# Long-term treatment of antiphospholipid syndrome with intravenous immunoglobulin in addition to conventional therapy

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## Abstract

### Objective

*This work aims to prospectively assess the long-term effects of intravenous immunoglobulin (IVIG Flebogamma®) in a small cohort of patients affected by primary or secondary antiphospholipid syndrome (APS), in addition to conventional therapy.*

### Methods

*Three primary and four secondary APS patients (6 women and 1 man), aged between 40 and 62 years, were treated with IVIG in addition to conventional therapy with anticoagulants or antiplatelets, while six primary and one secondary APS patients (6 women and 1 man), aged between 31 and 61 years, continued their regular conventional therapy. One infusion of IVIG was administered at a dose of 0.4 g/kg/day every month to the first group of patients for two years. Patients were assessed at baseline, after 1 year and 2 years from the beginning of the study and were evaluated for the occurrence of any thromboembolic events and by laboratory measurement of antiphospholipides antibodies (aPL).*

### Results

*No venous or arterial thromboses occurred in patients treated with IVIG, whereas in the control group two patients presented cerebral ischaemic attacks and one patient reported a deep vein thrombosis during the follow-up. At the end of the study, in the group treated with IVIG, we observed a statistically significant decrease of anticardiolipin antibodies (IgG and IgM) and of IgM anti-β2-glycoprotein I antibodies.*

### Conclusion

*Our results show the efficacy of IVIG in addition to conventional therapy, in primary and secondary APS patients, preventing the occurrence of thromboembolic events. However, further clinical studies on a larger group of patients are necessary to fully understand the mechanisms of action and the optimal doses of IVIG in APS.*

**Key words**

antiphospholipid syndrome, intravenous immunoglobulin, antiphospholipid antibodies

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## Introduction

Antiphospholipid syndrome (APS) is an autoimmune disease characterised by vascular thrombosis and/or pregnancy morbidity in the presence of circulating antiphospholipid antibodies (aPL) (1). Currently recognised laboratory criteria for APS include lupus anticoagulant (LA), immunoglobulin (Ig)G or IgM anticardiolipin antibodies (aCL), or IgG or IgM anti-$\beta_2$-glycoprotein I antibodies (anti$\beta_2$-GPI) (2, 3). The management of patients with APS is a subject of controversy (4). In systemic lupus erythematosus (SLE) patients with positive LA or isolated persistent aCL at medium-high titers, primary thromboprophylaxis with hydroxychloroquine (HCQ) and low-dose aspirin is recommended, whereas in non-SLE individuals with aPL and no previous thrombosis, the prophylaxis with low-dose aspirin is suggested. Concerning the secondary thromboprophylaxis in patients with definite APS and a first venous event, oral anticoagulation therapy to a target INR 2-3 is recommended, whereas in patients with arterial thrombosis, an INR more than 3.0 or a combination with antiaggregant treatment is needed (4). Alternative treatment modalities, which include low-molecular weight heparin (LMWH), statins, and HCQ, have been implemented to manage APS refractory to conventional thromboprophylaxis (4). Data on the use of intravenous immunoglobulin (IVIG) in patients with APS focused on the obstetric complications and on catastrophic APS, while the use of IVIG in other clinical manifestations is reported in few studies (5-7).

The purpose of this study was to prospectively assess the long-term efficacy and safety of IVIG (Flebogamma®) in a small cohort of patients affected by primary or secondary APS in addition to anticoagulant or anti-aggregant conventional therapy.

## Patients and methods

We conducted an open-label prospective, comparative study, evaluating 14 consecutive outpatients with primary or secondary APS who accessed our department. The diagnosis of APS was based on a history of venous and/or arterial thrombosis or recurrent miscarriages in the presence of aPL, according to the 2006 updated APS criteria (2). Patients were considered to have secondary APS if they had concurrent SLE as defined by the American College of Rheumatology criteria (8).

Three primary and four secondary APS patients, aged between 40 and 62 years, were treated with IVIG, in addition to conventional therapy with anticoagulants or antiplatelets (Group I) (Table I). Six primary and one secondary APS patients, aged between 31 and 61 years, continued their conventional therapy (Group II) (Table I).

Data on the concomitant therapies and autoantibody positivity are reported in Table I.

Thirteen overall patients (seven in Group I and six in Group II) had a high risk aPL profile, according to the APS Risk Scale (9).

Group I patients received IVIG therapy (Flebogamma®) at a monthly dose of 0.4 g/kg/day for two consecutive years, from September 2010 to August 2012.

All patients were assessed at baseline, after one and two years from the beginning of the study and were clinically evaluated for any occurrence of thromboembolic events. Instrumental investigations (including venous doppler ultrasonography, magnetic resonance imaging, computed tomographic scan) were performed as appropriate if any signs and symptoms of thrombosis were noted. Furthermore, we performed laboratory measurement of aPL (aCL and anti$\beta_2$-GPI IgM and IgG class) according to international guidelines through ELISA assays (2, 10, 11). In the case of aCL and anti$\beta_2$-GPI, only patients with titers of 40 U/mL or higher were selected, in accordance with the current diagnostic criteria for APS (2). LA was detected by coagulation assays adhering to the International Society of Thrombosis and Haemostasis (12).

Possible side effects were carefully assessed at each infusion. Patients were monitored through physical examination (heart and respiratory rate and blood pressure), routinary blood tests (creatinine, electrolytes, complete blood count analysis, IgA, IgG, IgM, erythrocyte sedimentation rate, C3 and C4 components) and urinalysis.

*Statistical analysis*

Student's *t*-test or $\chi^2$ test were used to demonstrate the homogeneity of the two groups variables in basal condition. Because of the small number of patients and the skewness of our data, a statistical non-parametric analysis was performed. On each group, the statistical evaluation was performed using the non-parametric 2-way Friedman test with multiple comparisons for all variables studied among times.

Comparison between groups was performed using the analysis of variance after rank transformation. Differences were considered statistically significant when $p<0.05$.

## Results

Baseline comparison of the two groups showed no statistically significant differences in demographic and clinical characteristics, except for the number of patients with primary APS that is greater in Group II and for the concomitant therapy (Table I).

During the follow-up period, no further clinically or instrumentally confirmed thrombosis occurred in Group I. In Group II, after eight month of follow-up, one patient presented an episode of deep vein thrombosis (DVT) localised in the popliteal vein of his left leg; Prothrombin Time-International Normalised Ratio (PT-INR) value at time of the DVT was 2.67. Furthermore, one patient developed, after one year of follow-up, three consecutive epileptic seizures during the period of one month; magnetic resonance imaging confirmed the ischaemic nature of these attacks (PT-INR at time of first epileptic seizure was 2.82). Another patient, after seven months of follow-up, presented an episode of acute left hemiplegia (PT-INR 2.73); computed tomography scan showed two new small hypodense focal lesions in the posterior branch of the internal capsule and one in the knee of the internal capsule, compared to a previous examination. Figure 1A displays the time course for aCL IgG titer in both groups studied. At the end of the study, we observed a statistically significant decrease ($p<0.01$) of aCL IgG, *versus* baseline, in Group I, but no statistically significant differences were found in Group II. As shown in Figure 1B, the trend difference of aCL IgG after rank transformation was statistically significant ($p<0.01$) between the two groups at the end of follow-up. In Figure 2A, we reported the time course of aCL IgM. At the end of follow-up, the decrease of this variable was statistically significant ($p<0.05$), *versus* baseline, in group I, whereas no statistical significant differences were found in Group II. Figure 2B shows the statistically significant ($p<0.05$) trend difference of aCL IgM after rank transformation, between the two groups. Regarding the measurement of anti$\beta_2$-GPI IgM, in both groups, no statistically significant differences were found at two years' follow-up *versus* baseline. The results obtained for anti$\beta_2$-GPI IgM assessment showed a statistically significant decrease ($p<0.05$) at the end of follow-up *versus* baseline in Group I (data not shown). LA measurement switched from positive to negative, at the end of follow-up, in 3 patients of group I, whereas no similar changes occurred in patients of Group II. According to APS Risk Scale, at the beginning of the study, all patients belonging to Group I, presented a high risk, whereas, after the

**Table I.** Patients' characteristics at baseline.

| Variables | Group I | Group II |
|---|---|---|
| Age (mean±SD) | 47.2±6.5 | 45±10.7 |
| Gender M/F | 1/6 | 1/6 |
| Disease duration (years) (mean±SD) | 10.3±3.58 | 12.4±4.5 |
| Primary/secondary APS (n.) | 3/4* | 6/1* |
| APL profile (n positive patients) | | |
| LA | 7 | 7 |
| aCL IgG | 7 | 7 |
| aCL IgM | 2 | 2 |
| anti$\beta_2$GPI IgG | 6 | 6 |
| anti $\beta_2$GPI IgM | 4 | 3 |
| ANA positive patients (n.) (%) | 6/7 (85.7%) | 6/7 (85.7%) |
| Anti-dsDNA positive patients (n.) (%) | 2/7 (28.6%) | 2/7(28.6%) |
| APS risk scale (n. patients) | | |
| High | 7 | 6 |
| Medium | 0 | 1 |
| Low | 0 | 0 |
| Pregnancy morbidity (n.) | | |
| Miscarriage | 1 | 4 |
| Prematury birth | 2 | 1 |
| Previous thrombotic events (n.) | | |
| DVT | 2 | 3 |
| DVT+PE | 2 | 1 |
| Stroke | 4 | 3 |
| Jugular vein thrombosis | 0 | 1 |
| Renal artery thrombosis | 1 | 0 |
| Anticoagulant therapy (n.) | | |
| Warfarin | 3 | 4 |
| ASA | 2 | 0 |
| Warfarin+ASA | 2 | 1 |
| Acenocoumarol | 0 | 2 |
| Concomitant therapy (n.) | | |
| HCQ | 0 | 2* |
| Azathioprine | 3 | 0* |

Group I: patients treated with intravenous immunoglobulin in addition to conventional therapy; Group II: patients treated only with conventional therapy; SD: standard deviation; M: male; F: female; APS: antiphospholipid syndrome; APL: antiphospholipid antibodies; LA: lupus anticoagulant; aCL: anti-cardiolipin antibodies; antiβ2GPI: anti-β2-glycoprotein I antibodies; ANA: antinuclear antibodies; anti-dsDNA: anti-double strand DNA; DVT: deep vein thrombosis; PE: pulmonary embolism; ASA: acetylsalicylic acid; HCQ: hydroxychloroquine.
*$p<0.05$ $\chi^2$ test.

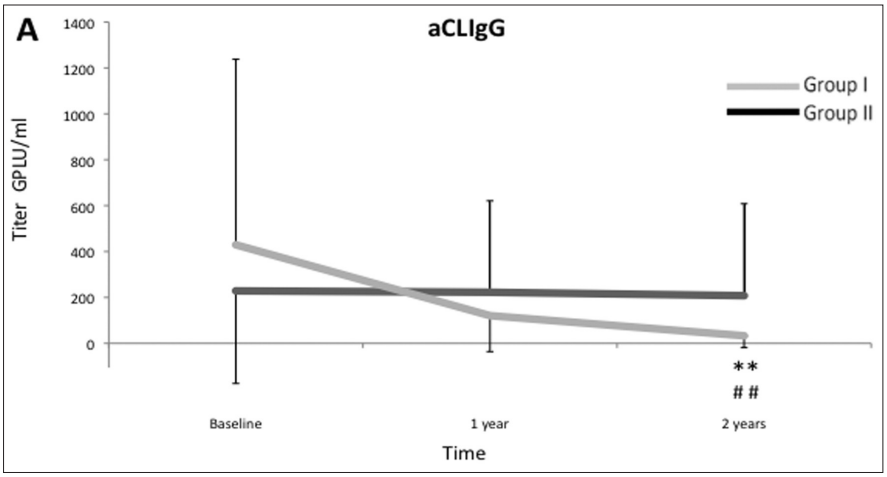


**Fig. 1 A.** aCLIgG titer course over time (mean±SD). Friedman test.
$^{\#\#}p<0.01$ Group I *vs*. Group II. $^{**}p<0.01$ 2 years *vs*. baseline.

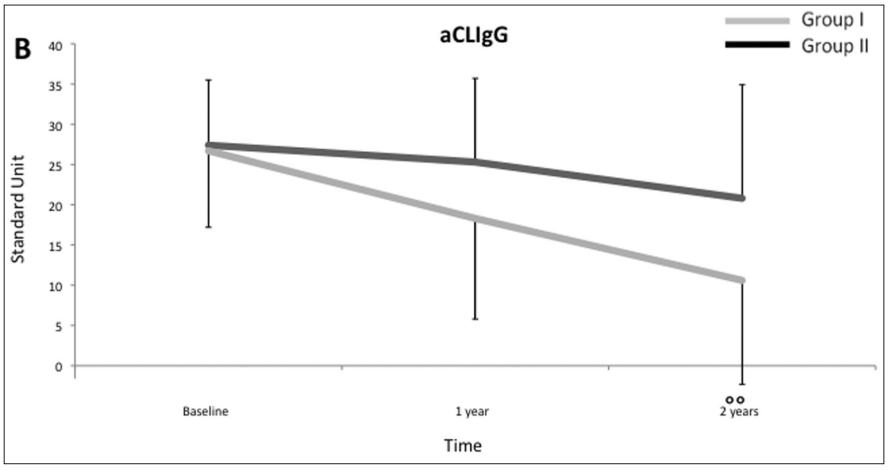


**Fig. 1 B.** ANOVA after Rank Transformation for aCLIgG (mean±SD) over time.
$^{\circ\circ}p<0.01$ Group I *vs*. Group II.

two-year follow-up period, a high-risk class persisted only in 2 patients.

No significant modifications concerning routinary blood tests were found during the follow-up period.

About tolerability of IVIG treatment, during the infusion, only one patient presented side effects, such as headache and facial flushing, so light that he did not interrupt the therapy. Furthermore, no alterations were found in routine blood investigations.

## Discussion

APS is associated with recurrent arterial or venous thrombosis, and pregnancy loss with the presence of aPL and/or LA (2, 3). Nowadays, treatment of APS and its complications remain a challenge. Among alternative treatment modalities to manage APS refractory to conventional thromboprophylaxis, IVIG administration could be useful (5-7). Currently, IVIG is used in the treatment of a wide variety of autoimmune or inflammatory diseases, although the FDA-approved indications are limited (13). Previous experience with IVIG in APS has only included patients with obstetric complications in whom anticoagulation was contraindicated or those with catastrophic APS (5-7).

The aim of the present study was to evaluate efficacy, and tolerability of IVIG at a monthly dosage of 0.4 g/kg/day for two consecutive years in 7 APS patients in addition to conventional therapy with anticoagulants or antiplatelets compared with 7 APS patients who assumed only conventional therapy.

Among commercially available IVIG products, we selected Flebogamma®; because of the absence of sucrose, it can be safely used in diabetic patients and in those with mild renal impairment. Furthermore, Flebogamma® formulation, characterised by liquid 5% concentration, leads to a lower thrombotic risk, not increasing plasma viscosity (14). No current guidelines exist about the duration, frequency or optimal dose of IVIG. Although most studies described a high dose protocol (0.4 g/kg/day for five consecutive days once a month or 1 g/kg/day for two consecutive days once a month) for autoimmune diseases, we selected a low dosage on the basis of previous experience in SLE (15). This dosage allowed us to reduce costs and adverse events and to continue IVIG administration for a long-term period. The results of our study demonstrate the clinical efficacy of IVIG therapy, in fact, thromboembolic events did not occur in Group I, in contrast to what was observed in Group II. Interestingly, we pointed out a statistically significant decrease between basal and final value of aCL IgG and aCL IgM titers in patients treated with IVIG; furthermore, we also observed a negativisation of LA measurement, in 3 subjects of the same group. Despite the good clinical outcome in Group I, we did not observe a statistically significant decrease of anti$\beta_2$-GPI IgG during the follow-up. It is now generally accepted that these antibodies are the most pathogenic in APS, but some diagnostic weaknesses of ELISA test have been reported. In fact, despite the theoretically higher specificity compared to the aCL ELISA, the anti$\beta_2$-GPI ELISAs detect all antibodies reactive with $\beta_2$GPI, including non-pathogenetic antibodies, phospholipid-independent and low affinity a$\beta_2$-GPI, which makes ELISA less suitable as a general diagnostic test (16).

Furthermore, this therapy was safe and well tolerated.

To our knowledge, this is the first comparative study that assesses the efficacy of long-term IVIG treatment in addition to conventional therapy in primary or secondary non pregnant APS patients.

Before us, only Sciascia *et al*. (7) described the use of IVIG in non pregnant APS patients; in this open, prospective non comparative long-term (>5 years) study the authors emphasised the pos-

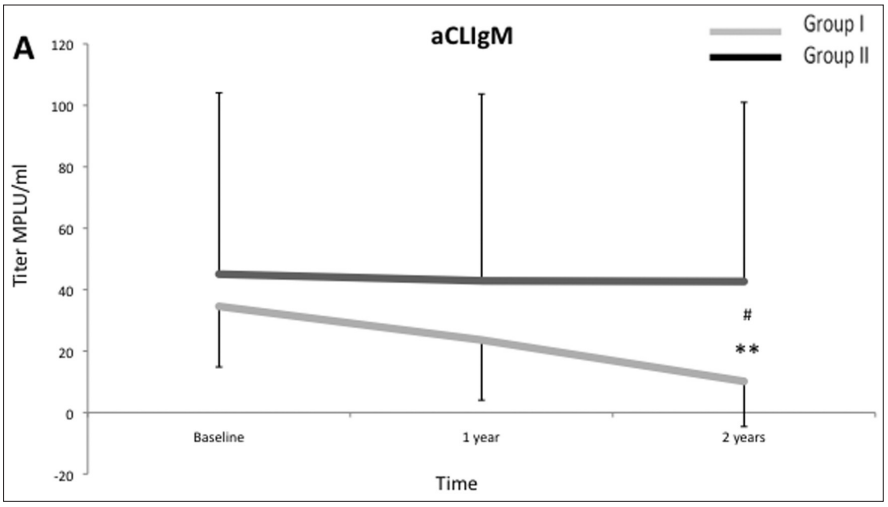


**Fig. 2 A.** aCLIgM titer course over time (mean±SD). Friedman test. $^{\#}p<0.05$ Group I *vs*. Group II. $^{**}p<0.01$ 2 years *vs*. baseline.

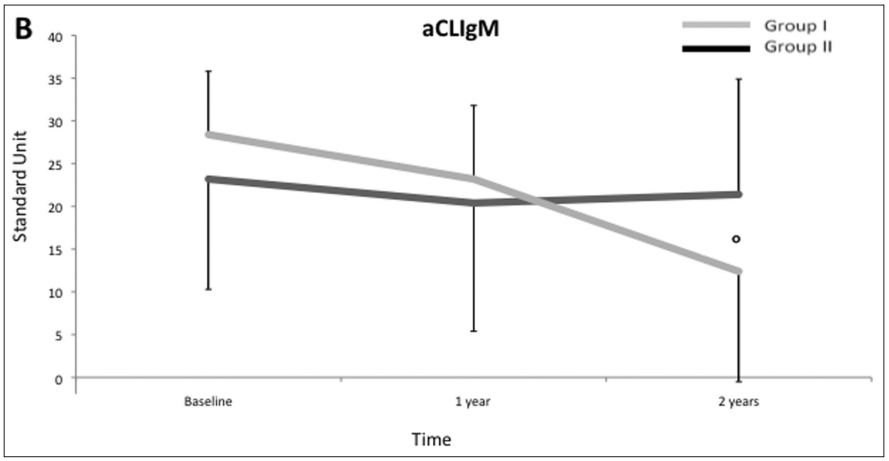


**Fig. 2 B.** ANOVA after Rank Transformation for aCLIgM (mean±SD) over time. °$p<0.05$ Group I *vs*. Group II.

sible effectiveness of IVIG administration in preventing recurrent thrombosis, among a small cohort of high-risk APS patients. Clinical results of this study are in agreement with ours; however Sciascia *et al*. did not observe any statistically significant decrease in the aPL profile after IVIG treatment at 6, 12 and 24 months (7). This unexpected decrease of aCL, anti$\beta_2$-GPI IgM and the negativisation of LA in 3 IVIG-treated patients could be explained with anti-idiotypic activity of IVIG. Two different studies have already reported the inhibition of aCL binding to cardiolipin by Fab from IVIG, and LA activity (17, 18). The decrease in aPL titers could be the result of inactivation of idiotype bearing B-cell clones with the subsequent decrease in aPL production and increase of degradation of these antibodies.

Other potential anti-inflammatory and immunomodulatory effects of IVIG therapy may be related to IgG antigen-binding fragment (Fab) mediated activities, such as neutralisation of autoantibodies, cytokines and activated complement components, modulation of dendritic cell maturation and restoration of idiotypic-anti-idiotypic networks (13). Anti-idiotypic activity of IVIG is probably the most important mechanism of action in the treatment of APS and it results in short-term neutralisation of aPL. Furthermore, other possible mechanisms include the blockade of the crystallisable fragmented (Fc) portion of IgG and the increased catabolism of IgG (13).

Some limitations of our study need to be discussed. First, because of the rarity of the disease, the small sample size limits our study results. In addition, the population of our cohort was heterogeneous for what concerns the concomitant therapy and the number of patients with primary or secondary APS and it might also affect the results of our study. In particular, patients with APS secondary to SLE could represent a subset of cases potentially more susceptible for IVIG therapy, considering the efficacy of IVIG in SLE reported by various authors (15, 19). Furthermore, this is an open-label prospective, comparative study, with all the limitations inherent to a non-randomised trial.

## Conclusion

In conclusion, in APS patients IVIG may provide an additive or rescue therapy, in selected patients, for recurrent thrombosis, although widespread use may be limited by expense and reduced availability. Moreover, further clinical studies on a larger group of patients are necessary to fully understand the mechanisms of action and the optimal doses of IVIG in APS.

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