SEED Haemostasis

Antiphospholipid syndrome

Introduction

Haemostasis is a complex process that helps to keep the blood in a fluid state and prevent blood loss at the site of injury. While the intact endothelium of blood vessels has an anti-thrombogenic function that prevents blood coagulation, in the case of vessel wall damage, the exposed sub-endothelial components initiate the formation of a clot that will stop blood loss. Under healthy conditions, the mechanism of clot formation (pro-coagulant) and clot destruction (anti-coagulant) are well balanced. An abnormal increase of the pro-coagulation and/or decrease of the anti-coagulation mechanisms results in thrombotic disorders. These are medical conditions characterised by the formation of an unwanted clot, mostly in veins, but also in the arteries. On the other hand, an abnormal decrease of the pro-coagulation and/or increase of the anti-coagulation mechanisms results in bleeding disorders. In both disorders, we distinguish between acquired and congenital disorders.

What is the 'antiphospholipid syndrome'?

Antiphospholipid syndrome (APS) is an acquired prothrombotic autoimmune disorder defined by arterial or venous thrombosis and/or pregnancy morbidity in patients who exhibit a persistent presence of antiphospholipid antibodies (aPL) – mostly IgG and IgM subtypes, but more rarely also IgA. [1] According to Sydney revised Sapporo criteria, APS is diagnosed based on clinical and laboratory criteria summarised in Table 1. Definite APS is considered present if at least one of the clinical and one of the laboratory criteria are met.

Table 1 Criteria for APS according to Sydney revised Sapporo criteria classification

Clinical criteria	
Vascular thrombosis	One or more episodes of arterial, venous or small vessel thrombosis in any tissue or organ (confirmed by imaging or histopathology).
Pregnancy complications	Recurrent pregnancy loss (after > 10 weeks' gestation) or one or more premature births due to pregnancy complications.

Laboratory criteria	
Detection of lupus anticoagulant	Lupus anticoagulant (LA) in plasma on two occasions at least 12 weeks apart.
Detection of anti-cardiolipin antibodies	Anticardiolipin/antiphospholipid antibodies (ACA/APA) of IgG and/or IgM isotype on two occasions at least 12 weeks apart.
Detection of anti-β2 glycoprotein 1 antibodies	Anti-β2GPI antibodies of IgG and/or IgM isotype on two occasions at least 12 weeks apart.

The syndrome can either be associated with an existing auto-immune disease, in which case it is called 'secondary APS' or, if there is no evidence of an existing underlying disease, it is called 'primary APS'. Most of the patients have a primary APS (53 %), while the others have a secondary APS with underlying auto-immune diseases summarised in Figure 1.

APS patients associated with autoimmune diseases

- 1%
- 1%
- 1%
- 4%
- 5%
- 11%
- 77%

- Systemic lupus erythematosus
- Lupus-like syndrome
- Primary Sjogren's syndrome
- Rheumatoid arthritis
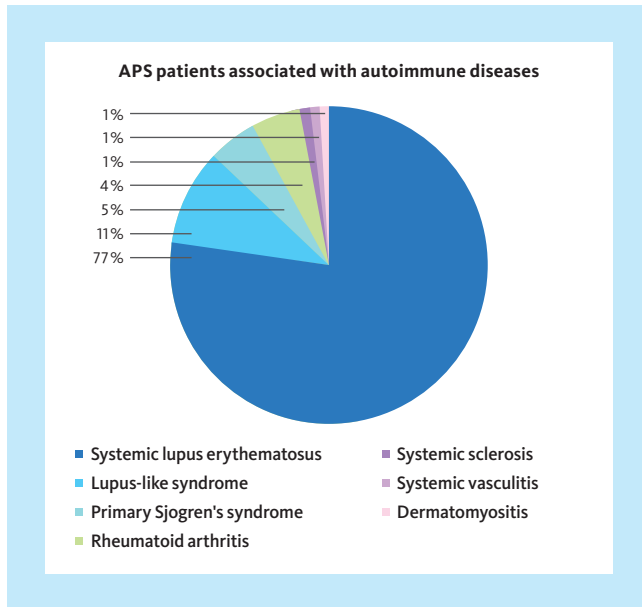- Systemic sclerosis
- Systemic vasculitis
- Dermatomyositis

Fig. 1 Distribution of primary autoimmune diseases in patients with secondary APS

Apart from the primary autoimmune diseases mentioned above, several other diseases are associated with the development of aPLs. Most of them come from bacterial, viral or parasitic infections, and lymphoproliferative disorders (e.g. lymphoma). But also, some medications may increase the risk of developing antibodies against phospholipids. In most cases, these aPLs are transient and not associated with a clinical APS. [2]

Who is affected?

Up to 5–10 % of the healthy population carry antiphospholipid antibodies in their blood without exhibiting a clinical sign of an APS.

The APS can be developed at any age, regardless of it being primary or secondary. Women are more frequently affected than men. The ratio between both sexes varies in studies between 5:1 and 7:1 for females to males. However, with 7:1, this ratio is higher in patients with systemic lupus erythematosus (SLE) than in patients with primary APS (3.5:1). In one third of the cases, individuals with primary SLE develop antibodies against phospholipids.

What are the clinical symptoms?

The most common clinical manifestations are venous or arterial thrombosis or pregnancy complications in the presence of aPLs.

Deep vein thrombosis (DVT) occurs in up to 40 % of APS patients and, with almost 50 % of the DVT cases, the rate of subsequent pulmonary embolism is higher in APS patients than in non-APS patients with DVT. Frequent clinical symptoms are summarised below and the risk of developing symptoms based on the anti-body profiles is listed in Table 2.

Clinical symptoms in APS patients

Arterial thrombosis
- Stroke
- Transient ischaemic attack
- Ocular vascular diseases
- Gastrointestinal ischaemia
- Myocardial infarction and intra-cardiac thrombosis
- Peripheral artery thrombosis including digital or critical ischaemia of lower limbs with extreme gangrene
- Renal artery thrombosis

Venous thrombosis
- DVT of lower limbs
- Pulmonary embolism
- Cerebral vein thrombosis
- Renal vein thrombosis
- Ocular vascular disease
- Gastrointestinal thrombosis

Small vessel thrombosis
- Renal thrombotic microangiop-athy (TMA) in renal glomeruli
- Myocardial microthrombosis
- Encephalic microthrombosis
- Adrenal microthrombosis leading to a paradoxical haemorrhage

Obstetrical manifestation
- Foetal loss typically after 10 weeks' gestation
- Pre-embryotic or embryotic pregnancy loss (< 10 weeks' gestation)
- Placental insufficiency

Non-thrombotic manifestation
- Hyperplasia
- Focal cortical atrophy
- Kidney failure
- Cardiopulmonary manifestation
- Cutaneous manifestation
- Haematologic manifestation (e.g. thrombocytopenia)
- Non-thrombotic neurological manifestation

Table 2 Risk of clinical complications based on antibody profile

Risk level	Antibody profile presentation
High risk	Triple positive antibody profile: - LA tested positive and/or - medium to high ACA titre of IgG or IgM subtype and/or - medium to high anti-β2GPI antibody titre of IgG or IgM subtype
Medium risk	Double positive antibody profile: - LA negative, but - medium to high ACA titre of IgG or IgM subtype and/or - medium to high anti-β2GPI antibody titre of IgG or IgM subtype
Low risk	Single positive antibody profile: - LA negative, - low titre of ACA and/or - low titre of anti-β2GPI antibodies
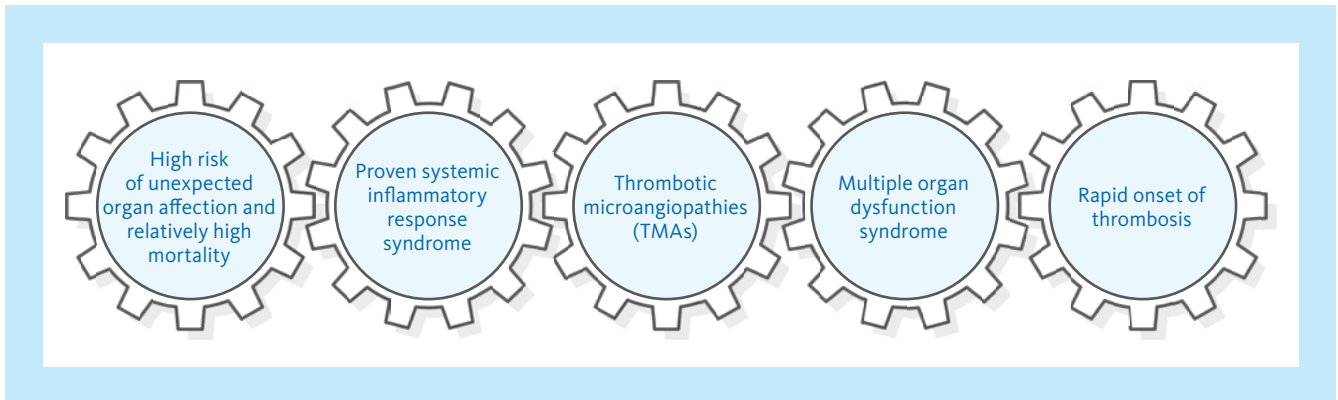
Fig. 2 Clinical characteristics of CAPS

A severe and very rare form of APS is the catastrophic antiphospholipid syndrome (CAPS). In this state, despite therapy, patients have a high tendency to develop thrombosis and bleeding, which can lead to death within a short time. The CAPS is mainly defined by thromboembolic complications in three or more organ systems of which particularly the kidneys, lungs and central nervous system (CNS) are most commonly affected. In CAPS, thrombi are found in many small vessels and not in the large ones, which is in contrast to the usual forms of APS. [3]

The cause has not yet been fully clarified, but previous infections, surgeries, the termination of anticoagulant therapy and the use of hormonal contraceptives are considered triggers of CAPS.

How is APS developed?

The mechanisms leading to the formation of aPLs and why they are a risk factor for thrombophilia is not exactly known in detail. However, various pathophysiological mechanisms are involved.

Antibodies directed against β2GPI are considered to be most relevant because anti-β2GPI antibodies are highly associated with the clinical presentation of APS. However, the detection of anti-β2GPI antibodies in healthy individuals suggests that the antibody alone is insufficient for a pathogenesis of APS. Apart from β2GPI, prothrombin, annexin A5, thrombomodulin, protein C, protein S and low and high kininogen are targets of aPLs. [4]

The most common explanation of the pathogenesis is given by the two-hit theory proposing a primary injury, such as a vessel wall injury, infection or recent surgery, irritates the endothelium first (first hit) and then a second injury triggers the thrombosis (second hit). [5, 6] The second injury can be a condition such as smoking, immobilisation, pregnancy or malignancy. [7, 8]

Mechanisms that trigger thrombosis
The mechanisms of thrombosis in APS patients are largely dependent on the patients. A frequently reported explanation is that anti-

bodies directed to domain I of β2GPI are produced by B cells after the 'first hit'. [9, 10] β2GPI is a protein able to bind to negatively charged molecules such as phospholipids, cardiolipin, phosphatidylserine and other phospholipids of activated endothelial cells, trophoblasts, monocytes and platelets. After the antibodies have bound to β2GPI, the β2GPI-antibody complexes can bind to receptors on the cell surface [9, 10, 11], leading to cellular activation and the interaction with phospholipid-dependent coagulation factors on the surface of the cells. This is subsequently causing the induction of a prothrombotic cellular phenotype with tissue factor (TF) expression and the inhibition of tissue factor pathway inhibitor (TFPI) expression. [12, 13] The inhibition of protein C activity [14], complement activation, release of cytokines, adhesion molecules and the release of interleukin 8 as well as the contents of neutrophil extracellular traps (NETs) from neutrophilic granulocytes furthermore inactivate the antithrombotic surface of endothelial cells and create a prothrombotic situation. [15–17]

Mechanisms that trigger pregnancy complications
The mechanisms that trigger pregnancy complications in APS patients are largely dependent on the patients. One trigger is anti-β2GPI antibodies stimulating the trophoblasts to increase the secretion of vascular endothelial growth factor, placental growth factor, and endoglin, which significantly increases the risk of obstetric complications. The β2GPI antibody complexes also disrupt the anticoagulant barrier formed by annexin A5 on vascular cells. All these factors can lead to placental thrombosis, which leads to foetal growth restrictions and/or loss of pregnancy.

How to treat APS? Which medication is available?

The treatment of APS must be individualised according to the patient's current clinical status and history of thrombotic events. Asymptomatic individuals with positive blood test findings may not require specific treatment, while patients with CAPS require intense monitoring and treatment, often in an intensive care environment. [18]

Prophylactic treatment

Risk factors, such as oral contraceptives, smoking, hypertension, diabetes mellitus or hyperlipaemia must be treated consistently. Pharmacologic prophylaxis is mainly required during surgery or hospitalisation. Low-dose aspirin is used in most patients without a history of thrombosis or pregnancy complications. However, the effectiveness of low-dose aspirin as primary prevention for APS is not yet fully proven and studies are still ongoing. [19] Clopidogrel might be an alternative to aspirin in persons with APS intolerant to aspirin.

Treatment with hydroxychloroquine, which may have antithrombotic properties, is considered in patients with primary SLE and APS. [20]

Treatment of thrombosis in APS patients

Following a thrombotic event, full anticoagulation with intravenous or subcutaneous heparin followed by warfarin therapy is recommended. The target for the international normalised ratio (INR) is dependent on the patients, but the therapeutic ranges are 2.0–3.0 for venous thrombosis and 3.0 for arterial thrombosis. Patients with recurrent thrombotic events may require an INR of 3.0–4.0, while severe or persistent cases might require a combination of warfarin and aspirin. Treatment for patients with major, recurrent thrombotic events is generally lifelong, while in other cases treatment might be stopped at least if the aPLs are no longer detectable on two examination appointments at three-month intervals. However, a secondary antiplatelet treatment is recommended.

Single therapy with aspirin is an alternative to warfarin therapy in elderly patients after stroke with only low titres of antibodies. [21, 22]

Treatment of APS patients with new oral anticoagulants (i.e., direct thrombin inhibitors (DTI) and factor Xa inhibitors (DiXaI)) is currently lacking some data but might be considered in patients who are warfarin-intolerant or have poor anticoagulant response. [19, 20]

Prophylaxis of APS in pregnancy

Prophylaxis during pregnancy is provided with low molecular weight heparin (LMWH) in combination with low-dose aspirin. Women with APS and a history of thrombosis in previous pregnancies receive prophylactic anticoagulation during pregnancy and for six weeks postpartum. For women with APS and without a history of thrombosis, clinical monitoring or prophylactic use of LMWH is recommended antepartum, along with six weeks of postpartum anticoagulation. Generally, anticoagulant therapy is stopped at the time of delivery and restarted after delivery. The treatment is then continued for up to 6–12 weeks or remains lifelong in patients with a history of thrombosis. Warfarin is generally contraindicated during pregnancy. The use of heparin, aspirin and warfarin is not contraindicated with breast feeding. [23, 24]

Treatment in patients with CAPS

CAPS patients are at risk for life. Commonly, CAPS patients are treated with a combination of anticoagulant, corticosteroids, intravenous immunoglobulin, and plasma exchange. [25] Additional agents such as cyclophosphamide are beneficial in patients with SLE with some exceptions. [24] The use of hydroxychloroquine and intravenous immunoglobulin (IVIG) has been associated with good outcomes in pregnant women with APS who develop recurrent episodes of thrombosis or CAPS despite receiving adequate antithrombotic treatment. [26] Rituximab has shown some benefit in controlling severe thrombocytopenia, skin ulcers, and cognitive dysfunction that can be associated with APS and CAPS. [20]

What lab tests may I perform?

The most frequently tested antiphospholipid antibodies (aPLs) are LA, ACA and anti-β2GPI antibodies. Lab testing of asymptomatic individuals is not mandatory. [27] The presence of aPLs should be tested using a combination of different test systems due to the heterogeneity of the antibodies, but it is recommended to measure ACA and anti-β2GPI IgG and IgM on the same platform. At least two different functional tests (e.g. a lupus-sensitive APTT and the diluted Russel's viper venom time (dRVVT)) to detect LA and two immunologic test methods should be used. [28] It is recommended to run assays to detect LA, ACA and anti-β2GPI antibodies in patient samples obtained by the same sampling. All immunologic and functional test procedures must be repeated and confirmed after at least 12 weeks in order to be able to safely rule out the transient presence of antiphospholipid antibodies.
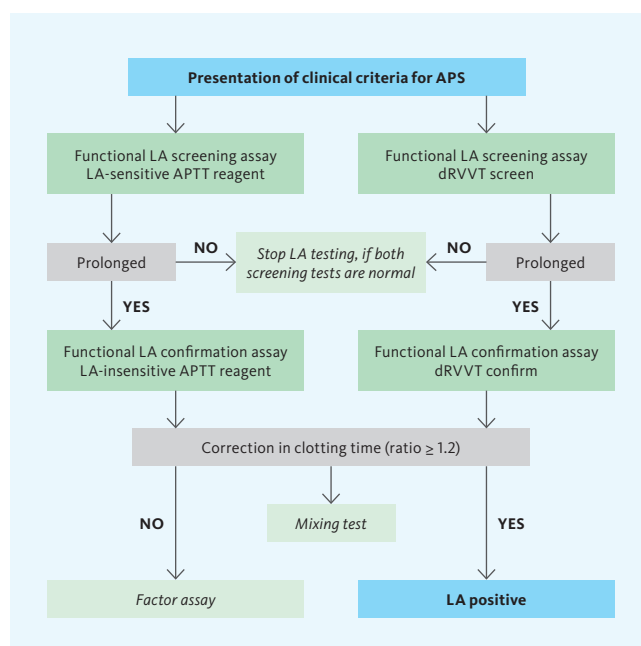

Fig. 3 *LA test scheme for individuals suspected of APS according to CLSI guideline*

Lupus anticoagulant (LA)

LAs are different types of antibodies directed against negatively charged phospholipids/protein complexes, therefore leading to prolonged clotting times in phospholipid-dependent tests. APTT assays using an LA-sensitive reagent and dRVVT screening reagent, each with a low concentration of phospholipids, are the preferred tests for assessing patients suspected of having APS. LA present in the sample inhibits the phospholipids from the reagent, leading to a prolonged clotting time. It is recommended each laboratory should determine their own cut-off levels for positive results by measuring LA in at least 40 healthy controls and determining the 99th percentile. Normalised ratios (test sample/the mean in the normal distribution) are recommended to compensate for inter- and intra-assay variation, and clotting time ratios (test/mean) > 1.2 for dRVTT are considered positive. [27] Positive results must be confirmed by running an LA-insensitive APTT and dRVVT confirmatory reagent. These reagents contain higher phospholipid concentrations expected to neutralise LA present in the test plasma and shorten the clotting time. Non-phospholipid-dependent inhibitors will still prolong the clotting time of the confirmatory test as they did in the screening test and prompt the adoption of a different diagnostic pathway. [29]

Non-phospholipid-dependent inhibitors can be further excluded by performing the mixing test. Mixing equal amounts of test plasma and normal pooled plasma (NPP) will replenish any clotting factors that are deficient in the test plasma sufficiently to restore the clotting time of the screening test to within normal limits. Conversely, any inhibitor present in the test plasma will maintain its inhibitory properties in the mixture with NPP such that an elevated clotting time persists. An elevated screening test result followed by a mixing test result correcting to within normal limits would be expected to initiate further tests for factor deficiencies, whilst a non-correcting mixing test result would initiate performing the corresponding confirmatory test to assess whether the inhibitor is phospholipid-dependent or not.
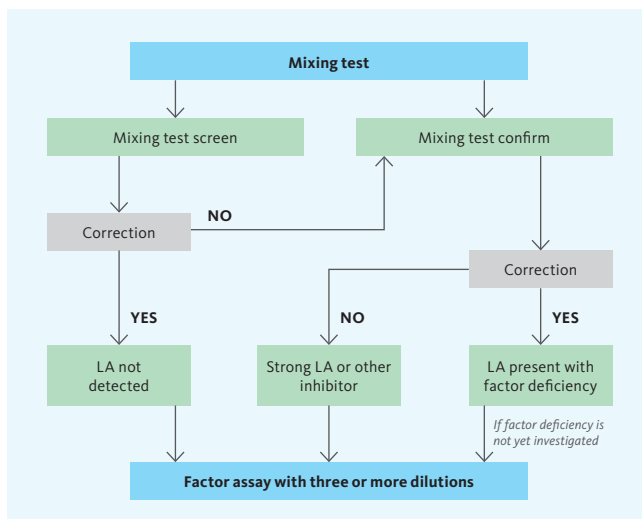
Performing a mixing test after screening tests is recommended in the SSC 2009 guideline for each patient in question while it is optional in the CLSI 2014 guideline. [27, 30] Other assays to detect LA in patient plasma are kaolin clotting time (KCT), diluted pro-thrombin time (dPT) or other snake venom assays.

Anti-cardiolipin antibody (ACA) assays

ACA assays are ELISA-based immune assays specifically measuring human anti-cardiolipin / antiphospholipid antibodies. ACA from the sample reacts with immobilised and saturated cardiolipin from the reagent. ACA assays usually show a poor inter- and intra-assay agreement mainly because of cut-off, calibration, and other meth-odological issues. [31–33] Expression of ACA assays in ranges of positivity achieves better interlaboratory and inter-run agreement than quantitative reports. [33] While IgM ACA tends to give false-positive results, particularly in the low-positive range, especially in the presence of rheumatoid factor or cryoglobulins, IgA ACA are not considered as a laboratory criterion for APS. [1, 35, 36]

Anti-β2GPI antibody assays

Anti-β2GPI antibodies are an independent risk factor for thrombosis and pregnancy complication. [37, 38] Anti-β2GPI assays are ELISA-based immune assays specifically measuring human auto- and alloantibodies to β2GPI. Anti-β2GPI antibodies from the sample react with immobilised and saturated β2GPI from the reagent. The anti-β2GPI assay shows higher specificity than ACA for APS diagnosis and is associated with pre-eclampsia and/or eclampsia in unselected pregnant women who tested negative for ACA. [39] The limitations of methodology and standardisation given for ACA also apply for anti-β2GPI. Interference of cryoglobulins and rheumatoid factors should be considered in the interpretation of IgM anti-β2GPI. In contrast to IgG and IgM, IgA anti-β2GPI anti-bodies are not associated with any clinical manifestation of APS. [1]
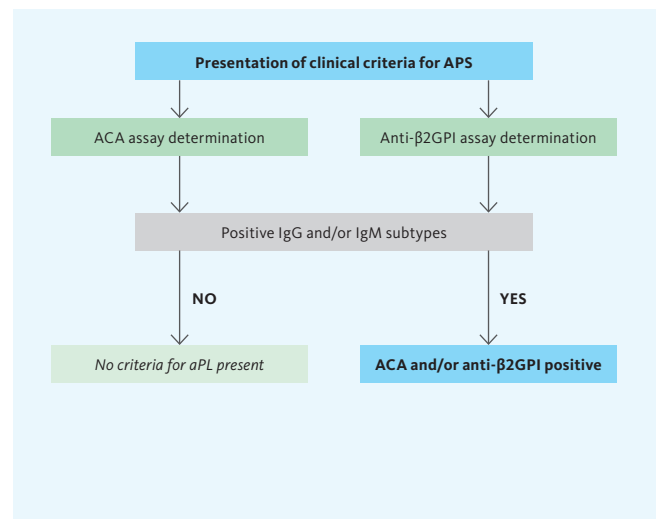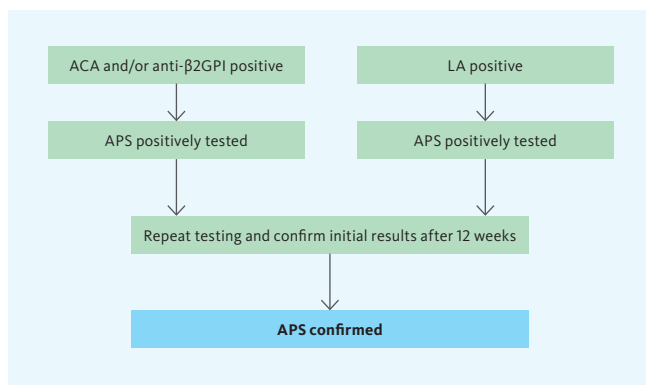

Fig. 4 Mixing test scheme for individuals suspected of APS according to CLSI guideline

Fig. 5 Solid phase assay scheme for individuals suspected of APS according to CLSI guideline

Fig. 6 Confirmation test scheme for individuals suspected of APS according to CLSI guideline

Platelets and red blood cells

Thrombocytopenia is common in individuals with APS and is associated with an ongoing thrombotic event. However, patients with platelet counts of less than 50,000/µL may have an increased risk of bleeding and require the administration of platelet concentrates. Haemolytic anaemia is frequently observed in patients with APS and associated with the presence of IgM ACA antibodies. [1]

Prognosis

Adequate medication and a lifestyle tailored to the disease presentation are essential for a good prognosis. A crucial factor here is patient education. Most patients with primary APS can lead a normal healthy life under a good therapy and with an appropriate lifestyle. However, despite extensive therapies, some patients still have thrombotic events, often with a devastating course of the disease, significantly increased morbidity or early death. In large European cohort studies, the overall 10-year survival rate is around 90–94 %. [40] However, every second patient who has developed a CAPS dies.

Before you leave…

Antiphospholipid syndrome is an acquired prothrombotic auto-immune disorder defined by arterial or venous thrombosis and/or pregnancy morbidity in patients who exhibit a persistent presence of antiphospholipid antibodies, mainly lupus anticoagulant, anti-cardiolipin antibodies and anti-β2GPI antibodies. It can affect individuals regardless of their age or gender and develops as a primary or secondary disease. Individuals are tested positive for APS if at least one clinical and one laboratory criteria is present, and the results are confirmed on two occasions 12 weeks apart. For patients suspected of APS, lab tests include functional and immunologic assays run in parallel. Treatment is highly patient-dependent and includes pharmacologic treatment as well as lifestyle adjustment.

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