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PREDICTIVE ROLE OF hs-C-REACTIVE PROTEIN AND PLATELETS ACTIVATION MARKERS FOR RECURRENT THROMBOSIS IN PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME

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Abstract

Introduction. Antiphospholipid syndrome (APS) is an autoimmune disorder causing thrombosis with a potential risk for recurrence. Thrombotic APS therapy is based on long-term coagulation, still, new therapies are under study: targeting inflammation, immune modulation and complement inhibition. However, before establishing the right therapy, it is important to understand the mechanisms of APS.
Objective of the study. The aim of the study was to evaluate the significance of high-sensitivity C reactive protein (hs-CRP), P selectin and sCD40L serum levels as predictors of recurrent thrombotic events in patients with antiphospholipid syndrome.
Material and method. Forty-one patients with APS, diagnosed according to the revised Sapporo classification for APS criteria were included in the study.
Results. High titers of hs-CRP were correlated to high titers of aCL (r = 0.38; p = 0.014) and to high P selectin levels (r = 0.296; p = 0.05). There was a significant correlation between the serum levels of P selectin and the number of recurrent thrombotic events (r = 0.368; p = 0.018).

Résumé

Le rôle prédictif de la protéine C- réactive et des marqueurs d'activation des plaquettes pour la thrombose récurrente chez les patients atteints de syndrome antiphospholipidique

Introduction. Le syndrome antiphospholipidique (APS) est un trouble auto-immun qui cause la thrombose, présentant un risque potentiel de récidive. Le traitement d'APS thrombotique est basé sur la coagulation à long terme. De nouvelles thérapies sont étudiées: le traitement ciblant l'inflammation, la modulation immunitaire et l'inhibition du complément. Cependant, avant d'établir la bonne thérapie, il est important de comprendre les mécanismes de l'APS.
Objectif de l'étude. L'objectif de l'étude était d'évaluer la signification des taux sériques de la protéine C à haute sensibilité (hs-CRP), de la P sélectine et de sCD40L comme prédicteurs d'événements thrombotiques récurrents chez les patients atteints du syndrome antiphospholipidique.
Matériel et méthode. Quarante et un patients atteints d'APS, diagnostiqués selon la classification

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Conclusion. Platelet activation and inflammation occur in patients with APS and P selectin and hs-CRP proved to predict the evolution with recurrent thrombosis in patients with APS.

Key words: hs-CRP, antiphospholipid syndrome, aCL, P selectin.

List of abbreviations:
APS: Antiphospholipid syndrome
hs-CRP: high-sensitivity C reactive protein
aPL: antiphospholipid antibodies
SLE: systemic lupus erythematous
IL-1β: Interleukin 1β
sCD40L: Soluble CD40-ligand
PAPS: primary antiphospholipid syndrome
aCL: anticardiolipin antibodies

Résultats. Les titres élevés de hs-CRP ont été corrélés avec les titres élevés d'aCL (r = 0,38; p = 0,014) et avec les niveaux élevés de P sélectine (r = 0,296; p = 0,05). Nous avons identifié une corrélation significative entre les taux sériques de P sélectine et le nombre d'événements thrombotiques récurrents (r = 0,368; p = 0,018).
Conclusion. L'activation des plaquettes et l'inflammation se produisent chez les patients atteints d'APS et de P sélectine et hs-CRP, ayant prouvé qu'ils prédisent l'évolution de la thrombose récurrente chez les patients atteints d'APS.

Mots-clés: hs-CRP, syndrome antiphospholipidique, aCL, P sélectine.

Introduction

Antiphospholipid syndrome (APS) is an autoimmune disorder causing thrombosis with a potential risk of recurrence. It can affect different vascular beds, but the mechanisms underlying this condition are not yet completely defined[1].

However, the need for a better understanding of individual clinical manifestations provoked by antiphospholipid antibodies (aPL) requires a more precise risk assessment. The current practice is limited to evaluation of venous and cardiovascular risk and aPL profile which are not enough anymore for good clinical practice. Nonetheless, the medical field is evolving and new tests are being used to stratify risk in APS: biologic risk assessment, platelet activation, aPL-specific thrombosis risk calculators are some of them[2].

Thrombotic APS therapy is based on long-term coagulation, still, new therapies are under study: targeting inflammation, immune modulation and complement inhibition[3,4,5]. However, before establishing the right therapy, it is important to understand the mechanisms of APS. Inflammation activation has been demonstrated in vivo in a mouse model. Also, patients with APS present cells with increased expression of caspase-1 and NLRP3 with high three-fold serum concentration of IL-1β. This finding could suggest chronic inflammation activation and contribution of cofactor independent aPL to APS pathogenesis[6,7].

Objectives of the study

The aim of the study was to evaluate the significance of high-sensitivity C reactive protein (hs-CRP), P selectin and sCD40L serum levels as predictors of recurrent thrombotic events in patients with antiphospholipid syndrome (APS).

Material and methods

Forty-one patients with APS, diagnosed according to the revised Sapporo classification for APS criteria, mean age was 47,67 ± 12 years, 30 women, were evaluated for a history of thrombosis and were followed-up for 12 months for recurrent (venous or arterial) thrombosis (Group 1). Twenty healthy subjects demographic similarly served as controls (Group 2). Fourteen of the APS patients group had primary antiphospholipid syndrome (PAPS), 20 patients had secondary APS associated with systemic lupus erythematous (SLE), and other 7 patients had secondary APS not associated with SLE (non-SLE).

Patients were excluded in case of aspirin or clopidogrel therapy prior to blood sampling, as the potential impact of these drugs on platelet activity could not be determined. In patients experiencing an acute thrombotic event, soluble adhesion molecules were measured at the time of aPL assessment (2-3 days after the acute thrombosis).

Serum IgG or IgM anticardiolipin antibodies (aCL), serum P selectin, sCD40L and hs-CRP 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, Pearson's correlation test. The study was approved by the local Ethics Committee and each enrolled patient signed an informed consent.

Results

Seven patients experienced an acute thrombotic event till the end of the follow-up period: 5 patients with PAPS (of which 4 had venous thrombosis and 1 was diagnosed with pulmonary embolism); 1 patient with LES (venous thrombosis); and 1 patient with secondary APS non-SLE (arterial thrombotic event). Patients with APS showed significantly higher serum V1 P-selectin and V1 sCD40L levels than healthy controls [195.4(94.51; 316.27) vs. 102.92 (86.06; 109.17), *P*<0.0003], respectively, [16871,6(5727.90; 25670.40) vs. 4031.79(2710.62; 4301.50), *P*=0.000008]. There were no significant differences between hs-CRP values determined at V1 and V2 in patients with APS and hs-CRP values in Group 2.

APS patients with a history of recurrent thrombosis had higher P selectin levels compared to APS patients without a history of recurrent thrombosis (207.7 ± 145.79 *versus* 169.72 ± 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 recurrent thrombosis (256.63 ± 145.79 *versus* 121.85 ± 101.47 ng/dL; p=0.04). Hs-CRP serum values were more elevated in patients with a background of acute thrombotic events than in those with a background of recurrent thrombosis, without reaching statistical significance. There were no significant differences between hs-CRP values determined at the time of enrolment in patients with APS secondary to SLE who experienced recurrent thrombosis, and hs-CRP values in patients with APS secondary to SLE who experienced acute thrombotic events.

High titers of hs-CRP were correlated to high titers of aCL (r = 0.38; p = 0.014) and to high P selectin levels (r = 0.296; p = 0.05). There was a significant correlation between the serum levels of P selectin and the number of recurrent thrombotic events (r = 0.368; p = 0.018). There was no statistically significant difference between titers sCD40L in patients with antiphospholipid syndrome with a history of recurrent thrombosis, with no history of thrombotic recurrence and those with acute thrombotic event.

Discussion

Antiphospholipid syndrome (APS) is defined as a noninflammatory disease, however, in recent years, evidence suggests a preexisting proinflammatory state[1,2]. The existence of a proinflammatory endothelial response induced by antiphospholipid antibodies (aPL) was previously demonstrated and it is characterized by up-regulation of adhesion molecules, chemokines synthesis and secretion of proinflammatory cytokines. The role of platelet activation is also an essential pathogenic mechanism of APS[1,2,8]. In fact, activated endothelium in the disease provokes an increased leukocyte adhesion to endothelial cells. Moreover, proinflammatory response triggers leukocytes which are an important source of tissue factor, a major component of the clotting cascade. For instance, in catastrophic antiphospholipid syndrome, the most severe form of APS, systemic inflammatory response caused by excessive release of cytokines from necrotic tissue is described as a new possible pathogenic mechanism. Furthermore, activation of the complement system plays a critical role in the evolution of pregnancy complications in patients with antiphospholipid syndrome and could be incriminated in its pathologic development[5,6,7].

In this study, hs-CRP values were significantly higher in the study group compared to the control group. Persistence of elevated hs-CRP at the second screening visit confirmed the existence of a pro-inflammatory state in these patients. A two-hit hypothesis has been proposed to explain clinical manifestations of APS, considered as the second blow, in the pathogenesis of the disease. Nevertheless, it is not known if inflammation secondary to infectious causes is responsible for a new thrombotic event, or if it is the result of an expression of proinflammatory phenotype in APS patients.

Conclusions

Platelet activation and inflammation occur in patients with APS and P selectin and hs-CRP proved to predict the evolution with recurrent thrombosis in patients with APS.

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