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Prevalence of IgG and IgM Anti-prothrombin Antibodies in
Healthy and Diseased Populations Measured by ELISA

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Antiphospholipid antibodies are a heterogeneous group of autoantibodies frequently associated with thrombosis, thrombocytopenia, and fetal loss (e.g. APS). "Autoimmune" antiphospholipid antibodies require plasma protein cofactors for optimal immunologic binding. Some of these antibodies bind to cofactors in the absence of phospholipids. B2GPI and prothrombin are recognized as the most common antiphospholipid cofactors and antibodies to B2GPI have been shown to be more specific for thrombosis than anticardiolipin antibodies. The prevalence of antibodies to human prothrombin (aPT) was studied in serum and plasma from healthy and diseased populations by ELISA. Using a pre-established cut-off of 20 units for IgG and IgM aPT antibodies, the results for 249 healthy blood donors were: mean IgG aPT 6.5 G units and 94.4% negative; mean IgM aPT 8.7 M units and 95% negative. Syphilis samples (n=42): IgG aPT 7.1 G units and 96% negative; IgM aPT 10.3 M units and 88% negative. Rheumatoid arthritis (RA) samples (n=42): IgG aPT 6.6 G units and 95% negative; IgM aPT 4.2 M units and 100% negative. Unselected SLE (n=83): IgG aPT 10.8 G units and 15% positive; IgM aPT 11.8 M units and 12% positive (healthy vs SLE $p < 0.01$). A group of primary APS and lupus anticoagulant (LA) patients were studied for aPT antibodies. Primary APS (n=8): IgG aPT 15 G units and 25% positives; IgM aPT 19 M units and 37% positive (healthy vs APS $p < 0.001$). LA samples (n=20): IgG aPT 19.6 G units and 45% positive; IgM aPT 32 M units and 45% positive (healthy vs LA $p < 0.001$). The prevalence of high serum levels IgG and IgM aPT antibodies in SLE, APS and LA was significantly higher than the healthy controls, syphilis and RA samples. The highest prevalence was found in LA samples. These results suggest that measuring aPT antibodies may be useful in the serologic evaluation of APS and LA.

Introduction

Antiphospholipid antibodies are a heterogeneous group of autoantibodies directed to anionic phospholipids, phospholipid-protein complexes or to proteins in the absence of phospholipids. The protein targets, commonly referred to as antiphospholipid cofactors, are thought to play an important role in the pathogenesis of thrombosis in the antiphospholipid syndrome (APS). Several plasma proteins have been described as cofactors with a focus on beta-2 glycoprotein I (B2GPI) and prothrombin.

Recent studies on the role of antibodies to B2GPI in APS have shown anti-B2GPI antibodies to be more specific for thrombosis than anticardiolipin (aCL) antibodies. Many clinical laboratories now include assays for the detection of anti-B2GPI antibodies as part of their diagnostic panel for APS. The exact role and clinical significance of antibodies to prothrombin is now under intensive investigation. Early findings suggest that the determination of anti-prothrombin (aPT) antibody levels may be an important component in the laboratory evaluation of APS in the near future.

Objective

- Study aPT antibody prevalence in healthy (control) versus diseased (APS) serum samples.
- Determine the prevalence of aPT antibodies in plasma samples with lupus anticoagulant (LA) activity.

Material and Methods

Serum Samples

- 252 healthy blood bank donors
- 42 infectious disease (syphilis) patients
- 42 rheumatoid arthritis (RA) patients
- 42 progressive systemic sclerosis (PSS)
- 83 systemic lupus erythematosus (SLE)
- 8 Primary antiphospholipid syndrome (APS)

Plasma Samples

- 126 healthy blood bank donors
- 5 patients with abnormal coagulation profiles, which did not fulfill LA criteria, were used as controls.
- 20 samples selected for strong LA activity. LA diagnosis based on published criteria.

Brandt et al. Thromb Haemost 74:1185, 1995

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.

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

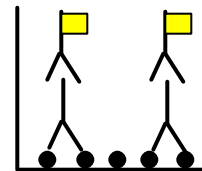
Samples diluent: BSA* in PBS

Wash: PBS + Tween 20

Conjugate: HRP goat anti-human IgG/IgM/IgA

Substrate: 1-component TMB

Stop: 0.36N sulfuric acid



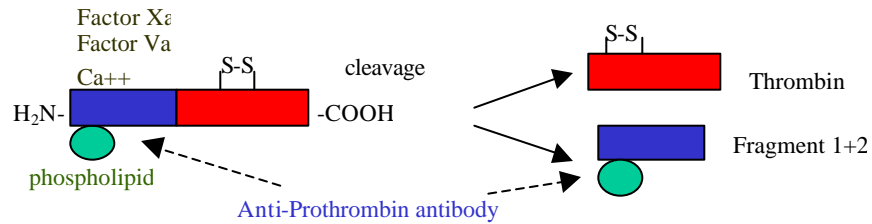
Standardization: in-house patient samples (panel) Cut-off established at 20 units (arbitrary) for IgG, IgM, IgA (mean +2SD)

*BSA without lipids or B2GPI

Single-chain glycoprotein (MW 72 kDa)

γ -carboxyglutamic acid (GLA) domains bind anionic phospholipids

Plasma concentration ~100ug/ml with half life of 2-4 days



In vitro: High affinity prothrombin-antiprothrombin complexes compete with other coagulation factors for available phospholipid binding sites (LAC activity)

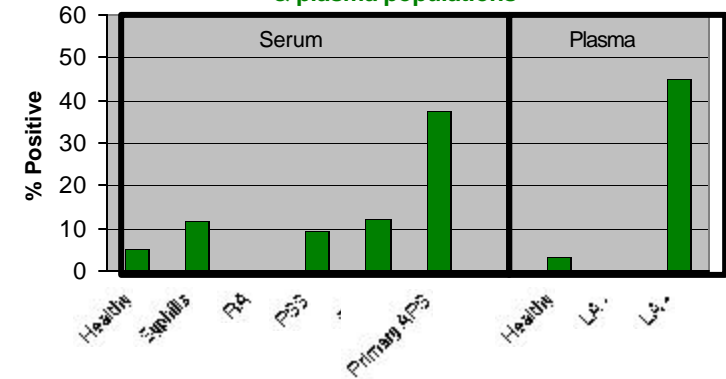
In vivo: aPT antibodies increase thrombin generation at sites of vascular injury with exposed phospholipids (? decrease thrombomodulin), leading to increased fibrin (clot) formation. ? impairment of Protein C anticoagulant system.

Summary of aPT Antibodies in Various Healthy and Diseased Populations Determined by ELISA

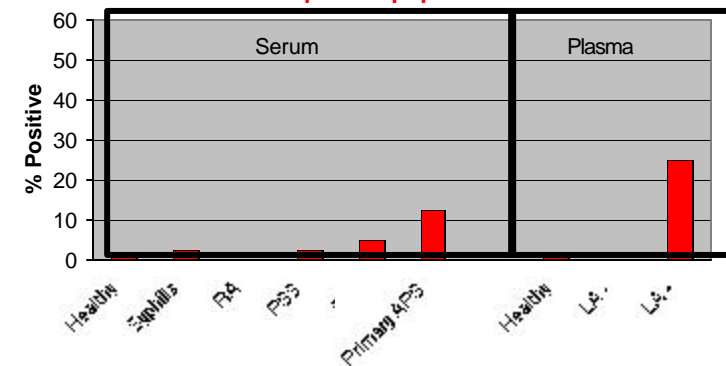
Table #1		IgG		IgM		IgA	
SERUM	n=	mean	% Pos	mean	% Pos	mean	% Pos
Healthy	252	6.4	5.5%	8.6	5.1%	5.6	1.2%
Syphilis	42	7.1	4.8%	10.3	11.9%	4.2	2.4%
RA	42	6.6	4.8%	4.2	0	4.2	0
PSS	42	9.8	14.3%	11.2*	9.5%	5.5	2.4%
SLE	83	10.6*	13.3%	10.9*	12.1%	6.6	4.8%
Primary APS	8	14.9*	25.0%	18.9*	37.5%	6.7*	12.5%
PLASMA	n=	mean	% Pos	mean	% Pos	mean	% Pos
Healthy	126	4.9	0.8%	7.2	3.2%	3.3	1.2%
LA Negative	5	4.6	0	6.3	0	4.9	0
LA Positive	20	19.6*	45.0%	32.5*	45.0%	12.1*	25.0%

* Statistically significant (p-value<0.05)

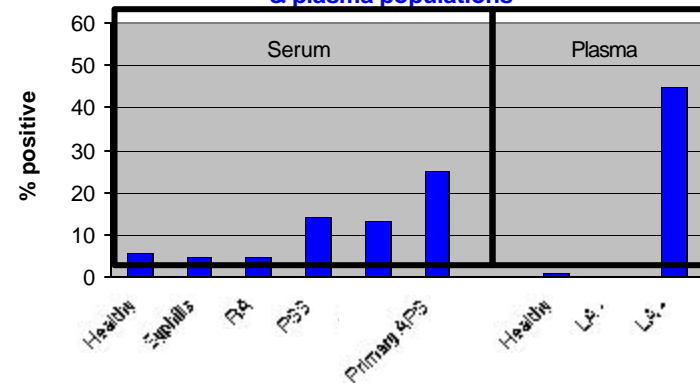
Graph #2 **IgM anti-Prothrombin antibodies in various serum & plasma populations**



Graph #3 **IgA anti-Prothrombin antibodies in various serum & plasma populations**



Graph #1 **IgG anti-Prothrombin antibodies in various serum & plasma populations**



Summary and Conclusions

- IgG, IgM, and IgA aPT antibodies were detected in higher occurrence and levels in the primary APS group.
- IgG, IgM, and IgA aPT antibodies were elevated in LA+ plasma samples compared to the control groups.
- Testing for aPT along with other assays (aCL, aPS, and anti-B2GPI) may add valuable additional information used to assess the risk of thrombosis related to the antiphospholipid syndrome.
- Testing LA positive patient samples for aPT antibodies may be a valuable tool for confirmation of LA activity.