

Hypercoagulability resulting from opposite effects of lupus anticoagulants is associated strongly with thrombotic risk

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Hypercoagulability resulting from opposite effects of lupus anticoagulants is associated strongly with thrombotic risk

Interference of antiphospholipid antibodies (aPL) with coagulation was investigated in 40 aPL-patients (24 with thrombosis) using thrombography. Impairment of the activated protein C anticoagulant pathway was partially offset by the genuine anticoagulant effect. The net result, a procoagulant phenotype, was associated with a 7-fold increased risk of thrombosis in aPL-patients.

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Antiphospholipid antibodies (aPL) are associated with thrombosis and/or pregnancy morbidity in the setting of the antiphospholipid syndrome (APS).¹ Some aPL-positive patients remain asymptomatic suggesting that improved assessment of the thrombotic risk is still required. While aPL-induced inhibition of thrombin formation has been reported,^{2,3} acquired activated protein C (APC) resistance and is thought to be the main cause of aPL-associated thrombosis.^{2,4} However, this remains the subject of debate.⁴⁻⁶ Previously, we demonstrated that thrombography could confirm both the extent of the lupus anticoagulant (LA) effect and APC resistance of thrombin generation.⁷ Given the range of laboratory aPL characteristics and the complexity of thrombin formation and inhibition, composite interference of immune complexes on pro- and anti-coagulant complexes may determine the overall result. We studied 40 persistently aPL-positive patients and 19 aPL-negative healthy controls. The study aimed to investigate if the net *in vitro* phenotype is hypercoagulability, and to determine whether total generated thrombin activity, given the two opposite effects of aPL, is associated with an increased risk of thrombosis, to determine whether total generated thrombin activity is associated with an increased risk of thrombosis given the two opposite effects of aPL.

Twenty-four of the aPL-positive patients had experienced thrombosis but were not treated with anticoagulants for medical reasons independent of this study. Two patients were undergoing bridging therapy with low-molecular-weight heparin. In these 2 cases, plasmas were obtained when heparin levels were undetectable. Patient characteristics are summarized in Table 1. Thrombin concentration over time in blood specimens stimulated with phospholipid-free tissue factor was recorded in the absence or the presence of APC. Total generated thrombin activity was quantified by the endogenous thrombin potential (ETP) as previously described.⁸

On average, there was no statistical difference in ETP without APC (ETP₀) between patients and controls whereas ETP in the presence of any APC concentration was significantly higher ($p < 0.005$) for patients than for controls (mean increase of 1.7-fold; see Figure 1A). Overall response to APC was evaluated using APC concentration at half the ETP₀ value (IC₅₀ APC).⁹ Analysis showed significantly higher values for patients compared with controls (32.0±3.4 vs 9.1±0.9 nM, $p < 0.0001$). In contrast, ETP₀ was lower for LA-positive patients (n=23) than for LA-negative patients (1227±65 vs 1680±131 nM.min, $p < 0.005$) and the respective IC₅₀-APC was higher (42.2±4.6 vs 18.2±2.7 nM, $p < 0.001$) (see Figure 1B). Overall, APC inhibition was greater (elevated ETP in

Table 1. Patients' characteristics.

	APS- (n=16)	APS+ (n=24)
Men/Women	3/13	8/16
Age, years	43±4 (23-69)	41±3 (21-76)
aPL-associated thrombocytopenia (platelet count <100 G/L) (blood platelet count, G/L)	0	1 severe (30)
SLE or lupus-like	6	8
Primary APS	—	16
Thromboembolic events		
Vascular thrombosis	—	21
Pregnancy morbidity	—	4
Catastrophic APS	—	2
Laboratory criteria		
Category I (more than one laboratory criteria present)	5	13
Category IIa (lupus anticoagulants present alone)	3	4
Category IIb (anti-cardiolipin present alone)	8	7

Continuous variables denoted as mean±SEM (range), categorical variables as number. SLE indicates systemic lupus erythematosus; APS, antiphospholipid syndrome; APS-, asymptomatic aPL-positive patients, APS+, patients with APS; - not applicable. Categories I, IIa and IIb refer to classification accorded to Sydney criteria. Lupus anticoagulant testing was in agreement with the International Society on Thrombosis and Haemostasis recommendations, taking into account the Sydney revision. Anticardiolipin and anti-β₂-glycoprotein I antibodies were detected by home-based ELISA with cut-off levels locally defined by the method of percentiles with more than 50 healthy volunteers. Antibody values were distributed up to 35-fold, 70-fold, 5-fold and 22-fold the cut-off value for IgG anticardiolipin, IgG anti-β₂-glycoprotein I, IgM anti-cardiolipin and IgM anti-β₂-glycoprotein I respectively.

presence of APC) than prothrombin activation (low ETP₀), resulting in a net procoagulant phenotype. This may be due to differences in membrane requirements and binding kinetics between pro- and anti-coagulant factors. Patients with and without thrombosis were compared to examine whether APC resistance alone may favor thrombosis. IC₅₀-APC was higher in APS-patients than in asymptomatic aPL-positive patients (37.2±4.6 vs 24.1±4.6 nM, $p=0.05$). However, odds ratio (OR) of thrombosis associated with IC₅₀-APC did not reach significance (Figure 1C).

The extent to which a phenotype integrates the two opposite effects of LA is associated with thrombosis was assessed by using two combined thrombographic parameters, APC sensitivity ratio (APCsr) and ETP₀×IC₅₀-APC. In fact, APCsr based on ETP ratios was reported to be associated with thrombosis elsewhere previously.⁹ We observed a negative correlation between APCsr and ETP₀ and a positive one between APCsr and ETP in the presence of 13.9 nM added APC. Seventeen of the 24 APS-patients displayed APCsr >99th percentile compared to 5 of the 16 asymptomatic aPL-positive patients ($p=0.02$). A significantly elevated thrombotic risk was therefore found for APCsr values exceeding the 99th percentile, OR was 5.34; 95% CI=1.35-21.1 (Figure 1C). The use of ETP ratios is limited by the fact that the response to APC is investigated with only one arbitrary concentration of APC. Considering that IC₅₀-APC globally assessed the response to APC and had to be combined with ETP₀, we

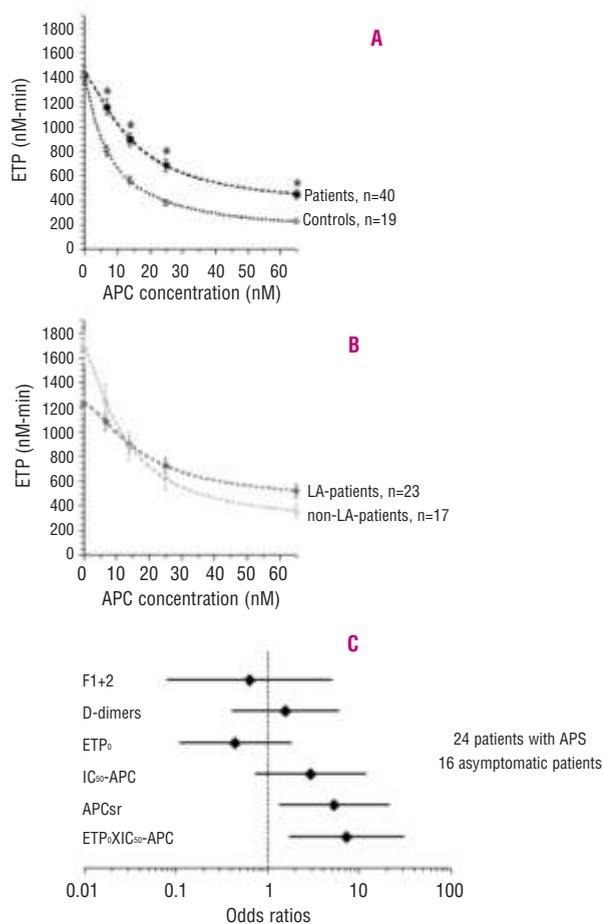


Figure 1. Association of the *in vitro* phenotype of the clotting system with definite antiphospholipid syndrome. **A.** Values obtained with platelet-rich plasma (PRP) of the 19 controls (open symbols) and PRP of the 40 patients (closed symbols). For each subject, measurements are means of triplicate. * $p < 0.005$ compared with controls. **B.** Values obtained with PRP of the 23 patients with lupus anticoagulants (dark grey symbols) and PRP of the 17 patients without lupus anticoagulants (light grey symbols). **C.** Association of the *in vitro* phenotype or *in vivo* activation of coagulation and risk of thrombosis when comparing patients with (APS⁺) and without (APS⁻) thrombosis. Results are expressed as odds ratios with accompanying 95%CI for a cut-off level set at the 99th percentile of the control values. The markers of *in vivo* activation of coagulation were F1 + 2 fragments (thrombin formation) and D-dimers (degradation products of fibrin formed by thrombin). ETP₀, ETP value without APC; IC₅₀-APC, APC concentration reducing ETP₀ by 50%; APC_s_r, APC sensitivity ratio: ETP in presence of 13.9 nM APC/ETP₀.

used their arithmetic product and thus confirmed that a net procoagulant phenotype was associated with an increased risk of thrombosis, OR was 7.29; 95% CI=1.74-30.6 (Figure 1C). In contrast, OR of thrombosis associated with plasma markers of *in vivo* activation of coagulation (F1+2 and D-dimers) was not significant. This had been previously reported for patients with inherited thrombophilia.¹⁰ Clotting system reactivity is assessed by the amount of thrombin that can be formed *in vitro* in

response to a defined stimulus, while plasma markers reflecting thrombin generation *in vivo* depend on both this reactivity and intermittent triggers of different intensities.

In conclusion, changes in sensitivity of thrombin activity to APC, taking into account its modulation by the genuine anticoagulant effect of aPL, is associated with an increased risk of thrombosis in aPL-positive patients.

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