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ORIGINAL ARTICLE

The introduction of anti-phosphatidylserine/prothrombin autoantibodies in the laboratory diagnostic process of anti-phospholipid antibody syndrome: 6 months of observation

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Abstract
Purpose To evaluate the impact of the introduction of the anti-phosphatidylserine/prothrombin autoantibodies (aPS/PT) in the laboratory diagnostic process of anti-phospholipid antibody syndrome (APS).
Methods Four hundred and twenty-one patients (71.5 % females; 53 ± 15 years) presenting a medical prescription for aPS/PT antibodies were consecutively enrolled in the study from March 2013 to August 2013. During the same period, aPS/PT were additionally investigated in a selected series of 62 patients characterized by difficult lupus anticoagulant (LA) tests interpretation and in a retrospective series of 52 LA positive cases with available data about anti-prothrombin (aPT) antibodies. The aPS/PT antibodies, as well as the anti-cardiolipin (aCL), the anti-β2 glycoprotein I (aβ2GPI) and the aPT antibodies were analyzed by ELISA. LA was tested according to the recommended criteria, performing both the screen and the confirm steps.
Results Overall, aPS/PT IgM positive (>30 U/ml) and/or IgG frankly positive (>40 U/ml) antibodies were found in 49/421 (11.6 %) cases. Among the LA positive patients, we found 56.1 % aPS/PT positive versus 31.7 % aCL and/or aβ2GPI positive cases, with limited (17.1 %) simultaneous positivity. The PS/PT complex resulted the newly recognized specificity in about 27 % of patients recruited from the subset with difficult LA test interpretation. Compared to aPT antibodies, the aPS/PT antibodies displayed a much higher sensitivity (55.8 versus 15.4 %) in LA positive patients.
Conclusions The introduction of aPS/PT antibodies in the diagnostic process of APS is highly recommended, since they disclose a notable diagnostic performance and a high correlation with LA activity, such that they can be a viable alternative.

Keywords Anti-phospholipid antibody syndrome · Anti-phosphatidylserine/prothrombin autoantibodies · Lupus anticoagulant · Anti-cardiolipin antibodies · Anti-beta2-glycoprotein I antibodies

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Introduction

Antiphospholipid syndrome (APS) is defined by the presence of a hypercoagulable disorder, (clinically displayed by venous or arterial thrombosis and/or adverse obstetric outcomes), accompanied by persistent and elevated levels of antiphospholipid (aPL) antibodies [1]. According to the 2006 revised international diagnostic criteria [1], the presence of one among anti-beta2 glycoprotein I (aβ2GPI) IgG or IgM, anti-cardiolipin (aCL) IgG or IgM and the lupus anticoagulant (LA) is indicated for a definite diagnosis of APS. In some cases aCL did not associate with LA activity; not infrequently LA activity remains isolated or can not be demonstrated. Only recently the so-called "seronegative APS" was definitely recognized as a distinctive setting [2], or better redefined by the demonstration of new classes of aPL antibodies, such as anti-vimentin/cardiolipin antibodies [3] and

anti-prothrombin/phosphatidylserine (aPS/PT) antibodies [4]. Atsumi et al. [5], already in 2000, showed that aPS/PT antibodies and aCL have similar diagnostic value for APS patients, but only recently aPS/PT antibodies were recommended as helpful for the diagnosis of APS in a clinical setting [6]. Numbers of recent papers underlined such an important role in primary APS, lupus and other systemic inflammatory disorders frequently associated with APS manifestations [7–10] and showed better performance compared to anti-prothrombin (aPT) antibodies [11, 12]. Of note, the combination of aβ2GPI, aPS/PT and LA demonstrates the best diagnostic accuracy for APS as a whole and individually for both thrombosis and pregnancy loss [13]. In addition, aPS/PT antibodies were recently recommended as a surrogate of LA when specific inhibitors and/or analytical variables may affect its interpretation, (i.e., oral anticoagulant therapy, OAT), [14]. Despite these recommendations, nowadays very few clinical laboratories in Italy still include aPS/PT antibodies in routine analyses.

We herein report the results obtained during the first 6 months after the introduction of aPS/PT antibodies in clinical laboratory practice, analysing the prevalence, the relationship with to aCL, aβ2GPI and aPT antibodies, the association with LA and the specific additional contribution in APS diagnostic process.

Patients

Four hundred and twenty-one patients (71.5 % females; mean age 53 ± 15 years, range 18–88) were consecutively enrolled in the study, as patients attending the Laboratory of Immunopathology and Allergy of the University Hospital of Udine from March 2013 to August 2013, presenting a medical prescription for aPS/PT antibodies. During the same period of time, integrating the medical prescription, aPS/PT were also analyzed in a selected series of 62 patients referred by the Unit of Haemostasis, to improve the interpretation of uncertain LA test results (i.e., borderline results, OAT, inherited or acquired deficits of coagulant factors, contradictory results between screen and confirm steps). aPS/PT antibodies were also investigated in a retrospective series of 52 LA positive samples with historical data of aPT antibodies and in 52 healthy donors (HDs; mean age 37 ± 13 years, range 18–65; 29 females and 23 males) from the Department of Transfusion Medicine as controls. All patients and controls gave their informed consent to this retrospective study according to the Declaration of Helsinki and to the Italian legislation (Authorization of the Privacy Guarantor No. 9, 12 December 2013).

Methods

The aPS/PT IgG and IgM antibodies were analyzed by ELISA using the Quanta Lite aPS/PT IgG/IgM ELISA kit (Inova Diagnostics Inc, San Diego, CA). The aCL IgG and IgM, the aβ2GPI IgG and IgM and the aPT IgG and IgM were analyzed by ELISA (Orgentec Diagnostika, Mainz, Germany) considering the following cut-offs: aCL IgG >10 U/ml; aCL IgM >7 U/ml; aβ2GPI IgG and IgM >8 U/ml; aPT IgG and IgM >20 U/ml.

Plasma samples were tested for the presence of LA according to the recommended criteria from the ISTH Subcommittee on lupus anticoagulant-phospholipid-dependent antibodies [15, 16]. All samples were screened using a sensitive activated partial thromboplastin time (aPTT) test performed with silica as an activator (HemosIL Silica Clotting Time—SCT, Instrumentation Laboratory, Italy) and confirmed by a dilute Russell viper venom time dRVVT coagulation test (HemosIL dRVVT, Instrumentation Laboratory, Italy).

Ratios higher than 1.23 for SCT and higher than 1.20 for dRVVT which did not correct with the 50:50 mixture with normal plasma were considered diagnostic of LA.

Statistic analyses (2 × 2 contingency tables using Fisher exact test) were performed using the Graph Pad Prism and Instat softwares (San Diego, CA).

Results

aPS/PT antibodies: specificity and sensitivity compared to other aPL assays

As a first step to set in our population the cut-off of the new tests for aPS/PT IgG and IgM antibodies, we performed the analysis in a series of 52 healthy donors. The cut-off suggested by the manufacturer was ≥30 U/ml both for IgG and IgM. Considering this value, the specificities were, respectively, 98.1 % for aPS/PT IgM and 86.5 % for aPS/PT IgG. Setting the cut-off of IgG at 40 U/ml, the specificity improved (94.2 %), thus we finally decided to consider frankly positive aPS/PT IgG when ≥40 U/ml, borderline between 30 and 40 U/ml.

Overall, aPS/PT IgG and/or IgM positive antibodies were found in 49/421 (11.6 %) cases, comprising 37 (75.5 %) IgM positive, 11 (22.4 %) IgG frankly positive (≥40 U/ml) patients and one aPS/PT IgM/IgG double positive case. Borderline aPS/PT IgG (30–40 U/ml) antibodies were present in 16 cases (two of which were also positive for aPS/PT IgM). After adding these borderline IgG cases, the ultimate aPS/PT sensitivity was assessed at 15 % (63/421).

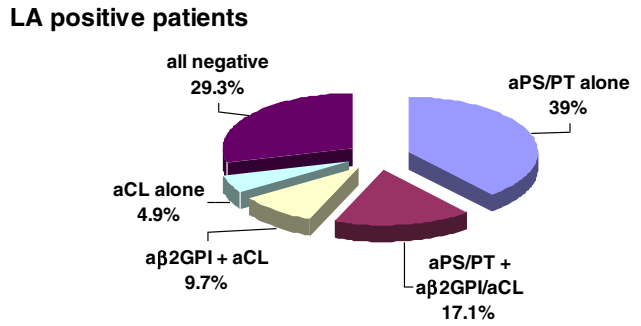

Fig. 1 Prevalence of aPS/PT, aCL and aβ2GPI antibodies, alone or combined, in LA positive patients

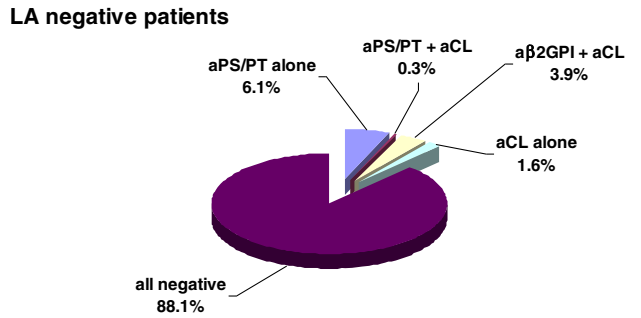

Fig. 2 Prevalence of aPS/PT, aCL and aβ2GPI antibodies, alone or combined, in LA negative patients

aCL antibodies were simultaneously analyzed in 403 of the 421 cases, resulting in 4 (1 %) IgG positive, 20 (4.9 %) IgM positive and 5 (1.2 %) IgG/IgM double positive cases.

The aβ2GPI antibodies were simultaneously analyzed in 398 of the 421 patients, resulting in 25/398 (6.3 %) positive cases: 4 IgG, 19 IgM and 2 IgG/IgM double positive cases.

Data about LA were available in 367 of the overall 421 cases, of which 41/367 (11.2 %) resulted positive. Among the LA positive patients, 23/41 (56.1 %) resulted aPS/PT positive: of note all these cases were IgM positive, while only one was also IgG frankly positive. In contrast, in the same subgroup of LA positive subjects, only 12/41 (31.7 %) resulted aCL ($p = 0.023$, 95 % CI 1.24–7.69, OR 3.09) and 11/41 (26.8 %) aβ2GPI positive ($p = 0.013$, 95 % CI 1.38–8.80, OR 3.49).

In general, the simultaneous presence of aPS/PT and aCL or aβ2GPI antibodies was infrequent; in particular, only one of the aPS/PT IgG frankly positive patients (1/12, 8.3 %) resulted also aCL and/or aβ2GPI positive, while among the aPS/PT IgM positive patients, 9/38 (23.7 %) were also aCL and/or aβ2GPI positive.

Table 1 Newly discovered aPS/PT antibody positive cases among patients referred to the haemostasis unit and characterized by difficult LA test interpretation

Subgroups	aPS/PT alone Pos % (no.)	aCL and/or aβ2GPI Pos % (no.)	aPS/PT + aCL and/or aβ2GPI Pos % (No.)
LA positive but aCL and aβ2GPI negative (no. 21)	47.6 (10)	0 (0)	0 (0)
Patients in OAT (no. 17)	17.6 (3)	11.7 (2)	11.7 (2)
LA negative (no. 19)	21.1 (4)	10.5 (2)	5.3 (1)

Further important observations came from the analysis of LA positive and negative patients.

In LA positive patients, testing of aPS/PT antibodies allowed to identify 39 % previously unrecognized aPL antigenic specificities, while the simultaneous presence of the aPS/PT and the aCL and/or aβ2GPI antibodies was noticed in 17.1 % of cases, the aCL plus the aβ2GPI antibodies in 9.7 % and the sole aCL antibodies in 4.9 % (Fig. 1).

The analysis of the 310 LA negative cases in which all the aPL antibodies were tested (Fig. 2), revealed 6.1 % aPS/PT positive patients (equally distributed between IgG and IgM) and 3.9 % aCL plus aβ2GPI antibodies positive cases (the large majority IgM).

aPS/PT antibodies testing helps to interpret uncertain LA test results

Patients referred by the Haemostasis Unit to improve the interpretation of LA tests were eventually classified as 26 LA positive, 19 LA negative and 17 inconclusive because of OAT. The majority (50/62, 80.6 %) of these patients presented repetitively aCL and aβ2GPI negative antibodies in their follow-up. Our attention was initially focused on sera that were negative for aCL and aβ2GPI antibodies and positive for LA (no. 21): in this subgroup, we disclosed 10/21 (47.6 %) aPS/PT positive cases (4 IgG and 6 IgM), (Table 1).

Important results came also from the analysis of the subgroup of patients in OAT (Table 1): 5/17 (29.4 %) patients showed positive aPS/PT IgG or IgM antibodies, while 4/17 (23.5 %) were aCL and/or aβ2GPI positive (two of which also aPS/PT positive). Therefore, testing for aPS/PT antibodies allowed us to find out 3/17 (17.6 %) previously unrecognized aPL positive subjects.

Finally, among the LA negative patients (Table 1), we discovered 4/19 (21.1 %) aPS/PT positive, only two aCL positive and one aPS/PT plus aCL positive cases.

Table 2 Comparison between aPS/PT and aPT antibody sensitivity in a retrospective cohort of LA positive patients

aPT	aPS/PT	
	Neg % (no.)	Pos % (no.)
Neg % (no.)	38. 5 (20)	46.1 (24)
Pos % (no.)	5.8 (3)	9.6 (5)

Comparison between aPS/PT and aPT antibodies in LA-positive patients

We retrospectively analyzed the prevalence of the aPS/PT antibodies compared to the prevalence of the aPT antibodies in a series of 52 LA-positive patients previously investigated for aPT antibodies. As illustrated in Table 2, aPT IgG and/or IgM antibodies were positive in 8 patients (15.4 %), while the aPS/PT IgG and/or IgM antibodies in 29 (55.8 %; $p < 0.0001$; 95 % CI 2.73–17.60, OR 6.94). Both aPS/PT and aPT antibodies were positive in 5 cases (9.6 %), while aPT alone in 3 (5.8 %) and aPS/PT alone in 24 (46.1 %).

Discussion

In this study, we analyzed the impact of the introduction of the aPS/PT IgG/IgM antibodies among the routinely investigated aPL antibodies, showing a significant improvement of the APS laboratory diagnostic performance. Among the 421 samples analyzed during the 6 months of observation, we recognized nearly 12 % of aPS/PT positive cases. This result appeared of a particular interest if compared with those observed for the so-called "criteria" aPL antibodies. The prevalence of positive aCL and/or aβ2GPI antibodies in the same series of patients stood around 7 % and LA performed positive in about 10 %. Moreover, LA positive patients presented aPS/PT positive antibodies in nearly 56 % of cases, while aCL antibodies were positive in about 30 %. According to these observations, in a series of cases reported as "difficult LA interpretation", aPS/PT antibodies allowed to discover a significant number of previously unrecognized aPL positive cases, either in the subset of LA positive, but aCL and aβ2GPI negative patients, and, more importantly, in the subset of patients in OAT, where LA testing is precluded.

During the same 6 months of observation, aCL antibodies were requested and analyzed in more than 2,500 patients and aβ2GPI antibodies in more than 1,500, with positive aβ2GPI antibodies in 9.6 % of cases (data not shown). In our experience, nowadays, aCL and aβ2GPI antibodies represent, together with anti-nuclear antibodies, the most requested analysis in autoimmunology. It is of considerable importance the choice of the method between the large number of those available in the market, never forgetting the limits of each method and the not negligible possibility of false positive and negative results. Despite the increasing number of aPL antigenic specificities, anyone apart aCL and aβ2GPI, has acquired the sufficient strength to be inserted in the classification criteria yet.

The results of this short observational study lend further support to the recent encouraging indications appeared in the 2010 task force report in Galveston [6]. In our hands, testing also for aPS/PT antibodies allowed to finally reclassify a significant percentage of patients, previously negative for the classic aPLs. These patients will be then followed in a more appropriate way, taking advantage from the identification of the antigenic specificity responsible for their clinical manifestations and, in some cases, for the LA positivity. Thanks to their elevated correlation with LA, aPS/PT antibodies may help in cases where immunologic deficits or anti-coagulant drugs avoid a correct LA interpretation. Thus, as recently proposed by Pregnolato et al. [14], aPS/PT antibodies are on track to represent a very useful complementary tool in the APS diagnostic route.

In our experience, aPS/PT antibodies rarely associate with aCL and/or aβ2GPI antibodies, especially the IgG, possibly identifying a different subset of APS patients that merit further studies. Moreover, as for the other classic aPL antibodies, also for aPS/PT antibodies, IgG differed from IgM in terms of prevalence (2.6 % frankly positive versus 8.8 %) and correlation with LA (9 versus 62 %). These last observations also deserve further investigations in a larger series.

The significant lower performance demonstrated by aPT compared to aPS/PT antibodies in a series of LA positive retrospective cases, led us to substitute aPT with aPS/PT antibodies in the routine approach of APS laboratory diagnosis, deserving the analysis of aPT IgG antibodies only to the selected patients with venous thrombosis [12], when all the other aPL antibodies resulted negative.

In conclusion, the introduction of aPS/PT antibodies in the diagnostic process of APS is highly recommended, since they disclosed diagnostic laboratory performances at least equal to the aCL and aβ2GPI antibodies and a high correlation with LA activity, such that they can be a viable alternative.

Conflict of interest All Authors declare no conflict of interest.

Informed consent All patients and controls gave their informed consent to this retrospective study according to the Declaration of Helsinki and to the Italian legislation (Authorization of the Privacy Guarantor No. 9, 12 December 2013).

Human and animal rights All Authors declare that in this study we have respected human and animal rights.

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