

Antiphospholipid Antibodies in Medical Practice: A Review

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ABSTRACT

Background: Antiphospholipid antibodies are autoantibodies that have been associated with thrombosis and recurrent foetal loss. The purpose of this review is to highlight the history of these antibodies, their epidemiology, to document what is known of their pathogenesis, clinical features, diagnosis and available treatment modalities.

Methods: Literature on the subject was reviewed using manual library search, articles in journals, internet search and conference abstracts.

Result: Antiphospholipid antibodies have been detected in all age groups; incidence increases with age. They are antibodies to protein-phospholipid complexes and not to phospholipid alone. The most commonly detected antiphospholipid antibodies are lupus anticoagulant (LA), anticardiolipin antibodies and anti- β -2 glycoprotein-1 antibodies.

Apart from thrombosis and recurrent foetal loss, they are also associated with neurologic disorders, cutaneous manifestations and thrombocytopenia.

Conclusion: Early detection requires a strong index of suspicion especially when thrombosis is seen at unusual sites. Several modalities of treatment such as anticoagulants and antiplatelet agents have been developed, though it is difficult to monitor level of anticoagulation as the antibodies may interfere with coagulation studies. Grey areas remain in the management of antiphospholipid antibodies; for instance it is not certain if patients with a positive laboratory test without any clinical feature should be treated.

KEYWORDS: Antiphospholipid antibodies; Review.

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INTRODUCTION

Antiphospholipid antibodies (APLA) are autoantibodies that, recognize various combinations of phospholipids, phospholipid-binding proteins or both and thus prolong the phospholipid-dependent coagulation tests, they interfere with the coagulation reactions which depend on protein phospholipid complexes *in vitro*¹. They could be immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA) or combinations of these isotypes.

The most commonly detected subgroups of APLA are LA antibodies, anticardiolipin antibodies (ACAs) and anti-beta 2 glycoprotein 1 (anti- β -2 GP-1) antibodies

based on the method of detection¹. The presence of APLA is associated with recurrent thrombotic episodes; repeated foetal loss and thrombocytopenia. Cutaneous symptoms and varied neurological disorders can also occur.

Amongst Nigerians, APLA has been found in non-pregnant multiparous women², women with pre-eclampsia³, sickle cell disease (SCD) patients⁴ as well as in the normal population^{3,4}.

HISTORICAL PERSPECTIVES

The first antiphospholipid antibody was detected in patients with syphilis in 1906⁵, and the antigen to which this antibody was formed was later identified to be cardiolipin⁶. The lupus anticoagulant (LA) was first described in 1952⁷. In 1963 it was shown that LA was related to clinical thrombosis⁸. Thiagarajan and co-workers were the first in 1980 to explain the differences between the laboratory and clinical observations⁹, though Feinstein and Rapaport had earlier used the name lupus anticoagulant in 1972. However, most patients with LA do not have systemic lupus erythematosus (SLE).

The triad of LA, recurrent spontaneous abortions and thromboembolism was described in 1980¹⁰, other clinical manifestations of LA have subsequently been described. In 1983, solid phase immunoassay for ACAs was developed and the ACAs detected were strongly associated with LA, false positive venereal disease research laboratory (VDRL) tests and thrombosis¹¹.

The early 1990s led to the discovery of the fact that some ACAs require the presence of the plasma phospholipid binding protein: beta-2 glycoprotein 1 (β -2 GP-1) in order to bind cardiolipin, and subsequently to the discovery that some autoantibodies bind directly to β -2 GP-1 in the absence of phospholipids¹². This resulted in the change of focus from phospholipids to phospholipid binding proteins¹³.

EPIDEMIOLOGY

Antiphospholipid antibodies (APLA) may be found in up to 2% of apparently normal adults; though only about 0.2% have high titres¹⁴. Like other autoantibodies, prevalence increases with age¹⁵. They have been described in children, Von Landenberg et al found APLA in 2% of control children in their study¹⁶. Although isolated LA and anticardiolipin

antibodies (ACAs) in children who are asymptomatic do not lead to clinical complications and are usually transient¹⁷. Primary APLA occurs equally in both sexes while the secondary form is more frequent in females, APLA has been described in all parts of the world¹⁸. In patients with SLE, the prevalence of APLA is much higher, ranging from 12-30 % for anticardiolipin antibodies¹⁹ and 15-34 % for LA^{19,20}. Many patients have laboratory evidence of APLA without clinical consequences¹. In healthy control subjects it is not known what percentage of those with APLA will develop the antiphospholipid syndrome¹.

Since prospective studies have shown an association between APLA and the first thrombotic event such as venous thrombosis, myocardial infarction and recurrent stroke²¹, attempts have been made to identify patients with APLA who are at an increased risk for a thrombotic event¹. The important risk factors found include: history of thrombosis, presence of LA and an elevated level of IgG anticardiolipin antibody, each of which increases the risk of thrombosis up to five times²². Some investigators have demonstrated that there is a seasonal influence on the prevalence of these antibodies in normal healthy populations, with a higher prevalence in the winter than summer months²³. Familial clustering of raised APLA levels and HLA linkages²⁴ indicate that the antibodies may occur in genetically susceptible hosts in response to some antigenic challenge.

APLA has been found in SCD patients by various investigators and the prevalence ranged from 8-68%^{25,26}. A study in Benin found LA in 11.4% of adult homozygous SCD patients⁴, it has also been described in patients with -thalassaemia.

LA was present in 8% of normal non-pregnant multiparous women in a study carried out in Benin-City, Nigeria², it was also present in 15.4% of Nigerian women with pre-eclampsia compared with 2% among apparently healthy pregnant Nigerian women³.

PATHOGENESIS

The genesis of antiphospholipid antibodies (APLA) is not yet established, though the disorder is considered to be autoimmune²⁷. Pathogenetic mechanisms involved are largely unknown, recent data suggest that lupus anticoagulant and ACA are antibodies to protein-phospholipid complexes rather than to phospholipids as had originally been thought and that other protein phospholipid complexes not recognized by standard assays for LA or ACAs may also exist in patients with the antiphospholipid syndrome (APS)²⁸.

APLA generated in response to infections generally recognize phospholipids epitopes directly (no cofactor

required) while those generated in patients with the APS recognize epitopes on phospholipid-binding proteins, which is primarily β -2 GP-1, and are thus cofactor dependent²⁹.

Several hypotheses have been proposed to explain the cellular and molecular mechanisms by which APLA promote thrombosis. These include the suggestion that APLA interferes with or modulates the function of phospholipid binding proteins involved in the regulation of coagulation such as β -2 GP-1¹.

β -2 GP-1 is a glycosylated single chain plasma protein composed of 326 amino acids with a molecular mass of 50 kDa, it is the major cofactor for the recognition of anionic phospholipids by APLA³⁰. It is a member of the short consensus repeat (SCR) superfamily. It has been shown that APLA can recognize β -2 GP-1 directly in the absence of phospholipids³¹.

Probably most APLA recognize domain 1 of β -2 GP-1 which binds to phospholipids via the cationic portion of its fifth SCR domain and APLA binding to domains I and II promotes the increased binding of the protein to membrane phospholipids³². β -2 GP-1 has been suggested to play a scavenging role for exposed anionic phospholipid after apoptosis³³. Binding of β -2 GP-1 to endothelium is mediated by annexin II, which also serves as a receptor for plasminogen and tissue plasminogen activator³⁴. Again it is possible that the antibodies are an effect and not a cause of thrombosis²⁷. Additional cofactors and antigenic targets that have been identified include: coagulation factors II and V, proteins C and S, annexin-V, high molecular weight kinninogen and low molecular weight kinninogen³⁵.

The oxidation of phospholipids may be necessary for APLA recognition³⁶, thus some affinity purified cardiolipin binding antibodies in sera from patients with SLE have been shown to cross react with oxidized low density lipoprotein (LDL)³⁷.

Disruption of Annexin-v Anticoagulant Shield.

The annexin family of proteins was first recognized in 1990³⁸, most of them have four domains each domain consists of about 70 amino acids²⁷. Annexin-V has a high affinity for anionic phospholipids and is able to displace coagulation factors from phospholipid surfaces; these features are responsible for its potent anticoagulant activity in vitro. Annexin-V is shaped like a concave disc with the phospholipid and calcium binding domains present on the convex surface, and clusters on exposed membrane phospholipids³⁹ on which it forms 2-dimensional crystalline arrays⁴⁰. This crystallization forms a lattice of annexin-V over the phospholipids surface, blocking its availability for

coagulation reactions²³.

Annexin-V is expressed by placental trophoblasts being abundant on the apical surfaces of syncytiotrophoblasts. Experiments with pregnant mice have shown that infusion of polyclonal anti-annexin-V antibody will result in placental infarction and pregnancy wastage, showing that annexin-V is necessary for placental integrity⁴¹.

Annexin-V is expressed on cultured human umbilical vein endothelial cells⁴² and treatment of these cells with a chelator or with polyclonal antihuman annexin-V antibody resulted in the acceleration of the coagulation of plasma exposed to these cells⁴³. In the placentas of pre-eclamptic women annexin-V expression is decreased on their trophoblasts⁴⁴.

Available data supports the hypothesis that annexin-V has a thrombomodulatory function on the surfaces that line the intervillous space through which maternal blood circulates and the expression of annexin-V by endothelial cells indicates that it may play a similar role at the vascular blood interface of systemic circulation by shielding anionic phospholipids from participating in coagulation reactions. Since both APLA and annexin-V have affinity for anionic phospholipids it was hypothesized that APLA might interfere with the formation of the antithrombotic annexin-V shield over phospholipids on apical cytoplasmic membranes²⁷. Rand *et al* found that IgG fractions from patients with the APS reduce the quantity of annexin-V on cultured trophoblasts and endothelial cells and that APLA IgG also accelerate the coagulation of plasma that is incubated with these cells after their exposure to the antibodies⁴³.

The reduction of annexin-V on the surfaces of placental trophoblasts and vascular endothelial cells, which come in contact with flowing blood, may provide a thrombogenic mechanism²⁷. Inhibition of the tenase (Xase) and prothrombinase complex assembly on the phospholipid surface by APLA is responsible for the LA effect⁴⁵, in the absence of significant levels of annexin-V, so high affinity antibody-cofactor complexes reduce the amount of phospholipid available for coagulation reactions and will indicate an apparent anticoagulant effect. On the other hand, when annexin V is present in the system, APLA accelerates coagulation by disrupting the structure of annexin-V shield, increasing the availability of phospholipids for coagulation reaction²⁷.

Another hypothesis involves the activation of endothelial cells with up regulation of the expression of adhesion molecules, secretion of cytokines and metabolism of prostacyclins, APLA have been found to recognize, and /or activate cultured vascular endothelial cells⁴⁶. Cultured endothelial cells incubated with APLA express increased levels of cell adhesion molecules⁴⁵, an effect mediated by β -2 GP-1⁴⁷ and may increase the

Adhesion of leucocytes to the vascular wall and promote inflammation and thrombosis²⁷. It has also been demonstrated that incubation of cultured endothelial cells with APLA results in the increased expression of tissue factor⁴⁸ and that a subset of APLA that recognizes annexin-V induces apoptosis in endothelial cells⁴⁹. While LA have been shown to stimulate the release of micro particles and possible prothrombotic activity from endothelial cells⁴⁹.

In addition to the expression of tissue factor by cultured endothelial cells, APLA also promotes tissue factor synthesis by leucocytes⁵⁰. APLA may also increase tissue factor activity and generate activated factor X (Xa) by antibody mediated inhibition of tissue factor pathway inhibitor activity⁵¹.

They may also act via oxidant-mediated injury of the vascular endothelium.

Oxidized low-density lipoprotein (LDL) is taken up by macrophages leading to their activation and subsequent damage to endothelial cells⁵². Autoantibodies to oxidized LDL occur in association with anticardiolipin antibodies and some APLA may show cross-reactivity against oxidized LDL³⁶ and may thus be associated with increased risk of atherosclerosis⁵³.

APLA can interfere with the protein C pathway⁵⁴, in addition, patients with antiphospholipid syndrome frequently have protein S deficiency⁵⁵. Also it has been shown that LA positive children who present with haemorrhage are more likely to have an acquired deficiency of prothrombin⁵⁵.

Lastly, thrombosis in the antiphospholipid syndrome has been likened to that in heparin induced thrombocytopenia⁵⁶. Both syndromes induce thrombosis in multiple arterial and venous beds⁵⁶ and vascular injury may be necessary for thrombosis to occur in both syndromes¹. It has been shown that some APLA cross-react with heparin and heparinoid molecules and inhibit the acceleration of anti-thrombin III activity⁵⁷.

The absence of anionic phospholipids on the cell surface and lack of reactivity of APLA with intact cells suggests that perturbations of the cell membrane may be required for APLA to bind to cells¹. Some APLA react with activated platelets⁵³ and apoptotic cells⁵⁸ which have undergone a loss of normal asymmetric distribution of membrane phospholipids and expose anionic phospholipids on their cell surface. Binding of APLA to apoptotic cells is dependent on β -2 GP-1⁵⁸ as is induction of APLA by apoptotic cells.

ANTIPHOSPHOLIPID ANTIBODY SYNDROME

The antiphospholipid antibody syndrome (APS) is

an acquired autoimmune disorder of unknown aetiology in which patients present with vascular thrombosis or recurrent pregnancy losses along with laboratory evidence for antibodies against phospholipids or phospholipid binding protein cofactors in their blood²⁷. The term was first coined to denote the clinical association between APLA and a syndrome of hypercoagulability.

Recently, a consensus statement provided simplified criteria for the diagnosis of the APS. A patient must meet at least one of two clinical criteria, which are: vascular thrombosis or complications of pregnancy, and at least one of two laboratory criteria¹: demonstration of the presence of LA or ACA. None of the other laboratory manifestations of the APS such as thrombocytopenia are included in the clinical criteria⁵⁹.

APS can be divided into two categories. Primary APS occurs in patients without clinical evidence of other autoimmune disease, while secondary APS occurs in association with autoimmune or other diseases¹.

Clinical Features

There are no major differences in the clinical consequences of APLA between patients with primary or secondary APS⁵⁹ and any organ in the body can be involved¹.

Venous thrombosis is the most common manifestation of the APS while arterial thrombosis is less common⁵⁹ and most commonly manifest as ischaemia or infarction.

Thrombotic episodes associated with APLA may occur in vascular beds that are infrequently affected by prothrombotic states¹.

Organ involvement in patients with the APS can present in a spectrum from rapidly progressive to clinically silent and indolent. The central nervous system may be involved with arterial occlusion of larger veins leading to stroke and transient ischaemic attacks. Varied neurological disorders such as dementia, epilepsy, chorea and migraine headaches⁶⁰ have been described. Thrombosis of the inferior vena cava and hepatic veins may lead to the Budd-Chiari syndrome⁶¹. The lungs and heart may also be involved leading to pulmonary embolism, pulmonary hypertension⁶¹, and myocardial infarction especially in the young⁶².

Other prominent manifestations of the syndrome include thrombocytopenia, cutaneous symptoms include leg ulcers, livedo reticularis, widespread cutaneous necrosis and distal cutaneous ischaemia⁶³ and haemolytic anaemia⁵⁹. In patients with renal involvement, hypertension is almost invariably present⁶⁴. Positive results of a direct Coomb's test and thrombocytopenia may be included as laboratory

manifestations of this syndrome⁶⁵. The clinical course of the secondary syndrome is independent of the activity and severity of SLE but the presence of the APS worsens the prognosis of patients with lupus⁶⁶. Children who present with thrombosis and LA are found to have underlying disorders similar to those of adults⁶⁷.

The main pathologic lesion has been found to be a non-inflammatory thrombotic occlusion of small or large vessels; arterial or venous and occasionally both. The loss of blood supply to the brain, skin, heart or other organs or to the placenta due to arterial or venous thrombosis explains the clinical symptoms of the APS⁶⁸.

Women with APLA have a higher proportion of pregnancy losses within the foetal period when compared with unselected women⁶⁹ and their pregnancies can also be complicated by premature delivery due to pregnancy associated hypertension and uteroplacental insufficiency⁶⁹. Localized placental thrombosis may occur as a result of interference with trophoblastic annexin-V⁴³.

LABORATORY DETECTION OF CLINICALLY RELEVANT ANTIPHOSPHOLIPID ANTIBODIES

Based on method of detection, the most common APLA are LA, ACAs and anti β -2 GP-1 antibodies¹. LA antibodies are identified by coagulation assays, in which they prolong clotting times while anticardiolipin and anti β -2 GP-1 antibodies are detected by immunoassays that measure immunologic reactivity to a phospholipid or a phospholipid-binding protein. In spite of the fact that there is often concordance between LA antibodies and either anticardiolipin⁷⁰ or anti β -2 GP-1⁷¹ antibodies, they are not identical. Generally, LA antibodies are more specific for the APS, while ACA are more sensitive⁷². There is however, no definite association between specific clinical manifestations and particular subgroups of APLA.

Despite the name, LA antibodies are associated with thromboembolic events rather than clinical bleeding¹. APLA can interfere with both anticoagulant and procoagulant mechanisms. Though the phospholipid surface used in most in-vitro coagulation assays favours inhibition of procoagulant pathways and thus prolongation of clotting, the microenvironment of cell membranes in vivo may promote greater inhibition of anticoagulant pathways and therefore may favour thrombosis⁷². Current criteria for detection of LA antibodies require prolongation of at least one phospholipid dependent coagulation assay¹.

The method of detection of APLA has remained a source of much debate and disagreement. Several

screening methods have been described for the detection of LA.

They include activated partial thromboplastin time (APTT), kaolin clotting time (KCT), dilute Russell's viper venom time (DRVVT), tissue thromboplastin inhibition test (TTI), APTT correction ratio⁷³ and platelet neutralization test. The KCT is simple, sensitive to the presence of LA⁷⁴, with high specificity⁷⁵ and it is affordable. Specific immunological assays for APLA are also available; these are based on either radioimmuno-assay (RIA) or enzyme linked immunosorbent assay (ELISA)¹⁵.

KCT has been shown to have a specificity of up to 93% for LA⁷⁵ and it is able to detect LA at a much greater dilution in normal plasma than TTI or DRVVT⁷⁶. KCT is also more sensitive to the presence of LA than TTI⁷⁷. The ELISA method was found to be comparable to KCT in its ability to detect high dilutions of LA⁷⁶. KCT has been automated, the result of which are comparable with the manual method, automation provides a quick, inexpensive way of screening patients for LA⁷⁸.

TREATMENT

Patients with persistently positive test for LA or ACA and who have a past history of thrombosis are at an increased risk for recurrence of about 50% over a 5-year period¹⁵.

While attempts are being made to formulate appropriate treatment modalities, it is generally agreed that in secondary APLA, the underlying disease should be treated where possible.

Modalities of treatment currently available range from immunosuppression with steroids, high dose intravenous immunoglobulin and use of antiplatelet drugs to anticoagulant drugs and plasmapheresis. However, Optimal treatment of these patients is unclear¹⁹, treatment decisions fall into these main areas¹:

Prophylaxis

It has been shown that aspirin at a dose of 75mg daily may provide protection against thrombosis in women with the APS and previous pregnancy loss^{19,79}

Hydroxychloroquine used alone may be protective against thrombosis in patients with SLE and secondary APS⁸⁰. Factors predisposing to thrombosis should, if possible, be eliminated.

Treatment After a Thrombotic Event:

A beneficial role for anticoagulation in decreasing the rate of recurrent thrombosis has been shown in retrospective studies^{19,81}. It has also been shown that intermediate intensity {International normalized ratio (INR) 2.0-2.9} and high intensity (INR 3.0 or more) warfarin therapy significantly reduced the rate of recurrent thrombosis while low-intensity treatment (INR

1.9 or less) did not confer significant protection⁸¹.

Treatment with warfarin should be long term or probably life long¹, since among patients whose anticoagulant therapy was stopped, the rate of recurrence was 50 percent at two years and 78 percent at eight years⁸¹. Aspirin alone was ineffective in reducing the rate of recurrent thrombosis¹⁹. Treatment with high intensity warfarin (producing an INR of >3) +/- low dose aspirin (75mg/day) was significantly more effective than treatment with low intensity warfarin (INR <3) +/- low dose aspirin or treatment with aspirin alone in preventing further thrombotic events, it was thus concluded that the risk of recurrent thrombosis in patient with APLA is high and long term anticoagulant treatment with INR >3 is advisable in these patients⁸².

Patients with acute thrombotic microangiopathy (which usually affects small vessels of multiple organs with the kidney being most frequently affected); have been treated with a combination of anticoagulants, steroids and either plasmapheresis or intravenous immune globulin⁸³. Intravenous immunoglobulin was shown by some investigators to result in transient suppression of LA but not ACAs⁸⁴, however, a randomised controlled study found it to be of no benefit when compared with heparin and aspirin in reducing adverse obstetrical outcomes in women with APLA⁸⁵.

Treatment of pregnancy in association with antiphospholipid antibodies:

It has been shown by investigators in prospective trials that intravenous high dose low molecular weight heparin plus low dose aspirin is more effective than aspirin alone for achieving live births among these women^{81,83,86,87}. Combinations of aspirin and heparin have been shown to improve the outcome of pregnancy in women who have had at least two foetal losses^{69,88}. However, the dose and duration of treatment with heparin remains a subject of much debate.

CONCLUSION

Antiphospholipid antibodies (APLA) have been described by several investigators in Nigeria like in other parts of the world. Since APLA can masquerade as other diseases ranging from neurological impairment to acute abdomen, it is necessary that for early diagnosis doctors maintain a strong index of suspicion, especially when thrombosis is seen at unusual sites in younger patients, in women with repeated foetal loss and patients with unexplained thrombocytopenia.

In spite of the fact that the more sophisticated laboratory tests may not be widely available in a developing country like ours, several assay methods

such as the KCT which is relatively inexpensive have been shown to be sensitive and specific in demonstrating the presence of LA if properly carried out^{74,75}, the APTT correction ratio is another method that has been used⁷³. ELISA its are also available for detection of ACAs though they are often quite expensive.

The role of APLA as a risk factor in arterial and venous thrombosis in primary and secondary APS have been demonstrated. However, no specific APLA has been found to be the most important. For this reason, multiple assays are necessary to identify patients at risk .It is noteworthy that it is difficult to monitor the level of anticoagulation in these patients as the APLA may interfere with coagulation studies.

Grey areas still remain in the management of APLA because appropriate treatment for patients with a past history of just one foetal loss or only thrombocytopaenia is yet to be determined. It is also not yet certain whether there should be a different approach in patients with primary or secondary syndromes, and if patients who demonstrate the presence of APLA alone without any other symptom should be offered treatment and if so, which form of treatment . The same question may be asked about women who are positive for APLA without any prior history of foetal loss who want to get pregnant. A lot of research is required in the future to answer these questions and others that may arise.

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