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# **Prevalence and significance of anti-phosphatidylserine antibodies: a pooled analysis in 5992 patients**

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**Short Title:** Prevalence of anti-phosphatidylserine antibodies

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## **Highlights**

1. The prevalence of aPS antibodies and their role when assessing clinical risk of APS is unknown.
2. Data from 5992 patients tested for aPS antibodies included in 20 retrieved studies were analysed.
3. aPS antibodies are frequently detected in patients with known APS, especially primary APS.
4. The added diagnostic value and clinical role of aPS antibodies remains uncertain.

## Introduction

The antiphospholipid syndrome (APS) is an autoimmune disorder characterized by vascular thrombosis (both venous and arterial) and/or pregnancy morbidity (including miscarriages, foetal deaths, premature births, and preeclampsia) associated with a persistent positivity for antiphospholipid antibodies (aPL). The syndrome can arise alone as Primary APS (PAPS) or in association with other autoimmune conditions, more typically with Systemic Lupus Erythematosus, that represents a Secondary form of APS (SAPS). The current classification criteria for APS include three laboratory tests: lupus anticoagulant, anti-cardiolipin and anti- $\beta$ 2glycoprotein-I antibodies[1]. To prevent detection of transient antibodies, tests must be positive on  $\geq 2$  occasions, at least 12 weeks apart[1]. The clinical value, the sensitivity, and the specificity of the 'criteria' aPL in association with recurrent fetal loss and thrombosis has been extensively demonstrated [2].

Nevertheless, some patients present clinical manifestations highly suggestive for APS but result to be persistently negative for classical aPL tests. In order to fill this gap, researchers are examining the usefulness of testing for new aPL specificities in patients with clinical manifestations of APS, particularly in those who are repeatedly negative for the criteria aPL[3]. In this context, the clinical utility of aPL assays for autoantibodies other than the routinely used is currently under debate[4].

Among the *so-called* 'extra-criteria' aPL tests, anti-phosphatidylserine (aPS) antibodies are one of the most promising and have been proposed as an additional tool to be considered when investigating a patient suspected for having APS, especially when other aPL tests are negative[5]

However, to date, the exact prevalence of aPS antibodies and their role when assessing the risk of developing clinical manifestations of APS is uncertain.

In this study, we aim to estimate the prevalence of aPS antibodies in patients with clinical manifestations of APS, by systematically reviewing the literature.

### **Methods:**

A detailed literature search has been developed *a priori* to identify articles that reported findings from clinical and laboratory studies that tested for aPS antibodies. Inclusion criteria included: a) clinical data referring to aPL-related manifestations; b) laboratory data including aCL, LA and/or anti- $\beta$ 2GPI testing; c) anti-aPS antibodies testing with detailed assay methodology, analyzed isotype, defined cut offs of positivity. Key words and subject terms included: "anti-phosphatidylserine[All Fields] AND ("antibodies"[MeSH Terms] OR "antibodies"[All Fields])". The search strategy was applied to Ovid MEDLINE, In-Process and Other Non-Indexed Citation and Ovid Medline 1989 to present. Abstracts from EULAR and ACR/ARHP Annual Meetings (2011-2015) were screened and included in the analysis when meeting the inclusion criteria and not replicating studies published elsewhere.

Studies that met the criteria to evaluate the prevalence of aPS antibodies were systematically analysed by two independent reviewers (MR and IC). Disagreements were resolved by consensus; if consensus could not be achieved, a third party (SS) would provide an assessment of eligibility. As the data on eligibility were dichotomous (eligible: yes / no), agreement at both the title and abstract review and the full article review stages was determined by calculation of Cohen's kappa coefficient ( $k=0.93$ ).

Literature search strategy is shown in *Figure 1*.

The prevalence of aPS antibodies was compared between populations by Fisher's exact test, two-tailed, whether results for association of positivity and risk of thrombosis were compared using odds ratios reported in the studies analysed.

The present study has been performed according to PRISMA guidelines.

## Results:

Data from 5992 patients included in 20 retrieved studies were analysed. These studies had different design and varying sample size. Table 1 summarizes the characteristics of the studies included in the analysis and the results of the prevalence study of aPS antibodies, references are provided as supplementary material.

In patients with APS (either PAPS or SAPS), we report an overall estimated median prevalence of aPS antibodies of 55% [range 29-87%] and 35% [16-65%] for IgG and IgM, respectively. Overall, aPS antibodies were more frequently found in patients with known diagnosis of APS, when compared to patients with thrombosis (IgG mean 55%±28.9; IgM 35±4.3), pregnancy loss (IgG mean 30±19.6; IgM 1±2.8) or Systemic Lupus Erythematosus (IgG mean 22±13; IgM 14±8.3) ( $p<0.05$ ) (Table S1). aPS positivity was distributed as follows. Out of 366 patients with known APS retrieved in 7 studies, we found a mean rate of aPS positivity of 55%±21.1 and 35%±17.9 for IgG and IgM, respectively. Albeit all 366 patients were positive for at least one aPL criteria (thus meeting the classification criteria for APS), retrieved data do not allow to stratify the aPS prevalence when dividing for LA, aCL and/or anti-beta2GPI antibodies positivity. Pooling together the 2 studies investigating 78 patients with primary APS (PAPS), we estimated a mean prevalence of 64%±18.4 aPS IgG and 48%±1.3 aPS IgM, higher when compared to the rate observed in the 29 patients with secondary APS (SAPS) (37% aPS IgG and 24% aPS IgM positive,  $p<0.05$ ). In 259 APS cases the eventual concomitant presence of another autoimmune condition was not specified.

When analysing patients without thrombosis and pregnancy morbidity, seven (cross-sectional) studies found a mean prevalence of 22%±13 for IgG; 14%±8.3 for IgM in patients

with SLE (n = 787), and one single study[6] reported a prevalence of 21% IgG;25% IgM in 24 aPL asymptomatic carriers.

In 3565 patients with cardiovascular events retrieved in 4 studies[7–10], we estimated a mean aPS prevalence of 18%±28.9 for IgG and 7%±4.3 for IgM. Six studies counting for 1250 women with a previous history of pregnancy morbidity showed a mean aPS prevalence of 30% (±19.6) for IgG and 1% (±2.8) for IgM.

Only 2 studies analysed the role of aPS in APS patients when computing separately thrombosis and pregnancy loss (supplementary material, Lopez et al. 2004; Roggenbuck et al. 2016). In brief, when computed together these two studies, we found a mean aPS positivity of 5.4% IgG/21.6% IgM in patients with obstetric APS and 45.9% IgG/28.6% IgM in thrombotic APS.

## **Discussion:**

In this study, we report an overall median prevalence of aPS antibodies in APS patients of 55% [range 29-87%] and 35% [16-65%] for IgG and IgM, respectively. We observed a significant higher frequency of aPS in APS, both in PAPS andSAPS, when compared with SLE patients (22% for IgG and 14% for IgM). We noted an even statistically significant higher prevalence of aPS positivity in patients with PAPS (64% for IgG and 48% for IgM), compared with that of patients with SAPS (37% IgG and 24% IgM).

However, we should acknowledge that this observation, although statistically significant, might be biased by the smaller sample size of included patients with SAPS.



When separating data for inclusion criteria, in a sub-analysis including more than 3500 patients with cardiovascular accidents and no known diagnosis of APS, we observed an aPS prevalence of 18% ( $\pm 28.9\%$ ) for IgG and 7% ( $\pm 4.3\%$ ) for IgM. These observations might suggest that approximately a fifth of subjects with cardiovascular events could be positive for aPS. Nevertheless, the lack of properly designed studies aiming to assess the value of these autoantibodies as an independent risk factor when compared to current criteria aPL testing, leaves the questions unanswered. Similarly, albeit approximately a third of the women with a previous history of pregnancy morbidity were found positive for aPS, we were not able to estimate how many of those were positive also for traditional aPL testing, and, more critically, for aPS only.

Taken the above together, while aPS seem to be frequently found in patients with thrombosis and/or pregnancy morbidity and in up to half of patients with APS, with the level of evidence currently available, in this systematic review of the literature we are not able to establish an independent association between aPS and thrombosis and/or pregnancy morbidity. Future perspective studies are needed to clarify if aPS (isolated or in addition to criteria aPL) confer an increased risk for thrombosis and/or pregnancy morbidity in subject without previous event. The strengths of this analysis lie on *a priori* designed search strategy, and the inclusion of gray literature searches (e.g. EULAR and ACR/ARHP Annual Meetings) and manual review of reference lists minimized the risk of missing eligible studies. We performed independent and duplicate review for study selection and data extraction.

However, our analysis also suffers for limitations. All the included studies were observational, and therefore subject to the biases inherent in such study designs. Additionally, there was heterogeneity in the data in terms of inclusion criteria, clinical outcome definition, assay heterogeneity, cut-off values definition, detected Ig isotypes, clinical details, and control groups. Moreover, none of the studies reported data comparing persistent versus transient

aPS positivity. Also, none of the studies confirmed their results by multivariate analysis. Furthermore, when analyzing the control groups used in the studies included in the analysis, only a minority of studies included a control group consisted of event-free aPL carriers, limiting the generalizability of the role of aPS positivity in the different subgroups (e.g., thrombotic APS, obstetric APS, isolated aPL carriers, aPL carriers in patients with SLE). Finally, while significant international improvements have been achieved in the standardization of IgG and IgM aPL measurement, due to the heterogeneity in used methods (both in house ELISA and commercial available kits), some intrinsic differences due to the techniques might be speculated.

**Conclusions:** While aPS are frequently detected in patients with known APS, especially PAPS, their added independent diagnostic value and the clinical relevance in patients with thrombosis/pregnancy loss and/or concomitant autoimmune disease remain unknown. Future studies are needed to determine if aPS positivity (isolated or concomitant to other aPL specificities), might confer an increased risk in subject without previous events.

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**Authorship Contributions:** MR, IC and ER searched the literature, assisted with the organization of the manuscript, interpreted and collected data, and wrote and edited the Review. DR, MM and SS interpreted and collected data, helped to design the figures and panel, and wrote and edited the Review.

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## **Legend of Tables and Figures:**

*Table 1. Characteristics of the studies included in the analysis*

*Figure 1. Literature search strategy on prevalence of positivity for aPS antibodies*