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Does seronegative obstetric APS exist? "pro" and "cons"

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ABSTRACT

Antiphospholipid Syndrome (APS) is the commonest treatable cause of recurrent miscarriage and pharmacological treatment of pregnant patients with antiphospholipid antibodies (aPL) should aim at preventing obstetric complications and maternal thrombotic events. Conventional treatment for patients with an established diagnosis of obstetric APS (OAPS), generally resulting in over 70–80% successful pregnancies. Since seropositive (SP)-APS and seronegative (SN)-APS patients had shown similar clinical profiles, patients with SN- OAPS, as well as SP-OAPS, should receive combined treatment in order to improve the pregnancy prognosis; indeed, current standard of care increased good pregnancy outcome in SN-APS, with similar effect to confirmed APS.

The above data suggest that there are patients with the clinical manifestations of OAPS but persistently negative to conventional aPL that need to be identified to ensure adequate therapy and therefore a better prognosis. The clinical utility of non-criteria aPL in the diagnosis of SN-APS is still a matter of debate. In the last decade more and more studies have reported the presence of patients suffering from SN-APS in which non-conventional ("non-criteria") aPL might be present or antibodies may be detected using methodological approaches different from the traditional assays. To improve test standardization large prospective, multicenter, and multinational studies are needed. Therefore, when assessing a patient with clinical manifestations consistent with OAPS but aPL negative using the conventional available assays, the clinician should consider the possibility that the patient is affected with SN-APS.

1. Introduction

Antiphospholipid Syndrome (APS) is a systemic autoimmune disease characterized by obstetric morbidity and/or vascular thrombosis mediated by a family of pathogenic autoantibodies called "antiphospholipid antibodies" (aPL). Since the first description of APS in 1983 [1], international collaborative research has focused on both clinical and laboratory definition. According to 2006 Sidney classification criteria [2] (an update of the original 1999 Sapporo criteria), a patient can be classified as having APS in presence of one clinical criteria and one laboratory criteria (Table 1). A number of features (both clinical and laboratory) have been recognized to be associated with APS but they were not included in the classification criteria because of lack of specificity [2]. Due to the possible clinical and prognostic relevance of these 'non-criteria' manifestations and antibodies, in 2013 a task force was carried out to analyze these features of APS and also to discuss about the so called 'seronegative APS' (SN-APS) [3]. This term, used for the first time by Hughes and Khamashta in 2003 [4], indicates the presence of the typical clinical features of APS but without aPL.

Among these 'non criteria' aPL there are IgA isotypes of anti-cardiolipin (aCL) and anti-beta-2-glycoproteinI antibodies (aβ2GP1), anti-phosphatidylserine antibodies (aPS), antiphosphatidilethanolamine antibodies (aPE), anti-prothrombin antibodies (aPT), anti-phosphatidylserine-prothrombin complex antibodies (aPS/PT), anti-Annexin A5 antibodies (aAnnA5), antibodies towards the Domain 1 of β2GP1 (aDI) [5,6]. Regarding obstetric morbidity, there is increasing interest in understanding the value of different aPL profiles in the occurrence of clinical events [7], as it seems that severe obstetric phenotypes can be associated not only with multiple, high titer aPL positivity but also with single-positive, low titer aPL. On the other hand, many women who are faced with recurrent pregnancy failures whose cause is unknown could benefit from a diagnosis of APS if any serological clue can be found [8]. Therefore, research has been underway to detect "novel aPL" which could be the hallmarks of what we are currently calling "SN-APS" [9].

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Table 1
Classification criteria for antiphospholipid syndrome.

Clinical criteria[a]
Vascular thrombosis • One or more clinical episodes of arterial, venous or small vessel thrombosis, in any tissue or organ. • Thrombosis must be confirmed by appropriate imaging studies or histopathology. • Thrombosis should be present without significant evidence of inflammation in the vessel wall.
Pregnancy morbidity • One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus. • One or more premature births of a morphologically normal neonate before the 34th week of gestation due to eclampsia and severe pre-eclampsia, or to recognized features of placental insufficiency. • Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomical or hormonal abnormalities, and paternal and maternal chromosomal causes excluded.
Laboratory criteria[a] • Lupus anticoagulant (LA). Present in plasma, on more than two occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies). • Anticardiolipin antibody of IgG and/or IgM isotype. Present in serum or plasma at medium or high titer (> 40 GPL or MPL, or > 99th percentile), on more than two occasions at least 12 weeks apart, measured by a standardized ELISA. • Anti-β2GPI antibody of IgG and/or IgM isotype. Present in serum or plasma (titer > 99th percentile), on more than two occasions at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures.

Anti-β2GPI: anti-β2 glycoprotein I; GPL: IgG phospholipid units; GPM: IgM phospholipid units.
[a] Antiphospholipid syndrome is present if at least one clinical criterion together with one laboratory criterion are met.

Methodological issues have been haunting aPL assays over decades [5] and these limitations should be taken into account also when dealing with "non-classical" aPL.

This article will summarize the debate "Does seronegative obstetric APS exists?" which took place at the 5th International Congress on Controversies in Rheumatology and Autoimmunity (Florence, Italy; 14th–16th March 2019).

2. Does seronegative obstetric APS exist? "pro"

A close relationship between autoimmunity and autoantibodies is self-evident; nevertheless, some patients with autoimmune diseases are persistently negative for all known disease-specific autoantibodies. Seronegative autoimmune diseases are the definition for these conditions. Although these are infrequent, they may represent a challenge for physicians [10]. In clinical practice, it is not rare to find individuals with clinical signs highly suggestive of APS, who are persistently negative for the recommended tests for the detection of aPL – i.e. aCL and aβ2GP1, measured by standardized enzyme-linked immunosorbent assay (ELISA), and the lupus anticoagulant (LA), detected by clotting assays, [2]. Therefore, in cases of high suspicion, some experts proposed for this population the term of SN-APS [4,11,12]. In 2003 Hughes & Khamashta proposed three possible explanations for this condition: wrong diagnosis; presence of aPL not detected by current "criteria" tests or previously positive aPL tests reverted to negative [4,13]. The inadequacy of the tests could depend on the limits of the traditional methods or on the existence of different antigenic targets [14].

2.1. Thin-layer chromatography immunostaining and anti-cardiolipin/vimentin antibodies in SNAPS

Regarding the limits of the traditional methods, the results of ELISA tests for aPL are typically reported in arbitrary units, that reflect the level of reactive immunoglobulins (class G or M) based on calibrator standards. The lack of agreed standards leads to extraordinarily high inter-laboratory or inter-method variability [15]. Therefore, additional techniques have been developed to detect aPL, such as thin-layer chromatography (TLC) immunostaining, which is a non-quantitative assay method based on three main phases: antigen separation, immunostaining with patients' sera and detection of immunoreactivity [16–18].

TLC immunostaining is based on the different separation characteristics of the phospholipids between the stationary phase and the mobile phase of the solvent, which occurs according to the different polarities of the solvent [14]. The binding of phospholipid to solid phase mainly involves both electrostatic and hydrophobic interactions. Thus, the antigen exposure is quite different as compared to that on the surface of microtiter wells, where phospholipids are coated in a layer of immobilized lamellar phospholipids [19]. These features make TLC immunostaining another tool that could be used for aPL testing [9,20,21]. In 2012 TLC immunostaining was used for the first time to investigate aPL in a cohort of 36 SN-APS patients allowing to detect aCL in 47.2%, anti-lysobisphosphatidic acid (aLBPA) in 41.7% and aPE in 30.5% of patients [20]. In the aforementioned cohort, 58% of the patients had features of obstetric APS (OAPS); in this population, TLC immunostaining allowed to detect aCL and aLBPA in 42.8%, aPE in 28.6% of patients [20].

In addition, a proinflammatory and procoagulant effect in vitro of purified IgG from SN-APS was demonstrated as they were able to induce phosphorylation of interleukin-1 receptor-associated kinase 1 (IRAK) and consequently the activation of NF- kB, the expression of VCAM-1 (Vascular Cell Adhesion Molecule-1) and tissue factor (TF) release in Eahy926, a human-derived endothelial cell line [20]. The same pathway was already demonstrated for conventional aPL [22–24]. Furthermore, TLC-immunostaining was used for aCL detection in a case of suspected catastrophic SNAPS; the positive test allowed to undertake an appropriate therapy [25].

In addition, a proteomic approach identified anti-cardiolipin/vimentin antibodies (aCL/Vim) as a "new" target for APS, also detectable in SN-APS patients [26]. In patients with SLE, antibodies directed against vimentin are strongly associated with the presence of aCL antibodies [27]. Ortona et al. have shown that vimentin is able to bind cardiolipin in vitro, probably owing to electrostatic interactions between vimentin and negatively charged aminoacids on cardiolipin [26]. Anti-CL/Vim IgG and IgM were detected in 55% and 38% of SN-APS and the occurrence of both IgG and IgM antibodies was significantly greater in patients with APS, SN-APS, and SLE compared with healthy donors ($P < .0001$). Moreover, the occurrence of aCL/Vim reactivity was significantly greater in APS patients compared with SLE and rheumatoid arthritis patients ($P < .0001$) [26]. Furthermore, affinity-purified aCL/Vim antibodies from SN-APS seem to have a pathogenic role as they were able to induce IRAK1 phosphorylation and to activate NF-kB in endothelial cells [26].

More recently, TLC-immunostaining was used to detect aPL in a monocentric cohort of 61 patients with suspected "seronegative" OAPS; using these approaches, the authors demonstrated the presence of aCL antibodies in the 62.2% of cases [9]. Specifically, aCL antibodies were positive respectively in 41%, 29.5% and 4.9% of patients that experienced three or more unexplained consecutive miscarriages before the 10th week of gestation, an intrauterine death of a normal fetus and premature births. The concordance of the antibodies positivity detected by TLC-immunostaining between first and second test (at least 12 weeks apart) showed a substantial agreement as demonstrated by the Cohen's kappa test ($K = 0.696$). These data suggest that the use of TLC-

immunostaining can be useful for accurate and timely diagnosis of patients with SN-OAPS. The patients enrolled in this study had strongly suggestive characteristics of APS, and other possible causes of thrombosis and pregnancies morbidity were excluded [9].

Fifty out of 61 SN-OAPS patients (82%) were positive for at least one of the tests used. Thirty-eight out of 50 patients (76%) showed the presence of aCL antibodies detected by TLC-immunostaining, 27/50 (54%) were positive for aCL/Vim. In 35 out of 61 patients the tests were repeated on two occasions, at least 12 weeks apart; in only 2 patients (5.7%) the previously positive test was not confirmed with the second test [9].

2.2. Other autoantibody specificities

Many studies have shown that aPL represent a heterogeneous family of antibodies [28–30]. Regarding the existence of different antigenic targets, several antibody specificities have been described in APS patients, with a variable prevalence. Different authors described antibodies directed towards phospholipid-binding cofactor proteins, including not only β2GP1 [31,32] but also different proteins, or phospholipid-protein complexes, such as prothrombin [33], protein S [34,35], protein C [36], annexin V [14,37,38], annexin II [39], oxidized low-density lipoprotein, LBPA [20], PE [38,40]; PS/PT [9,14,38,40–43] and sulfatides [44–46]. In the last decade several of such unconventional autoantibodies have been investigated in the obstetric form of the SN-APS.

Increasing evidence has highlighted that aPS/PT might enhance the diagnostic performance for the diagnosis of SN-OAPS. In most of the studies the prevalence of aPS/PT ranged from 4 to 12% of SN-OAPS patients [9,38,43]. On the contrary, only one study reported a higher prevalence (48%) of aPS/PT in patients with SN-OAPS [47]. The different prevalence could depend on the methods used to detect aPS/PT.

The possible diagnostic and prognostic role of aDI has been investigated in APS. Anti-DI were found in 27% of seropositive APS (SP-APS) [40] and in 60% of OAPS resulting in an odds ratio of 2.4 (1.4–4.3) [17]. So far, only 2 studies evaluated the presence of aDI in SN-APS patients with a prevalence ranging from 0 to 4% of patients [38,40].

AnnA5 is an anticoagulant protein mainly found in trophoblasts and vascular endothelial cells. Anti-AnnA5 seems to be associated with the clinical features of OAPS and aAnnA5 were found up to 88% of patients with SN-OAPS [38].

The association of aPE with pregnancy morbidity is a matter of interest. Anti-PE seem to be associated to recurrent early pregnancy losses and mid-to-late pregnancy losses [48]. The role of aPE in patients with the SN-OAPS is a hot topic, still under investigation. Recently, in a single study, aPE IgG and IgM were detected respectively in 60% and 12% of patients with SN-OAPS [38].

The possible role of IgA aCL and IgA aβ2GPI antibody in patients with pregnancy morbidity was evaluated in a cohort of patients with pregnancy morbidity: patients with well-defined PAPS, patients with unexplained pregnancy morbidity and SLE patients. Overall, IgA aCL were detectable in 38% and IgA aβ2GPI in 4% of APS patients; 11% of patients with unexplained pregnancy morbidity and 14% of SLE patients had IgA aCL alone [49].

Furthermore, the role of aCL IgA isotype in SN-OAPS was evaluated in two studies showing a low prevalence ranging between 0% to 4% [9,38].

Although the presence of anti-protein S and anti-protein C has been reported in numerous studies on patients with APS [34,50] and the levels of such autoantibodies correlate with obstetric manifestations and preeclampsia [51] these antibodies have not been tested in SN-APS.

The study by Zohoury et al. evaluating the non-criteria tests in a cohort of SN-APS and SP-APS patients, showed that aCL/Vim together with aPS/PT were the most sensitive non-criteria biomarker in the SN-APS group; aCL/Vim were present in 16% of SN-OAPS patients [40]. In a monocentric Italian cohort of SNAPS patients, the prevalence of aCL/Vim was 45.8% [14].

3. Does seronegative obstetric APS exist? “cons”

The potential reasons for the diagnosis of SN-APS is that the “criteria aPL” are negative or have become negative, or that the classic aPL tests are not sufficient to diagnose APS in some cases.

3.1. Classic aPL are persistently negative or previous positive aPL have become negative

a. In the updated APS classification criteria, the term “consistently positive aPL” was added to avoid overdiagnosis of APS due to false-positive initial readings, e.g. due to infections [2]. Accordingly, to confirm that the classic aPL are “consistently negative”, all previous aPL measurements should be evaluated. More specifically, it should be checked whether: a. all three aPL types (aCL, aβ2GPI and LA) have been tested given that LA testing is often ignored; b. both IgG and IgM isotypes have been examined for aCL and aβ2GPI antibodies, since IgM is often considered as “not important”; c. the laboratory methods used were accurate. According to the current classification criteria for APS, LA should be measured by expert laboratories according to the International Society on Thrombosis and Haemostasis (ISTH) guidelines [2]. In addition, IgG and IgM aCL and aβ2GPI antibodies should also be measured using a standardized ELISA with established cut-off points, given the high variability among several available aPL assays. If aPL were detected negative in the first aPL testing, aPL might be negative at that specific time and positive if we repeat the tests. In cases with high clinical suspicion, aPL testing can be repeated at some reasonable time point (3–6 months) after the APS related (e.g. thrombotic) event.

b. Previously positive aPL can become negative, although this is uncommon for high titer aPL.

3.2. “Criteria” aPL tests (aCL, LA, aβ2GPI) are not sufficient to diagnose APS

a. Previous studies have shown that in patients with SN-APS, antibodies may be detected using methodological approaches different from the traditional assays, such as the TLC-immunostaining [14,52]. However, the feasibility and the cost-effectiveness of more advanced techniques for their detection, and whether not commercially available tests should be used, remain questionable.

b. Non-conventional (“non-criteria”) aPL might be present. Other variants in aPL testing have emerged including IgA isotypes of aPL (aCL, aβ2GPI), or binding to alternative antigenic targets such as vimentin, negatively charged phospholipids (e.g. PS), AnnA5, plasma proteins involved in the coagulation cascade (PT or PS/PT complexes), and the domain I of β2GPI [2].

3.2.1. IgA isotypes of aCL and anti-aβ2GPI antibodies

Data from a literature review performed in the context of the 15th international congress on aPL showed that the presence of IgA aCL and aβ2GPI antibodies usually coexists with the presence of “criteria” aPL, raising questions about the clinical significance of their additional evaluation [53]. In addition, lack of standardization in the assay methods used for their detection still exists.

3.2.2. Anti-vimentin/cardiolipin antibodies

Vimentin is a protein that is shown to bind cardiolipin in vitro. Previous studies have demonstrated a correlation between aCL/Vim and aCL, thrombotic APS and OAPS but their specificity for APS is considered low since they have also been detected in other systemic autoimmune diseases such as SLE and rheumatoid arthritis [14,26].

3.2.3. Antibodies against negatively charged phospholipids, phosphatidylethanolamine, and annexin A5

The ISTH does not recommend the inclusion of antibodies against negatively charged phospholipids other than cardiolipin such as PS or phosphatidylinositol (PI), PE, and AnnA5 in the standard panel of aPL due to lack of laboratory assay standardization and unconfirmed evidence about their clinical utility in APS [54].

3.2.4. aPT and aPS/PT

In a systematic literature review, < 50% of the associations between aPT antibodies and thrombosis reached statistical significance [55]. The presence of aPS/PT antibodies was associated with a 2-fold-increased risk for arterial thrombosis but there were several methodological issues in primary studies such as the retrospective design of studies, the small samples, and a high heterogeneity among studies associated with a "Low Level of Evidence" according to GRADE criteria. These limitations have been addressed by a recent international multicenter study of 247 patients that included for the first time a validation study in addition to the initial study [56]. The sensitivity, specificity, positive likelihood ratio (LR) and negative LR of aPS/PT antibodies for the detection of thrombotic APS in the validation study were 47%, 88%, 3.9% and 0.6%, respectively. For OAPS, although the initial study showed a predictive association between aPS/aPT and pregnancy complications, the validation study did not confirm this association.

3.2.5. Anti-β2GPI Domain I antibodies

The working group on non-criteria aPL in the 15th International congress on aPL concluded that the additional value of aDI antibodies vs aβ2GPI antibodies and the type of aβ2GPI assay to which the aDI assay is compared should be further clarified [8]. In a cohort study of 101 patients with APS, 123 with autoimmune disorders, 82 diseased controls and 120 healthy individuals, the clinical performance characteristics, the additional diagnostic value, and the contribution to APS risk stratification of an automated chemiluminescence aDI assay was analyzed [57]. Multivariable logistic regression showed that the agreement between aDI and aβ2GPI IgG is high, limiting the added value of aDI antibodies to APS diagnosis and risk stratification. A systematic literature review of 11 studies including 1585 patients showed that aDI positivity doubled the risk for thrombotic events [58]. The limitations of the study included the analysis of only observational studies and the heterogeneity among studies in the inclusion criteria, laboratory tests, cutoff values definition, clinical characteristics, site of thrombosis, and control groups. No multivariate analysis and no comparison between persistent vs transient antibodies performed by most of studies. In addition, an analysis on the association between aDI antibodies and OAPS outcomes was performed.

3.2.6. Combination of non-criteria aPL

In a recent study of non-supervised hierarchical clustering of all criteria and non-criteria aPL, no association between conventional aPL and PS or PT alone, domain I or IgA β2GPI, and AnnA5 was observed. A high correlation between aPS/PT antibodies and LA and triple aPL was demonstrated [42]. Nakamura et al. showed that a combination of IgG aβ2GPI-DI and IgG/M aPS/PT antibodies had 100% positive predictive value for the diagnosis of APS and similar sensitivity but higher specificity vs IgG aCL and IgG aβ2GPI antibodies for APS diagnosis [59]. Further investigation is needed to confirm these results due to several limitations of the study including a retrospective cross-sectional design, a small sample size that affects also the predictive values, a single measurement of tested antibodies, and the involvement of only one center with lack of internal and external validation cohorts.

In a collaborative USA/UK study of 175 patients including 68 patients with clinical criteria for APS but persistently negative laboratory criteria, 1/3 of the 'seronegative' sera gave positive results for one or more tests using a comprehensive panel of 'non-criteria' antibodies. [40]. A question that arises is if this group of patients should be considered as SN-APS and be treated as APS. Incorrect diagnosis might result to wrong or unnecessary (usually long-term) treatments (e.g. lifelong anticoagulation in the case of thrombotic SN-APS or during whole pregnancy period in OAPS) with potential adverse events (e.g. anticoagulation-related) or incorrect direction of treatment in cases that different treatment might needed.

3.3. How well can non-criteria aPL classify subjects into disease or non-disease group?

Prior studies have suggested that since failure to diagnose APS can result in severe clinical consequences, patients with clinical features of APS but negative for conventional aPL should undergo additional testing for non-criteria biomarkers, and an update to the current classification criteria incorporating new serological markers is needed [8,55].

The results of the previous studies are based on the detection of higher levels of some of non-criteria aPL in patients with APS than healthy controls. However, comparisons using healthy individuals as controls typically overestimate sensitivity and specificity of tests. The clinical utility of tests used for diagnosis/classification of a clinical entity requires the use as controls of individuals with conditions that mimic or that could be confused with the disease in question. Importantly, this has not been done in any study of non-criteria aPL but it has been a part of the methodology in the development of classification criteria for SLE [55].

3.4. How tests can correctly classifying individuals by disease status?

Ideally, biomarkers for diagnosis should provide information not available from currently available tests and they should be tested as they would be used in clinical practice [60]. Biomarkers could be affected by many variables, such as patient's age, gender, disease activity, therapeutic intervention, or the presence of comorbidities. However, no adjustment for these variables has been performed in the majority of studies addressing the role of non-criteria aPL as potential new biomarkers for the diagnosis/classification of APS. A meta-analysis assessing the validity of clinical associations of biomarkers in translational research studies included studies on 6 systemic autoimmune diseases published between 2004 and 2009 in 10 high impact journals for translational studies [61]. The results showed that less than half of studies reported associations between laboratory markers and the presence of disease incorporated design features needed for valid interpretation of the potential biomarkers.

Typically a test is evaluated against a reference diagnosis to calculate its sensitivity, specificity, positive predictive value, negative predictive value, and the analytical performance of the test [62].The above mentioned procedure has not been followed in most of studies of non-criteria aPL, with only a few exceptions (isolated studies of aPS/PT antibodies, and aDI antibodies). A key aspect of diagnostic tests is their stability over time and under different clinical conditions, however, no prospective studies are available in the non-criteria aPL field. In addition, internal and external validation is required that has not been previously performed for non-criteria aPL tests with only sporadic exceptions [56].

In a systematic literature review of the relevance of "non-criteria clinical manifestations" of APS it was concluded that although the consequences of SN-APS diagnosis are categorized as very critical for decision-making, the overall quality of evidence was low to support any conclusions [63]. Considering the trade-off between misdiagnosis and the risk of future events, it was strongly recommended to conduct further well-designed studies to determine its real significance.

3.5. SN-OAPS specific data

Although a high number of candidate non-criteria aPL have been

evaluated, there is a paucity of studies examining their associations with OAPS manifestations, independently of thrombotic APS events. In addition, although more promising data exist for aPS/PT and aDI antibodies, the majority of studies have examined their associations with thrombotic APS raising the question about their validity in OAPS. Another important question is if there is similar validity in different presentations/types of OAPS. It has been well recognized that women with a history of exclusively early losses, but no thrombosis carry a different risk of future obstetric and/or thrombotic events compared to those with a history of second or third trimester loss(es), and/or a history of thrombotic events.

A major limitation of the studies of SN-OAPS is their high heterogeneity regarding the inclusion criteria, proportion of "criteria aPL" and "non-criteria" aPL, aPL assays, and the control groups used. In addition, most of studies included mixed populations of vascular and obstetric manifestations, or different OAPS types (miscarriages and fetal losses) with lack of stratified data.

4. Conclusions

APS is the commonest treatable cause of recurrent miscarriage [64] and pharmacological treatment of pregnant patients with aPL should aim at preventing obstetric complications and maternal thrombotic events. Combination therapy of low-dose aspirin (LDA) and heparin is regarded as conventional treatment for patients with an established diagnosis of obstetric APS, generally resulting in over 70–80% successful pregnancies [65–67].

Since SP-APS and SN-APS patients had shown similar clinical profiles [68], patients with SN-OAPS, as well as obstetric SP-APS, should receive combined treatment in order to improve the pregnancy prognosis; indeed, current standard of care increased good pregnancy outcome in SN-APS, with similar effect to confirmed APS.

The above data suggest that there are patients with the clinical manifestations of OAPS but persistently negative to conventional aPL that need to be identified to ensure adequate therapy and therefore a better prognosis. The clinical utility of non-criteria aPL in the diagnosis of SN-APS is still a matter of debate. In the last decade more and more studies have reported the presence of patients suffering from SN-APS in which non-conventional ("non-criteria") aPL might be present or antibodies may be detected using methodological approaches different from the traditional assays. To improve test standardization large prospective, multicenter, and multinational studies are needed. Therefore, when assessing a patient with clinical manifestations consistent with OAPS but aPL negative using the conventional available assays, the clinician should consider the possibility that the patient is affected with SN-APS.

5. Take home messages

- Since APS is the commonest treatable cause of recurrent miscarriage in patients with recurrent foetal loss it is essential to look for aPL.
- Several studies have shown that with a variable prevalence "non-criteria" aPL can be found in patients with recurrent miscarriage supporting a diagnosis of SN-OAPS.
- Large prospective, multicentre, and multinational studies are needed to improve standardization test and to validate the use of non-criteria aPL in clinical practice of O-APS.

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