Prevalence of Antiphospholipid Antibodies Negativisation in Patients with Antiphospholipid Syndrome: A Long-Term Follow-Up Multicentre Study

Massimo Radin[1,*] Karen Schreiber[2,3,*] Savino Sciascia[1] Dario Roccatello[1] Irene Cecchi[1] María Ángeles Aguirre Zamorano[4] Maria Jose Cuadrado[5]

[1] Department of Clinical and Biological Sciences, Center of Research of Immunopathology and Rare Diseases, Coordinating Center of Piemonte and Valle d'Aosta Network for Rare Diseases, S. Giovanni Bosco Hospital, University of Turin, Turin, Italy
[2] Department of Thrombosis and Haemophilia, Guy's and St Thomas' Hospital, London, United Kingdom
[3] Department of Rheumatology, Copenhagen University Hospital, Copenhagen, Denmark
[4] Department of Rheumatology, University Hospital "Reina Sofia," Cordoba, Spain
[5] Department of Rheumatology, Clinica Universidad de Navarra, Madrid, Spain

Address for correspondence Savino Sciascia, MD, PhD, Center of Research of Immunopathology and Rare Diseases, Coordinating Center of Piemonte and Valle d'Aosta Network for Rare Diseases, and SCDU Nephrology and Dialysis, S. Giovanni Bosco Hospital, Piazza del Donatore di Sangue 3, Turin 10154, Italy
(e-mail: savino.sciascia@unito.it).

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Abstract

Objective This article aims to analyse the rate of antiphospholipid antibodies (aPL) negativisation in patients with antiphospholipid syndrome (APS), and to evaluate potential new clinical manifestations after negativisation and/or aPL fluctuations in a long-term follow-up.

Methods Inclusion criteria are (1) any patients with an APS diagnosis according to the current Sydney criteria and (2) patients in whom aPL negativisation occurred. aPL negativisation was defined as repeated aPL measurements on at least two consecutive occasions at least 12 weeks apart, with a follow-up of at least 1 year since aPL first turned negative.

Results Out of 259 APS patients, a total of 23 patients (8.9%) met the inclusion criteria for persistent aPL negativisation. Patients were followed-up for 14.4 ± 8.1 years, experienced aPL negativisation after a mean of 5.3 ± 3.5 years and were followed-up after experiencing the aPL negativisation for a mean of 7.6 ± 5.8 years. Seventeen patients (73.9%) presented with thrombotic APS, 2 with pregnancy morbidity (8.7%) and 4 (17.4%) with both. Most of the patients (18; 78.3%) had a single aPL positivity, 5 (21.7%) double, while no triple aPL positivity was observed. At the time of data collection, after aPL negativisation, anticoagulation was stopped in 8 patients with previous thrombotic venous event (8/21, 38%) according to the treating physicians' judgements. None of the patients experienced any recurrent thrombotic event during the follow-up period after their aPL negativisation.

Conclusion In our patient cohort consisting of 259 patients with definitive APS, we observed over a mean observation period of > 5 years, that aPL negativisation occurred in approximately 9% of patients. Negativisation occurred most often in patients who were previously found to be positive for only one aPL.

Keywords
- antiphospholipid syndrome
- antiphospholipid antibodies
- negativisation
- anticoagulation
- thrombosis

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Introduction

The antiphospholipid syndrome (APS) is an autoimmune disorder characterised by vascular thrombosis (arterial and/or venous) and/or pregnancy morbidity associated with the persistent presence of antiphospholipid antibodies (aPL). The current classification criteria for APS include three laboratory tests: lupus anticoagulant (LA), anti-cardiolipin (aCL) and anti-β2 glycoprotein-I (β2GPI) antibodies.[1] To prevent detection of transient antibodies, tests must be positive in two occasions, at least 12 weeks apart.[1]

The therapeutic mainstay of thrombotic APS is long-term anticoagulation with vitamin K antagonists (VKAs). Recent studies have created new ground for discussion about the possible discontinuation of anticoagulation therapy in patients with a history of thrombotic APS in whom aPL are not detected any longer (i.e., aPL *negativisation*).[2,3] In particular, a recent systematic review has described a total of 4 studies that included 47 thrombotic APS patients with persistent aPL negativisation.[3] Three out of four studies, including 23 thrombotic APS patients, also described discontinuation of anticoagulation therapy after aPL negativisation.

To date, there are no data on the frequency of aPL negativisation among APS patients and very little clinical information is available about the significance of persistent aPL negativisation.

In this long-term multicentre study, we aimed to analyse the rate of aPL negativisation in APS patients and aPL carriers, to evaluate any changes that must have occurred regarding anticoagulant therapy after negativisation and to describe any clinical events appearing after anticoagulation cessation.

Methods

We retrospectively retrieved data from patients who attended from 2010 to 2018 the S. Giovanni Bosco Hospital, Turin, Italy, the Lupus Unit, Department of Rheumatology at St Thomas' Hospital, London, United Kingdom, and Hospital Reina Sofia, Córdoba, Spain. Inclusion criteria to the study included: (1) previously diagnosed as APS due to persistent aPL positivity (positive in two or more occasions, at least 12 weeks apart) and fulfilled the clinical Sydney criteria for APS,[1] negative screening for inherited thrombophilia; (2) follow-up of at least 2 years; and (3) subsequent negativisation of aPL antibodies defined as: at least two negative aPL consecutive tests, at least 12 weeks apart, with a follow-up of at least 1 year since aPL first turned negative.

As control group, we included a second group of patients who were persistently aPL positive, who fulfilled the inclusion criteria (2) and (3), but did not fulfilled the clinical criteria for APS.[1]

Clinical and laboratory data were retrospectively collected from patient notes. Diagnosis and aPL positivity profile were systematically analysed by three independent reviewers (M.R., I.C. and K.S.). ►**Fig. 1** resumes screening and selection strategy.

Categorical variables are presented as number (%) and continuous variables are presented as mean (standard deviation [SD]). Categorical agreement and degree of linear association was analysed. The significance of baseline differences was determined by the chi-square test, Fisher's exact test or the unpaired *t*-test, as appropriate. A two-sided *p*-value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 19.0 (IBM, Armonk, New York, United States).

Results

A total of 259 APS patients were screened (aPL profile at diagnosis: 39.1% single aPL positivity, 24.6% double aPL positivity and 36.2% triple aPL positivity). A total of 23 patients (8.9%) (mean age at data collection 52.7 [SD ± 14]; females 65%), met the inclusion criteria of the study, with persistently aPL negativisation. Mean time of follow-up was 14.4 years (SD ± 8.1) since the diagnosis of APS and mean to negativisation was 5.3 years (SD ± 3.5). Patients were followed-up after aPL negativisation for a mean time of 7.6 years (SD ± 5.8). When analysing the clinical manifestations of APS, 21 patients (91.3%) presented with history of thrombotic APS (9 patients [42.8%] experienced a previous arterial event, 11 patients [53.4%] experienced a previous venous event and 1 patient [4.8%] both arterial and venous), 2 patients with history of pregnancy morbidity (8.7%) and 4 patients (17.4%) had a history of both thrombotic and pregnancy-related events.

When focusing on the confirmed aPL positivity profile at the diagnosis, most of the patients (18; 78.3%) had a single aPL positivity, while no triple aPL positivity were observed and only 5 patients (21.7%) presented with double aPL positivity. When analysing the cardiovascular risk factors at diagnosis of the cohort, the patients had a mean adjusted global antiphospholipid score (aGAPSS) of 6.4 (SD ± 2.7), 10 patients (43.5%) presented with hypertension, 7 patients (30.4%) with hyperlipidaemia, 2 patients (8.7%) with diabetes and 2 patients (8.7%) with a history of smoking habit.

The characteristics of retrieved patients with aPL negativisation are illustrated in ►**Table 1**.

For comparison, asymptomatic persistently aPL positive patients were screened. Three patients out of 47 (6.4%) met the inclusion criteria of the study, with aPL negativisation (confirmed by at least by two aPL determinations, 12 weeks apart) and with a follow-up of at least 1 year. When considering the aPL profile, one patient had isolated LA positivity, one patient had isolated aCL positivity (immunoglobulin G [IgG], medium titer 40–80 U/mL) and one patient had double aPL positivity (anti-β2GPI and aCL [IgG medium titer 40–80 U/mL]). No statistical differences were observed between the patients with APS and aPL carriers, in terms of time to negativisation (aPL+ group: 4.5 years; SD ± 3.8), risk factors at diagnosis (aPL + group: mean aGAPSS 6 [SD ± 2.6], one patient presented with hypertension and one patient with hyperlipidaemia) and rate of aPL positivity (two patients had single aPL positivity and one patient presented with double positivity).

Follow-Up and Anticoagulation Therapy

After the first thrombotic manifestations, 16 patients were treated with VKA (2 patients lately switched to direct oral anticoagulants [DOACs] during the follow-up), 4 patients with low molecular weight heparin (for erratic time in therapeutic

Table 1 APS patients with aPL negativisation: Clinical and laboratory characteristics

	Negativised patients	% (23 patients)
Anagraphic		
Mean age (SD) at data collection	52.7 (±9.4)	
Sex (females)	15	65.2
Mean time of follow-up (years) (SD)	14.4 (±8.1)	
Diagnosis		
PAPS	20	86.9
SAPS	3	13
Clinical manifestations of APS prior to aPL negativisation		
Thrombosis (*n*)	21	91.3
Arterial thrombosis (*n*)	10	43.8
Stroke (*n*)	8	34.8
Myocardial infarction (*n*)	2	8.7
Venous thrombosis (*n*)	12	52.2
Deep vein thrombosis (*n*)	9	39.1
Pulmonary embolism (*n*)	2	8.7
Cerebral venous thrombosis (*n*)	1	4.3
History of multiple thrombosis prior aPL negativisation (*n*)	5	26.1
Venous (*n*)[a]	2	8.7
Arterial (*n*)[b]	3	13
Obstetric APS (*n*)	6	26.1
Three or more spontaneous abortions (< 10 weeks of gestation)	4	17.4
One or more episodes of foetal death (> 10 weeks of gestation)	2	8.7
aPL profile at diagnosis		
LA (positive, *n*)	13	56.5
aCL (IgG/M, *n*)	9	39.1
Anti-β2GPI (IgG/M, *n*)	5	21.7
Single aPL positivity	18	78.3
Isolated LA (*n*)	11	47.8
Isolated aCL (IgG, high titer ≥ 80 U/mL, *n*)	1	4.3
Isolated aCL (IgM, high titer ≥ 80 U/mL, *n*)	1	4.3
Isolated aCL (IgG, medium titer 40–80 U/mL, *n*)	2	8.7
Isolated aCL (IgG and IgM, medium titer 40–80 U/mL, *n*)	1	4.3
Isolated anti-β2GPI (IgG, medium titer 40–80 U/mL, *n*)	2	8.7
Double aPL positivity	5	21.7
aCL (IgG, high titer ≥ 80 U/mL) and anti-β2GPI (IgG, medium titer 40–80 U/mL)	1	4.3
Anti-β2GPI (IgG, high titer ≥ 80 U/mL) and aCL (IgG, medium titer 40–80 U/mL)	2	8.7
LA + anti-β2GPI (IgG medium titer 40–80 U/mL)	1	4.3
LA + aCL (IgG medium titer 40–80 U/mL)	1	4.3
Triple aPL positivity	0	0

(*Continued*)

Table 1 (*Continued*)

	Negativised patients	% (23 patients)
Cardiovascular risk factors at diagnosis		
Hypertension	10	43.5
Hyperlipidaemia	7	30.4
Diabetes	2	8.7
Smoking	2	8.7
Mean aGAPSS (SD)	6.4 (±2.7)	
Negativisation		
Mean time to negativisation (mo) (SD)	62.4 (±41.8)	
Mean time of follow-up after negativisation (mo) (SD)	91.2 (±79.6)	

Abbreviations: aCL, anti-cardiolipin antibodies; aGAPSS, adjusted Global AntiPhoSholipid Score; anti-β2GPI, anti-β2glycoprotein I antibodies; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; IgG/M, immunoglobulin G/M; LA, lupus anticoagulant; PAPS, primary antiphospholipid syndrome; SAPS, secondary antiphospholipid syndrome; SD, standard deviation.
[a]The patients experienced, while on anticoagulation, prior to aPL negativisation a recurrence of deep vein thrombosis.
[b]The patients experienced, while on anticoagulation, prior to aPL negativisation an episode of ischaemic stroke. One patient had a prior episode of ischaemic stroke, while two patients experienced an episode of deep vein thrombosis prior to the arterial recurrence.

Table 2 Therapy's characteristics of patients with thrombotic history and aPL negativisation

	Therapy after first thrombosis	% (21) patients	Therapy at data collection, after negativisation	% (21) patients
VKA (warfarin)	16	76.2	10	47.6
LMWH	4	19.1	0	0
DOAC	1	4.8	3	14.3
LDA	14	66.7	9	42.9
Statins	8	38.1	8	38.1
Anti-hypertensive drugs	10	47.6	10	47.6
HCQ	5	23.8	6	28.6
Prednisone	4	19.1	5	23.8
Immunosuppressive drug	0	0	0	0

Abbreviations: aPL, antiphospholipid antibodies; DOAC, direct oral anticoagulant; HCQ, hydroxychloroquine; LMWH, low molecular weight heparins; LDA, low-dose aspirin; VKA, vitamin K antagonists.

rage while on VKA when DOAC were not available) and 1 patient was treated with 20 mg rivaroxaban once daily. Additional treatments to anticoagulation were as follows: 14 patients received low-dose aspirin, 5 patients received hydroxychloroquine (HCQ), 8 patients were treated with statins and 10 with anti-hypertensive medications. At the time of data collection, after aPL negativisation, 10 patients were still receiving VKA and 3 patients were treated with DOAC. In 2 patients (9.5%; 1 patient with previous venous event and 1 patient with previous arterial event) the anticoagulation therapy was switched from VKA to DOACs. In 8 patients (38%), anticoagulation was suspended, according to treating physicians' judgement. All 8 patients in whom anticoagulation was stopped had a history of thrombotic venous events and they were all started on low-dose aspirin. After a median follow-up time of 3.2 years (±1.5) after withdrawal of anticoagulation, no recurrence of thrombotic events was observed.

Interestingly, when analysing previous clinical manifestations of patients in whom anticoagulation was stopped, most of them (7 patients out of 8), had a single venous thrombotic event and had a mean aGAPSS lower than patients in whom anticoagulation was still on-going (mean aGAPSS 5.4 ± 2.2 vs. 6.9 ± 2.9).

►**Table 2** illustrates the therapy of thrombotic APS patients after the first thrombosis and at the time of data collection, after aPL negativisation.

Discussion

In this retrospective multicentre study, including a total of 259 patients with definite APS, we observed that 9% of the patients experienced aPL negativisation.

aPL can be found with vast heterogeneity in terms of type, titers, and persistence in a large range of autoimmune diseases[4]

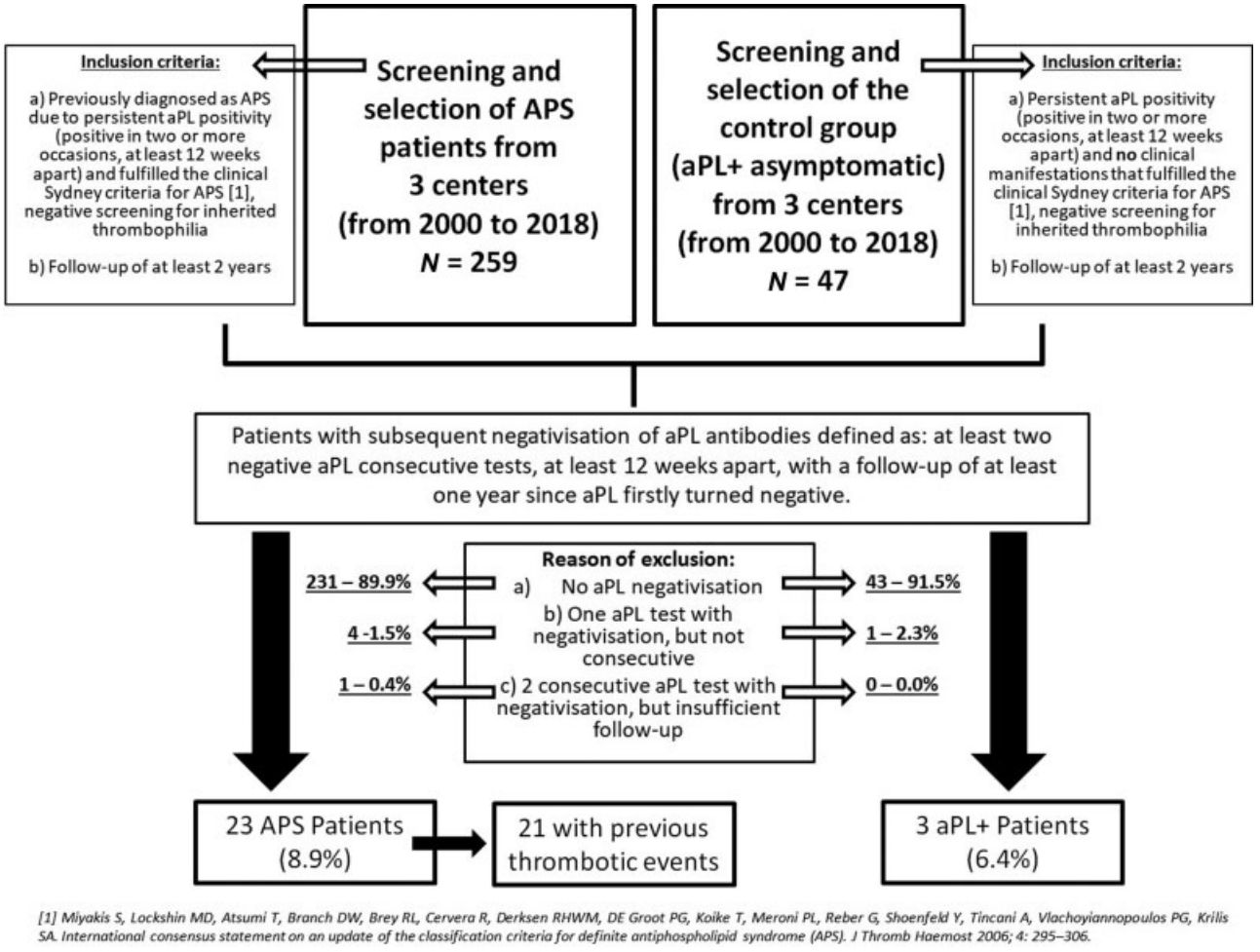

Fig. 1 Screening and selection strategy.

and non-autoimmune diseases.[5] To date, some authors suggest that aPL testing should remain stable for at least three-quarters of sequent tests, regardless of the laboratory performing the tests, to consider the laboratory finding clinically significant.[6] However, no adequate consensus exists on the accurate definition of aPL negativisation, as timing for aPL testing and length of follow-up to consider when evaluating a previously aPL patient who turns 'persistently' negative are still a matter of discussion. In clinical practise, it is not unusual to find patients whose aPL profile turns negative during follow-up and our study further supports that aPL negativisation can be observed in a not negligible percentage of patients with previous diagnosis of APS, posing some clinical considerations.

First, it is worth noting that approximately 80% of the patients who turned negative in our cohort were positive for only one aPL at the time of the diagnosis. This observation is in line with recent evidence supporting that persistence of initial aPL positivity depends on the antibody profile, with a percentage of re-confirmation as high as 98% in patients with triple aPL profile.[7] Interestingly, we failed to observe any difference after aPL negativisation when comparing APS patients and aPL carriers: no difference in cardiovascular risk and aGAPSS mean, rate of aPL positivity and time to negativisation were the same in the two cohorts, as well as there were not any discrepancies in immunomodulatory or cardioprotective drugs used in the two groups. Consequently, especially in patients with low-risk aPL profile, other mechanisms rather than aPL persistence alone might play a role in determining the occurrence of clinical manifestations.[8] Notably, the majority of the patients of the cohort presented with single LA positivity (11 out of 23). While it is well known that testing patients during treatment with VKA or DOAC remains a contentious issue and has been discouraged by official guidelines[9–11] because of interpretational problems affecting the mixing test, patients of our cohort were not treated with anticoagulants when LA was tested.

Second, the disappearance of aPL might raise therapeutic question, whether or not to continue anticoagulation. Medina et al,[12] investigating 24 primary APS patients with aPL negativisation, after 60 months of follow-up, observed 45.8% of recurrence of thrombosis despite the continuation of the anticoagulant treatment. Notably, more than a third of the patients had a past history of arterial thrombotic events (eight strokes and one mesenteric thrombosis) before aPL disappearance. Further, 87.5% of patients in Medina's cohort were positive for aCL antibodies and 29% were both positive for LA and aCL. Anti-ß2GPI antibodies were tested only in a subset of patients who were initially negative to aCL/LA. The rate of double or triple aPL positivity might have been considerably higher in Medina's cohort.

These facts could place this group of patients at a higher thrombotic risk category when compared with those who experienced aPL negativisation in our cohort. In fact, on one hand, we observed no recurrence of thrombotic events despite stopping anticoagulation; on the other hand, in our cohort, anticoagulation was only stopped in patients with previous low-risk aPL profile and one venous event. However, while these observations are in line with data discussed elsewhere,[3,13] it was out of the scope of this study to trial the safety of stopping anticoagulation when the aPL turn negative.

Third, to date, laboratory criteria for APS include the assays' test for the presence of LA, aCL and anti-β2GPI antibodies[1]; however, in patients with persistent disappearance of aPL, some authors suggested a second level screening of non-criteria aPL to guide the decision of stopping anticoagulant treatment.[2] Among others,[2,14] very recently, Conti et al[15] described a patient with catastrophic APS, the most severe variant of the disease, who tested negative to the conventional aPL but positive for aPL in thin layer chromatography immunostaining and vimentin/cardiolipin antibodies by enzyme-linked immunosorbent assay test, highlighting how relevant non-criteria antibodies, in selected cases, can be potentially useful in guiding the therapeutic choices. Further, one should not forget the importance of traditional cardiovascular risk factors and triggers for thrombosis, that increase the risk of recurrence, independently of aPL, and should always kept in consideration when risk stratifying these patients and planning therapeutic approaches and follow-up.

Fourth, as high as 24% of patients that experienced aPL negativisation were treated with HCQ. HCQ has been shown to be able to reduce the level of aPL[16] and to directly interfere with the binding of antibody–β2GPI complexes to phospholipid bilayers.[17] Consequently, an immunomodulatory role of HCQ alone or in synergic action with other therapy is very intriguing and should be considered.[18–21]

This work presents several limitations, mainly relaying on its retrospective design, potential heterogeneity in laboratory testing on three different sites and the chart reviewing-based methodology. Importantly, changes of sensitivity of different assays might have an important impact on aPL testing detection, especially when considering aPL negativisation. To date, standardisation of aPL testing is an important matter of discussion and great effort by international groups such as APS ACTION or the European Forum on aPL are on their way.[22,23]

Nevertheless, it has some strengths, namely the sample size, the stringent inclusion criteria, the precise definition of aPL negativisation, the length of follow-up before and after aPL negativisation and the long-term experience of the enrolling centres in performing aPL testing.

In conclusion, we found that aPL negativisation is not a negligible fact in the routine clinical practise and it mainly occurs in patients with low-risk aPL profile at diagnosis. The stratification of risk based on aPL positivity is also stressed in the recent EULAR task force recommendations that further stress the importance of multiple aPL positivity.[24] While there is no current agreement on the timing for repeating aPL testing once the APS diagnosis is established, one could consider a sensible approach to test for aPL in patients with stable APS at least every 12 to 18 months to detect eventual changes in aPL profile, or when clinical changes occur (e.g. planning a pregnancy; changes in immunosuppressive therapy; new clinical event, not limited to thrombosis). Until further studies are available, in selected groups of patients, the discontinuation of anticoagulation therapy, when aPL turn persistently negative, might be discussed, especially in those with single venous event, single aPL positivity at diagnosis and controlled conventional cardiovascular risk factors. Testing for extra criteria aPL tests might also have a role in supporting the therapeutic decision.

What is known about this topic?

- The therapeutic mainstay of thrombotic antiphospholipid syndrome (APS) is long-term anticoagulation with vitamin K antagonists.
- Recent studies have created new ground for discussion about the possible discontinuation of anticoagulation therapy in patients with a history of thrombotic APS in whom antiphospholipid antibodies (aPL) are not detected any longer (i.e. aPL *negativisation*).

What does this paper add?

- In this long-term observational follow-up study, we analysed the rate of aPL negativisation in patients with APS and we evaluated potential new clinical manifestations after negativisation and/or aPL fluctuations.
- When considering the 259 patients with definite APS that met the inclusion criteria of the study, we observed a rate of 9% of aPL negativisation, most of patients (78.3%) had a single aPL positivity.
- aPL negativisation is not a negligible fact in the routine clinical practise and it mainly occurs in patients with low-risk aPL profile at diagnosis.

Authors' Contributions
M.R., S.S., I.C. and K.S. retrieved data from patients' files, drafted the manuscript and tables and critically reviewed the manuscript. M.R., S.S., I.C., D.R., M.A. and M.C. drafted the manuscript and critically reviewed the manuscript.

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None declared.

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