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Diagnosis and treatment

Diagnosis of antiphospholipid syndrome: From an historical perspective to the emergence of new autoantibodies☆

Diagnóstico en el síndrome antifosfolipídico: desde una perspectiva histórica a la aparición de nuevos autoanticuerpos

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Introduction

Antiphospholipid syndrome (APS) is defined as an autoimmune disease characterised by the presence of antiphospholipid antibodies (AAF) and at least one thrombotic clinical manifestation or history of recurrent foetal loss.[1] Although the association between the phenomenon of lupus anticoagulant (LA), the presence of cardiolipin antibodies (aCL) and the development of various thrombotic events date back to the mid-twentieth century, it was not until 1983 that these findings were grouped as a separate clinical entity on the basis of the description of a greater number of thrombotic and obstetrical adverse events in a cohort of patients with systemic lupus erythematosus (SLE) with LA, compared to those who did not have LA.[2] This disease would temporarily be given the name "Hughes syndrome" because of the main researcher of this publication. The first consensus meeting for its classification was held in the Japanese city of Sapporo in 1999 and later revised in 2006 in Sydney,[3] encompassing clinical and laboratory findings that may precede clinical practice, provided an alternative explanation is not found (Table 1).

Background Wasserman reaction and cardiolipin

In 1906 a method to diagnose syphilis was developed by detecting circulating antibodies against *Treponema pallidum*. A "syphilitic antigen" was added to the patient's serum, whose main ingredients were the livers of children who died from congenital syphilis and a purified protein from the complement system. The last one was sheep erythrocytes. If the patient had antibodies against *T. pallidum*, they would join the "syphilitic antigen" and exhaust the added complement through the formation of immune complexes. Therefore, no haemolysis of the sheep erythrocytes occurs, resulting in a positive test. If there were no antibodies against *T. pallidum* in the serum, the added complement generated detectable lysis from the sheep erythrocytes and the reaction for syphilis would be declared negative.[1] The passing of the years would show that the "syphilitic antigen" could be replaced by various animal tissues not infected with syphilis and similar results would be obtained, without a logical explanation, until in 1941 when a phospholipid was identified in a bovine heart that would be christened "cardiolipin", as the cross-reactivity of antibodies against spirochete.[4] The *Venereal Disease Research Laboratory* test developed a few years later had (and still has) the disadvantage of obtaining false positives when there are many other infectious and non-infectious diseases including SLE[5] present, again due to cross-reactivity with cardiolipin, of which its location was recognised in the mitochondria membrane inside the eukaryotic cells, as was its role in the exchange of molecules from

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Table 1
Criteria for classification of antiphospholipid syndrome.

Clinical criteria	
1. Vascular thrombosis	One or more episodes of arterial, venous or small vessels thrombosis in any organ or tissue, except superficial venous thrombosis. Thrombosis must be confirmed by images, Doppler studies or histopathology. For a histopathologic confirmation, thrombosis should be present without significant evidence of inflammation of the blood vessel wall
2. Obstetric morbidity	a) One or more unexplained foetal deaths during or after the 10th week of gestation, with normal foetal morphology documented by ultrasound or direct examination of the foetus b) One or more premature births of morphologically normal neonates at or before 34 weeks due to severe pre-eclampsia, eclampsia or severe placental insufficiency c) Three or more consecutive abortions, unexplained, before week 10 of gestation, excluding maternal anatomical and hormonal abnormalities, as well as chromosomal causes
Laboratory criterion	
1. Lupus anticoagulant	Present in plasma in 2 or more occasions separated by at least 12 weeks and detected according to *International Society on Thrombosis and Haemostasis* guidelines
2. Cardiolipin antibodies	IgG or IgM in serum or plasma, present in average or high titers (>40 GPL or MPL, or above the 99th percentile) on 2 or more occasions, separated by at least 12 weeks, measured by a standardised ELISA method
3. Anti-beta2 glycoprotein I antibodies	IgG or IgM in serum in titers over the 99th percentile, on 2 or more occasions, separated by at least 12 weeks, measured by a standardised ELISA method

and into the interior of this organelle, including that the association between aCL and thrombotic phenomena became evident through multiple observations.

Cardiolipin antibodies

These antibodies are currently detected by using the traditional ELISA method which includes a standardised antigen and comparing the results with an internationally accepted standard. The first standard was developed by Dr. Hughes' research group and consists of a mixture of normal sera mixed with a defined concentration of antibodies. These mixtures were named Harris or Louisville standards.[6] Later the "Sapporo" and "Koike" standards were developed, also known under the timely designations of EY2C9 and HCLA for subtypes IgM and IgG respectively, monoclonal chimeric antibodies officially recognised by the CDC and which the manufacturers should use to compare their products.

Quantification of aCL is performed in GPL and MPL units for IgG and IgM, respectively, expressing the amount in micrograms of antibodies in a given volume of serum. Then comes the difficulty of establishing an abnormal cut-off point for autoantibodies that should not normally circulate in the body. According to the Sydney criteria, the cut-off should be 40 GPL or MPL, or use the 99th percentile of the general population. This percentile calculation must be performed on at least 120 healthy people. Notably, there are significant quantitative differences between the 99th percentile of a population, usually a low value, and the other cut-off point of 40 GPL or MPL, which may prove to be much higher. To illustrate this, in a cohort the 99th percentile stood at 17 GPL, with 73% of patients who met clinical criteria for APS having between 17 and 40 GPL.[7] The arbitrariness of defining a cut-off point for considering a result to be positive or negative can be solved by assuming that the persistent presence of these autoantibodies is always pathological and that an increased serum concentration of these is associated with an increased risk of adverse clinical events and low titres do not mean a low risk of having them. Therefore, any persistent positive result should alert the clinician, giving greater significance to higher results.[8] The division of a positive result in categories of high, medium or low titres is also arbitrary, but is accepted nomenclature naming a result above 40 GPL or MPL as an average titre, and above 80 as a high titre, although these values may vary according to the literature. In case of an isolated positive result for IgM aCL and negative for other autoantibodies in APS, the possibility of a false positive must be considered, caused by the presence of circulating rheumatoid factor.[9] Unfortunately, and because of the aforementioned limitations, a great variability is observed in the GPL and MPL titres reported by different laboratories when measuring the same sample, which could lead to misclassification of patients within the syndrome according to the cut-off point used to define a positive or negative test result.[10]

Beta2 glycoprotein I

During the second half of the 1980s the following phenomenon was studied: by adding plasma with aCL to phospholipid substrates, the aforementioned antibodies bind with their antigen, but if a purified aCL was prepared, they did not react with cardiolipin in the absence of plasma. It was postulated that there must be a circulating molecule that would act as a cofactor or binding bridge for these antibodies, later identified as apolipoprotein H, also called beta2 glycoprotein I (B2GPI) involved in haemostasis.[11]

The B2GPI is a polypeptide chain of 50 kD divided into five domains, synthesised in the liver. Its role has been associated with a decreased activation and platelet adhesion, neutralisation of the von Willebrand factor and limitation of the activation of endothelial cells.[12] In its current form, the domain v binds to i, thus creating an inactive closed chain. It has been shown that antibodies only develop against the molecule when it is *extended*, that is, when the v domain is separated from i giving the molecule a linear structure. It is in this linear state that the B2GPI binds to receptors on the molecules mentioned (GPIb/IX/V on platelets and annexin A2 in the endothelium).[13]

Antibodies against B2GPI were included as APS criteria in the Sydney consensus from previous observations of thrombotic events associated with its presence. The document does not specify a cut-off point to consider them positive, so this value should be obtained from the 99th percentile of the general healthy population, estimated to be at least 20 individuals.[3] Although detection of anti-B2GPI antibodies is performed by ELISA methods, quantification is done in units arbitrarily defined by laboratories (units/ml, ng/ml, optical density values, etc.) and there is no international standard against which to compare the results of each institution.[14] As is the case with aCL, the clinician should assume that the significance of these antibodies is continuous – its positivity always involves disease (it should not exist under normal conditions) – and that the higher the concentration, the more clinical impact it will have, and that because of the variability due to the lack of standardisation in measurement, the close to normal values should be interpreted cautiously.

IgA anticardiolipin and anti-B2-glycoprotein I

The use of subtype IgA for detecting AAF is not standardised. Its measurement is only recommended in patients with high clinical suspicion and negative results for subtype IgG and IgM tests, since in certain isolated clinical cases its presence has been shown in the absence of other antibodies. The clinical relevance and that of the