IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Evaluation of novel assays for the detection of autoantibodies in antiphospholipid syndrome

This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1754544 since 2020-09-01T10:07:42Z

Published version:

DOI:10.1016/j.autrev.2020.102641

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

17 September 2026

Evaluation of novel assays for the detection of autoantibodies in antiphospholipid syndrome

Sciascia S[1], Radin M[1],Ramirez C[2],Seaman A[2], Bentow C[2],Casas S[2], Cecchi I[1], Rubini E[1], Foddai SG[1], Baldovino S[1], Fenoglio R[1], Menegatti E[1], Roccatello D[1], Mahler M[2]

1. Center of Research of Immunopathology and Rare Diseases- Coordinating Center of Piemonte and Valle d'Aosta Network for Rare Diseases, Department of Clinical and Biological Sciences, and SCDU Nephrology and Dialysis, S. Giovanni Bosco Hospital, Piazza del Donatore di Sangue 3, 10154, Turin, Italy.

2. InovaDiagnostics, San Diego, CA, USA.

Short Title: Novel assays for the detection of autoantibodies in APS

Key words: Antiphospholipid syndrome; antiphospholipid antibodies; non criteria aPL; Thrombosis; Aptiva; PMAT

Corresponding Author: Savino Sciascia MD, PhD

Center of Research of Immunopathology and Rare Diseases- Coordinating Center of Piemonte and Valle d'Aosta Network for Rare Diseases, and SCDU Nephrology and Dialysis, S. Giovanni Bosco Hospital, Piazza del Donatore di Sangue 3, 10154, Turin, Italy. Email savino.sciascia@unito.it Tel +390112402056 Fax +390112402052

Funding:MR is funded by a grant from the Italian Ministry of Health SG-2018-12368028

Abstract:

Patients with antiphospholipid syndrome (APS) present with clinical features of recurrent thrombosis and pregnancy morbidity and persistently test positive for the presence of antiphospholipid antibodies (aPL). At least one clinical (vascular thrombosis or pregnancy morbidity) and one lab-based (positive test result for lupus anticoagulant, anticardiolipin antibodies and/or anti-β2-glycoprotein 1 antibodies) criterion have to be met for a patient to be classified as having APS. Nevertheless, the clinical variety of APS encompasses additional signs and symptoms, potentially affecting any organ, that cannot be explained exclusively by a prothrombotic state. Those manifestations, also known as extra-criteria manifestations, include haematologic (thrombocytopenia and haemolytic anaemia), neurologic (chorea, myelitis and migraine) manifestations as well as the presence of livedo reticularis, nephropathy and valvular heart disease. The growing body of evidence describing the clinical aspect of the syndrome has been paralleled over the years by emerging research interest focusing on the development of novel biomarkers that might improve the diagnostic accuracy for APS when compared to the current aPL tests. This review will focus on the clinical utility of extra-criteria aPL specificities.Besides, the promising role of a new technology using particle based multi-analyte testing that supports aPL panel algorithm testing will be discussed. Diagnostic approaches to difficult cases, including real-world case studies investigatingthe diagnostic added value of extra criteria aPL, particularly anti-phosphatidylserine/prothrombin,will also be examined.

1.1 Introduction - Filling the gaps in APS diagnosis

The antiphospholipid syndrome (APS) is a unique form of acquired autoimmune thrombophilia initially described in 1980s [1], referring to individuals who presented with recurrent thrombosis (arterial and/or venous) and/or pregnancy complications and who tested persistently positive for antiphospholipid antibodies (aPL).

The current aPL included in the laboratory classification criteria [2] are lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti-β2-glycoprotein 1 (aB2GPI) antibodies. Most of the autoantibodies found in the patients' serum are directed against the plasma apolipoproteins that bind the phospholipids, especially β2-glycoprotein-1 and prothrombin. Since its first description, the number of antibodies that have been associated to APS is constantly increasing[3]. While the tests currently included in the classification criteriaare able to correctly detect the great majority of the cases, some patients at high clinical suspicion of APSmay be not identified. The diagnostic utility of tests non included in the criteria (so-called extra criteria aPL tests) is currently debated [4] and, among the others, the relevance of extra criteria aPL such as anti-phosphatidylserine/prothrombin (aPS/PT) antibodies and IgA isotypes has been proposed as an additional tool to be considered when investigating a patient suspected of having APS, particularly in the absence of routine aPL[5,6], or as a part of risk assessment strategies [7].

The importance of early diagnosis is now a well-recognized notion in the management of many rheumatic diseases, and it is still an unmet need in patients with APS. Ideally, the prompt identification of aPL could modify the strategy of treatment, impacting on both pregnancy-related and thrombotic events management.

Herewith, we aimto discuss the added diagnostic value of testing for extra criteria aPL. Besides, a special focus on the emerging role of particle-based multi-analyte technology(PMAT) that embraces aPL panel testing and profiling will be examined.

2. New frontiers in aPL testing: The panel approach

The advent of new diagnostic platforms combined with deep learning artificial intelligence constitutes a new frontier in aPL testing that holds the promise of closing serological gaps in autoantibody diagnostics. This has been demonstrated by recent studies showing the potential clinical utility of this profiling approach in rheumatoid arthritis an idiopathic inflammatory myopathy[8–11].

In recent years, a full automated digital system using PMAT has been developed which allows for the simultaneous detection of autoantibodies and proteins. More specifically, the APS reagents detect antibodies of IgG, IgA and IgM isotypes to CL, ß2GPI and PS/PT, resulting in a profile of 9 different aPL tests.

The analytes in the assays are created by covalently binding antigens to paramagnetic microparticles. Each analyte is associated with a discrete population of particles with a unique signature that allows for their classification by an optical module. The optical module is composed of two light-emitting diodes (LEDs) units set to different wavelengths and one charge-coupled device sensor. One diode is used to classify the particles into discrete sets (populations) that are assigned to a specific analyte, this is achieved by shining light at a specific electromagnetic wavelength, while the second diode shines light at a different wavelength, selected specifically, to excite the fluorochromes present in the phychoerythrin conjugated to anti-human IgG, IgM or IgA detection antibodies.

Multiple images are generated by the system in order to identify and count the three unique analyte particles, as well as determine the amount of conjugate on each particle. A fourth particle, coated with goat anti-human antibodies (IgG, IgM or IgA, depending on isotype), is present in the reagent as a control to detect that the patient serum sample and the conjugate have been added. The median fluorescent intensity (MFI) for each analyte is proportional to the concentration of conjugate bound to human IgG, IgM or IgA, which is proportional to the concentration of IgG, IgM or IgA bound to the corresponding particle population.

Each analyte in the PMAT APS reagent is assigned a predefined lot specific master curve. The analyte specific master curve is stored on the reagent's cartridge radio frequency identification label. Based on results obtained by running calibrators (supplied separately), the system creates individual working curves used by the software to calculate fluorescent light units for each analyte from the MFI values obtained for each sample.

Prior to use in the full automated digital instrument, the isotype-specific reagent cartridge, containing all required components, is prepared by piercing the sealed reagent tubes with the cartridge lid. Once place onboard, the instrument will automatically rehydrate the microparticles. A patient serum sample is pre-diluted by the instrument with sample buffer in a small disposable plastic cuvette. Small amounts of the diluted patient serum, the microparticles, and the assay buffer are all combined into a second cuvette, mixed, and then incubated for 9.5 minutes at 37ºC. The magnetized microparticles are washed repeatedly, before being transferred to the optical module for quantitation.

3. Case Study: Added clinical value of the aPL panel approachin patients suspected for APS

3.1 Patients selection

We chart-reviewed patients with thrombotic events and/or pregnancy morbidity who tested persistently positive for at least one aPL (more than 2 occasions over a time ofmore than 12 weeks) that presented at San Giovanni Bosco Hospital in the last 5 years. The study was performed in compliance with the Declaration of Helsinki.

We enrolled 80 patients who met one of the following inclusion criteria:

1) Fulfilled the diagnosis of APS defined as per Sydney criteria [2].

2) Patients with thrombosis and/or pregnancy morbidity and suspected APS not completely fulfilling the laboratory criteria [2], as follows: a) inconsistent previous LA positivity; and/or b) low-medium titersaPL [defined as levels of aCL IgG/IgM or aβ2GPI IgG/IgM between 10-30 GPL/MPL]. Clinical and laboratory characteristics were retrospectively collected.

3.2 Previous autoantibody detection and testing with PMATplatform

The aPL routine testing at diagnosis included LA as well as aCLa, β2GPIand aPS/PT (IgG and IgM isotypes) antibodies. Venous blood was collected using a 21-gauge butterfly needle, with minimal venous stasis, into Vacutainer® tubes (BD, Plymouth, UK). Serum was collected after double centrifugation at ambient temperature (2000 g for 15 minutes) and stored in aliquots at -80 °C.

The aCL and aß2GPI and aPS/PT(IgG and IgM) antibodieswere detected by commercial enzyme-linked immunosorbent assay (ELISA) (QUANTA Lite®, Inova Diagnostics). Plasma samples were tested for the presence of LA according to the recommended criteria from the International Society on Thrombosis and Haemostasis (ISTH) Subcommittee on Lupus Anticoagulant/Phospholipid-Dependent Antibodies [12,13]. The samples were then tested with the Aptiva™, full automated digital system using PMAT, andAptiva™ APS reagents (Inova Diagnostics, San Diego, CA; under development and for research use only).

3.3 Results

A total of 80 patients were included for analysis. Seventy-four (92.5%) patients presented with previous thrombotic events, 6(7.5%) presented with previous history of pregnancy morbidity (fulfilling the clinical criteria for APS [2]). Four(5%) patients presented with both. Further, 65 (81.3%) patients fulfilled the diagnosis of thrombotic APS defined as per Sydney criteria [2] and 15 (18.8%) patients did not completely fulfilling the laboratory criteria [2], as previously defined in the inclusion criteria of the study.

When testing with the PMAT APS reagents, 70 patients (87.5%) tested positive for at least one aPL, as illustrated in Figure 1. Thirty-height (47.5%) patients tested positive for aCL IgG. The number of positive patients increased to 55 (68.8%) patients when testing for aβ2GPI and aCL IgG/M. Interestingly, the number of positive patients further increased to 59 (73.8%) and 69 (86.3%) when adding the IgA isotype for aCL and aβ2GPI antibodies as well as aPS/PT IgG/IgM antibodies, respectively.

When limiting the analyses to the patients with thrombosis and/or pregnancy morbidity and suspected APS not completely fulfilling the laboratory criteria, 9/15(60.0%) were found positive for aPL when tested with PMAT(7/15 positive for aCL IgG/M and/or aβ2GPIIgG/M). Remarkably, two patients with thrombosis were positive for aPS/PT only (one patient with aPS/PT IgG and IgM, one patient withIgM alone).

4. Discussion

Careful evaluation of autoantibody assays for the detection of aPL is of utmost importance since some of these antibodies are included or being considered for APS classification criteria [2,3]. The markers are not only relevant for establishing the diagnosis, but also in the stratification into risk specific subsets [7].

The reliability of autoantibody measurements (i.e. reproducibility of the test) and the diagnostic accuracy (i.e. ability to identify patients at higher risk for a specific condition) are therefore crucial for optimum patient care and management. Historically, ELISA-based assays have been used to confirm and quantify the concentration of autoantibodies in patient samples. These measurements have traditionally been performed in specialized immunology laboratories. However, autoantibody testing is now commonplace with an increasing tendency towards more automated methods, in larger number of laboratories. As the number of testing increases, the number of available techniques for immunological testing is increasing as well.On the other hand, as medical intervention moves to disease prediction and a model of "intent to PREVENT", diagnostics will need to include an early symptom/risk-based, as opposed to a disease-based approach. With the potential of simultaneously testing several analytes on a small sample size, newer diagnostic platforms based on multi-analyte technology has the potential to facilitate the shift to a personalized medicine approach.aPL testing mighttherefore undergo a paradigm shift, moving from being solely a diagnostic marker to be consider a biomarker to help in different clinical settings, including disease prediction and prevention; early and accurate diagnosis; and effective and timely treatment. Here we presented a case study to provide evidence to support the use of PMATto identify patients suspected for APS. Which is most interesting of our preliminary findings is that 60% of patients with thrombosis and/or pregnancy morbidity and suspected APS not completely fulfilling the laboratory criteria were found positive for at least one aPL when tested with PMAT. More intriguingly, 2/15 (13.3%) of them were positive only for aPS/PT.

From this perspective, our study helps to provide some further evidenceaiming to address the question: "Should aPS/PT be incorporated into the routine serological tests in the diagnosis of APS?" Some considerations are worth mentioning when addressing this point.

Firstly, aPS/PT covers a significant proportion of patients clinically suspected for having APS but negative LA, aCL and aβ2GPI, and the combination of aPS/PT with traditional aPL further enhances the diagnostic power. Secondly, the introduction of aPS/PT further strengthens risk stratification in patients with APS. Thirdly, when the 2006 international consensus statement[2]was proposed, the detection of aPS/PT was mainly based on in-house ELISA, resulting in large variability among different studies.Over the past 10 years, the performance of ELISA-based systems for detection of aPS/PT has substantially improved, and commercially available assays with improved sensitivity and specificity have been evaluated in many studies[14]. Data coming from 2 available systematic reviews[15,16] involving about 10.000subjects have shown a strong association between aPS/PT and the clinical manifestations of APS. With the available level of evidence, aPS/PT testing can be considered as a robust test applicable in the management of patients suspected for APS, also beyond the research settings. One significant limitation of our study is that we did not include a control population that would allow for assessing the specificity. However, the specificity of the assays has previously been established[17].

While additional studies based on larger cohorts are needed to fully assess the assay performance on the novel PMAT system for the measurement of autoantibodies in APS, the approach PMAT offers for detecting a spectrum of antibodies in patients suspected for APS represents a leading way to biomarker disease profiling and to improving our management of patients suspect for APS.

References

[1] Hughes GR. The anticardiolipin syndrome. Clin Exp Rheumatol n.d.;3:285–6.

[2] Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4:295–306. doi:10.1111/j.1538-7836.2006.01753.x.

[3] Sciascia S, Amigo M, Roccatello D, Khamashta M. Diagnosing antiphospholipid. Nat Publ Gr 2017. doi:10.1038/nrrheum.2017.124.

[4] Hughes GR V, Khamashta MA. "Seronegative antiphospholipid syndrome": an update. Lupus 2019;28:273–4. doi:10.1177/0961203319826358.

[5] Rodríguez-García V, Ioannou Y, Fernández-Nebro A, Isenberg DA, Giles IP. Examining the prevalence of non-criteria anti-phospholipid antibodies in patients with anti-phospholipid syndrome: a systematic review. Rheumatology (Oxford) 2015;54:2042–50. doi:10.1093/rheumatology/kev226.

[6] Nayfe R, Uthman I, Aoun J, Saad Aldin E, Merashli M, Khamashta MA. Seronegative antiphospholipid syndrome. Rheumatology (Oxford) 2013;52:1358–67. doi:10.1093/rheumatology/ket126.

[7] Sciascia S, Sanna G, Murru V, Roccatello D, Khamashta MA, Bertolaccini ML. GAPSS: the Global Anti-Phospholipid Syndrome Score. Rheumatology (Oxford) 2013;52:1397–403. doi:10.1093/rheumatology/kes388.

[8] Martinez-Prat L, Lucia D, Ibarra C, Mahler M, Dervieux T. Antibodies targeting protein-arginine deiminase 4 (PAD4) demonstrate diagnostic value in rheumatoid arthritis. Ann Rheum Dis 2019;78:434–6. doi:10.1136/annrheumdis-2018-213818.

[9] Darrah E, Martinez-Prat L, Mahler M. Clinical Utility of Antipeptidyl Arginine Deiminase Type 4 Antibodies. J Rheumatol 2019;46:329–30. doi:10.3899/jrheum.180905.

[10] Richards M, Garcia-De La Torre I, Gonzalez-Bello YC, Vazquez-Del Mercado M, Andrade-

Ortega L, Medrano-Ramirez G, et al. Autoantibodies to Mi-2 alpha and Mi-2 beta in patients with idiopathic inflammatory myopathy. Rheumatology (Oxford) 2019;58:1655–61. doi:10.1093/rheumatology/kez092.

[11] Mahler M, Betteridge Z, Bentow C, Richards M, Seaman A, Chinoy H, et al. Comparison of Three Immunoassays for the Detection of Myositis Specific Antibodies. Front Immunol 2019;10:848. doi:10.3389/fimmu.2019.00848.

[12] Brandt JT, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. Thromb Haemost 1995;74:1185–90.

[13] PENGO V, TRIPODI A, REBER G, RAND JH, ORTEL TL, GALLI M, et al. Update of the guidelines for lupus anticoagulant detection. J Thromb Haemost 2009;7:1737–40. doi:10.1111/j.1538-7836.2009.03555.x.

[14] Sciascia S, Sanna G, Murru V, Khamashta MA, Bertolaccini ML. Validation of a commercially available kit to detect anti- phosphatidylserine/prothrombin antibodies in a cohort of systemic lupus erythematosus patients. Thromb Res 2014;133. doi:10.1016/j.thromres.2013.12.001.

[15] Sciascia S, Sanna G, Murru V, Roccatello D, Khamashta MA, Bertolaccini ML. Anti-prothrombin (aPT) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies and the risk of thrombosis in the antiphospholipid syndrome. A systematic review. Thromb Haemost 2014;111:354–64. doi:10.1160/TH13-06-0509.

[16] Radin M, Foddai SGF, Cecchi I, Rubini E, Schreiber K, Roccatello D, et al. Anti-phosphatidylserine/prothrombin antibodies: an update on their association with clinical manifestations of antiphospholipid syndrome. Thromb Haemost 2020 n.d.

[17] Elbagir S, Mohammed NA, Kaihola H, Svenungsson E, Gunnarsson I, Manivel VA, et al. Elevated IgA antiphospholipid antibodies in healthy pregnant women in Sudan but not

Sweden, without corresponding increase in IgA anti-β_2 glycoprotein I domain 1 antibodies. Lupus 2020:096120332090894. doi:10.1177/0961203320908949.

Legend of Figures and Tables

Figure 1. Added progressive percentage of patients positive for antiphospholipid antibodies (divided for isotypes)

Figure 1. *Added progressive percentage of patients positive for antiphospholipid antibodies*

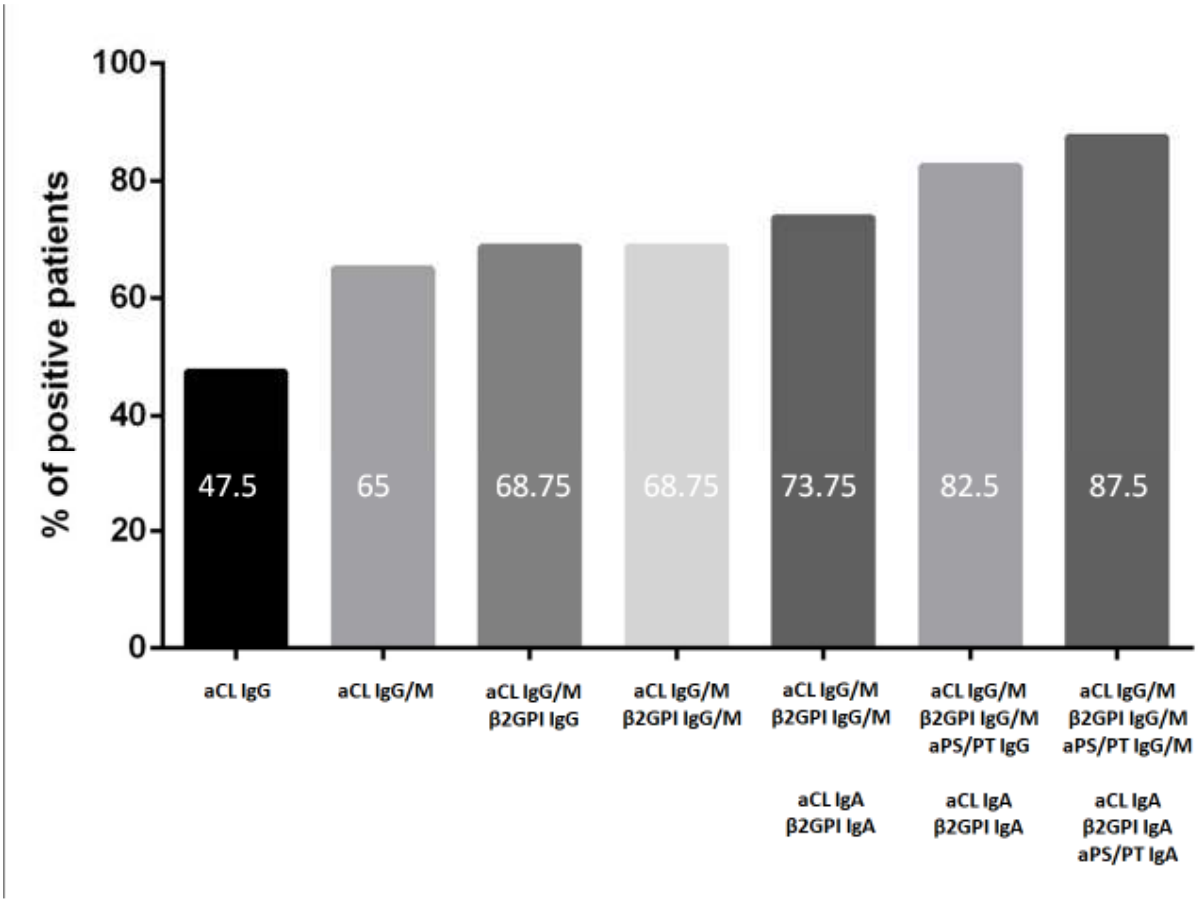

Figure 1. Added progressive percentage of patients positive for antiphospholipid antibodies (divided for isotypes)