Published in final edited form as:

Ann Rheum Dis. 2014 June 1; 73(6): 1176–1180. doi:10.1136/annrheumdis-2013-203622.

A Prospective Open-label Pilot Study of Fluvastatin on Pro-inflammatory and Pro-thrombotic Biomarkers in Antiphospholipid Antibody Positive Patients

Doruk Erkan¹, Rohan Willis², Vijaya L. Murthy², Gurjot Basra², JoAnn Vega¹, Patricia Ruiz Limón², Ana Laura Carrera², Elizabeth Papalardo², Laura Aline Martínez-Martínez², Emilio B. González², and Silvia S. Pierangeli²

¹Barbara Volcker Center for Women and Rheumatic Diseases, Hospital for Special Surgery, Weill Medical College of Cornell University, New York, NY.

²University of Texas Medical Branch, Galveston, TX.

Abstract

Objective: To determine if pro-inflammatory and pro-thrombotic biomarkers are differentially upregulated in persistently antiphospholipid antibody (aPL)-positive patients, and to examine the effects of fluvastatin on these biomarkers.

Methods: Four groups of patients (age 18-65) were recruited: a) Primary Antiphospholipid Syndrome (PAPS); b) Systemic Lupus Erythematosus (SLE) with APS (SLE/APS); c) Persistent aPL positivity without SLE or APS (Primary aPL); and d) Persistent aPL positivity with SLE but no APS (SLE/aPL). The frequency-matched control group, used for baseline data comparison, was identified from a databank of healthy persons. Patients received fluvastatin 40 mg daily for three months. At three months, patients stopped the study medication and they were followed for another three months. Blood samples for 12 pro-inflammatory and pro-thrombotic biomarkers were collected monthly for six months.

Results: Based on the comparison of the baseline samples of 41 aPL-positive patients with 30 healthy controls, 9/12 (75%) biomarkers (interleukin [IL]-6, IL1 β , vascular endothelial growth factor [VEGF], tumor necrosis factor [TNF]- α , interferon [IFN]- α , inducible protein-10 [IP10], soluble CD40 ligand [sCD40L], soluble tissue factor [sTF], and intracellular cellular adhesion molecule [ICAM]-1) were significantly elevated. Twenty-four patients completed the study; fluvastatin significantly and reversibly reduced the levels of 6/12 (50%) biomarkers (IL1 β , VEGF, TNF α , IP10, sCD40L, and sTF).

Corresponding author: Silvia S. Pierangeli, Ph.D. Division of Rheumatology/Internal Medicine University of Texas Medical Branch Brackenridge Hall 2.108, 301 University Boulevard Galveston, TX 77555-0883. Phone: 409-772-0222 - Fax: 409-772-0223 - sspieran@utmb.edu.

COMPETING INTERESTS:

None

CONTRIBUTORSHIP:

DE, SP and JV contributed to the conception and design of the study

DE, SP and RW contributed to the writing of the manuscript

JV, RW, VM, BG, EP, PRL, ALC, LAM and EG contributed to analysis and interpretation of data and critical review of the manuscript

All authors contributed to the final approval of the manuscript

Conclusion: Our prospective mechanistic study demonstrates that pro-inflammatory and pro-thrombotic biomarkers, which are differentially upregulated in persistently aPL-positive patients, can be reversibly reduced by fluvastatin. Thus, statin-induced modulation of the aPL effects on target cells can be a valuable future approach in the management of aPL-positive patients.

Keywords

Antiphospholipid antibodies; Antiphospholipid syndrome; inflammation; treatment; cytokines

INTRODUCTION

Antiphospholipid syndrome (APS) is an autoimmune disorder of thromboses and pregnancy losses associated with persistent antiphospholipid antibodies (aPL) (lupus anticoagulant [LA] test, anticardiolipin antibodies [aCL], and anti- β_2 glycoprotein-I antibodies [a β_2 GPI]). [1] Antiphospholipid antibodies can occur in otherwise healthy individuals as well as in 30-40% of systemic lupus erythematosus (SLE) patients

Antiphospholipid antibody-mediated clinical events occur due to complex interaction of pro-inflammatory and pro-thrombotic cells. *Firstly*, aPL increase endothelial cell (EC) expression of the cellular adhesion molecules (CAMs) such as intracellular CAM-1 (ICAM-1), vascular CAM-1 (VCAM-1), and E-selectin (E-sel) [2-6]. *Secondly*, tissue factor (TF) upregulation is as an important mechanism of the pro-thrombotic effects of aPL [7-9]. *Thirdly*, aPL induce significant increase in pro-inflammatory cytokines (interleukin [IL]-6, IL-8, and tumor necrosis factor- α (TNF- α)) on EC [8, 9]. Fluvastatin diminishes aPL-mediated upregulation of adhesion molecules and TF in vitro in endothelial cells, as well as the in vivo thrombogenic and pro-inflammatory effects of aPL in mice [10-12].

Given the relationship between thrombosis and increased expression of CAMs, TF activity, and pro-inflammatory cytokines in APS, we hypothesize that patients with persistently positive aPL have increased levels of pro-inflammatory and pro-thrombotic biomarkers when compared with healthy controls, and fluvastatin treatment for three months decreases significantly and reversibly, the level of these biomarkers.

METHODS

Study Design

The primary objective of this open-label prospective pilot intervention trial was to determine if pro-inflammatory and pro-thrombotic biomarkers are differentially upregulated in persistently aPL-positive patients with or without SLE. The secondary objective was to determine the effects of fluvastatin on pro-thrombotic and pro-inflammatory biomarkers in aPL-positive patients with or without SLE.

Study Population and Inclusion/Exclusion Criteria

Four groups of patients (age 18-65) were recruited: a) Primary APS (PAPS); b) SLE with APS (SLE/APS); c) Persistent aPL positivity without SLE or APS (Primary aPL); and d) Persistent aPL positivity with SLE but no APS (SLE/aPL). Systemic Lupus Erythematosus

was defined based on the America College of Rheumatology Classification Criteria [13]. Antiphospholipid Syndrome was defined based on the Updated Sapporo Classification Criteria. Positive aPL was defined as persistently [at least 12 weeks apart] positive LA test, aCL ≥ 40 GPL/MPL, and/or a β_2 GPI ≥ 20 SGU/SMU) [1].

Exclusion criteria were age less than 18 years-old, pregnancy, statin or any other cholesterol lowering agent within three-month prior to the screening, underlying liver or muscle disease, chronic renal failure requiring dialysis, active infections requiring antibiotics; systemic autoimmune disease other than SLE; routine non-steroidal anti-inflammatory drug (NSAID), prednisone > 10 mg/day, or immunosuppressive use (except hydroxychloroquine) within one month prior to the screening; biologic agents within six months prior to the screening; treatment with protease inhibitors, rifampin, rifabutin, cholestyramine, fluconazole, itraconazole, ketoconazole, synercid, delavirdine, erythromycin, or clarithromycin within one week prior to screening; history of an allergic reaction to statins; and active illegal drug use or alcohol abuse within the last 52 weeks.

The frequency-matched control group (n: 30) were identified from a databank of healthy persons (no autoimmune or inflammatory diseases) at UTMB.

Study Interventions

All subjects had provided informed consent approved by the Internal Review Boards at UTMB and HSS (ClinicalTrials.gov NCT00674297). Baseline data collection included demographics, general and aPL-specific medical history, medications, blood for specialized outcome measures, and safety outcome measures (aspartate transaminase [AST], alanine aminotransferase [ALT], creatinine phosphokinase (CPK), and urine pregnancy test for premenopausal patients). Within a week of the screening visit, all patients were started on Fluvastatin 40 mg daily for three months. At three months patients were instructed to stop the study medication and they were followed for another three months. Blood samples for specialized outcome measures were collected at the baseline visit, and one, two, three, four, five, and six months. Blood samples for safety outcome measures were collected at the baseline visit and two months after. A window period of ± 4 days was allowed for each study visit.

After the enrollment, if an immunosuppressive medication and/or > 10 mg of prednisone are indicated for the treatment of any disease activity, patients were withdrawn from the study. Patients were instructed not to use NSAIDs regularly during the study period and any occasional NSAID use was recorded during the monthly study visits. If patients reported the use of an NSAID for more than seven consecutive days they were withdrawn from the study.

Specialized Outcome Measures

An in-house enzyme-linked immunosorbent assay (ELISA) and a commercial ELISA (INOVA Diagnostics, San Diego, CA) were used to measure aCL and a β_2 GPI, respectively. Lupus anticoagulant activity was determined as recommended by the International Society of Thrombosis and Haemostasis Subcommittee.[14]

The MILLIPLEXMAP human cytokine/chemokine panel assay (Millipore, Billerica, MA), which utilizes Luminex xMAP technology, was used to determine levels of IL-1 β , IL-6, IL-8, TNF- α , IFN- α , IP10, VEGF, and sCD40L. Interleukin 1, E-selectin, and VCAM-1 serum levels were assessed using a commercial ELISA kit (R&D systems, Minneapolis, MN). Plasma samples were used to detect sTF using a chromogenic assay (American Diagnostics, Stamford, CT). Cut-offs for the cytokines, cellular adhesion molecules, and TF were determined as the 95th percentile of 30 healthy controls for each assay.

Safety Outcome Measures

The safety of fluvastatin was evaluated immediately after starting the medication at one, two, three, four, five, and six months. An adverse event (AE) was defined as any untoward medical occurrence in a study patient regardless of causality assessment. A serious adverse event (SAE) was defined as an AE occurring at any dose which met one or more of the following criteria: death; life-threatening; requiring or prolonging inpatient hospitalization; disabling; or resulting in a congenital anomaly/birth defect.

In case of persistent AST, ALT, and/or CPK elevations, i.e., more than two times the normal values twice in one week, the patient was withdrawn from the study.

Statistical analyses

The Kruskal-Wallis test was used to compare the levels of biomarkers in aPL-positive subjects to the controls. Spearman test was used to analyze the significance of monthly changes in biomarker levels and to correlate the levels of the biomarkers in the different subgroups of patients before and after treatment.

RESULTS

Patients Demographics

Forty-one aPL positive patients (female: 31 [74%], mean age: 43.5 ± 12.5 , Caucasian: 28 [68%]; Primary APS: 18, SLE/APS: 7, Primary aPL: 9, and SLE/aPL: 7) were enrolled and their baseline samples were compared to 30 frequency-matched healthy controls (female: 25 [85%]; mean age: 43.5 ± 12.5). All APS patients had history of thrombosis except two SLE/APS patients who had only obstetric APS. In patients with vascular events, the mean time between the event and the first blood collection was 54.6 ± 79.2 months (range 1-240 months). Out of the 22 patients that were not on anticoagulation at the time of the enrollment, 16 (73%) were LA test positive. Twenty-three (56%) patients were on hydroxychloroquine, 7 (17%) on prednisone (mean dose: 5.8 ± 1.1), and 19 (56%) on low-dose aspirin.

Baseline Biomarkers of Patients Compared to Healthy Controls

Table 1 demonstrates the median levels and interquartile ranges (IQR) of specific outcome measures. While the serum or plasma levels of all the biomarkers were above the cut-off of each assay, in 20-100% of the aPL-positive subjects, IL-6, IL-1 β , VEGF, TNF- α , IFN- α , IP-10, sCD40L, sTF and sICAM-1 were significantly elevated compared to healthy controls.

Many of the biomarkers correlated well among each other, the most significant being TNF α and IL8 ($r=0.848$, $p<0.001$) and IL6 and VEGF ($r=0.506$, $p=0.001$).

Based on a subgroup analysis, the levels of: a) IL-8, TNF- α , and IP10, were significantly higher in PAPS, SLE/APS and SLE/aPL when compared to primary aPL; b) VEGF, sICAM-1, and sVCAM-1 were significantly higher in PAPS when compared to the other groups; and c) sTF and sCD40L were elevated in all subgroups when compared to controls (Table 1)

Effect of Fluvastatin on Specialized Outcome Measures in Persistently aPL-positive Patients

Of 41 patients recruited, 24 completed the study (mean age: 44.6 ± 13.6 ; female: 70%; Primary APS: 8, SLE/APS: 7, Primary aPL: 5; SLE /aPL: 4). Nine (43%) patients were on anticoagulation, 15 (61%) on hydroxychloroquine, four on prednisone (mean dose: 4.5 ± 1.1), and 10 (41%) on low-dose aspirin. The early withdrawal reasons for 15 patients were: five lost to follow-up or refused treatment after the baseline visit; four stopped treatment due to myalgia; three wanted to continue fluvastatin after three months; one did not receive the treatment due to baseline elevated liver function tests; and one stopped treatment due to insomnia. Adverse events occurred in eight of 38 (21%) patients during a mean of 74 ± 26 days of fluvastatin treatment were: arthralgia (n:1); lupus flare (n:1); myalgia with high CPK (n: 1); myalgia with normal CPK (n: 3); recurrent deep vein thrombosis (n: 1); headache (n: 1); and insomnia (n: 1). There were no serious adverse events.

Figure 1 shows the effects of fluvastatin on the biomarkers within 3-months of fluvastatin treatment. The levels of 8/12 (66%) biomarkers (IL-6, IL-1 β , VEGF, TNF- α , IFN- α , IP-10, sCD40L, and sTF) significantly decreased with fluvastatin; mean maximum reduction of biomarkers was achieved between 30 to 70 days of fluvastatin treatment. More than 80% of the subjects with elevated levels of sTF, TNF- α , and IFN- α showed a significant reduction with fluvastatin.

Table 2 shows the effects of stopping fluvastatin on the biomarkers during the second half of the study. The levels of 6/8 (75%) biomarkers (IL-1 β , VEGF, TNF- α , IP-10, sCD40L, and sTF) significantly increased after stopping the fluvastatin treatment; 14 to 90% of the patients with fluvastatin-induced reduction of the biomarkers showed an increase in the levels of the biomarker.

Clinical Observations

A 36 year-old female with SLE/APS developed diffuse arthritis at week 8. The baseline IL-6, IL-1 β , IL-8, TNF- α , IP-10, sCD40L, and sVCAM-1 levels were significantly elevated when compared with controls; a significant reduction of IFN- α (75%), IL-6 (82%), IL-8 (84%), TNF- α (65%), and VEGF (53%) occurred after four weeks of fluvastatin. At week eight, when the patient had a lupus flare, there was a significant increase in these biomarkers (IFN- α [500%], IL-6 [226%], IL-8 [246%], TNF- α [837%], and VEGF [67%]) compared to week 4; in addition IL-1 β and sTF were significantly increased compared to baseline (186% and 75%, respectively) even if the change between baseline and week 4 was not significant.

A 30 year-old male patient with SLE/APS developed recurrent deep vein thrombosis (DVT) at week 12. The baseline IFN α , TNF α , IP10, and IL6 levels were elevated when compared with controls; a significant reduction of IL6, IFN α , sTF and IP10 was observed after four weeks of fluvastatin. At week 12, when the patient developed a recurrent DVT, the IL6, TNF α , IP10, and sTF levels were significantly elevated.

DISCUSSION

Our prospective mechanistic study investigating the effect of fluvastatin on pro-inflammatory and pro-thrombotic biomarkers demonstrated that IL-6, IL-1 β , VEGF, TNF- α , IFN- α , IP-10, sCD40L, sTF, and sICAM are differentially upregulated in aPL-positive patients with or without vascular events and/or SLE; the majority of these biomarkers (IL-1 β , VEGF, TNF- α , IP-10, sCD40L, and sTF) can be significantly and reversibly reduced by fluvastatin.

A commonly accepted theory for thrombosis in aPL-positive patients is that aPL increase the thrombophilic threshold as the 'first hit' (induce a pro-inflammatory/thrombotic phenotype via the cytokines and chemokines), and then clotting takes place only when a 'second hit' (infection, inflammation, surgical procedures or use of estrogens) exists [15-20]. Our findings, especially elevated levels of sTF and sCD40L in persistently aPL-positive patients independent of the APS or SLE diagnosis, strengthen this theory, and suggest that these biomarkers may have a predictive role in aPL-positive patients for the development APS or SLE.

Fluvastatin prevents the expression of cellular adhesion molecules, TF, and IL-6 in aPL-treated endothelial cells in vitro.[11] In the only human mechanistic study published, utilizing a proteomic analysis, López-Pedrerá *et al.* showed that inflammatory proteins can be reversed in aPL-positive patients following one month of daily 20 mg fluvastatin [21] In our study, we extended the treatment with fluvastatin to three months, and also monitored biomarkers for additional three months after discontinuation of the treatment. All the biomarkers were reduced by fluvastatin within two months suggesting that the potential thrombosis risk in persistently aPL-positive patients also decreases within that the same time frame. Furthermore, the prospective and self-controlled nature of the study allowed us to demonstrate the rebound elevation of the majority of the biomarkers after cessation of the therapy.

Interestingly, one patient experienced a lupus flare with concomitant and significant elevation of selected pro-inflammatory and pro-thrombotic markers indicating that these biomarkers are sensitive to fluctuations in disease activity despite statin treatment. This observation is important in a sense that the beneficial effects statins in aPL-positive can be mitigated in the setting of a lupus flare.

Our study has several limitations. Firstly, aPL-positive patients with diverse clinical manifestations were included in the study; the cytokine pattern of our patients could therefore reflect, at least in part, differences in the molecular mechanisms of clinical phenotypes. Secondly, the sample size is relatively small and thus we were not able to

perform a subgroup analysis of the effects of fluvastatin on the biomarkers. Thirdly, different statins may have diverse pleiotropic effects; given that all the *in vitro/vivo* studies in APS were completed using fluvastatin, we used fluvastatin in this study for consistency purposes. And lastly, our study cannot fully elucidate the association between other comorbidities and change in biomarker levels.

In summary, our prospective mechanistic pilot study with frequency-matched controls demonstrates that pro-inflammatory and pro-thrombotic biomarkers, which are differentially upregulated in aPL-positive patients with or without vascular events and/or SLE, can be reversibly reduced by fluvastatin. Thus, statin-induced modulation of the aPL effects on target cells can be a valuable future approach in the management of aPL-positive patients.

Acknowledgments

The study has been supported partially by NIH R01 AR056745-04 and partially by the Barbara Volcker Center at the Hospital for Special Surgery, New York, NY.

REFERENCES

1. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost.* 2006; 4:295–306. [PubMed: 16420554]
2. Simantov E, LaSala J, Lo SK, et al. Activation of cultured vascular endothelial cells by antiphospholipid antibodies. *J Clin Invest.* 1995; 96:2211–9. [PubMed: 7593607]
3. Pierangeli SS, Liu X, Espinola R, et al. Functional analyses of patient-derived IgG monoclonal anticardiolipin antibodies using *in vivo* thrombosis and *in vivo* microcirculation models. *Thromb Haemost.* 2000; 84:388–95. [PubMed: 11019960]
4. Gharavi AE, Pierangeli SS, Colden-Stanfield, et al. GDKV-induced antiphospholipid antibodies enhance thrombosis and activate endothelial cells *in vivo* and *in vitro*. *J Immunol.* 1999; 163:2922–7. [PubMed: 10453040]
5. Del Papa N, Guidali L, Sala A, et al. Endothelial cell target for antiphospholipid antibodies. Human polyclonal and monoclonal anti- β_2 glycoprotein I and induce endothelial cell activation. *Arthritis Rheum.* 1997; 40:551–61. [PubMed: 9082944]
6. Kaplanski G, Cacoub P, Farnarier C, et al. Increased soluble vascular cell adhesion molecule 1 concentrations in patients with primary or systemic lupus erythematosus-related antiphospholipid syndrome: correlations with the severity of thrombosis. *Arthritis Rheum.* 2000; 43:55–64. [PubMed: 10643700]
7. Zhou H, Woldberg AS, Roubey RA. Characterization of monocyte tissue factor activity induced by IgG antiphospholipid antibodies and inhibition by dilazep. *Blood.* 2004; 104:2353–8. [PubMed: 15226179]
8. Vega-Ostertag M, Casper K, Swerlick R, et al. Involvement of p38 MAPK in the up-regulation of tissue factor on endothelial cells by antiphospholipid antibodies. *Arthritis Rheum.* 2005; 52:1545–54. [PubMed: 15880836]
9. Forastiero RR, Martinuzzo ME, De Larranaga G. Circulating levels of tissue factor and proinflammatory cytokines in patients with primary antiphospholipid syndrome or leprosy related antiphospholipid antibodies. *Lupus.* 2005; 14:129–36. [PubMed: 15751817]
10. Meroni PL, Raschi E, Testoni C, et al. Statins prevent endothelial cell activation induced by antiphospholipid (anti- β_2 -glycoprotein I) antibodies: effect on the proadhesive and proinflammatory phenotype. *Arthritis Rheum.* 2001; 44:2870–8. [PubMed: 11762948]
11. Ferrara DE, Swerlick R, Casper K, et al. Fluvastatin inhibits up-regulation of tissue factor expression by antiphospholipid antibodies on endothelial cells. *J Thromb Haemost.* 2004; 2:1558–63. [PubMed: 15333031]

12. Ferrara DE, Liu X, Espinola RG, et al. Inhibition of the thrombogenic and inflammatory properties of antiphospholipid antibodies by fluvastatin in an in vivo animal model. *Arthritis Rheum.* 2003; 48:3272–9. [PubMed: 14613293]
13. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997; 40:1725. [PubMed: 9324032]
14. Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. *J Thromb Haemost.* 2009; 10:1737–1740. [PubMed: 19624461]
15. Linker-Israeli M, Deans RJ, Wallace DJ, et al. Elevated levels of endogenous IL-6 in systemic lupus erythematosus: a putative role in pathogenesis. *J Immunol.* 1991; 147:117–23. [PubMed: 2051017]
16. Gabay C, Cakir N, Moral F, et al. Circulating levels of tumor necrosis factor soluble receptors in systemic lupus erythematosus are significantly higher than in other rheumatic diseases and correlate with disease activity. *J Rheumatol.* 1997; 24:303–8. [PubMed: 9034987]
17. Camargo JF, Correa PA, Castiblanco J, et al. Interleukin-1beta polymorphisms in Colombian patients with autoimmune rheumatic diseases. *Genes Immun.* 2004; 5:609–14. [PubMed: 15470475]
18. Kornberg A, Blank M, Kaufman S, et al. Induction of tissue factor-like activity in monocytes by anti-cardiolipin antibodies. *J Immunol.* 1994; 153:1328–32. [PubMed: 8027560]
19. Redecha P, Tilley R, Tencati M, et al. Tissue factor: a link between C5a and neutrophil activation in antiphospholipid antibody induced fetal injury. *Blood.* 2007; 110:2423–31. [PubMed: 17536017]
20. Desai-Mehta A, Lu L, Ramsey-Goldman R, et al. Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. *J Clin Invest.* 1996; 97:2063–73. [PubMed: 8621796]
21. Lopez-Pedreria C, Ruiz-Rimon P, Aguirre MA, et al. Global effects of fluvastatin on the prothrombotic status of patients with antiphospholipid syndrome. *Ann Rheum Dis.* 2011; 70:675–82. [PubMed: 21173016]

Figure 1. Effects of Fluvastatin on Pro-inflammatory and Pro-thrombotic Biomarkers (BMR) in Antiphospholipid Antibody (aPL) Positive Patients

The percentage of patients with elevated (elev.) BMR levels at baseline (■) and with subsequent reduced (red.) levels following fluvastatin (■) is shown on the left primary vertical axis. The mean ($\pm$ SD) maximum (max) level of BMR concentration (conc) reduction following fluvastatin is shown on the right secondary vertical axis (bars (□)). * $p < 0.05$. IL: interleukin; IFN: interferon; IP10: inducible protein 10; sCD40L: soluble CD 40 ligand; sEsel: soluble E-selectin; sICAM-1: soluble intercellular adhesion molecule; sTF: soluble tissue factor; sVCAM-1: soluble vascular cell adhesion molecule 1; TNF α : tumor necrosis factor α ; and VEGF: vascular endothelial growth factor.

Table 1

Levels of Pro-inflammatory and Pro-thrombotic Biomarkers in Antiphospholipid Antibody (aPL) Positive Patients Compared to Healthy Controls: Combined and Subgroup Analysis.

Biomarkers Median (IQR)	Controls n:30	Combined n:41	PAPS n: 18	SLE/APS n: 7	SLE/aPL n: 7	Primary aPL n: 9
IL6 (pg/ml)	0.7 (0.0)	38.0* (47.3)	31.2* (48.4)	12.2* (103.5)	2.7* (76.8)	0.4 (0.7)
IL 1 β (pg/ml)	0.3 (0.1)	4.7* (25.1)	3.0* (7.7)	11.4* (20.1)	0.5 (5.1)	0.3 (0.6)
IL8 (pg/ml)	27.4 (30.0)	42.6 (43.9)	24.5 ∇ (48.4)	27.4 ∇ (53.7)	21.6 ∇ (48.9)	7.2 (50.4)
VEGF (pg/ml)	88.3 (85.2)	225.1* (318.4)	242.2 $\square$ (399.6)	109.1 (348.4)	67.2 (499.4)	74.6 (158.3)
TNF α (pg/ml)	0.5 (0.0)	29.9* (27.3)	21.5* ∇ (50.5)	11.6* ∇ (24.1)	53.9 ∇ (62.5)	8.9* (15.7)
IFN α (pg/ml)	0.1 (10.2)	12.9* (115.7)	10.1 (88.4)	0.3 (367.1)	13.2 (558.5)	0.3 (512.2)
IP10 (pg/ml)	96.2 (58.0)	584.4* (551.8)	427.2* ∇ (569.8)	656.2* ∇ (454.8)	472.5* ∇ (690.5)	249.7 (698.9)
sCD40L (pg/ml)	16.4 (14.6)	230.1* (2730.8)	276.5* (676.0)	145.6* (5159.8)	76.9* (970.0)	149.7* (17035.6)
sTF (pM)	13.0	134.0* (206.0)	153.6* (381.0)	329.2* (447.0)	102.1* (177.0)	190.4* (139.0)
sICAM-1 (ng/ml)	9.5	151.3* (300.2)	281.6* $\square$ (406.8)	55.1* (70.3)	2.8 (32.9)	163.5 (341.7)
sVCAM-1 (ng/ml)	33.7	41.9 (444.2)	1128.4* $\square$ (1152.8)	156.4 (19.3)	41.1 (27.2)	321.3 (1015.1)
sE-sel (ng/ml)	10.1	14.1 (27.5)	27.7* (36.7)	14.7 (20.4)	4.1 (21.5)	10.9 (26.4)
# of biomarker elevated*		9/12	9/12	7/12	4/12	3/12

The mean levels of aCL IgG (53.3 GPL), aCL IgM (30.9 MPL), aCL IgA (13.7 APL), antiB2GPI IgG (42.3 SGU), antiB2GPI IgM (44.7 SMU), and antiB2GPI IgA (29.8 SAU) were significantly higher compared to healthy controls ($p<0.05$).

* = $p<0.05$ compared with controls,

∇ = $p<0.05$ compared to primary aPL patients,

$\square$ = $p<0.05$ in PAPS vs all other patient groups, IL-interleukin, IFN α - interferon α , IP 10-inducible protein 10, IQR-interquartile range, sCD40L – soluble CD 40 ligand, sEsel – soluble E-selectin, sICAM-1 – soluble intercellular adhesion molecule, sTF soluble tissue factor, sVCAM-1 – soluble vascular cell adhesion molecule 1, TNF α – tumor necrosis factor α , VEGF – vascular endothelial growth factor

Table 2

Pro-inflammatory and Pro-thrombotic Biomarkers in Antiphospholipid Antibody-positive Patients After Stopping Fluvastatin:

Biomarker (BMR)	# of Patients with Increased BMR After Stopping Fluvastatin (%)	Mean $\pm$ SD Maximum BMR Increase After Stopping Fluvastatin	Mean time (days) to Maximum BMR Increase after Stopping Fluvastatin
IL6 (pg/ml)	9/12 (75%)	56.7 $\pm$ 34.5	35 $\pm$ 12
IL1 β (pg/ml)	5//6 (83%)	89.0 $\pm$ 23.5 *	43 $\pm$ 15
IL8 (pg/ml)	3/5 (60%)	45.6 $\pm$ 34.1	60 $\pm$ 10
VEGF (pg/ml)	5/10 (50%)	57.8 $\pm$ 28.5 *	48 $\pm$ 15
TNF α □ (pg/ml)	6/9 (67%)	90.3 $\pm$ 4.5 *	58 $\pm$ 17
IFN α □□ (pg/ml)	6/8 (75%)	56.7 $\pm$ 21.0	60 $\pm$ 20
IP 10 (pg/ml)	8/12 (67%)	87.5 $\pm$ 14.5 *	55 $\pm$ 15
sCD40L (pg/ml)	9/10 (90%)	90.6 $\pm$ 4.3 *	45 $\pm$ 15
sTF (pM)	13/20 (65)	80.4 $\pm$ 10.3 *	50 $\pm$ 12
sICAM-1 (ng/ml)	1/7 (14)	23.4 $\pm$ 12.0	60
sVCAM-1 (ng/ml)	3/4 (75)	47.6 $\pm$ 10.4	46 $\pm$ 15
sE-sel (ng/ml)	0/2 (0)	N/A	N/A

N/A: not applicable, IL-interleukin, IFN α – interferon α , IP10-inducible protein 10, sCD40L – soluble CD 40 ligand, sEsel- soluble E-selectin, sICAM-1 – soluble intercellular adhesion molecule, sTF soluble tissue factor, sVCAM-1 – soluble vascular cell adhesion molecule 1, TNF α – tumor necrosis factor α , VEGF – vascular endothelial growth factor

* p<0.05;