INTRODUCTION

Definition

APS is an auto immune disease in which auto antibodies are detected against cell membrane phospholipids and is clinically characterised mainly by thrombocytopenia, thrombosis, neurologic and dermatologic manifestation and recurrent fetal loss. More recently it is found that these antibodies react with specific plasma proteins associated with phospholipids (e.g. β2 glycoprotein1 β2gp1) rather than against the anionic phospholipids directly.

Antiphospholipid - protein auto antibodies (aPL or APA)
Currently 3 of these proteins are recognized to have important clinical significance. They are
1. anti cardiolipin antibodies (aCL)
2. Lupus anticoagulant (LA)
3. Anti β 2 glycoprotein 1 antibodies

CLASSIFICATION

1. Primary APS. When APS is not associated with any other well recognized conditions especially immunological diseases and form about 50%.
2. Secondary APS. When APS is secondary to other conditions Table I
3. Catastrophic APS - in which there is an acute severe multiple organ APS illness.

PATHOGENESIS

The exact mechanisms by which antiphospholipid antibodies (aPL antibodies) produce the various clinical manifestations including thrombosis are not well understood but there are many proposed mechanisms.

<table>
<thead>
<tr>
<th>Clinical diagnoses associated with antiphospholipid - protein antibodies</th>
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<tbody>
<tr>
<td><strong>Primary antiphospholipid-protein antibody syndrome</strong></td>
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<tr>
<td>- Autoimmune disorder with no apparent cause</td>
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<td><strong>Secondary autoimmune disorders</strong></td>
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<td>- Systemic lupus erythematosus; other autoimmune and connective tissue diseases; drug induced: Procainamide, hydralazine, quinidine, phenothiazines, penicillin</td>
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<tr>
<td><strong>Malignancies</strong></td>
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<tr>
<td>- Leukemia, lymphoproliferative and plasmacytic disorders, solid tumors, essential thrombocytosis</td>
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<tr>
<td><strong>Infections</strong></td>
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<tr>
<td>- Viral, bacterial, protozoal, fungal</td>
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<tr>
<td><strong>Neurologic disorders</strong></td>
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<tr>
<td>- Liver disease Valvular heart disease Peripheral arterial disease Chronic renal failure Sickle cell disease Ethylenediaminetetraacetic acid-dependent pseudothrombocytopenia No apparent disease</td>
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Potential mechanisms are
1. aPL antibodies cause endothelial activation. They directly bind to the endothelial cells and upregulate the secretion of adhesion molecules and cytokine secretion (e.g., tissue factor expression which is very important in initiation of thrombosis).
2. Oxidant mediated vascular injury. Autoantibodies to oxidized LDL occur with aCL antibodies and aCL antibodies recognize oxidized phospholipids and phospholipid-binding proteins causing vascular injury.
3. The aPL antibodies interfere with the function of phospholipid binding proteins in regulating coagulation. They interfere with the anticoagulant function of Protein C or annexin V or enhance procoagulant activity.

In many immunological studies it has become clear that the aPL antibodies play a direct role in initiation, propagation or maintenance of thrombosis. The aPL antibodies act by interfering with coagulation, possibly involving dysfunction or apoptosis of endothelial cells, platelets or coagulation factors. Specific mechanisms of thrombosis that have been implicated are inhibition of Protein C, acquired Protein S deficiency, platelet activation and abnormalities in the antigenic levels or activity or endothelial derived hemostatic factors. These include inhibition of prostacycline secretion, fibrinolysis or disruption of annexin A5 or A2. The aPL antibodies also may interfere with endothelial cell phospholipids required for antithrombin and Protein C and S anticoagulant activity and prostacycline synthesis. They also increase endothelial cell expression of the procoagulants like tissue factor, vWF, platelet activating factor and plasminogen activator inhibitor type 1.

Pathogenic mechanisms in recurrent fetal loss
Thrombotic events at the placental level will explain many of the clinical manifestations. The aPL antibodies may induce intervillous thrombosis and intravillous infarction resulting in poor placental perfusion. They also affect cytotrophoblastic tissue in vitro. aPL antibodies inhibit prostacyclines, reduce Protein C, produce chronic villitis and or decidual plasma cell infiltration. Annexin A5 & A2 present in the placenta function as a good anticoagulant. aPL antibodies act against annexin promoting thrombosis. Normally annexin A5 may cover thrombogenic anionic surfaces and prevents the activation of Factor X and prothrombin. aPL/β2gp1 antibodies might disrupt such a shield on trophoblast and endothelial cell monolayers.

It is also demonstrated that the aPL antibodies evoke a local inflammatory response which culminates in fetal loss. There is also evidence supporting the ability of aPL antibodies to target maternal decidua and the invading the trophoblast directly. This leads to defective placentalation of the fetus. The antibodies may induce cell injury, apoptosis, inhibition of proliferation and syncytia formation, decreased human chorionic gonadotropin production and defective invasiveness. All of these aPL antibodies mediated effects might play a role in causing defective placentation.

Mechanism of prolonged PTT in APS
In this coagulation test phospholipids that are added from outside binds to prothrombin during the test. When there is LA, these antibodies impair the function of phospholipid bound prothrombin prolonging APTT. This prolongation is not corrected by normal plasma during mixing studies.

Epidemiology and clinical associations
Prevalence of aPL antibodies in young apparently healthy control subjects are reported to be 1 - 5%. In SLE aPL occurs in 12 - 34%. If SLE patients are followed up for longer periods up to 50% of the patients might show aPL antibodies. LA and aCL antibodies have been detected in a variety of medical conditions including SLE and other autoimmune and connective tissue disorders. They are also seen with drug administration, malignancies, infections and in a number of systemic diseases. The frequency and the interpretation depend upon the sensitivity of the different assay systems used. APA positivity is a common finding in patients with ITP, apparently healthy elderly people and 5 - 10% of normal blood donors.

Clinical manifestations
Clinical manifestations associated with all the three types of aPL antibodies are same although the antibodies are immunologically different.

Clinical experience show that LA antibodies are more associated with venous and aCL antibodies with arterial thrombosis.

A wide variety of clinical manifestations are seen with APS. However many patients with APS remain asymptomatic and a proportion of the asymptomatic patients develop SLE or other disorders. The various clinical manifestations of APS are given in Table II.

Arterial and venous thromboembolic disease
This is the most common clinical manifestation of APS.
Asymptomatic Arterial and venous thromboembolism
- Avascular osteonecrosis

Hematologic
- Cytopenias: Thrombocytopenia, autoimmune hemolytic anemia, leukopenia
- Coagulopathy: Platelet dysfunction, prothrombin deficiency, lupus anticoagulant

Neurologic
- Acute ischemia (cerebrovascular accident, transient ischemic attack, encephalopathy); severe migraine; multiple infarct dementia; cognitive dysfunction; seizures

Dermatologic
- Livedo reticularis; acrocyanosis (distal cutaneous ischemia, ulceration, gangrene); widespread cutaneous necrosis; pyoderma gangrenosum-like skin lesions; anetoderma

Cardiopulmonary
- Marantic endocarditis; myocardial ischemia and infarction; intracardiac thrombotic mass; peripheral arterial disease; thromboembolic and nonthrombotic pulmonary hypertension

Obstetric
- Recurrent spontaneous abortion; intrauterine growth restriction; preeclampsia; chorea gravidarum; low Apgar scores; prematurity

Catastrophic antiphospholipid syndrome

Table II. Clinical Manifestations Of Antiphospholipid Antibodies

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Frequency</th>
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<tr>
<td>Most common site of involvement is lower extremity deep veins.</td>
<td>1.</td>
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<tr>
<td>Unusual sites like axillary retinal and hepatic veins and cerebral venous sinuses are sometimes involved.</td>
<td>2.</td>
</tr>
<tr>
<td>Cerebral thrombosis</td>
<td>3.</td>
</tr>
<tr>
<td>Mesenteric artery occlusion, adrenal occlusion, gastrointestinal ischemia, subclavian thrombosis</td>
<td>4.</td>
</tr>
<tr>
<td>Multiple cerebral infarcts with dementia</td>
<td>5.</td>
</tr>
<tr>
<td>Coronary occlusion</td>
<td>6.</td>
</tr>
<tr>
<td>Avascular osteonecrosis</td>
<td>7.</td>
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Antibodies to β2gp1 are found to be highly associated with venous thrombosis.

Thrombocytopenia

In about 50% of cases, moderate immune thrombocytopenia is found. It is no longer considered as a clinical criterion for the diagnosis of APS. It is important to note that thrombocytopenia of obscure etiology including ITP like syndromes should be screened for aPL antibodies. Sometimes patients with APS may present with bleeding. This may be due to thrombocytopenia, functional or other underlying coagulopathies. Other cytopenias like auto immune hemolytic anemia and leukopenia may be associated with APS.

Neurological manifestations

Many disorders which are not associated with ischemia and thrombosis can occur. The exact reason for these manifestations is not fully understood. These include Dementia, migraine, chorea, seizures, transverse myelitis, GBS, mononeuritis and myasthenia gravis.

Dermatological manifestations

1. Livedo reticularis which is more prevalent in females with APS secondary to SLE
2. Acrocyanosis leading to distal cutaneous ischemia, ulceration and gangrene
3. Wide spread cutaneous necrosis
4. Pyoderma gangrenosum-like skin lesions

Cardiac Manifestations

It is good to screen patients undergoing reconstructive vascular surgery and in whom antiplatelet drugs are considered after the procedure. There is a high incidence of aPL antibodies in some of these patients who experience an associated high risk of early graft thrombosis. A variety of valvular heart lesions are associated with APS in addition to myocardial ischemia and infarction.

Pulmonary disease

APS is linked to ischemic and thrombotic pulmonary disease including pulmonary embolism. Pulmonary hypertension, intra alveolar hemorrhage and ARDS are also noted.
Obstetric manifestations
APS is one of the commonest causes for Recurrent Spontaneous Fetal Loss (RSFL). The definition of RSFL is three or more consecutive pregnancy loss at 20 weeks or less or with fetal weight less than 500g. In 40 - 50% of RSFL, the cause remains unidentified. In about 16 - 36% of patients with RSFL, APS is identified.

RSFL in APS is due to a variety of reasons which include intrauterine growth retardation (IUGR), pre eclampsia, premature placental separation, DVT, HELLP (Hemolysis Elevated Lever Enzymes and Low Platelets) and DIC. Normal fetal karyotype is the rule.

Obstetric criteria for diagnosis of APS listed by international consensus are
a. One or more unexplained deaths of a morphologically normal fetus at or beyond the tenth week of gestation (with normal fetal morphology) or
b. One or more premature births of a morphologically normal neonate before the 34th week of gestation because of pre eclampsia, eclampsia or placental insufficiency or
c. Three or more unexplained consecutive spontaneous abortions before the tenth week of pregnancy (with exclusion of maternal anatomic and hormonal abnormalities and paternal and maternal chromosomal causes)

Patients with RSFL should be tested for aPL antibodies. Many studies have shown that repeated testing may be necessary to increase the sensitivity and specificity of the diagnosis. Elevated maternal alpha feto protein and human chorionic gonadotropin are common in women with aPL antibodies (APA) and are significantly associated with fetal loss.

CATASTROPHIC APS (CAPS)
Some of the patients develop an acute severe form of APS characterized by diffuse small vessel occlusion and ischemia with extensive tissue damage and multiorgan dysfunction, this condition has a high mortality of 50%. Usually there is no precipitating event. The small vessel thrombotic occlusions occur in the kidney, lung, CNS, heart and skin. The syndrome is defined by clinical involvement of at least three different organ systems with histological evidence of thrombosis. Differential diagnosis includes acute severe lupus vasculitis, TTP and DIC. In some studies 50% of patients had underlying SLE and 40% were primary APS.

DIAGNOSIS OF APS
Currently the diagnosis of APS is based on the international consensus statement on an update of the classification criteria for the definition of APS. Table III. This includes clinical events and laboratory abnormalities. A diagnosis

<table>
<thead>
<tr>
<th>Clinical Event</th>
<th>Laboratory Abnormality</th>
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<tbody>
<tr>
<td>Venous thrombosis</td>
<td>Positive lupus anticoagulant test</td>
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<tr>
<td>or Arterial thrombosis</td>
<td>Positive anticardiolipin antibody test (moderate-titer or high-titer IgG or IgM antibodies)</td>
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<tr>
<td>or Small-vessel thrombosis</td>
<td>Positive BS2 antibodies test (titer &gt;99th percentile, IgG or IgM antibodies) and Laboratory abnormality should persist for two or more occasions at least 12 wk apart</td>
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<tr>
<td>Complications of pregnancy (One or more unexplained deaths of normal fetuses or after 10 wk of gestation with normal fetal morphology or one or more premature births of normal neonates before 34 wk of gestation or three or more unexplained consecutive spontaneous abortions before 10 wk of gestation, excluding anatomic, hormonal, and chromosomal abnormalities)</td>
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Table III. Criteria For Diagnosis Of The Antiphospholipid - Antibody Syndrome (ASP)
of definite APS requires the presence of at least one clinical event and at least one laboratory abnormalities. These are mainly required for a standardization of research activities. Sometimes a more practical approach for the diagnosis is made at the bedside depending on the clinical situation and limited laboratory investigations especially in India.

**Lab diagnosis of Antiphospholipid - Protein antibodies**

The usual tests done are for
1. LA
2. aCL IgG & IgM
3. β2gp1 IgG & IgM

There are various methods of doing these tests. In India we need to standardize these tests in a much better way. The ideal test to identify LA is controversial. Some investigators have reported the Kaolin clotting time to be the most sensitive test. On the other hand some DRVVT (direct Russell Viper Venom test) assays are more able to identify LA associated with thrombosis. aCL and β2gp1 antibodies are detected by ELISA or by radioimmunoassay. Commercial ELISA systems are reasonably well standardized.

For the diagnosis of APS in addition to a clinical event (arterial, venous, or small vessel thrombus/ pregnancy morbidity, mortality) positive laboratory tests for LA, aCL or β2gp1 antibodies (in medium or high trite) should be found on two occasions at least 12 weeks apart. Measurement of β2gp1 antibodies is now recommended as a definite diagnostic test for APS. The consensus criteria require persistence of laboratory abnormality for at least 12 weeks. But in clinical practice a functional definition taking into account the number & nature of clinical manifestations and the titre of aPL antibodies on even a single occasion may help to categorize patients having definite, probable or doubtful APS. According to this practical method immediate treatment options can be implemented without waiting for a confirmation after 12 weeks. A triple positive profile (all the three antibodies being positive) has been identified as a strong independent risk factor for thrombosis.

There are many reports where clinical manifestations may occur without the detection of antibodies (SNAPS sero negative APS). The treatment options in such a situation become controversial and there is no consensus.

**TREATMENT OF APS**

The optimal treatment (especially the duration and the intensity) of APS is still undefined though randomized treatment trials have given us useful information.

Asymptomatic patients with positive antibodies are not treated by most of the investigators prophylactically. But they are given short term anticoagulant interventions when additional thrombophilic hazards such as immobilization or surgery are anticipated.

Patients with significant thrombotic events like deep venous thrombosis, arterial ischemia or fetal loss are appropriate patients for antithrombotic treatment. Most of these patients are initially given heparin. The usual regimen is a Low molecular weight heparin (ENOXAPARIN or NADROPARIN 0.4 ml (40 mg - 3800 Iu)) given sub cutaneously twice daily. Patients should not have a contra indication like high risk of bleeding or prior history of heparin induced thrombocytopenia. Patients with renal insufficiency are given unfractionated heparin which can be stopped immediately in the event of bleeding.

Oral anti coagulants like warfarin should overlap the initial heparin treatment for 4 - 5 days till a therapeutic range of INR is reached. Clinical trials have shown that standard intensity warfarin treatment (target INR between 2 and 3) is the best option rather than high intensity warfarin (INR between 3.1 to 4 or 4.5). After 12 weeks of initial treatment aPL antibodies should be tested again to confirm APS. If the antibodies are still positive, treatment with oral anti coagulants should be continued indefinitely. Diagnostic tests for LA may be falsely positive in some warfarin treated patients. So the physician should interrupt warfarin treatment (several days) prior to obtaining a plasma sample for repeat LA testing after 12 weeks. Long term treatment with warfarin is currently strongly recommended because of the high rate of recurrence. It was clear from clinical trials that patients continuing oral anti coagulants over 8 years did not have recurrence. Patients who discontinued oral anti coagulants had a 50% probability of recurrent thromboembolic episode after 2 years and an almost 80% probability of recurrence after 8 years. So oral anti coagulation is recommended indefinitely- even life long.

If the initial high titres of antibodies continually become absent for at least 6 months one can consider stopping the anticoagulation but such patients should have no other thrombophilic risks and close surveillance is feasible. There is no high quality published evidence that clearly defines a time interval beyond which the risk of anticoagulation outweighs the benefits.
Literature review of treatment of APS patients with ischemic stroke concluded that treatment with either aspirin or standard intensity warfarin (2-3) is effective. Another recommendation based on Warfarin-Aspirin Recurrent Stroke Study (WARSS) is antiplatelet therapy instead of anticoagulation for most patients. For non cerebral arterial thrombus, combined treatment with standard intensity warfarin (2.0 to 3.0) and aspirin 75mg is recommended. Patients with uncommon LA hypo prothrombinemia syndrome who may develop bleeding or need surgery may benefit from steroid therapy or intravenous IgG.

In patients with LA and acute thrombus difficulties may arise in monitoring unfractionated heparin treatment with APTT. Since APTT is usually prolonged in LA option is either using LMWH which does not require monitoring or using heparin assays for monitoring. Sometimes LA can interfere with PT also. In such situations prothrombin preconversion assay or a Chromogenic Factor X assays may be used. Corticosteroid therapy often abolished the coagulation abnormalities and immune thrombocytopenia of APS.

Additional therapies like aspirin, corticosteroids and pulse doses of immuno suppressive drugs like cyclophosphamide are not usually recommended in patients receiving warfarin. They are used only when recurrent thrombotic or ischemic events are seen despite warfarin therapy.

In patients with CAPS, intensive treatment with corticosteroids, immuno suppressive drugs, intravenous IgG or plasmapheresis is used but mortality is very high. Rituximab is found to be useful in one study.

Obstetric treatment
Satisfying and gratifying results are obtained when women with RSFL are treated. Patients with recurrent abortions and fetal loss should be intensively tested for aPL antibodies. If they are tested positive for aPL antibodies and are not treated, pregnancy loss may be up to 90%. Many drugs including aspirin low dose corticosteroids and anticoagulation have been tried. Intravenous IgG is of no benefit for these patients.

Patients with RSFL with positive aPL antibodies should be counselled regarding the treatment. The pregnancy should be planned. Aspirin 75mg may be started one or two months before the planned conception. Low molecular weight heparin should be started as soon as the pregnancy is confirmed by an ultrasound examination. There are still controversies regarding the intensity of heparin treatment. For patients with prior history of thrombosis therapeutic doses of heparin is definitely indicated. Low molecular weight heparin for example ENOXAPARIN or NADROPARIN 0.4 ml (40 mg, 3800 Iu) s/c is given twice daily (12H) till the onset of labour. Heparin is restarted after delivery. Warfarin is started and overlaps with heparin treatment till INR target is between 2.0 and 3.0. At this point heparin is stopped and oral anticoagulants are continued for 3 to 6 months.

For patients without a prior thrombosis heparin is given prophylactically (this means LMWH once daily) by many physicians and also at a therapeutic dose by others. In our centre we give therapeutic doses of heparin for this group of patients.

For patients who cannot afford low molecular weight heparin conventional or unfractionated heparin can be used in a dose of 5000 - 10000 units s/c daily or twice daily. This treatment has to be monitored by APTT which should be two to two and a half times the normal value. LMWH does not require monitoring.

By treatment live birth rate is improved in this group of patients from 0 - 40% to 70 - 80%. Our centre has a success rate of about 90%. Heparin treatment in RSPL who are aPL negative is debated.

Aspirin increases prostacyclines and reduces thromboxane A2. Heparin is found to have anti inflammatory actions in addition to anticoagulation. It is also found that the beneficial effects of anticoagulants in APS associated pregnancy loss result from inhibition of complement activation.

Special issues in treatment
In patients taking warfarin there can be artifactual elevations of INR which may be depending on the type of instrument and thromboplastin. So INR reagent insensitive to the effect of aPL should be used.

Spinal hematoma may be associated with LMWH when used in pregnancy especially with advanced age, concomitant antiplatelet therapy and traumatic needle/catheter placement. The most important factor which influences the bleeding risk is the interval between LMWH injection and the time of needle/catheter placement or removal. Patients with aPL antibodies undergoing major surgery should have LMWH (prophylactic dose) or injection fondaparinux 2.5mg daily started 6 - 8 hours after surgery. Extended use
of thromboprophylaxis is given for surgeries like total hip arthroplasty (28 - 35 days).

Some patients may develop symptoms of thrombosis while on anticoagulation in therapeutic doses. The various options in such difficult resistant cases is to increase the INR target from 3.0 to 4.0, adding antiplatelet drugs like aspirin, combining the above two or treat the patient with therapeutic dose of LMWH. Published experience suggests that treatment with LMWH in such a situation may be as long as 6 years without overt complications. Recent studies have shown the benefits of hemopoietic stem cell transplantation (HSCT) especially for refractory SLE and Rituximab in resistant cases of APS where aPL antibodies cleared in majority of patients.

FUTURE
Better diagnostic methods, safer anticoagulation by newer drugs and more targeted treatment for the antibodies (monoclonal antibodies) are all in the horizon.

In summary APS is a common auto immune disease which can sometimes be devastating. The common antibodies detected are LA, aCL and β2gp1 antibodies. APS can be primary or secondary to various conditions. Patients with APS can remain asymptomatic. Others may mainly develop acute arterial or venous thrombosis or RSFL. More light is now thrown into the pathogenesis of APS. Diagnostic criteria are updated. Treatment options and methods have become clearer and evidence based. The future depends on evolution of newer oral anticoagulant drugs and targeted therapy for the auto antibodies.

REFERENCES
5. Gracia A D, Kamashta MA, Crowther MA. How we diagnose and treat thrombotic manifestations of the antiphospholipid syndrome: a case based review. Blood 2007; 110: 3122 - 3127