Laboratory tests for antiphospholipid syndrome diagnosis in pregnant women

Pruebas de laboratorio empleadas para el diagnóstico del síndrome antifosfolípidos durante la gestación

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Abstract

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of autoantibodies against proteins bound to negatively charged phospholipids. Obstetric antiphospholipid syndrome (APS) is characterized as an acquired autoimmune disorder associated with different obstetric complications, defined as a state of hypercoagulability, which causes a wide range of complications associated with placental insufficiency including recurrent gestational loss, fetal death, preeclampsia, preterm delivery, among others. Its diagnosis is based on the Sidney criteria which include adverse obstetric history such as: (i) three consecutive miscarriages and spontaneous abortions prior to 10 weeks gestation (ii) fetal loss on one or more occasions at 10 weeks gestation and (iii) fetal death or preterm delivery due to eclampsia or severe preeclampsia or placental insufficiency prior to 34 weeks gestation, and laboratory findings such as (i) two positive tests for lupus anticoagulant (LA) at least 12 weeks apart (ii) two positive results for acL IgG or IgM at least 12 weeks apart and (iii) two positive results for 2GPI IgG or IgM at least 12 weeks apart. The laboratory tests give rise to an antibody profile related to the risk of complications; thus establishing as a high-risk profile, the presence of AL accompanied or not by high titers for acL or aβ2GPI. On the other hand, it is important to perform a differential diagnosis with other thrombotic microangiopathies with implications in pregnancy and to rule out the presence of different entities that may course with production of antiphospholipid antibodies. Based on these aspects and the severity of the syndrome under study, articles are required to determine the importance of the laboratory tests used for the diagnosis of antiphospholipid syndrome in pregnant women.

Key words: Antibodies, pregnancy, thrombosis, diagnosis, antiphospholipid syndrome.

Resumen

El síndrome antifosfolípido (SAF) es una enfermedad autoinmune que se caracteriza por la presencia de autoanticuerpos contra proteínas unidas a fosfolípidos de carga negativa. El síndrome antifosfolípido obstétrico (SAFO), se caracteriza por ser una alteración autoinmune adquirida asociada con diferentes complicaciones obstétricas, definiéndose como un estado de hipercoagulabilidad; que provoca una amplia gama de complicaciones que se asocian con insuficiencia placentaria incluyendo, pérdida gestacional recurrente, muerte fetal, preeclampsia, partos prematuros, entre otros. Su diagnóstico se basa en los criterios de Sidney los cuales incluyen antecedentes obstétricos adversos como: (i) tres abortos consecutivos y espontáneos previos a la semana 10 de gestación (ii) perdida fetal en una o más ocasiones a las 10 semanas de gestación y (iii) muerte fetal o parto prematuro por eclampsia o preeclampsia grave o por insuficiencia placentaria antes de las 34 semanas de gestación y resultados de laboratorio como (i) dos pruebas positivas para anticoagulante lúpico (AL) con al menos 12 semanas de diferencia (ii) dos resultados positivos para acL IgG o IgM con al menos 12 semanas de diferencia y (iii) dos resultados positivos para 2GPI IgG o IgM con 12 semanas de diferencia como mínimo. Las pruebas de laboratorio dan origen a un perfil de anticuerpos relacionado con el riesgo de complicaciones; estableciendo de esta manera como un perfil de alto riesgo, la presencia de AL acompañado o no de títulos altos para acL o aβ2GPI. Por otro lado, es importante realizar un diagnóstico diferencial con otras microangiopatías trombóticas con implicaciones en el embarazo y descartar la presencia de diferentes entidades que puedan cursar con producción de anticuerpos antifosfolípidos. Partiendo de estos aspectos y de la gravedad del síndrome en estudio se requiere de artículos que determinen la importancia de las pruebas de laboratorio utilizadas para el diagnóstico del síndrome antifosfolípidos en mujeres embarazadas.

Palabras clave: Anticuerpos, embarazo, trombosis, diagnóstico, síndrome antifosfolípido.

Introduction

Antiphospholipid syndrome (APS) is a systemic autoimmune disorder, characterized by arterial and venous thrombosis, where there is an unfavorable perinatal evolution (for the mother and fetus), this syndrome is associated with antiphospholipid antibodies (AAF), where there is a clinical picture of hypercoagulability. Antiphospholipid antibodies are a family of autoantibodies that recognize various combinations of phospholipids, phospholipid-bound proteins or both. Of these, the most studied are anticardiolipin antibodies (AAC), lupus anticoagulant (LA) and anti-ß2-glycoprotein antibodies (B2GPI)[1].

Initially it was thought that AAF were directly related to negatively charged phospholipids. However, it has been shown that they are directed against epitopes of certain proteins which are called cofactors, among which proteins C and S (natural proteins), annexin A2 and A5 and high molecular weight criminogen stand out. However, the two cofactors most related to the pathogenicity of FFA are beta2-glycoprotein I (B2GPI) and prothrombin[2].

These antibodies can occur in isolation and are called primary FAS or in association with other systemic autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis. This syndrome occurs mainly in young women of childbearing age. In addition, AAF can also be found in other situations, such as infections, neoplasms or in relation to the intake of drugs. Recent studies have described a subgroup of APS where patients develop multiple episodes of thrombosis, occurring mainly in small caliber vessels of various organs, occurring over a short period of time, which has been termed catastrophic APS and is responsible for a mortality of up to 30%. This condition is more frequent in pregnant women and is more likely to occur again in this population compared to others[3].

The antibodies involved in FAS are described below:

Anticardiolipin antibody

Anticardiolipin antibodies (aCL) are antiphospholipid antibodies that specifically recognize the phospholipids that form cell membranes.

The aCL recognize and attack cardiolipin, which is an anionic phospholipid present in the mitochondrial inner membrane, but absent in the membrane of platelets and endothelial cells.

The aCLs can also react with phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, β2 glycoprotein I (β2 GPI), prothrombin or annexin V.

These antibodies are currently detected by using the traditional ELISA methodology which is based on the detection

of IgG and IgM aCL isotype. Cardiolipin β2GPI complex (bovine or human) should be used as antigen.

Beta2 glycoprotein I

The B2GPI antibody is a plasma protein identified as one of the cofactor molecules required for optimal binding of FFA and negatively charged phospholipids. B2GPI is synthesized in the liver, its main role has been associated with decreased platelet activation and adhesion, neutralization of von Willebrand factor and limitation of endothelial cell activation. Laboratory determination of B2GPI can help to investigate inappropriate blood clot formation, either thrombotic episode or venous thromboembolism; to help determine the cause of recurrent miscarriages; as part of an evaluation for FAS.

Lupus Anticoagulant

It refers to a phenomenon caused by multiple circulating antibodies directed against multiple phospholipids (B2GPI, prothrombin, plasmin, plasminogen activating factor, annexin A2, thrombin, phosphatidyl serine, phosphatidyl inositol, phosphatidyl ethanolamine). LA have the property of lengthening phospholipid-dependent clotting times, they are circulating anticoagulants.

The determination of lupus anticoagulant is made in citrated plasma poor in platelets, i.e., double centrifuged, in order to eliminate the maximum number of platelets, since these are rich in anionic phospholipids, particularly membrane phospholipids[4].

The cause of the production of autoantibodies against phospholipid-linked proteins such as anti-beta 2 glycoprotein I is largely unknown, FFAs affect the coagulation and inflammation cascade. In a process mediated by beta 2-glycoprotein I, AAFs bind to platelets and endothelial cells, become activated, and induce a procoagulant state. Antibody binding also stimulates complement, leads to recruitment of other inflammatory cells, tissue factor activation, endothelial damage and, finally, thrombosis.

During gestation, women with PFS and FPA experience an increased risk of recurrent miscarriage and late complications such as preeclampsia and preterm delivery. Some clinical and experimental studies suggest that inflammation of the maternal-fetal interface and disruption of trophoblast function are more implicated as pathophysiological factors than a previous prothrombotic event in pregnancy failure in patients with PBS[5].

Epidemiologically, antibodies and FAS are more frequent in women and general prevalences of 1-5% for FFA and 40-50 cases per 100,000 inhabitants for FAS, however, they may be present in higher proportions in patients with frequent thrombotic events or recurrent miscarriages, situations in which the frequency of FFA may be 40%[6,7].

The presence of AAF is associated as a cause of 7-25% of unexplained miscarriages. The prevalence of AAF in women with fetal loss is estimated to be in the range 4.6 - 50.7% and in the case of AL it is between 0-14%, being 30% when the loss occurs after 20 weeks of gestation[8].

PHYSIOPATHOLOGY

The pathogenesis of thrombosis is based on the two-hit model, in which a first hit affects the endothelium and a second one favors thrombus formation[9]. Adverse pregnancy outcomes due to AAF alone remain under ongoing debate. In some experimentation in mice, fetal losses have been observed to occur in variable ways[10,11]. FFA may interfere with the normal in vivo function of phospholipids or phospholipid-bound proteins important for coagulation[12].

AAF can activate endothelial cells, due to increased expression of adhesion molecules, secretion of cytokines and production of arachidonic acid metabolites. At the same time, AAF cross-react with oxidized low-density lipoproteins generating oxidative damage of the vascular endothelium[13].

Recent advances in reproductive biology and observations in early normal pregnancies allow the classification of pregnancy loss into three developmental periods:

1. Pre-embryonic period, from the moment of conception until the beginning of the fifth week after menstruation.

2. Embryonic period, which runs from the fifth week to the ninth week, and

3. Fetal period, which starts from the tenth week (approximately 70 days after conception) and lasts until delivery.

Signs and symptoms

Approximately one quarter of women who suffer recurrent miscarriages are positive for one or more of the FNAs. In these women the risk of fetal loss is greatest after 10 weeks of gestation, as opposed to losses in the general population, which occur during the first 9 weeks of gestation. Pregnant women with FNA positivity may have complications leading to intrauterine growth failure (IUGR), preterm delivery and preeclampsia. Furthermore, if combined with hemolysis, increased liver enzymes and decreased platelet count, it leads to the so-called HELLP syndrome, which can also occur in patients with FPA. Additionally, regarding the role of these antibodies in cases of infertility, the issue is still controversial, since FPA can affect placental growth and embryo implantation, where it could theoretically cause infertility[14].

As mentioned above, recurrent miscarriages occur before 10 weeks of pregnancy with no other known causes, including fetal loss after 10 weeks, preeclampsia, eclampsia, elevated liver enzyme and thrombocytopenia, and fetal growth retardation.

It is common for patients to have more than one episode of preeclampsia before being diagnosed with obstetric antiphospholipid syndrome (APFS). Early-onset preeclampsia and prematurity are considered to be related to so-called

placental vascular insufficiency. Women with AAF in obstetric APS who are not treated are at much higher risk than the general population for gestational losses at any time during pregnancy[15].

Complications of FAS in pregnant women

Obstetric complications are classified among the main clinical manifestations linked to maternal-fetal morbimortality and thrombosis-dependent manifestations that develop in venous and arterial territories. There are reports of extremely severe forms of PAS such as the so-called Catastrophic PAS, characterized by successive multiple thrombosis in various territories and HELLP Syndrome associated with this entity, which are difficult to differentiate and have high mortality[16].

In addition to recurrent miscarriages before 10 weeks' gestation and maternal thrombosis, the revised classification criteria for FAS include reaffirmed preterm delivery in association with preeclampsia and severe eclampsia, recognized features of placental insufficiency such as growth restriction or oligohydramnios (insufficient amniotic fluid volume), and late pregnancy loss as recognized clinical criteria for the diagnosis of this syndrome.

On the other hand, it is well established that a significant proportion of pregnancy losses secondary to APS are fetal deaths in the second or third trimester, with estimates of up to 50%. Approximately one-third of women with APS will develop preeclampsia during pregnancy. Another study has reported a risk of up to 50% associated with women with severe early-onset preeclampsia before 34 weeks' gestation. FFA may be particularly associated with a variant of severe preeclampsia, such as HELLP syndrome, which is characterized by hemolysis, elevated liver enzymes, and thrombocytopenia. HELLP syndrome is estimated to occur in 10 to 20% of cases of severe preeclampsia. Although the actual incidence of HELLP syndrome in women with FAS is difficult to approximate, 78.4% of women diagnosed with HELLP syndrome were found to have FAS. Several studies have demonstrated a significant association between FAS and preeclampsia. Additionally, these pregnancies are subject to other complications, such as gestational systemic and pulmonary hypertension, in addition to placental insufficiency which is a frequent complication requiring immediate delivery[17].

APS is also among the most important autoimmune diseases capable of causing infertility/sterility in addition to pregnancy-associated hypertension, preeclampsia, fetal growth retardation, among others. FAS can impact both the mother and the fetus, and although rare, some pregnant women can develop extremely severe forms such as refractory antiphospholipid syndrome (RFAS) and life-threatening catastrophic FAS (CFAS). These disorders can also be difficult to differentiate clinically from HELLP syndrome, leading to high maternal and fetal mortality, and pose a high risk for future pregnancies[18].

LABORATORY DIAGNOSIS

The diagnosis is made, to a large extent, by clinical suspicion and laboratory findings. When arterial or venous thromboses occur in patients who do not have risk factors or when thrombotic events are recurrent, FAS should be considered[19]. LUISA

Lupus nnticoagulant evaluation

The criteria for its evaluation include a four-step sequence: prolongation of phospholipid-dependent coagulation assays (screening tests), evidence of the presence of an inhibitor by mixing with normal plasma, confirmatory testing, and differential diagnosis with other coagulopathies that may lead to diagnostic errors.

A recommendation at the time of the test is not to take antithrombotic anticoagulants or anti-factor Xa or heparin, since they can interfere with the test by prolonging the coagulation times, in case the patient is under administration of these drugs it is advisable to suspend them at least 72 hours before the test.

For the study of LA, screening tests should be used, including the Russell's viper venom test, which is an in vitro test based on venom-induced thrombosis, performed with low concentrations of phospholipids; if the sample contains a lupus anticoagulant, it generates a lengthening of the coagulation time.

An additional test that can be added is the dilute prothrombin time (dPT), which makes use of the comparison of prothrombin time in normal and dilute concentrations; the result is expressed as the ratio between (PT sec patient/TP sec control) and the (PT sec patient/TP sec control), with a ratio <1.30 being normal and >= 1.30 being abnormal.

If the above tests show an abnormal or prolonged prothrombin time (PT), a correction test is performed, in which the patient's plasma is mixed with normal control plasma to identify whether or not the prolongation of the PT is due to coagulation factor deficiency; if the correction does not occur, confirmation tests are performed using elevated concentrations of phospholipids, which in the case of correction allows for evidence of phospholipid dependence and therefore the presence of an AL.

Reporting for AL can be done by relating the screening test to the confirmatory test.

Interferences: Positive results for LA are frequently found in inflammatory states such as in recent coronavirus disease, on the other hand, some medications such as antibiotics, antiarrhythmics, chlorpromazine and some vaccines can be associated with the presence of lupus anticoagulant[20].

Another possible interference can be generated by the presence of C-reactive protein, which interferes with the phospholipid-dependent reagents used in the test and can generate false positives by lengthening coagulation

times; this protein can be present in inflammatory states or in autoimmune disorders[21-23].

Evaluation of anticardiolipin and anti-β2 glycoprotein I antibodies.

The identification of aCL and aβ2GPI antibodies in the laboratory is performed by solid phase immunoassays, in which an antigen in this case cardiolipin or β2-glycoprotein I is immobilized in a solid phase, which will be exposed to the patient's serum or plasma, which in case of containing antibodies against cardiolipin or β2-glycoprotein I will form an antigen-antibody complex, which could be visualized by adding a conjugate between an anti-human IgG and a substrate that will trigger a color, chemiluminescent or fluorescent reaction that can be quantified by means of different systems, in this way the signal emitted by the conjugate will be proportional to the concentration of the antibody under study[24].

The test can be classified as positive or negative taking into account the cut-off points; according to Sydney's criteria, antibody titers toβ2GPI above the 99th percentile calculated with reference subjects and above 40 GPL or MPL for aCL antibodies are considered as positive. However, based on the variability of the assays used, it is recommended that each laboratory establish its own population cut-off points.

There are some situations that should be considered when interpreting the results, for example, the negativity of antibodies to β2GPI, which could indicate that the antibodies identify antigens other than β2GPI, which have unknown clinical implications; on the other hand, antibodies against the domain of β2GPI do not represent pathogenicity; this is not the case for those directed against aDI, whose importance lies in its role in obstetric complications.

Regarding the immunoglobulin isotype, it is suggested that the tests determine both IgG and IgM; IgA antibodies should be used when there is clinical doubt of FAS or in black individuals in whom it prevails over the other two isotypes.

According to the literature, IgG type antibodies are more frequent in clinical events and commonly occur together with IgM type antibodies, the latter being infrequent in FAS and more relevant in OFAS. Additionally, different investigations show that the presence of aCL and aβ2GPI antibodies with the same isotype intensify the clinical possibility of FAS[25].

Interferences: Interference by cryoglobulins and rheumatoid factor are causes of false positives, especially for IgM aCL antibodies in low titers. Other situations that can lead to the production of IgM aCL antibodies are infectious processes, neoplasms and some drugs[26].

1.8. Laboratory criteria in obstetric antiphospholipid syndrome.

The Sidney criteria have been established for obstetric FAS, according to which obstetric FAS is defined under clinical and laboratory test conditions; the criteria for defining obstetric morbidity related to FAS (OMAPS) have also been determined, as shown in Table 1[27-29].

Table 1. Clinical and laboratory criteria for the diagnosis of OSAHS.

	Clinical criteria	Laboratory criteria
SAFO	Three consecutive miscarriages prior to 10 weeks of gestation	Two positive tests for lupus anticoagulant at least 12 weeks apart
	Fetal loss on one or more occasions at 10 weeks gestation.	Two positive results for acL IgG or IgM at least 12 weeks apart
	Fetal death or premature delivery due to eclampsia or severe preeclampsia or placental insufficiency before 34 weeks of gestation.	Two positive 2GPI IgG or IgM results at least 12 weeks apart
OMAPS	Two consecutive miscarriages of properly formed embryos Three or more non-successive miscarriages of properly formed embryos Eclampsia after 34 weeks of gestation or during puerperium Placental abruption. Delayed preterm labor. Frequent rupture of membranes. Unsuccessful implantation following in vitro fertilization	Same as SAFO

The determination of antibodies against the different proteins involved in antiphospholipid syndrome gives rise to an antibody profile that is in turn related to the risk of thrombosis and complications in pregnancy; thus defining a high-risk profile as the presence of AL with or without high titers for acL or aβ2GPI[30]; broader recommendations define this profile as the persistent presence of AL, double aPL (any union between LA, aCL and aβ2GPI) or triple aPL (LA, aCL and aβ2GPI[31].Likewise, the low-risk profile is defined as the presence of antibodies to aCL and/or aβ2G in low or medium titers[32].

Another aspect to be considered in the laboratory diagnosis is the possibility of facing a seronegative FAS, in which despite the obstetric history there are negative laboratory tests, which can be triggered by different factors such as: the decrease in the levels of antiphospholipid antibodies after a thrombotic event[33]; treatment with corticosteroids and anti-inflammatory drugs; urinary elimination of IgG antibodies, as occurs in the nephrotic syndrome.

interference with platelets present in the sample since these are rich in phospholipids at membrane level; IgA type antibodies against AAC or β2GPI which are not routinely determined[34,35].

Differential diagnosis

A differential diagnosis is suggested with diseases that cause unexplained arterial or venous thrombosis, thrombosis due to hereditary causes, including those presented in Table 2.

Table 2. Differential diagnosis for FAS [36]
Coagulation disorders
Factor V Leiden
Protein C deficiency
Protein S deficiency
Antithrombin III deficiency
Thrombosis of other causes
Hyperhomocysteinemia
Nephrotic syndrome
Oral contraceptives containing estrogens
Myeloproliferative syndromes
Systemic vasculitis

Additionally, a differential diagnosis should be made with other thrombotic microangiopathies that may develop in pregnancy, such as those shown in Table 3[37].

On the other hand, it is important to recognize that there are several entities in which AAF can be found such as infections, autoimmune diseases, chronic diseases and with the administration of some drugs, these entities are listed in Table 4[38].

Table 3. Microangiopathies for differential diagnosis with SAFO	
Disease	Features
HELLP Syndrome	Elevation of liver enzymes usually during pregnancy
Thrombotic thrombocytopenic purpura	Schistocytes, anti-ADAMTS13 antibodies
Hemolytic uremic syndrome	History of gastrointestinal infection, existence of Shiga toxin
Disseminated intravascular coagulation (DIC)	Prolongation of PT and aPTT, hypofibrinogenemia

Differential diagnosis PBS

Autoimmune diseases	Chronic diseases and neoplasms	Infections	Medications
Systemic lupus erythematosus	Leukemias	Syphilis	Chlorpromazine
Rheumatoid arthritis	Diabetes	Lyme disease	Phenytoin
Sjögren's syndrome	Multiple myeloma	Malaria	Quinine
Autoimmune hemolytic anemia	Hodgkin's disease	AIDS	Streptomycin
Idiopathic thrombocytopenic thrombocytopenic purpura		Adenovirus	Hydralazine
		Measles	
		Tuberculosis	
		Toxoplasmosis	

It should be noted that there are few data to guide the management of uncomplicated pregnant women with the presence of isolated PSA and who therefore do not meet the above-mentioned criteria for PBS. More than 50% of such women have uncomplicated pregnancies[39].

Due to the complex pathophysiology that defines APS and the multiple complications that are generated in both maternal and fetal health, there is a need to implement studies that describe in detail both the clinical and laboratory aspects in order to guide a timely diagnosis of antiphospholipid syndrome in high-risk populations, so the objective of this study is to determine the importance of laboratory tests used for the diagnosis of APS in pregnant women.

Conclusions

PAS is a condition of great clinical importance, since maternal morbidity and mortality due to thromboembolic events is becoming an increasingly important cause of death in pregnant women in particular. In addition, the incidence of obstetric events in patients with FAS is a topic of great interest in medicine because FAS is not only a thrombotic disease, but is also associated with microangiopathic features, so that FAS is a condition characterized by thrombotic phenomena that is associated with obstetric complications and the expression of medium and high titers of FAS. MADELEIN

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