

# Critical Update of the Antiphospholipid Syndrome Criteria

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**Abstract:** Diagnosis of Antiphospholipid Syndrome (APS) is made when vascular thrombosis or pregnancy morbidity occur in patients with circulating antiphospholipid antibodies [lupus anticoagulant (LA) and/or IgG/IgM anticardiolipin (aCL) and/or IgG/IgM anti- $\beta_2$ glycoprotein I ( $\text{a}\beta_2\text{GPI}$ ) antibodies]. Although each test may identify different autoantibodies, a single positive test makes the diagnosis possible when positive on two or more occasions at least 12 weeks apart. However, single test positivity may be unrelated to pathogenic antibodies that are now considered a subclass of  $\text{a}\beta_2\text{GPI}$  directed to Domain I of the protein. Conversely, all the test positive identify a single class of  $\text{a}\beta_2\text{GPI}$  antibodies and identify high risk patients with APS.

**Keywords:** Antiphospholipid syndrome, thrombosis, pregnancy morbidity.

## INTRODUCTION

According to the International Consensus Document that updated the classification criteria for antiphospholipid syndrome (APS) [1], a definite diagnosis implies vascular thrombosis and/or pregnancy morbidity and at least one of the following antibodies: lupus anticoagulant (LA) and/or IgG/IgM anticardiolipin (aCL) and/or IgG/IgM anti- $\beta_2$ glycoprotein I ( $\text{a}\beta_2\text{GPI}$ ) antibodies. Laboratory tests must be positive on two or more occasions at least 12 weeks apart. APS is unlikely if less than 12 weeks or more than 5 years separate the positive antiphospholipid antibodies (aPL) test and the clinical manifestation. Patients are to be allocated to classification categories on the basis of positivity to aPL – patients belong to category I if more than one test is positive and to category II if a single test is positive. ACL titres must be medium/high – thus should exceed 40 GPL or MPL units, or the 99<sup>th</sup> percentile calculated in normal subjects.  $\text{a}\beta_2\text{GPI}$  antibody titre must be above the 99<sup>th</sup> percentile of that of normal subjects [1].

Differently from LA and aCL,  $\text{a}\beta_2\text{GPI}$  ELISA is the only test that identifies antibodies directed to a specific protein. When used for immunization, this autoantigen leads to production of antibodies and induces experimental APS thus characterizing a specific autoimmune disease [2].

However, clinical manifestations do not occur in all the subjects with  $\text{a}\beta_2\text{GPI}$  antibodies and indeed there is increasing evidence that  $\text{a}\beta_2\text{GPI}$  positivity is not associated with thromboembolic events and only some types of  $\text{a}\beta_2\text{GPI}$  antibodies [i.e. anti Domain I (DmI) of  $\beta_2\text{GPI}$ ] are associated with the clinical manifestations of APS [3, 4]. The concept that a specific subtype of  $\text{a}\beta_2\text{GPI}$  antibodies are pathogenic is supported by studies in patients strongly positive for all the three tests exploring the presence of aPL in whom there is a strong association with clinical events of APS. When antibodies to  $\beta_2\text{GPI}$  are affinity purified from plasma of these

patients and spiked into normal plasma, they reproduce the positivity of all the three tests found in the original plasma [5]. This means that the type of  $\text{a}\beta_2\text{GPI}$  present in patients with LA is probably the relevant one. Therefore the detection of  $\text{a}\beta_2\text{GPI}$  antibodies only with negative results for LA and aCL (classification category IIc in Miyakis criteria) may not be sufficient to diagnose APS because only specific subtypes of these antibodies are pathogenic. The interpretation of these data highlights the fact that a definite autoimmune disease probably exists in patients with clinical manifestations testing positive for  $\text{a}\beta_2\text{GPI}$  antibodies and LA. Positivity for the same isotype in aCL ELISA in these patients confirms for the presence of pathogenic  $\text{a}\beta_2\text{GPI}$  antibodies.

Positivity for the same isotype in aCL and  $\text{a}\beta_2\text{GPI}$  ELISAs associated to a negative result in LA test may be seen in patients with pathogenic  $\text{a}\beta_2\text{GPI}$  antibodies whose titre is not sufficient to produce LA activity in plasma. Positivity for dRVVT becomes evident only when the aCL concentration exceeds 50 GPL Units [6]. The clinical significance of lower amount of antibodies remains unclear but might be relevant in pregnancy morbidity where lower titres of antibodies are usually encountered [7].

As far as category II in the Miyakis laboratory criteria is concerned, single test positivity may be unrelated to pathogenic  $\text{a}\beta_2\text{GPI}$  antibodies. In fact the sole aCL positivity is non specific, as detected antibodies may be directed against bovine  $\beta_2\text{GPI}$ , other cardiolipin-binding plasma proteins or directly binding cardiolipin (authentic aCL antibodies) [8-10]. In a recent study of a large cohort of APS patients we found that when IgG aCL is the sole positive test, its titre lies between 17 GPL (99<sup>th</sup> percentile) and 40 GPL, and the most common clinical feature is pregnancy morbidity (74%). Patients with thromboembolic events or both thrombosis and pregnancy morbidity have more than one positive test and IgG aCL values are well above 40 GPL in most cases [7]. The rationale behind the presence of lower titres of antibodies in APS patients with pregnancy morbidity remains unclear. Present data [11, 12], seem to confirm the hypothesis that the pathological mechanism involved in the placental injury [13, 14] is different from that involved in thrombosis

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[15]. Thus, despite the lack of consistent evidence to this effect, it would seem that the quantity and perhaps even the binding characteristics of IgG aCL in APS patients might differ in the two groups. A significant association was found between vascular thrombosis and multiple aPL positivity and IgG aCL titres >40 GPL units as well as between pregnancy morbidity and sole IgG aCL positivity and titres <40 GPL units. In the light of these findings it seems like these conditions - both considered part of the clinical manifestations of the APS syndrome - are distinct disorders originating from distinct pathogenic mechanisms.

Besides, while a clear association with clinical events has been confirmed in patients positive to LA as well as to aCL and/or a $\beta_2$ GPI (classification category I), LA positivity alone is apparently not associated with clinical manifestation of APS [16].

Therefore the elucidation of the clinical significance of aPL in the APS syndrome lies in the path of clinical studies considering cohorts of patients with homogeneous aPL profile. This means that when performing clinical trials on aCL positive patients a distinction should be made between different aPL profiles [aCL positive only (LA and a $\beta_2$ GPI ELISA negative) or aCL positive with other tests not performed or data not available or incomplete]. Studies in homogeneous cohorts of patients [17] indicate that high risk patients are only those with triple aPL positivity.

In conclusion APS diagnosis requires the following criteria to be taken into account:

#### Patients with Thromboembolic Events

- Consider triple positive patients (LA positive, IgG or IgM aCL >40 GPL, IgG or IgM a $\beta_2$ GPI >99<sup>th</sup> percentile) as a high risk population related to a single pathogenic autoantibody (definite APS).
- Consider double positivity (LA negative and aCL >40 GPL, a $\beta_2$ GPI >99<sup>th</sup> percentile, same isotype) as probably APS in low risk patients.
- Consider single positivity for LA, aCL or a $\beta_2$ GPI antibodies as non APS patients. More information from clinical studies on homogeneous cohort of patients with single positivity is needed.

#### Patients with Pregnancy Morbidity

- Consider triple positive patients (LA positive, IgG or IgM aCL >40GPL, IgG or IgM a $\beta_2$ GPI >99<sup>th</sup> percentile) as a high risk population related to a single pathogenic autoantibody (definite APS).
- Consider double positivity (LA negative and aCL and a $\beta_2$ GPI >99<sup>th</sup> percentile, same isotype) or single positivity for aCL (>99<sup>th</sup> percentile) or for a $\beta_2$ GPI (>99<sup>th</sup> percentile) as possible obstetric APS. More information from clinical studies on homogeneous cohort of patients with single positivity is needed.

Diagnosis of APS is more likely if:

- The patients is less than 50 years of age.
- Thromboembolic events are idiopathic (other causes are excluded).

- The predominant immunoglobulin isotype is IgG.
- Anti DmI ELISA is positive [4].
- Obstetric complications are late pregnancy loss or premature birth for preeclampsia/eclampsia, HELLP syndrome or there are signs of placental insufficiency.

#### CONCLUSION

Diagnosis of APS is essentially based on three laboratory tests that may be positive in various combinations. Clinical studies do not distinguish between patients with different laboratory profiles. Triple positivity appear the only pattern that identify a high risk population of patients with APS.

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