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Hematological perspective of antiphospholipid antibody syndrome - An updated overview

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Abstract
Background: The antiphospholipid antibody syndrome (APS) is a systemic, acquired, immune-mediated disorder characterized by episodes of venous, arterial, or microcirculation thrombosis and/or pregnancy abnormalities, associated with the persistent presence of autoantibodies, confirmed at least in two occasions 12 weeks apart, directed to molecular complexes consisting of phospholipids and proteins. It comprises 1% of the general population.
Discussion: APS is a prothrombotic condition characterized by the presence of a lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti-β2-glycoprotein-1 antibodies in patients with recurrent pregnancy morbidity and/or thromboembolic complications. Thrombocytopenia is a frequent finding in patients with APS, and balancing the need for anticoagulationو when faced with significant thrombocytopenia can be a considerable challenge for clinicians managing such patients.
APS can cause preeclampsia (18%), pregnancy-induced hypertension, foetal death (7%), retardation (31%), premature labor (43%), stillbirth, and ultimately sterility. It is generally recommended that a panel of tests be done for diagnosis of APS. Patients who test positive for all three of the major assays—positive LAC, elevated anticardiolipin antibodies and elevated anti- beta 2GPI antibodies (referred to as **"**triple positivity"), are at markedly increased risk for thrombosis and for pregnancy complications.
Conclusion: APS is a prothrombotic disorder with various manifestations, most commonly venous and arterial thromboembolism and recurrent pregnancy loss. Diagnosis of APS can be challenging due to evolving criteria, potential limitations in the laboratory assays used to measure antiphospholipid antibodies and overlapping characteristics with other prothrombotic thrombocytopenic disorders. APS should always be considered as a potential diagnosis, especially for young patients presenting with a history of thrombotic events, in particular when they occur without any obvious external trigger conditions or/and for women with recurrent pregnancy loss or later fetal deaths. Many other disorders are able to mimic APS, so a broad range of alternative diagnoses should be investigated and ruled out during clinical work-up.

Keywords: Anticardiolipin antibodies, antiphospholipid antibodies, intrauterine foetal death, lupus anticoagulant, recurrent.

Introduction

The antiphospholipid antibody syndrome (APS) is a systemic, acquired, immune-mediated disorder characterized by episodes of venous, arterial, or microcirculation thrombosis and/or pregnancy abnormalities, associated with the persistent presence of autoantibodies, confirmed at least in two occasions 12 weeks apart, directed to molecular complexes consisting of phospholipids and proteins [1].

APS is a prothrombotic condition characterized by the presence of a lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti-β2-glycoprotein-1 antibodies in patients with recurrent pregnancy morbidity and/or thromboembolic complications [1].

Unexplained recurrent pregnancy loss is consistently detailed as more than two successive pregnancy losses. It found in about 1% of the general worldwide population focusing to have children. Autoimmunity performs an important role in recurrent pregnancy loss. It has been concluded that immunologic peculiarity may be a cause in many of such cases. The affected women have usually no other signs or symptoms referring an autoimmune disease.

APS cause preeclampsia (18%), pregnancy-induced hypertension, foetal death (7%), retardation (31%), premature labor (43%), stillbirth, and ultimately sterility [1].

Summary of the Sydney Consensus Statement on Investigational Classification Criteria for the AP

Antiphospholipid antibody syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met.

Clinical criteria

1) Vascular thromboses

One or more documented episodes of arterial, venous, or small vessel thrombosis—other than superficial venous thrombosis in any tissue or organ. Thrombosis must be confirmed by objective validated criteria.

For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.

2) Pregnancy morbidity

(a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or

(b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe pre-eclampsia defined according to standard definitions, or (ii) recognized features of placental insufficiency, or

(c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

In studies of populations of patients who have more than one type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects according to a, b, or c above.

Laboratory criteria

1. Lupus anticoagulant (LAC) present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LACs phospholipid dependent antibodies)
2. Anticardiolipin antibody (aCL) of IgG and/or IgMisotype in serum or plasma, present in medium or high titer (i.e., > 40 GPL orMPL, or > the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.
3. Anti-Beta 2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures.

Results of various studies compelled the authors to classify APS patients in following categories [3].

I, more than one laboratory criteria present (any combination);
IIa, LAC present alone;
IIb, aCL present alone;
IIc, anti- beta 2GPI antibody present alone

The sensitivities and specificities of the tests are variable, and a single negative test cannot rule out the diagnosis in a patient of APS

Phenomenon of Triple positivity [4]

It is generally recommended that a panel of tests be done for diagnosis of APS.

Patients who test positive for all three of the major assays—positive LAC, elevated anticardiolipin antibodies and elevated anti- beta 2GPI antibodies (referred to as **"triple positivity"**), are at markedly increased risk for thrombosis and for pregnancy complications.

Types of patients having antiphospholipid (aPL) antibodies [3, 4, 5].

I. Antiphospholipid syndrome

A. "Primary" – in the absence of SLE
B. "Secondary" – in patients with SLE

II. aPL antibodies stimulated by infection

A. No known association with thrombosis
(e.g., syphilis, Lyme disease, cytomegalovirus, Epstein-Barr virus)
B. Possible association with thrombosis
(e.g., varicella, HIV, hepatitis C)

III. Drug-induced aPL antibodies

(E.g. chlorpromazine and other phenothiazines)

IV. aPL antibodies prevalent in the general population

Tests used for diagnosis of the antiphospholipid syndrome [3].

Immunoassays

Biologic false-positive serologic test for syphilis
Anticardiolipin antibodies (cofactor-dependent assay)
Anti-beta 2GPI antibodies
Antiphosphatidylserine antibodies
Antiprothrombin antibodies

Coagulation Tests

Dilute Russell viper venom time (DRVVT) with confirmatory tests
aPTT:
—evidence of inhibitor with mixing studies
—panel of aPL-sensitive and insensitive aPTT reagents
—platelet neutralization procedure
Kaolin clotting time
Tissue thromboplastin inhibition test

Factors affecting the test results of APS [5, 6]

Preanalytical variables

The collection and preparation of blood samples for detection of LA, requires minimal venous stasis, rapid draw and immediate anticoagulation are essential. Plasma should be prepared within 1 h of blood collection by centrifugation at room temperature at 2000 g for 15 min. Contamination with platelets and other blood cells must be minimized as these will limit the sensitivity of tests, particularly after freezing plasma samples.

This may be achieved by: pipetting the plasma into a polypropylene tube and repeating the centrifugation. The control should be from 20 healthy normal subjects.

An ideal sample for LA should have a platelet count of less than 10 x10^9/l.

Screening tests for LA

The characteristics of the phospholipid component of the APTT reagent appear to be critical in determining its LA sensitivity, and reagents vary in both the types of phospholipid present and in their relative concentrations (Kelsey *et al*, 1984)

[5]. This variability results in inconsistent sensitivity of the test (Brandt *et al*, 1991) [7]. The acute phase reactions and pregnancy are associated with increased levels of fibrinogen and factor VIII, which tend to shorten the APTT and could mask a weak lupus anticoagulant. Therefore, a normal APTT is insufficient to exclude LA and additional tests must be performed.

A number of modifications of the APTT have been described for use as screening tests for LA, including the use of dilute APTT reagents (Alving*et al*, 1992), ratios of the APTT performed with LA-sensitive and -insensitive reagents (Brancaccio *et al*, 1997) and hexagonal phase phospholipids, which correct the clotting time (Rauch *et al*, 1989) [5, 6].

(ii) Dilute Russell's viper venom time (DRVVT). It activates factor X, which in turn activates prothrombin phospholipid, leading to the formation of a fibrin clot. If the venom is diluted to give a suitable clotting time and the phospholipid reagent is diluted so that its concentration becomes rate limiting, any inhibition of the coagulant active phospholipid by LA results in a prolonged DRVVT.

A number of commercial DRVVT kits are now available but they vary considerably in sensitivity and specificity(Medical Devices Agency, 1998) and performance is influenced by the type of coagulometer used (Lawrie *et al*, 1999) [8].

With regard to the methodological considerations, generally the upper limit of normal for the ratio with dilute phospholipid is between 1.1 and 1.2. A method is described

in the BCSH guidelines (Machin *et al*, 1991) [5, 6]. If in-house' reagents are used, the RVV reagent must be titrated to give a clotting time suitable for the end-point method in use (usually 20±40 s). The correct dilution of phospholipid must then be determined to obtain optimal sensitivity and specificity of the test. If the concentration is too high, the test may be insensitive to weak LA and specificity may be lost with variations in factor levels having too great an effect on the results.

The composition of the phospholipid reagent is also important and affects sensitivity. The optimal dilution can be selected by titrating the reagent with a normal plasma and a known LA-positive plasma. The dilution of phospholipid reagent that just starts to give a prolonged clotting time with normal plasma and shows a significant prolongation with LA plasma should be selected. The stability of the reagents also varies and some deteriorate significantly at 48C during half a working day.

(iii) Other snake venoms used for LA testing. Several venoms that activate prothrombin directly have been assessed for LA testing. Taipa (Oxyuranus scutellatus)

Venom activates prothrombin in the presence of phospholipid and calcium ions, so that if a suitable dilute phospholipid is used, the test becomes sensitive to LA

(Rooney *et al*, 1994) [9]. the specificity can be improved by the use of mixing tests and/or a confirmation step (unpublished observations) using a platelet neutralization procedure.

Confirmatory tests

i. Demonstration of inhibitory activity the presence of an inhibitor can be confirmed by the performance of mixing tests with normal plasma. In factor deficiency, the clotting time is corrected by the addition of small volumes of normal plasma, but in the presence of LA relatively large volumes of normal plasma must often be added to correct the clotting time. The addition of normal plasma may even prolong the clotting time, a phenomenon known as the lupus cofactor effect. A 50% mix with normal plasma is generally used. [5, 6]

ii. Demonstration of phospholipid dependence. The platelet neutralization procedure (PNP) is commonly used. Washed normal platelets are activated with calcium ionophore or lysed by repeated freezing and thawing (which exposes procoagulant phospholipid). These platelets are added to plasma and their effect is compared with a buffer control. Platelets appear to bypass LA and correct the clotting time, whereas in factor deficiency the clotting time remains prolonged. This principle has been used for confirmation of LA in the APTT, the DRVVT and the Taipan venom time [5, 6]

The method used for the confirmation of LA uses a modified APTT reagent containing hexagonal phase lipids, which specifically bind LA and remove its effect on the APTT reagent.

In the DRVVT, an acceptable alternative to the PNP is the use of a `confirmation' reagent which contains a high concentration of phospholipid or an LA-insensitive phospholipid; several reagents are available commercially. Confirmation reagents can alter the clotting time of LA-negative plasmas and it is therefore advisable to calculate DRVVT ratios (patient time divided by control time) for each reagent [5, 6].

(a) Percentage correction of ratio. The DRVVT ratio of test´control plasma for the dilute phospholipid reagent (DPL ratio) and for the correction or confirm reagent (CORR ratio) are calculated. Percentage correction is equal to [(DPL Ratio: CORR ratio) -100]/DPL ratio. A result above the normal range (e.g. 1:1) with dilute phospholipid, which corrects to within the normal range, or by. 10% with the high phospholipid reagent or platelet neutralization procedure is considered indicative of LA.

(b) Percentage correction of clotting time. The control DRVVT clotting time is subtracted from the test DRVVT and the product is divided by the control DRVVT to yield a weighted ratio for dilute phospholipid (DPL) and for the correction reagent (CORR). The percentage correction is then calculated as [(DPL 2 CORR) _ 100]/DPL. In general, corrections of 65% are indicative of LA.

Tests for LA in subjects treated with oral anticoagulants.

Accurate detection of LA may not be possible in a subject treated with warfarin. One approach is to perform the DRVVT on equal volume mixtures of normal and test plasmas.

Taipan and Textarin venom times may be particularly useful diagnostically in plasma from patients receiving oral anticoagulants as they are relatively insensitive to prothrombin deficiency (Triplett *et al*, 1993; Rooney *et al*, 1994).[10]

Pathogenesis of APS

APS syndrome aggregate a heterogeneous group of circulating antibodies against anionic phospholipids with the most important ones are antiphosphatidyl choline, anticardiolipin antibodies (ACA), antiphosphatidyl serine, lupus anticoagulants and antinuclear antibodies [11].

As for the pathogenesis of APS, there are several known interpretations relating to the pathogenic role of aPLs which shows displaced placental function. In pregnancy, placenta is the target organ of APS, where ischemic infarctions evolve due to intimal proliferation, fibrinoid necrosis and intraluminal thrombosis of the spiral arteries [11].

Arachidonic acid metabolites fluctuation establish in the placenta in presence of aPLs. In the endothelial cells, aPLs disrupt the production of prostacyclins which plays an important role by helping vasodilatation and obstructing plate-

let aggregation following in the aggregation of the procoagulant thromboxane A2 [11, 12]

Thrombocytopenia in APS

Thrombocytopenia, defined by a platelet count less than 100–150 × 10^9/L, is found in approximately 20% of patients with APS and is found in more than 40% of patients who have APS associated with underlying systemic lupus erythematosis

Mechanisms of thrombocytopenia:- immune-mediated clearance of platelets, However, the frequent finding of thrombocytopenia and thrombosis in patients with APS suggests that antiphospholipid antibodies interact with platelets in a manner that triggers platelet aggregation and thrombosis. activation of arachidonic acid, thromboxane production and expression of glycoprotein IIb/IIIa have all been shown to occur following antiphospholipid antibody binding to platelets.

Mechanisms for Thrombosis in Patients with APS

The mechanism of thrombosis in patients with antiphospholipid antibodies is still unknown, although several mechanisms have been proposed

1) antiphospholipid antibody interference with endogenous anticoagulant mechanisms (disruption of the annexin A5 anticoagulant shield, inhibition of protein C pathway, inhibition of antithrombin),
2) binding and activation of platelets,
3) interacting with endothelial cells and inducing expression of adhesion molecules and tissue factor, and
4) activation of the complement cascade [11].

Bleeding Versus Thrombotic Risk in Patients with APS

Even though the APTT is increased APS predominantly presents as a prothrombotic disorder. It is one of few conditions that can manifest with both arterial and venous thromboembolism and can affect both large and small vessels. Interestingly, the presence of thrombocytopenia in patients with APS is not typically associated with hemorrhagic complications

In the retrospective Italian Registry of Antiphospholipid Antibodies, there were no bleeding complications among 44 patients with moderate thrombocytopenia (50–100 × 10^9/L), and 14 (32%) of these patients actually suffered thrombotic events. In the 32 patients [11], with severe thrombocytopenia (less than 50 × 10^9/L), 2 (6%) patients had bleeding complications and 3 (9%) patients had thrombosis. Although limited, this suggests that thrombotic events still occur in severely thrombocytopenic patients with

APS, although the risk may be lower compared to patients with moderate thrombocytopenia.

Distinguishing APS from Other Prothrombotic and Thrombocytopenic

The differential diagnosis of APS

Thrombotic thrombocytopenic purpura (TTP),

Heparin-induced thrombocytopenia (HIT) and

Disseminated intravascular coagulation (DIC) [11].

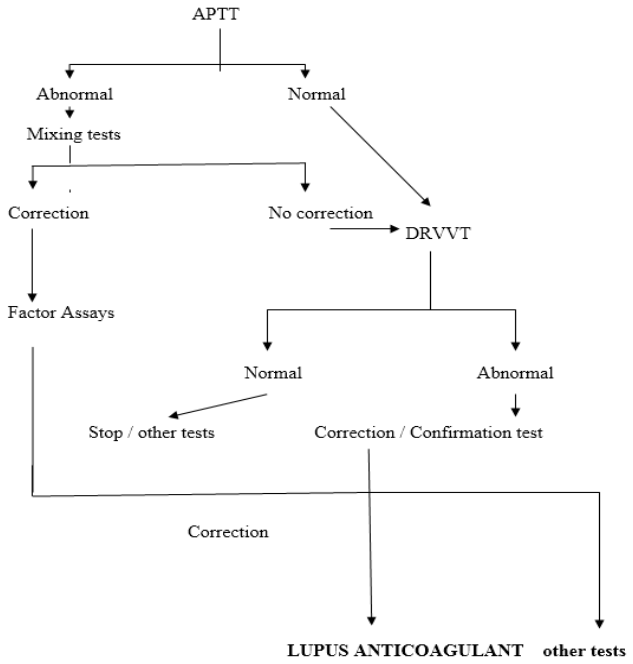
Distinguishing these conditions can be challenging. The diagnosis of APS requires documentation of persistent antiphospholipid antibodies in combination with compatible clinical features of thrombosis or pregnancy morbidity. However, antiphospholipid antibodies have been documented in TTP and other thrombotic microangiopathies including hemolytic uremic syndrome and HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome as well as in cases of HIT [4]

Patients with TTP present with microangiopathic hemolytic anemia, manifest with schistocytes on the blood smear and evidence of hemolysis, which is not a typical feature seen in APS. Measurement of the ADAMTS-13 metalloprotease, if available, may be helpful in these situations.

Patients with HIT have a history of heparin exposure, a typical decrease in platelet count occurring 5 to 10 days following this exposure and serologic evidence of the HIT antibody [12, 13]. y

Patients with DIC typically test negative for antiphospho-lipid antibodies and frequently present with evidence of thrombocytopenia, coagulopathy and thrombotic or hemorrhagic complications in the setting of a compatible clinical picture precipitating DIC [14].

Summary of Recommended Approach for the detection of LA.



A suggested flow chart for LA testing.- a prolonged APTT which fails to correct alone is insufficient for confirmation of LA; a weakly positive test may correct with normal plasma; alternatives to the DRVVT as the second screening test are other venom tests, the KCT or the TTI

Conclusions

APS is a prothrombotic disorder with various manifestations, most commonly venous and arterial thromboembolism and recurrent pregnancy loss. Diagnosis of APS can be challenging due to evolving criteria, potential limitations in the laboratory assays used to measure antiphospholipid antibodies and overlapping characteristics with other prothrombotic thrombocytopenic disorders.

APS should always be considered as a potential diagnosis especially for young patients presenting with a history of thrombotic events, in particular when they occur without any obvious external trigger conditions or/and for women with recurrent pregnancy loss or later fetal deaths.

Many other disorders are able to mimic APS, so a broad range of alternative diagnoses should be investigated and ruled out during clinical workup

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