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Regular Article

Prevalence and significance of anti-prothrombin (aPT) antibodies in patients with Lupus Anticoagulant (LA)

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ABSTRACT

Objective: Anti-prothrombin (aPT) antibodies have been found in Lupus Anticoagulant (LA) positive patients. Their prevalence and relative contribution to thromboembolic risk in LA-positive patients is not well defined. The aim of this study was to determine their presence and association with thromboembolic events in a large series of patients with confirmed LA.

Methods: Plasma from LA-positive patients was collected at Thrombosis Centers and sent to a reference central laboratory for confirmation. Positive plasma was tested using home-made ELISA for the presence of aPT and anti-β_2GPI antibodies.

Results: LA was confirmed in 231 patients. Sixty-one of 231 (26%, 95%CI 22-33) LA positive subjects were positive for IgG aPT and 62 (27%, 95% CI 21-33) were positive for IgM aPT antibodies. Clinical features of Antiphospholipid Syndrome (APS) were not associated with the presence of IgG aPT [43 APS in 61 (70%) positive and 109 APS in 170 (64%) negative IgG aPT subjects, p = ns] or IgM aPT. Rate of positivity of IgG and IgM aβ_2GPI was significantly higher than that of IgG and IgM aPT. Clinical events accounting for APS occurred in 97 of 130 (75%) IgG aβ_2GPI positive and in 55 of 101 (54%) IgG aβ_2GPI negative patients (OR 2.4, 95% CI 1.4 to 4.3, p = 0.002). No significant association with clinical events in patients positive for both IgG aPT and IgG aβ_2GPI as compared to those positive for one or another test was found. When patients negative for both IgG aPT and IgG aβ_2GPI (LA positive only) were compared with remaining patients, a significantly lower association with clinical events was found (OR = 0.4, 95% CI: 0.2 to 0.7, p = 0.004).

Conclusions: As compared to IgG aβ_2GPI, the prevalence of IgG aPT in patients with LA is significantly lower and not associated with the clinical features of APS.

1. Introduction

Lupus Anticoagulant (LAC) is a blood coagulation inhibitor that includes circulating antibodies directed mainly against two phospholipid (PL)-binding plasma proteins, β_2-glycoprotein I (β_2GPI) and prothrombin (PT) [1]. Despite the name, the presence of LAC is associated with thromboembolic rather than hemorrhagic events [2,3], and this association defines the Antiphospholipid Syndrome (APS) [4]. Both anti-β_2GPI (aβ_2GPI) and anti-PT (aPT) antibodies, when affinity purified from patient plasma, show LA activity when spiked with normal plasma [5–7] but the exact subset of antibodies that is *per se* causally related to the thrombotic event is not known. It seems that aβ_2GPI antibodies are more specific for thrombosis in patients with APS [8] while the role of aPT antibodies remains

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[1] The participating centers are listed in the addendum

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controversial [9]. However, some recent prospective studies suggest that aPT antibodies predict subsequent thromboembolic events in APS patients [10,11].

The aim of this study was to evaluate the frequency and association of aβ_2GPI and aPT antibodies in LA positive patients with APS.

2. Materials and Methods

The study design is described in detail elsewhere [12]. Briefly, centers affiliated to the Italian Federation of Thrombosis Centers (FCSA) were asked to identify LA positive patients and confirm the results after 6 weeks. Plasma of these patients was then sent for centralized LAC determination. Collected plasma samples were thawed at 37 °C and tested for dRVVT and KCT according to internationally accepted recommendations [13]. Demographic and clinical data of patients were obtained by means of a questionnaire sent to the participating centers. Aliquots of LA positive plasma were stored at -80 °C at the time of collection and used thereafter for the determination of specific autoantibodies using ELISA. All the patients gave their informed consent to participate in this study.

2.1. Anti-human prothrombin ELISA

aPT antibodies of IgG and IgM isotype were measured using a home made ELISA as described earlier [14]. Human prothrombin was purified according to the method of Miletich *et al.* [15]. A polyclonal rabbit anti-human prothrombin was used as positive reference control in each plate, and color development was blocked when the reference control reached an OD405 of 0.8 units. Results are expressed as home units based on the dilution curve of a reference high positive plasma sample to which the value of 100 Units was arbitrarily assigned. Cut-off values (10 arbitrary U for IgG and 5 U for IgM) were set on the 99th percentile of the values obtained in 40 normal age- and sex matched healthy subjects.

2.2. Anti-human β2-Glycoprotein I ELISA

IgG and IgM aβ2GPI antibodies are measured by ELISA as previously described [16] following the proposals of the Standardization Group of the European Forum on antiphospholipid antibodies [17,18]. Upper normal value, calculated using the 99th percentile obtained in 40 normal age- and sex matched healthy subjects is 15 arbitrary units for IgG and 10 for IgM.

2.3. Statistics

Fisher's Exact Test (using the approximation of Woolf) was performed for the comparison of categorical variables. aβ2GPI and aPT antibody levels were compared using the nonparametric Mann-Whitney *U test*. All the statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA).

3. Results

The reference laboratory confirmed LA positivity in 231 patients [12]. Sixty-one of 231 (26%, 95%CI 22-33) LA positive subjects were positive for IgG aPT and 62 (27%, 95% CI 21-33) were positive for IgM aPT antibodies. Patients positive for aPT antibodies had similar clinical features as negative patients for both IgG and IgM isotypes (Table 1). In particular, the rate of APS-defining clinical events in aPT positive and negative groups was similar.

Of 231 LA positive patients, 130 (56%) were positive for IgG aβ_2GPI antibodies and 93 (40%) were positive for IgM aβ_2GPI; a rate significantly higher than that for IgG and IgM aPT ($p<0.001$ and $p=0.003$, respectively). Clinical events accounting for Antiphospholipid Syndrome (APS) occurred in 97 of 130 (75%) IgG aβ_2GPI positive and in 55 of 101 (54%) IgG aβ_2GPI negative patients (OR 2.4, 95% CI 1.4 to 4.3, $p=0.002$). No association between thrombosis and IgM aβ2GPI positivity was found. The association between the presence of LA and both IgG aPT and IgG aβ_2GPI with APS-related clinical events [31 of 43 (72%)] was not stronger than that of patient positive for LA and the sole IgG aPT or the sole IgG aβ_2GPI [76 of 104 (73%); OR = 1.4, CI 0.6 to 3.0, p = ns]. Conversely, patients with LA positive only (IgG aPT and IgG aβ_2GPI negative) were at lower risk: 45 of 84 (53%) as compared with 107 of 147 (73%) of the remaining patients that had APS-related clinical events (OR = 0.4, 95% CI: 0.2 to 0.7, $p=0.004$).

3.1. Antibody titre

IgG aPT antibody titres are shown in Fig. 1. Median value of aPT was 6 U (interquartile range 3-12) in 152 LAC + /APS + and 6 U (interquartile range 3.7-10) in 79 LAC + /APS- patients (p = ns). IgM aPT antibody titres are shown in Fig. 2. Median value of aPT was 4 U (interquartile range 2-6) in 152 LAC + /APS + and 4 U (interquartile range 2-6.5) in 79 LAC + /APS- patients (p = ns).

4. Discussion

Antiphospholipid antibodies are a rather wide and heterogeneous family of immunoglobulins and among these LAs are those that are more strongly associated with thromboembolic events. The most commonly investigated antigenic targets in aPL-positive patients are β2GPI and PT; still, β2GPI is the most relevant one. However, not all subjects with aβ2GPI antibodies develop APS clinical manifestations. Indeed, there is increasing evidence that autoantibodies directed against the Domain I (DmI) epitope of β2GPI molecule, are associated with clinical manifestations of APS [8,19]. In this study we found that in APS patients the rate of positivity of aβ2GPI antibodies is significantly higher than that of aPT antibodies, indicating that the

Table 1
Characteristics of anti-prothrombin positive and negative patients.

	IgG aPT positive N = 61	*IgG aPT negative* N = 170	p	*IgM aPT positive* N = 62	*IgM aPT negative* N = 169	p
Age–yr (mean ± SD)	44 ± 18	45 ± 15	ns	42 ± 16	46 ± 16	ns
Male gender–N (%)	27 (44)	51 (30)	ns	15 (24)	57(33)	ns
Previous TE or pregnancy morbidity–N (%)	43 (70)	109 (64)	ns	40 (64)	112 (66)	ns
Venous thromboembolism–N	23	48	ns	22	49	ns
Arterial thromboembolism–N	13	36	ns	11	38	ns
Venousarterial thromboembolism–N	3	16	ns	2	17	ns
Pregnancy morbidity–N	4	9	ns	5	8	ns
Associated autoimmune diseases–N (%)	22 (36)	47 (28)	ns	18 (29)	49 (29)	ns