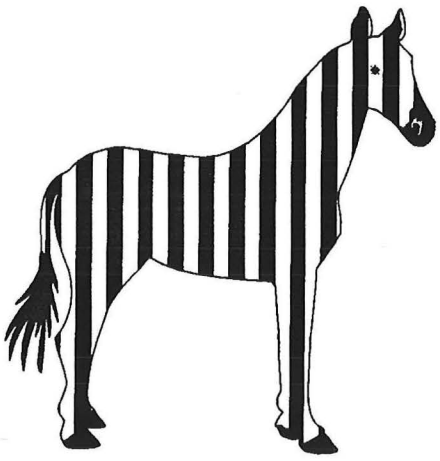
Anti-Phospholipid Antibody Syndrome

Medical Zebra:

Or, Just The Stripes



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Internal Medicine
Grand Rounds
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Introduction

Case Presentation

A 17 y/o Hispanic woman was admitted to PMH with complaints of leg swelling and dyspnea. One month prior to admission, she had fallen and sprained her left ankle, and the leg was put in a splint. Soon thereafter she noted lower extremity swelling, followed by the onset of sharp substernal chest pain that was pleuritic in nature accompanied by dyspnea. There was no cough or hemoptysis. These symptoms waxed and waned until she sought medical care while visiting relatives in California. The doctor there suggested she obtain care at home and she rode for three days in a car to Dallas.

In the PMH ER, she was afebrile with tachycardia and tachypnea. The left calf was 4 cm larger that the right. Ultrasonography revealed a left common femoral vein thrombosis; the chest radiograph showed bilateral basilar infiltrates. A ventilation/perfusion scan was interpreted as high probability for pulmonary embolism. There were no other signs or symptoms of SLE, and no family history of thrombosis or autoimmune diseases. Of note, the patient had recently been put on oral contraceptive pills for irregular menses.

Admitting laboratory data showed the patient to be mildly hypoxemic (pO2 of 71, 95% saturation), anemic (Hct 33), and thrombocytopenic (Plt 111,000). The initial aPTT was 53 sec. The VDRL and RPR were positive; but MHA-TP was negative. She was anticoagulated with heparin. She developed recurrent chest pain with a drop in pO2. Because of the fear of recurrent pulmonary embolism while on heparin, a Greenfield filter was placed in the inferior vena cava. The venogram obtained at that time showed clot in the vena cava, but patent renal veins.

Subsequent evaluation revealed ESR >130, ANA 1:5120 (speckled), anti-DNA was absent, but anti-Ro and anti-La were present. The Russell's viper venom time was prolonged without correction, and the platelet neutralization procedure was positive. IgG anticardiolipins were 8.9, IgM - 10.8, and IgA 8.9 (low positive values). The direct anti-globulin test was positive. There were 9 grams of protein per 24 hr in the urine. C3 and C4 were normal. The patient developed a sterile, exudative, left pleural effusion that resolved with NSAIDS. She was converted to warfarin and discharged from the hospital with an INR of 2.0.

What is this patient's diagnosis and why did she develop thrombosis? How should she be managed now? What if she wishes to become pregnant? This Grand Rounds will attempt to answer these question with a review of the antiphospholipid syndrome.

Historical Aspects

In 1940, Keil documented the occurrence of false-positive syphilis serology in ten patients with SLE when an extract of cardiac muscle (cardiolipin) was used as the antigen (1). With the development of more specific serology using trepo-

nemal antigens, Moore and Lutz confirmed in 1955 the association of chronic false-positive tests with patients having SLE or other autoimmune disorders (2).

At about the same time, it was recognized that many patients with SLE have a circulating inhibitor of *in vitro* coagulation, and the name 'lupus anticoagulant' was applied. In 1963, Bowie noted the increased incidence of thrombosis in these patients (3). In 1983, a solid-phase assay was developed that could measure antibodies to cardiolipin (4), as well as other phospholipids. Such antibodies were found more frequently and at higher titer in patients with autoimmune disorders and recurrent, unexplained thrombosis.

The term antiphospholipid syndrome has now been applied to these patients. In the past decade, there has been an extraordinary amount of study of antiphospholipid syndrome. Many retrospective and observational studies have been performed. Some of the antibodies associated with antiphospholipid syndrome have been identified and their pathogenicity confirmed in animal models. Currently, investigators are prospectively analyzing patients with antiphospholipid syndrome to develop more specific diagnostic studies, elucidate pathogenic mechanisms *in vivo*, and improve therapy.

Definition

There is currently no consensus or 'workshop' definition of antiphospholipid syndrome like there is for SLE or rheumatoid arthritis. A working definition was proposed by Harris in 1987 (5):

Criteria for the Antiphospholipid Syndrome

Clinical	Serology
Venous thrombosis	IgG anticardiolipin antibody (>20 GPL units)
Arterial thrombosis	Positive lupus anticoagulant test*
Recurrent Fetal Loss	IgM anticardiolipin antibody (>20 MPL units) and lupus anticoagulant
Thrombocytopenia	

Patients with antiphospholipid syndrome should have at least one clinical and one serological feature at some time in their disease. The assay for antiphospholipids should be present on at least two occasions greater than 8 weeks apart. As with all definitions of this type, these criteria should be used as guidelines to classify patients, not to dictate therapy.

Prevalence of Antiphospholipid Syndrome

Antiphospholipid syndrome can occur in the absence of other autoimmune disorders. There is no good estimate of the prevalence of this "primary antiphospholipid syndrome," although hundreds of patients have been described in retrospective analyses. The other major category of antiphospholipid syndrome, that associated with SLE is easier to quantify. 20-50% of SLE patients have significantly elevated titers of antiphospholipid antibodies. Approximately on-third of these patients have a history of thrombosis or recurrent fetal loss. Up to 7.5% of the normal population will have measurable anticardiolipins by solid-phase assays. In a study of 1,449 pregnant women who carried to term, low titer IgG antibodies were found in

1.7% and IgM in 4.3%. However, populations selected for vascular events have much higher frequencies of these antibodies. 10-30% of women with recurrent spontaneous abortion have evidence of antiphospholipids. Up to 19% of patients with idiopathic venous or arterial thromboses have antiphospholipids, as well as 45% of patients under the age of 50 with cerebral vascular accidents. In this type of analysis, however, the questions of selection bias and whether antiphospholipids are the cause or effect of vasculopathy. Some of these concerns are addressed below.

Laboratory Tests in the Antiphospholipid Syndrome

There are three general categories of laboratory tests that have been used to diagnose patients with antiphospholipid syndrome. They are: biological false-positive serological tests for syphilis (BFP-STS), functional assays for lupus anticoagulant, and immunological assays for the presence of antibodies to phospholipids. Although related, these laboratory tests are not redundant. That is, an individual patient may have an abnormal value in one test, but not another. Therefore, a thorough, yet well-reasoned approach must be undertaken in every patient considered for the diagnosis of antiphospholipid syndrome.

Biological False-Positive Serological Test for Syphilis

Although the false-positive test for syphilis was historically the first laboratory abnormality found in patients with antiphospholipid syndrome, and is often the first clue to the diagnosis based on routine VDRL or RPR screening, it lacks specificity and sensitivity. Several studies in the early 1950's documented that patients with a false-positive test for syphilis were at increased risk of developing lupus or another connective tissue disorder. In these patients, an increased risk of thrombosis or fetal loss was not appreciated.

The association of BFP-STS with antiphospholipid antibodies stems form the fact the test antigen used in the VDRL and other serologic screening tests contains cardiolipin, an acidic phospholipid found in alcohol extracts of bovine heart. In the VDRL, cardiolipin is mixed with lecithin and cholesterol. Sera from individuals with positive tests, but with no evidence of infection are tested against authentic treponemal antigens. Those patients with repeatedly false-positive tests (>6 months) were often found to have SLE, rheumatoid arthritis or Sjögren's syndrome.

Comparison of SLE and Syphilis Anticardiolipins

	SLE	Syphilis
Usual titer	>1:100	<1:10
Isotype	IgG>IgM	IgM>IgG
Avidity	High	Low
Preferred antigen	cardiolipin	cardiolipin-cholesterol-lecithin
IgG subclass	All	IgG1 and IgG3
Light chain	λ	κ
Anticoagulant activity	Yes	No

Modified from (6)

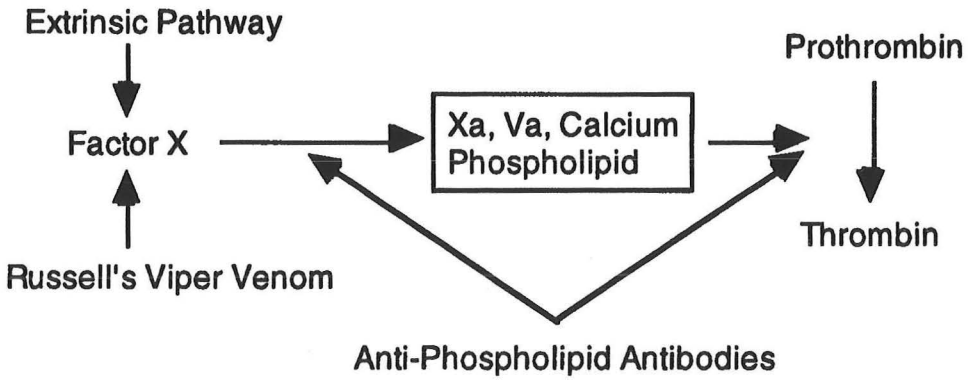
There are certain differences between the anticardiolipin antibodies that are found in syphilis and those in antiphospholipid syndrome associated with SLE. They include titer, isotype, antigenic specificity, and ability to act as a lupus anticoagulant.

One of the most interesting differences between anticardiolipins found in syphilis and in antiphospholipid syndrome is the effect of β2 glycoprotein I. The binding of antibodies to cardiolipins in syphilis is decreased by this serum protein. In contrast, it is a cofactor for the binding of antibodies from patients with antiphospholipid syndrome to cardiolipin. The role of β2 glycoprotein I in antiphospholipid syndrome is discussed in detail below.

The BFP-STS lacks of both sensitivity and specificity. Only 5 - 19% of such patients have SLE or other autoimmune disease (2). It can occur transiently in a variety of infectious diseases, including other spirochetal infections, tuberculosis, and viral hepatitis. Finally, the fact that the VDRL does not correlate with pathogenic antiphospholipid antibodies makes it an unnecessary test in light of the availability of more appropriate studies.

Lupus Anticoagulants

The lupus anticoagulant is an immunoglobulin that interferes with the phospholipid-dependent assays of blood coagulation. Ironically, the presence of these antibodies are associated clinically with thromboses. In addition, many patients with the lupus anticoagulant do not have a classical autoimmune syndrome. The name has stayed for historical reasons. Current recommendations (7) suggest that the diagnosis of a lupus anticoagulant be based on: 1) An abnormality in a phospholipid-dependent coagulation test, 2) Demonstration that the defect is due to an inhibitor and not a factor deficiency, and 3) Proof that the inhibitor is directed toward phospholipid.



***In vitro* action of antiphospholipid antibodies.**

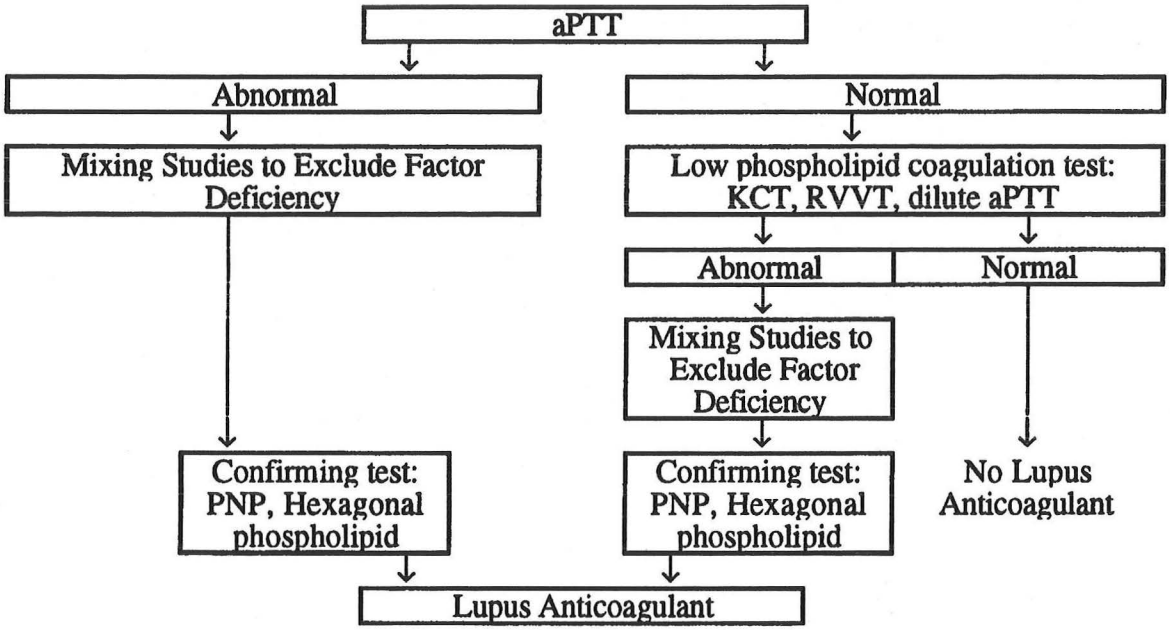
Recognition of a lupus anticoagulant requires an understanding of how blood coagulation is measured. The intrinsic and extrinsic coagulation pathways converge at the assembly of a "prothrombinase" complex that is comprised of factors Va and Xa in association with calcium on a phospholipid substrate such as platelet or endothelial cell membranes. Of the standard tests for blood clotting, the activated partial thromboplastin time (aPTT) is the most dependent on access of the enzymes to phospholipid. In the assay, platelet-poor plasma is mixed with

calcium, a standardized platelet substitute (partial thromboplastin), and an activator such as kaolin. After suitable mixing, the time to clot formation is measured. Certain antiphspholipid antibodies interfere with the formation of the prothrombinase complex and thus prolong the aPTT. Mixing the test plasma with normal plasma will not correct the defect as the inhibitor is still present. This will distinguish an lupus anticoagulant from a factor deficiency.

Although a suitable screening test, the aPTT will not detect weak lupus anticoagulants. The most common secondary test for a lupus coagulant is the diluted Russell's viper venom time (dRVVT). The venom of *vipera Russelli* contains an enzyme that act directly activates factor X, thus by-passing both the intrinsic and extrinsic clotting cascades. The sensitivity of the procedure is enhanced by decreasing the phospholipid present. In the test, diluted venom and platelet substitute (phospholipid) are added to test and control plasma. Calcium is added to clot formation is measured. Typically a ratio of the test and normal plasma times is given, with a value greater than 1.1 indicative of an inhibitor. This will not correct with the addition of normal plasma, however, the addition of either freeze-thawed platelets (platelet neutralization procedure) or purified hexagonal phase phospholipid will shorten the RVVT due to adsorption of antiphospholipid antibodies. Genetic deficiencies of factors V, X, prothrombin, or fibrinogen will also cause an abnormal dRVVT which is corrected by the addition of normal plasma.

The other secondary screening test sometimes used to detect lupus anticoagulants is the kaolin clotting test. This test has been said to be the most sensitive test in pregnancy. In this test, plasma is centrifuged twice and ultrafiltered to remove all traces of platelets or platelet membranes and mixed with kaolin and calcium. The time to clot is measured. Tests performed with different ratios of normal and test plasma can reveal combinations of both lupus anticoagulant and factor deficiency.

An algorithm for the detection of a lupus anticoagulant is given below:

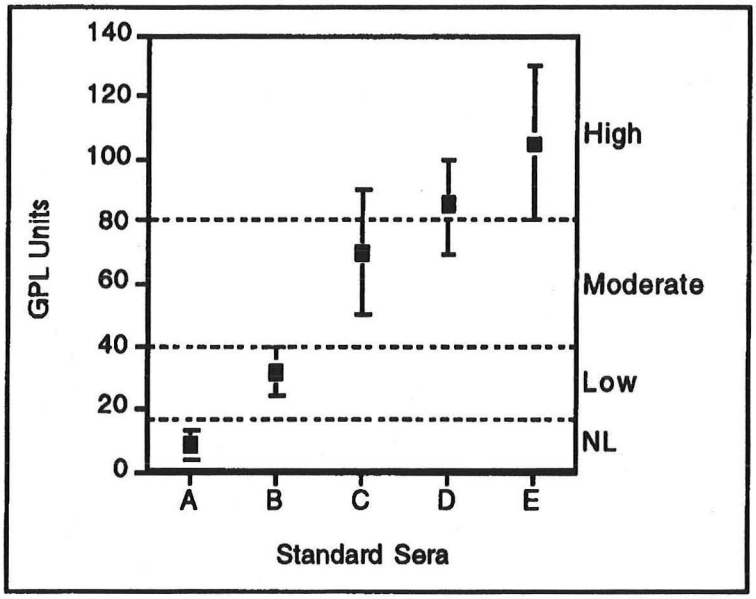


Algorithm for Detection of a Lupus Anticoagulant. Although relatively insensitive, the initial screening test is usually the activated partial thromboplastin test (aPTT). If this is abnormal, mixing studies will confirm the presence of an inhibitor of coagulation. If normal, more sensitive assays (dilute Russell's viper venom time, Kaolin clotting time, or diluted aPTT can be used. The identity of the inhibitor as an antiphospholipid antibody can be assessed by neutralizing it with platelet membranes or purified phospholipids.

Immunologic Tests for Antiphospholipid Antibodies

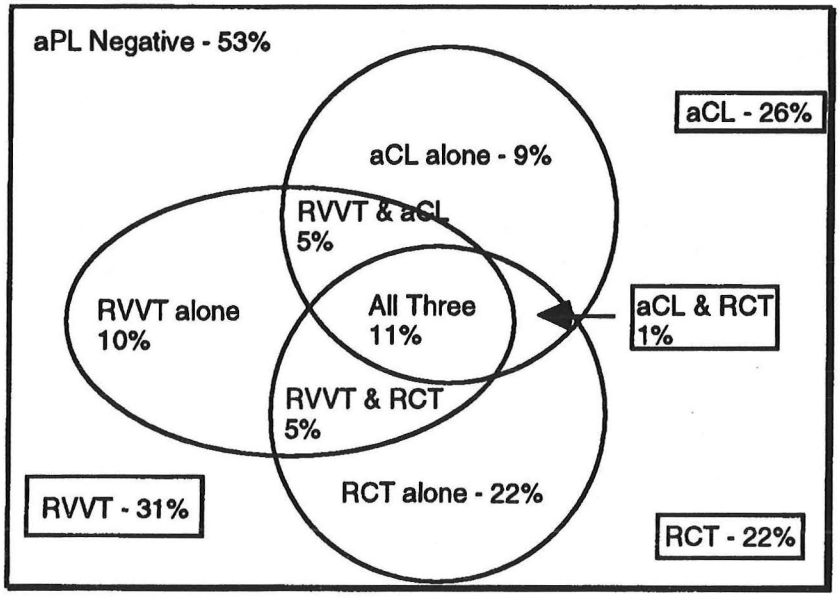
While the presence of a lupus anticoagulant is functional evidence for antiphospholipid antibodies, direct measurement of these proteins can be done by ELISA, or less commonly RIA. Phospholipids are coated on the surface of microtiter wells. The antigen used is often cardiolipin, but can be pure phospholipids such as phosphatidyl serine, phosphatidyl ethanolamine, or mixtures of phospholipids. Diluted test sera are added, and the bound antibodies quantified spectrophotometrically. Sera that test positively are usually assayed for the fraction of IgG, IgM, and IgA antiphospholipid. Since antiphospholipid titers are not normally distributed, they are expressed relative to reference preparations. These are reported in GPL (IgG phospholipid units), MPL, and APL. In this semiquantitative scheme negative or low titers are 0 - 16 GPL; intermediate 17 - 40 GPL; low positive 41 - 80 GPL; high positive >80 GPL. IgM titers are considered low, 0 - 10 MPL; medium, 11 - 40 MPL; or high, >41 MPL. Other measures, such as "multiples of the mean" have also been used. Titers of antiphospholipid determined by ELISA do not correlate with arterial or venous thrombosis. High titers of antiphospholipid, particularly the IgG isotype, are associated with pregnancy loss. The clinical utility of titers of antibodies to specific phospholipids is not clear, although experimental animal models have demonstrated that antiphosphatidyl serine alone cause fetal wastage.

The precise values of serial antiphospholipid titers may not be clinically meaningful due to the semi-quantitative nature of the determination. However, titers tend to remain within sub-groups for stable patients. Sera from five patients representing different levels of IgG antiphospholipid antibodies were tested ten separate times over one year (8). There is less than 10% variation in the test for each sample on a single day, although there is considerable day-to-day variation. Therefore, modest fluctuations in antiphospholipid titer should not be the basis for changing therapy.



Variation in Antiphospholipid Antibody Titer Over Time Means ± std. dev. of IgG antiphospholipids are plotted.

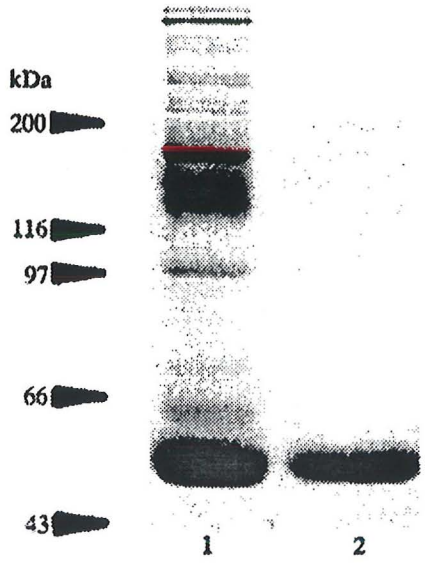
Different Tests for Antiphospholipids May Identify Separate Groups of Patients



An individual patient with antiphospholipid syndrome may be detected by one or more of the different tests for the antibodies. The antibodies responsible for the lupus anticoagulant phenomenon may or may not be the same as a particular patient's antiphospholipids detected by ELISA. An example of this is a prospective study done on a cohort of 227 lupus patients followed in the Johns Hopkins Rheumatology Clinics (9) Each time the patients were seen over a five year period, three tests for antiphospholipids were obtained: Russell's viper venom time (RVVT), the recalcified clotting time (RCT), and an anticardiolipin ELISA. Over half the patients never had an positive test for antiphospholipid. Only eleven percent of the patients were positive for all three tests at any time during the five years. Other sub-sets of patients were identified by one or more of the evaluations. This study supports the hypothesis that different antibodies are responsible for different features of the antiphospholipid syndrome. In addition, it underscores the need for comprehensive testing when the clinician believes that a patient has antiphospholipid syndrome.

The Role of β2 Glycoprotein I

In 1990, two groups showed that the binding of highly purified anticardiolipin antibodies to cardiolipin required a serum co-factor (10, 11). ELISA assays done with purified antibody in the absence of human or bovine serum were negative. The co-factor was purified and identified as β2 glycoprotein I (apolipoprotein H) (10).



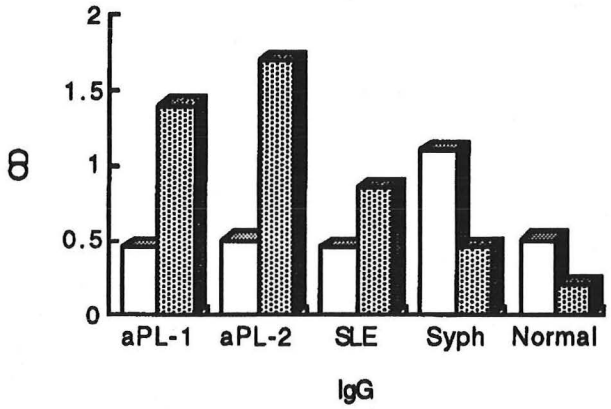
Isolation of β2 glycoprotein I in association with anticardiolipin. Serum from a patient with antiphospholipid syndrome was passed over a cardiolipin-Sepharose column. The eleuant from this column (lane 1 contains IgG anticardiolipin at ~150 kDa and β2-glycoprotein I at ~50 kDa. Further fractionation by gel filtration and ion-exchange chromatography yields pure β2-glycoprotein I (lane 2). Adapted from (10).

This protein has a molecular mass of 50 kDa, and binds to lipoproteins, anionic phospholipids, platelets, DNA, and mitochondria. Its physiologic role is unclear. It does inhibit the intrinsic pathway of blood coagulation and ADP-dependent platelet aggregation.

Several studies have demonstrated that a large fraction of the anticardiolipin antibodies isolated from patients with antiphospholipid syndrome will not bind to either purified phospholipid or β2 glycoprotein I alone in the fluid phase, but will react with a mixture of the two (12, 13). This raises the possibility that the true antigenic epitope is cryptic on either phospholipid or β2 glycoprotein I, and is revealed in the complex, or that the epitope is formed by both factors. There is evidence that β2 glycoprotein I could be the sole antigen of some

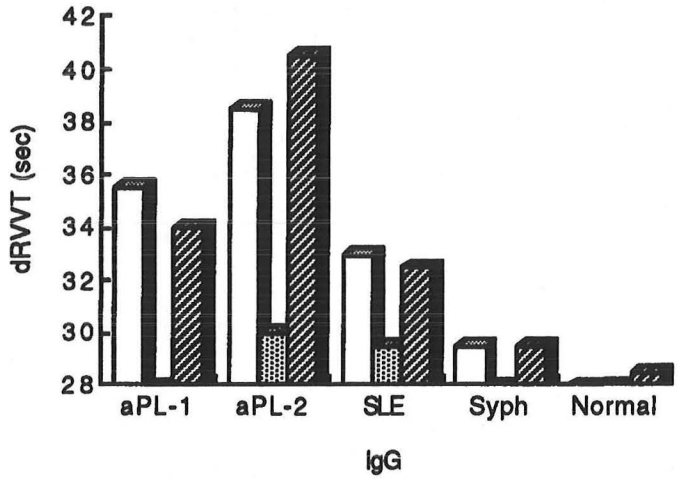
antiphospholipid antibodies. Sera from four patients with antiphospholipid syndrome bound to β2 glycoprotein I immobilized on plastic. The binding was not diminished by the presence of soluble β2 glycoprotein I (14). In another study, the binding of anticardiolipin antibodies from patients to plastic immobilized β2 glycoprotein I was shown to be increased by irradiating the plastic, thereby increasing the oxygen content in the plastic (15). The authors concluded that the epitope recognized by these anticardiolipins is expressed by a conformational change that occurs when β2 glycoprotein I contacts the oxygen-substituted surface. This conformational change may also occur when β2 glycoprotein I contacts phospholipids *in vivo*.

β2 glycoprotein I has also been shown to affect the functional activity of antiphospholipids purified from patients (12). IgG was isolated from two patients with primary antiphospholipid syndrome, one with SLE, one with syphilis, and a normal control. The method of isolation was designed to obtain immunoglobulin in the absence of β2 glycoprotein I. These IgG samples were first tested for their binding to cardiolipin-coated plates in the presence or absence of exogenous β2 glycoprotein I. The antiphospholipid syndrome and SLE patient's IgG binding was enhanced by β2 glycoprotein I, while the syphilis IgG was inhibited.



Binding of IgG to cardiolipin in the absence (open columns) or presence (shaded columns) of 40 μg/ml β2 glycoprotein I. Modified from (12).

The effect of β2 glycoprotein I on the prolongation of the dilute Russell's viper venom time by these immunoglobulins was determined. IgG at 1 mg/ml was added to normal plasma, β2 glycoprotein I-depleted plasma, and depleted plasma reconstituted with 40 μg/ml β2 glycoprotein I.



Anticoagulant activity of various IgG preparations on normal plasma (open columns), β2 glycoprotein I-depleted plasma (shaded columns), and depleted plasma plus exogenous β2 glycoprotein I (hatched columns). Modified from (12).

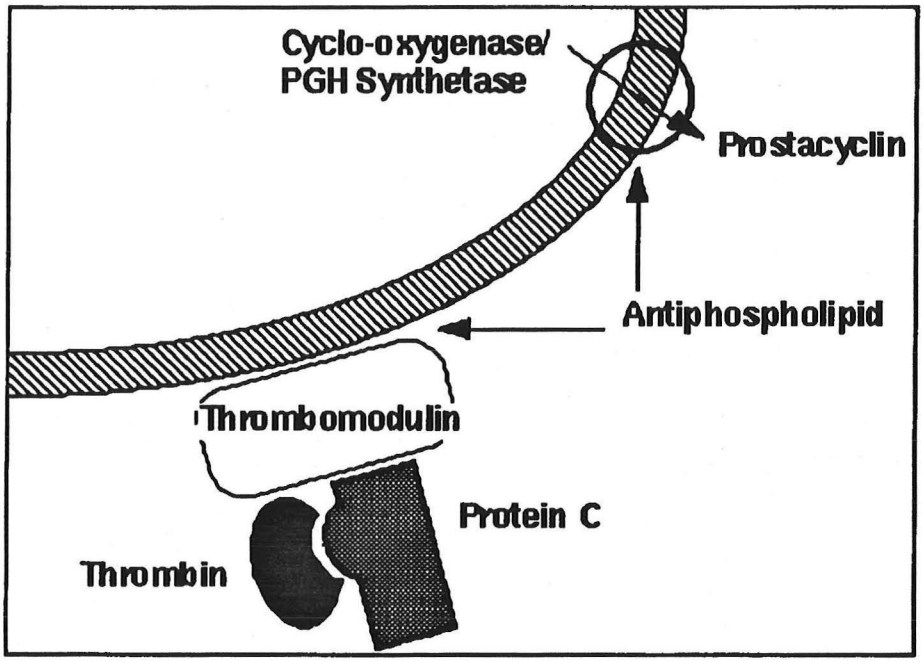
The removal of β2 glycoprotein I from the test plasma eliminated the lupus anticoagulant effect of the IgG. Restoration of β2 glycoprotein I caused the dRVVT time to once again become abnormally long.

The final piece of evidence that β2 glycoprotein I is an important antigen in antiphospholipid syndrome comes from the immunization of rabbits and mice with purified protein (16). Rabbits that received immunizations with β2 glycoprotein I in Freund's complete adjuvant developed both antiphospholipid as well as anti-β2 glycoprotein I antibodies. Mice immunized with β2 glycoprotein I also developed antiphospholipid antibodies while mice immunized with cardiolipin mixed with human serum albumin did not. Taken together, these studies suggest that a large fraction of antiphospholipid antibodies in patients react with a plasma apolipoprotein. This protein, β2 glycoprotein I, may be a natural anticoagulant *in vivo*. Therefore one of the procoagulant effects of antiphospholipid antibodies is the sequestration and functional inactivation of β2 glycoprotein I.

In Vivo Action of Antiphospholipid Antibodies

Initially, it may seem paradoxical that antibodies that are associated with thrombosis are measured by their anticoagulant effect. This is because *in vitro* assays of coagulation only see part of a complex homeostasis involving both procoagulant factors as well as natural anti-coagulants. Although no formal proof exists, these natural anticoagulants are thought to be the *in vivo* targets of antiphospholipid antibodies. On the surface of endothelial cells, activated thrombin binds to the transmembrane protein thrombomodulin. This complex activates protein C which acts with protein S to inactivate factors Va and VIIIa. Genetic deficiency of either protein C or S is associated with familial thrombotic episodes. Antibodies from 7 of 30 patients with antiphospholipid syndrome were found to interfere with the inactivation of factor Va by activated protein C

(17). Some of these antibodies were directed at phospholipid-bound protein C, while others required the association of protein S. As mentioned above, β2 glycoprotein I is thought to have anticoagulant activity, in part by inhibiting factor Xa production. Antibodies that bind to β2 glycoprotein I complexed to phospholipids may lead to increased local Xa generation. Finally, there is limited data that antiphospholipid antibodies interfere with prostacyclin production by endothelial cells (18). This disruption of local prostacyclin / thromboxane ratios may lead to vasoconstriction and platelet aggregation. The data in this regard are conflicting.



Possible role of antiphospholipid antibodies in pathogenesis of the thrombophilic state. Thrombin and thrombomodulin assemble on the surface of cells to activate Protein C which degrades Factors Va and VIIIa in the presence of phospholipid and Protein S. aPL may disrupt the Protein C activating complex. In addition, aPL may prevent the synthesis and secretion of prostacyclin by endothelial cells, promoting platelet aggregation.

Animal Models of Antiphospholipid Syndrome

The most convincing argument for the pathogenicity of anticardiolipin antibodies stems from the mouse models of this disease. Both spontaneous and induced models exist. The spontaneous model of antiphospholipid syndrome occurs in mice prone to systemic lupus-like disease (19). (NZB×NZW)F_1 mice develop several autoantibodies in an age-dependent manner. These are associated with immune complexes and lupus nephritis. Thrombocytopenia, thrombosis and myocardial infarction are also seen (20). Antibodies to cardiolipin and phosphatidyl serine have been isolated from these mice. Like human antiphospholipid antibodies, these require the β2-glycoprotein I cofactor. A subset of these natural autoantibodies bind to platelets, suggesting a potential role in thrombocytopenia. Other autoimmune mouse strains, such as Mrl/lpr also produce natural antiphospholipid antibodies (21).

The induced model of antiphospholipid syndrome has produced some very interesting results. Monoclonal antiphospholipid antibodies have been produced from mice with experimental SLE. Normal were immunized by repeated injection with human monoclonal anti-DNA antibody. These mice develop murine lupus with elevated ESR, leukopenia, proteinuria, anti-DNA antibodies, and antiphospholipid antibodies (22). They also develop other features of the antiphospholipid syndrome including thrombocytopenia (the normal platelet count for a mouse is 700,000 - 1,000,000).

Mice Immunized With Anti-DNA Antibodies

Monoclonal Antibody	Anticardiolipin Antibody	Platelet Count $x10^{-3}$	aPTT, sec
Anti-DNA	+	254 ± 41	94 ± 8
Control	-	981 ± 55	34 ± 4

Modified from (22)

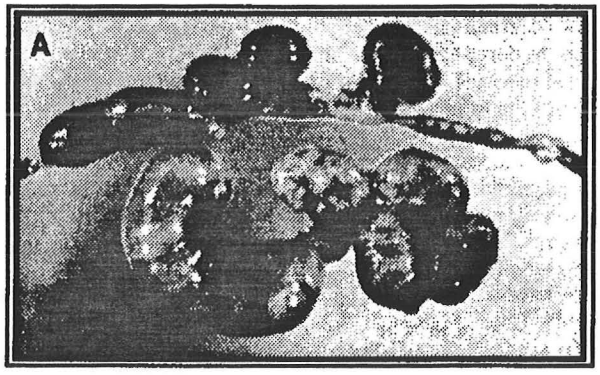
Other mice were infused intravenously with the purified mouse monoclonal antibody (10 μg/mouse), a control antibody, or polyclonal human anticardiolipin antibodies. One to four days after the infusions, the mice were mated and the outcome of their pregnancies determined. The mice were dissected prior to giving birth and the uteri examined for evidence of fetal resorption, the equivalent of fetal death in animals with multiple gestations.

Effect of Anticardiolipin Antibody on Pregnancy

Monoclonal Antibody	Percent Resorption	Platelet Count $x10^{-3}$	aPTT, sec	Placenta weight, mg	Embryo weight, mg
Anti-cardiolipin	68 ± 7	268 ± 12	83 ± 7	143 ± 8	790 ± 110
Control	1 ± 0.02	842 ± 38	48 ± 5	179 ± 9	1410 ± 220
None (PBS)	0	1,123 ± 299	46 ± 6	183 ± 11	1520 ± 180

Modified from (22)

In addition to the hematological findings, there was significant loss of embryo viability, embryo size, and placental weight. Immunofluorescence studies found evidence of deposition of the anticardiolipin, but not the control, antibody in the placentas.

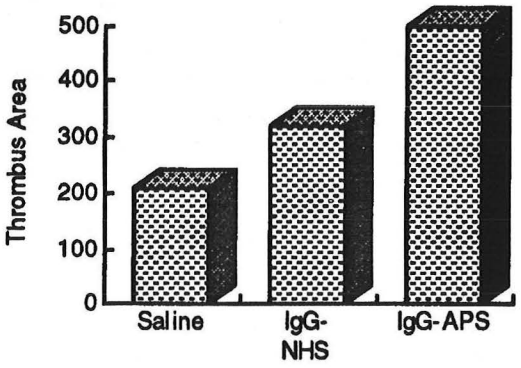


The upper uterus is from an animal given antiphospholipid antibodies during pregnancy. The embryos are small and there are regions of fetal resorption. A uterus from an animal given control antibodies is shown below for comparison.

Similar results were seen with the infusion of polyclonal human anticardiolipins. Embryo weights decreased from a mean of 1,157 mg to 693 mg, and there was evidence of resorption of up to 62% of all embryos. This model system has been used successfully to test interleukin-3 as a possible therapeutic for antiphspholipid syndrome in pregnancy (23).

This model allows the testing of individual antiphospholipids such as anti-phosphatidyl serine, anti-phosphatidyl ethanolamine, etc. Antibodies to a variety of antigens are found in patients with antiphospholipid syndrome and it is assumed that part of the difficulty correlating antibody presence with disease is this heterogeneity. Polyclonal antibodies to phosphatidyl serine were isolated from patients with antiphospholipid syndrome and recurrent fetal loss (24). Normal mice were given these antibodies intravenously. As with polyspecific antiphospholipid antibodies, these anti-phosphatidyl serine antibodies produced thrombocytopenia, prolongation of the aPTT, and fetal wastage. These antibodies could be isolated from the placentas of the mice. This correlates with the finding that human cytotrophoblast as well as syncytiotrophoblast bind anti-phosphatidyl serine antibodies, and may lead to a better understanding of fetal loss.

Although most of the animal models have focused on hematological effects and fetal wastage, a system has been developed to study thrombosis in mice (25). This involves the infusion of human antiphospholipid antibodies in mice followed by the application of a standardized crush injury to the femoral vein. The extent of thrombus formation was determined by trans-illumination and real-time video microscopy. Both the thrombus area and persistence of thrombi were greater in the mice infused with antiphospholipid IgG when compared to control IgG.



The small, but significant, increase in thrombus size was seen in 17 mice infused with this antibody. While this study looked at only one antibody source, it demonstrated the feasibility of this system to study the relationship of specific phospholipid-dependent autoantibodies *in vivo*.

Clinical Syndromes Associated with Antiphospholipid Antibodies

Elevated levels of anticardiolipin antibodies or the presence of a lupus anticoagulant has been seen in patients with occlusion of nearly any vascular bed.

Arterial Occlusion Syndromes

- Aortic arch syndrome, claudication
- Stroke, TIA, multi-infarct dementia
- Retinal artery thrombosis
- Digital and extremity gangrene
- Myocardial infarction, coronary artery bypass graft occlusion
- Mesenteric infarction
- Hepatic infarction
- Renal artery thrombosis, thrombotic microangiopathy
- Adrenal Infarction

Aortic and mitral valve abnormalities

- Vegetations
- Valvular incompetence

Catastrophic Occlusion Syndrome
Venous Occlusion Syndromes

- Deep venous thrombosis
- Pulmonary embolism
- Budd-Chiari syndrome
- Saggital vein and cavernous sinus thrombosis
- Retinal vein thrombosis

Dermatologic Syndromes

- Livedo reticularis
- Non-healing skin ulcers

Obstetric Syndromes

- Recurrent Fetal Loss
- "Complicated" pregnancy - intrauterine growth retardation, preterm delivery, maternal hypertension, etc.

Most of these associations have been based on sporadic case reports or selected series. There are several large retrospective studies, but a paucity of prospective data regarding the predictive value of a positive antiphospholipid determination. Over the past ten years, most of the clinical data has been gathered from obstetric patients and patients with cerebral ischemia. These topics are discussed separately below.

Deep venous thrombosis and pulmonary embolism are frequently associated with antiphospholipid antibodies.

Defects Associated with DVT in 100 Consecutive Patients

Defect	%
Anticardiolipin antibodies	24
Malignancy	11
Arthroscopy	10
Major trauma	8
Protein S deficiency	8
Anti-thrombin III deficiency	8
Orthopedic surgery	7
Obesity	6
Lupus anticoagulant	4

Adapted from (26)

Bick, *et al.* looked for etiologic factors in 100 consecutive unselected patients with DVT (26). Anticardiolipin antibodies were the most common, occurring in 24% of patients. Estrogen use, diabetes mellitus, and other factors were identified in less than 4% of their sample.

The Physicians Health Study was a randomized, double-blind trial of aspirin and β-carotene in 22,071 male physicians. Subjects were identified who had suffered either ischemic stroke (100 patients) or DVT or pulmonary embolus (90 patients) during the 5 year period following randomization (27). Serum samples from these patients as well as controls taken from the study (matched as to age, smoking status, and length of follow-up) were evaluated for the presence of anticardiolipin antibodies by ELISA.

Characteristics of Patients and Controls

Variable	Patients with Stroke(n=100)	Controls (n=100)	Patients with DVT/PE (n=90)	Controls (n=90)
Age, yr.	61	61	58	58
Smoking, %	24	24	48	48
Aspirin (study)	49	47	46	46
Anticardiolipin titer, IgG units				
Mean ± SD	3.7 ± 8.5	2.9 ± 5.5	16.3 ± 24.2	9.6 ± 12.5
P value		0.42		0.01

Adapted from (27)

The presence of IgG anticardiolipin was associated with deep venous thrombosis or pulmonary embolus, but not stroke. The study treatment of aspirin did not appear to be protective against these events in a logistic regression model. Further analysis of the data demonstrated an increasing risk for venous thrombosis with increasing titer of IgG anticardiolipin. The relative risks for DVT or pulmonary embolus in patients with antibody titers over the 95th percentile was 4.0; over the 98th percentile it was 7.5. These risks were moderated slightly when they were calculated using the control, rather than thrombosis patient distribution. The authors conclude that IgG anticardiolipin titers above the 98th percentile impart high risk for venous thrombosis, these between the 95th and 98th percentiles may carry moderate risk, and values below 95th percentile, little excess risk.

Both of these studies illustrates the central question concerning antiphospholipid antibodies and venous thrombosis. Each study was well designed, and without the selection biases that are more blatant in other reports. Both demonstrate an increased prevalence of anticardiolipin antibodies in patients with DVT or pulmonary embolism. However, these are both common conditions with multiple other causes. Are the autoantibodies just markers of endothelial damage from other causes, or are they the causes themselves? The animal models described above may help decide this. If a high-titer antiphospholipid antibody is detected should these persons be treated before an event? Unfortunately, there is no data on this point.

Antiphospholipids in Peripheral Vascular Disease

In a study designed to evaluate the occurrence of antiphospholipids in peripheral vascular disease, 234 patients undergoing vascular surgery were tested for anticardiolipins and lupus anticoagulant (28). This was a select group of older patients referred to a university vascular surgery clinic. Twenty-six percent of patients had antiphospholipid antibodies (determined by either an elevated anti-cardiolipin or positive lupus anticoagulant). While there were no demographic differences between those patients with antiphospholipids and those without, this study is flawed by the lack of a control group without a history of vascular occlusion. The authors cite historical data that show antiphospholipids at a significant level present in 1% of matched controls. Despite this potential source of bias, there was a correlation between the presence of antiphospholipid antibodies and both previous lower extremity vascular procedure, and the failure of such a procedure.

Logistic Regression Analysis of Antiphospholipid Antibodies in Vascular Surgery Patients

Factor	Odds Ratio	95% Confidence interval	p Value
Previous lower extremity vascular procedure	1.8	1.0 - 3.3	0.047
Occlusive failure of previous lower extremity procedure	5.6	1.9 - 16.8	0.003

The obvious question from this analysis is whether the original arteriosclerosis, the presence of a vascular graft, or the occlusion of such a graft causes antiphospholipid antibodies. Even if these antibodies are only a marker for other event in these patients they may have predictive value. Over the eight years prior to the initiation of this study, patients without antiphospholipids had higher levels of graft patency. The authors suggest that screening be done in vascular surgery patients to identify persons at higher risk for graft failure, although they stop short of recommending more agressive anticoagulation therapy.

Catastrophic Antiphospholipid Syndrome

A small number of patients (less than 50) have been described with a malignant vasculopathy in association with antiphospholipid antibodies. These patients suffer vascular occlusion in multiple organ systems occurring suddenly, and over a short period of time (29). There is usually no identifiable precipitating event. The differential diagnosis includes thrombotic thrombocytopenic purpura and disseminated intravascular coagulation. In 1992, Asherson (30) reviewed ten such patients.

Clinical Features of the "Catastrophic" Antiphospholipid Syndrome	
Age	13 - 43 yr.
Gender	7 F, 3 M
SLE or lupus like illness	90%
Livedo reticularis	50%
Digital infarcts	30%
Multiple cerebral infarcts	40%
Myocardial infarction	30%
Hepatic or adrenal infarction	20%
DVT	40%
Malignant hypertension	60%
Renal disease (thrombi or ARF)	90%
CNS dysfunction	90%
Survival	60%

Most of the patients have SLE that is well-controlled until presentation. All of them had high titers of anticardiolipin antibodies or the presence of a lupus anticoagulant. The symptoms appeared and progressed over the course of days. Treatment with high-dose corticosteroids was ineffective. Although all reports of this condition are anecdotal, treatment with plasmapheresis (as for TTP) and heparin had the most success. In one case, circulating levels of plasminogen activator were unmeasurable at presentation with multiple CNS and renal thrombi. This patient was successfully treated with intravenous Russell's viper venom (Ancrod). This suggests a basic defect in fibrinolysis. Treatment of such patients rests on the recognition of the syndrome and heroic efforts to reverse the coagulopathy.

Treatment of Patients with Thromboses in the Antiphospholipid Syndrome

The first goal in treating patients with venous or arterial occlusion is to rule out other, more common causes than antiphospholipid syndrome. Hyperlipidemia, hypertension, diabetes mellitus, nephrotic syndrome, malignancy, immobility/trauma, and mechanical obstruction are all common risk factors for vasculopathy. Inherited defects of coagulation such as protein C, protein S, or anti-thrombin III deficiency are present at nearly the same frequency as antiphospholipid antibodies in the general population and deserve consideration. It is especially important to think of these conditions prior to initiating anticoagulant therapy so that baseline samples can be obtained.

There is no evidence that treatment with immunosuppressive drugs is beneficial in antiphospholipid syndrome (8). Like most autoantibodies, anticardiolipin levels can be lowered by high-dose steroid therapy. Once the steroids are stopped, the antibodies recur, and the physician is faced with the prospect of lifelong immunosuppressive therapy and its attendant complications. Steroid therapy is indicated for those patients with SLE and antiphospholipid syndrome whose lupus manifestations (e.g. vasculitis) require it. In addition, corticosteroids have been used with success in the treatment of recurrent fetal loss in women with antiphospholipid syndrome (see below).

Therapy should be directed first to the thrombosis, first with heparin and then with oral anticoagulation. The length of anticoagulation is problematic. Roseve and Brewer (31) reviewed the charts of seventy consecutive patients with

antiphospholipid syndrome. The mean length of follow-up was 5.2 ± 5.6 years. 53% of the patients had recurrent events during that time. In nearly each case of recurrence, an arterial event was followed by another arterial event and venous event followed venous events. Two patients died from thrombosis. The analysis of treatment options during the follow-up period suggested decreasing recurrence with intense anticoagulation.

Value of Various Anti-Thrombotic Treatments

Variable	Patient-Years of Follow-up	Number of Events (Venous/Arterial)	Events per Year of Follow-up
No Treatment	161.2	31 (17/14)	0.19
Aspirin alone	27.5	10 (2/8)	0.36
Aspirin plus intermediate-intensity warfarin	5.3	2 (0/2)	0.38
Aspirin plus high-intensity warfarin	5.0	0	0
Heparin	7.5	4 (3/1)	0.53
Low-intensity warfarin	11.3	6 (4/2)	0.57
Intermediate-intensity warfarin	40.9	3 (0/3)	0.07
High-intensity warfarin	110.2	0	0

Adapted from (31)

Patients on low-intensity warfarin had an INR ≤ 1.9; intermediate intensity, INR 2.0 - 2.9; high intensity, INR ≥ 3.0. Five non-fatal bleeding complications occurred in the patients on warfarin. In two of the cases the INRs were > 4.0 at the time. Of the four recurrences on heparin, two occurred during high-intensity therapy and consisted of multiple pulmonary emboli and complete occlusion of the common iliac vein. No bleeding complications occurred in patients treated with aspirin or heparin. Due to the high rate of recurrence in this population, the authors recommend indefinite anticoagulation in reliable patients with a target INR of 3.0.

A second study was performed by Derksen, *et al.*, (32) who followed nineteen patients after their first venous thromboembolic episode. The median follow-up in this group was 93 months. There were 34 recurrent venous thrombotic episodes during this time. These second or third thrombi occurred anywhere from months to years after oral anticoagulation was stopped. The target INR for treated patients was 2.5 - 4.0. One of the patients with a recurrence while on warfarin with an INR of 2.2. 100% of the patients taking oral anticoagulants were free of thrombi during the first eight years of the study, while discontinuing anticoagulation produced a 50% probability of recurrence at two years and a 78% probability at eight years. These authors also recommend aggressive life-long anticoagulation. As always, it is difficult to apply retrospective data directly to prospective treatment decisions. Currently, one must weigh the seriousness of likely recurrence (CVA vs. DVT, for example) and the morbidity of long-term anticoagulation in a given patient.

Neurologic Features of Antiphospholipid Syndrome

The brain is the most common site for arterial thrombosis in patients with antiphospholipid syndrome, and cerebral ischemia is the most common neurological symptom in patients with antiphospholipid antibodies (33). Patients with Sneddon's syndrome, the association of recurrent stroke with livedo reticularis are almost always antiphospholipid positive, as are young patients with multi-infarct dementia. High titers of antiphospholipids have been found in 18-46% of stroke patients less than 50 years old (34, 35). Over three-quarters of pediatric stroke victims are positive for these antibodies (36). Other neurological syndromes associated with antiphospholipid syndrome are ocular ischemia, chorea, migraine-associated neurological deficits (though not headaches, *per se*), seizure disorder, and transverse myelopathy.

Antiphospholipid antibodies are often associated with neuroradiological abnormalities, even in asymptomatic persons. The lesions are best detected by MRI and are high signal intensity, particularly on T2-weighted images. Most often they are in the sub-cortical white matter, and are similar to the lesions seen in multiple sclerosis, Behçet's disease, and Sjögren's syndrome. In one prospective study (37), 37 patients with SLE were studied by MRI. Only seven patients had a history of CNS symptoms. 45 healthy age- and gender-matched controls were also studied. MRI images were scored for the presence of abnormal high intensity signal by a single observer, blinded as to patient status. Abnormal MRIs were found in 14 of the SLE patients and 4 of the controls. The difference was most striking when the study subjects were divided by age.

Incidence of Abnormal MRI in SLE Patients and Controls

Age (Years)	< 50	≥ 50
SLE	10/31*	4/6
Healthy Controls	1/35	3/10

* p = 0.008; Adapted from (37)

Comparison of Patients With and Without Abnormal MRI

	High Intensity Signal		
	Present	Absent	p Value
Number of patients	10 (all F)	21 (18 F)	NS
Age (yr.)	36.3 ± 7.7	36.9 ± 8.7	NS
Age at onset (yr.)	21.4 ± 8.5	26.1 ± 8.9	NS
Disease duration	14.9 ± 6.1	11.1 ± 8.4	NS
CNS lupus	5	2	0.011
Thrombotic events	1	1	NS
Fetal loss	4	1	NS
Renal involvement	3	11	NS
Hypertension	3	6	NS
Livedo reticularis	6	3	0.008
IgG anticardiolipin	8	4	0.002
IgM anticardiolipin	2	1	NS
Lupus anticoagulant	2	2	NS

Adapted from (37)

The records of the patients who were less than 50 years old were reviewed. Comparison of patients with abnormal MRI findings to those with normal MRIs revealed several significant differences. There were no differences in other skin rashes or autoantibodies. While this study does not prove a causal relationship, it indicates the level of unappreciated CNS pathology in patients with SLE. The relationships of antiphospholipid antibodies to these lesions and of these lesions to symptoms is also problematic.

Despite a large body of data correlating stroke with antiphospholipid antibodies, there are continuing debates over the validity and meaning of the association. Most of the studies have been retrospective small case series or from selected populations. Other factors for cerebral ischemia such as hypertension, hypercholesterolemia, and diabetes are not always taken into account. This raises the question of whether antiphospholipids are merely markers for endothelial damage from other causes. The argument that young stroke patients, even children, are unlikely to have co-morbid causes of ischemia, and yet have high titers of antiphospholipids may not valid if thrombotic events themselves cause antibody production. In patients followed prospectively for vascular events after the finding of high-titer antiphospholipid antibodies, there was an association with deep venous thrombosis and pulmonary embolism, but not stroke (27).

Two recent studies can be compared to illustrate the differences in interpretation of antiphospholipid data. The Antiphospholipid Antibody in Stroke Study Group obtained data on 248 first stroke patients of all ages and 257 matched controls (38, 39). When the data were controlled for age, gender, hypertension, diabetes mellitus, coronary artery disease, and cigarette smoking, there was still and independent risk associated with anticardiolipin antibody positivity.

Prevalence of Anticardiolipin Antibody Among Stroke and Control Patients

	Anticardiolipin +			
Patient Group	Number	Percent	Odds Ratio (CI)	P
First Stroke (n=248)	24	9.7		
Control (n=257)	11	4.3	2.33 (1.10 - 4.94)	0.028
Prior Stroke (n=119)	14	11.8		

In this group, the mean age of first stroke patients was 66. Thus, antiphospholipids appear to be a risk for both young and old patients with cerebral ischemia. The authors compared the risk of antiphospholipid antibodies to other risks for stroke.

Risk Factors for First Stroke for Patients Aged > 50

Risk Factor	Relative Risk (Odds Ratio)	Risk Difference per 1000	Prevalence per 1000	Population-Attributable Risk (%)
Non-valvular atrial fibrillation	3.6	11.8	14	3.5
Hypertension	2.6	4.1	531	45.9
Anticardiolipin antibody	2.3	5.4	51	5.9

As the prevalence per 1000 for these (and other factors) decreases with age, the population-attributable risk for anticardiolipin antibodies rises. These authors use this data to point out the public health consequences of anticardiolipin antibodies, and estimate that approximately 40,000 strokes per year in the United States associated with the antibodies.

In contrast, a study of an unselected stroke population in Scotland revealed an association of antiphospholipids with stroke only in the oldest patients, and most strongly with the number of other risk factors for cerebral ischemia (40). Although this study has been criticized on methodological grounds, it points out the problem with recommending large-scale screening of stroke patients without a clear-cut treatment plan based on the outcome.

The association of antiphospholipids and recurrent cerebral ischemia is stronger, and is more consistent with the definition of antiphospholipid syndrome. This risk of recurrent stroke in young patients with antiphospholipid antibodies was eight times that of patients without the antibodies in one prospective study (34). 46 patients younger than 50 with cerebral ischemia were compared to patients with other neurological diagnoses. 46% of the stroke/TIA patients were positive for antiphospholipids. Both IgM and IgG antibodies were present within three days of the index event. 28% of antiphospholipid positive patients had multiple ischemic episodes versus 8% in the antibody negative group. A similar study evaluated 55 patients aged 15-44 with stroke or TIA. 18% of these patients had antiphospholipid antibodies (35). 70% of these antibody-positive patients had a history of stroke or TIA (versus 27% of the controls), and 40% had new events during follow-up (versus 4% of controls). The Antiphospholipid Antibodies and Stroke Study Group reported similar rates of recurrence for 74 of their patients followed prospectively.

In the face of these conflicting or potentially biased data what recommendations for evaluation of stroke patients can be made? As with any disease entity undergoing as much study as antiphospholipid syndrome, any guideline must be rather loose. It is unlikely that routine screening of all stroke patients for antiphospholipid antibodies or lupus anticoagulants will identify unique sets of patients for whom special therapies can be tailored. Assessment of antiphospholipid status is part of the evaluation of <u>unexplained</u> cerebral or ocular ischemia, particularly in younger patients. However, more common causes of endothelial damage should be considered in all age groups. The risk of recurrence in these patients may be higher than in those without antiphospholipid antibodies. Therefore, extended anticoagulation, perhantiphospholipid syndrome for life, should be considered. Immunosuppressive therapies in these patients are of unknown value, and will require evaluation by properly devised clinical studies.

Antiphospholipid Syndrome and SLE

The occurrence of antiphospholipid antibodies in patients with systemic lupus erythematosus is clear. A false-positive serologic test for syphilis, one measure of antiphospholipids, fulfills one criterion for the classification of SLE. Of note, these criteria were revised in 1982, one year prior to the initial description of solid phase assays for antiphospholipid antibodies. The prevalence of lupus anticoagulant and anticardiolipin antibodies in large groups of patients with SLE are between 31-34% and 40-44%, respectively (9). Conversely, SLE or "overlap" autoimmune syndromes account for 50-60% of patients with moderate or high titer antiphospholipid antibodies. A large fraction of patients with antiphospholipid antibodies do not have other features of lupus. These patients felt to have a "primary antiphospholipid syndrome". This raises the question of whether primary antiphospholipid syndrome is a prelude to SLE, or a separate entity. In a European multi-center study (41), 114 patients were evaluated over two years. Fifty-six of them had antiphospholipid syndrome in association with SLE, and Fifty-eight had primary antiphospholipid syndrome. Historically, the patients in each group had similar numbers of thrombotic episodes, but were different in the other hematological manifestations of SLE.

Historical features of patients with primary and SLE-associated antiphospholipid syndrome

	Primary (n=58)	SLE-Associated (n=56)	p value
Venous thrombosis	50%	61%	NS
Pulmonary embolism	24%	21%	NS
Arterial thrombosis	36%	36%	NS
Fetal loss	53%	48%	NS
Livedo Reticularis	24%	20%	NS
Thrombocytopenia	40%	41%	NS
Pulmonary hypertension	3.5%	1.8%	NS
Hemolytic anemia	7%	21%	<0.05
Neutropenia	0	11%	<0.01
Endocardial valve disease	37%	63%	<0.005

Risk factors for thrombosis (hypertension, diabetes mellitus, hyperlipidemia, and cigarette smoking) were similar in both groups. However, more patients with primary antiphospholipid syndrome used oral contraceptives (21% vs. 6%). The isotypes and titers of anticardiolipin antibodies were not significantly different between the two groups. Not surprisingly, the patients with SLE had significantly higher prevalence of anti-nuclear antibodies, anti-DNA antibodies, direct anti-globulin test, hypocomplementemia, neutropenia, and lymphopenia. The patients with SLE continued to have a higher prevalence of abnormal echocardiographic findings, primarily related to the mitral valve.

The patients were followed for a period of 4 months to 5 years (mean, 2 yr.). there were ten thrombotic episodes, six in the primary antiphospholipid syndrome patients and four in the SLE patients. In each group, six women became pregnant and there was one fetal loss. Nine of the ten patients who suffered recurrent thrombosis were on oral anticoagulant therapy at the time, with international normalized ratios of 2-3. No patient with the primary syndrome developed SLE during the study. These data emphasize that antiphospholipid

syndrome is not different when it occurs in the setting of SLE, and should not be treated differently. Manifestations of SLE should be treated separately, and in addition to, thrombotic complications of antiphospholipid syndrome.

The presence of antiphospholipid syndrome in lupus patients does affect their long-term survival (42). 658 patients with SLE were followed for a total of 2039 person-years. Forty-nine patients died during the study. In general, these patients were sicker, with more frequent hematological, renal, and neurological manifestations of SLE. They also had higher frequencies of arterial and venous occlusion. A stepwise Cox multivariate analysis identified thrombocytopenia and arterial occlusion as independent risks for mortality, regardless of other measures of disease activity. Other measures of the antiphospholipid syndrome did not correlate with mortality.

While prospective studies of antiphospholipid syndrome in SLE have emphasized the repeated documentation of anticardiolipin antibodies over time to verify the diagnosis, this may not be of practical value. 127 lupus patients in three Australian rheumatology clinics (one university clinic; two private practices) had anticardiolipin antibodies measured at the earliest opportunity, without regard to clinical history (43). 24% of their patients had an antiphospholipid antibody present on this single determination. They then reviewed all the charts to determine the clinical utility of this test.

Clinical utility of positive anticardiolipin:

Probability of Feature: %	Pretest	Post-Test Anticardiolipin >15 units		
		(+)	(–)	Relative Risk
Recurrent fetal loss	6	20	1	20
Thrombosis	14	35	5	7
Thrombocytopenia	22	40	14	2.9
Cerebral ischemia	12	30	5	6
Digital infarcts	25	45	10	4.5

In this analysis of patients with SLE, a single positive anticardiolipin determination was strongly predictive of **past** clinical events such as fetal loss or thrombosis. The obvious question is what clinical utility does such a determination have prospectively? Such screening is not useful in the general population or low-risk obstetric patients (see below), since the incidence of these events is low. However, the incidence of antiphospholipid antibodies in SLE patients is substantial. The need for such a prospective study to identify patients at risk for thrombosis is tempered by the lack of safe and effective preventative therapy. Long-term anticoagulation for patients who have not yet had a thrombosis is drastic, immunosuppression carries significant side effects, and anti-platelet therapy may not be sufficient.

Antiphospholipid Syndrome in Pregnancy

The most controversial area of the antiphospholipid syndrome literature deals with obstetric patients. The concerns of the obstetricians and their patients are obvious. If antiphospholipid antibodies predispose to fetal loss, should screening for these antibodies be a routine prenatal test? Who should get treated? How? Unfortunately, the data from many large studies are not entirely

in concert. This can be explained on the basis of different patient selection techniques, different testing and interpretation methods, and different end-point determinations. In addition to the problem of fetal loss, there is data to suggest that these antibodies contribute to infertility (44), and may be responsible for neonatal antiphospholipid syndrome (45).

To review this literature rationally, it can be approached by the type of patient studied: normal pregnancy, women with a history of fetal demise, and women with autoimmune disorders such as SLE.

Antiphospholipids in "normal" pregnancy

The outcome of several large studies of patients without a history of antiphospholipid syndrome or fetal loss is outlined in the table below:

Effect of Antiphospholipids in Normal Pregnancies

Author	Population	Results	Conclusions
Harris (46)	1449 normal pregnancies	1.8% IgG+ (0.2% high+) 4.3% IgM+ (0.5% high+)	α–phospholipid rare; no correlation with fetal death
Lockwood (47)	737 normal pregnancies	0.2% lupus anti-coagulant; 2/22 had fetal death 0.2% α-cardiolipin; 12/16 had fetal death	α-cardiolipin and lupus anticoagulant rare; fetal death correlates with titer
Rix (48)	2856 pregnancies	aPTT high in 7% of normals, 19% of early abortions, 18% of IUGR	Lupus anticoagulant rare; low titer IgM common; α-phospholipid correlates with complications
Lynch (49)	389 pregnancies	24% α-phospholipid + of whom 16% had fetal loss	α-phospholipid common and correlate with fetal loss

Adapted from (8)

The last study (49) looked at low-risk, nulliparous women (78 of whom had previous spontaneous abortions) who had blood drawn for antiphospholipid determinations at their first prenatal visit. Six determinations were made: IgG, IgM, and IgA anticardiolipins (CL), aPTT, false-positive syphilis serology, and dRVVT. Patients were antiphospholipid positive if any of the six were abnormal (antiphospholipid profile). Even after correction for maternal age, race, cigarette use, and gestational age at first visit, the relative risk of having fetal loss with an IgG anticardiolipin was 1.92 (95% CI, 1.07 - 3.44), and for having fetal loss with any abnormal value (antiphospholipid profile) was 1.57 (CI, 1.08 - 2.29). Factors that did not increase the risk of fetal loss in this population were: more than one positive test, having a positive test both at the first prenatal visit and at delivery, and having a history of fetal loss.

Fetal Loss Related to Antiphospholipid Testing at First Prenatal Visit

Test	Patients with Fetal Loss (%)		Relative Risk	P Value	95% CI
	Test Abnormal	Test Normal			
IgG α–CL	27.7	7.8	3.54	0.014	1.56 - 8.07
IgM α-CL	5.0	9.0	0.56	1.0	0.88 - 3.87
IgA α-CL	0.0	9.0	0	1.0	
aPTT	21.4	8.5	2.51	0.123	0.87 - 7.23
False + RPR	50	8.5	5.86	0.167	1.41 - 24.35
dRVVT	11.5	8.5	1.35	0.44	0.59 - 3.11
α-PL profile	15.8	6.5	2.44	0.011	1.29 - 4.62

Adapted from (49)

Several interesting facts emerge from this study. First, over 24% of healthy women had one or more abnormal antiphospholipid tests. Most of these were prolonged dilute Russell's viper venom times. These were done without a platelet neutralization procedure. The normal values for pregnant women have not been established. Therefore, the true percentage of antiphospholipid positive patients is probably in the 2 -7% range found in other studies. Serial antiphospholipid measures were obtained from 239 patients. Only a positive determination at the first prenatal visit was correlated with fetal loss, suggesting variability in antiphospholipid titers. Lastly, most (85%) of the women with positive tests for antiphospholipid antibodies had uneventful pregnancies without maternal or fetal complication. Until there are better markers of pathogenic antiphospholipid antibodies and a better understanding of the other factors that contribute to fetal demise, it is inappropriate to screen healthy women in their first pregnancies.

Antiphospholipid Antibodies in Patients with a History of Fetal Loss

Effect of Antiphospholipids in Women with Recurrent Abortion

Author	Population	Results	Conclusions
Infante-Rivard (50)	331 post-first abortions	1.8% lupus anticoagulant	no correlation between α-phospholipid and first abortion
	993 post normal delivery	3.8% lupus anticoagulant	
Parke (51)	81 women 3+ abortions	16% α-phospholipid	correlation with recurrent abortion
	88 normal pregnancies	7% α-phospholipid	
Out (52)	102 women with 3+ abortions	5% lupus anticoagulant; 21% α-phospholipid	α–phospholipid correlates with fetal demise
	102 normal pregnancies	0% lupus anticoagulant; 10% α-phospholipid	
Petri (53)	44 women with 3+ abortions	9% lupus anticoagulant; 21% α-phospholipid	correlation was not statistically significant (sample size too small)
	40 women with 0-1 abortion	0% lupus anticoagulant; 2.5% α-phospholipid	

Adapted from (8)

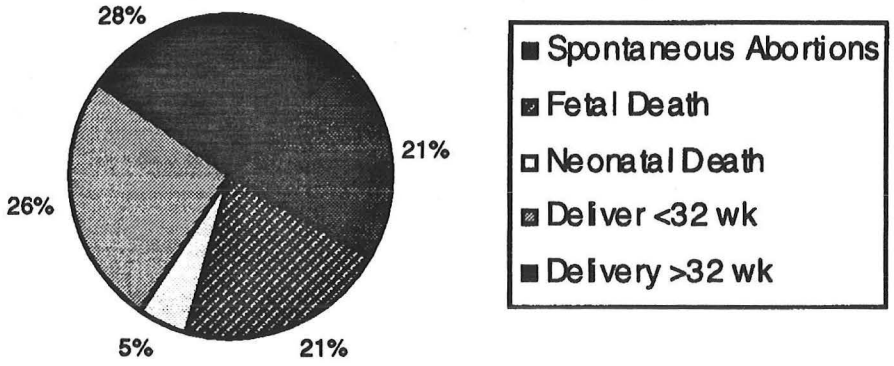
As one begins to select patient groups, more obvious patterns emerge. There are many causes of pregnancy loss, most of which are more common than antiphospholipid antibodies: genetic and developmental factors and maternal metabolic factors are most significant. Taken together, the fact that most predicts future pregnancy loss is prior pregnancy loss. This has been documented in the studies of antiphospholipids in patients with previous fetal demise.

In these patients, there is a greater statistical association between recurrent fetal loss and antiphospholipid antibodies as the number of prior fetal deaths increase.

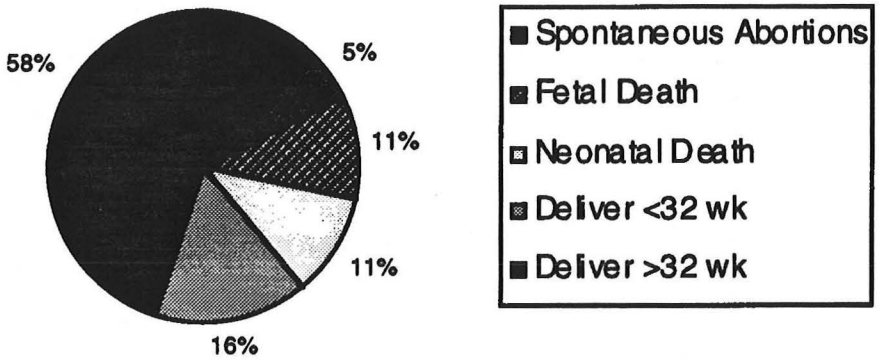
A careful, retrospective analysis was done by Branch, *et al.* (54) who have followed 54 women with antiphospholipid syndrome since 1983. 32% of these women have SLE. 96% have a lupus anticoagulant; 88% have high-titer IgG anticardiolipin; 79% a false-positive RPR. Previously these women had 195 total pregnancies. 92 of these resulted in spontaneous first-trimester abortion and 79 in fetal death beyond the 10th week of gestation. Four of the resulting live infants died of prematurity. Thus, only 10% of the previous untreated pregnancies resulted in a surviving infant.

These patients were followed through 82 pregnancies. 63% of these pregnancies resulted in a surviving newborn. It is illustrative to look at these pregnancies by treatment group. In 39 pregnancies, the women received prednisone (median = 40 mg/d) and low-dose aspirin. In 19 pregnancies, patients were treated with subcutaneous heparin (median = 15,000 U/day) and low-dose aspirin. 12 patients received prednisone, heparin, and aspirin, and 12 patients received other treatments including aspirin , prednisone, or heparin alone, prednisone and intravenous immunoglobulin, or prednisone plus heparin.

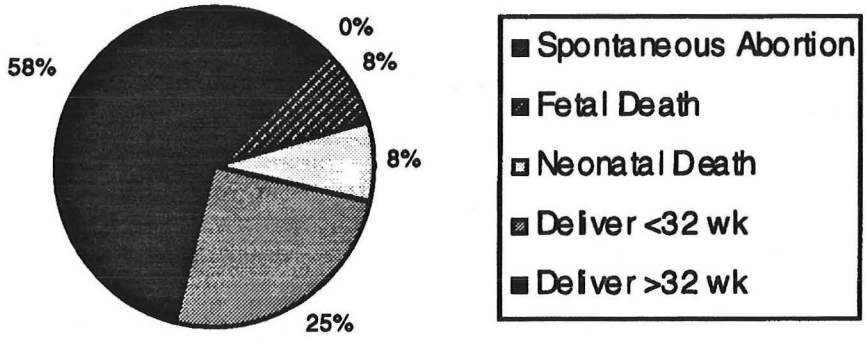
Patients Treated with Prednisone and Aspirin



Patients Treated with Heparin and Aspirin



Patients Treated with Prednisone, Heparin, and Aspirin



The differences in the percentages of surviving neonates in each group were not statistically significant. Of the possible factors related to pregnancy loss, only the total number of previous pregnancy losses or fetal deaths were significant. Other factors including SLE, history of pre-eclamsia, history of thrombosis, false-positive RPR, and titer of IgG anticardiolipin were not significant. These patients were also at risk for complications of their pregnancies.

Percent of Pregnancy Complications in Antiphospholipid Syndrome Patients

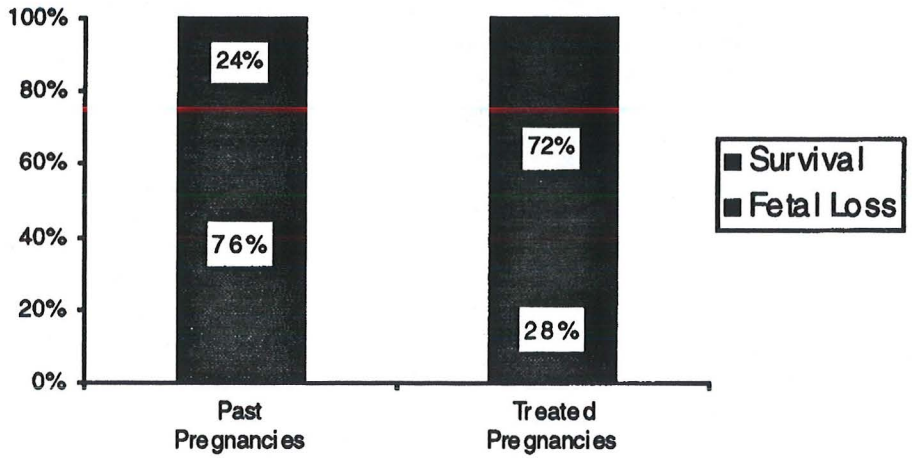
Treatment	Preeclamsia	Severe Preeclamsia	Gestational Diabetes	Fetal Distress	SGA	PROM
Pred/Aspirin	65%	35%	10%	61%	43%	13%
Heparin/Aspirin	41%	29%	0	60%	20%	7%
Pred/Heparin/Aspirin	50%	17%	42%	33%	22%	25%
Other	30%	10%	0	43%	29%	14%
Total	51%	27%	11%	53%	31%	14%

Adapted from (54)

There are obvious limitations in a study that compares patients to themselves as controls and does not have a group of untreated patients. Any treatment regimen improved the outcome. However, all treatment groups had complicated pregnancies. Success in one pregnancy did not always correlate with success in the next, even if identical treatments were used. Finally the authors note that although they identified patients on the basis of pregnancy loss, 41% had a history of thrombosis. 85% of the thrombotic episodes occurred during pregnancy, in the immediate post-partum period, or in association with oral contraceptive use. They emphasize the need for thrombosis prophylaxis in these patients.

Antiphospholipid Antibodies in Pregnant SLE Patients

Patients with lupus have a higher prevalence of antiphospholipid antibodies. When this is factored into analyses of women with a history of recurrent fetal loss, the presence of SLE is not significant. As in other groups, the most significant risk for fetal loss is a previous history of such an event. Treatment regimens for SLE patients with antiphospholipid syndrome have been similar to those used for non-SLE patients, with similar results. For example, Buchanan *et al.*, followed 100 consecutive pregnancies in 76 women with antiphospholipid syndrome and a history of fetal loss (55). They were treated with low-dose aspirin (75 mg/day). Subcutaneous heparin was added if there was a history of thrombosis, and other aspects of their lupus were managed with prednisolone, azathioprine, or hydroxychloroquine.



This treatment regiment resulted in a dramatic decrease in the number of surviving infants. Again, this was not a controlled study of treatment options. The authors note, however, that patients were not treated with steroids unless required for flares of their lupus activity.

Treatment of Pregnant patients with Antiphospholipid Syndrome

The issues surrounding treatment of pregnant women have been touched upon above. This is a very controversial area where emotion often overcomes logic. The goal of therapy should be increase the chances of a surviving infant with as little maternal morbidity as possible. No therapeutic alternatives are entirely safe. Prednisone, designed to suppress pathologic autoantibodies, carries

the risk of hyperglycemia, infection, and osteonecrosis. Aspirin and heparin carry risk of bleeding or heparin-induced osteopenia. Retrospective series have examined treatments with aspirin alone, aspirin plus prednisone (56,59) low-dose heparin plus prednisone, low-dose-heparin plus aspirin, and intravenous immunoglobulin therapy (57). Only one prospective trial has been done. Cowchock *et al.* (58) randomized twenty patients and obtained data from twenty-five more who would not or could not be randomized. All patients were treated with 80 mg aspirin per day throughout pregnancy. Prednisone was given at a dose of 20 mg twice a day. Heparin was self-administered at twice a day at a mean daily dose of 17,000 units that did not result in prolongation of the aPTT. These women also received calcium and vitamin D. None of these patients had SLE. No woman in either group had an uncomplicated live birth without treatment in the previous five years. There were live births in 75% of patients in both treatment groups. There were no differences between the randomized group and the groups that refused or were ineligible for randomization.

Comparison of Pregnancy Outcome and Complications in Patients Treated with Either Prednisone or Heparin

Feature	Randomized		All Patients		p value
	Prednisone (n = 8)	Heparin (n = 12)	Prednisone (n = 19)	Heparin (n = 26)	
Live births	75%	75%	68%	73%	0.8
Fetal death > 12 wk	25%	8%	21%	4%	0.15
Pre-term delivery	100%	25%	77%	12%	0.0002
Premature rupture of membranes	50%	0	46%	0	0.006
Pre-eclampsia	50%	0	41%	5%	0.01

Adapted fro (58)

These data reinforce that of the retrospective studies. Heparin and aspirin appear to be as effective as prednisone and aspirin with fewer maternal complications. The effect of aspirin alone has not been tested prospectively, nor have other potentially more practical or less toxic therapies such as low molecular weight heparin or intravenous immunoglobulin.

Conclusions and Recommendations

The field of antiphspholipid antibody research continues to evolve. New diagnostic and therapeutic strategies are being developed. As such, recommendations for therapy are subject to interpretation of current literature and may change as better, more precise studies are done.

1. The term antiphospholipid antibodies encompasses a heterogeneous group of autoantibodies. Their antigenic targets can be phospholipids themselves or plasma proteins in association with phospholipids. The full range of antiphospholipid specificities is not known.
2. Although gross generalizations can be made, it is impossible to predict the clinical course of a given patient based on the type of antiphospholipid (lupus anticoagulant vs. anticardiolipin antibody) or the titer of the antibody.
3. Clinically significant antiphospholipids are relatively rare. It is not prudent to screen healthy individuals. This includes pregnant women with no history of recurrent fetal loss.
4. Tests for antiphospholipid antibodies are only part of the diagnostic evaluation of **unexplained** venous or arterial thrombosis, **unexplained** recurrent fetal loss, or the **classification** of systemic lupus erythematosus. Other, often more common causes should be considered as well.
5. Therapy is directed at the thrombotic episode. There is no evidence that immunosuppression is of additional benefit. Life-long anticoagulation should be considered in patients with arterial thromboses or life-threatening venous thromboses.
6. The treatment of pregnant patients with aspirin and heparin is as efficacious as treatment with aspirin and prednisone, with fewer side effects. The risk of fetal loss is lowered, but not eliminated by therapy.

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