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Antibodies to Prothrombin and Beta-2-Glycoprotein I
in Selected Lupus Anticoagulant Positive Samples

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Lupus anticoagulants (LA) are a heterogeneous group of immunoglobulins (IgG, IgM, IgA) which inhibit *in vitro* phospholipid-dependent coagulation assays. LA require prothrombin or beta-2 glycoprotein I (B2GPI) as cofactors for optimal immunologic binding. Antibodies to these cofactors were determined in 20 selected plasma samples with strong LA activity. Anti-prothrombin (aPT) and anti-B2GPI ELISAs results were reported in arbitrary units and the cut-off was pre-established at 20 units for both assays. The prevalence of aPT antibodies in healthy controls was 4% for IgG and 2% for IgM and IgA. Six plasma samples with abnormal coagulation profiles, which did not fulfill LA diagnostic criteria, tested negative for aPT, anti-B2GPI, anti-phosphatidylserine and anti-cardiolipin antibodies confirming the specificity of the assays. Of the 20 LA plasma samples, 16 (80%) were positive for aPT and 17 (85%) for anti-B2GPI antibodies. Ten samples (50%) were positive for IgG, 12 (60%) for IgM and 7 (35%) for IgA aPT antibodies. The four aPT negative samples tested positive for anti-B2GPI antibodies. All 20 LA samples were positive for aPT or anti-B2GPI antibodies, 13 (65%) to both, 4 (20%) to anti-B2GPI only, and 3 (15%) to aPT only. In summary, aPT and anti-B2GPI antibodies are frequently found in LA samples as single isotype or in combination. All the LA samples had antibodies to prothrombin or to B2GPI. The most common occurrence was the combination of aPT with anti-B2GPI antibodies. In 3 LA samples aPT was the only contributor to LA activity, and in 4 samples it was anti-B2GPI. Testing for both aPT and anti-B2GPI antibodies may be a valuable tool to confirm the presence of LA. The clinical profiles of patients with antibodies against one versus both cofactors should be further investigated.

Introduction

Antiphospholipid antibodies are a heterogeneous group of autoantibodies directed to anionic phospholipids, phospholipid-protein complexes and/or to plasma proteins (cofactors) in the absence of phospholipids, which have been associated with the antiphospholipid syndrome (APS) and increased risk of thrombosis.

Lupus anticoagulants (LA) are a subgroup of antiphospholipid antibodies that inhibit *in vitro* phospholipid-dependent coagulation assays. LA have been reported to be more specific for thrombosis than aCL antibodies, and also shown to require protein cofactors B2GPI and/or PT) for optimal immunological binding.

The role of antibodies to these cofactors in lupus anticoagulant LA activity was studied in a group of 20 plasma samples referred to our laboratory as LA positive.

Objective

- Determine the presence of anti-prothrombin (aPT) and anti-B2GPI antibodies by ELISA and evaluate their contribution to LA activity.

Material and Methods

Plasma Samples

- Twenty (20) patient samples selected for strong LA activity. LA diagnosis based on published criteria.
- *Brandt et al. Thromb Haemost 74:1185, 1995*
- Six (6) samples from patients with abnormal coagulation profiles, which did not fulfill LA criteria, were used as controls.
- One hundred twenty six (126) samples from healthy blood bank donors were used as controls.

Anti-Prothrombin ELISA:

Purified human prothrombin was coated onto 96 well micro-plates, blocked and stabilized. 100uL of diluted patient serum (1:51) in sample diluent was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG, IgM, or IgA was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and optical density read at 450/650 nm.

anti-Beta 2 Glycoprotein I ELISA:

Purified human B2GPI (purity > 95% SDS-PAGE) was coated onto 96 well micro-plates, blocked, and stabilized in the absence of exogenous B2GPI. 100uL of diluted patient serum (1:50) in sample diluent containing no B2GPI was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG, IgM, or IgA was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and optical density read at 450/650 nm.

Table #1 IgG, IgM and IgA Antiphospholipid Antibodies in Lupus Anticoagulant (LA+) Plasma Samples

Cut off	aPT			aB2GPI			aCL			aPS		
	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM	IgA
LA-1	97	62	54	305	401	434	76	67	39	61	99	63
LA-2	47	21	10	308	23	289	79	5	8	57	22	12
LA-3	9	124	73	6	11	18	7	6	8	5	25	6
LA-4	3	5	9	2	18	23	6	7	11	3	19	10
LA-5	13	26	13	77	41	367	19	7	16	20	23	22
LA-6	77	126	98	27	205	170	40	61	19	18	89	25
LA-7	6	96	34	11	27	52	5	3	6	6	11	5
LA-8	71	117	114	62	5	91	84	14	11	32	38	9
LA-9	67	33	13	51	27	17	14	4	5	10	11	4
LA-10	54	80	18	5	16	8	38	10	8	11	36	9
LA-11	15	2	7	6	24	106	5	2	6	6	7	8
LA-12	5	6	9	2	7	64	21	5	16	11	7	**
LA-13	77	13	17	225	17	64	85	6	31	57	36	**
LA-14	22	11	6	237	104	212	84	32	18	58	71	**
LA-15	37	12	8	2	5	6	8	2	4	8	11	**
LA-16	38	8	13	156	7	143	32	1	10	46	9	**
LA-17	12	36	3	71	66	6	18	17	1	23	53	**

LA-18	7	24	70	311	137	395	98	18	33	58	30	56
LA-19	14	111	33	178	236	245	17	29	11	20	63	11
LA-20	4	12	7	57	411	237	17	62	9	14	96	14
%pos	50%	60%	35%	65%	60%	75%	45%	40%	15%	55%	65%	20%

Positive samples denoted by shading.

LA plasma samples fulfilled diagnostic criteria for Lupus Anticoagulants (Brandt et al. *Thromb Haemost* 74:1185,1995)

Table #2

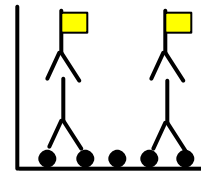
**IgG, IgM and IgA Antiphospholipid Antibodies
in Plasma Samples with Prolonged Coagulation Times (LA -)**

Cut off	aPT			aB2GPI			aCL			aPS		
	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM	IgA
	20	20	20	20	20	20	23	11	22	16	22	20
PC-1	10	4	4	8	5	9	20	2	4	14	6	4
PC-2	10	18	17	1	7	11	8	7	14	7	24	**
PC-3	5	6	6	2	2	4	7	2	6	5	21	**
PC-4	12	13	7	2	2	6	10	2	9	15	11	**
PC-5	6	6	17	3	9	15	6	1	5	2	4	4
PC-6	4	6	19	2	8	13	6	2	5	3	5	3

Positive samples denoted by shading.

Plasma samples with abnormal (prolonged) coagulation profiles - did not fulfill LA criteria

**ELISA for Detection of Antibodies
to Human Prothrombin**



- Solid surface:** medium binding polystyrene
- Antigen:** purified human Prothrombin @ 15ug/ml (>95% SDS PAGE) in acetate buffer in the presence of Ca⁺⁺
- Blocking:** BSA* in PBS
- Samples diluent:** BSA* in PBS
- Wash:** PBS + Tween 20
- Conjugate:** HRP goat anti-human IgG/IgM/IgA
- Substrate:** 1-component TMB
- Stop:** 0.36N sulfuric acid

- Standardization:** in-house patient samples (panel)
- Cut-off established at 20 units (arbitrary) for IgG, IgM, IgA (mean +2SD)
- *BSA without lipids or B2GPI

Table #3

Summary Results:

	LA Positive samples	LA Negative Controls
aPT	80% (16/20)	0%(0/6)
aB2GPI	85% (17/20)	0% (0/6)
aCL	60% (12/20)	0% (0/6)
aPS	70% (14/20)	16% (1/6)

- 100% of LA samples were + for aPT and/or aB2GPI
- 65% + for both, 20% + for aB2GPI only and 15% for aPT only
- 75% of LA samples + for aCL and/or aPS

Summary and Conclusions

- IgG, IgM, and IgA aPT antibodies are frequently found in LA+ samples as single isotype or in combination.
- 100% of patients studied with positive LA had antibodies to prothrombin or Beta 2 glycoprotein I.
- The most common occurrence was the combination of aPT and anti-B2GPI antibodies.
- In 3 LA samples, aPT was the possible sole contributor, and in 4 samples, it was anti-B2GPI.
- Testing for aPT and anti-B2GPI antibodies may be a valuable tool to confirm the presence of LA.
- The presence of one versus both antibodies may have differential clinical implications and/or different mechanisms of action for the development of thrombosis.

