Clinical Significance of Antiphospholipid Protein Antibodies. Receiver Operating Characteristics Plot Analysis

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ABSTRACT. ***Objective.*** To investigate the utility of receiver operating characteristic (ROC) analysis in determining the strength of association between various antiphospholipid and anti-protein cofactor antibodies (aPA) and thrombosis, pregnancy morbidity, and thrombocytopenia.

Methods. Clinical and laboratory variables were retrospectively studied in 204 patients: 160 with systemic lupus erythematosus (SLE), 22 with lupus-like syndrome (SLE-LS), and 22 with primary antiphospholipid syndrome (APS). Laboratory evaluation included detection of lupus anticoagulant (LAC) and measurement of IgG and IgM anticardiolipin (aCL), antiphosphatidylserine (aPS), antiphosphatidylinositol (aPI), anti-ß2 glycoprotein I (aß$_2$GPI), and antiprothrombin (aPT) antibodies. ROC plot analysis was used to determine the clinical accuracy of aPA tests, and calculate cut-off values which best associate with clinical symptoms typical for APS.

Results. The LAC was associated with a history of thrombosis [odds ratio (OR): 3.04; 95% confidence interval (CI): 1.5-6.2] and even more strongly with recurrent fetal loss (OR: 8.7; 95%CI: 2.8-26.7). ROC plot analysis revealed that the most accurate test for thrombosis was aCL IgG (ROC-derived cutoff value > 17.2 GPL; OR: 3.69; 95% CI: 1.8-7.4), for recurrent fetal loss, aPI IgG [> 22.1 theoretical units (TU); OR: 6.21; 95%CI: 2.1-18.5], closely followed by aCL IgG and aß$_2$GPI IgG, and for thrombocytopenia aPS IgM (> 6.7 TU; OR: 1.9; 95%CI: 1.04-3.4). Among 182 autoimmune patients (SLE + SLE-LS), 6.6% presented clinical symptoms of APS without classic aPA (LAC and/or aCL), but with elevated levels of antibodies against other phospholipids, mainly aPI IgM. ***Conclusion.*** A laboratory that evaluates APS patients should establish its own threshold values for aPA tests. We suggest that ROC plot analysis is a valuable tool in establishing cutoff values. LAC and aCL determinations seem sufficient for the majority of laboratories. However, in specialized centers other tests should be available to detect those patients with clinical symptoms for APS but who are positive for antiphospholipid antibodies other than aCL and the LAC. (J Rheumatol 2003;30:723-30)

Key Indexing Terms:
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Antiphospholipid syndrome (APS) is an autoimmune disorder that remains mysterious almost 20 years after its first description[1]. It is an example of antibody-mediated thrombosis[2]. The presence of a heterogeneous group of autoantibodies directed against certain plasma proteins bound to negatively charged phospholipids (aPL) is associated with both venous and arterial thrombosis as well as recurrent miscarriage. The risk of these clinical complications seems to be strongest with the presence of the lupus anticoagulant (LAC)[3], detected by the prolongation of phospholipid-dependent coagulation tests, and high titers of the IgG anticardiolipin antibodies (aCL)[4-6], measured by enzyme-linked immunosorbent assays (ELISA).

Recently, based on these and other studies, preliminary criteria for the classification of APS were proposed[7]. Medium or high titers of aCL IgG and/or IgM were included among the laboratory criteria. The lack of generally accepted standardization procedures make such terms as medium or high titers imprecise and leads to large differences between various laboratories[8,9]. Individual laboratories currently develop internal standardization procedures. Detection of the LAC is subject to less variation (although it is by no means free of standardization problems), when accepted criteria and confirmatory tests are properly followed[10]. The use of the cardiolipin as a target antigen in immunoassays detecting aPL is well known. However, partially cross-reacting with these antibodies are less well studied autoantibodies against other negatively charged

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phospholipids, e.g., phosphatidylserine (PS) or phosphatidylinositol (PI)[11]. It is now generally accepted that the vast majority of aPL are directed against phospholipid-binding proteins, mainly ß$_2$-glycoprotein I (anti-ß$_2$-GPI) and prothrombin (aPT). It is not clear if any of these antibodies are associated with a particular risk and/or localization (i.e., venous vs arterial) of thrombotic complications. In the majority of studies only LAC and/or aCL have been measured[3-5] and no such associations have been described. On the other hand, a recent report suggested that antiphosphatidylinositol antibodies may be particularly associated with cerebrovascular disease in young patients[12].

We wondered how useful it might be to detect antibodies against other negatively charged phospholipids (PS and PI), or their protein cofactors (ß$_2$-GPI and PT), in addition to aCL and the LAC, in determining their association with the clinical symptoms of APS.

We used receiver operating characteristics (ROC) plot analysis[13] to measure test accuracy and to calculate threshold concentrations of aPL with the optimal discriminatory power for the existence of clinical symptoms of APS in a large population of patients with systemic autoimmune diseases.

MATERIALS AND METHODS

Patients and controls. We studied 204 consecutive patients referred to our tertiary outpatient clinic for autoimmune diseases at the Jagiellonian University School of Medicine. The diagnosis of SLE was established whenever at least 4 American College of Rheumatology criteria (1982) were fulfilled[14], while the SLE-LS was defined as the presence of 2 or 3 of these criteria including antinuclear antibodies, detected on HEp2 cells. Primary antiphospholipid syndrome (APS) was diagnosed as proposed[15] in patients with none or only one criterion of SLE. The clinical profile of these patients is presented in Table 1.

The medical records of each patient were carefully reviewed according to a uniform protocol to reconfirm the existence of all the analyzed clinical manifestations. Deep vein thrombosis was always confirmed by Doppler ultrasound and ischemic stroke by computerized tomography scans. An episode of TIA was always confirmed by a neurologist, based on commonly accepted criteria[16]. Recurrent fetal loss was defined as at least 2 episodes of miscarriage[17]. Only women with at least 2 successful pregnancies were included for comparison with the recurrent fetal loss group.

The patients with primary APS were excluded from analysis of the associations between aPA and clinical symptoms of APS, because they were present in this group by definition.

One hundred healthy volunteers served as a control group for laboratory tests. This group was matched by sex and age with the examined groups (80 women and 20 men; mean age 33.5 years, range 19 to 70 years).

Laboratory analysis. Blood samples for ELISA determinations were obtained by clear venipuncture and collected into glass tubes. Samples were allowed to clot at room temperature. Serum was stored at -70°C until further use. For coagulation studies blood was collected into plastic tubes containing 1/10th volume of 3.8% trisodium citrate and centrifuged twice (2,000 × g for 10 min, and 10,000 × g for 10 min) to obtain platelet poor plasma (PPP). For LAC determination, PPP was used within 2 h after collection. Patients were considered positive for LAC and/or aCL if the tests gave abnormal results on 2 occasions at least 8 weeks apart.

Measurement of antibodies against phospholipids and their protein cofactors. All these antibodies were detected in serum using home-made ELISA methods for both IgG and IgM. aCL were measured as described[6]. In brief, microtiter plates with medium bind capacity (Polysorp Immunoplate, Nunc, Denmark) were coated with cardiolipin (Sigma, USA) dissolved in absolute ethanol and evaporated overnight at 4°C. Plates were washed with phosphate buffered saline (PBS) (pH 7.4) and blocked with 1% bovine serum albumin. Sera were diluted 1:50 in PBS and adult bovine serum. F(ab)$_2$ goat anti-human IgG or IgM fragments conjugated with peroxidase were used and absorption was measured at 492 nm. Harris standards (Aphl Louisville, USA) were used for constructing standard curves. Results were expressed as GPL and MPL units. Upper limits of normal were established using sera from 100 healthy volunteers and set at the 95th percentile of control population levels.

ELISA for aPS and aPI antibodies were prepared as a modification of aCL assay. Microplates were coated with PS or PI (Sigma, USA). OD values obtained by a stepwise serum dilutions (1:50, 100, 200, 400, 800, 1600, 3200, and 6400) of a strongly positive sample were used to construct the standard curve and results were expressed in arbitrary units. The first dilution of standard serum was arbitrarily considered as 100 theoretical units (TU). Upper limits of normal values were calculated as mentioned above. Anti-ß$_2$-GPI and aPT antibodies were detected as described[6]. In brief, gamma irradiated plates (Maxisorp Immunoplate, Nunc, Denmark) were coated overnight with human purified ß$_2$-GPI and prothrombin (Diagnostica Stago, France) dissolved in Tris buffered saline (TBS) (pH 7.4). Plates were washed with TBS containing 0.1 % Tween 20 and blocked with 0.1% gelatine. Sera were diluted 1:50 in TBS mixed with Tween 20 and 0.1 % gelatine. F(ab)$_2$ goat anti-human IgG or IgM fragments conjugated with peroxidase were used and absorption was measured in 492 nm. The standard curve and upper limits of normal values were established as above.

LAC was detected according to the guidelines of the ISTH Scientific and Standardization Subcommittee on LAC[10]. Two screening assays were performed: PTT-LAC (Diagnostica Stago, France) and DVV (American Diagnostica, USA). If one of these tests was abnormal, mixing studies were performed followed by confirmatory procedures (Staclot PNP for PTT-LAC prolongation and DVV confirm for DVV prolongation, respectively).

Statistical methods. Comparisons between groups were made by chi-square analysis followed by odds ratio (OR) calculations. Correlations were determined using the Spearman's rank correlation test. Statistical significance was accepted at a level of $p < 0.05$. Analyses were performed using Statistica software package (StatSoft Inc., Tulsa, OK, USA).

Analysis of ROC plots was performed using Stats Direct software (CamCode, England).

ROC plots provide a view of the whole spectrum of sensitivities and specificities giving all possible pairs for a particular test on a graph (e.g., Figure 1). Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold.

The area under the ROC curve (AUC) is the best measure of the diagnostic accuracy of the test. The area of 1.0 means that the test perfectly separates subjects with a particular symptom from those without it (an ideal situation: 100% sensitivity and 100% specificity). When the 95% CI of the area is < 0.5 the test does not separate 2 groups of patients, and for this reason such results are omitted in Table 4.

ROC plot analysis is used to find threshold values that best discriminate between patients who have a given symptom and those who do not. Such a calculated cutoff value provides an optimum trade-off between sensitivity and specificity of the test for a particular clinical symptom. The optimum cutoff point is the point nearest to the left upper corner on the graph of ROC curve (Figure 1).

RESULTS

Clinical symptoms of APS. The frequency of various clinical symptoms associated with APS detected in our group of 204 patients is shown in Table 1.

Altogether, 69 patients had thrombotic events in the past:

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Table 1. Clinical profile of 204 patients with systemic autoimmune diseases.

Variable, n (%)	SLE, n = 160	SLE-LS, n = 22	PAPS, n = 22
Mean age, yrs (range)	39.9 (13–72)	42.2 (21–64)	40.9 (15–69)
Female/male	148/12	17/5	15/7
Thrombosis n (%)	48 (30.0)	7 (31.8)	14 (63.6)
Venous	37 (23.1)	6 (27.3)	9 (40.9)
Arterial	13 (8.1)	2 (9.1)	6 (27.3)
Recurrent fetal loss*	12 (16.2)	4 (44.4)	4 (40.0)
Thrombocytopenia < 150,000/mm^3	56 (35.0)	7 (31.8)	10 (45.4)
Malar rash	110 (68.7)	3 (13.6)	0
Discoid rash	43 (26.9)	1 (4.5)	0
Photosensitivity	119 (74.4)	5 (22.7)	0
Oral ulcers	48 (30.0)	0	0
Arthralgia	144 (90)	13 (59.1)	2 (9.1)
Serositis	47 (28.1)	2 (9.1)	0
Renal disorder	72 (45.0)	2 (9.1)	0
Neurologic disorder	23 (14.4)	1 (4.5)	1 (4.5)
Hematologic disorder			
Thrombocytopenia < 100,000/mm^3	26 (16.2)	4 (18.2)	7 (31.0)
Hemolytic anemia	7 (4.4)	0	1 (4.5)
Leukopenia	100 (62.5)	5 (22.7)	1 (4.5)
Lymphopenia	29 (18.1)	1 (4.5)	0
Immunologic disorder			
Anti-dsDNA	58 (36.2)	2 (9.1)	0
Anti-Sm	11 (6.9)	0	0
False-positive VDRL	22 (13.7)	3 (13.6)	5 (22.7)
Antinuclear antibodies	160 (100)	22 (100)	3 (13.6)**

* The percentage of women with recurrent fetal loss were calculated in comparison to all women who were pregnant at least twice (SLE = 74, SLE-LS = 9, PAPS = 10). ** Low titer (≤ 1:80; not identifiable by radial immunodiffusion).

52 venous and 21 arterial (4 had thrombosis in both vascular beds). Recurrent thrombotic episodes were observed in 20 patients.

Among venous episodes the most common was deep vein thrombosis (DVT) of the lower extremities (34 episodes; 5 with accompanying pulmonary embolism). Among arterial episodes the most common were ischemic strokes (15 episodes; 5 manifested only as transient ischemic attacks).

Antiphospholipid-protein antibodies. Among all our patients, 77% showed the presence of at least one antiphospholipid or protein-cofactor antibody measured. The frequency of various antibodies is shown in Table 2. The most common among systemic autoimmune patients was aCL IgG (41.7%); LAC was present in 17%.

As the presence of several antibodies in the same patient was a frequent finding, we decided to analyse possible correlations between them (Table 3).

As could be expected, multiple associations (with widely varying strength) were found, the strongest being within the same isotype class of aPL. As a rule, autoantibodies against negatively charged phospholipids showed stronger correlation between themselves than with antibodies against protein cofactors. Of note, some correlations, although weak, have also been found between antiprothrombin and anti-ß$_2$-GPI antibodies.

Table 2. Frequency of different antiphospholipid and anti-protein-cofactor antibodies in the patients studied.

Antibody	SLE, n = 160 n (%)	SLE-LS, n = 22 n (%)	Primary APS, n = 22 n (%)
LAC	23 (14.4)	8 (35.4)	9 (41.0)
aCL IgG	60 (37.5)	10 (45.0)	15 (68.2)
aCL IgM	39 (24.4)	8 (35.4)	14 (63.6)
aPS IgG	29 (18.1)	7 (31.8)	10 (45.4)
aPS IgM	30 (18.7)	6 (27.3)	11 (50.0)
aPI IgG	43 (26.9)	8 (35.4)	12 (54.5)
aPI IgM	54 (33.7)	12 (54.5)	12 (54.5)
anti-ß2-GPI IgG	24 (15.0)	5 (22.7)	7 (31.8)
anti-ß2-GPI IgM	32 (20.0)	7 (31.8)	5 (22.7)
aPT IgG	48 (30.0)	6 (27.3)	11 (50.0)
aPT IgM	19 (11.9)	7 (31.8)	4 (18.2)

Clinical significance of antiphospholipid-protein antibodies (aPA). None of the single antibodies measured was preferentially linked to any of the clinical symptoms associated with APS, or for thrombotic complications, with its localization (results not shown).

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Table 3. Correlation coefficients (r) between different types of antiphospholipid and anti-protein-cofactor antibodies (Spearman rank correlation test) ($p < 0.05$).

	aCL IgM	APSIgG	aPS IgM	aPI IgG	aPI IgM	anti-ß2-GPI IgG	anti-ß2-GPI IgM	aPT IgG	aPT IgM
aCL IgG	0.38	**0.63**	0.31	**0.73**	0.21	0.52	0.28	0.34	0.22
aCL IgM		0.30	**0.80**	0.33	**0.68**	0.29	0.60	0.14	0.55
aPS IgG			0.28	**0.71**	0.14	0.43	0.22	0.32	0.20
aPS IgM				0.30	0.60	0.24	0.55	0.11	0.54
aPI IgG					0.12	0.53	0.28	0.44	0.20
aPI IgM						0.19	0.46	0.02	0.48
anti-ß2-GPI IgG							0.44	0.30	0.13
anti-ß2-GPI IgM								0.13	0.48
aPT IgG									0.12

Strong associations (r > 0.6) are marked in bold.

We identified a group of 24 patients with thrombosis (n = 22) and/or recurrent fetal loss (n = 3) but without classic antiphospholipid antibodies, i.e., LAC or aCL. Twelve of these patients (50%) had other aPA (9 aPI IgM, 1 aPI IgG, 2 aPT IgG, 1 aPT IgM, and 1 aPS IgM). Of interest, no patient in this group had anti-ß2-GPI or aPS IgG antibodies, while 9 of the 12 tested positive for IgM aPI antibodies. This group with clinical features of APS but without LAC or aCL constituted 6.6 % of our patients with systemic autoimmune diseases.

ROC plot analysis was used to compare accuracy of all the tests measured (Table 4, Figure 1). Patients with primary APS were excluded (see Materials and Methods). Such comparisons were independent of any particular threshold values and were based on the AUC. The AUC for all tests were very similar with overlapping CI, which means that they were roughly comparable. The assays seem to be the most accurate as markers associated with recurrent fetal loss and the least accurate as markers associated with thrombocytopenia. For thrombosis and recurrent fetal loss the presence of IgG isotypes always showed better accuracy than IgM, with aCL being the most accurate for the former and aPI closely followed by anti-ß$_2$-GPI and aCL for the latter. In contrast, thrombocytopenia seemed to be associated with the presence of IgM aPS.

ROC plot analysis was further used to find threshold values for all aPL and anti-protein cofactor antibodies measured, which discriminated between patients who experienced an APS clinical symptom from those who did not. OR were calculated for these ROC-derived threshold values. The results are shown in Table 5. OR for positive LAC and autoantibody levels exceeding normal values (above the 95th percentile of the control group) were included for comparison.

When analysis was based on the presence of LAC or autoantibody values above the normal level (so called low positive values), the highest OR for thrombosis and recurrent fetal loss was associated with the LAC, closely followed by anti- ß$_2$-GPI. The sensitivity of the latter, especially for thrombosis was quite low. Thrombocytopenia was not associated with the presence of these low positive values.

ROC plot-derived threshold values were usually higher than those calculated for healthy controls. They also brought

Table 4. Clinical accuracy of antiphospholipid-protein tests based on ROC plot analysis.

Autoantibody	Thrombosis AUC (95% CI)	Recurrent Fetal Loss AUC (95% CI)	Thrombocytopenia AUC (95% CI)
aCL IgG	0.66 (0.58–0.74)	0.70 (0.56–0.85)	
aCL IgM	0.63 (0.54–0.71)	0.65 (0.50–0.80)	
aPS IgG	0.62 (0.53–0.70)	0.67 (0.52–0.82)	
aPS IgM	0.59 (0.51–0.68)		0.59 (0.50–0.67)
aPI IgG	0.62 (0.53–0.70)	0.74 (0.60–0.88)	
aPI IgM	0.59 (0.51–0.68)		
anti-ß2-GPI IgG	0.62 (0.53–0.70)	0.72 (0.57–0.86)	
anti-ß2-GPI IgM			
aPT IgG		0.66 (0.50–0.80)	
aPT IgM			

AUC: area under the ROC curve; 95% CI: 95% confidence interval for the calculated AUC; results are omitted if 95% CI for AUC was < 0.5.

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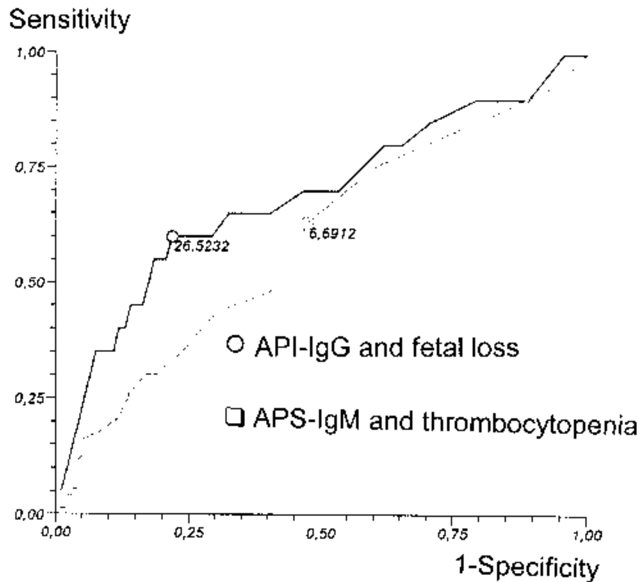

Figure 1. ROC plots with the highest (IgG aPI for recurrent fetal loss) and lowest (IgM aPS for thrombocytopenia) correlations.Corresponding calculated threshold values are included. See also Tables 4 and 5.

about a stronger association with APS clinical manifestations (e.g., thrombosis and aCL IgG or aPI IgG) than OR based solely on the presence of autoantibody levels above normal values. For certain antibodies, the association was significant, e.g., IgM aCL, aPS, and anti-ß$_2$-GPI antibodies, and thrombocytopenia. Anti-ß$_2$-GPI had high specificity for thrombosis, even when below the 95th percentile of the healthy population. Quite high OR were obtained for recurrent fetal loss and IgG aCL, aPS, aPI and anti-ß$_2$-GPI antibodies.

DISCUSSION

There is a general agreement that the presence of aPL in medium and high titers is associated with higher risk of thrombosis and recurrent miscarriage[18]. We were unable to find any specific associations between various antiphospholipid protein antibodies or antibodies to protein cofactors alone, and the particular type or location of clinical symptoms of APS. This is in agreement with other studies that analyzed primarily the LAC and aCL[3,4,19], and other aPL[20]. Using various techniques, these antibodies are frequently detected together, confirming a substantial crossreactivity[21]. However, the exact property (-ies) of these autoantibodies that determines the clinical manifestation and/or its location, still remains elusive.

Another question we addressed was the potential usefulness of performing multiple antiphospholipid protein antibody tests in patients with systemic autoimmune diseases in whom a diagnosis of APS is suspected, based on suggestive clinical manifestations. In 13% of such patients, no LAC and/or aCL (the most frequently assayed aPL) were detected. In half of them (almost 7% of all systemic autoimmune patients) we found other antiphospholipid protein antibodies, mainly IgM aPI. These antibodies as the sole laboratory marker of APS have been reported by others in a limited number of patients[21,22]. In some, an isolated presence of anti-ß$_2$-GPI has been previously identified[23]. A new clinical entity, antiphospholipid/cofactor syndrome has been proposed[24,25]. In our population there were no such subjects. Recently, a high prevalence of IgM antibodies to a zwitterionic phospholipid [anti-phosphatidylethanolamine (aPE)] has been found as the only aPL in patients with unexplained thrombosis[26]. Antibodies to aPE were not determined in our study. Taken together, from a clinical standpoint, these data suggest it may be useful to perform assays for non-conventional aPL when APS is suspected, until the exact nature of the antibodies specifically leading to thrombotic phenomena and the epitope(s) towards which they are directed have been identified[27].

Doubts about the clinical significance of low positive values for aCL[4,5,28] lead to the modification of laboratory criteria for the classification of APS[7]. Currently, only medium or high titer antibodies are considered clinically relevant. Such an approach has been recently validated in a one-center study including a selected population of patients similar to ours[29] and found to be useful for clinical studies. However, in their report Lockshin, *et al*[29] did not state exactly which values were considered moderate or high or how were they determined. Any arbitrarily chosen threshold values, suggested even by the most experienced centers in the field, are vulnerable to criticism. It would be very difficult to adopt such values, when one considers the variety of tests used by different laboratories to determine the presence of aCL.

Here we propose a different approach that takes advantage of ROC plots. They provide an index of diagnostic accuracy for the tests and help to select the best decision thresholds. They are also especially useful for comparisons of multiple tests in the same patients[13]. We applied ROC plots and their analysis to APS patients for these 2 purposes: (A) to compare the relative accuracy of different aPL tests in identifying patients with particular symptoms; and (B) to select optimal cutoff values for various aPL that discriminate best between the presence and absence of clinical symptoms of APS.

In general, our results highlight the fact that we are all currently limited to a rather poor set of laboratory tests for the classification of APS (the greatest AUC was 0.74). Moreover, none of them is clearly superior to any other, as their CI closely overlap. Our analysis does indicate that IgG aCL seems to be the most accurate test for thrombosis, confirming its usefulness in the identification of this syndrome[7,29]. For recurrent fetal loss, the most accurate tests in addition to IgG aCL were IgG aPI and IgG anti-ß$_2$-GPI. Less accurate for thrombocytopenia were aPS IgM anti-

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bodies. If confirmed by others, these results may indicate that each clinical symptom related to APS has its own set of most accurate tests.

When analyzing these data, one must always take into consideration the widely different sensitivities and specificities between the tests as well as the differences in correlation between these tests and various clinical symptoms of APS (see Table 5), which result in their different clinical accuracy (Table 4).

It appears that for any given clinical symptom and antibody analyzed there is a different threshold value above which the risk of experiencing such symptom rises substantially. For aCL (and most other aPL tested) it is higher than the normal values, established as the 95th percentile of the healthy population. It is, however, lower than the 40 GPL used by some as a threshold titer for thrombosis risk assessment[4]. Our results confirm that for the majority of aPL tested, the diagnostic value of slightly elevated titers is very low. Therefore, we suggest that in laboratories dealing with large numbers of autoimmune patients ROC-derived threshold concentrations (different for each different clinical symptom) should be considered equivalent to proposed, and thus far arbitrary, moderate aCL levels[7]. Still, the real diagnostic usefulness of such an approach in predicting the risk of developing APS clinical symptoms requires a large prospective trial.

For some antibodies tested (e.g., anti-$ß_2$-GPI, and IgM aPT) this threshold value could be close or even below the 95th percentile of a healthy population. In both cases it could result from very high specificity of anti-protein-cofactor antibodies for APS symptoms[23,30,31]. However, their disappointingly low sensitivity limits the use of anti-$ß_2$-GPI and aPT antibodies as sole tests for the diagnosis of APS[23,32,33].

The clinical value of the LAC could not be directly compared to other measurements using ROC-curve analysis, as this is not a quantitative test, when performed according to ISTH recommendations[10]. The LAC was associated with the highest OR for the appearance of APS clinical symptoms (especially thrombosis) when compared to

Table 5. Risk of APS clinical symptoms (OR; chi square test) associated with elevated levels of antiphospholipid and anti-protein cofactor antibodies.

		Thrombosis		Recurrent Fetal Loss		Thrombocytopenia	
		Normal	ROC Calculated	Normal	ROC Calculated	Normal	ROC Calculated
LAC	OR (CI)	3.04 (1.5–6.2)	NA	8.70 (2.8–26.7)	NA		NA
	sn/sp	32/87		55/88			
aCL IgG	Cutoff	11.0	17.2	11	18.4		
	OR (CI)	2.49 (1.3–4.6)	3.69 (1.8–7.4)	5.06 (1.6–16.0)	8.12 (2.5–26.1)		
	sn/sp	57/66	38/86	74/64	50/89		
aCL IgM	Cutoff	22.7	32.9	22.7	22.3		28.9
	OR (CI)		3.26 (1.6–6.1)	4.02 (1.4–11.5)	4.03 (1.4–11.6)		2.57 (1.3–5.1)
	sn/sp		35/86	55/76	55/77		33/84
aPS IgG	Cutoff	24.2	17.4	22.4	38.1		
	OR (CI)	2.75 (1.4–5.5)	3.51 (1.9–6.6)	4.00 (1.4–11.7)	11.17 (3.2–38.4)		
	sn/sp	35/83	52/76	50/80	50/92		
aPS IgM	Cutoff	14.2	10.0	14.2	11.3		6.7
	OR (CI)	2.32 (1.2–4.6)	2.05 (1.1–3.8)	3.22 (1.1–9.8)	3.45 (1.2–10.1)		1.90 (1.04–3.4)
	sn/sp	34/82	46/70	40/83	45/81		62/52
aPI IgG	Cutoff	22.1	30.9	22.1	26.5		
	OR (CI)	2.69 (1.4–5.1)	4.57 (2.2–9.4)	6.21 (2.1–18.5)	12.00 (3.7–39.1)		
	sn/sp	46/76	40/87	60/81	60/89		
aPI IgM	Cutoff	16.6	13.9				
	OR (CI)		1.93 (1.06–3.5)				
	sn/sp		55/61				
aß2GPI IgG	Cutoff	2.6	1.8	2.6	2.8		
	OR (CI)	2.96 (1.4–6.3)	4.07 (2.0–8.3)	7.0 (2.2–22.0)	8.00 (2.5–25.7)		
	sn/sp	29/88	42/83	50/88	50/89		
aß2GPI IgM	Cutoff	1.9	1.0				8.5
	OR (CI)		1.90 (1.02–3.5)				3.31 (1.1–9.7)
	sn/sp		44/70				13/95
aPT IgG	Cutoff	4.4	10.6	4.4	5.1		
	OR (CI)		3.13 (1.3–7.6)	4.95 (1.4–17.4)	3.88 (1.3–11.2)		
	sn/sp		33/81	60/70	55/76		
aPT IgM	Cutoff	18.6	12.4				
	OR (CI)		2.40 (1.8–4.9)				
	sn/sp		62/48				

OR: odds ratio; Normal: autoantibody level set at 95th percentile of the control group (100 subjects); sn/sp: sensitivity/specificity; CI: 95% confidence interval; NA: not applicable. Results are omitted if 95% CI < 1.0.

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the low-positive values of various antiphospholipid and protein-cofactor antibodies. This superiority became less evident when ROC-derived threshold values for aPA were used for comparisons. It may also explain why some authors claim superior clinical importance associated with the presence of the LAC[3,34,35], while others place higher reliance upon IgG aCL[4-6]. Precise comparisons of clinical utility and accuracy in the detection of both aPL require the development of generally accepted methods to quantitate the LAC effect. It must be stressed that in the majority of studies only thrombotic complications of the syndrome were analyzed. Our findings further support the important clinical role of detecting higher values of aCL.

Thrombocytopenia is no longer among the clinical classification criteria for APS[7]. There is, however, an ongoing discussion about the relevance of nonthrombotic features of APS in the diagnostic process of the syndrome[9]. It might be further fueled by a recent report showing that patients with immune thrombocytopenic purpura, who are positive for aPL, are at increased risk of developing APS[36]. Using ROC analysis, we were able to show some associations between high levels of various aPL of the IgM class and thrombocytopenia, although except for IgM aPS, their sensitivity was very low. Others found similar associations for hemolytic anemia[20], but not for thrombocytopenia. Recently, it has been reported that thrombocytopenia correlated with IgM anti-$ß_2$GPI positivity[37]. In both studies, however, the relation of hemocytopenias to the level of antibodies was not reported. Altogether, these data may indicate that there is an as yet unexplained connection between aPL of the IgM class and hemocytopenias often found in APS.

In summary, our results indicate that in a laboratory specialized in antiphopshoplipid protein antibody detection, ROC plot analysis may be an optimal tool to establish clinically relevant threshold levels of these antibodies. Such analysis may also help in the selection of the most accurate set of tests for the risk assessment of APS clinical symptoms. It should be stressed, however, that, in agreement with recently proposed classification criteria for APS[7] in the majority of laboratories, the detection of the LAC following proper procedures[10] and determination of aCL is sufficient and should be recommended. Only in a few reference centers should multiple tests be available to detect a rare group of patients in which non-conventional aPL are the only laboratory markers of APS.

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