

# The antiphospholipid syndrome: a large elephant with many parts or an elusive chameleon disguised by many colours?

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**Abstract** The antiphospholipid syndrome (APS) is characterized by a range of clinical features (primarily thrombosis and/or obstetric-related), together with the presence of antiphospholipid antibody (aPL) as detected by a diverse range of laboratory tests. APS remains a significant diagnostic and management challenge for clinicians across a wide range of specialties, some 30 years after APS was first described as a discrete clinical entity. This is due to ongoing issues regarding nomenclature, the diagnosis of APS in individual patients, the expanding range of recognized clinical manifestations and of APS-related laboratory tests, and management issues in particular APS patient subgroups (including obstetric and catastrophic APS). In addition to the presence of appropriate clinical features, the diagnosis of APS fundamentally requires the finding of positive aPL test result(s), which is hampered by ongoing problems with assay reproducibility and standardization. This review focuses on ongoing dilemmas and issues related to clinical and laboratory aspects of APS including: (1) diagnostic challenges posed

by the protean clinical manifestations of APS; (2) current nomenclature and recent proposals for revision of the 2006 international classification criteria; (3) an overview of some key issues related to aPL testing; (4) potential pitfalls of applying the APS classification criteria as diagnostic criteria; and (5) the controversial subgroups of seronegative APS and non-APS aPL positivity.

**Keywords** Anti-beta-2-glycoprotein-I antibodies · Anticardiolipin antibodies · Antiphospholipid antibodies · aPL · Antiphospholipid syndrome · APS · Clinical features · Criteria · Diagnosis

## Introduction

The antiphospholipid syndrome (APS) is a challenging multifactorial and multifaceted condition primarily characterized by vascular thromboses (arterial and/or venous) and/or pregnancy morbidity, and associated with the presence of antiphospholipid antibodies (aPL) [1]. A current Medline search of the term “antiphospholipid syndrome” will yield over 6,700 papers and over 1,800 reviews, consistent with ongoing clinical and research interest in this disease. This review focuses on ongoing dilemmas and issues related to clinical and laboratory aspects of APS including: (1) diagnostic challenges posed by the protean clinical manifestations of APS; (2) current nomenclature and recent proposals for revision of the 2006 international classification criteria; (3) an overview of some key issues related to aPL testing; (4) potential pitfalls of applying the APS classification criteria as diagnostic criteria; and (5) the controversial subgroups of seronegative APS (SNAPS) and non-APS aPL positivity.

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### APS: an elephant with many parts or a chameleon with many colours?

The history and nomenclature of APS has been extensively covered elsewhere [2–5] but reminds us that APS is a relatively new disease entity, having been identified within the past 30 years. Because of the wide clinical features present in APS, the disease can present perceptually differently to a wide range of clinical specialties (Table 1), as well as primary care physicians and general practitioners. The importance of making the correct diagnosis of APS is underscored by the fact that this determines subsequent management, including the potential for prolonged anticoagulation therapy. We have previously applied to APS the analogy of several people trying to describe an elephant by touch alone, and having done so each describe a completely different creature, because each was only able to feel a small part of the elephant [6]. Perhaps another suitable analogy is that of a chameleon disguised by many colours, depending on the surrounding environment.

Many patients with APS have a “primary” form of disease (PAPS), with no features closely associated with other autoimmune diseases, in particular systemic lupus erythematosus (SLE). However, a proportion of APS patients have another associated disease entity (including SLE; [7]), and may therefore be identified as having “secondary” APS (SAPS), though this terminology may be changing as discussed in the next section. In addition, some PAPS patients may develop features of definite SLE over a period of time [8], and it is therefore recommended that PAPS patients be clinically and serologically monitored for such potential development [4]. As mentioned above, a myriad of clinical manifestations can develop in patients with APS, and these patients can therefore initially present to a range of different clinicians (Table 1). These clinical manifestations may affect

virtually any organ in the body, including the lungs, skin, brain, liver, kidneys, adrenal glands, heart and eyes [2, 6, 9–12]. The “classical” clinical presentations include deep venous thrombosis (DVT), pulmonary embolism (PE) and/or arterial thrombotic events, frequently but not always accompanied by thrombocytopenia and livedo reticularis. Obstetric presentations include pregnancy morbidity such as recurrent miscarriage and fetal growth restriction. However, there are a number of other clinical presentations not intuitively linked with APS, such as ischaemic bone fractures, renal and coeliac artery stenosis, and a possible tendency toward accelerated atherosclerosis. Dermatological manifestations other than livedo reticularis can also occur and may be the first presenting physical feature of APS [11]. Adrenal insufficiency is another “unusual” presentation of APS [10]. The association of APS with a range of malignancies has also been described [13]. This wide diversity in the clinical presentations of APS not only causes problems in its diagnosis and subsequent treatment, but provides different clinical specialties with differing perceptions of APS. An initial presentation with dermatological manifestations would typically result in referral to a dermatologist, recurrent miscarriage would be seen by an obstetrician, recurrent migraines would probably lead to referral to a neurologist, and so on. Alternatively, the patient may present to the emergency department of a hospital with one of the more severe “classical” manifestations of APS (such as PE, lower limb DVT or stroke). Differential management approaches might subsequently be applied in each of these different scenarios.

While vascular thrombosis is a key pathogenic mechanism that explains many of the clinical features of APS, some clinical manifestations (e.g. chorea) do not appear to have a purely thrombotic or ischaemic aetiology [12]. Obstetric manifestations including recurrent miscar-

**Table 1** Initial presenting manifestations of the antiphospholipid syndrome and the clinical specialties to which the patient may present or be referred

Presenting manifestations of APS	Possible specialist(s) for referral
Major thrombosis (arterial and/or venous)	Emergency physician; general physician (internist); haematologist; vascular physician/angiologist; rheumatologist; clinical immunologist
Obstetric-/pregnancy-/infertility-related	Obstetrician
Most neurological (e.g. migraine, chorea, memory loss)	Neurologist
Some neurological (e.g. balance disorder, hearing loss)	Neurologist; ear, nose and throat surgeon
Nonthrombotic haematological (e.g. thrombocytopenia)	Haematologist
Suspected specific organ involvement (e.g. adrenal gland, pituitary gland, kidney, liver, eyes)	Endocrinologist; nephrologist; gastroenterologist/hepatologist; ophthalmologist; etc
Dermatological	Dermatologist
Skeletal (e.g. ischaemic bone fractures)	Orthopaedic surgeon; rheumatologist
Any of the above (excluding obstetric) in a patient less than 16 years of age	Paediatrician

Adapted from reference [6]

riages, early onset and severe preeclampsia, may be the initial presenting feature in a significant proportion of APS patients and may remain the only clinical manifestation(s) in these individuals [14]. Although it is intuitive to invoke thrombotic mechanisms for these obstetric manifestations, nonthrombotic mechanisms also appear to be important pathogenically, in particular related to defective placentation and primary infertility [15].

### Nomenclature issues

Another twist in the life history of APS is that it now faces a potential significant change in nomenclature (Table 2). According to the recent 2006 Sydney classification criteria [1] and consensus opinion from the 2007 International Congress on Anti-Phospholipid Antibodies in Florence, Italy [16], the “primary/secondary” nomenclature should no longer be used, and instead patients with “primary APS” should be

described simply as having “APS”, while the term “secondary APS” should be replaced with APS and specific mention of the autoimmune disorder with which it is known to be associated. For example, when SLE is present with APS, the new recommendations would identify the patient as having “APS associated with SLE” rather than “secondary APS”.

However, these proposals have not been universally accepted by all APS experts, and the proposed changes may also pose significant problems in terms of patient classification for ongoing and future APS studies [6]. Patients already entered into prospective studies will need to be “relabelled” from the “primary/secondary APS” nomenclature to the “APS/APS with another autoimmune disease” terminology. Raising the large elephant analogy, not only are workers in the APS field describing different parts of the elephant, but some are also speaking different languages. Undoubtedly, this issue will be extensively discussed at the forthcoming 2010 APLA meeting in Texas.

**Table 2** Nomenclature used in the field of APS

Category	Term (abbreviation)	Description
Clinical condition	Antiphospholipid syndrome (APS)	A disease associated with the presence of antiphospholipid antibodies; may present with a wide variety of clinical manifestations
	Primary antiphospholipid syndrome (PAPS)	APS not associated with another disease process (e.g. SLE or other associated systemic autoimmune disorders). PAPS can in time develop features of a secondary disease. A recent recommendation is to change this terminology to just “APS”
	Secondary antiphospholipid syndrome (SAPS)	APS associated with another disease process (e.g. SLE). A recent recommendation is to change this terminology to “APS associated with [a description of the secondary disease]” (e.g. APS associated with SLE)
	Catastrophic antiphospholipid syndrome (CAPS)	An aggressive form of APS characterized by widespread thrombosis with clinical consequences that occur at several sites in the vasculature, typically involving multiple organ sites over a short period of time, often associated with significant morbidity and/or mortality
	Seronegative antiphospholipid syndrome (SNAPS)	Refers to patients with typical clinical manifestations of APS but who are found to be negative in a range of aPL tests/assays. Various explanations for these findings have been suggested
Laboratory testing	Antiphospholipid antibodies (aPL)	Describes the range of tests used to identify APS. A positive aPL test in a patient with clinical features of APS can be used to help diagnose APS. However, an isolated positive aPL test result may be found in clinically asymptomatic (non-APS) individuals, in particular (but not limited to) IgM isotype antibodies
	Anticardiolipin antibodies (aCL)	A sensitive but relatively nonspecific test for APS. Can be performed in a $\beta$ 2GPI-dependent or $\beta$ 2GPI-independent manner, with the former being more specific for APS. Suffers from high interassay and interlaboratory variability and some methodological issues that compromise interlaboratory portability and clinical utility
	Anti-beta-2-glycoprotein-I antibodies (anti- $\beta$ 2GPI)	A more specific but relatively less sensitive test for APS. Suffers from high interassay and interlaboratory variability and some methodological sensitivity issues that compromise interlaboratory portability and clinical utility
	Lupus anticoagulant (LA)	A clot-based assay which shows the strongest association with thrombotic features of APS, but which is also subject to a wide variety of preanalytical and analytical issues that compromise interlaboratory portability and clinical utility
	Antiprothrombin (aPT), antiphosphatidylserine (aPS), antiphosphatidylserine-prothrombin complex (aPS/PT)	Additional aPL assays sometimes utilized in APS investigations. Rarely offered by most routine diagnostic laboratories. Evidence base regarding clinical correlations is limited by small patient groups and a limited range of disease controls in published studies

### Key laboratory issues related to aPL testing

Clinicians face the ongoing dilemma of which aPL tests to request to facilitate or exclude the diagnosis of APS. Many will get around this dilemma by simply ordering “aPL”, thus shifting the decision regarding the choice of tests/assays to the diagnostic laboratory. In 2010 there are a wide panel of available tests, including a range of lupus anticoagulant (LA) assays, and three isotypes (IgG, IgA and IgM) of antibodies against cardiolipin (aCL), Beta-2-glycoprotein-I (anti- $\beta$ 2GPI), prothrombin and phosphatidylserine. There is ongoing vigorous debate regarding: which of these tests/assays should constitute a preliminary “routine” screen, which tests/assays should comprise a “confirmatory” or “full” panel, and which isotype(s) of the various antibodies should be included in these panels [17, 18]. For example, some experts recommend only IgG aCL and LA as a preliminary “routine” screen, some add IgG anti- $\beta$ 2GPI to these two tests, while others recommend a wider range of tests (IgG/IgM aCL, IgG/IgM anti- $\beta$ 2GPI and LA) [19]. It would be expected that the former panel of only two tests may miss some cases of APS, whilst the latter panel of five tests may produce a number of “false-positive” results.

A recently published retrospective Italian study [20] and the accompanying editorial [21] add further weight to the opinion that the combination of positive LA, IgG (>40 GPL) and/or IgM (>40 MPL) aCL, and IgG and/or IgM (level of positivity was not defined) anti- $\beta$ 2GPI tests are more strongly correlated with the clinical thrombotic events and/or pregnancy morbidity than if only one or two of these tests were positive [22–24]. However, all patients in the study had defined definite clinical APS as well as triple positivity for laboratory aPL, thus introducing a significant positive selection bias that is demonstrated by the high cumulative incidence (44.2% after 10 years) of thromboembolic events [20]. Consequently, these results cannot necessarily be generalized to patients with less definite or only “probable” clinical features of APS. Finally, while the authors and the accompanying editorial describe these patients as “triple positive”, all study patients actually had five rather than three tests performed, namely LA, IgG aCL, IgM aCL, IgG anti- $\beta$ 2GPI and IgM anti- $\beta$ 2GPI).

The editorial also specifically points out that only a minority of patients (ten, 6%) had predominately IgM rather than IgG isotype aCL and anti- $\beta$ 2GPI tests, these patients were significantly older and were more likely to have arterial (rather than venous) thrombosis, and only one had a recurrent event over the follow-up period [21]. The editorial then suggests that risk factors other than the IgM isotype aCL and anti- $\beta$ 2GPI may have been the primary contributory factors for the thrombotic events in

these patients, and that these observations support the exclusion of IgM isotype aCL and anti- $\beta$ 2GPI testing to “simplify the laboratory work-up of aPL antibodies”. However, the concluding comment regarding the “antiphospholipid triangle” does not clarify whether this is actually a “antiphospholipid pentagon” that includes IgG and IgM isotypes of the aCL and anti- $\beta$ 2GPI tests, as actually performed in the study. In any case, this nicely illustrates the ongoing controversy regarding what should constitute, in these cost-vigilant times, the most comprehensive, sensitive and most cost-effective panel of tests for aPL.

Reflecting further on the above, a recent survey revealed a wide variety in the tests/assays requested, recommended or provided by clinicians and laboratory personnel alike [25]. In this survey, most respondents (97/130, 75%) ordered or recommended tests for solid-phase aPL testing, and most also attempted to grade these tests. Most were familiar with aCL and anti- $\beta$ 2GPI testing, and tended to request primarily IgG and IgM isotypes of these antibodies. However, a small number of respondents requested/recommended IgA isotype testing of these antibodies or the other solid-phase aPL assays (e.g. antiprothrombin, aPT). A similar number of respondents (104/130, 80%) also ordered or recommended tests for LA, and most also attempted to grade these tests. Some discipline-related biases were evident, in that 32 of 34 of immunology-based respondents (94%) ordered or recommended specific solid-phase tests for aPL, compared with only 62 of 94 of haematology-based respondents (66%). In contrast, 83 of 94 of haematology-based respondents (88%) ordered or recommended specific LA test procedures, compared with only 18 of 34 of immunology-based respondents (53%).

It is also important to acknowledge the disappointing fact that many aPL tests are neither robust nor appropriately standardized, leading to much frustration amongst experts in the field as well as potential diagnostic errors in clinical and laboratory practice [18, 19, 26–29]. In cross-laboratory studies, coefficients of variation approaching 50% in aCL and anti- $\beta$ 2GPI results have been consistently reported [29–31]. This means that the range of reported aCL and anti- $\beta$ 2GPI test results can differ substantially from the median result obtained from a group of diagnostic laboratories, including those using the same in-house or commercial test/assay. Consequently, it is not uncommon for laboratories to disagree on whether a test sample is positive or negative for aPL, and the reported grade of positivity will also differ between laboratories. For example, a single cross-laboratory tested sample that yielded a median test result of 40 GPL (i.e. “moderately positive”) could feasibly generate a range of test results between 20 GPL (i.e. “equivocal”)



cal” or “weakly positive”) and 60 GPL (i.e. “strongly positive”). Despite these pessimistic findings, attempts to improve the standardization of all aPL tests/assays continue [32, 33].

The posttest laboratory report is also an important (postanalytical) variable affecting the diagnosis of APS. A survey on aCL reporting practices in Australia and New Zealand in 2001 found that many laboratories do not provide any interpretative comments in their reports, or when they do, many are not sufficiently comprehensive or informative for clinicians not intimately familiar with APS [34]. Because clinicians are likely to place considerable reliance on such interpretative comments, this observed variation in their provision and content could significantly determine whether an APS patient with a nonclassical clinical presentation is appropriately diagnosed or not. For example, a clinician requesting a single IgG aCL test as a screen for APS, may make or exclude a diagnosis of APS based on this test result alone, especially if the associated interpretative comment does not mention that a single positive or single negative aCL test result does not necessarily make or exclude (respectively) the diagnosis of APS, and/or does not indicate that other aPL tests (e.g. LA) should be performed. Accordingly, the Australasian Anticardiolipin Antibody Working Party considered it a priority to include sample interpretative comments in its published consensus guidelines on aCL [35] and anti- $\beta$ 2GPI [36] antibody testing and reporting.

After the initial clinical investigation based on the initial presenting clinical feature(s), the patient may subsequently be referred to a specialist for further assessment and management. Depending on the location and availability of specialist units with an interest in APS, the patient may be referred to a haematologist, rheumatologist, clinical immunologist or even a vascular physician/angiologist. Subsequent further diagnostic and management practices might then again be differentially applied. The above-mentioned survey [25] provides further evidence for this as it revealed that haematologists were more likely to investigate APS with haematology-based liquid-phase assays (i.e. LA), whereas immunologists were more likely to investigate APS by means of solid-phase assays (e.g. aCL and anti- $\beta$ 2GPI). The differing sensitivity and specificity of the liquid-phase and solid-phase aPL assays for clinical features of APS [37, 38] may thus affect the likelihood of APS being diagnosed.

### Limitations of the APS classification criteria

It is important to recognise that the original 1999 Sapporo [39] and revised 2006 Sydney [1] Classification

Criteria for APS *are* primarily classification (as opposed to diagnostic) criteria, developed and refined to maximize the possibility that patients who satisfy these criteria actually have APS and can therefore be appropriately included in prospective studies on APS (i.e. to minimize the number of “false-positive cases” in these studies). Accordingly, some patients with clinical APS will simply not satisfy these classification criteria [3, 6, 40]. The clinical significance of this is that some clinicians may inappropriately apply these classification criteria “strictly” to make or exclude a clinical diagnosis in a patient suspected of having APS. Hence, a proportion of patients with clinical APS (almost 30% based on the validation study of the 1999 Sapporo criteria by Lockshin et al. [40]) may have a diagnosis of APS incorrectly excluded, and thus may not be given appropriate therapy (including anticoagulation) leading to potentially significant adverse consequences for the patient. Therefore, the important take-home message is that even if such a patient does not fully satisfy the Consensus Classification criteria, he/she may still have APS and thus require appropriate management based on the clinical scenario [3].

Moreover, whilst the clinical criteria were left unchanged in the 2006 revised criteria [1], the laboratory criteria were expanded to include persistently positive IgG and/or IgM anti- $\beta$ 2GPI antibody findings, and perhaps most importantly, the requirement that aCL testing be performed in a  $\beta$ 2GPI-dependent manner was removed. Many APS experts have expressed concern that these changes (especially the latter) may result in a reduction in the specificity of the revised 2006 criteria for APS, due to the inclusion of patients with false-positive, noncofactor-related, aCL results as their laboratory criteria [41, 42].

Finally, it is important to recognize that the consensus process used to create and revise such classification criteria has its limitations, having a significant component of “eminence-based” rather than “evidence-based” input, largely due to the relative paucity of good quality prospective clinical studies in the area. Accordingly the consensus process typically produces broad (e.g. 70–90%) rather than complete (100%) agreement on the more contentious issues, as discussed in greater depth elsewhere [6, 19, 43].

### Seronegative APS and non-APS aPL positivity: are they a part of the elephant or a colour of the chameleon?

The term seronegative antiphospholipid syndrome (SNAPS) has been coined to describe patients with typi-

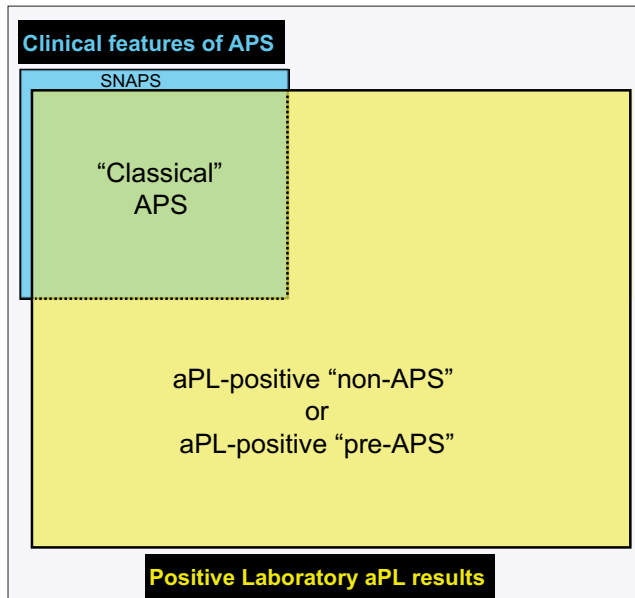
cal clinical manifestations of APS but who are still found to be negative for detectable aPL [4]. It has been recommended that for SNAPS to be diagnosed, the patient should be seronegative for all aPL types (i.e. LA, aCL, anti- $\beta$ 2GPI, etc) and all antibody isotypes (i.e. IgG, IgM and IgA) at the time of the thrombotic event and following its resolution, and careful exclusion of other prothrombotic conditions is also required. The importance of repeat testing for aPL following resolution of the thrombotic event has been emphasized elsewhere, with a number of possible explanations for transient aPL seronegativity in APS patients having been proposed [4, 44]: (1) 20–30% of APS patients are positive only for aCL or LA, thus leading to the recommendation that both tests should always be performed in all patients with suspected APS; (2) aCL and LA may transiently fall to undetectable levels due to “consumption” at the time of the thrombotic event; and (3) some APS patients may only have antibodies directed against phospholipids other than cardiolipin (e.g. phosphatidylserine, phosphatidylethanolamine).

While it would be naive to believe that the currently available laboratory testing processes would identify all potential aPL, the requirement to investigate APS, and more specifically a potential case of SNAPS, raises the significant perpetual dilemma regarding how many aPL assays/tests a diagnostic laboratory should have available and/or routinely offer to clinicians. This extends the previous discussion regarding the observation that the clinical thrombotic events and/or pregnancy morbidity correlate best with laboratory findings when more than one or two of the “standard” aPL tests are positive. Most diagnostic laboratories offer IgG and IgM isotypes of aCL and LA testing, with anti- $\beta$ 2GPI testing (primarily IgG isotype) being offered by a smaller number of diagnostic laboratories [25, 34]. However, very few would offer antiprothrombin, antiphosphatidylserine, antiphosphatidylethanolamine and/or other aPL assays/tests. Thus, when assessing a patient with a clinical picture consistent with APS but who is negative for the more commonly available aPL assays (i.e. aCL, anti- $\beta$ 2GPI and LA), the treating clinician(s) and/or diagnostic laboratory(ies) have to decide whether to spend the time and money (potentially quite significant) to get additional aPL tests performed (possibly overseas) or simply to make a diagnosis of SNAPS based on the routinely and locally available aPL assays.

Conversely, it is also very important to recognize that aPL positivity in isolation is not sufficient to make a diagnosis of APS. It is well recognized that the more laboratory tests performed, the greater the risk of obtaining a “false-positive” test result simply by chance alone. As 5% of normal test results will fall outside a standard

derived normal reference range of mean  $\pm 2$  standard deviations, one in 20 tests would be expected to be falsely positive by chance alone [45]. Indeed, non-APS-related aPL positivity is well recognized by experts working in the field of APS. Thus, although aPL positivity is an integral part of making the diagnosis of APS (with the exception of the concept of SNAPS), aPL positivity has been found to occur in approximately 1–6% of normal healthy individuals [46]. In these asymptomatic individuals, a positive aCL (usually low positive, but occasionally moderate or even high positive) result may simply be observed as a chance event when these individuals are used as controls for reference range evaluations. Similarly, LA may be found to be positive following elevated coagulation test results performed for routine (e.g., preoperative) screening. Moreover, aPL positivity can occur in patients with other autoimmune conditions (including SLE) who do not display clinical features of APS. aCL positivity, especially of the IgM isotype, can also occur with a wide range of other (non-APS) conditions including infections such as syphilis (which is how the cardiolipin test came to be named [26]). Thus, it is important that clinicians make the diagnosis of APS based on appropriate clinical features and subsequent relevant laboratory assessment for aPL, as opposed to positive aPL test results that are unexpectedly obtained in asymptomatic patients with a low pretest probability of APS (Fig. 1).

As mentioned above, the requirement for aCL assays to be performed in a  $\beta$ 2GPI-dependent manner was not included in the Sydney APS classification criteria [1]. Some APS experts have subsequently pointed out that this is likely to increase the number of  $\beta$ 2GPI-independent (“false positive”) aCL results [42]. Partially prompted by this change, a number of APS experts have therefore recommended that the aCL test be completely abandoned and replaced by the anti- $\beta$ 2GPI test, in order to reduce the potential number of these false-positive (largely  $\beta$ 2GPI independent) aCL test results [17, 42]. However, while the anti- $\beta$ 2GPI assay is more specific for APS than the aCL assay (about 99% and 90%, respectively, according to Helbert et al. [47]), this is at the expense of significantly lower sensitivity for APS (about 75% and over 95%, respectively, in the same study). In other words, a proportion of APS patients will be negative for anti- $\beta$ 2GPI even though they may be positive for aCL. Although some of the anti- $\beta$ 2GPI assays used in previous studies may have been relatively insensitive compared to currently available anti- $\beta$ 2GPI assays, the potential consequences of using a less sensitive diagnostic test in patients with a moderate to high pretest probability for APS are significant. If the aCL test was no longer routinely available to evaluate patients with sus-



**Fig. 1** The majority of APS patients will have a “classical” form of APS, as defined by the presence of clinical features of both APS and positive laboratory aPL test results [1]. A small proportion of APS patients will be identified with clinical features of APS but without any positive aPL test results. These “seronegative” APS patients (SNAPS) probably do express a form of aPL which may not be identifiable given the limitations in currently available aPL tests/assays. Another subgroup of individuals will be identified that yield positive aPL test results but do not have any clinical features of APS. It is possible that some of these patients will have clinical features of related autoimmune disorders (such as SLE) and/or will ultimately develop clinical features of APS (i.e. represent an aPL-positive “pre-APS” group). However, probably most of these individuals will remain clinically asymptomatic (i.e. represent an aPL-positive “non-APS” group)

pected APS who are negative for anti- $\beta$ 2GPI and LA, these patients would have to be considered as having SNAPS. Alternatively, and of more concern, clinicians who are less familiar with the differing diagnostic properties of anti- $\beta$ 2GPI and aCL tests, may mistakenly exclude the diagnosis of APS entirely and not institute appropriate therapy (including anticoagulation) with potentially significant adverse outcomes for the patient.

The complex issues related to the management of such patients with non-APS-related aPL positivity are beyond the scope of this review. However, the presence of aPL in the absence of clinical features associated with APS is generally considered to be associated with a low risk of thrombotic events [46]. Accordingly, the potential benefits of what is essentially prophylactic treatment have to be weighed against the potential risks (e.g. bleeding), inconvenience and costs of the treatment. Indeed, a recent randomized double-blind placebo-controlled APLASA study by Erkan and colleagues [48] found that low-dose aspirin (81 mg/day) was not superior to placebo in preventing the first thrombotic episode in asymptomatic persistently aPL-positive individuals, although

there was a relatively low incidence rate of the first thrombosis in the study population. Interestingly the concomitant presence of SLE appeared to be an independent risk factor for the development of thrombosis in these persistently aPL-positive subjects, leading the authors to suggest that these patients should be managed more aggressively. The authors also recommended that the ideal primary thrombosis prevention strategy in asymptomatic persistently aPL-positive individuals needs to be risk-stratified and determined according to the individual’s age, traditional cardiovascular risk factors, presence or absence of other systemic autoimmune diseases, as well as which particular aPL tests were positive.

It therefore seems reasonable that similar considerations should be applied in non-SLE aPL-positive individuals with no clinical features associated with APS. A recently published clinical vignette included a literature review and an analysis of the small number of relevant studies comprising retrospective, cohort and prospective designs [46]. Given the uncertain benefit of aspirin and the small associated risk of bleeding, the authors concluded that there was no current good quality evidence to support the use of routine aspirin as primary thromboprophylaxis in such patients who did not have other cardiovascular or thrombotic risk factors.

## Conclusion

This review outlines the tight interplay between laboratory tests for aPL and the clinical diagnosis of APS, given that the fundamental concept of APS requires that these patients must have appropriate clinical manifestations and positive aPL test result(s). Laboratories providing aPL testing need to be fully aware of the potentially profound implications that significant changes in laboratory testing panels and practices may have for the vast majority of clinicians, especially those less familiar with the laboratory aspects of aPL testing. These problems also illustrate why APS remains one of the more challenging diagnostic entities in clinical medicine, in terms of both making the diagnosis and the subsequent management of the patients identified [6, 19, 49]. This review has also focuses on several aspects of the laboratory/clinical interface in terms of identification and diagnosis of “typical” APS, but does not cover other subgroups of APS that have been reviewed elsewhere (e.g. paediatric APS [50] and catastrophic APS [51]). It is apparent that the range of possible pathogenic mechanisms, protean clinical manifestations, possible changes in nomenclature, reliance on “unreliable” aPL assays to make the diagnosis (with the exception of SNAPS), uncertainty regarding the optimal number of aPL tests to offer/perform, and the

**Table 3** Important ongoing diagnostic issues in APS

Issue	Current situation	Comments
Revision of “primary/secondary” nomenclature	The 2006 Sydney classification criteria and 2007 Phospholipid Conference in Florence (Italy) recommend that the “primary/secondary” nomenclature be replaced with “APS” and specific mention of any autoimmune disorder known to be associated with APS	Patients currently enrolled in prospective trials will need to be relabelled. Some concepts of APS (e.g. “primary plus APS”) are not accommodated in the proposed new nomenclature
Use of APS classification criteria for diagnostic purposes	Clinicians may use the APS classification criteria when trying to make a diagnosis of APS in patients suspected of having the disease, as opposed to their intended purpose to appropriately classify APS patients for inclusion into prospective trials	Patients who only partially satisfy the APS classification criteria may be misdiagnosed as not having APS and thus denied appropriate therapy, with potentially significant adverse outcomes for the patient
Seronegative APS	How many aPL tests should be performed before considering a patient with clinical features consistent with APS as truly being seronegative?	Many routine diagnostic laboratories offer IgG aCL, IgG anti- $\beta$ 2GPI and LA testing. Expanding the number of isotypes (including IgA) of these antibodies and other aPL tests will increase the complexity and cost of routine testing, and may also increase the proportion of false-positive results
Seronegative APS	Some authors have proposed that the aCL test be removed and replaced with the anti- $\beta$ 2GPI test to improve overall specificity	Because of its higher specificity, the anti- $\beta$ 2GPI test is less sensitive than the aCL test, and therefore some APS patients may be anti- $\beta$ 2GPI-negative but aCL-positive. If the aCL test is no longer routinely available, these patients may be diagnosed as having SNAPS, or more worryingly, not be recognized as having APS at all
Lack of standardization of laboratory assays and of consensus regarding tests, test panels and antibody isotypes to utilize in the diagnosis of APS Establishment of large registries of APS patients	Most tests have high interassay and interlaboratory variability; some tests are more sensitive and others more specific for APS; some tests and isotypes are more highly correlated to clinical features (e.g. thrombosis) than others The 1999 Sapporo and 2006 Sydney Classification Criteria for APS have enabled the establishment of large registries of APS patients in both North America and Europe	Much more work is required to standardize these assays and make them truly useful and portable for the diagnosis of APS. A better evidence base is required to enable appropriate consensus on the best tests, best panels and most appropriate isotypes for testing The registries enable pooling of clinical experience, and identification of differing clinical manifestations as well as various treatment modalities used in APS
Internet-based registries for rarer subgroups of APS	For example, the internet-accessible CAPS registry ( <a href="http://www.med.ub.es/MIMMUN/FORUM/CAPS.HTM">http://www.med.ub.es/MIMMUN/FORUM/CAPS.HTM</a> )	Enables pooling of clinical experience of otherwise rare conditions which any individual clinician or unit might only see once or twice per year. This has led to various insights into the frequency of differing clinical manifestations well as the various treatment modalities used in CAPS patients

Adapted from reference [6]

relative lack of prospective clinical trials, makes APS a fertile ground for ongoing basic, clinical and diagnostic laboratory-based research and vigorous discussion well into the 21st century (Table 3). The different clinical presentations and different specialist perceptions also mean that many of us will only ever perceive certain parts of the very large elephant or only see some of the many vibrant colours of the elusive chameleon that is APS.

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