

TEN-MONTH FOLLOW-UP OF VARIOUS ANTIPHOSPHOLIPID ANTIBODIES LEVELS IN A PATIENT WITH ANTIPHOSPHOLIPID SYNDROME

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ABSTRACT

Antiphospholipid antibodies are a heterogeneous group of autoantibodies associated with the antiphospholipid syndrome (APS) clinically characterized by arterial or venous thrombosis, thrombocytopenia and fetal loss. Elevated serum levels of these antibodies are serologic markers for the diagnosis of APS. Current criteria require lupus anticoagulant (LA) and/or IgG or IgM anticardiolipin (aCL) positive tests. Borderline or weak positive results may require retesting in 8 - 10 weeks for confirmation of results. Antibodies to beta 2 glycoprotein I (B2GPI) have been reported as more specific for thrombosis than aCL antibodies. However, their determination has not yet been incorporated into the serologic criteria for APS. A 31 y/o female patient hospitalized for acute renal failure and past history of SLE with lupus nephritis as well as 2 cerebrovascular accidents was studied. She presented skin ulcers on both ankles and livedo reticularis. Her SLE serology (ANA and anti-dsDNA) was negative and the coagulation profile was interpreted as positive for LA with increased D-dimer levels. These findings prompted the determination of aCL antibodies that was reported as borderline for IgG (24 GPL, cut-off <23) and negative for IgM and IgA aCL. Due to the clinical picture suggestive of APS, antiphosphatidylserine (aPS) and anti-B2GPI antibodies were also determined. IgG aPS was strongly positive (69 GPS, cut-off <16) and negative for IgM aPS. Similarly, IgG (152 units) and IgA (160 units) anti-B2GPI antibodies were strongly positive (cut-off <20 units). This aCL borderline and aPS (and anti-B2GPI) positive patient was followed for 10 months and the results summarized below (positives in bold):

Antibody (Month/Year)	Cut-off	9/99	3/00	7/00
IgG aCL	<23	24	89	80
IgM aCL	<11	8	3	3
IgG aPS	<16	69	81	102
IgM aPS	<22	20	9	12
IgG anti-B2GPI	<20	152	137	131
IgM anti-B2GPI	<20	16	6	5
IgA anti-B2GPI	<20	160	156	154

IgG aCL levels increased from borderline to strong positive when retested after 6 months and remained elevated. IgG aPS as well as IgG and IgA anti-B2GPI levels remained elevated during the follow-up period. This case illustrates the following points: If the serologic diagnosis would have been based only on aCL results, the diagnosis of APS would have been missed or delayed for several months until the patient seroconverted. Because ELISA tests for aCL and aPS antibodies contain exogenous B2GPI, it is commonly believed that these antibodies are directed to B2GPI, and therefore, are similar. These results clearly show that aCL and aPS are not only distinct but behaved differently over time. In addition, the presence of anti-B2GPI antibodies provided additional information toward the initial diagnosis of APS in this patient.

INTRODUCTION

Antiphospholipid antibodies are a heterogeneous group of autoantibodies with reactivity toward:

- 1.- negatively-charged (anionic) phospholipids i.e. cardiolipin (CL), phosphatidylserine (PS),
- 2.- phospholipid-protein complexes, and
- 3.- certain plasma proteins (cofactors) on a solid surface in the absence of phospholipids.

Elevated levels of these antibodies are serologic markers for the diagnosis of the **antiphospholipid syndrome (APS)**, clinically characterized by recurrent arterial or venous thrombosis, thrombocytopenia and/or fetal abortion.

Anticardiolipin (aCL) ELISA tests use bovine serum as source of cofactor (B2GPI) and are the most commonly used for the diagnosis of APS, however, this test may detect both “infectious” (cofactor independent) as well as “autoimmune” (cofactor-dependent) antibodies.

The **anti-phosphatidylserine (aPS) ELISA** tests also use bovine serum and a more physiologically relevant phospholipid antigen, however, as with the aCL ELISA, it may also detect both types of antibodies.

It is now widely accepted that the antigen for “autoimmune” antiphospholipid antibodies is located on the protein (cofactor) molecule and if so, one can speculate that the same “autoimmune” antibody would react in both assays (aCL and aPS) as they contain cofactor (B2GPI). Therefore, it has been suggested that aPS are the same as aCL antibodies and do not add to the diagnosis of APS.

As recently shown, “autoimmune” antibodies also react on ELISA tests using purified cofactor (B2GPI) in the absence of phospholipids, and **anti-B2GPI ELISA** tests have been described as more specific for thrombosis (and APS) than aCL or aPS ELISAs.

OBJECTIVES

- To follow over time the serum levels of 3 antiphospholipid antibodies of a patient with APS since diagnosis to study their serologic behavior.
- To test the usefulness of an algorithm for the laboratory evaluation of antiphospholipid antibodies in the diagnosis of APS.

MATERIAL AND METHODS

Antiphospholipid Syndrome (APS) patient

A 31 year-old female patient was hospitalized for renal failure and a history of being diagnosed with SLE, lupus nephritis as well as 2 strokes several years before. On exam, skin changes suggestive of livedo reticularis and ulcers on both ankles were recorded. SLE serology (ANA and anti-dsDNA) was negative at this time. Her coagulation profile was interpreted as positive for Lupus Anticoagulant and D-dimer levels were increased. No evidence of clinical thrombosis was found. These findings prompted an aCL antibody evaluation, which was reported as negative/borderline for IgG aCL (24 GPL with cut-off of 23 GPL), negative for IgM and IgA aCL.

Because the clinical picture was suggestive of APS, aPS and anti-B2GPI antibodies were also tested on this sample.

Antiphospholipid antibody assays

All the serum samples taken at different time intervals during the follow-up were stored at -70°C until tested. IgG, IgM and IgA antibodies to CL, PS and human B2GPI were determined by ELISA (REAADS) following the manufacturer’s instructions:

anti-Cardiolipin ELISA: Purified bovine cardiolipin was coated onto 96-microwell plates, blocked and stabilized. 100uL of diluted patient serum (1:50) in sample diluent containing bovine 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 absorbance read at 450/650 nm.

anti-Phosphatidylserine ELISA: Purified bovine phosphatidylserine was coated onto 96-microwell plates, blocked and stabilized. 100uL of diluted patient serum (1:50) in sample diluent containing bovine 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 absorbance 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.

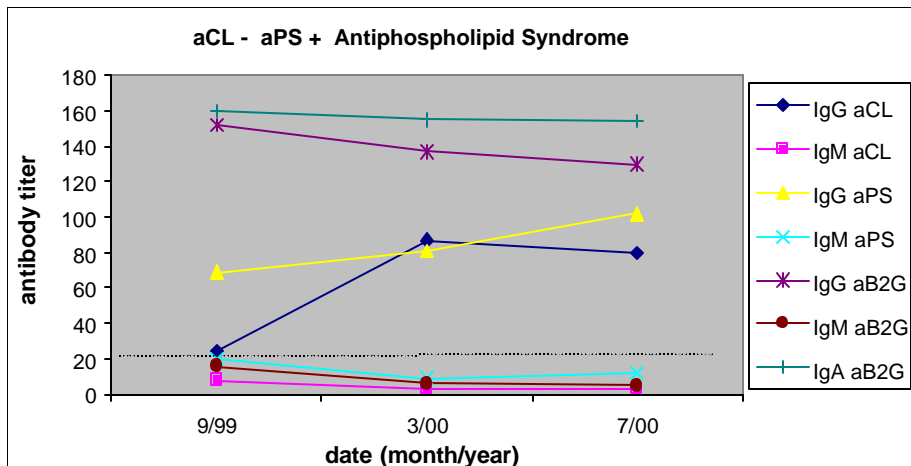
RESULTS

At presentation, the aCL antibody test was reported as negative/borderline for IgG aCL (24 GPL with cut-off of 23 GPL) and negative for IgM and IgA aCL. IgG aPS was strong positive (69 GPS with a cut-off of 16 GPS), negative for IgM aPS. This **IgG aCL negative and aPS positive** patient was followed for 10 months and the results are summarized in the following table and graph.

Table 1: Antiphospholipid antibody levels in a patient with APS

APL Antibody	Cut-off	month/year		
		9/99	3/00	7/00
IgG aCL	<23 GPL	24	89	80
IgM aCL	<11 MPL	8	3	3
IgG aPS	<16 GPS	69	81	102
IgM aPS	<22 MPS	20	9	12
IgG anti-B2GPI	<20 units	152	137	131
IgM anti-B2GPI	<20 units	16	6	5
IgA anti-B2GPI	<20 units	160	156	154

This graph shows that IgG aCL levels increased with time from negative/borderline to strong positive (87 GPL) and remained high several months after diagnosis. IgM aCL remained negative throughout the follow-up period. IgG aPS continued a gradual increase from the initial 69 GPS positive level to 102 GPS while IgM aPS remained negative. When the anti-B2GPI ELISA test was available, the samples were retested. As shown in the graph, this patient was strong positive for both IgG and IgA anti-B2GPI from the beginning.



SUMMARY AND CONCLUSIONS

This case illustrates the following points:

- If the serologic diagnosis were based only on aCL results, the diagnosis of APS would have been missed or delayed for 6 months when the patient seroconverted to strong aCL positive.
- Because IgG aPS was strongly positive when IgG aCL was low, it can be concluded that these antibodies not only are different but behaved differently over time.
- In the proposed algorithm, it was suggested to screen for antiphospholipid antibodies with both aCL and aPS assays to minimize the number of patients missed. Interestingly, IgG and IgA anti-B2GPI antibodies were strongly positive from the beginning, indicating that one test (i.e. aCL) may not be enough.
- Following the algorithm would have led to an earlier diagnosis of APS and treatment in this patient. This is only one patient, but the serologic follow-up presented suggests that the diagnosis of APS can be missed or delayed in some patients if the diagnosis is based on only one test (i.e. aCL) and one testing point in time.

