



## Advances in Basic, Laboratory and Clinical Aspects of Thromboembolic Diseases\*

### THE ITALIAN REGISTRY OF ANTIPHOSPHOLIPID ANTIBODIES

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#### ABSTRACT

**Background and Objective.** The clinical importance of antiphospholipid antibodies (APA) derives from their association with a syndrome of venous and arterial thrombosis, recurrent fetal loss and thrombocytopenia known as the *antiphospholipid syndrome* (APS). The *Italian Registry of Antiphospholipid Antibodies* was set up in 1989 for the purpose of collecting a large number of patients with lupus anticoagulant (LA) or anticardiolipin antibodies (ACA) for clinical studies in order to obtain more information on the clinical features of APS.

**Evidence and Information Sources.** The Italian Registry has completed two clinical studies and proposed an international trial on the treatment of APS patients. These activities of the Registry are reviewed herein. Additional information has been obtained from pertinent articles and abstracts published in journals covered by the Science Citation Index<sup>®</sup> and Medline<sup>®</sup>.

**State of art.** The first study of the Registry was a retrospective analysis of enrolled patients which showed that: a) the prevalence of thrombosis and thrombocytopenia was similar in cases with idiopathic APA or APA secondary to systemic lupus erythematosus, and b) the rate of thrombosis was

significantly reduced in patients with severe thrombocytopenia but not in those with only a mild reduction of the platelet count. The second study was a *prospective* survey of the natural history of the disease, showing that a) previous thrombosis and ACA titer > 40 units were independent predictors of subsequent vascular complications; b) a history of miscarriage or thrombosis is significantly associated with adverse pregnancy outcome; c) hematological malignancies can develop during follow-up and patients with APA should be considered at increased risk of developing NHL. Thus the possibility of a hematologic neoplastic disease should be borne in mind in the initial evaluation and during the follow-up of these patients.

**Perspectives.** The latest initiative of the Registry was the proposal of an international, randomized clinical trial (WAPS study) aimed at assessing the efficacy and safety of high-dose warfarin in preventing recurrent thrombosis in patients with APA and vascular disease. The study is scheduled to start in March 1997.

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**A**ntiphospholipid antibodies (APA) are a heterogeneous family of immunoglobulins directed against different protein-phospholipid complexes.<sup>1</sup> They include lupus anticoagulants (LA) and anticardiolipin antibodies (ACA). The former recognize the (human) prothrombin-phospholipid complex, in this way inhibiting the phospholipid-dependent coagulation reactions,<sup>2</sup> whereas the latter are directed towards  $\beta$ 2-glycoprotein I ( $\beta$ 2-GPI) bound to anionic lipid surfaces.<sup>3,4</sup> ACA can be divided into two groups:<sup>5</sup> ACA-type A, which inhibit coagulation reactions by enhancing

the binding of  $\beta$ 2-GPI to the procoagulant phospholipid surface; and ACA-type B, which are devoid of anticoagulant properties. The two types of ACA can be differentiated by simple laboratory tests and might be associated with a different prevalence of thrombotic complications.<sup>6</sup>

The clinical importance of APA derives from their association with a syndrome of venous and arterial thrombosis, recurrent fetal loss and thrombocytopenia known as the *antiphospholipid syndrome* (APS)<sup>7</sup> (Figure 1). APS may occur in patients with an underlying systemic autoimmune disease like

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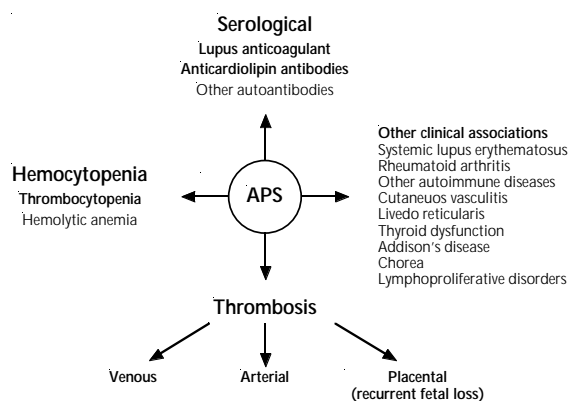


Figure 1. Clinical, serological and hematological manifestations of the antiphospholipid syndrome (APS) (modified from ref. #8).

systemic lupus erythematosus and lupus-like disease, or in patients without manifestations of a systemic disease, the so-called primary APS (PAPS).<sup>8</sup> Cumulative literature<sup>9,10</sup> indicates that a history of thrombosis is present in approximately 30-40% of patients with APA. However, the risk factors for thrombosis and the optimal treatment of the syndrome are still uncertain.

To obtain more information on the clinical features of APS, a Registry of APA patients has been running in Italy since 1989. To date, the Italian Registry has completed two clinical studies and proposed an international trial on the treatment of APS patients. These activities of the Registry will be briefly reviewed herein.

#### **Retrospective analysis of thrombosis and thrombocytopenia in patients with primary or secondary APS**

The purpose of the retrospective analysis was twofold: first, to establish whether the prevalence and type of vascular complications were different in patients with the primary or the secondary syndrome; and, second, to assess whether the presence of thrombocytopenia influenced the rate of thrombosis.<sup>11</sup>

Three hundred and nineteen patients (M/F 80/239, median age 31 ys., range 2-76) with primary APA (n=207, 65%) or APA secondary to overt SLE (n=112, 35%) were evaluated. Diagnosis of LA was established according to recommended criteria:<sup>12</sup> a) prolongation of at least one phospholipid-dependent clotting test; b) persistent abnormality (ratio patient:normal >1.2) of the test(s) after 1:1 mixing of patient's plasma with normal pooled plasma; c) modification of the clotting time on changing the phospholipid concentration (i.e. cor-

rection on increasing the phospholipid concentration and/or prolongation with phospholipid dilution). IgG ACA were assayed with the ELISA procedure described by Loizou *et al.*<sup>13</sup> Values were expressed as GPL Units and considered negative (<10 U), low but positive (10-40 U) or highly positive (>40 U).

Arterial and venous thromboses were registered in 71 idiopathic and 47 secondary cases (34% vs. 42%, n.s.). In both groups, arterial thromboses were most frequently cerebral (81% vs. 85%) and venous thromboses were localized in the deep leg veins (64% vs. 58%). Thrombocytopenia (platelets <100×10<sup>9</sup>/L) was present in 26% of cases and was severe (<50×10<sup>9</sup>/L) in 11%. The prevalence of bleeding and thrombosis in patients with different platelet numbers is shown in Table 1. The rate of vascular complications was similar in patients with mild thrombocytopenia and in non-thrombocytopenic patients (32% vs. 40%). However, severe thrombocytopenia was associated with a significantly lower prevalence of thrombosis (9%, p<0.01).

In conclusion, this retrospective analysis of Italian APA patients demonstrated that the occurrence of thrombosis was independent of the presence of underlying autoimmune disorders or moderate thrombocytopenia, whereas it was significantly lower in patients with severe thrombocytopenia.

#### **Prospective study of the natural history of the disease**

All APA patients enrolled in the Registry were followed over a five-year period to assess the natural history of the syndrome and the risk factors for the occurrence of thrombotic complications.<sup>14</sup>

In all, 360 consecutive patients (M/F 118/242, median age 39, range 2-78 ys) who fulfilled the above defined criteria for a diagnosis of lupus anticoagulant (n=326) and/or raised IgG anticardiolipin (n=185) were collected from 16 Italian institutions and prospectively observed at least every six months as outpatients. Each check-up included clinical and laboratory examinations and, if necessary, instrumental investigations. No mandatory guidelines for therapy were established, but treatment was left to the responsibility of the physician in charge in each center. Twenty-three patients (6.4%) were lost to follow-up. Median follow-up was 3.9 years (range 0.5-5).

Main end points were: the occurrence of arterial or venous thrombosis, the outcome of pregnancies and any severe complications leading to hospitalization or death.

Thirty-four patients suffered thrombotic complications during the follow-up, with a total incidence of 2.5% pt-yr. Thromboses were arterial in 17 (16 cerebral and 1 peripheral) and venous in 17 cases

Table 1. Bleeding and thrombosis in APA patients enrolled in the Registry according to platelet number (retrospective study).

plt no. $\times 10^9/L$	total, n (%)	Thrombosis, n. (%)	Bleeding, n. (%)
< 50	32 (11)	3 (9)*	2 (6)
50-100	44 (15)	14 (32)	—
>100	213 (74)	87 (40)	2 (1)
Total	289	104 (36)	4 (1.4)

\* $p < 0.01$

Table 2. Multivariate analysis of factors related to risk of thrombosis in 360 APA patients enrolled in the Registry (prospective study).

Variable	Relative risk (95% CI)	p
Age < 40 yrs	1.02 (0.54-1.90)	0.96
Female sex	1.41 (0.49-4.06)	0.52
Previous thrombosis	4.90 (1.76-13.7)	<0.005
Underlying SLE or SLE-like	1.72 (0.81-4.02)	0.10
ACA titer > 40U	3.66 (1.24-10.8)	< 0.01
Participating center	0.97 (0.90-1.04)	0.42

Previous abortions and cigarette smoking not included because not significant in univariate analysis (not shown).

(9 deep vein thromboses of the legs, 4 pulmonary embolisms, 3 superficial thrombophlebitis and 1 Budd-Chiari syndrome). One ischemic stroke and one occlusion of the hepatic veins were fatal. Twenty-six thromboses (76%) were apparently spontaneous, whereas 8 were triggered by infections (three), pregnancy (two), surgery (two) or immobilization. Twenty-four thromboses (73%) were recurrences and four (12%) were observed during oral anticoagulant prophylaxis.

Multivariate logistic regression analysis identified previous thrombosis and IgG ACA titer above 40 units at enrollment as independent risk factors for subsequent thrombotic events (Table 2).

A total of 28 pregnancies were observed in 25 women and 11 were unsuccessful (39%). Women with a history of miscarriages or vascular occlusions had a significantly higher rate of adverse pregnancy outcome (9/16, 66%) than asymptomatic women (2/12, 17%; Fisher's exact test  $p=0.035$ ).

Five patients (age range 42-70 yrs.) developed a malignant neoplastic disease during the follow-up, after a median of 32 months (range 12-88) from the first diagnosis of APA. One patient (42 yrs., idiopathic APA) developed a breast carcinoma and four presented non-Hodgkin lymphomas (NHL), including: small lymphocytic NHL; Waldenström's disease; diffuse, small cleaved cell NHL and diffuse, large cell NHL.

Eighteen patients (age range 22-73 yrs.) died during the follow-up. Hematological cancers and vas-

cular complications (including three sudden deaths) were the most frequent causes, amounting to five cases each.

The main result of this study was to identify previous thromboses and high ACA titer as independent predictors of thrombosis. Patients with a history of vascular events showed a 5.4% pt-yr incidence of further complications as compared with 0.95% pt-yr in asymptomatic subjects. Age, sex, previous miscarriages, underlying SLE or related disease and smoking were not risk factors for thrombosis.

Surprisingly, hematological malignancies were a major cause of morbidity and mortality in our study. In particular, four new cases of non-Hodgkin lymphoma were recorded, with an estimated rate of 0.28% pt-yr. This is far higher than the expected incidence of NHL, which ranges between 5 and  $15 \times 100,000/yr$ . Cancer is not usually considered a characteristic feature of patients with APA. However, an association of APA and malignancies has been described in some cross-sectional studies.<sup>15</sup> These findings suggest that patients with APA should be considered at increased risk of developing NHL and that the possibility of a hematologic neoplastic disease should be borne in mind in the initial evaluation and during the follow-up of these patients.

#### **Management of the antiphospholipid syndrome and proposal for a clinical trial of high-dose warfarin (WAPS study)**

The results of our prospective follow-up study clearly showed that asymptomatic patients are at low risk of developing thrombotic complications, supporting the general agreement that they need no active treatment. Thrombocytopenia in APS rarely requires therapy either.<sup>16</sup> However, when it is necessary the same treatment policy as for autoimmune thrombocytopenia should be considered<sup>17</sup> (Figure 2).

Pregnant women with a previous history of recurrent fetal loss need to be treated. Current evidence seems to suggest that standard (or low molecular weight) heparin combined with low-dose aspirin is a relatively safe and effective treatment. Prednisone and other corticosteroids may be needed for treatment of associated autoimmune disorders, but the combination of heparin and prednisone should be limited as much as possible because of the increased risk for vertebral fractures<sup>18</sup> (Figure 2).

Secondary prevention of vascular complications in APS patients is a difficult task. No prospective clinical trial has been published so far and current recommendations are based on retrospective series of consecutive patients<sup>19</sup> (Figure 2). In two studies,<sup>20,21</sup> high intensity warfarin (PT INR > 3) conferred better antithrombotic protection than lower intensity warfarin or aspirin. However, there is con-

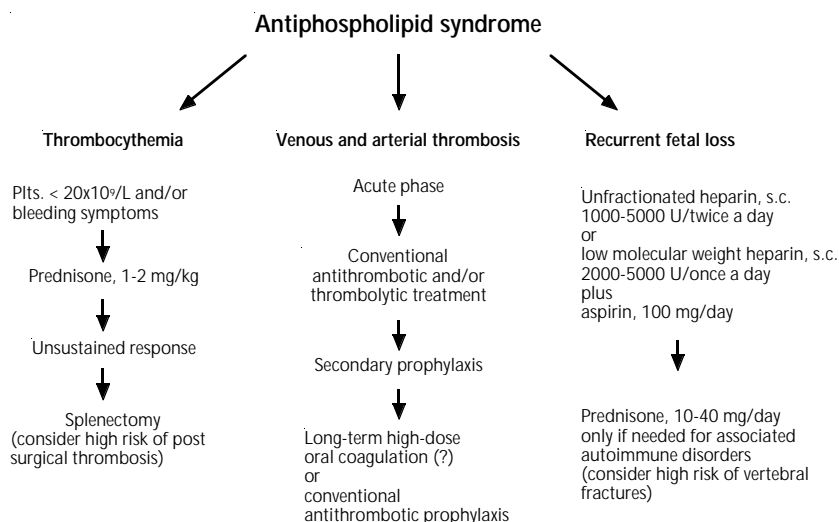


Figure 2. Summary of recommendations for the management of patients with the antiphospholipid syndrome.

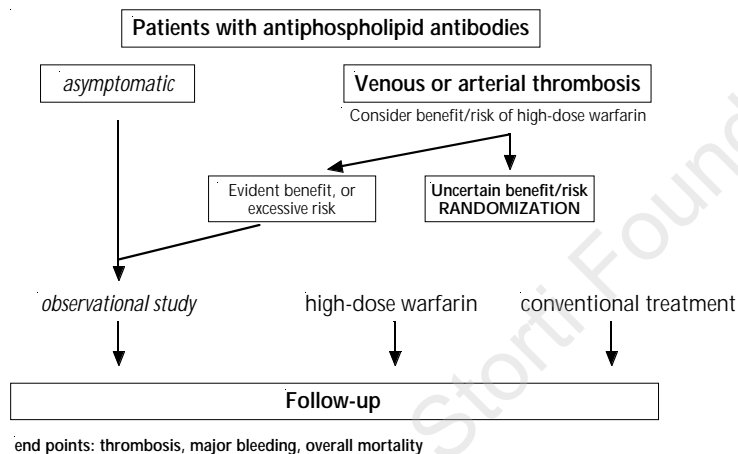


Figure 3. General design of the WAPS (Warfarin in the AntiPhospholipid Syndrome) trial.

cern about the implications of recommending this therapy on the basis of retrospective and nonrandomized data.<sup>22-24</sup> Fatal, cerebral or uncontrollable bleeding was reported during anticoagulation and the cumulative risk of hemorrhage is expected to increase with the duration and intensity of treatment. Therefore, the *Italian Registry of APA* proposed a randomized, prospective clinical trial, referred to as the WAPS study (Warfarin in AntiPhospholipid Syndrome), with the aim of assessing the efficacy and safety of high-dose warfarin in controlled conditions.<sup>25</sup>

The general design of the WAPS study is summarized in Figure 3. Men and women aged 14-65 ys. with primary or secondary APS diagnosed within the last five years are eligible for the study. APS is defined by the presence of: a) lupus anticoagulant or moderate to high titer of anticardiolipin antibodies, and b) one or more previous thromboses (myocardial infarction, ischemic stroke, TIA, acute

peripheral arterial thrombosis, venous thromboembolism). Eligible patients will be randomized to high-dose warfarin (PT INR target range 3.0-4.0) or to standard management, that is the current clinical practice in each participating Center (for example, aspirin for stroke or low-dose warfarin, PT INR 2-3, for venous thrombosis). Endpoints of the study are: a) mortality from all causes; b) recurrences of thrombosis, and c) major bleeding. Since our previous prospective survey suggested that the rate of recurrent thrombosis in the standard arm is at least 5% per year, if high-dose warfarin can reduce the incidence by 50%, then 450 patients per arm should be evaluated in a 2-year follow-up. Multicenter, worldwide collaboration is planned to enroll this number of patients and the trial is presently in the organizational phase in the setting of the *SSC Subcommittee for Standardization of Lupus Anticoagulant of the International Society on Thrombosis and Hemostasis*.

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