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Review Article

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Non-Criteria Antiphospholipid in Seronegative Antiphospholipid Syndrome: A Systematic Review

Kianoosh Shahideh, Zahra Rezaieyazdi and Mandana Khodashahi*

Rheumatic Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

Background: Given the catastrophic results of antiphospholipid syndrome (APS) left untreated, the possible role of non-criteria antiphospholipid (aPL) in the diagnosis of seronegative (SN)-APS patients, and its impact on the timely treatment of people with SN-APS, the present review aimed to gather the available literature up to 2022 focusing on the role of non-criteria aPL in the diagnosis of SN cases suspected of APS using non-criteria aPL.

Methods: All published papers focusing mainly on the diagnosis of the SN-APS patients by non-criteria aPL were searched in four databases of PubMed, Scopus, Medline, and Web of Sciences, followed by manual search up to 20 February 2022 using such keywords as "Antiphospholipid Syndrome", "Seronegative", and "Non-Criteria". Finally, 15 studies were selected after the detailed evaluation of the full-text versions.

Results: Based on the obtained results of our study, 24% to 81.9% of the SN-APS cases were positive for at least one isotype of non-criteria aPL. Thrombophilia events were more frequent in the APS population, compared to SN-APS; however, obstetric manifestations were more frequent in the SN-APS population, compared to seropositive-APS. Antivimentin/cardiolipin and antiphosphatidylserine/prothrombin IgG/IgM were introduced as the best non-criteria aPL to detect seronegative cases that were correlated with clinical manifestations.

Conclusion: It seems that a combination of testing for non-criteria aPL should be performed for cases suspected of APS with negative criteria markers.

Keywords: Antiphospholipid syndrome, Antiphospholipid antibodies, Seronegative

*** Corresponding author**

Mandana Khodashahi, MD
Rheumatic Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
Tel: +98 51 3801 2753
Email: khodashahimn@mums.ac.ir

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Introduction

Antiphospholipid Syndrome (APS) is a systemic autoimmune disorder and a type of autoantibody with the simultaneous presence of thrombophilia manifestations, pregnancy complications, and antiphospholipid (aPL) antibodies. [(1)] Thrombosis, abortion, preterm delivery, cardiac valvular disease, renal thrombotic microangiopathy, thrombocytopenia, haemolytic anaemia, and cognitive impairment are the known manifestations of the APS (2).

The persistent presence of anti-IgG/IgM anticardiolipin antibodies (aCL), anti-β2-glycoprotein I antibodies (aβ2-GPI), and lupus anticoagulant (LA) is introduced by 2006 Sidney classification criteria as the main assays to detect aPL antibodies (3). However, some patients with persistent negative conventional aPL have clinical situations highly suggestive of APS. The term "seronegative APS" (SN-APS) has been dedicated to describing the patients who are persistently negative for the routine aPL tests, and the non-criteria aPL concept refers to this issue (4). In this regard, testing for new aPL specificities is suggested to more clearly identify the syndrome in patients with strongly suspected APS.

The presence of SN-APS could be dependent on the imperfection of the conventional tests due to the insufficient and limited traditional approach. In this regard, the role of non-criteria aPL in diagnosing SN-APS patients has been supported by some evidence.[6-12] Based on the 13th International Congress on Antiphospholipid Antibodies, aβ2-GPI IgA could be considered a "non-criteria" test for the detection of aPL in the APS patients who are negative for IgG and IgM isotypes (5). Antiphosphatidylserine/prothrombin (aPS/PT) antibodies and their association with clinical manifestation of APS, including thrombosis and pregnancy morbidity, have been observed in some studies (6-8). Antivimentin/cardiolipin (aVim/CL) complex antibodies have been described as a possible detectable target in the sera of patients with SN-APS (9). Moreover, different methodological approaches, including immunostaining on thin-layer chromatography (TLC), could be used to detect non-criteria aPL in SN-APS (10,11).

Since the accurate diagnosis of APS is necessary for optimal treatment, the assessment of new approaches with high sensitivity and specificity that minimize diagnostic errors is highly crucial; however, there is controversy on the acceptance, as well as treatment of non-criteria APS as a spectrum of APS. Given the catastrophic results of APS, if left untreated, the possible role of non-criteria aPL in the diagnosis of SN-APS patients, and its impact on the timely treatment of people with SN-APS, the present review aimed to gather the available literature up to 2022 focusing on the role of non-criteria aPL in the diagnosis of SN cases suspected of APS using non-criteria aPL.

Materials and Methods

This systematic review was arranged based on the recommendations of the Cochrane Handbook, including seven domains consisting of presenting a question, determining the eligibility criteria, searching the data, excluding the unrelated papers, determining the quality assessment, gathering the information, and discussing (12). In this review, all published papers conducted on the diagnosis of SN-APS patients by non-criteria aPL were searched up to 20 February 2022 in PubMed, Scopus, Medline, and Web of Sciences databases.

Inclusion and exclusion criteria

The eligibility criteria were selected based on the participants-intervention-comparison-outcome-study design and included 1) provision of a clear description of methodological approaches to detect SN-APS by non-criteria aPL, 2) examination of human samples, and 3) publication in English. On the other hand, the articles with insufficient data, narratives, reviews, systematic/meta-analyses, editorial letters or consensus statements, case reports or case series, and qualitative studies were excluded from this analysis. On the other hand, all prospective and retrospective articles with a descriptive nature, as well as case-controls on human subjects were entered in this review

Literature search

Data were gathered considering the predetermined goals by two trained researchers. The papers were searched in four electronic databases, including PubMed, Scopus, Medline, and Web of Sciences. It is worth mentioning that the manual research was also conducted up to 20 February 2022. The search

process was carried out using the following keywords "Antiphospholipid Syndrome", "Seronegative", and "Non-Criteria".

Quality assessment

The quality assessment of each article was assessed using Cochrane instructions (13). All the entered papers were investigated in terms of seven domains, including bias due to confounders, the selection of subjects, the measurement of variables, missing data, incomplete outcome data, selective reporting, and other sources (14). We used checklists' report compliance of each study with each item to assess the quality of studies based on the QUADAS instrument. To determine the quality assessment, low, high, and unknown risks of bias were recorded in a Table as "Yes", "No", and "Unclear", respectively (Table 1).

2.4. Study selection, data extraction, and design

In the first step, all the articles focusing on the role of non-criteria aPL in the diagnosis of SN cases suspected of APS using non-criteria aPL were searched with the predetermined keywords up to 20 February 2022. The titles and abstracts of all the studies were separately reviewed by each researcher, and the irrelevant articles were removed based on the eligibility criteria. The researchers were in touch with each other in all stages of the study. The full-text version of all entered papers was extracted and reviewed precisely for final screening. PRISMA flow diagram showed the selection process of the entered articles (Figure 1). In the next stage, two researchers who were continuously in contact with each other in order to exchange information extracted the main information, including sample size, age of the study population, male-to-female ratio, clinical manifestations of APS, the used test for determining the aPL, and correlation between variables. The required results were recorded in a checklist, and the gathered information was finally recorded in a researcher-made form.

Table 1. Quality assessment of entered studies in the review

Author reference	Bias due to confounders	Bias due to the selection of participants	Bias due to the measurement of variables	Bias due to missing data	Incomplete outcome data	Free of selective reporting	Other sources of bias
Ortona *et al* (23)	Yes	Yes	No	Yes	Yes	No	Yes
Conti *et al* (11)	Yes	No	No	No	No	No	No
Conti *et al* (9)	Yes	No	No	No	No	No	No
Baleva *et al* (26)	Yes	Yes	No	Yes	Yes	No	Yes
Mattia *et al* (18)	Yes	No	No	Yes	No	No	Unclear
Mekinian *et al* (24)	Yes	Yes	No	Yes	Yes	No	Yes
Zohoury *et al* (21)	Yes	No	No	No	No	No	No
Shi *et al* (7)	Yes	No	No	Yes	No	No	No
Omar *et al* (17)	Yes	No	No	Yes	No	No	Unclear
Litvinova *et al* (16)	Yes	No	No	No	No	No	Unclear
Truglia *et al* (10)	Yes	No	No	No	No	No	Unclear
Liu *et al* (20)	No	No	No	No	No	No	Yes
Ferreira *et al* (15)	Yes	No	No	Yes	Yes	No	Unclear
Capozzi *et al* (19)	Yes	No	No	No	No	No	Unclear
Truglia *et al* (22)	Yes	No	No	No	No	No	Unclear

Results

In general, 1,079 papers were extracted in the first search of the databases. Among them, 795 papers were excluded due to irrelevancy or lack of focus on SN patients. Of the 281 remaining studies, 119 duplicate papers were removed, and 162 papers remained. In the next step, the papers with inaccessible full-text versions (n=2) and those published in other languages, except for English (n=3) were excluded from the study. Moreover, editorial letters (n=17), books (n=5), case reports (n=34), qualitative and narrative review articles (n=62), systematic reviews (n=2), in vitro studies (n=5), and radiographic studies (n=2) were removed from the present review. Furthermore, the studies focusing only on clinical manifestations in SN-APS patients were also removed from the review (n=13). Moreover, three studies comparing the primary APS with secondary studies were removed due to the lack of focus on SN patients and insufficient data (n=2). Finally, 15 articles were entered into the review process (Figure 1). All papers investigating the non-criteria assays for the diagnosis of APS in SN patients with clinical presentation of APS were entered in this review.

All cross-sectional and comparative studies, in which non-criteria assays of APS were described or compared between seropositive (SP)-APS and SN-APS cases were also included. The majority of the articles were retrospective, observational, and comparative case-controls (n=13; 86%), and only 2 (13.3%) studies were retrospective observational case-controls. The data extracted from each article (sample size, age of the study population, male-to-female ratio, thrombophilia, obstetric manifestations, the used test for determining the APS antibodies, correlation between clinical manifestations and non-criteria APS, and correlation among various non-criteria APS antibodies) were included in this review (Table 2). The majority of the entered articles were performed in Europe, including Italy (n=6; 40%), France (n=3; 20%), the UK (n=2; 13.3%), and Bulgaria (n=1; 6.7%). Moreover, 2 (13.3%) articles

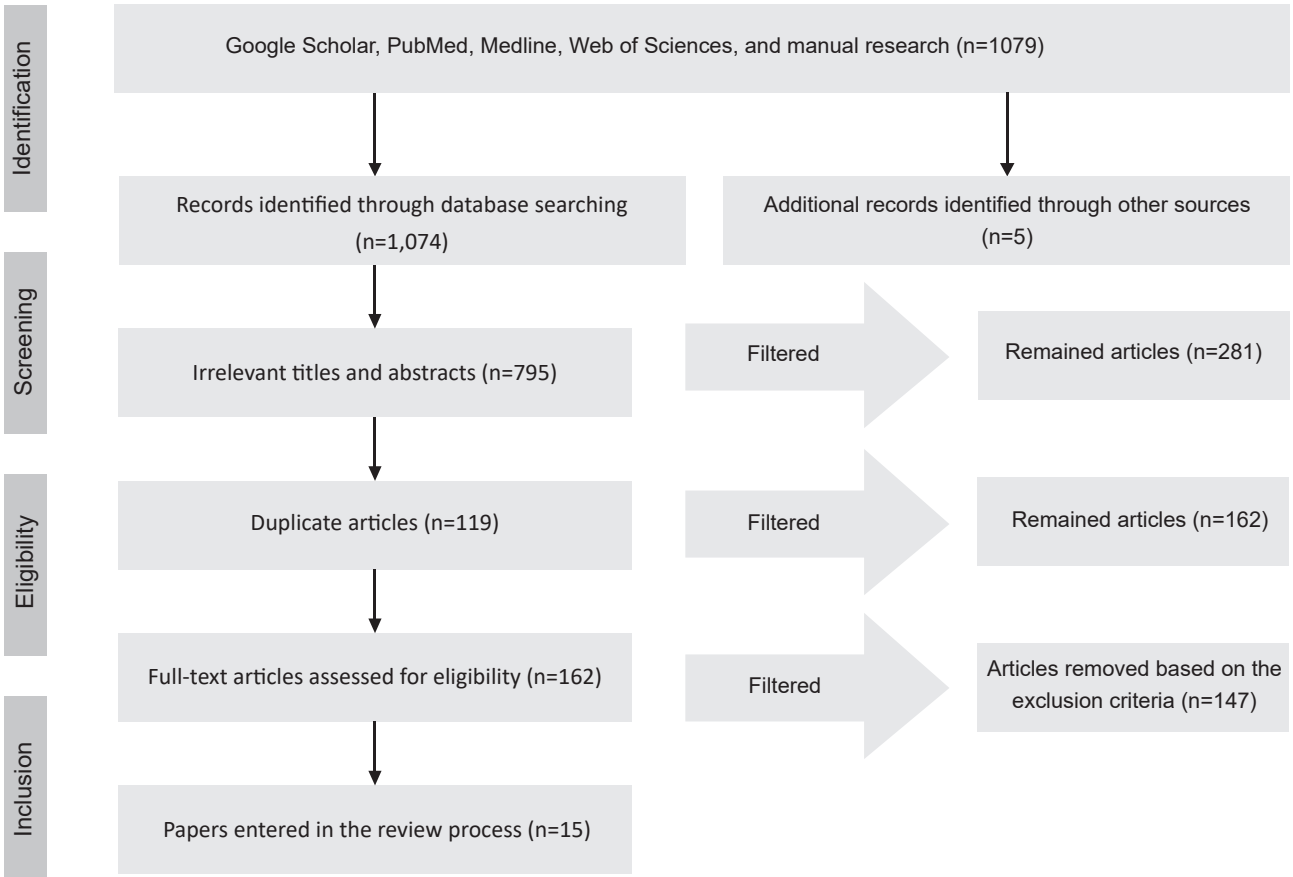

Figure 1. PRISMA flowchart representing the study selection process.

were performed in China and 1 (6.7%) in Egypt. The entered articles were conducted on a total of 919 cases with APS with conventional criteria and 792 SN cases with clinical presentation of APS. The mean ages of the patients with SP-APS and SN-APS in various studies were from 33.1 to 47, and from 29 to 47.4 years, respectively. In general, the male ratio was obtained at 16.4/1 in the SP-APS patients, and it was determined at 12/1 in SN-APS cases. Out of 15 entered studies, 5 (33.3%) articles used ELISA and TLC to measure non-criteria assays of APS, and 10 (66.6%) studies used only ELISA to measure non-criteria assays.

Thrombophilia events and obstetric manifestations

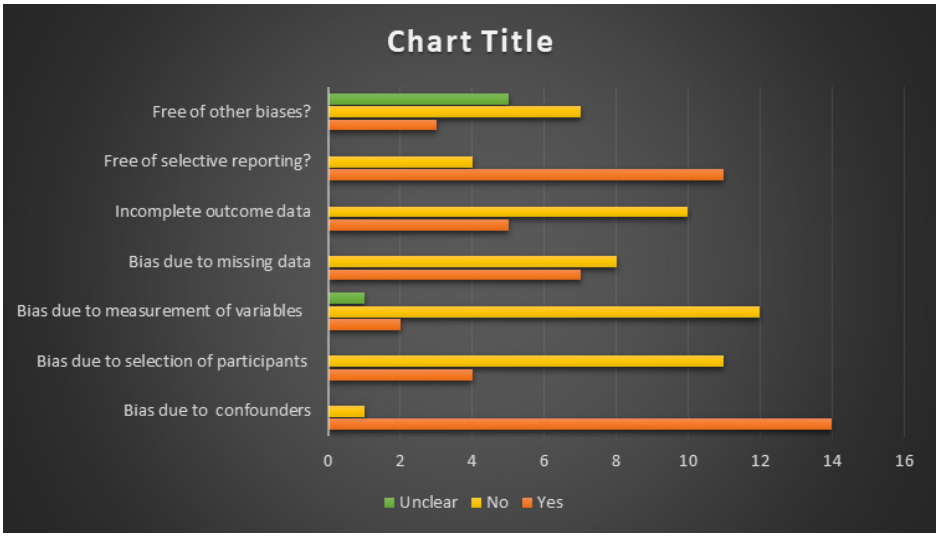

Figure 2. Quality assessment of included articles in review process.

Table 2. Extracted data from each included study

Author (years) reference	Country	Type of study	Sample Size	Age mean (year)	Female	Thrombophilia	Obstetric	Tests	Correlation between clinic manifestations	Correlation between clinic manifestations and non-criteria APS	Correlation between non-criteria APS antibodies
Ortona *et al* (2010) (23)	Italy	Case control study	APS[1]:40 SN[2]:30 SLE[3]:30 Control:32	SN:47.4 Groups were matched	Only female	No Comparison	No	ELISA and TLC	--	No correlation between IgG or IgM Vim/ CL[4] antibodies with clinical manifestations.	--
Conti *et al* (2012) (11)	UK	Case control study	APS:19 SN:36 SLE:18	APS:43·4 SN:46·4 SLE:38·8	APS:17 SN:Only female SLE:Only female	More frequent in the APS population compared to SN-APS	More frequent in the SN-APS population compared to SP-APS	ELISA and TLC	Vascular thrombosis was correlated with pregnancy morbidity in SN-APS	--	aCL, aLBPA[5] and aPE[6] were correlated positivity.
Conti *et al* (2014) (9)	UK	Case control study	APS:25 SN:24 SLE:18 Control:13	APS:45.7 SN:43.9 SLE:36.8 Control: Merged	APS:18 SN:22 SLE:Only female Control: Merged	More frequent in the APS population compared to SN-APS	Nearly similar in SP-APS and SN-APS	ELISA and TLC	Vascular thrombosis was correlated with pregnancy morbidity in SN-APS.	The prevalence of the clinical features was not correlated with specific autoantibodies in SN-APS patients.	--
Baleva *et al* (2014) (26)	Bulgaria	Case control study	APS:22 SN:54	29	Only female	No compare	No compare	ELISA	--	--	--
Mattia *et al* (2014) (18)	Italy	Case control study	APS:84 SN:66 Control:78	APS:44 SN:40	APS:75 SN:57	More frequent in the APS compared to SN-APS	More frequent in the SN-APS population compared to SP-APS	ELISA	--	IgA anti-β2GPI[7] antibodies was associated with thrombosis and pregnancy morbidity.	Relationship between conventional aPL risk profile and IgA aCL and IgA anti-β2GPI antibody.
Mekinian *et al* (2014) (24)	France	Case control study	APS:38 SN:73 Control:45	--	Only female	No compare	No compare	ELISA	--	--	IgG and/or IgM aPS/PT[8] were associated to LAC in SP-APS patients.
Zohoury *et al* (2017) (21)	Italy	Case control study	APS:107 SN:68	APS:46.2 SN:45.8	APS:99 SN:67	More frequent in the SN-APS population compared to SP-APS	More frequent in the SN-APS population compared to SP-APS	ELISA	--	--	

Cont table 2

Shi *et al* (2018) (7)	China	Case control study	APS:186 SN:48	APS:34.2 SN:38.9	APS:164 SN:41	More frequent in the SN-APS population compared to SP-APS	More frequent in the APS population compared to SN-APS	ELISA	--	aPS/PT IgG was associated with total thrombosis events, aPS/PT IgG and/or IgM were associated with arterial thrombosis events and fetal loss.	IgG and/or IgM aPS/PT were associated with LAC.
Omar *et al* (2018) (17)	Egypt	Case control study	APS:30 SN:30	APS:33.1 SN:32.7	APS:29 SN:28	More frequent in the APS compared to SN-APS	Nearly similar in SP-APS and SN-APS	ELISA	Thrombotic events were not different between two groups.	Anti-AnxA5[9] IgG and IgM were correlated with clinical manifestations in the SP-APS and SN-APS groups.	--
Litvinova *et al* (2018) (16)	France	Case control study	APS:41 SN:17	15 to 92	50	More frequent in the APS compared to SN-APS	More frequent in the SN-APS population compared to SP-APS	ELISA	--	--	The presence of anti-PA antibodies was correlated with anti-PS antibodies.
Truglia *et al* (2018) (10)	Italy	Cross sectional	SN:61	Median age of 39	Only female	SN:14.6%	85.4%	ELISA and TLC	--	--	A correlation was found between anti-PS/PT and anti- Vim/CL.
Liu *et al* (2020) (20)	China	Case control study	APS:192 SN:90 SLE:103	APS:35 SN:39 SLE:34	APS:162 SN:77 SLE:92	More frequent in the SN-APS population compared to SP-APS	More frequent in the APS compared to SN-APS	ELISA		aβ2GPI IgG was the best predictor of arterial and thrombosis stroke and aPS/PT IgG was the best predictor of venous thrombosis. aPS/PT IgM was associated with pregnancy morbidity.	Anti-PS/PT antibodies of IgM isotype was correlated with the presence of LA.
Ferreira *et al* (2020) (15)	France	case control study	APS: 15 SN: 21 Control: 20	APS: 39 SN: 34.5 Control: 42	APS: 13 SN: 17 Control:11	More frequent in the APS population compared to SN-APS	More frequent in the SN-APS population compared to SP-APS	ELISA	--	--	--
Capozzi *et al* (2021) (19)	Italy	Prospective case control study	APS: 30 SN: 60 Control: 40	APS: 44.7 SN: 40.8 Control: 39.2	APS: 26 SN: 52 Control: 34	More frequent in the APS population compared to SN-APS	More frequent in the SN-APS population compared to SP-APS	ELISA		The prevalence of arterial thrombosis was correlated with IgA aVim/CL.	
Truglia *et al* (2022) (22)	Italy	Retrospective case control study	APS:49 SN:114 Control:90	APS:47 SN:42.4 Control: 39.5	APS:34 SN:101 Control:75	Nearly similar in SP-APS and SN-APS	Nearly similar in SP-APS and SN-APS	ELISA and TLC		Thrombotic events and obstetrical features were higher among SN-APS patients with double positivity of aVim/ CL antibodies.	aCL by TLC-immunostaining was correlated with aVim/CL.

1- Seropositive Anti-Phospholipid Syndrome, 2- Seronegative Anti-Phospholipid Syndrome, 3- Systemic Lupus Erythematosus, 4-Vimentin/Cardiolipin Complex, 5-Antibodies Lyso (bis) Phosphatidic Acid, 6-Antibodies Phosphatidylethanolamine (aPE), 7-Antibodies -β2 glycoprotein, 8-Antiphosphatidylserine/prothrombin antibodies, 9-Anti-annexin A5 Antibodies

were compared between SP-APS and SN-APS in 11 studies. Among them, thrombophilia events were more frequent in the APS population, compared to SN-APS in 7 (63.6%) studies (9, 11, 15-19); however, in 27.2% (n=3) of the articles, it was more frequent in the SN-APS population (7, 20, 21). On the other hand, obstetric manifestations were more frequent in the SN-APS population, compared to SP-APS, as reported in 6 (41.3%) studies (11,15,16,18,19,21,22). In 3 studies, the frequency of obstetric manifestations was nearly similar in SP-APS and SN-APS (18.2%) (9,17,22). The remained articles (n=2) showed that obstetric manifestations were more frequent in the SP-APS population than SN-APS cases (7,20).

In total, 3 studies assessed the correlation between aVim/CL antibodies and clinical manifestations. Among them, in one study, the thrombotic events were correlated with IgA aVim/CL (19), and one of them showed no correlation between IgG/IgM aVim/CL antibodies and clinical manifestations in the SN-APS patients (23). The association of aPS/PT IgG/IgM with thrombotic events and pregnancy manifestations was reported in two studies (7,20). Moreover, one study introduced aβ-۲GPI IgG as the best predictor of arterial thrombosis and stroke (20). The other study confirmed the association of IgA anti-β2GPI antibodies with thrombosis and pregnancy morbidity (18).

One study demonstrated the correlation between anti-AnxA5 IgG and IgM with clinical manifestations

Table 3. Prevalence of each non-criteria APS antibody in SP-APS and SN-APS patients

Author (years) Reference	Anticardiolipin (aCL)			Anti-Beta2 Glycoprotein I (aβ2GPI)			Anti-lysobisphosphatidic acid (LBPA)	Vimentin/ Cardiolipin Complex			Antiphosphatidylserine/ prothrombin antibodies (aPS/PT)		Antibodies Phosphati-dylethanolamine (aPE)		At least positivea PL[1]
	IgA	IgG	IgM	IgA	IgG	IgM		IgA	IgG	IgM	IgG	IgM	IgG	IgM	
Ortona *et al* (2010) (23)	--	--	--	--	--	--	--	--	APS[2]:92% SN[3]: 55.2% SLE[4]: 43.3% Control: None	APS:80 SN:37.9% SLE: 43.3% Control: None	--	--	--	--	--
Conti *et al* (2012) (11)		By ELISA APS:100% SN: None SLE: 77·7% Control: None By TLC APS: 68·4% SN: 47·2% SLE: 61·1%		--	By ELISA APS: 73·6% SN: None SLE: 38.8% Control: None By TLC APS: 63·1% SN:41·7% SLE: 61·1%		By ELISA APS: SN: None	--	By ELISA APS: 88% SN: 45.8% SLE: 38.8% Control: None	--	By ELISA SN: None	--	By TLC APS: 42·1% SN: 30·5% SLE: 33·3%		58·3%
Conti *et al* (2014) (9)		APS: 100% SN:0 SLE: 77.7% Control: None			APS: 72% SN: 0%		--		APS: 72% SN:0 SLE: 38.8% Control: None		APS: 36% SN: 12.5% SLE: 5.5% Control: None		APS: (36% SN: 12.5% SLE:5.5% Control: None		79.2%
Baleva *et al* (2014) (26)		By TCL APS: 68% SN: 54.2% SLE: 61.1% Control: None		--	--	--	By TCL APS: 56% SWN: 37.5% SLE: 61.1% Control: None		By TCL APS: 88% SN: 45.8% SLE: 38.8% Control: None		BY ELISA APS:18% SN::3.7%	BY ELISA APS:13.6% SN:5.5%	APS:4.5% SN:9.2%	APS: None SN:3.7%	54%
Mattia *et al* (2014) (18)	APS:19% SN:4.5% Control:2.6%	APS:57.1% SN: None Control:1.3%	APS:21.4% SN: None Control:2.6%	APS:50% SN:10.6% Control:1.3%	APS:73.6% SN: None Control:1.3%	APS:47.6% SN: None Control:1.3%	--	--	--	--	--	--	--	--	--
Mekinian *et al* (2014) (24)		APS:72% SN:32% Controls: 13%		APS: 77% SN:0% Control:0%	--	--	--	--	--	--	APS:48% SN:4.4% Control:2.2%	--	SN:13% Control:2%		33%
Zohoury *et al* (2017) (21)	APS: 9.3 SN: None P<0.05	APS:38% SN: 1.5%	APS:18.7% SN: 1.5%	APS: 22.4% SN: 1.5%	APS: 26.2% SN: None	APS: 23.4% SN: 1.5%	--	--	APS: 44.8% SN:16.1%	--	APS: 23.4% SN: 11.8%	APS: 25.2% SN: 1.5%	APS: 41.1% SN: 5.9%	APS: 35.5% SN: 5.9%	30.9%
Shi *et al* (2018) (7)	--	--	--	--	--	--	--	--	--	--	APS:70% SN:28.6%	APS:67% SN:34.7%	--	--	50%
Omar *et al* (2018) (17)	--	APS: 76.7% SN:	APS: 21; 7% SN:	--	APS: 76.7% SN:	APS: 73.3% SN:	--	--	--	--	--	--	--	--	--
Litvinova *et al* (2018) (16)		APS: 95% SN: None Control:None		APS:51% SN: None Control:None	--	--	--	--	--	--	APS: 43.9% SN: 5.6% Control:None	APS: 65.8% SN: 16.7% Control: 6.7%	--	--	--
Truglia *et al* (2018) (10)	By ELISA:4% TLC:None	--	--	By ELISA: 2% TLC: None	--	--	--		By TLC: 54%		By TLC: 12%		--	--	81.9%
Liu *et al* (2020) (20)	APS:42% SN:12% Control:None	--	APS: 60% SN: None Control: None	--	--	APS: 68% SN: None Control: None	APS: 39% SN: 10% Control: None	--	--	--	APS: 71% SN: 36% Control: None	APS: 73% SN: 36% Control: None	--	--	60.9%
Ferreira *et al* (2020) (15)	APS: SN: Control: None	--	--	--	--	--	--	--	--	--	APS: 60% SN: 5% Control: None	APS: 73% SN: 5% Control: None	APS: 40% SN:14% Control: None	APS: 60% SN: None Control: None	24%
Capozzi *et al* (2021) (19)	APS:36.6% SN:5% Control:None	--	--	APS:26.6% SN: 3.3% Control: None	--	--	--	APS: 40% SN: 26.7% Control: None	APS: 80% SN: 36.7% Control: None	--	--	--	--	--	28.3%
Truglia *et al* (2022) (22)	By TLC APS:69.3% SN:58.8% Control:None	--	--	--	--	--	--		By TLC APS:93.9% SN:31.5% Control: None		By TLC APS:40.8% SN: 10.5% Control: None		--	--	60.5%

1- Antiphospholipid antibodies, 2-Seropositive Anti-Phospholipid Syndrome, 2- Seronegative Anti-Phospholipid Syndrome, 3- Systemic Lupus Erythematosus

in the SP-APS and SN-APS patients (17). On the other hand, one study revealed that the prevalence of the clinical features was not correlated with any specific autoantibodies in SN-APS patients (9). The assessment of correlation between non-criteria autoantibodies showed that aCL, aLBPA, and antiphosphatidylethanolamine (aPE) were correlated positively in one study (11). The correlation between aPS/PT IgG/IgM antibodies and LAC in SP-APS patients was confirmed in 3 studies (7,20,24). One study confirmed the correlation between aVim/CL and aCL (22), and another study found a correlation between aPS/PT and aVim/CL (10). Table 3 presents the prevalence of each non-criteria APS antibody in SP-APS and SN-APS patients. Quality assessment of the entered articles is depicted in figure 2.

Discussion

This study aimed to determine the diagnostic value of non-criteria aPL in the detection of APS in SN cases.

Non-criteria aPL in SN-APS Cases and its relationship with clinical manifestation

Recently, positivity heterogeneous antibodies against various phospholipids, such as aPS/PT antibodies has been suggested as a part of the Global APS Score (GAPSS). However, the antibodies are not included yet in the APS laboratory criteria (25). Search results in the literature have shown that 24%-81.9% of the SN-APS cases were positive for at least one isotype of non-criteria aPL (9,15,19,21,22,26). Moreover, Liu *et al* suggested that 93.5% of the SP-APS patients have at least one positive non-criteria aPL (20). The majority of studies assessing the clinical manifestations among SP-APS and SN-APS indicated that thrombophilia events were more frequent in the APS population, compared to SN-APS (9,11,15-19,22); however, obstetric manifestations were more frequent in the SN-APS population, compared to SP-APS (11,15,16,18,19,21,22). Some studies showed that thrombotic events were correlated with aVim/CL IgA, aPS/PT IgG/IgM, and β۲GPI IgG antibodies (7,18-20,22). Moreover, some studies confirmed the association of anti-Annexin V IgG/IgM, aPS/PT IgG/IgM, and aβ2-GPI IgA antibodies with pregnancy morbidity (17,18). The information on five concepts was gathered as follows

Role of lupus anticoagulant, aCL, and aβ2-GPI antibodies in the detection of APS

Based on a study performed by Shi *et al*, the performance of aβ2-GPI IgA was better than that of aCL IgM and aβ2-GPI IgM in the detection of SP-APS, SN-APS, and systemic lupus erythematosus (SLE) patients (7). Furthermore, the sensitivity of aβ2-GPI IgA was higher than that of aCL IgM and aβ2-GPI IgM to detect the SP-APS (7). Consistent with other evidence, it seems that IgA aβ2-GPI is more useful than aCL IgM and aβ2-GPI IgM to detect APS (27-29). Since there is some evidence on the potential pathogenic role of aβ2-GPI IgA, it seems that aβ2-GPI IgA pushed to the margins unfairly (30-32).

However, a low prevalence (lower than 3%) of both aCL and aβ2-GPI IgA in a large sample size of patients with APS is reported by a study carried out by Hu *et al* that showed the rejection of the diagnostic role of aPL IgA in the Chinese population (33). The low prevalence of IgA isotype of aCL and aβ2-GPI in the SN-APS patients has been also confirmed by other studies (19, 20). Therefore, it seems that the isotypes appear along with other antibodies. Based on a study conducted by Liu *et al*, the best sensitivity and specificity of single biomarkers to detect APS belonged to aβ2-GPI IgG (0.67) and LAC (0.98), respectively. Although aβ2-GPI domain 1 IgG has better specificity (0.97), it has much lower sensitivity (0.489). It appears that a combination of aPS/PT IgG, aCL IgG, and LAC with high sensitivity (82.35%) and specificity (89.97%) are valuable tools to detect APS (20).

Role of antiphosphatidylserine/prothrombin antibodies in the detection of APS

Based on the literature, aPS/PT antibodies could be used as a strong prognostic factor for both arterial and venous thrombosis. According to Liu *et al*, the most frequent non-criteria aPLs detected in both SP-APS and SN-APS cases were aPS/PT IgG and aPS/PT IgM. Moreover, aPS/PT IgG is a more balanced biomarker with moderate sensitivity (0.63) and high specificity in the detection of APS (0.94) (20). Based on a study

performed by Zohoury *et al*, the most sensitive non-criteria biomarker was the aPS/PT (IgG/IgM) in cases with SP-APS, which was detected positive in 56.1% of the cases (21).

Litvinova *et al* indicated a high prevalence of aPS/PT antibodies in SP-APS patients; moreover, aPS/PT IgG and IgM were positive in all catastrophic APS patients. However, the positivity of these antibodies was not associated with the type of thrombosis in the patients. The highest sensitivity of non-conventional markers was aPS/PT antibodies of IgM isotype, which was comparable to the lupus anticoagulant sensitivity (81.8%), whereas positivity for IgG isotype was reported only in 18.2%. Positivity for aPS/PT antibodies of IgM isotype was strongly correlated with LA presence. Moreover, both IgG and IgM isotypes were detected in SN-APS patients (16). It was confirmed by previous studies that the aPS/PT of IgM isotype may be less pathogenic, compared to aPS/PT of IgG isotype (34,35). There is some evidence demonstrating a strong correlation among aPS/PT antibodies related to ischemic/thrombotic cerebrovascular events (5,25,36). A strong association of aPS/PT was also found with thrombosis and obstetric complications in a study performed by Shi *et al*.

aPS/PT IgG/IgM antibodies were positive in the majority of SP-APS and SN-APS patients with a history of stroke. However, LAC was positive in less than half of these patients.

The prevalence of aPS/PT IgG was higher in the SN-APS cases, compared to those with SLE and other autoimmune diseases, as well as healthy control. Moreover, the prevalence of aPS/PT IgM was more in the SN-APS cases, compared to healthy controls. Moreover, high levels of aPS/PT IgG/IgM antibodies were diagnosed in some SLE patients. Since SLE patients may develop APS, the presence of the antibody levels may indicate unrecognized APS or its development in the future. The highest sensitivity (72%) and specificity (95.8%) belonged to aPS/PT IgG among non-criteria assays. Moreover, the sensitivity and specificity of a combination of a-PS/PT IgG and IgM were 86% and 81.2%, respectively. Due to the specificity of 100% in LAC, it seems that a combination of LAC and aPS/PT IgG/IgM assays leads to high sensitivity and specificity. The aforementioned study confirmed the diagnostic value of aPS/PT IgG for the evaluation of patients suspected of APS (7).

It appears that aPS/PT antibodies (IgM and IgG) could be considered an important alternative to LAC in the detection of APS since it is not affected by any anticoagulant treatments (37). Litvinova *et al* reported a strong correlation between LA and aPS/PT IgM antibodies in asymptomatic APS carriers (16). The data is very important, especially when LA could not be measured. Since patients with a thrombotic event started an anticoagulant therapy, the accurate detection of the LAC test may interfere with anticoagulation treatment that often results in false positives (38,39). Some reports showed that up to 86.7% of the LAC-positive specimens are aPS/PT-positive (39). LAC has been introduced as a very useful assay to detect SN cases; however, in the absence of the LAC test, aPS/PT could be used since it is not affected by any anticoagulation treatments (5,35).

Role of anti-phosphatidylethanolamine antibody in the detection of APS

Although there are limited studies assessing the aPE isotype role in the detection of APS in SN-APS cases, some evidence confirmed the effectiveness of the non-criteria aPL for the diagnosis of SN cases. According to Mekinian *et al*, among non-criteria aPL, only the aPE IgG was higher in SN-APS patients, compared to the control group. Conti *et al* reported a correlation among aCL, aLBPA, and aPE positivity using TLC immunostaining and ELISA. Other phospholipids tests showed no reactivity in this regard (11). In a study conducted by Zohoury *et al*, a similar sensitivity was reported for three biomarkers of aVim/CL IgG, aPS IgG/IgM, and aPE in SP-APS cases. In the SN-APS group, 11.8% of the patients were positive for aPE IgG/IgM (21).

In a study by Ferreira *et al*, non-criteria aPL was reported in 24% of the patients with SN-APS, while it was not detected in any of the healthy controls. The frequency of non-criteria aPL was higher in APS cases, compared to SN-APS and healthy controls for all aPL, except for aPE IgG, whereas none of the patients were positive for aPE IgM (15). Moreover, based on a study by Baleva *et al*, aPE IgM was higher in the recurrent pregnancy losses (RPL) alone, compared to the control group. One or more positive antibodies of

Anti-prothrombin (aPT) were reported among RPL along with APS and RPL alone groups (26).

Role of antivimentin/cardiolipin antibodies in the detection of APS

Up to certain levels, various antigenic targets could be used to explain the seronegative existence. Based on a study by Conti *et al*, about two-thirds of SN-APS patients with thrombosis or pregnancy morbidity could be detected by a combination of TLC immunostaining for aCL and ELISA for aVim/CL complex antibodies (9). Truglia *et al* suggested aVim/CL and aPS/PT antibodies as the most sensitive non-criteria biomarkers to detect SN-APS cases (22). These findings were supported by other studies (10,19,21,23), and Truglia *et al* showed an association between aVim/CL and aCL positive in TLC immunostaining leading to identifying a subset of patients with a higher prevalence of mixed thrombotic and obstetrical features.

Moreover, a positive likelihood ratio of 8.136 to thrombotic events was reported in cases with double positivity by aCL in TLC immunostaining and aVim/CL (22). This is a valuable finding since a higher rate of positivity of aCL can be detected by TLC immunostaining, and these results can be completed by aVim/CL, which is easier to perform. It seems that more than half of SN-APS cases could be diagnosed with a combination of TLC immunostaining for aCL and ELISA for aVim/CL complex. However, TLC immunostaining for aCL is not available in all laboratories and unlike aVim/CL, its standardization is difficult due to non-reproducibility. Therefore, a combination of the tests should be performed to obtain a more accurate result.

Based on the obtained results of a study by Capozzi *et al*, the prevalence of aVim/CL IgA was higher than that of aCL and aβ2-GPI IgA among SN-APS patients. A higher prevalence of arterial thrombosis was observed among patients with SN-APS who were positive for aVim/CL IgA; accordingly, the chance of thrombosis was 5.7 times higher among SN-APS with positive aVim/CL IgA, compared to those with negative aVim/CL IgA.

Moreover, the chance of livedo reticularis (likelihood positive ratio: 4.07) and thrombocytopenia (likelihood positive ratio: 5.86) was higher among SN-APS patients with positive aVim/CL IgG, compared to those with negative aVim/CL IgG. Moreover, a higher rate of pregnancy morbidity and thrombocytopenia was observed among APS patients with positive tests for aVim/CL IgG, compared to those with negative aVim/CL IgG. They introduced aVim/CL IgG as the most prevalent non-criteria aPL in SN-APS patients (19). The findings showed that in addition to a high prevalence of aVim/CL IgA and IgG in SN-APS patients, compared to other non-criteria aPL, APS-related clinical characteristics were higher among cases with positive aVim/CL antibodies.

Based on a study by Ortona *et al*, both IgG and IgM aVim/CL antibodies were greater in patients with APS, SN-APS, and SLE, compared to healthy controls. They showed no correlation between IgG/IgM aVim/CL antibodies and clinical manifestations or thrombotic risk factors in SN-APS patients. The presence of aVim/CL complex antibodies has been confirmed in almost all the SP-APS patients and a large number of SN-APS cases. Therefore, Vim was identified as a strongly immunoreactive autoantigen (23). Due to the correlation between aVim and aCL antibodies, it seems that Vim could be proposed as a "new" antigenic cofactor for aPL in APS. However, the question remains unanswered: how can aVim/CL complex become antigenic?

Role of anti-annexin V antibodies in the detection of APS

There are limited studies assessing the anti-Annexin V role in the detection of APS in SN-APS cases. According to Ferreira *et al*, no difference was observed between the APS patients and healthy controls regarding the anti-Annexin V values in seronegative (15). However, the obtained results of one study by Omar *et al* indicated no difference in terms of IgG and IgM isotypes of anti-Annexin V levels between SP-APS and SN-APS. The sensitivity and specificity of anti-Annexin V IgG in the prediction of pregnancy morbidity were 71.2% and 75.9%, respectively. Moreover, anti-Annexin V IgG sensitivity in the prediction of venous and arterial morbidity was higher than 70%; moreover, its specificity was higher than 77%. The sensitivity and specificity of anti-Annexin V IgM in the prediction of pregnancy morbidity, arterial morbidity, and venous

morbidity were lower than those in the anti-Annexin V IgG (17). Similarly, other studies indicate that only the IgG isotype of Anti-Annexin V was related to fetal losses (40,41).

In addition, in a study by Litvinova *et al,* Annexin V IgG and IgM isotypes were found in patients with SN-APS (16). Baleva *et al* showed that Annexin V IgG was higher in patients with APS, compared to females with RPL. However, Annexin V IgG was higher among RPL alone, compared to the controls (26). These findings confirm the role of Annexin V IgG in the detection of seronegative APS; however, future studies should be performed to obtain more reliable information.

The need to create a new diagnostic criterion for APS

Future updates to the APS classification criteria are very necessary. In this regard, two systems based on testing a profile of antibodies (GAPSS) and providing an algorithm for scoring (antiphospholipid score) have been suggested (42,43). However, both systems are required to specifically measure the aPS/PT antibodies in addition to other biomarkers, including LAC, aCL, and aβ2-GPI antibodies. The use of the new diagnostic algorithms and biomarkers helps us to timely diagnose the APS cases with negative serology for APS, which is beneficial in the prediction of serious clinical consequences owing to delayed treatment and reducing healthcare expenditure. These antibodies may be proper diagnostic tools in SN cases with clinical features suggestive of APS who are persistently negative for the routine aPL tests. Future studies in larger and well-characterized populations should be performed on the non-criteria antibodies in order to be included in the approved classification criteria for APS.

Study limitations and risk of bias in outcomes

In this systematic review, all papers were investigated in terms of quality assessment in seven domains based on the Cochrane guidelines. Except for bias due to confounders, a low-to-moderate risk of bias was observed in the other domains in the majority of the entered studies. The results of assessing the quality of the entered studies were shown in table 2 and figure 2. Firstly, interfering factors, such as different demographic characteristics of participants (age, gender, and race) and other unknown intervention variables in the various studies may affect our results. The majority of studies were single-center retrospective and only the relationship, not causality, has been investigated, which can affect the outcomes of the present study. Therefore, the data cannot be generalized to similar populations. Finally, there were no homogeneous studies to convert this study to a meta-analysis. Due to insufficient data to provide a definite result, it is suggested to perform future studies assessing the role of non-criteria aPL in diagnosing SN-APS patients considering possible confounding variables.

Conclusion

Based on the obtained results of our study, 24 to 81.9% of the SN-APS cases were positive for at least one isotype of non-criteria aPL. Thrombophilia events were more frequent in the APS population, compared to SN-APS, whereas obstetric manifestations were more frequent in the SN-APS population, compared to SP-APS. aVim/CL and aPS/PT IgG/IgM were introduced as the best non-criteria aPL to detect SN cases that were correlated with clinical manifestations. The findings emphasize using new antigenic targets, including aVim/CL and methodological approaches, especially TLC immunostaining to detect patients with SN-APS. Although the data supporting aPE and anti-Annexin V biomarkers for the detection of SN-APS is weaker than those on aPS/PT, the potential value of these biomarkers should not be neglected. Moreover, despite the availability of aPS/PT, the other assays are less standardized. Therefore, future studies should be performed to assess the validity using controlled studies. It seems a combination of testing for non-criteria aPL should be performed for cases suspected of APS with negative criteria markers.

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