tion should undergo bacterium eradication before or after CyA as reported,[12] at least in areas with a high prevalence of Hp infection.

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IgM anti-protein S antibodies as a risk factor for venous thrombosis

Lupus anticoagulant (LA) and antiphospholipid antibodies (APA) are immunoglobulins directed at phospholipid-protein complexes that have been associated frequently with thrombophilia. Protein S (PS) is a protein with high affinity for phospholipids and can be a target for APA. It is a cofactor in the Protein C (PC) anticoagulant pathway and facilitates the inactivation of coagulation Factors Va and VIIIa. We performed a case-control study to assess the prevalence of anti-protein S antibodies (anti-PS) in a Spanish population and to determine if they are a risk factor for venous thrombosis (VT).

Patients were included when they had been referred to our hospital from November 1997 to April 2002. This case-control study has been described previously in detail.[1] Briefly: from the initial case-control study that included 250 patients and 250 controls, we obtained plasma for anti-PS analysis from 244 patients (108 males, 136 females) and 246 controls (107 males, 139 females). The patients were included in the study if they had suffered their first thrombotic event when under 70 years of age. Patients' clinical characteristics are shown in Table 1. The control subjects were recruited according to the following criteria: similar age (±10 years), same sex, no genetic relationship to the patients and no personal or family history of VT. All procedures were approved by the Institutional Review Board of the Hospital de la Santa Creu i Sant Pau in Barcelona. Written informed consent was obtained from all participants.

Blood samples were obtained at least six months after the most recent thombotic event. IgG and IgM anti-PS antibodies were determined by an ELISA kit from Hyphen BioMed (Neuville-sur-Oise), specific for the IgG or IgM isotype. Normal range ≤8.2 AU for IgM and ≤8.9 AU for IgG (99th percentile of the distribution in the control population). The variation coefficient according to the manufacturer ranged from 3 to 6% for intra-assay and 4 to 8% for inter-assay. Tests for LA used Russell viper venom, (Life Diagnostics Frenchs Forest NSW Australia). IgG and IgM APA, including anticardiolipin, antiphosphatidylserine and anti-β2 Glycoprotein I were determined by ELISA methods. Antithrombin, PC, activated protein C resistance, total and free PS, Factor VIII clotting activity, Factor V Leiden (FVL), PT20210A of prothrombin gene, and *F12C46T* polymorphism were analyzed as previously described.[1] Age was expressed as mean ± standard error (SE). The frequencies of variables are expressed in percentages and were compared with the χ^2 test. A logistic regression method was used to estimate both the crude and adjusted Odds Ratio (OR) as a measure of risk. Adjustments were made for sex, age, LA and for other APA as covariables. Also, adjustments were made for those factors previously associated with VT in our population including: FVL, PT20210A, *F12C46T* (*T/T*), and levels of FVIII >90th percentile. The SPSS 14.0 software package was used for statistical analyses.

We found that 12 patients (4.9%) and 2 controls (0.8%) had IgM anti-PS antibodies. The associated thrombotic risk was 6.3 (95% CI: 1.4-28.5). After including LA and APA as covariables, the adjusted OR was 6.1 (95% CI: 1.3-28.0) (Table 2). The risk did not change when other thrombotic risk factors were included in the analysis (adjusted OR: 7.0; 95% CI: 1.5-33.5). After excluding patients with recurrent thrombosis, we studied 183 patients; 8 of them (4.4%) had IgM anti-PS. The associated risk was similar;

Table 1. Patients' clinical characteristics.

	n (%)
Family history of thrombosis	96 (39.3)
Sex	
Male	108 (44.3)
Female	136 (55.7)
Age at first thrombosis (mean±SE)	42.0 ± 0.9
Multiple thrombosis	61 (25)
Spontaneous	105 (43)
Secondary	139 (57)
Site of thrombosis	
Deep vein thrombosis	149 (61)
Pulmonary embolism *	73 (30)
Upper arm thrombosis	17 (7)
Intracranial sinus thrombosis	5 (2)

**Deep venous thrombosis was diagnosed in 42 patients with pulmonary embolism.*

Table 2. Thrombotic risk of anti-PS antibodies and of other thrombotic risk factors in our population.

	Crude OR (95% CI)	Adjusted* OR (95% CI)	Adjusted° OR (95% CI)
IgG anti-PS	2.5 (0.5-13.3)		
IgM anti-PS	6.3 (1.4-28.5)	6.1 (1.3-28.0)	7.0 (1.5-33.5)
LA	3.6 (1.3-9.9)	3.2 (1.1-8.9)	3.7 (1.3-10.6)
APA	2.0 (0.9-4.5)	2.0 (0.9-4.8)	2.1 (0.9- 5.1)
Factor V Leiden	7.0 (2.7-18.4)		6.9 (2.6-18.5)
PT 20210 A	2.5 (1.1-5.9)		2.2 (0.9-5.5)
FVIII > p90	3.7 (2.2-6.0)		4.4 (2.6-7.5)
F12 C46T (T/T)	3.2 (1.1-8.8)		4.1 (1.4-12.0)
PC deficiency	5.1 (1.1-23.6)		
PS deficiency	1.3 (0.6-2.9)	–	
AT	–	–	

OR: odds ratio. Adjusted OR: odds ratio adjusted by age, sex, lupus anticoagulant (LA), and antiphospholipid antibodies. Adjusted OR°: OR adjusted for risk factors in our population.*

the crude OR was 5.6 (95% CI:1.2-26.6). IgG anti-PS antibodies were present in 5 patients (2.0%) and in 2 controls (0.8%). The crude OR was 2.5 (95% CI: 0.5-13.3). Only 2 patients with IgM anti-PS presented LA or other APA. The remaining 10 patients and 2 controls with IgM anti PS did not present LA or other APA.

Early reports[2,3] have described anti-PS antibodies as complications of varicella associated with a transitory decrease of PS and thrombosis. Later, these antibodies were described in patients with systemic lupus erythematosus (SLE) that had suffered arterial or VT.[4,5] Nojima *et al.*[6] studied only the IgG anti-PS antibodies and reported that they constituted a strong risk factor for DVT also in non-SLE patients. Recently, Galli *et al.*[7] reported that IgM anti-PS was associated with a high risk of prospective thrombosis (OR 6.58; 95% CI: 1.19-36.36) in the WAPS study (patients with persistent LA or APA). In our study, we found a 6-fold increased risk of thrombosis associated to IgM anti-PS and this risk was independent of LA, APA or other thrombotic risk factors.

The effect of these antibodies on hemostasis and their possible influence on thrombotic risk is still inconclusive. They may bind to the free form of PS and thereby inhibit its function[8] or they may form immune complexes with PS causing an increased clearance of this protein.[9] The possible link of these antibodies to thrombotic risk was suggested by Regnault *et al.*[10] who reported one case in which, after varicella infection, there was a temporal relationship between PS deficiency, antibodies to PS and hypercoagulability in the Calibrated Thrombin Generation Test.

Our study has some limitations. First, we did not study the persistence of these antibodies in a second determination 12 weeks apart. However, since the blood of patients was obtained at least six months after their last thrombotic event, it is not likely that the antibodies that we detected were a transitory consequence of thrombosis. Second, since we are a reference center in our region, it is possible that the patients we studied represented a sample with higher thrombotic risk than a random group of patients. In this case, the thrombotic risk observed might be slightly lower in the general population. Finally, more studies of different populations would be desirable to confirm our results.

In conclusion, our study supports the hypothesis that IgM anti-PS antibodies could be an independent risk factor for thrombosis in a Spanish population.

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Antiplatelet drugs and risk of venous thromboembolism: results from the EDITH case-control study

Previous studies suggested that aspirin provided some protection against VTE.[1,2] However, the role of antiplatelet agents in venous thrombus initiation or propagation is still discussed. With recent studies highlighting a possible link between arterial diseases and venous thrombosis,[3,4] new perspectives exist for the role of antiplatelet drugs in the management of VTE. We focused on the association between antiplatelet drug exposure and risk for a first venous thromboembolic event using a case-control study of hospitalized patients, and taking into account other potential associations.

Methods of the case-control study have been described in detail elsewhere.[5] Briefly, all patients ≥18 years hospitalized with a well-documented symptomatic VTE, were eligible for enrolment. For this report, we retained only outpatients, i.e. patients admitted for the VTE and selected those with a first venous thromboembolic event not related to major acquired risk factors, i.e. in the absence of surgery, plaster cast, pregnancy or delivery in the past three months, or active cancer. Controls were matched to the cases by age, gender and had no major acquired risk factors of VTE. Subjects with a previous episode of VTE or lifelong anticoagulant therapy were not eligible as controls. The protocol was approved by our institutional scientific and ethics board.

Cases and controls were interviewed during their hospital stay in a one-to-one standardized way. Atherothrombosis was defined as a past or recent history of either myocardial infarction or stroke or lower limb arteriopathy. For cases and controls drug exposure was defined as current use of drugs at the time of admission. All drugs recorded had to be taken at admission for more than one week. We retained as antiplatelet agents: aspirin (daily dose from 75 to 300 mg), dipyridamole and thienopyridines. We also recorded the use of lipid-lowering drugs, and the use of anti-inflammatory drugs: non steroidal anti-inflammatory drugs (NSAIDs) other than aspirin, and steroids. The reliability of our study database concerning drug exposure was validated by comparison to information provided by the National Health Service of France. A matched case control design was used to estimate relative risks of VTE according to drug exposure. Multivariate conditional logistic regression was performed to adjust for atherothrombosis and statin use, suspected as the main confounding factors, and for all other potential confounders detected through the univariate analysis.

Table 1. Characteristics of cases and matched controls.

Variables	*Cases n=402*	*Controls n=402*	*p value*
Mean age (±SD)	67.0 (±18.3)	67. 2 (±18.2)	0.91
Gender (% female)	232 (57.7)		
Pulmonary embolism (PE), n (%)	81 (20.1)		
Deep vein thrombosis (DVT), n (%)	162 (40.3)		
DVT+PE	159 (39.6)		
Mean Body Mass Index (± SD)	25.8 (±4.3)	24.8 (± 5.6)	<0.0001
Family history of VTE, n (%)	93 (23.1)	36 (9.0)	<0.0001
Chronic pulmonary disease, n (%)	14 (3.5)	30 (7.5)	0.02
Cardiac insufficiency, n (%)	20 (5.0)	28 (7.0)	0.29
Myocardial infarction, n (%)	25 (6.2)	40 (10.0)	0.05
Lower limb arteriopathy[a], n (%)	27 (7.7)	23 (7.2)	0.84
Stroke, n (%)	16 (4.0)	23 (5.7)	0.25
Atherosclerothrombosis[b], n (%)	60 (16.8)	75 (22.8)	0.05

[a]Lower limb arteriopathy was not evaluated in 49 cases and 84 controls; [b]atherosclerothrombosis was defined as history of myocardial infarction and/or stroke and/or lower limb arteriopathy.

Table 2. Matched conditional odds ratios and 95% confidence intervals for venous thromboembolism in relation to drugs of interest.

Exposure	*N. exposed patients among 402 cases n (%)*	*N. exposed patients among 402 controls n (%)*	*OR (95% CI)*
Aspirin*	51 (12.7)	88 (21.9)	0.50 (0.34-0.74)
Thienopyridine*	18 (4.5)	24 (6.0)	0.74 (0.40-1.38)
NSAID**	13 (3.2)	14 (3.5)	0.93 (0.44-1.98)
Steroid	36 (9.0)	33 (8.2)	1.12 (0.66-1.89)
Statin	32 (8.0)	55 (13.7)	0.52 (0.32-0.85)
Fibrate	48 (11.9)	30 (7.5)	1.72 (1.05-2.82)

**One control was current user of aspirin + thienopyridine. Two controls were current users of aspirin + dipyridamole. **NSAID: non steroidal anti-inflammatory drug.*

Four hundred and two cases were included in this analysis. Baseline characteristics are shown in Table 1.

Table 2 shows estimated risks for VTE in relation to the various drugs recorded for this report. One control used an association of aspirin + thienopyridine. None of the patients used dipyridamole alone, and 2 controls were current users of an association aspirin + dipyridamole. Aspirin use was associated with a significant 50% decreased risk of VTE (OR=0.50, 95% CI O.34-0.74). Neither thienopyridines, nor NSAIDs and steroids were associated with the risk of VTE.

The multivariate conditional logistic regression adjusting on statin use, atherothrombosis, chronic pulmonary disease, BMI, and family history of VTE did not alter the association between aspirin and VTE (OR=0.40, 95% CI 0.24-0.68). This association did not significantly differ across age groups or according to gender (Breslow-Day test values were 0.84 and 0.79 respectively).

Our data suggested that aspirin use was associated