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**MULTICENTRIC STUDY OF ANTI-PROTHROMBIN AND  
ANTI-B2GPI ANTIBODIES IN LA SAMPLES**

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Lupus anticoagulant (LA) are immunoglobulins that inhibit *in vitro* phospholipid dependent coagulation assays. LA require the presence of a protein cofactor for optimal immunologic binding. IgG and IgM antibodies to prothrombin (aPT) and B2GPI (anti-B2GPI) were measured by ELISA in 61 LA positive plasma samples collected from 3 independent reference coagulation labs. 100 plasma samples from healthy blood donors and 56 plasma samples with abnormal coagulation profiles which did not meet the diagnostic criteria for LA were used as controls. The diagnosis of LA was based on published diagnostic criteria, however, each institution followed their own tests and procedures to establish the presence of LA. 23, 41 and 43% (mean 38%) of the LA positive plasma samples from each institution were found reactive to aPT antibodies while only 11% and 2% of the samples were positive from the abnormal coagulation control and healthy groups. Similarly, 43,74 and 92% (mean 67%) of the LA positive plasma samples from each institution were found reactive to anti-B2GPI antibodies while only 12% and 0% of the samples were positive from the abnormal coagulation control and healthy groups. 55.7% of the samples in the LA positive group had elevated levels of aPT and/or anti-B2GPI antibodies compared to 21.4% of the samples in the abnormal coagulation control group and 0% in the healthy blood donors. In summary, increased plasma levels of aPT and anti-B2GPI antibodies were found in the LA positive samples compared to the controls. Unlike a previous study with strictly selected LA samples, these groups did not show ~100% reactivity to aPT and/or anti-B2GPI antibodies. In addition, each institution showed different profiles of aPT or anti-B2GPI antibody reactivity, most likely due to differences in the LA classification criteria. The abnormal coagulation group showed high positive rates for aPT and anti-B2GPI antibodies compared to healthy controls. Determination of aPT and anti-B2GPI antibodies in plasma samples suspected of having LA activity may help in establishing the diagnosis of LA.

**Introduction**

Lupus anticoagulants (LA) are a heterogeneous subgroup of antiphospholipid antibodies detected by coagulation assays that are directed to the protein targets like prothrombin or beta-2 glycoprotein I (B2GPI) bound to phospholipids. Most patients with LA have both anti-prothrombin (aPT) and anti-B2GPI antibodies.

We investigated the prevalence of these antibodies in a larger lupus anticoagulant and additional control populations. In this study, IgG and IgM aPT antibodies were measured by ELISA in 61 LA positive plasma samples collected from 3 independent coagulation laboratories. The diagnosis of LA was based on published diagnostic criteria, using the routine tests and procedures employed by each institution to establish the presence of LA. One hundred (100) plasma samples from healthy blood donors, and 56 plasma samples with abnormal coagulation profiles not meeting the diagnostic criteria for LA were used as controls. In addition, all samples were tested for IgG and IgM anti-beta-2 glycoprotein I (anti-B2GPI) antibodies.

**Objective**

- Determine the prevalence of increased antibody levels to anti-prothrombin and anti-B2GPI in patients with positive lupus anticoagulants from three (3) independent institutions.

**Material and Methods**

**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, or IgM 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, or IgM 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.

**Plasma Samples**

- **61 LA positive samples collected from 3 independent reference coagulation laboratories.**
  - site #1; n = 21
  - site #2; n = 27
  - site #3; n = 13
- **56 samples from patients with abnormal coagulation profiles, which did not meet the diagnostic criteria for a positive LA (samples from site #3).**
- **100 Healthy blood donors**
  - Brandt et al. *Thromb Haemost* 74:1185, 1995

## Summary and Conclusions

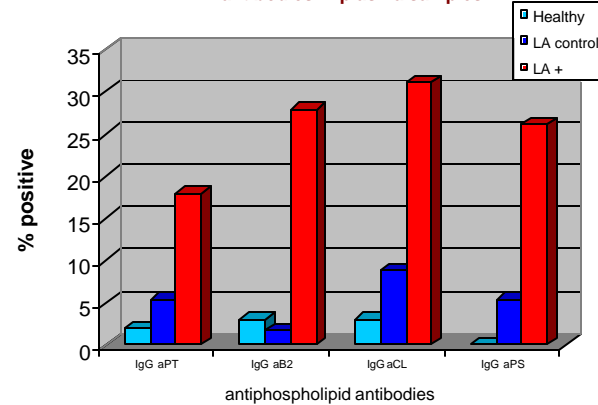
### Summary of IgG and IgM Combined Data for aPT and anti-B2GPI Results with LA Positive and Control Sample Groups

Table #1	aPT IgG or IgM % positive	aB2GPI IgG or IgM % positive	aPT &/or aB2GPI IgG or IgM % Positive
<b>LA Positive Group</b>	<b>36%</b>	<b>38%</b>	<b>54%</b>
<b>Site 1</b>	<b>23%</b>	<b>15%</b>	<b>31%</b>
<b>Site 2</b>	<b>38%</b>	<b>29%</b>	<b>48%</b>
<b>Site 3</b>	<b>41%</b>	<b>56%</b>	<b>70%</b>
<b>Controls:</b>			
<b>Abnormal Coag.</b>	<b>11%</b>	<b>13%</b>	<b>21%</b>
<b>Healthy Donors</b>	<b>4%</b>	<b>4%</b>	<b>5%</b>

- Statistically significant increased plasma levels of aPT and/or anti-B2GPI antibodies were found in these LA positive samples compared to the controls.
- Approximately half of the positive LA population tested positive for aPT and/or anti-B2GPI antibodies.
- Each institution showed different profiles of aPT or anti-B2GPI antibody reactivity, most likely due to differences in LA diagnostic methods.
- The abnormal coagulation group showed higher positive rates for aPT and anti-B2GPI antibodies when compared to the healthy controls. This suggests that some of the abnormal coagulation samples may indeed have antiphospholipid antibodies.
- Determination of aPT and anti-B2GPI antibodies in plasma samples suspected of having LA activity may help in confirming the presence of LA.

Graph #1

#### Percent (%) positives of IgG antiphospholipid antibodies in plasma samples



Graph #2

#### Percent (%) positives of IgM antiphospholipid antibodies in plasma samples

