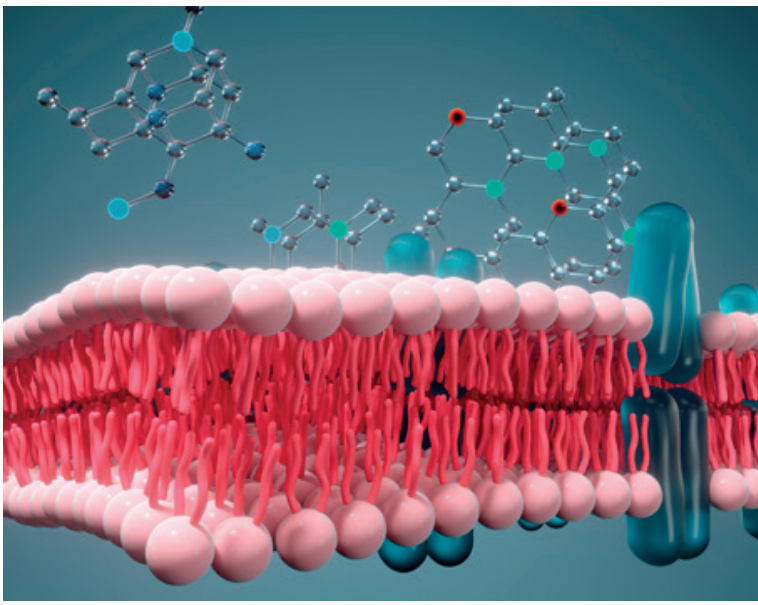
Weighted analysis of autoantibodies in new classification criteria for antiphospholipid syndrome

By Dr Jacqueline Gosink

New American College of Rheumatology and European Alliance of Associations for Rheumatology classification criteria for antiphospholipid syndrome have been developed to aid cohort selection for research and clinical trials. The classification system includes both clinical and laboratory criteria, which are hierarchically clustered, weighted and risk-stratified to ensure high specificity.



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Autoimmune blood disorder

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by persistent presence of autoantibodies against phospholipids or their binding proteins. The hallmark manifestation of APS is arterial, venous or microvascular blood clots, which can lead to pulmonary embolism, stroke and myocardial infarction. Women with APS are at higher risk for pregnancy-related problems, including fetal loss and pre-eclampsia. APS can also manifest non-thrombotically, for example as cardiac valve disease or thrombocytopenia [1]. APS is classified as either a primary disease or secondary to other autoimmune diseases such as systemic lupus erythematosus. Catastrophic APS is a rare but severe disease form with thrombotic complications in multiple organs and a high risk of mortality. APS is generally managed using anticoagulation therapies, most commonly vitamin K antagonists [2].

Heterogeneous autoantibodies

Anti-phospholipid antibodies (aPL) are a heterogeneous group of autoantibodies of IgG, IgM and IgA isotypes. Target antigens include cardiolipin and phosphatidylserine, as well as phospholipid-binding co-factors such as β_2-glycoprotein 1 (β_2GPI) and prothrombin. Antibodies against cardiolipin (aCL) and β_2GPI (aβ_2GPI) of classes IgG and IgM as well as lupus anticoagulant are the core laboratory parameters used for disease classification.

Updated classification criteria

In 2023, new classification criteria were published with international multidisciplinary input and support from the American College of Rheumatology (ACR) and the European Alliance of Associations for Rheumatology (EULAR) [1]. These replaced the previous Sapporo criteria from 2006. The goal of the criteria was to define a threshold for APS classification for use in research on disease pathophysiology and treatment effects. The new classification system is based on an up-to-date understanding of APS, including aPL-associated non-thrombotic clinical manifestations, the role of traditional thrombosis risk factors in aPL-positive individuals, and risk stratification by aPL.

Weighted point system

The probability of an individual having APS is assessed using an additive, weighted system based on clinical and laboratory criteria. Entry into the classification system requires at least one documented clinical criterion plus a positive aPL result that occurs within 3 years of the clinical feature. Individual criteria carry scores of 1 to 7, and a total of 3 points each from the clinical and laboratory domains is required for classification as APS.

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Table 1. Laboratory criteria for antiphospholipid syndrome classification

aPL by lupus anticoagulant test	**Weight**
Positive (single – one time)	1
Positive (persistent)	5
aPL by solid-phase assay	**Weight**
Moderate or high positive (IgM) (aCL and/or $a\beta_2$GPI)	1
Moderate positive (IgG) (aCL and/or $a\beta_2$GPI)	4
High positive (IgG) (aCL *or* $a\beta_2$GPI)	5
High positive (IgG) (aCL *and* $a\beta_2$GPI)	7

>> The clinical criteria encompass six domains: macrovascular venous thromboembolism, macrovascular arterial thrombosis, microvascular, obstetric, cardiac valve and hematologic. The laboratory criteria are divided into two distinct domains: positive lupus anticoagulant test and solid-phase detection of aCL or $a\beta_2$GPI (IgG or IgM) at moderate to high titres (Table 1). The laboratory results must be confirmed after an interval of at least 12 weeks, since aPL may also develop during infections. Novel features of the laboratory criteria include quantification of single, double and triple aPL positivity based on different domains and weights, separation of IgM and IgG isotypes of aCL and $a\beta_2$GPI, and definition of two levels of antibody positivity.

Validation of the new criteria in international patient cohorts revealed a specificity of 99% compared to 86% for the previous Sapporo criteria. The very high specificity, even at the cost of sensitivity, enables stringent selection of homogeneous patient cohorts to enable comparability across clinical studies and trials.

In the diagnostic consensus used for managing patients in a clinical setting, inclusion of a low-positive or intermediate zone in the interpretation ranges for aCL and $a\beta_2$GPI in addition to moderate-positive and high-positive categories is recommended. This is important because the risk associated with these antibodies increases on a continuous scale, and the presence of multiple aPL antibodies also carries a higher risk than single positivity. Furthermore, different levels of antibodies may be associated with different symptoms [3].

Further laboratory parameters

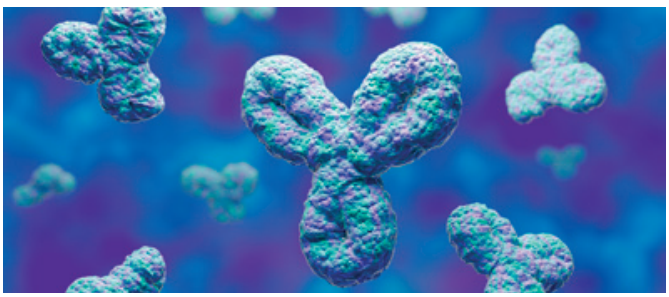
Class IgA aCL and $a\beta_2$GPI occur mainly in combination with IgG and/or IgM. However, isolated IgA positivity has been described. IgA antibodies are not included in the classification criteria, since their pathogenic and prognostic significance is not yet sufficiently understood. However, in the diagnostic consensus, determination of aCL and $a\beta_2$GPI of class IgA is recommended in cases in which IgM and IgG isotypes are negative but APS is still suspected [3]. Antibodies against phosphatidylserine have also been shown to have a significant association with APS [4]. Research on various noncriteria aPL to clarify their clinical relevance and potential role in diagnostics is ongoing [5].

Laboratory methods

The lupus anticoagulant test is based on the identification of prolonged clotting times in patient plasma samples. However, anticoagulants frequently used to treat clotting disorders may interfere with the assay, generating false-positive or -negative findings. Other disadvantages of the test include the impact of preanalytical factors such as sample collection and transport on detection accuracy, as well as poor assay standardization [6].

aCL and $a\beta_2$GPI assays are calibrated immunological tests which enable quantitative measurement of the antibody titres. They do not require fresh plasma, and results are not affected by anticoagulant therapy. In the ACR/EULAR classification criteria, ELISA is the stipulated method for measurement of aCL and $a\beta_2$GPI owing to established titre thresholds. If only alternative technologies are available, researchers must identify and validate the moderate/high thresholds for their system and correlate these with the ELISA thresholds.

Of note, the Steering Committee recognizes the increasing usage of new laboratory automation platforms. In its research agenda for future updates of the classification criteria, it recommends studying other technologies to determine the thresholds corresponding to ELISA. Chemiluminescence immunoassay technology using magnetic particles as solid phase is among the promising testing modalities for further validation.



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aPL assays from EUROIMMUN

A range of ELISA and chemiluminescence immunoassays (ChLIA) for determination of aPL is available from EUROIMMUN. The ELISA range encompasses detection of aCL or $a\beta_2$GPI of classes IgG, IgM or IgA. Additional assays for anti-phosphatidylserine antibodies are also available. In clinical evaluation with a panel of APS patients, the prevalences of aCL determined using the ELISAs amounted to 67% (IgG), 38% (IgM) and 10% (IgA), whereas the prevalences of $a\beta_2$GPI were 43% (IgG), 52% (IgM) and 52% (IgA). ELISAs for simultaneous measurement of all three antibody classes yielded prevalences of 81% for aCL (IgAGM) and 86% for $a\beta_2$GPI (IgAGM). In control panels of samples from patients with HIV, HBV or HCV infections, healthy pregnant women and healthy blood donors, the specificities of all ELISAs lay between 97% and 100%.

ChLIAs are available for rapid random-access analyses of aCL or $a\beta_2$GPI of isotypes IgG or IgM. In a panel of patients with APS, the clinical sensitivities using the ChLIAs amounted to 85% (IgG) and 40% (IgM) for aCL and 84% (IgG) and 32% (IgM) for $a\beta_2$GPI. In a control panel of patients with systemic autoimmune rheumatic disorders, patients with HIV, HBV or HCV infections and healthy blood donors, the ChLIAs yielded diagnostic specificities between 94% and 98%. ChLIA technology offers the advantage of a faster processing time than ELISA, with first results available in as little as 40 minutes. The continuous sample loading and walkaway automation also provide exceptional flexibility and efficiency in daily routines.

Conclusion

Laboratory tests play a central role in the diagnosis of APS and stratification of patients for clinical studies. Updated APS classification criteria for research studies are based on a weighted assessment which includes multiple aPL positivity, distinction of IgM and IgG isotypes of aCL and $a\beta_2$GPI, and two antibody titre levels. Future directions of research include elucidating the role of IgA isotypes of aCL and $a\beta_2$GPI, studying further test parameters, and assessing the feasibility of new automated technologies such as chemiluminescence immunoassay platforms.

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For further information see:
www.euroimmun.com

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