

Risk of recurrent thrombosis related to antiphospholipid antibodies, soluble CD40L and P selectin serum levels in patients with antiphospholipid syndrome secondary to systemic lupus eritematosus

Riscul de tromboză recurentă asociat cu nivelele serice de anticorpi antifosfolipidici, CD40L solubil și P selectină la pacienții cu sindrom antifosfolipidic secundar lupusului eitematos sistemic

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Abstract

Antiphospholipid antibodies (aPL) are often associated with thrombosis, defining the antiphospholipid syndrome (APS) but it remains unclear why many subjects who are positive for aPL do not develop thrombosis. Experimental observations suggest that the platelet is an important player in the pathogenesis of the APS, and CD62P (P selectin) and soluble CD40L (sCD40L) are independent markers of platelet activation. Objectives: to evaluate the significance of P selectin and sCD40L serum levels for an evolution accompanied with venous and arterial recurrent thrombosis in patients with APS secondary to systemic lupus erythematosus (SLE). The secondary aim was to identify the clinical and serological risk factors for the evolution with recurrent thrombotic events. Methods: 20 patients with APS secondary to SLE, diagnosed according to the revised Sapporo classification for APS criteria, whose mean age was 48 ± 11 years, and of which 16 were women, were evaluated for a history of thrombosis and followed-up 24 month for an evolution with new thrombotic events. Serum IgG or IgM anticardiolipin antibodies (aCL), serum P selectin and sCD40L levels were assessed at baseline (V1) and after 12 months (V2) using standardized ELISA methods (R&D Minneapolis USA). Statistics: t test /ANOVA, logistic regression. Results: APS patients with a history of thrombosis had higher P selectin levels compared to APS patients without a history of thrombosis (256.63 ± 145.79 versus 121.85 ± 101.47 ng/dL; $p=0.04$). V1 P selectin levels were significantly higher in patients with arterial recurrent events compared to patients without a history of arterial thrombosis (256.63 ± 145.79 versus 121.85 ± 101.47 ng/dL; $p=0.04$). V2 sCD40L levels were significantly higher in patients with venous recurrent events compared to patients without a history of venous thrombosis.

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is (13433.2 ± 8249.39 versus 5004.72 ± 3769.62 pg/dL; $p=0.015$). *Conclusions: Platelet activation assessed by P selectin and sCD40L is significantly increased in APS secondary to SLE patients compared to controls. P selectin is significantly increased in patients with a history of recurrent thrombosis.*

Key words: thrombosis, antiphospholipid syndrome, P selectin, sCD40L

Rezumat

Obiective: evaluarea semnificației valorilor serice ale P selectinei și sCD40L pentru evoluția cu tromboze recurente arteriale sau venoase la pacienții cu sindrom antifosfolipidic (APS) secundar lupusului eritematos sistemic (SLE). Evaluarea factorilor de risc clinici și serologici pentru evoluția cu tromboză recurentă. Pacienți și metodă: Studiul a inclus 20 pacienți cu APS secundar SLE diagnosticați conform clasificării Sapporo revizuite pentru criteriile de APS, cu vârsta medie 48 ± 11 ani, 16 femei. Pacienții au fost evaluați pentru istoricul de tromboză arterială sau venoasă și urmăriți 24 luni pentru evoluția cu noi evenimente trombotice. Anticorpții anticardiolipinici (aCL) IgG și IgM, valorile serice ale P selectinei și sCD40L (R&D Minneapolis, SUA) au fost determinate folosind metoda ELISA standardizată la includerea în studiu (V1) și peste 12 luni (V2). Statistica: t test /ANOVA, regresie logistică. Rezultate: Pacienții cu APS secundar SLE și istoric de tromboză au avut valori semnificativ mai mari ale P selectinei serice la V1 în comparație cu pacienții cu APS fără istoric de tromboză recurentă (256.63 ± 145.79 versus 121.85 ± 101.47 ng/dL; $p=0.04$). Valorile V1 P selectinei au fost semnificativ mai mari la pacienții cu tromboze arteriale recurente în comparație cu pacienții fără istoric de tromboză arterială (256.63 ± 145.79 versus 121.85 ± 101.47 ng/dL; $p=0.04$). Valorile V2 sCD40L au fost semnificativ mai mari la pacienții cu tromboze recurente venoase față de pacienții fără istoric de tromboze recurente venoase (13433.2 ± 8249.39 versus 5004.72 ± 3769.62 pg/dL; $p=0.015$). Concluzie: La pacienții cu APS secundar SLE există activare plachetară evaluată prin nivelele serice crescute ale P selectinei și sCD40L. Nivelele serice ale P-selectinei au fost semnificativ crescute la pacienții cu APS secundar SLE cu istoric de tromboză recurentă.

Cuvinte cheie: tromboză, sindrom antifosfolipidic, P selectina, sCD40L

Background

The risk of recurrent thrombosis in patients with antiphospholipid syndrome (APS) is high, but there are conflicting opinions on which antibodies should be measured to detect patients at risk of recurrent thrombosis (1-4). Experimental observations suggest that the platelet is an important player in the pathogenesis of the APS, and P selectin and sCD40L are independent markers of platelet activation (2-4).

There is now abundant evidence in the literature (5 - 9) that aPL are particularly associated with a risk of thrombosis, especially recurrent events and pregnancy-related morbidity. The risk appears to be higher for LA (lupus anticoagulant) than aCL but when high titers of aCL are considered, the risks are also high (1, 7, 10).

Research into the pathology of the syndrome has been blinded for a long time by the

idea that negatively charged phospholipids are the central theme around which the clinical manifestations of the syndrome exist (9, 10). Today the studies have focused on a concept of cells and of cellular activation (11, 12) as the key to unraveling the pathophysiology of the syndrome. Over the last 15 years, plasma proteins and cellular receptors had been identified to be involved in antiphospholipid antibody-mediated cellular activation. Several cells, such as platelets (13 - 17), monocytes and endothelial cells (18, 19), have been investigated for their possible contribution to antiphospholipid antibody-mediated disease. Platelet activation, tissue factor expression on the endothelial cells and leucocytes and activation of the complement system (19-24) are observed in the presence of the antiphospholipid antibodies, providing a possible explanation for the clinical manifestations of the syndrome. Probably the most widely accepted hypothesis is that some aPL

may activate platelet and other cells to promote thrombosis. A two-step scenario had been imagined: initial weak, subclinical platelet activation, favoring the binding of β 2-GPI, anti- β 2-GPI or other aPL, followed by full thrombotic activation, possibly involving Fc receptors (19, 25, 26).

The CD40-CD40 ligand (CD40L) system has been implicated in the pathophysiology of atherothrombotic complications and prognosis in cardiovascular disease, as well as in the processes of inflammation and thrombosis (27-29). The surface-expressed CD40L is cleaved from the platelets over a period of minutes to hours, subsequently generating a soluble fragment. Circulating soluble CD40 ligand (sCD40L) is considered to derive predominantly from activated platelets and, hence, may reflect platelet activation (27, 28, 30, 31). High plasma concentrations of sCD40L were associated with increased vascular risk (28, 30, 31).

Recent studies have shown that P selectin has procoagulant properties and reflects a prothrombotic state in human subjects (27, 32, 33). Elevated P selectin levels have been considered as risk factor for thromboembolism and as predictive biomarker for the recurrence of thromboembolic complications in patients with a first episode of unprovoked thrombosis and in patients with lupus anticoagulant with previous thrombotic event (32, 33). Increased serum levels of P selectin have been reported in acute venous thrombosis (32, 33) and have been identified as an independent risk factor for venous thrombosis in patients in whom LA was present (32, 33).

Determination of direct platelet membrane activation markers such as P selectin and sCD40L may provide a useful marker to identify patients at high thrombotic risk.

Objectives

The evaluation of the significance of P selectin and sCD40L levels for the occurrence of recurrent venous and arterial thrombosis in the outcome of patients with APS. We aimed to clarify whether determination of levels of P selectin and sCD40L could predict subsequent

thromboembolic events in patients with APS. The second aim was to identify the clinical and serological risk factors for the evolution with recurrent thrombotic events.

Methods

Study Population

Started in 2007, this ambispective (retrospective and 2-year prospective), nonrandomized cohort study enrolled 20 patients with APS secondary to SLE (4 men and 16 women) with a mean age 48 ± 11 years that were evaluated for thrombotic history and followed 24 months for an evolution with new thrombotic events. Thrombotic episodes or pregnancy-related morbidity were retrospectively identified using medical records. The patients were diagnosed according to revised Sapporo classification for APS criteria. Patients on aspirin or clopidogrel therapy prior to blood sampling were excluded, as the potential impact of these drugs on platelet activity cannot be assessed. Twenty age and gender matched healthy subjects served as controls. The study was approved by the local research Ethics Committee and informed written consent was obtained from all patients.

Laboratory tests

Serum P selectin, sCD40L (R&D Minneapolis, USA) and aCL levels were assessed at baseline (V1) and after 12 months (V2) using an ELISA technique.

IgG and IgM aCL levels were expressed in IgG or IgM phospholipid units (GPL, and, respectively, MPL). The laboratory detection of lupus anticoagulant (LA) was based on the initial use of phospholipid-depleted or platelet depleted coagulation tests, such as Kaolin clotting time, dilute activated partial thromboplastin time, dilute prothrombin time, and dilute Russell's venom viper time.

In patients with acute thrombotic events soluble adhesion molecules were measured at the same time with aPL and LA (2-3 days after thrombosis), i.e. V1 and after 12 months (V2).

Table 1. Demographic data and serologic P selectin and sCD40L levels in APS patients and control group

Characteristics	APS patients	Control Group	p
Mean age	48 ± 11	44 ± 9	NS
Gender (M/F)	4/16	7/13	NS
V1 P selectin ng/mL	209.46 ± 145.01	94.20 ± 20.75	p < 0.00011
V1 sCD40L pg/mL	13339.64 ± 12960.74	3277.66 ± 1435.73	p < 0.0021
V2 Pselectine ng/mL	198.52 ± 192.50	94.20 ± 20.75	p = 0.0210
V2 sCD40L pg/mL	5891.93 ± 4845.03	3277.66 ± 1435.73	p = 0.0266

Diagnosis of thrombotic events

Thrombotic episodes or pregnancy losses before the first visit were considered thrombotic events. Two episodes of thrombosis before the first visit were considered recurrent thrombosis. The new episodes of venous or arterial thrombosis were considered acute thrombotic events.

The diagnostic methods used to detect thrombosis during the follow-up were ultrasonography for deep vein thrombosis, computed tomography (CT) for cerebral thrombosis, arteriography for peripheral arterial occlusions and angio-CT for pulmonary embolism.

The clinical events occurring during follow-up were classified as: 1) venous thrombosis (lower limbs, pulmonary embolism, retinal, cerebral and mesenteric thrombosis); 2) arterial thrombosis (retinal, cerebral, renal, mesenteric, coronary or peripheral arteries); 3) pregnancy events (spontaneous abortion caused by an antiphospholipid syndrome-related indication); there was no patient with catastrophic event (clinical evidence of multiple organ involvement over a short period).

Statistical analysis was performed using the SPSS 17.0 statistical package (SPSS Inc., Chicago, IL). Quantitative values were expressed as mean ± standard deviation and qualitative values as percentage. While the χ^2 test was used for categorical values, the analysis of variance (ANOVA) was utilized to compare the continuous variables as appropriate. A p-value < 0.05 was considered statistically significant. In multivariable analysis independent variables for recurrent

thrombosis were P selectin, sCD40L levels, and aCL. Multiple logistic regression analyses were used to investigate whether high titers of P selectin, sCD40L and aCL were associated with recurrent thrombosis and acute thrombotic events.

Results Baseline characteristics and follow-up results of P selectin and sCD40L levels in the APS patients and control subjects are presented in Table 1.

Patients receiving coumadin had an INR of 2.1 at the time of enrollment. One single patient with SLE has experienced an acute thrombotic event until the end of follow-up (venous thrombosis).

Patients with APS secondary to SLE showed significantly higher V1 P selectin and V1 sCD40L levels than did healthy subjects (209.46 ± 145.01 versus 94.20 ± 20.75 ng/dL; p=0.0011) and (13339.64 ± 12960.74 versus 3277.66 ± 1435.73 ng/dL; p=0.0021) respectively. A significant positive correlations were observed between V1 sCD40L and V1 P selectin levels (R=0.55; p=0.0004). At V2 soluble P selectin and sCD40L were 198.52 ± 192.50 ng/mL and 5891.93 ± 4845.03 pg/mL in cases and 94.20 ± 20.75 ng/mL and 3277.66 ± 1435.73 pg/mL in controls respectively (p=0.0210; p=0.0266).

APS patients with a history of recurrent thrombosis had higher V1 P selectin levels than APS patients without a history of thrombosis (256.63 ± 145.79 versus 121.85 ± 101.47 ng/dL; p=0.04) (fig 1). APS patients with history of recurrent thrombosis had no significantly higher

sCD40L levels and aCL levels compared to patients without recurrent thrombosis (15402.29 ± 15290.62 versus 9508.99 ± 6164.03 pg/dL; $p=0.34$ respectively 194.25 ± 137.5 versus 52.57 ± 10.6 UPL; $p=0.13$).

V1 P selectin levels were significantly higher in patients with arterial recurrent events compared to patients without a history of arterial thrombosis (256.63 ± 145.79 versus 121.85 ± 101.47 ng/dL; $p=0.04$) (Figure 1).

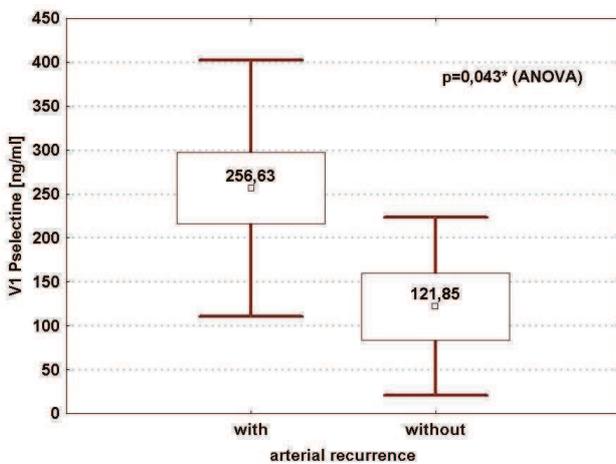


Figura 1. V1 P selectin levels in patients with versus without arterial recurrence

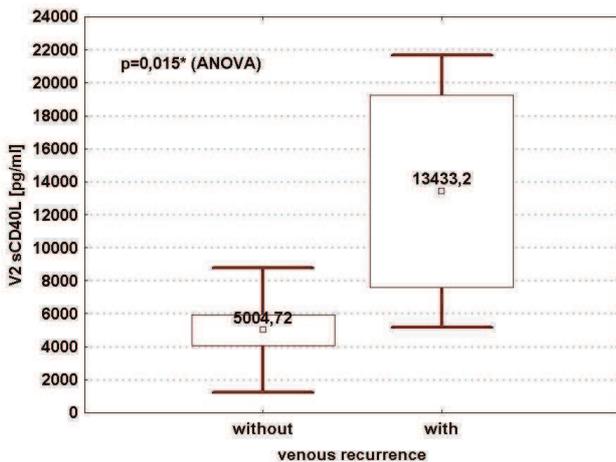


Figura 2. V2 sCD40L levels in patients with versus without venous recurrence

V2 sCD40L levels were significantly higher in patients with venous recurrent events compared to patients without a history of venous thrombosis (13433.2 ± 8249.39 versus 5004.72 ± 3769.62 pg/dL; $p=0.015$) (Figure 2).

Risk Factors for Thrombosis

The patients' clinical and laboratory characteristics at diagnosis were analyzed using univariate and multivariate models to evaluate their predictive value. Univariate analysis showed that the significant predictors for the recurrent thrombosis were a high V1 P selectin titer (>113 ng/mL), and a high sCD40L titer (> 11451 pg/mL) for the venous thrombosis. The multivariate logistic regression analysis confirmed only a V1 P selectin titer of >113 ng/mL as an independent risk factor for thrombosis in our patients.

There was no difference in frequency of aCL, either IgG or IgM, between the thrombotic and non-thrombotic groups. Increased levels (without statistical significance) of LA were found in the thrombotic group.

Discussions

The laboratory diagnosis of APS assigns patients with a common event (thrombosis) to a group with a high risk of recurrence, a prerequisite for long-term oral anticoagulant therapy, potentially exposing them to a high risk of bleeding. Recent studies could not identify clinical and immunologic predictors of thrombotic events or pregnancy morbidity and mortality in APS and current set of tests are still await to correctly identify patients with primary and secondary APS and thromboembolic risk (33).

Although a lot of studies showed that in patients with antiphospholipid syndrome the risk of arterial or venous thrombosis is associated with the presence of antiphospholipid antibodies (1, 6, 8) other studies have been not (9, 10). Differences in study characteristics and methodological limitations were incriminated to explain these conflicting results (9).

The persistence of the procoagulant state defined by continuous endothelial and

platelet activation (3, 4, 19, 26) was described in APS by increased levels of soluble adhesion molecules (27 - 30).

Recent studies have identified a high P selectin titer as an independent risk factor for venous thrombosis in patients with LA present. Although all subjects had evidence of endothelial activation, only platelet activation assessed by P selectin serum levels was different in thrombotic compared to non-thrombotic history. This supports the hypothesis that platelet activation predisposes to thrombosis in the presence of chronic endothelial activation (31, 33).

This study was undertaken with the aim of evaluating the platelet activation in APS patients, with and without thrombosis.

In our study a significant increase in platelet activation was observed in APS secondary to SLE patients compared to control group. This was observed by two platelet activation markers P selectin and sCD40L. The persistence of high levels of platelet activation markers at V2 suggests that platelet activation is chronic and ongoing.

In our study, patients with recurrent thrombotic events had significantly higher levels of P selectin (V1 and V2), reflecting an enhanced platelet activation. This finding raises the possibility that some aPL in the recurrent thrombotic group are interacting mainly with platelets. It is also possible that additional aPL not tested in this study may be specific to the recurrent thrombotic group.

It is, therefore, clear from the present study that additional procoagulant markers have to be analyzed in patients with APS, in order to reach clinically useful information about the risk of recurrent thrombosis. Our present study has identified a suitable marker in this way, i.e. P selectin.

Furthermore, P selectin may represent a target for the development of novel thromboprophylactic agents in APS. Determination of this membrane activation marker may provide a useful marker to identify patients at high thromboembolic risk. Although our study supports the

existence of an association between thromboembolic events and P selectin in APS, there are many issues that require further investigations.

It is the first study in which serial measurements of this membrane activation marker were performed.

The present study has several limitations. First, the sample size of the study was small. Finally, our study population has a relatively low-event rate, despite relatively high-risk patients.

Conclusions

Platelet activation assessed by P selectin and sCD40L occur in APS associated with SLE patients. Serum P-selectin, but not sCD40L, IgG or IgM anticardiolipin antibodies levels were associated with recurrent thrombosis.

Abbreviation list

aCL = anticardiolipin antibodies
 aCL = anticardiolipin antibodies
 anti-β2-GPI = anti-beta 2 glycoprotein I
 anti-β2-GPI = anti-beta 2 glycoprotein I antibodies
 β2-GPI = beta 2 glycoprotein I
 CT = computed tomography
 GPL = phospholipid units type G
 LA = lupus anticoagulant
 MPL = phospholipid units type M
 P selectin = CD62P
 sCD40L = soluble CD40L
 SLE = systemic lupus erythematosus
 V1 = baseline visit
 V2 = 12 months visit
 UPL = phospholipid units

Competing interests

The authors declare that they have no competing interests.

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