

EXPERT
REVIEWS

The current standing of diagnosis of antiphospholipid syndrome associated with systemic lupus erythematosus

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The antiphospholipid syndrome was first described in the early 1980s. The term was first coined to describe patients presenting with recurrent arterial and venous thrombosis or pregnancy complications. Antiphospholipid syndrome was first reported in systemic lupus erythematosus patients, but later on it became obvious that systemic lupus erythematosus is not a necessary condition for its occurrence. It has been shown that antibodies to phospholipids are the main causative agents of the disease, hence its name. The diagnosis of the disease has witnessed a remarkable evolution over the course of the past 25 years. With the observation that clinical parameters would not be enough to accurately diagnose the disease, antiphospholipid antibodies were recognized to play a central role in this regard. The main hindrance to an accurate diagnosis was the lack of standardization between different laboratory parameters that tested for the antiphospholipid antibodies. Lately, a combination of tests has been acknowledged to play a crucial role in diagnosis.

KEYWORDS: anti- β 2-glycoprotein I • anticardiolipin • antiphospholipid • lupus anticoagulant • revised Sapporo criteria • Sapporo criteria • seronegative antiphospholipid syndrome • systemic lupus erythematosus

The antiphospholipid syndrome (APS) was first described in the early 1980s [1,2]. The term was first coined to describe patients with recurrent arterial and venous thrombosis, or pregnancy complications. APS was first reported in systemic lupus erythematosus (SLE) patients, but later on it became obvious that SLE is not a necessary condition for its occurrence [1]. The first article proving the possible mutually exclusive nature of the two diseases was not published until the late 1980s [3]. It included 70 patients who had APS but did not fulfill the classification criteria for SLE [3]. The diagnosis of the disease has witnessed a remarkable evolution over the course of the past 25 years. It has been shown that antibodies to phospholipids are the main causative agents of the disease, hence its name. Antibody titers were found to closely correlate with the number of disease-related events [4]. The main hamper to advancement in understanding the disease was the lack of standardization between different laboratory parameters that tested for the antiphospholipid antibodies (aPLs) [5]. With the observation that clinical parameters would

not be enough to accurately diagnose the disease, aPLs were recognized to play a central role in this regard. The lupus anticoagulant (LA) was described as early as 1952; it was found to lead to defects in coagulation and to a false positive syphilis test in some SLE patients [6]. It was not until 1972 that the term LA was used to describe this phenomenon by Feinstein and Rapaport [7]. Later on, the anticardiolipin antibody (aCL) was employed in the classification of APS [1,8]. More recently, a new entity known as seronegative APS (SNAPS) has been described in which patients have typical APS symptoms but are negative for the conventional autoantibodies [9].

SLE-associated APS

As mentioned earlier, APS was primarily thought to be one of the manifestations of SLE [3]. Moreover, it has been shown that around 10% of the patients with SLE suffer from APS [10]. Even though SLE patients might have a particular proclivity to thrombosis by virtue of their disease, the presence of aPL was strongly associated with the development of thrombosis [11]. Distinguishing

between the two disease entities can be challenging and is usually based on the presence of clinical signs and antibodies specific to SLE, such as antibody to Sm protein, antibodies to native DNA or antinuclear antibodies [12].

Clinical presentation

Thrombosis and obstetric complications are the hallmark of APS. Venous thrombosis has been shown to be the most frequent of the APS complications [13]. Thrombocytopenia, fetal death, miscarriage, livedo reticularis and CNS manifestations, such as migraines and strokes, are considered common manifestations of the disease [2,14]. Occurring less frequently than the previously mentioned manifestations are preeclampsia, eclampsia, premature births, hemolytic anemia, valvular and coronary heart disease [14]. Other much less common manifestations of APS that have been reported are chorea, epilepsy, vascular dementia, pulmonary hypertension, leg ulcers, digital gangrene, retinal artery or vein thrombosis, amaurosis fugax, osteonecrosis, APS nephropathy and mesenteric ischemia [14–16].

Epidemiology

APS is considered to be a rare disease. Approximately, 10% of the women with recurrent miscarriages are diagnosed with APS [17]. The presence of the disease was shown to increase the risk of preeclampsia and placental insufficiency in pregnant women. Approximately one in three women diagnosed with APS will suffer from preeclampsia during gestation [18]. Intrauterine fetal death has been observed more frequently in APS patients; however, a causality association could not be deduced. Some authors suggest that this association should be interpreted with caution since a cornucopia of comorbidities that are not accounted for in such patients may contribute significantly to fetal death [14]. In a study enrolling 144 SLE patients who had positive aPL tests, 20.1% of the patients had thrombosis after a median follow-up time of 104 months. A systematic review has demonstrated that in patients younger than 51 years of age, presenting with stroke, the probability of testing positive for aCL and LA is 21 and 8%, respectively [19].

Pathophysiology

Many factors have historically been proposed to explain the mechanistic process that leads to this intriguing disease. A recent review tackling this topic clumps the causative mechanisms underlying the disease into categories [20]. Increased oxidative stress altering the structure of glycoprotein I was shown to be one the main prothrombotic factors. This is mainly elucidated by the observation that proteins with antioxidant properties such as paraxonase are decreased in APS patients, whereas oxidative stress markers seem to be increased [20,21]. This entails formation of previously absent disulfide bridges in β_2 -glycoprotein I (β_2 -GPI) [22]. The conformation of glycoprotein I (shifting from circular to linear) might also play a role in exposing a B-cell epitope to aPL [20,23]. Endothelial disruptions due to the disturbances in the redox status in the plasma of APS patients prime them for an eventual thrombotic event. Smoking constitutes an additional oxidative stress. It has been shown that among patients who were positive for LA and smoked, the odds ratio was 33.7 (95% CI: 6.0–189.0) versus 5.3 (95% CI: 1.4–20.8) for those

who did not, with regards to myocardial infarction occurrence [24]. Decreased activity of endothelial nitric oxide synthase has also been incriminated as a potential perpetuating factor for thrombosis in APS patients [25,26]. Increased tissue factor expression, along with an increase in the free form of factor XI, were postulated to favor thrombosis in APS patients [20,27,28]. Moreover, antibodies to β_2 -GPI were shown to activate a multitude of receptors that favor a thrombotic state; these include TLR2, TLR4, annexin A2 and apo E receptors [20]. An endogenous shield, created by annexin A5, usually binds to phosphatidylserine (which is usually expressed upon endothelial injury), preventing it from exerting its thrombogenic effects [15]. Some aPLs cause breakage of this shield, subsequently leading to thrombus formation [29]. Furthermore, complement activation and increased expression of TLR7 and TLR8 were also found to contribute to the pathogenesis of APS [20]. In addition, the release of thromboxane A2 from platelets after activation with aPLs also contributes to the prothrombotic state present in APS [20]. A large retrospective study demonstrated that 24% of the patients presenting with an idiopathic venous thromboembolism were positive for aPL [30]. Finally, a group of investigators showed that there were no significant differences from a clinical or laboratory point of view between APS and APS associated with SLE except for heart valve disease, hemolytic anemia, low C4 levels and neutropenia, which were more common in the latter group [31].

A word about treatment

It would be futile to delve into a discussion about the diagnosis of APS without understanding its ultimate purpose: the management of APS patients. Accurate assessment of patients is necessary as treatment could drastically affect prognosis. Prevention by thromboprophylaxis is the most important point in management. It is subsequently important to identify high-risk patients who are classified as such based on their lifestyle, age, diagnosis of SLE, non-APS-related risk factors (hypertension, diabetes, hyperlipidemia and medication) and serologic status [32]. The most commonly used management options are warfarin, low molecular weight heparin (LMWH), aspirin and hydroxychloroquine namely in SLE patients [33,34].

The 13th international meeting on aPLs suggests that high-risk APS patients should be placed on daily aspirin, with additional hydroxychloroquine in the event they carry a diagnosis of SLE [35]. The management of a first venous event is usually with lifelong warfarin (target international normalized ratio [INR]: 2–3) if they have definite APS, or warfarin for a duration of 3–6 months if they have low-risk APS. This is different when the thromboembolic event is arterial; warfarin should be given with a target of INR >3 or with a target INR of 2–3, but this has to be accompanied by aspirin. Statins, low molecular weight heparin or hydroxychloroquine have all been suggested for cases with refractory thrombosis [14,35].

With regards to obstetrical event prevention in APS patients without thrombosis who had a history of early miscarriages, the recommendation is to use low-dose aspirin concomitantly with either unfractionated heparin (5000–7500 IU subcutaneously every 12 h) or LMWH at a prophylactic dose [36]. In the event

that a pregnant APS patient suffers from a thrombosis, the recommendation is to use aspirin along with either heparin or LMWH (enoxaparin, dalteparin), each using a therapeutic dose, respectively [36].

A historic overview of APS diagnosis

An early retrospective study exploring the occurrence of the APS syndrome within 667 SLE patients attempted to classify patients with SLE into having definite, probable, doubtful or negative APS syndrome [10]. The classification relied on the number of clinical events occurring as well as the titers of aPLs present, which at the time were restricted to the aCLs. An interesting point to note is that LA, prolonged plasma thromboplastin time, Venereal Disease Research Laboratory test and Coombs test were all found to be more prominently present as the APS became more definite. The article came to a conclusion that APS is part of the SLE disease [10]. With advances in both the discovery of antibodies associated with APS and a clearer clinical presentation of the disease, new criteria were put into place. Each of the latter was adequate in view of the technologic advances in laboratory testing present at the time. This started with the introduction of the Sapporo criteria in 1999, which were later amended in 2006 during an international meeting in Sydney, Australia, becoming what came to be known as the revised Sapporo criteria [37,38]. With rapid advancements and the introduction of newer antibodies (the noncriteria antibodies), along with the discovery that autoimmunity to factors in the coagulation cascade is associated with APS, the Sydney criteria soon became obsolete (FIGURE 1) [39,40]. Unfortunately, most studies have looked into each aPL test separately in the context of APS, not taking into account possible combinations.

Sapporo criteria

The Sapporo criteria constitute the first internationally approved criteria for classification of definite APS (FIGURE 1). They were conceived in a workshop that followed the 8th international meeting for APS. The main aim behind their formulation was the standardization of definite APS classification criteria [37]. Criteria mainly relied on previous clear-cut evidence from prospective trials in the field. The classification criteria required patients to have at least one clinical manifestation and one positive serologic test. Clinical criteria were divided into pregnancy related and nonpregnancy related. For patients to meet the latter, they had to have an arterial or venous thrombus without evidence of vessel wall inflammation. Pregnancy-related complications were divided into three classes entitled A, B and C. Class A complications represented women presenting with fetal death beyond the 10th gestational week. Class B consisted of women having one child or

more born prematurely due to placental insufficiency, eclampsia or pre-eclampsia. Class C included patients having three abortions or more preceding the 10th gestational week, while excluding other possible causes. The laboratory criteria that were used were the aCLs (IgG or IgM) and LA only. An anticardiolipin test was considered positive if it persistently showed medium or high titers 6 weeks after initial testing by ELISA [41]. LA similarly needed to be present after a 6-week interval to be considered positive; however, the testing method varied. It was proposed that a test should be considered positive if there was evidence of a prolonged coagulation parameter that would only correct after adding excess phospholipid, while other coagulopathies were excluded [42]. Two studies at a later stage came to validate the Sapporo criteria as being both sensitive and specific for classifying APS; 71 and 98%, respectively, in one study [43,44]. It was also suggested that if other factors such as thrombocytopenia, hemolytic anemia and livedo reticularis were to be included in the criteria, then sensitivity would increase even further.

Revised Sapporo criteria

With more recent studies emerging and the realization that aPL becomes more common as people age, the scientific community has witnessed the development of newer criteria for APS classification (FIGURE 1) [38]. The main divergence with the Sapporo criteria was the 12-week time interval (instead of 6 weeks) after which patients had to test positive for aPL to be considered as carrying that antibody. Moreover, these criteria witnessed the addition of the anti- β_2 -GPI (both IgG and IgM) antibody to the two that were previously present. The Sydney criteria also aided in disambiguating the aPL levels that needed to be present in order for a test to be considered positive [38]. A review comparing the two

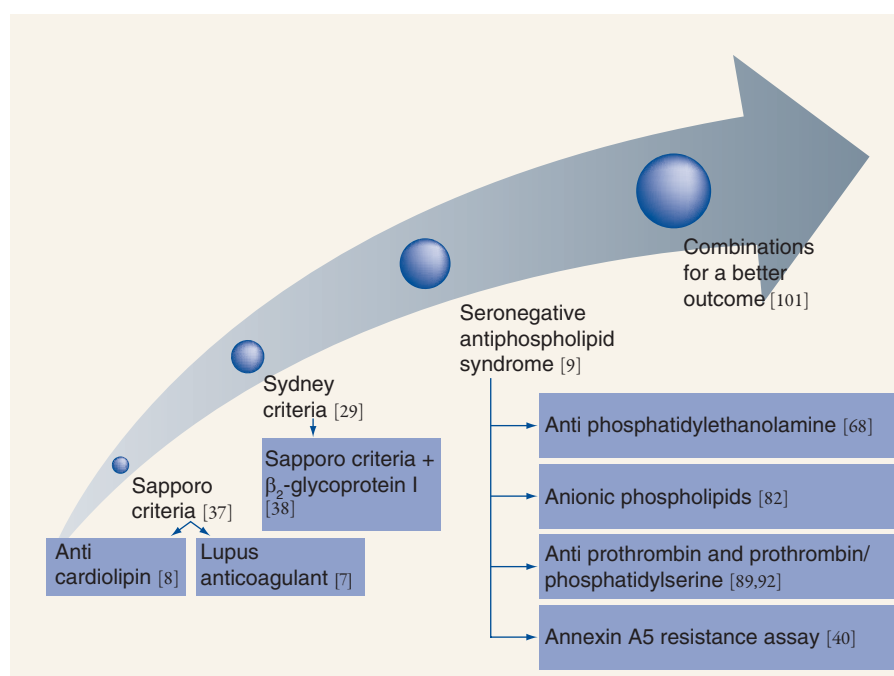


Figure 1. Evolution of diagnostic criteria for the antiphospholipid syndrome in systemic lupus erythematosus patients.

criteria found the 2006 criteria to be more valid than the original Sapporo criteria [45]. Patients considered positive for all three aPLs presented in the revised Sapporo criteria were later found to have a higher risk of thrombosis [46]. A retrospective study assessed the revised Sapporo criteria versus its predecessor and included 144 patients that fit the 1999 criteria. Authors found that only 59% of the original patients met the criteria of the Sydney revision. They came to a conclusion that the new guidelines would limit the number of false negatives and would better identify APS patients [47].

Anticardiolipin testing

Testing for this aPL is accomplished using ELISA and is considered positive whenever the IgG and/or the IgM antibodies are found to have a value that exceeds 40 GPL or MPL, or that is higher than the 99th percentile of values for that antibody in the general population [38]. Positivity to the aCL by those standards paradoxically was shown to be unrelated to thrombotic events in a large retrospective study [48]. However, another study, published at a later time, has shown that aCL positivity is a risk factor for thrombosis in SLE (OR: 2.02; $p = 0.037 \times 10^{-4}$) [49]. A persistently positive aCL was shown to represent an independent predictor of thrombosis in aPL-positive SLE patients (OR: 5.87; $p = 0.01$) [50]. This demonstrates the importance of this aCL as a cornerstone in APS diagnosis in SLE patients. Anticardiolipin appears to have a correlation with pregnancy complications [46]. Moreover, reproducibility of the test poses a challenge since ELISA kits are difficult to standardize. Variable results have been obtained on a same sample, tested at different places. This discrepancy was also present when using the same kit [51,52]. A large number of commercial or locally made enzyme-linked immunosorbent kits are available for aCL testing. Even though they theoretically follow the same principle of manufacture, a study has found major discrepancies in the results of different commercial kits [53]. A few measures have been suggested in order to minimize this cross-kit variation by using the same phospholipid in the kit preparation, using the same amount of bovine serum for blocking the nonspecific-binding sites, avoiding the use of the detergent Tween 20, and not heating the plasma [54]. Finally, some cardiolipin-binding proteins that are not routinely tested for, might be related to the manifestation of APS; this is the case for complement factor H, for example [55].

Lupus anticoagulant

The Sydney revision of the Sapporo criteria suggests that patients should have positive LA on initial examination and on follow-up tests 12 weeks later. Testing should follow the guidelines of the International Society on Thrombosis and Hemostasis [38,42,56]. A positive LA test by itself was not shown to be associated with thrombosis, although a previous meta-analysis had validated the opposite conclusion [37,57,58]. Some authors have suggested that single positivity for LA might be independent of β_2 -GPI. They suggest that tendency for thrombosis in those APS patients might be caused by a different process [46]. Issues with the reproducibility of the test have also been noted here. In addition, the test has been

criticized due to the difficulty in applicability [51]. Furthermore, LA seems to increase in some clinical situations such as old age, which might lead to erroneous results and invalid conclusions [16]. A cornerstone study demonstrated that only anti β_2 -GPI with LA activity was to be held responsible for the APS manifestation. This means that the LA test by itself might lead to many false positives [59,60]. It is recommended to test for LA by two assays that employ different principles. The most common screening tests are lupus-sensitive activated partial thromboplastin time, dilute Russell viper venom time, dilute prothrombin time and the kaolin clotting time. The common scenario is detection of a prolonged activated partial thromboplastin time that does not correct with mixing studies. This has to be followed by a LA confirmatory test [61]. A recent assay for LA has been suggested; it seems to confer higher specificity in testing and will probably solve the issue of standardization in the near future [62].

β_2 -GPI

This antibody, similarly to the LA and aCL, should also be present in the serum or plasma with a titer exceeding the 99th percentile by ELISA on two occasions (initially and 12 weeks later), for it to be considered positive [38]. Anti- β_2 -GPI is not considered an aPL *per se* since it is a protein involved in the coagulation pathway; it associates aCL as a cofactor to bind antiphospholipids [54]. Although counter-intuitive, single positivity for antibodies to β_2 -GPI was not associated with thrombosis from previous studies [24,48]. This was explained by the fact only the anti- β_2 -GPIs that targeted domain I were the ones associated with thromboembolic events [63]. Similarly to aCL, one of the shortcomings of the anti- β_2 -GPI was the lack of reproducibility of the tests among different centers due to lack of standard, uniform materials and procedures [52].

SNAPS

This concept was first introduced in 2003 by Graham Hughes [64]. A recent retrospective study compared 87 APS patients diagnosed according to the Sydney criteria versus 67 patients fulfilling the clinical criteria of APS in addition to two noncriteria manifestations with constantly negative phospholipid antibody values (SNAPS) [39,64]. The noncriteria manifestations consisted of migraines, livedo reticularis, Raynaud's disease, cognitive dysfunction, seizures, chorea, multiple sclerosis-like illness, one or two abortions before 10 weeks of gestational age, thrombocytopenia white matter lesions (on brain MRI) and cardiac manifestations (namely mitral or aortic valve disease). The article demonstrated that there was no significant difference in clinical manifestations between the two groups. Deep venous thrombi among others were present in 31.4% of the patients with SNAPS versus 31% in seropositive APS patients ($p = 0.96$). The authors concluded that patients from both groups should be treated the same since they constitute the same disease entity. Other authors have described occurrences similar to catastrophic APS in patients thought to have SNAPS [65]. The continuous negative aPL tests in the SNAPS patients were explained by the presence of other protein cofactors or noncriteria aPLs that were not tested for, such as annexin V, prothrombin, the vimentin/cardiolipin complex

and phosphatidylethanolamine. These were not included in the laboratory criteria of either the Sapporo or Sydney criteria, due to lack of standardization between different labs [66,67].

Antibodies to phosphatidylethanolamine

Phosphatidylethanolamine (PE), a zwitterionic (dipolar) phospholipid, was shown to be a prominent target of aPLs in the SNAPS group of patients [68]. PE's physiological role lies in both enhancing activated protein C's action and by inhibiting activated factor X–prothrombin, which ultimately promotes anticoagulation [69,70]. Paradoxically, PE was also found to play a role in promoting coagulation through multiple mechanisms involving inhibition of factor X, prothrombin or by inactivation of LA [71,72]. PE was shown by several studies to be associated with pregnancy complications and thromboembolic phenomena, in patients fulfilling the above mentioned SNAPS criteria [73–79]. An early report that shed the light on PE's role in APS pathogenesis demonstrated positivity to both LA and PE, while all other aPLs tested were negative [80]. One study subsequently demonstrated that roughly 18% of the patients suffering from a thrombotic event are only positive for antibodies to PE among all other phospholipids. The authors concluded that anti-PE testing could have a role in the diagnosis of patients with primary APS [73]. A study including 276 subjects, 140 patients with venous thrombi and 138 controls found that antibodies to β_2 -GPI, aPL and PE were significantly present in the former group compared to the latter (14.3%; $p = 0.014$ for antibodies to PE [aPE]). Another observation from that trial was that there was no overlap in the presence of the abovementioned antibodies among patients, insinuating that criteria antibodies might not be enough to identify all APS patients [79]. In a large case–control study, aPE IgM was found to be a significant risk factor for early fetal loss (OR: 6.0; 95% CI: [2.3–15.7]; $p = 0.0003$ [75]). The main obstacle for using aPE for the diagnosis of APS lies in the lack of standardization of the different ELISA kits used for testing, along with the absence of well-structured prospective trials offering indisputable evidence for its benefit in diagnosis [81].

Anionic phospholipids excluding anticardiolipin

Phosphatidylinositol, phosphatidylserine (PS) and phosphatidic acid are all grouped under this category [9]. They are ubiquitously found on the membranes of cells; however, they are seldom referred to for diagnosis due to insufficient evidence to justify their use [40]. A retrospective study including 866 females with recurrent pregnancy loss showed that 10.1% of women were positive for an antibody other than aCL, namely antibodies to phosphatidylinositol, PS, phosphatidic acid and PE [82]. Furthermore, a different study has shown that 49 out of 872 women with recurrent pregnancy loss were negative for aCL and LA while they had antibodies for PS [9]. This demonstrates their usefulness in testing; however, similarly to other aPLs, the lack of standardization constitutes a major obstacle to applicability. In addition, *in vitro* studies have shown that antibodies to PS may cause obstetrical morbidity not only through an increase in the thrombogenic status of the blood but also through inhibition of development of the trophoblast and syncytiotrophoblast [83,84]. It was also suggested that anticoagulation for patients with recurrent

pregnancy loss, who are positive for anionic phospholipids, could be beneficial [85].

Antibodies to prothrombin

Prothrombin is a protein of the coagulation cascade that gets converted to thrombin in the final stage of a thrombus formation, similarly to β_2 -GPI, it is not an aPL [86]. Its applicability in APS was suggested as early as 1995 [9]. Low reproducibility of detection for aPT constituted a major problem, since a multitude of ELISA kits were used [87,88]. This problem was successfully resolved by standardizing ELISA kits after a cornerstone study on 66 APS patients was performed [89]. An ELISA encompassing γ -irradiated plates in combination with a phosphate-buffered saline buffer and a coating antigen of 10 $\mu\text{g/ml}$ prothrombin, was found to be the most sensitive test [89]. Different IgG antibody to prothrombin (aPT) assays seem to have good concordance compared with different IgM aPT assays [90]. Many studies have confirmed that aPT plays a role in the diagnosis of APS. Although not yet included in the Sydney guidelines, studies have confirmed aPT's added value for thrombosis prediction alongside the three serologic tests mentioned in the 2006 guidelines (LA, antibodies to β_2 -GPI and aPL) [9,72]. Thrombosis was shown to be accurately predicted by IgG antibodies to aPT ($p = 0.001$) in a study that included 14 SLE patients that experienced a thromboembolic event [91].

Antibodies to prothrombin/phosphatidylserine

aPT can bind to the complex formed by prothrombin and phosphatidylserine. A study of 212 SLE patients versus 100 controls showed that 31% of the SLE patients were positive for antibodies to prothrombin/phosphatidylserine (aPT/PS) and that both IgG and IgM isotypes were associated with thrombosis [92]. Furthermore, other reports have confirmed that aPT/PS had a similar accuracy for diagnosing APS with LA, but surprisingly had a higher sensitivity and specificity when compared with aPL [40,93,94].

aPT/PS could be used as a confirmatory test in patients who are suspected of having APS but in whom a laboratory diagnosis cannot be established based on the last published criteria [38]. One should keep in mind, however, that further efforts should be made to standardize these tests so as to have clear cut reference ranges, beyond which patients are considered to be positive for the antibody.

Annexin A5 resistance assay

This is the only test that was developed based on an understanding of the pathophysiology of the disease. It was created by the Rand laboratory in an attempt to identify APS patients based on molecular pathways that favor the disease [40]. Annexin A5 is a protein found on primordial placental tissue and on endothelial cells of the body. It is thought to regulate thrombus formation by oligomerization and creation of a protective shield whenever it is put in contact with phospholipids. This process is thought to halt the coagulation cascade by secluding thrombogenic factors from the exposed area [95–97]. aPL were found to distort this shield and favor thrombosis [97]. The annexin A5 resistance test identifies

vulnerable patients who are thought to have a defective shield, also called annexin A5 resistant [29,98]. One study showed that 52% of the APS patients were positive to the above mentioned test whereas only 2–5% of the controls were annexin A5 resistant [38]. Clinical reports have emerged concerning antibodies to annexin A5, showing inconsistencies among different studies, with some even disclaiming the previously established relationship to thrombosis [89,99–101]. Following these disappointing results, the 13th international congress on aPL advised the adoption of the annexin A5 resistance assay instead of the older annexin A5 antibody test [9].

A new era

As previously demonstrated in the discussion, the topic of aPL antibody testing has been extensively explored. Whether referring to antibodies cited in the 2006 Sydney criteria or other noncriteria antibodies of the SNAPS, one could notice that there exists a cornucopia of laboratory and clinical data on each test individually. What are lacking, however, are data on combinations of these tests. The topic of APS diagnosis, namely in SLE patients, has been a very fertile terrain for research in the past 25 years, and has been witness to paradigm-changing discoveries. In terms of basic research, this scientific area can easily be described as booming, but one should also remember that at the end of the day, this is a medical topic where patients' lives are at stake. Accordingly, investigators have looked more into making use of all the data present so far and combining the different aPL tests that have been introduced to provide the best combination in terms of diagnostic accuracy and validity so as to maximally benefit patients.

Combining tests for a better outcome

A recent retrospective study enrolling 230 SLE patients, mostly women, explored the issue of diagnosis of APS by combining multiple antiphospholipid tests. The patients underwent testing for aCL, LA, anti β_2 -GPI, aPT/PS, aPT and aPE. Among the 23 possible combinations of those tests, LA with anti β_2 -GPI and aPT/PS turned out to be the best possible combination. In terms of diagnostic accuracy, the combination proved to have an AUC = 0.712; OR = 3.73 (95% CI: 1.82–5.38); $p = 0.0001$, with a Youden index of 0.32. Concerning thrombosis and pregnancy loss, the numbers were equally impressive: AUC = 0.709; OR = 3.75 (95% CI: 2.13–6.62); $p = 0.0001$; Youden index of 0.37 and AUC = 0.677; OR: 4.82

(95% CI: 2.17–10.72); $p = 0.0007$ and a Youden index of 0.38, respectively [102]. This was considered a revolutionary study since it dethroned the previously existing conviction that the Sydney criteria offered the best classification accuracy [38]. Similarly to other studies, this report confirmed that the risk of thrombosis is directly proportional to the number of aPLs present [48,103–105]. Positivity for all of LA, anti β_2 -GPI, and aPT/PS was associated with the highest risk of thrombosis (OR: 23.2; 95% CI: 2.57–46.2). This is in contrast to single positivity, where ORs equalled 7.3 (95% CI: 2.21–25.97), 5.7 (95% CI: 2.12–17.01) and 3.11 (95% CI: 1.56–7.8) for LA, aPL/PT and anti β_2 -GPI, respectively [102].

Expert commentary & five-year view

We tend to think that the near future of the diagnosis of APS in SLE patients lies in those few words. After the era of single aPL tests has gone, time has come for large prospective trials examining all different combinations of both aPL in APS and SNAPS. This ought to better delineate an accurate and precise diagnosis of the disease with the hope of generating new diagnosis guidelines to replace the now proven obsolete 2006 revised Sapporo criteria. That being said, a few other problems need to be addressed, namely the universal standardization of different tests on an international level in order to have comparable data. This would exponentially boost advancements in APS diagnosis in terms of antibody testing as it would allow accurate comparative data to be generated and interpreted with certainty. Furthermore, large prospective trials are of the essence. The majority of the conclusions drawn about APS have been deduced through the means of mainly retrospective or cross-sectional studies. Even though the difficulties that lie behind launching a prospective trial in APS are many (ethical, safety among others), this is a necessary step that the scientific community needs to undertake to better outline the disease characteristics so as to ultimately provide patients with better detection and early prevention.

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Key issues

- The diagnosis of antiphospholipid syndrome (APS) is challenging, owing to the common/nondiscriminative clinical presentation of the disease.
- APS occurs mainly in systemic lupus erythematosus patients; in fact, around 10% of diagnosed systemic lupus erythematosus patients suffer from APS.
- The Sapporo criteria provide a 71% sensitivity and 98% specificity for the classification of APS.
- The revised Sapporo criteria included anti β_2 -GPI as an additional antibody for serologic testing. They provide better classification accuracy than their predecessors.
- Noncriteria antiphospholipid antibodies might add diagnostic power if combined with aCL, anti β_2 -GPI and LA, and help identify the seronegative APS patients.
- The main obstacle for using noncriteria antiphospholipid antibodies is the lack of proper universal standardization.
- The number of positive aPLs is proportional to the risk of thrombosis in a single patient.
- Combining LA with anti- β_2 -GPI and aPT/PS provides the best diagnostic accuracy for APS.

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