**Patients with antiphosholipid syndrome and thrombotic recurrences: A real world observation (the Piedmont cohort study)**

# Patients with antiphosholipid syndrome and thrombotic recurrences: a real world observation (the Piedmont cohort study)

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**Short running  title: Thrombotic recurrences in APS.**

**Summary.**

*Background:* Patients with Antiphospholipid Syndrome (APS) often have thrombotic recurrences, sometimes despite appropriate ongoing anticoagulant treatment. Identifying APS vascular patients at high risk for thrombotic recurrences is still an unsolved issue.

*Objectives:* to report the real-life experience of thrombotic recurrences in APS patients included in Piedmont observational cohort study. Secondly, we aimed to evaluate clinical and laboratory risk factors for thrombotic recurrences in a wide.

*Patients:* A multi-center observational study was performed by enrolling 177 patients with vascular APS [primary APS in 99 subjects (56%)]; the median follow-up was 5 years (1-26).

*Results:* The observed thrombotic recurrence rate was about 7.5/100 patient years in the first 5 years after the first thrombotic event. While the first recurrence often occurred (45%) in patients who were not on oral anticoagulant therapy (OAT), the second recurrence mainly occurred despite ongoing OAT (80%). However, due to the real-life observational nature of this study, treatment was based on treating physician's judgment and no structured therapeutic protocol was applied. Moreover, compliance of OAT are not available. No differences in antiphospholipid antibodies (aPL) profile were observed between patients with or without thrombotic recurrences, but a high risk aPL profile (Miyakis type1 and 2a) was present in 96% of our patients, 26% of whom had triple positivity. Diabetes ($p< 0.01$, OR 10), inherited thrombophilia ($p< 0.0078$, OR 4) and *OAT withdrawal* were independent risk factors for recurrences.

*Conclusions:* With the limit of a real-life observational cohort study, the thrombotic recurrence rate in APS was as high as 7.5/100 patient years in the first 5 years after the first thrombotic event. OAT discontinuation**,** diabetes and inherited thrombophilia, when associated to a high-risk aPL profile, are risk factors for thrombotic recurrences.

**Keywords:** anticoagulation, antiphospholipid syndrome, thrombosis.

**Introduction**

The antiphospholipid syndrome (APS) is a systemic, acquired, immune-mediated syndrome characterized by thrombotic events (venous, arterial or of the microcirculation) and/or by pregnancy morbidities, which is associated to the persistent presence of auto-antibodies that target a specific phospholipid-binding protein. These antibodies can be detected by two different methods: a functional, aPTT-based method for Lupus Anticoagulant (LA), and an immune assay for both anticardiolipin antibodies (ACL) and anti-beta2 glycoprotein1 (antiB2GP1). Confirmed positivity of at least one of these three tests after three months, and the presence of a thrombotic event or an obstetric morbidity suffice to confirm the diagnosis of APS (1,2)**.** APS patients seem to have thrombotic recurrences more frequently than non-APS thrombotic patients, and recently some reviews have focused on this issue (3,4). In the literature there is consistent evidence that laboratory antiphospholipid antibodies (aPL) profile (number of positive tests) may correlate with thrombotic risk (5,6). More than one positive test or LA positivity alone seem to be the strongest risk factors for thrombosis; moreover the "triple" positivity in the aPL profile (LA+ACL+antibeta2GP1) is emerging as an even stronger risk factor for thrombosis (7,8,9,10,11)**.** In APS patients with thrombotic events, thrombosis recurrence rates have been described as ranging between 1 and 16/100 patients-year in the various studies applying different classification criteria and therapeutic strategies (12-17)**.** However, the quality of these studies is poor, mainly because of a lack of standardization of the laboratory tests and low adherence to the classification criteria. Therefore identifying APS patients at higher risk for recurrent

thrombosis is still an unsolved issue. A group of physicians and biologists from several University Departments and Hospitals in the Piedmont area of Italy ("APS Piedmont Consortium") have been working together in an effort to upgrade and standardize laboratory diagnoses and to improve and share therapeutic strategies for APS patients. A cohort of APS patients were diagnosed according to the ongoing guidelines (1). Piedmont, which is in northwestern Italy, is an area with about five million people. The general characteristics of this cohort have already been published (18). The "APS Piedmont Consortium" is part of the Rare Diseases Network of the National Health Service. Over the last few years, careful attention has been paid within the APS Piedmont Consortium to the problem of thrombotic recurrences, a serious and often invalidating clinical problem in this setting. The aim of our study was to report the real-life experience of thrombotic recurrences in APS patients included in Piedmont observational cohort study. Secondly, we attempt to: (1) to calculate the thrombotic recurrence rate; 2) to evaluate the ongoing treatment (if any) at the time of the recurrence (treatment failure); 3) to evaluate clinical and laboratory risk factors for thrombosis (aPL profile and other risk factors, including the association with concomitant other autoimmune conditions ); 4) to evaluate major bleedings and deaths).

## Methods

*Study design and population*

Two hundred-seventeen patients with APS included in the network of the Piedmont Consortium were screened, and, among these, 177 patients were enrolled as vascular APS

(defined as APS patients with a thrombotic index event) in this real word observation study; 40 obstetrical APS patients were excluded. Primary APS was diagnosed in 99 subjects (56%), while in 78 APS was associated to another autoimmune condition, mainly Systemic Lupus Erythematosus (Table 1). APS patients were diagnosed between January 1995 and December 2010. The study was performed according to the principles of the Declaration of Helsinki and written consent was obtained at the time of inclusion in the Rare Diseases Registry. A centralized board evaluated and confirmed both laboratory results and the objectively diagnosed thrombotic events. All the APS diagnoses that were made before 2006 were revised (and eventually confirmed) afterwards according to the ongoing International Society on Thrombosis and Haemostasis classification criteria. An external quality assessment (EQA) of laboratory diagnosis was periodically organized and performed by the participating laboratories. It was not among the aims of the centralized board to assess therapeutic decisions, as no standard structured protocol was applied according to the real life observational fashion of this study. Therapy was managed by treating physicians (Hematologist, Rheumatologist or Immunologist) on the base of their judgment and current literature recommendation (19-23). Main therapeutic strategies can be summariesed as follows (19-23). Overall, patients suffering their first venous event received oral anticoagulant therapy (OAT) with INR 2-3; patients suffering their first arterial thrombosis received either OAT (INR 2-3) or anti-platelet therapy (therapeutic option relies on treating physician's choice). Patients with recurrent events during anticoagulant treatment were then treated with either low-dose aspirin (ASA) associated to OAT (INR 2-

3), or with OAT (INR 3-4). All APS patients receiving OAT were followed-up by anticoagulant clinics.

Similarly, no standard bridging plan was provided when stopping OAT was planned and therapeutic options were chosen case by case by treating physicians.

*Data collection and clinical features at diagnosis*

Patients' data were anonymously entered into a database (Excel). The database was shared among all the hospitals of the APS Piedmont Consortium, and the reported characteristics included: sex, race, age, age at diagnosis, underlying autoimmune disease, if any, characteristics of the first thrombotic event, aPL profile, inherited thrombophilia assay, cardiovascular risk factors, site and characteristics of all thrombotic recurrences. Cardiovascular risk factors included: diabetes mellitus, blood pressure, total and HDL cholesterol, BMI, smoking habit and family history. Risk factors for venous thrombosis were: recent surgery, immobilization, oral estroprogestinic therapy, pregnancy, malignancy, family history, genetic thrombophilia (anti-thrombin, protein C and protein S, factor V Leiden and prothrombin G20210A mutations, hyperhomocysteinemia, high factor VIII:C levels). Ongoing therapy at the time of recurrences, if any, was also recorded.

*aPL assay*

Patients were classified as being LA positive if LA antibodies were detected on two or more occasions at least 12 weeks apart according to the revised International Society on Thrombosis and Haemostasis (ISTH) three step diagnostic strategy (24)**.** The following panel of coagulation tests was performed for each patient: a sensitive APTT [low phospholipids and silica as activator, Silica Clotting Time (SCT Screen, Instrumentation Laboratories Company, USA) or PTT-LA (Diagnostica Stago, France)] and the DRVVT [LAC Screen, Instrumentation Laboratories Company, USA or DRVVT Diagnostica Stago].

The confirmatory tests we used were commercially available assays that increased the phospholipid concentration in the screening tests. LA was diagnosed when at least one of the screening procedures and one of the confirmatory procedures were positive, regardless of the mixing study for patients with weak prolongation of the screening assay. Before 2009, the criteria proposed by the SSC Sub-committees for Standardisation of Lupus Anticoagulant were used for the diagnosis of the phospholipid-dependent inhibitors of coagulation (25). LA was diagnosed when at least one of the screenings and one of the confirmatory procedures were positive on two or more occasions at least 6 weeks apart. ACL and antiB2GP1 IgG and IgM isotypes were measured using commercially available enzyme linked immunosorbent assay (ELISA) kits (Orgentec Diagnostika, Aesku Diagnostics and Inova Diagnostics) and more recently (since 2009) an automated fluorescence enzyme immunoassay (EliA, Phadia Laboratory System). The results of ACL testing were expressed in GPL/ml and MPL/ml and the results of antiB2GP1 were reported in arbitrary units (U/ml). Prior to 2006, patients were considered positive to aPL immunoassays if IgG and IgM ACL values exceeded 15 GPL or 11 MPL units, respectively, and if IgG and IgM antiB2Gp1

exceeded 10 units in 2 or more occasions at least 6 weeks apart. Since 2006, the Sydney updated classification criteria (1) have been used for the cut-off values.

*Outcome events*

Venous thromboembolism (VTE) was diagnosed using compression ultrasonography for deep vein thrombosis, while computed tomography (CT) or ventilation-perfusion lung scans were used for pulmonary embolism. Intracerebral thrombosis was assessed by CT scan or by magnetic resonance. Retinal thrombosis was diagnosed by ophthalmologic examination and fluoroangiography. Peripheral or mesenteric artery thrombosis were documented by arteriography or at surgery. Acute myocardial infarction was diagnosed in the presence of a typical clinical presentation associated with electrocardiographic features and elevated cardiac enzymes (CK-MB or troponins). Stroke was defined according to standard definitions. Major bleeding was defined as any of the following: fatal bleeding, clinically overt bleeding associated with a >2g/dl drop in hemoglobin levels over 24 h and/or requiring an unplanned transfusion of >2 Units of packed red blood cells or whole blood, intracranial bleeding (documented by imaging), retroperitoneal bleeding, intraocular bleeding causing blindness, joint hemorrhage, or the need for surgery or angiographic intervention to stop the hemorrhage. All other bleeding events that did not match the criteria for major bleeding were considered minor bleeding. The causes of death were obtained from clinical or autopsy reports and/or death certificates.

**Statistics**

Demographics and clinical data were analyzed using descriptive statistics. Prospective observation of thromboembolic events was monitored by applying cumulative incidence occurrence measurements, according to the common epidemiological definition. Logistic regression was adopted in order to investigate the relationship between undesirable outcome and potentially explanatory variables, including: sex, race, age, age at diagnosis, underlying autoimmune disease, if any, characteristics of the first thrombotic event, aPL profile, inherited thrombophilia assay, cardiovascular risk factors (as detailed in *Data collection and clinical features at diagnosis).*

The model generates parameter estimates for each interest variable, finally β coefficients may easily be converted into odds ratio scores. Lastly, a log-rank test was applied to evaluate statistic significance of cumulative incidence distribution by therapy exposure.

## Results

The demographic and clinical characteristics of the APS patients are summarized in Table 1. The index event (the first thrombotic event) at diagnosis was: VTE in 97 cases (55%), arterial thromboembolism in 77 cases (43%), while 3 patients had both arterial and venous thromboses.

*Thrombotic recurrences and treatment*

The cumulative incidence of thrombotic recurrences, both arterial or venous, was 12.5% at 1 year, 29% after 3 years, 38% after 5 and 49% after 10 years (Fig.1). Fifty-five patients had a first recurrence (39% were arterial and 61% venous); at the time of the first recurrence 55% (n=30) were receiving treatment and 45% (n=25) were off therapy. Among patients off therapy, 14 had the diagnosis of APS in concomitance of the recurrence, and for this reason they were not under OAT at that time; 5 patients were not under OAT because of spontaneous withdrawal, or withdrawal for clinical choice and 6 stopped ASA after the first arterial thrombotic event. About patients on treatment 14 were on OAT (alone or plus ASA) and 16 were on anti platelet therapy.

Twenty patients had a second thrombotic recurrence (33% arterial and 67% venous), 80% (n=16) were under treatment and 20% (n=4) were off therapy: 2 patients spontaneously stopped OAT, 2 patients stopped OAT because of surgery. Among patients who were on treatment: 11 were on OAT (alone or plus ASA) and 5 were on anti platelet therapy (see Fig.2). If patients are splitted into two groups (Fig.3), according to long term OAT (with or without ASA) versus ASA or no treatment, calculated recurrences rate were significantly higher in the second group ($p<0.0001$), being respectively 12% and 37% at three years, 14% and 46% at five years, and 19% and 56% at eight years .

*Clinical and laboratory risk factors for thrombosis*

*Clinical characteristics of patients with and without thrombotic recurrences are shown in table 2.*

Among the cardiovascular risk factors for thrombosis (diabetes mellitus, blood pressure, total and HDL cholesterol, BMI, smoking habit and family history), multivariate analysis showed that diabetes (n=13, $p<0.01$, OR 10, CI 1.6-73.4) and thrombophilia (n=18, $p<0.008$, OR 4; CI 1.5-12.8) were both statistically significant and independent risk factors for the first recurrence.

When stratifying according to aPL positive tests, high risk aPL profiles, i.e. double positivity or LA positivity alone were highly represented (96%) . Moreover, 26% of patients had triple positivity (LA + ACA+ antibeta2 GP1). The distribution of double positivity or LA positivity alone and of triple positivity, did not differ between patients with one or two recurrences and patients with no recurrences (p=0.57).

*Major bleedings and deaths*

There were ten major bleeding events; 8 occurred while on OAT and 2 on ASA.

1.3/100 patients/year major bleedings were observed during the follow-up period.

There were 2 deaths: one was cardiovascular and one was due to neoplasia.

## Discussion

This study aimed to capture and report a real life scenario of thrombotic recurrences in a well-selected cohort of patients with APS from the Piedmont observational cohort study. Thrombotic recurrences are frequent and often invalidating in APS patients, for whom risk factors for thrombotic recurrences have not yet been well defined. This is a “real-life”

observational study, in which APS patients were also tested for inherited thrombophilia and cardiovascular risk factors.

The observed cumulative incidence of thrombotic recurrences was about 7/100 patient years in the five years after the first thrombotic event. Fifty-five % of patients were on treatment (ASA or OAT) at the time of the first recurrence, but only half were on anticoagulant treatment (OAT). On the contrary, at the time of the second recurrence, 80% of patients were on OAT treatment. As shown in Fig. 2, APS patients on warfarin treatment have statistically significant fewer thrombotic recurrences ($p<0.01$) than patients on antiplatelet therapy or off treatment. With limitations of non-randomized study, in our cohort thrombotic recurrences seem to be related to either early OAT discontinuation or, more infrequently, to a possible treatment failure (a thrombotic recurrence despite ongoing OAT treatment). However, although patients were followed in dedicated OAT clinic, INR values were not available at the time of recurrences for all the patents, thus making the diagnosis of treatment failure speculative in selected cases.

Accurate clinical and laboratory diagnosis allowed us to obtain a cohort with an "high risk aPL profile": 26% of patients had triple positivity, 24% had double positivity, and 46% had LA positivity alone. The aPL profile itself, including triple positivity, was not found to correlate with thrombotic recurrences. Multivariate analysis showed that diabetes (n=13, beta=2.37; p=0.02) and inherited thrombophilia (n=18; beta=1.46, p< 0.01) were both statistically significant, independent risk factors for thrombotic recurrences. Moreover, in

this cohort, the index event at diagnosis did not predict the type of recurrence (arterial or venous). This result is similar to what was reported by Pengo et al (7).

In summary, the strengths of this study relies on the presence of wide sample of APS patients, with laboratory and clinical diagnosis established according to ongoing (at the time) classification criteria and validated by a centralized board; OAT was monitored by OAT clinics for almost all patients. Laboratory diagnosis were performed in laboratories that periodically performed an EQA in this setting.

The limits of this study are the followings. a) quantitative value for aPL are not available, but results are expressed as "positive" or "negative" according to cut off values from ongoing guidelines; b) laboratory diagnosis were not centralized in a core laboratory; c) time in range for each OAT clinic is available (data not shown), but not the INR value at the time of the recurrent thrombotic event.

Most importantly, assessing the efficacy of any therapeutic approach or protocol to prevent recurrences was out of the scope of this very study. Finally, patients were not stratified for ongoing treatments other than OAT and anti-platelets nor for bridging therapy at the time of surgery as those data were not collected.

In conclusion, in our APS cohort the observed recurrence rate was as high as 7.5/100 patient years in the first 5 years after the first thrombotic event. Despite the intrinsic limit of a non-controlled observational study, the high recurrence rate in non-OAT treated patients underlines the need for long-term OAT in high risk APS patients (26). According to our results, patients at "high risk" would be defined not only by their aPL profile, but also by associated inherited pro-thrombotic conditions or cardiovascular risk factors, in particular

diabetes. Achieving a better understanding of the risk profile and defining new anticoagulant strategies (higher INR ? association of OAT to ASA ? new direct oral anticoagulants ?) or the association with other drugs (statins? Hydroxychloroquine? immunodepressant ?) is still an unmet clinical need in high risk APS patients, and must be the object of future studies.

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## Disclosure of Conflict of Interest

The authors declare no competing financial interests.

**Figure legends**

Figure 1. Cumulative incidence of recurrent thromboembolic events in 177 APS vascular patients

Figure 2: Flow chart concerning thrombotic recurrences of 177 APS vascular patients whit respect to ongoing antithrombotic treatment (if any) at the time of event.

Figure 3. Cumulative incidence of recurrent thromboembolic events in APS vascular patients according to treatment with or without oral anticoagulants. No OAT (dotted line) = no treatment or antiplatelet treatment. OAT (continuous line) = oral anticogulant treatment alone or associated with antiplatelet therapy.

* Log-rank test was applied to evaluate statistical significance of cumulative incidence distribution by therapy exposure ($P<0.001$)

Table 1. Demographic and clinical characteristics of 177 APS vascular patients at their first thrombotic event.

Table 2 Clinical characteristics of patients with and without thrombotic recurrences

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