

Bouquet variety of antiphospholipid antibodies in recurrent pregnancy loss

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Abstract

Seventy-six female patients with two or more recurrent pregnancy losses (RPL) during the 1st trimester were studied. Based on the results of the aCL and aB2GPI antibodies testing, patients were divided in two groups: 22 patients with RPL and elevated immunoglobulin (Ig) G/IgM aCL and/or aB2GPI [RPL + antiphospholipid syndrome (APS)] and 54 patients with RPL alone (without high antibodies). Immunoglobulin G aPS and IgG a-AnV in patients with RPL + APS were higher than in controls and IgG aPS were higher in RPL + APS than in RPL alone. Additionally IgG a-AnV and IgM aPE are higher in RPL alone than in controls. In 18/22 (81%) patients with RPL + APS and 29/54 (54%) patients with RPL alone, there were one or more positive antibodies: aPS, aPT, a-AnV or aPE. These results raise a question whether or not these antiphospholipid antibodies should be routinely tested in women with RPL and especially in the context of the so-called “seronegative APS”.

Key words: antiphospholipid syndrome, antiphospholipid antibodies, recurrent pregnancy loss.

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Introduction

As per a witty quote of Shoenfeld *et al.* [1], in the antiphospholipid syndrome (APS) there is an “explosion of autoantibodies”. Along with the “classical” antibodies such as the anticardiolipin (aCL), anti- β -2-glycoprotein I (aB2GPI) and lupus anticoagulant (LA), physicians can also detect antibodies against other phospholipids: phosphatidylserine (aPS), phosphatidylethanolamine (aPE), phosphatidylglycerol, phosphatidic acid, phosphatidyl inositol (aPI), to proteins such as annexin V (a-AnV), to glycoproteins such as prothrombin (aPT) and well as antibodies against 32 other epitopes. The exact diagnostic significance of the above antibodies still needs to be clarified, especially in the cases of an indeterminate or blurry autoimmune disease or accompanied by a specific clinical symptom.

The aim of the present study was to determine the diagnostic value of the aPS, aPE, aPT and a-AnV in female patients with recurrent pregnancy loss (RPL) with or without positive aCL and/or aB2GPI.

Material and methods

Seventy-six female patients, mean age 29 \pm 4 years (range 20-40), with a history of two or more RPL in the

1st trimester of pregnancy were investigated. Based on their aCL and aB2GPI status, patients were divided in two groups:

- 22 patients (RPL + APS) – with elevated immunoglobulin (Ig) G/IgM aCL and/or aB2GPI [2];
- 54 patients (RPL) – with negative IgG/IgM aCL and/or aB2GPI.

The study included 64 women without pregnancy losses, mean age 31 \pm 4 years (range 23-40).

The levels of aCL, aB2GPI, aPS, aPT and a-AnV (class IgG and IgM) were measured using ELISA method (Orgentec-Germany) and aPE – with ELISA method from IBL-Germany.

Results

The mean values of aPS, aPT, a-AnV and aPE in patients and controls are presented in Table 1.

As per the above results, IgG aPS and IgG a-AnV in patients with RPL + APS are higher than in controls and IgG aPS levels are higher in RPL + APS compared with patients with RPL alone. Additionally IgG a-AnV and IgM aPE are higher in patients with RPL alone compared with controls.

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In 18/22 (81%) patients with RPL + APS, there was at least one type of positive antiphospholipid antibodies (aPL): aPS, aPT, a-AnV or aPE (Table 2).

In 29/54 (54%) patients with RPL, who did not have positive aCL and/or aB2GPI expressed one or more aPL from the other types (Table 2).

Discussion

Vashisht and Regan report that aPL are positive in 15% of miscarriages (MC) during the 1st trimester, in 21% – during the 2nd trimester as well as are positive in HEELP, intrauterine retardation, pre-eclampsia and other pregnancy complications [3]. According to Coulam [4], the immunological factors, including the aPL actually account for 65% of all risk factors. These antibodies can be found in only 2% of women with normal pregnancies.

The group of aPL is very heterogeneous and includes antibodies against various phospholipids cardiolipin (CL), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidyl inositol (PI), etc., antibodies against proteins (AnV) or glycoproteins prothrombin (PT) and β-2-glycoprotein I (B2GPI). The cardiolipin is a negatively charged phospholipid at the inner surface of the cellular membrane. The activity of the aPL is largely dependent upon their interaction with the complex CL/B2GPI. The antibodies against that complex take part in the pathogenesis of APS by altering the fibrinolytic system and activating monocytes, endothelial cells and platelets thus leading to the production of tissue factor or thromboxane [5].

The B2GPI and PT have procoagulant properties and are well studied as antiphospholipid co-factors. They are preferred targets for the aPL. B2GPI (apolipoprotein H) is linked with various lipoproteins in human plasma as chylomicrons and very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL). It inhibits the contact activation of the intrinsic pathway of coagulation [6],

Table 1. Mean values of aPS, aPT, a-AnV and aPE in patients and controls

Antibody	RPL + APS	RPL	Controls
IgG aPT	9.59 ±7.4	8.59 ±7.76	7.59 ±7.18
IgM aPT	5.73 ±4.51	4.2 ±3.8	3.99 ±3.36
IgG aPS	8.84 ±8.65*	2.1 ±2***	2.21 ±1.94
IgM aPS	13.03 ±12.52	6.69 ±6.68	5.77 ±5.19
IgG aPE	9.76 ±9.15	5.48 ±5.21	4.02 ±2.31
IgM aPE	8.86 ±6.95	10.15 ±10**	5.61 ±5.19
IgG a-AnV	7.25 ±5.3*	6.38 ±5.3**	4.42 ±4.24
IgM a-AnV	5.46 ±4.46	3.57 ±3.12	3.11 ±3.1

*p < 0.05 – patients with RPL + APS/controls

**p < 0.05 – patients with RPL/controls

***p < 0.05 – patients with RPL + APS/RPL

the platelet prothrombinase activity [7] and ADP-induced platelet aggregation [8]. The antibodies against B2GPI inhibit this glycoprotein thus increasing the clotting activity and fetal loss observed in patients with highly positive aB2GPI.

Antibodies to prothrombin and phosphatidylserine

The PT (factor II) plays an active role in the human coagulation process. In the presence of thromboplastin and calcium ions PT transforms into thrombin. The antibodies against PT bind to PT, prethrombin I and fragment I but not thrombin [9]. It has been considered that LA recognizes the lipid-bound PT thus inhibiting the phospholipid dependent coagulation reactions [10]. In 1995, Arvieux *et al.* [11] found that the aPT can be detected by the means of regular ELISA and using PT, which is immobilized on an irradiated polystyrene surface and in 1997, Galli *et al.* detected antibodies against the PT/PS complex [12]. The exact clinical significance of aPT in pregnant women with RPL has been evaluated by numerous authors. The results of von Landerberg *et al.* [13], obtained using an ELISA assay by Aesku.lab Diagnostik, Wendelsheim, Germany and cut-off values greater than 15 U/ml reveal a significantly higher proportion positive IgG aPT (61%) in patients with APS and early pregnancy loss, however the authors did not find a link between the clinical symptoms and the presence of IgM aPT. Ailus *et al.* [14] showed that elevated values of aPT are associated with secondary abortions. In

Table 2. aPT, aPS, aPE and a-AnV antibodies in patients with RPL and RPL + APS

Antibodies	RPL + APS	RPL
IgG aPT	0	4
IgM aPT	1	0
IgG aPS	4	2
IgM aPS	3	3
IgG aPE	0	2
IgM aPE	1	5
IgG a-AnV	1	4
IgM a-AnV	0	1
IgG a-AnV + IgM a-AnV	2	1
IgG aPE + IgM aPE	1	2
IgG a-AnV + IgM a-AnV + IgG aPE	2	0
IgM aPS + IgG a-AnV + IgG aPE	1	0
IgG a-AnV + IgG aPE	1	0
IgG a-AnV + IgM aPE	0	3
IgG aPS + IgG aPT	1	2
Total	18/22 (81%)	29/54 (54%)

a group of women with miscarriages, Munoz-Rodriguez *et al.* [15] found no correlation between aPT positive and aPT negative patients. By using γ -irradiated microtiter plates, Donohoe *et al.* [16] found an association between fetal loss and IgM aPT, but not with IgG aPT. According to Atsumi and Koike [17], the clinical symptoms of APS, including pregnancy morbidity in patients with IgG aPS/aPT are similar to those in patients with positive aCL. Lopez *et al.* investigated a small group of patients with pregnancy morbidity [18] and found elevated IgG aPT/aPS in only 6% of patients. Vladea *et al.* [19] found IgG aPT/aPS complex in a large proportion of patients with obstetrical complications. The authors even suggest this complex to be used as a second level assay to confirm APS classification. According to Noxha *et al.* [20], the presence of IgG and/or IgM aPT/aPS is largely associated with obstetrical complications. However other researchers [21] did not find antibodies against that complex in pregnant women with unexplained recurrent miscarriages.

Our team used plates loaded only with PT or PS. The mean values of IgG/IgM aPT between our three investigated groups were not statistically different (Table 1). It is worth noting that in 4/54 patients with RPL alone, the IgG aPT were the only positive antibody, and in other 2/54 patients, there was a combination of positive IgG aPS + IgG aPT, i.e. 6/54 (11%) patients with RPL, who without aCL and/or aB2GPI are positive for aPT or aPT/aPS. This proportion is slightly higher in women with RPL + APS: one patient had positive IgM aPT and 1 – positive IgM aPS + IgG a-AnV + IgG aPT and 1 – IgG aPS + IgG aPT, respectively. So 3/22 (13.6%) had positive aPT (Table 2). This raises a question whether the use of another aPS/aPT assay would result in an even higher number and values of aPT. As per Bertolaccini *et al.*, 7/32 (22%) systemic lupus erythematosus (SLE) patients with a history of pregnancy loss had positive IgG or IgM aPT but this is not statistically higher than that in patients without such pregnancy problems [22].

There is a discussion on the significance and the benefit of testing aPS in patients with pregnancy problems. Based on the results of Rote *et al.* [23], published in 1990, 87% of patients with RPL have elevated IgG aPS and 40% – elevated IgM aPS. The authors specifically note that anti-PS assay is more sensitive than the determination of aCL.

Velayuthaprabhu and Archunan [24] found that 19% of women with recurrent abortion have elevated IgG aPS but negative aCL. The study of Alijotas-Reig *et al.* [25] did not find a link between the aPS and spontaneous pregnancy loss, either. Our results show that the levels of IgG aPS are significantly higher in women with RPL + APS compared to RPL alone and controls (Table 1). On the other hand, IgG and IgM aPS are elevated mainly in patients with RPL + APS – 7/22 (32%). In patients with only RPL positive for IgG and IgM aPS antibodies were in 5/54 (9%). It is very rare to have positive aPS in combination with other antibodies (Table 2).

Antibodies against phosphatidylethanolamine

The PE is a neutral phospholipid, localized in the inner surface of the cell membrane. It has anti- as well as procoagulant properties. The detection of aPE is still not very popular due to the lack of: 1. Suitable microtiter plate [26, 27]; 2. Suitable buffer and constituents such as fetal calf serum, adult bovine serum, adult bovine plasma [26, 27]; 3. Origin of PE – egg yolk, soybean, bovine brain, *Escherichia coli* [27]; 4. Commercial assays; 5. These antibodies are not a part of the APS standards of testing and care. According to some authors, aPE are an important risk factor for early and idiopathic fetal loss [28, 29]. Gris *et al.* [28] focus on the importance of testing the following 4 antibodies: IgM aPE, IgG a-AnV, IgG aB2GPI and LA as retrospective markers for otherwise idiopathic early fetal loss.

A multicentre study within the European Forum on antiphospholipid antibodies [30] revealed that a group of patients negative to LA, aCL and aB2GPI are actually positive to aPE. It is worth noting that in our study, the IgM aPE in patients with RPL alone were significantly higher than in controls (Table 1) but there is no significant difference in the mean levels of IgG aPE and IgM aPE in patients with RPL + APS and RPL alone. In two patients with RPL + APS, there were positive IgG aPE and IgG/IgM a-AnV, and in one patient – IgG aPE and IgG aAnV. In three patients with RPL alone, there were positive IgG a-AnV and IgM aPE (Table 2).

Antibodies against AnV

AnV is a 36 kDa glycoprotein abundant in a wide variety of tissues and cells, i.e. placenta, endothelium and peripheral blood cells. AnV participates in the regulation of two important physiological mechanisms: coagulation and apoptosis. In 1994, Matsuda *et al.* [31] for the first time described positive a-AnV in patients with habitual abortion and pre-eclampsia but without APS. In 2005, Bizzaro *et al.* [32] found positive IgG a-AnV antibodies in 17% of women with recurrent miscarriages and moreover 16% of the aCL-negative patients were positive only for IgG a-AnV. In 2006, Zammiti *et al.* [33] found that positive a-AnV and aB2GPI are independent risk markers for idiopathic RPL. Galli *et al.* [34] showed that the presence of IgG a-AnV is associated with a 9-fold increased risk of abortions. Arnold *et al.* [35] found positive a-AnV in 35% of the aCL-positive and in 19% of the aCL-negative female patients with fetal loss but did not assume that a-AnV are an independent risk marker for recurrent miscarriages. Siaka *et al.* [36] and Arai *et al.* [37] suggest that in patients with APS and spontaneous abortions a-AnV – are rarely found and are not a sensitive test for detection of antiphospholipid antibodies. Our data show that the mean levels of IgG a-AnV are significantly higher compared with the controls as in patients with RPL + APS, as in those without

APS (Table 1). According our results, 7/22 (32%) patients with RPL + APS had positive IgG/IgM a-AnV alone or in combination with aPE, aPS or aPT and 9/54 (16.6%) patients with RPL were positive for IgG/IgM a-AnV alone or in combination with aPE (Table 2).

The exact role of aPS, aPE, aPT, a-AnV and other antiphospholipids still has to be defined. According to Galli [38], the clinical significance of the above antibodies in the pathological pregnancy is considered to be unclear, thus emphasizing the therapeutic helplessness in such patients. According to the guidelines of the APS committee [2], there will be other antibodies to be included in the APS diagnostic panel. Schoenfeld and Blank [39] consider that a large number of antibodies such as aCL, aB2GPI, aPS, aPE, aPT, a-AnV, etc. are be linked to the reproductive failures. Makino [40] focuses on two of those antibodies: aPE and a-AnV and their role in recurrent reproductive wastage. In women with unexplained recurrent miscarriages and negative aCL and aB2GPI Bizzaro *et al.* [32] found positive aPT in 11% and positive a-AnV – in 19%. Yetman and Kutteh [41] described positive antibodies either against PI, or to phosphatidylglycerol, PS, PE in 10% of patients with RPL without aCL, thus emphasizing the need for standardization, quality control and clinical interpretation of the positive results.

In the recent years there has been a discussion on the so-called “seronegative APS” [42-45]. There has been a statement that it will be good to have a roadmap on the exact clinical significance [43] of the antibodies against other phospholipids (PE, phospholipid-binding plasma proteins, phospholipid-protein complexes as well as anionic phospholipids different than CL). Based on the statement of Schoenfeld [46], the APS is a more systemic condition than the SLE alone and is more than a syndrome, which initially included only two antibodies (aCL and LA) but now it is about to include multiple antibodies against PS, PE, phosphatidylcholine, AnV, PT, DNA, endothelial cells, platelets, ox-LDL, lipoprotein A and many others, which still need to be defined.

Our study shows that only 22 (29%) all 76 patients with RPL during the 1st trimester had positive aCL and/or aB2GPI. In the remaining 2/3 of patients with RPL as well as in a part of women with APS we detected other types of antiphospholipid antibodies: aPS, aPE, aPT and a-AnV. This galore of antibodies raises a question whether or not they should be routinely tested. Their presence in the serum does not necessarily confirm or reject the presence of APS but by all means speaks of pathology demanding further investigation.

The authors declare no conflict of interest.

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