

Measurement of antibodies to prothrombin and beta-2 glycoprotein I in Lupus Anticoagulant positive samples. K. J. Dier, L. W. Olsen, A. M. Whittier, C. A. Fink, L. R. Lopez. Research & Development, Corgenix, Inc., Westminster, CO.

Lupus anticoagulants (LA), which inhibit in vitro phospholipid dependent coagulation assays, have been associated with the antiphospholipid syndrome and increased risk of thrombosis. LA may require prothrombin (PT) or beta-2 glycoprotein I (B2GPI) as cofactors for optimal immunologic binding. We investigated the contribution of antibodies to these cofactors to LA activity in a group of 20 plasma samples referred to our laboratory as LA positive. Commercially available anti-B2GPI ELISAs for detection of IgG, IgM and IgA antibodies were performed as recommended by the manufacturer's instructions. New ELISAs to measure anti-prothrombin antibodies (aPT), IgG, IgM and IgA were developed. Ninety-six-well microtiter plates were coated with purified human PT (>95% SDS-PAGE), blocked and stabilized. Patient plasma samples (100 μ l) diluted into PBS (1:50) were incubated in the microwells. After washing, 100 μ l of HRP-conjugated anti-IgG, anti-IgM or anti-IgA was added followed by TMB substrate. The reaction was stopped with 0.36N H₂SO₄ and optical density read at 450/650 nm. Total incubation time was 40 minutes (15', 15', 10') at room temperature. The new aPT assays were standardized against in-house reference preparations and results reported in arbitrary units. The assay cut-off for each antibody isotype was established at 20 units (mean of 100 serum and 38 plasma samples from healthy subjects + 2 SD). The antibodies to PT in patient plasma were calculated from a single point calibrator containing a known concentration of antibodies to PT. The prevalence of aPT antibodies in healthy controls was 4% for IgG, 2% for both IgM and IgA. Six 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 aPT assay. Of the 20 LA plasma samples, 16 were positive and 4 were negative for aPT antibodies. Of the 16 positive samples, 10 were positive for IgG, 12 for IgM and 7 for IgA (i.e. several samples were positive for more than one isotype). All four aPT negative samples tested positive for anti-B2GPI antibodies, 1 for IgG, 2 for IgM and 4 for IgA. All 20 LA samples were positive for aPT or anti-B2GPI antibodies, 13 for both, 4 for anti-B2GPI only, and 3 for aPT only. In summary, aPT and anti-B2GPI antibodies are frequently found in LA samples. The results support isotype identification when testing for these antibodies as some samples were positive for only one isotype, while others were positive for two or three. Testing for anti-PT and anti-B2GPI antibodies may not only be a valuable tool to confirm the presence of LA, but also to distinguish patients with antibodies against only one versus both cofactors. These results may also suggest different mechanisms of action for the development of thrombosis in patients with LA.

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