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Antiphospholipid Antibodies Impact the Protein C (PC) Pathway Behavior

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Antiphospholipid antibodies may interfere with the PC pathway, displaying a resistance to the activated PC (resistant phenotype). This effect was evaluated by the APCR and the ProCG systems in 36 lupus anticoagulant samples, yielding abnormal results in 47% of $APCR_{original}$, 17% of $APCR_{modified}$, and 22% of ProCG test. ProCG values correlated with $APCR_{original}$ but not with $APCR_{modified}$. Most of lupus anticoagulants affecting the PC pathway showed abnormal $APCR_{original}$ results but not abnormal ProCG values. The different behavior between both systems may be due to the heterogeneity of the antibodies or could be attributed to the fact that, in the ProCG, a PC activator is added, while the APCR employs already activated exogenous PC. Am. J. Hematol. 71:128–130, 2002.

Key words: lupus coagulation inhibitor; anticardiolipin antibodies; protein C; activated protein C resistance

INTRODUCTION

Once activated by the thrombin–thrombomodulin complex on the surface of endothelial cells, the activated protein C (APC) exerts an inhibitory effect by degrading fVa and fVIIIa; protein S and factor V are required as cofactors for the APC activity in vivo [1]. Currently, different systems can be used to measure the PC pathway, e.g., activated protein C resistance (APCR) [2] and ProC global test (ProCG) [3].

It was reported that some antiphospholipid antibodies may interfere with the PC system, displaying an APC resistant phenotype [4] and associated mainly with lupus anticoagulant (LA) and lgG anticardiolipin antibodies (ACA-IgG) [5]. This effect may be diluted out by mixing samples with factor V depleted plasma [6] as in the $APCR_{modified}$ test.

To evaluate if the LA profile, ACA-IgG, and/or ACA-IgM titer could affect the PC pathway, we compare two systems: APCR, which employs exogenous APC, and ProCG, where a PC activator is added.

MATERIALS AND METHODS

Thirty six LA patients (25 females, 11 males), detected by established criteria [7] based on dRVVT were analyzed. We performed PTT-LA (Stago) and dRVVT (Sigma), including mixing studies and neutralization procedures; ACA-IgG and ACA-IgM by ELISA (The Binding Site®); APCR by the original ($APCR_{original}$) (Coatest APC Resistance, Chromogenix) [2] and the modified ($APCR_{modified}$) techniques (Coatest APC Resistance-V, Chromogenix) [8] and the ProCG (Dade Behring) [3].

Blood was collected in plastic tubes on 0.11 M sodium citrate (9:1) centrifuged twice at 1,500*g* for 15 min to obtain platelet-poor plasma ($<3 \times 10^9$/L), and divided in aliquots that were either tested immediately or frozen at −70°C.

Data were analyzed by SPSS 9.0 for Windows. Correlations were assessed by Spearman's test; comparison

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TABLE I. Antiphospholipid Antibodies Effects on PC System (*n* = 36)

		$APCR_{original}$ (NV = 2.40–4.16)		$APCR_{modified}$ (NV = 1.95–3.24)		ProCG (NV = 1.98–3.86)	
Correlations		*r*	*P*	*r*	*P*	*r*	*P*
PTT-LA (32–46 seg)		−0.662	<0.001	−0.401	0.010	−0.244	0.179
dRVVT (<0.1)		−0.591	<0.001	−0.281	0.083	−0.266	0.141
ACA-IgG (1–15 GPL)		−0.608	<0.001	−0.265	0.098	−0.195	0.285
Difference between groups		Median	*P*	Median	*P*	Median	*P*
PTT-LA	Normal	2.87		2.61		2.49	
			<0.001		0.016		0.578
	Abnormal	2.20		2.24		2.61	
ACA-IgG	(−)	2.55		2.56		2.85	
			0.001		0.262		0.194
	(+)	1.93		2.26		2.27	
ACA-IgG (+)	<50	2.59		2.76		2.25	
			0.023		0.011		1.000
	>50	1.83		1.73		2.43	

Normal values (NV) are shown in parentheses.

between groups (normal vs. abnormal) evaluated by Mann-Whitney *U*-test and association between tests analyzed applying χ^2 or Fisher's exact test when a cell value was less than 5. A $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

We found that 17/36 LA showed an abnormal $APCR_{original}$ (range: 1.47–2.33), but only 6/36 and 8/36 displayed a low response on $APCR_{modified}$ (range: 1.34–1.90) and ProCG (range: 1.22–1.87) results, respectively. ProCG correlated with $APCR_{original}$ ($r = 0.372$; $P = 0.043$) but not with $APCR_{modified}$ ($r = 0.120$; $P = 0.520$).

Table I shows a significant correlation between $APCR_{original}$ and either PTT-LA, dRVVT, or ACA-IgG titer but not between $APCR_{modified}$ and dRVVT or ACA-IgG; in addition, no correlation was observed between ProCG and either PTT-LA, dRVVT, or ACA-IgG values. Neither $APCR_{original}$ nor ProCG correlated with ACA-lgM (data not shown).

Patients were grouped according to normal or abnormal PTT-LA values, negative or positive ACA-IgG, and positive ACA-IgG lower or higher than 50 GPL. Lower $APCR_{original}$ values were observed in abnormal than in normal groups for both PTT-LA and ACA-IgG, and also in ACA-IgG >50 GPL compared with ACA-IgG <50 GPL (Table I). Abnormal $APCR_{original}$ was associated with prolonged PTT-LA (16/17) or positive ACA-IgG (13/17). This association was potentiated when both abnormalities were present together (12/17), compared with patients having either a prolonged PTT-LA (4/17; $P = 0.006$) or a positive ACA-IgG (1/17; $P < 0.001$). In contrast, no abnormal test was associated with a low response on ProCG.

It has been reported that some LA showed a prolonged basal time (PCAT/0) in the ProCG, suggesting that the test should not be used in these patients [9]. In our study, 21/36 samples presented this pattern; however, a few LA samples affected the ProCG (6/21 with abnormal and 2/15 with normal PCAT/0, $P = 0.498$). Recently, abnormal ProCG in LA (66%) non-related to PCAT/0 values were also reported [10].

As has been published [4], we found that not all LA displayed a resistant phenotype. The effect on PC system was mostly reflected by the $APCR_{original}$ (abnormal $APCR_{original}$ = 12; abnormal $APCR_{original}$ + ProCG = 5), and to a lesser extent by the ProCG (abnormal ProCG = 3).

Differences between tests may be due to the heterogeneity of the antibodies or attributed to the different principles on which each system is based.

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