Research Article

Seronegative Antiphospholipid Syndrome Existence in Clinical Practice

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Abstract

Background: In daily clinical practice, there appears to be a subset of patients with classical APS manifestations who test negative for the recommended criteria APA tests as a result of a lack of harmonization and/or standardization. Alternatively, they may have APA that target other antigens involved in the pathogenesis of APS. Therefore, the term '**seronegative APS'** (SN-APS) has been coined to include these patients. **Objectives**: To assess the clinical manifestations of a group of SN-APS patients comparing that with a group of seropositive APS (SP-APS). **Method**: ٦٠ patients were divided into two groups, ٣٠ Patients with seropositive APS (SP-APS) whether primary or secondary fulfilling the Sydney classification criteria for APS (group I) and ٣٠ Patients with SN-APS matched for sex and age which tested negative for LA, aCL and anti-β٢-GP١ twice at least ١٢ weeks apart . Each patient underwent a complete history taking and physical examination and laboratory investigations. **Results:** No significant differences in the frequency of obstetric and thrombotic morbidity between SN-APS versus SP-APS .**Conclusion:** our results should help clinicians bear in mind that the possibility of SN-APS can exist in patients with strong evocative clinical evidence of the disease and that appropriate treatment may prevent thrombosis from recurring and improve fetal and maternal outcome.

Keywords: Antiphospholipid Syndrome- Antiphospholipid antibodies (APA) Seropositive APS (SP-APS) -Seronegative APS (SN-APS).

Introduction

In daily clinical practice, we can find patients with clinical manifestations suggestive of antiphospholipid syndrome (APS) who are persistently negative for the routinely used assays to detect anticardiolipin antibodies (aCL), lupus anticoagulant (LA) and anti-β٢-glycoprotein-١ (anti-β٢-GP١) antibodies. Therefore, the term 'seronegative APS' (SN-APS) has been coined to include these patients[١].

Roubey, ٢٠٠٠, reported that the most important explanation is laboratory evidence of autoantibodies thought to be associated with APS, but not detected in conventional APA assays. These include autoantibodies to certain phospholipid-binding plasma proteins, as well as antibodies detected in immunoassays using phospholipids other than cardiolipin[٢].

Hughes and Khamashta high lightened the history of ''seronegative RA'' and ''seronegative lupus'' and suggests a positive approach towards "seronegative APS"[٣].

Subsequent comparative studies tried to specify characteristics of patients with seronegative APS[٤],[٥].

Ricard Cervera and his colleagues in ٢٠١٢ presented the arguments for and against the diagnosis of SN-APS aiming to help the clinician when approaching a patient with clinical manifestations consistent with APS diagnosis but with negative APA using the commonly available tests[٦].

Nayfe et al., ٢٠١٣ presented a literature review on the most promising antibodies of this heterogeneous APA family, which includes antibodies to a zwitterionic

phospholipid, namely phosphatidyle thanolamine, phospholipid-binding plasma proteins, phospholipid_ protein complexes and anionic phospholipids other than cardiolipin [٧].

These findings suggest that in sera from patients with seronegative APS, antibodies may be detected using new antigenic targets or methodological approaches different from traditional techniques[٨].

Aim the work

We aimed To study the clinical presentation of a group of seronegative APS (SN-APS) patients; who had major clinical manifestations of APS but tested negative for antiphosholipids antibodies at least twice on two separate occasions, comparing that with another group of seropositive APS (SP-APS).

Patients and methods

Two groups of patients were included; ٣٠ patients with seropositive APS (SP-APS) whether primary or secondary fulfilling the Sydney classification criteria for APS **as (Group I)** and ٣٠ patients matched for age and sex with obstetric and/or thrombotic morbidity persistently negative for conventional APA and presenting with at least two additional non-criteria manifest-tations of APS '(seronegative APS', SN-APS) as (**Group II).**

At least two of the following non-criteria manifestations associated with APS were required: (١) livedo reticularis. (٢) Raynaud's phenomenon. (٣) Migranous headache. (٤) Cognitive dysfunction. (٥) Seizures. (٦) Chorea. (٧) Multiple sclerosis-like illness. (٨) Brain MRI white matter lesions. (٩) Mitral valve disease. (١٠) Aortic valve disease. (١١) One or two spontaneous abortions <١٠ weeks. (١٢) Thrombo-cytopaenia (<١٠٠,٠٠٠/mm٣).

Patients with either a clinical evidence or history of vasculitis were excluded from the study.

In each group of patients, the number and type of clinical thrombotic events as well as pregnancy morbidity according to the clinical manifestations from the Sydney classification criteria for APS was recorded.

Statistical analysis

Analysis of data was done by personal computer using SPSS (Statistical program for social science) version ١٦. Data were expressed as mean ± SD for parametric variables and as number and percent for non-parametric variable. Comparison between groups for parametric data was done by independent samples t-test (unpaired t-test).Chi – square (X^2) test was used to compare qualitative variables. The difference was expressed as probability of value (P value). The difference was considered significant if $P < ٠.٠٥$.

Results

A) <u>Obstetric manifestations:</u>

The studied groups show no significant difference regarding the age at first pregnancy events (P=٠.٣٣).The frequency of abortions and recurrent abortions <١٠ weeks was represented more in patients of group II, however no significant difference was detected between the two groups. The frequency of abortions and recurrent abortions >١٠ weeks was reported more in group I, but still no significant difference was detected between the two groups.

Moreover, no significant difference was detected between the two groups regarding the frequency of abortions >٣٤ weeks, recurrent abortions >٣٤ weeks, pre-eclampsia and number live births referring to successful pregnancies (Table ١&٢).

<u>B) Thrombotic manifestations:</u>

<u>١- Venous thrombotic events:</u>

The studied groups show no significant difference regarding the age at first venous thrombotic events (mean ± SD was ٢٧.٨ ± ٣.١in group I and ٢٩.٢ ± ٢.٨ in group II). Although the frequency of deep venous thrombosis (DVT) and recurrent DVT was reported more in group I than group II, the difference still not statistically significant. Moreover, no significant difference was reported as regard the frequencies of pulmonary embolism, recurrent pulmonary embolism and cerebral venous thrombosis (Table ٣).

٢-Arterial thrombotic events :

The studied groups show no significant difference regarding the age at first arterial thrombotic events (mean ± SD was ٣٧.٦ ± ٣.٨ in group I and ٣٧. ٤ ± ٥.٩ in group II). No significant difference between the studied groups regarding frequencies of stroke, recurrent stroke , transient ischemic attacks (T.I.A) , recurrent transient ischemic attacks and chronic ischemic artery disease **(Table ٤).**

Laboratory investigations:

١) Haematologic profile:

There was no significant difference between the studied groups as regard anemia (P=٠.١٩), leucopenia (P=٠.٤٩) and thrombocytopenia (P=٠.٧٨) **(Table ٥).**

٢) Immunological profile:

As regard the immunological profile, a high significant difference was detected between the studied groups regarding ANA (P= ٠.٠٠) and anti-double strand DNA (P= ٠.٠٠) in favor of group I **(Figure ١)**.

٣) Antiphospholipid antibodies:

As regard the frequencies of standard Anti –phospholipid antibodies (APA) among patients of SP-APS was represented in with lupus anticoagulant was the most presented **(٨٣.٣٪)** and Anticardiolipin IgM was the least presented **(٧٠.٠٪) (Figure٢).**

Table (١): Obstetrical manifestations in group I and group II (%) (Mean ± SD:

Variable	Group (I) SP-APS (n=٢٩)	Group (II) SN-APS (n=٢٨)	P. value (Sig.)
Age of first pregnancy event (yr)	٢٢.٠ ± ١.٧	٢٢.٥ ± ٢.٠	٠.٣٣
Abortion > ١٠ weeks	١٨ (٦٢.١٪)	١٥ (٥٣.٦٪)	٠.٥٢
Abortion < ١٠ weeks	١٣ (٤٤.٨٪)	١٩ (٦٧.٩٪)	٠.٠٨
Abortion > ٣٤ wks.	٧ (٢٤.١٪)	٦ (٢١.٤٪)	٠.٨٠
Pre-eclampsia	٦ (٢٠.٧٪)	٥ (١٧.٩٪)	٠.٧٨

SP-APS=Seropsitive antiphospholipid syndrome.
SN-APS= Seronegative antiphospholipid syndrome.
Chi squre test. MannWhitney test. *Significant (P≤ ٠.٠٥) .

Table (٢): Obstetrical manifestations in group I and group II (%)(Mean ± SD):

Variable	Group (I) SP-APS (n=٢٩)	Group (II) SN-APS (n=٢٨)	P. value (Sig.)
Recurrent abortion > ١٠ wks.	١٢ (٤١.٤٪)	٨ (٢٨.٦٪)	٠.٣١
No. of recurrent abortion > ١٠ wks.	٠.٩٠ ± ١.١	٠.٥٧ ± ٠.٩٢	٠.٢١
Recurrent abortion < ١٠ wks.	١٣(٤٤.٨)	١٨(٦٤.٣)	٠.١٤
No. of recurrent abortion < ١٠ wks.	١.٧٦ ± ٢.٠	٢.٥٧ ± ٢.٣	٠.١٦
Recurrent abortion > ٣٤ wks.	٣ (١٠.٣٪)	٤ (١٤.٣٪)	٠.٦٥
No. of recurrent abortion > ٣٤ wks.	٠.٢٤ ± ٠.٦٣	٠.٢٩ ± ٠.٧١	٠.٨١
No. of live births	١.٦٢ ± ٠.٩٤	١.٥٠ ± ٠.٦٩	٠.٥٨

SP-APS=Seropsitive antiphospholipid syndrome.
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Table (٣): Venous Thrombotic events in group I and group II (%) (Mean ± SD):

Variable	Group (I) SP-APS (n=٣٠)	Group (II) SN-APS (n=٣٠)	P. value (Sig.)
Age of first venous thrombotic event (yr)	٢٧.٨ ± ٣.١	٢٩.٢ ± ٢.٨	٠.٢٣
Deep venous thrombosis (DVT)	١٣ (٤٣.٣٪)	١٠ (٣٣.٣٪)	٠.٤٢
Recurrent DVT	٩ (٣٠.٠٪)	٧ (٢٣.٣٪)	٠.٥٥
Pulmonary embolism	٧ (٢٣.٣٪)	٧ (٢٣.٣٪)	١.٠
Recurrent Pulmonary embolism	٢ (٦.٧٪)	١ (٣.٣٪)	٠.٥٥
Cerebral venous Thrombosis (CVT)	٥ (١٦.٧٪)	٣ (١٠.٠٪)	٠.٤٨

SP-APS=Seropsitive antiphospholipid syndrome.
SN-APS= Seronegative antiphospholipid syndrome.
Chi squre test . MannWhitney test. * Significant (P≤ ٠.٠٥) .

Table (٤): Arterial thrombotic events in group I and group II (%) (Mean ± SD):

Variable	Group (I) SP-APS (n=٣٠)	Group (II) SN-APS (n=٣٠)	P. value (Sig.)
Age of first arterial thrombotic event (yr)	٣٧.٦ ± ٣.٨	٣٧. ٤ ± ٥.٩	٠.٩٥
Stroke	٨ (٢٦.٧٪)	٥ (١٦.٧٪)	٠.٣٥
Recurrent stroke	٢ (٦.٧٪)	١ (٣.٣٪)	٠.٥٥
Transient ischemic attacks	٥ (١٦.٧٪)	٣ (١٠.٠٪)	٠.٤٤
Recurrent transient ischemic attacks	٢ (٦.٧٪)	١ (٣.٣٪)	٠.٥٥
Coronary artery disease	٢ (٦.٧٪)	٣ (١٠.٠٪)	٠.٦٤

SP-APS=Seropsitive antiphospholipid syndrome.
SN-APS= Seronegative antiphospholipid syndrome.
Chi squre test. MannWhitney test. *Significant (P≤ ٠.٠٥) .

Table (٥): Haematologic Profile in group I and group II (%):

Variable	Group (I) SP-APS (n=٣٠)	Group (II) SN-APS (n=٣٠)	P. value (Sig.)
Anemia<١٢ g/dl	١٩ (٦٣.٣٪)	١٤ (٤٦.٧٪)	٠.١٩
Leucopenia<٤٠٠٠ g/dl	٢ (٦.٧٪)	١(٣.٣٪)	٠.٤٩
Thrombocytopenia <١٠٠٠٠٠٠	١١ (٣٦.٧٪)	١٠ (٣٣.٣٪)	٠.٧٨

SP-APS=Seropsitive antiphospholipid syndrome.
SN-APS= Seronegative antiphospholipid syndrome.
Chi squre test. *Significant (P≤ ٠.٠٥)

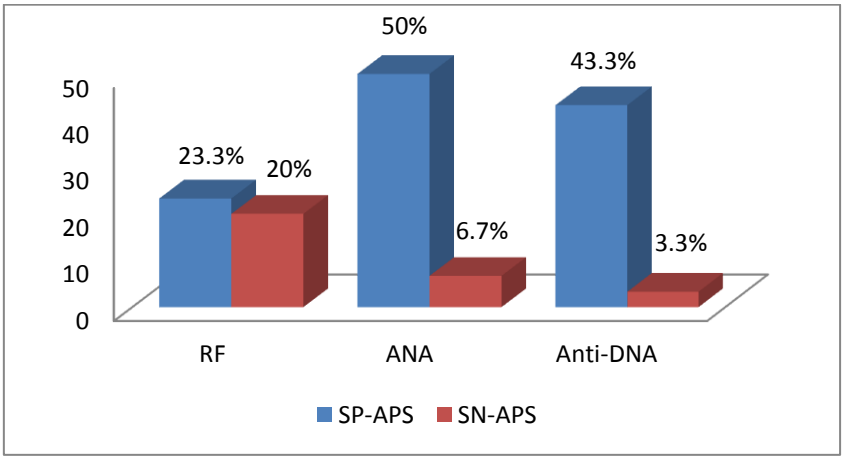

Fig. (١): Immunological profile in SP_APS and SN-APS patients.

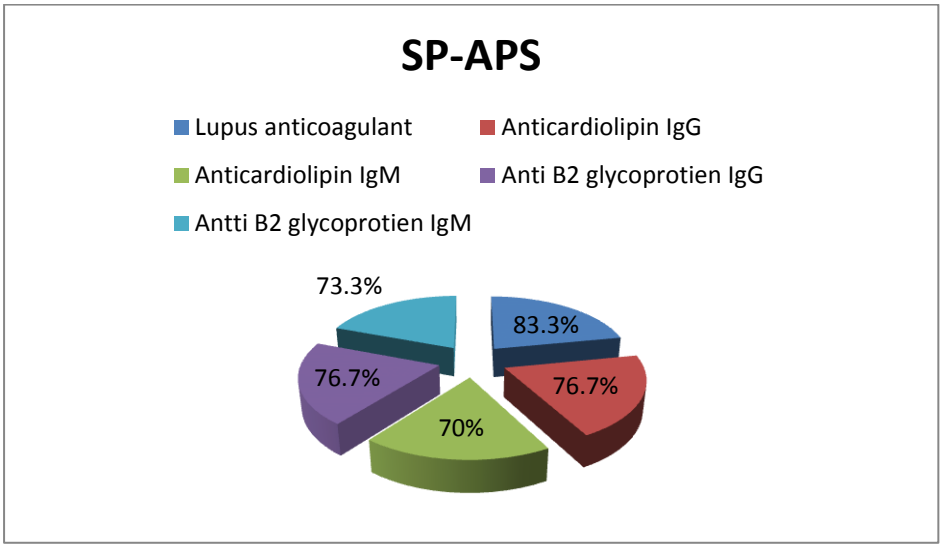

Figure ٢: Anti-phospholipide antibodies positivity in SP-APS patients.

Discussion

The term 'seronegative APS' (SN-APS) has been supposed to include patients with clinical manifestations suggestive of antiphospholipid syndrome (APS) but are persistently negative for the routinely used assays to detect anticardiolipin antibodies (aCL), lupus anticoagulant (LA) and anti-β٢-glycoprotein-١ (anti-β٢-GP١) antibodies.

Our study shows no significant difference between the studied groups as regard the frequency of obestetricl and thrombotic manifestations of APS and increase the rate of recurrence in patients with previous venous thrombotic events in the studied groups on withdrawal of anticoagulant treatment, or when the values of the international normalized ratio (INR) were below the recommended targets (٨٣.٣٪ in SP-APS vs ٨٠٪ in SN-APS, P=٠,٠٨٤).

Our results are in concordance with that of Rodriguez-Garcia et al., ٢٠١٢ and that of Conti et al., ٢٠١٤ who show no significant differences regarding the frequencies of obstetrical, arterial and venous thrombotic events between the studied groups.

In conclusion, our results should help clinicians bear in mind that the possibility of SN-APS can exist in patients with strong evocative clinical evidence of the disease and that appropriate treatment may prevent thrombosis from recurring and improve fetal and maternal outcome.

Disclosure statement: The authors have declared no conflict of interest.

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