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**COMPARISON OF THREE ELISA METHODS FOR THE
DETECTION OF ANTI-B2GPI ANTIBODIES**

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ABSTRACT

Antibodies to B2GPI have been reported to be more specific for thrombosis than classic anticardiolipin antibodies and very useful in the diagnosis of antiphospholipid syndrome (APS). We evaluated the performance of three commercial IgG anti-B2GPI ELISA methods. A group of 22 selected APS serum samples were tested by each method. All assays performed within their expected quality control specifications. Method 1 classified 16 as positive (73%, mean value = 87.9 units), Method 2 classified 10 (45%, mean = 43.5 units) and Method 3 classified 12 (54%, mean = 65.0 units). When the individual values from Method 1 were compared with Methods 2 and 3, coefficients of correlation (r) of 0.432 and 0.588 were obtained. Methods 2 and 3 showed a correlation of 0.765, however, these two methods detected 6 and 4 less positives than Method 1. Four samples that classified as positive with the three methods differed significantly in values and interpretation (i.e. weak versus strong positive). Since the majority of APS patients with history of thrombotic episodes should present with these antibodies, Method 1 had the best performance by identifying more patients (73%) as positive. In addition, Method 1 was tested using a proposed chimeric IgG monoclonal antibody standard (HCAL), which resulted as strong positive (124 units). Although all of these assays use purified human B2GPI, similar testing procedures, cut-offs and possibly similar reporting units, the standardization and clinical performance seems to be different. These results demonstrate inter-method variability for detecting aB2GPI antibodies and the need for better standardization.

INTRODUCTION

The association between elevated serum levels of “autoimmune antiphospholipid antibodies and recurrent venous or arterial thrombosis (antiphospholipid syndrome) has been well established and is now widely recognized. These antibodies frequently require the presence of a serum protein as a cofactor for optimal in vitro binding activity. Many studies have demonstrated that these antibodies recognize a newly formed antigenic site on beta 2 glycoprotein I (B2GPI), a natural anticoagulant protein found in normal serum, when bound to phospholipids. This protein cofactor has a high binding affinity for negatively-charged phospholipids forming phospholipid-protein complexes. Research on the role of protein cofactors has increased our understanding of the heterogeneous nature of antiphospholipid antibodies and their possible in vivo pathogenic role in thrombosis.

The detection of antibodies that bind to B2GPI can be performed directly by binding purified B2GPI to a negatively charged plate surface in the absence of phospholipid, but still produce a similar antigenic site. The direct detection of anti-B2GPI antibodies provides enhanced clinical sensitivity for thrombosis.

OBJECTIVE

Evaluate anti-B2GPI levels in samples from patients with the antiphospholipid syndrome and/or with thrombosis with different commercially available ELISA assays.

Compare individual values of each assay along with the performance of a proposed anti-B2GPI standard (HCAL, mAb) to determine its usefulness related to standardization issues.

MATERIAL AND METHODS

Samples

A selected group of 22 serum samples from patients with antiphospholipid syndrome (APS) and/or thrombosis was tested by each method. Listed below is the summary of patient characteristics. Detailed clinical history was not available for all of the specimens, but all had a clinical and serologic diagnosis of APS.

Sample ID	SLE	Thrombosis	Other/misc.	Additional Lab Results
APS #1		+		LA+, IgG aCL+, IgG/A aPS+, IgA aB2GPI+
APS #2	+	+	Preg. losses	LA+, ANA+, IgG/M/A aCL+, IgG/M aPS+, IgM/IgA aB2GPI+
APS #3		+	Preg. losses	LA+, IgG aCL+, IgG/M aPS+, IgG/M aB2GPI+
APS #4		+		LA+, IgG/M aCL+, IgG/M aPS+, IgM/A aB2GPI+
APS #5				
APS #6				IgG/M/A aCL+
APS #7				IgA aCL+, IgG aPS+, IgA aB2GPI+
APS #8				IgG/A aCL+, IgG/M aPS+, IgA aB2GPI+
APS #9				IgG/M/A aCL+, IgM/A aB2GPI+, IgM aPT+
APS #10				LA+
APS #11		+		LA+, IgG/A aCL+, IgG/M aPS+, IgA aB2GPI+
APS #12		+		LA+, IgG/M aCL+, IgG/M/A aPS+, IgA aB2GPI+, IgM aPT+
APS #13				IgG/M aCL+, IgG/M aPS+, IgA aB2GPI+
APS #14				IgG aCL+, IgG aPS+, IgM aB2GPI+, IgA aB2GPI+
APS #15			Cancer	LA+, IgG aCL+, IgA aB2GPI+, IgM aPT+
APS #16		+		IgG aCL+, IgG/A aPS+, IgA aB2GPI+, IgG aPT+
APS #17		+		IgM aPS+, IgM/A aB2GPI+, IgM aPT+
APS #18	+	+		IgG aCL+, IgG/M/A aPS+, IgM/A aB2GPI+, IgM aPT+
APS #19	+			IgM/A aCL+, IgM/A aPS+, IgM/A aB2GPI+, IgM aPT+
APS #20		+		IgG/M/A aCL+, IgM/A aPS+, IgG aPT+
APS #21	+			IgG aCL+, IgG aPS+, IgM aB2GPI+, IgA aB2GPI+
APS #22				IgG aCL+, IgG aPS+, IgM aB2GPI+, IgA aB2GPI+

Test kits

Method #1 – REAADS IgG Anti-Beta 2 Glycoprotein I Semi-Quantitative Test Kit, Corgenix, Westminster, CO

Method #2 – Bindazyme Anti-B2GPI Enzyme Immunoassay Kit, Binding Site, Birmingham, England

Method #3 – Quanta Lite B2GPI IgG, Inova, San Diego, CA

HCAL IgG anti-B2GPI Standard

HCAL is a monoclonal chimeric antibody, which consists of a human constant region and a mouse monoclonal variable region with specificity to beta 2 glycoprotein I either bound to a negatively charged phospholipid or to an oxygenated surface.

Comparison of Methods

	Method #1	Method #2	Method #3
Format	96 well (8x12) plate	96 well (8x12) plate	96 well (8x12) plate
Plate Mfg	Nunc	Greiner	Labsystems
Plate Type	oxygenated	?	?
Antigen	Hu B2GPI	Hu B2GPI	purified B2GPI
Conjugate	goat anti-Hu-IgG	Rabbit anti-Hu-IgG	goat anti-Hu-IgG
Calibration	1 Point (lot dependent)	5 point (300,100,33,11,3.7)	5 Point (150,75,37.5,18.8,9.4)
Substrate/WL	TMB	TMB	TMB
Stop solution	0.36N sulfuric acid	3M phosphoric acid	0.334M sulfuric acid
Wavelength	450nm	450nm	450nm
Sample Dilution	1:50	1:100	1:101
Incubations	15/15/10 min	30/30/30 min	30/30/30 min
Reported Units	G units	U/mL	SGU
Assay Range	0 – 200 G units	0 – 300 U/mL	0 – 150 SGU
Assay Cutoff	20 G units	20 U/mL	20 SGU

RESULTS

IgG anti-B2GPI Antibodies

Method #1 Method #2 Method #3

Cut off	20 units	20 units	20 units
APS #1	101	8.7	8.5
APS #2	126	27.5	95.3
APS #3	119	19.9	54.5
APS #4	166	58.9	200
APS #5	0.5	10.8	2.3
APS #6	0.9	11.0	2.7
APS #7	76.3	45.5	32.3
APS #8	159.9	122.6	172.1
APS #9	119.0	95.8	27.6
APS #10	2.0	3.4	2.6
APS #11	160.8	142.6	266.6
APS #12	160.8	81.1	135.3
APS #13	141.7	24.9	65.5
APS #14	50.9	4.6	6.2
APS #15	18.1	3.7	3.0
APS #16	166.9	230.4	276.9
APS #17	2.6	3.6	2.3
APS #18	125.2	24.6	29.3
APS #19	37.0	17.6	5.7
APS #20	17.7	6.0	4.6
APS #21	124.9	8.5	29.6
APS #22	57.3	4.2	7.7

Mean Value	87.9	43.5	65.0
Std Dev	62.5	58.3	88.0
% Positive	73% (16/22)	45% (10/22)	55% (12/22)

Reactivity of HCAL mAb (18.8 ug/mL)*

	aB2GPI
Method #1	142.1
Method #2	233.6
Method #3	112.0

*HCAL mAb was diluted and tested according to each methods instructions

IgG anti-B2GPI Discrepant Samples

	Method #1	Method #2	Method #3	Additional positive assays
Cut off	20 units	20 units	20 units	
APS #1	101	8.7	8.5	LA, IgG aCL, IgG aPS, IgA aPS, IgA aB2GPI, thrombosis
APS #3	119	19.9	54.5	LA, IgG aCL, IgG aPS, IgM aB2GPI, IgA aB2GPI, thrombosis
APS #14	50.9	4.6	6.2	IgG aCL, IgG aPS, IgM aB2GPI, IgA aB2GPI
APS #19	37.0	17.6	5.7	IgM/A aCL, IgM/A aPS, IgM/A aB2GPI, IgM aPT, SLE
APS #21	124.9	8.5	29.6	IgG aCL, IgG aPS, IgM aB2GPI, IgA aB2GPI, SLE
APS #22	57.3	4.2	7.7	IgG aCL, IgG aPS, IgM aB2GPI, IgA aB2GPI

SUMMARY

- In a population of well defined patients with antiphospholipid syndrome (APS) and/or thrombosis, there were significant differences in the performance of the three anti-B2GP assays evaluated.
- Because anti-B2GPI antibodies are more specific for thrombosis, it was expected that most would result positive for the presence of IgG anti-B2GPI.
- Method #1 detected a total of 16 positive samples out of the 22 tested (73%) compared to 10 (45%) with Method #2 and 12 (55%) with Method #3. Four samples that resulted as positive with all three methods differed significantly in values and interpretation (i.e. weak versus strong positive).
- Methods #2 and #3 did correlate better with each other compared to Method #1, though both methods did result negative for 4 and 6 APS patient samples that Method #1 reported as positive.
- The HCAL mAb resulted as strong positive with all three methods, resulting highest on method #2 which had the fewest number of positive samples.

CONCLUSION

- Although all of these assays use purified human B2GPI, similar testing procedures, cut-off; and possibly similar reporting units, the standardization and clinical performance seems to be different.
- Clinical laboratories measuring anti-B2GPI antibodies should evaluate this inter-method variability to avoid under reporting patient results.
- The HCAL mAb works well to confirm reactivity to a specific binding site on B2GPI, but did not explain the differences seen in detecting anti-B2GPI antibodies in many of the clinical patients between the three methods in this study.