatol 1998;41:701–709
70. Ziporen L, Shoenfeld Y. Antiphospholipid syndrome: from patient's bedside to experimental animal models and back to the patient's bedside. Hematol Cell Ther 1998;40:175–182
71. Brey RL, Cote S, Barohn R, et al. Model for the neuromuscular complications of systemic lupus erythematosus. Lupus 1995;4(3):209–212
72. Vaarala O. Atherosclerosis in SLE and Hughes syndrome. Lupus 1997;6:489–490
73. Matsuura E, Kobayashi K, Yasuda T, Koike T. Antiphospholipid antibodies and atherosclerosis. Lupus 1998;7(Suppl 2):S135–S139
74. Vaarala O. Antiphospholipid antibodies and myocardial infarction. Lupus 1998;7(Suppl 2):S132–S134
75. George J, Afek A, Gilburd B, et al. Atherosclerosis in LDL-receptor knock-out mice is accelerated by immunization with anticardiolipin antibodies. Lupus 1997;6:723–729
76. Pierangeli SS, Harris EN. Antiphospholipid antibodies in an in vivo thrombosis model in mice. Lupus 1994;3:247–251
77. Abbas AK, Lichtman AH, Pober JS. The major histocompatibility complex. *In:* Cellular and Molecular Immunology. Boca Raton, FL: WB Saunders Company, 1991:98–114
78. Wilson WA, Scopelitis E, Michalski JP, et al. Familial anticardiolipin antibodies and C4 deficiency genotypes that coexist with MHC DQBI risk factors. J Rheumatol 1995;22:227–235
79. Bentolila S, Ripoll L, Drouet L, et al. Lack of association between thrombosis in primary antiphospholipid syndrome and the recently described thrombophilic 3′-untranslated prothombin gene polymorphism. Thromb Haemost 1997;78:1415–1421
80. Bertolacini ML, Atsumi T, Hunt BJ, et al. Prothrombin mutation is not associated with thrombosis in patients with antiphospholipid syndrome. Thromb Haemost 1998;80:202–203
81. Dagenais P, Urowitz MB, Gladman DD, et al. A family study of the antiphospholipid syndrome associated with other autoimmune diseases. J Rheumatol 1992;19:1393–1396
82. Hudson N, Busque L, Rauch J, et al. Familial antiphospholipid syndrome and HLA-DRB gene associations. Arthritis Rheum 1997;40:1907–1908
83. Goel N, Ortel TL, Bali D, et al. Familial antiphospholipid antibody syndrome criteria for disease and evidence for autosomal dominant inheritance. Arthritis Rheum 1999;42(2):318–327
84. Goldstein R, Moulds JM, Smith CD, Senger DPS. Studies of primary antiphospholipid antibody syndrome and of antiphospholipid antibodies in systemic lupus erythematosus. J Rheumatol 1996;23:1173–1179
85. Arnett FC, Thiagarajan P, Alm C, Reveille JD. Associations of anti-β2 glycoprotein 1 autoantibodies with HLA class II alleles in three ethnic groups. Arthritis Rheum 1999;42(2):268–274
86. Levine SR, Deegan MJ, Futrell N, Welch KMA. Cerebrovascular and neurologic disease associated with antiphospholipid antibodies: 48 cases. Neurology 1990;40:1190–1196
87. Levine SR, Brey RL, Salowich-Palm L, et al. Antiphospholipid antibody associated stroke: prospective assessment of recurrent event risk. Stroke 1993;24:188
88. Shah NM, Khamashta MA, Atsumi T, Hughes GRV. Outcome of patients with anticardiolipin antibodies: a 10 year follow-up of 52 patients. Lupus 1998;7:3–6
89. Triplett DA, Brandt JT, Musgrave KA, Orr CA. The relationship between lupus anticoagulants and antibodies to phospholipids. JAMA 1988;259:550–554
90. Levine SR, Salowich-Palm L, Sawaya KL, et al. IgG anticardiolipin antibody titer >40 GPL and the risk of subsequent thrombo-occlusive events and death. Stroke 1997;28:1660–1665
91. Escalante A, Brey RL, Mitchell BD, Dreiner U. Accuracy of anticardiolipin antibodies in identifying a history of thrombosis among patients with systemic lupus erythematosus. Am J Med 1995;98:559–567
92. Verro P, Levine SR, Tietjen GE. Cerebrovascular ischemic events with high positive anticardiolipin antibodies. Stroke 1995;26:160
93. Wilson WA, Gharavi AE, Koike T, et al. International Consensus Statement On Preliminary Classification Criteria for Definite Antiphospholipid Syndrome: Report of an International Workshop. Arthritis Rheum 1999;2(7):1309–1311
94. Kalashnikova LA, Nasonov EL, Kushekbaeva AE, Gracheva LA. Anticardiolipin antibodies in Sneddon's syndrome. Neurology 1990;40:464–467
95. Zelzer B, Sepp N, Stockhammer G, et al. Sneddon's syndrome; a long-term follow-up of 21 patients. Arch Dermatol 1993;129:437–444
96. Tourbah A, Peitte JC, Iba-Zizen MT, et al. The natural course of cerebral lesions in Sneddon syndrome. Arch Neurol 1997;54:53–60
97. Sneddon IB. Cerebral vascular lesions in livedo reticularis. Br J Dermatol 1965;77:180–185
98. Emmi L, Bergamini C, Spinelli A, et al. Possible role of activated platelets in the primary antiphospholipid syndrome involving the central nervous system. Ann NY Acad Sci 1997;823:188–200
99. Kent M, Vogt E, Rote NS. Monoclonal antiphosphatidylserine antibodies react directly with feline and murine central nervous system. J Rheumatol 1997; 24:1725–1733
100. Coull BM, Bourdette DN, Goodnight SH, et al. Multiple cerebral infarctions and dementia associated with anticardiolipin antibodies. Stroke 1987;18:1107–1112
101. Chinnery PF, Shaw PI, Ince PG, et al. Fulminant encephalopathy due to the catastrophic primary antiphospholipid syndrome. J Neurol Neurosurg Psych 1997;62:300–301
102. Asherson RA. The catastrophic antiphospholipid syndrome. A review of the clinical features, possible pathogenesis and treatment. Lupus 1998;7(Suppl 2):55–62
103. Toubi E, Ben-David J, Kessel A, et al. Autoimmune aberration in sudden sensorineural hearing loss: association with anticardiolipin antibodies. Lupus 1997;6:540–542
104. Naarendorp M, Spiera H. Sudden sensorineural hearing loss in patients with systemic lupus erythemaotsus or lupus-like syndromes and antiphospholipid antibodies. J Rheumatol 1998;25:589–592

105. Carhuapoma JR, Mitsias P, Levine SR. Cerebral venous thrombosis and anticardiolipin antibodies. Neurology 1997;28:2363–2369
106. Ameri A, Bousser MG. Cerebral venous thrombosis. Neurol Clin 1992;10:87–111
107. Montalban J, Arboix A, Staub H, et al. Transient global amnesia and antiphospholipid antibodies. Clin Exp Rheumatol 1989;7:85–87
108. Rafuse PE, Canny CLB. Initial identification of antinuclear antibody-negative systemic lupus erythematosus on ophthalmic examination: a case report with discussion of the ocular significance of anticardiolipin (antiphospholipid) antibodies. Can J Ophthalmol 1992;27:189–193
109. Labutta RJ. Ophthalmic manifestations in the antiphospholipid syndrome. *In:* Asherson RA, Cervera R, Piette JC, Shoenfeld Y, eds. The Antiphospholipid Syndrome. Philadelphia, PA: CRC Press, 1996:213–218
110. Ortel TL, Moll S. Monitoring oral anticoagulant therapy in patients with lupus anticoagulants. Br J Haematol 1998;101(2):390–392
111. Tan EM, Cohen AS, Fries JF et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–1277
112. Molad Y, Sidi Y, Gornish M, et al. Lupus anticoagulant: correlation with magnetic resonance imaging of brain lesions. J Rheumatol 1992;19:556–561
113. Provenzale JM, Heinz ER, Ortel TL, et al. Antiphospholipid antibodies in patients without systemic lupus erythematosus: neuroradiologic findings. Radiology 1994;192:531–537
114. Toubi E, Khamashta MA, Panarra A, Hughes GRV. Association of antiphospholipid antibodies with central nervous system disease in systemic lupus erythematosus. Am J Med 1995;99:397–401
115. Tietjen GE, Day M, Norris L, et al. Role of anticardiolipin antibodies in young persons with migraine and transient focal neurologic events. Neurology 1998;50:1433–1440
116. Schmidt R, Auer-Grumbach P, Fazekas F, et al. Anticardiolipin antibodies in normal subjects. Neuropsychological correlates and MRI findings. Stroke 1995;26:749–754
117. Sailer M, Burchert W, Ehrenheim C, et al. Positron emission tomography and magnetic resonance imaging for cerebral involvement in patients with systemic lupus erythematosus. J Neurol 1997;244:186–193
118. Hachulla E, Michon-Pasturel U, Leys D, et al. Cerebral magnetic imaging in patients with or without antiphospholipid antibodies. Lupus 1998;7:124–131
119. Specker CH, Perniok A, Brauckmann U, et al. Detection of cerebral microemboli in APS—introducing a novel investigation method and implications of analogies with carotid artery disease. Lupus 1998;7:S75–S80
120. Babikian V, Wechsler L. Recent developments in transcranial Doppler sonography. J Neuroimag 1994;4: 159–163
121. Babikian VL, Wijman CA, Hyde C, et al. Cerebral microembolism and early recurrent cerebral or retinal ischemic events. Stroke 1997;28:1314–1318
122. Del Sette M, Angeli S, Stara I, et al. Microembolic signals with serial transcranial Doppler monitoring in acute focal ischemic deficit: a local phenomenon? Stroke 1997;28(7):1311–1313
123. Georgiadis D, Preiss M, Lindner A, et al. Doppler microembolic signals in children with prosthetic cardiac valves. Stroke 1997;28:1328–1329
124. Padayachee TS, Parsons S, Theobold R, et al. The detection of microemboli in the middle cerebral artery during cardiopulmonary bypass: a transcranial Doppler ultrasound investigation using membrane and bubble oxygenators. Ann Thorac Surg 1987;44(3):298–302
125. Spencer MP, Thomas GI, Nicholls SC, Sauvage LR. Detection of middle cerebral artery emboli during carotid artery endarterectomy using transcranial Doppler ultrasonography. Stroke 1990;21:415–423
126. Russell D, Madden KP, Clark WM, et al. Detection of arterial emboli using Doppler ultrasound in rabbits. Stroke 1991;22:253–258
127. Sitzer M, Muller W, Siebler M, et al. Plaque ulceration and lumen thrombus are the main sources of cerebral microemboli in high-grade internal carotid artery stenosis. Stroke 1995;26(7):1231–1233
128. Brey RL, Carolin MK. Detection of cerebral microembolic signals by transcranial Doppler may be a useful part of the equation in determining stroke risk in patients with antiphospholipid antibody syndrome. Lupus 1997;6:621–624
129. D'Olhaberriague L, Levine SR, Saolwich-Palm L, et al. Specificity, isotype and titer distribution of anticardiolipin antibodies in CNS diseases. Neurology 1998;51:1376–1380
130. Abuaf N, Laperche S, Rajoely B, et al. Autoantibodies to phospholipids and to the coagulation proteins in AIDS. Thromb Haemostat 1997;77:856–861
131. Babikian VL, Levine SR. Therapeutic considerations for stroke patients with antiphospholipid antibodies. Stroke 1992;23(Suppl 2):33–37
132. Brey RL, Coull BM. Antiphospholipid antibodies: origin, specificity and mechanism of action. Stroke 1992;23(Suppl 2):15–18
133. Brey RL. Stroke prevention in patients with antiphospholipid antibodies. Lupus 1994;3:299–302
134. Feldmann E, Levine SR. Cerebrovascular disease with antiphospholipid antibodies: immune mechanisms, significance and therapeutic options. Ann Neurol 1995;37(Suppl):S114–S130
135. Brey RL, Escalante A. Neurological manifestations of antiphospholipid syndrome. Lupus 1998;7:567–574
136. Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Ann Intern Med 1990;112:682–698