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Pathogenic Mechanisms of Thrombosis in Antiphospholipid Syndrome (APS)

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1. Introduction

The term "antiphospholipid syndrome" (APS) was coined in the early '80s to describe a unique form of acquired autoimmune thrombophilia, with clinical features of recurrent thrombosis and pregnancy morbidity, combined with the presence of antiphospholipid antibodies (aPL). aPL consist a heterogeneous group of autoantibodies, which recognize phospholipid-protein complexes or rather proteins with high affinity for phospholipids, such as β2-glycoprotein I (β2GPI) and prothrombin. This heterogeneity reflects the nature of the antigen recognized by each antibody and it is expressed with different pathogenic mechanisms. Although a broad spectrum of aPL exists, the universally accepted diagnostic aPL tests are lupus anticoagulant (LA); anticardiolipin (anti-CL); and anti-β2-glycoprotein I (anti-β2GPI) antibodies (Table 1). These autoantibodies interfere with physiological mechanisms of coagulation and fibrinolysis, leading the hemostatic balance towards coagulation. Moreover, it seems to affect the physiological function of various cells such as platelets, monocytes and endothelial cells (EC). However, not all aPL are pathogenic; transient increase in aPL during several infections has been observed. Although aPL can cause clinical manifestations of almost every organ in the body (e.g. blood vessels, brain, kidneys, lungs, gastrointestinal tract, placenta), the common hallmark of the syndrome is thrombosis, either arterial, venous or in the microcirculation.

2. Epidemiology

2.1 aPL in healthy population and other conditions

In healthy population, the incidence of aPL ranges from 1 to 5%. It has been shown to increase with age and especially with coexistence of chronic diseases [1]. Among various studies, there is great variation in the incidence of anti-CL antibodies in apparently healthy elderly subjects, ranging from 12 to 64% [2]. The prevalence of anti-β2GPI antibodies is calculated at 31.8%. These variations may be attributed to both methodological differences and choice of sample population. In general population, aPL are detected in about one out of five patients who suffered from cerebrovascular events (strokes) at the age under 50 years. The clinical suspicion is clearly enhanced in young patients with additional features of the syndrome. Moreover, aPL can be detected in various conditions such as infections, malignancies, vaccination and use of certain drugs. In these cases, aPL are usually transient, low-titre and normally independent of the presence of β2GPI. The prevalence of aPL in
healthy obstetric population is difficult to be determined since aPL have been implicated in pregnancy morbidity. However, in two studies with large number of healthy pregnant women, aPL were identified in 0.7 and 5.3% respectively \[^3\]. It has been reported that aPL are detected in 11-29% of women with preeclampsia \[^4\].

<table>
<thead>
<tr>
<th>Symbol/acronym</th>
<th>Description/explanation</th>
<th>Definition based on the detection assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CL</td>
<td>Anti-cardiolipin</td>
<td>Antibodies detected against cardiolipin-a negatively charged phospholipid, which is used as antigen in an enzyme linked immunosorbent assay (ELISA). These antibodies recognize ß2GPI which exists in abundance in bovine serum; this serum is used as a blocking agent (blocks the non-specific binding sites) in ELISA plates. ß2GPI binds to cardiolipin.</td>
</tr>
<tr>
<td>Anti-ß2 GPI</td>
<td>Antibodies recognizing ß2 GPI independently of cardiolipin</td>
<td>Anti-ß2GPI antibodies are detected by ELISA, but the polystyrene ELISA plates have been γ-irradiated and negative charges are generated on their surface. ß2GPI binds to negatively charged irradiated plates in the absence of cardiolipin and is recognized by the anti-ß2GPI antibodies.</td>
</tr>
<tr>
<td>LA</td>
<td>Lupus Anticoagulant</td>
<td>Antibodies which interfere in vitro with the generation of thrombin from prothrombin, thus increasing the activated partial thromboplastin time (aPTT). The prolonged aPTT is not corrected by adding normal plasma in the detection system. These antibodies disrupt in vitro the prothrombinase complex (constituted by the activated coagulation factors V and X, prothrombin and phospholipids). Kaolin clotting time (KCT) and Dilute Russel Viper Venom Test (DRVVT) are two other ways to measure LA. Antibodies of the LA type usually recognize ß2GPI or Prothrombin.</td>
</tr>
<tr>
<td>BFP-STS &amp; VDRL test</td>
<td>Biological false positive serological tests for syphilis &amp; Venereal Disease Research Laboratory test</td>
<td>Antibodies against phospholipids/cholesterol complexes</td>
</tr>
</tbody>
</table>

Table 1. Classification of antiphospholipid antibodies defined according to detection assay.
2.2 aPL in other systemic autoimmune diseases

APS can occur in association with other systemic autoimmune diseases, particularly Systemic Lupus Erythematosus (SLE). About 40% of patients with SLE have aPL, but less than 40% of them will eventually have thrombotic events [5]. Particularly, 37% of patients in the "Euro-Phospholipid" study was suffering from SLE, while 4% was associated with SLE-like disease [6]. It is well documented that thrombotic complications appear more frequently in patients with SLE and aPL, as compared to aPL positive patients without other systemic autoimmune disease[7,8]. The diagnosis of secondary APS clearly leads to a threefold increase of miscarriages, especially after the 20th week of gestation [9]. aPL have also been detected in other autoimmune diseases, such as rheumatoid arthritis (RA), with a frequency up to 28%.

3. Classification Criteria for APS

An international consensus statement on the APS classification was published in 1999, after the 8th International Symposium on the aPL in Sapporo [10]. The diagnosis of the syndrome requires the presence of at least one clinical event and one positive laboratory test for aPL, including lupus anticoagulant (LA) or anti-CL antibodies, or both, in medium-high titers, detected at least twice within 6 weeks. The classification criteria were revised in 2006, in Sidney, Australia [11]. Essentially, the clinical criteria remained unchanged while the laboratory criteria were modified in two significant points: (a) the time between two positive determinations was extended to 12 weeks and (b) anti-β2GPI antibodies (IgG and IgM) were included in the laboratory tests (Table 2).

After 30 years of intensive clinical and basic research, it is now well documented that LA is the strongest predictor of thrombosis [12-14]. Moreover, anti-β2GPI antibodies appear to be mainly responsible for the clinical manifestations that characterize the syndrome [15,16]. More specifically, a subpopulation of anti-β2GPI antibodies, raised against a cryptic epitope in domain I β2GPI (Arg39-Arg43), is highly correlated with clinical events and prognosis of the syndrome [17]. Several research groups suggest that patients positive for all three classes of antibodies (LA, anti-CL and anti-β2GPI) are at the higher risk for venous/arterial thrombosis and pregnancy morbidity [18,19].

4. Clinical manifestations of APS

Clinical presentation of APS includes manifestations of various organs and systems, such as blood vessels, central nervous system (CNS), skin, kidney, gastrointestinal tract, heart, and placenta. The hallmark of the syndrome is thrombosis, either arterial or venous. Unlike the strict vaso-specific localization of thrombosis due to congenital thrombophilias (e.g. correlation between deficiency of protein C only with venous thromboembolic events), thrombosis in APS may occur in any vascular bed [20,21]. This "diffuse" thrombotic predisposition clearly indicates a multifactorial influence of autoantibodies in the haemostatic system. It is worth noting that thrombotic recurrences tend to occur in the same vascular distribution as the original event (i.e., arterial are followed by arterial and venous by venous thrombotic events)[22]. Venous thromboembolic events are the most common clinical manifestations of APS. The clinical expression of the syndrome can vary from mild (mild thrombocytopenia, livedo reticularis, leg ulcers, migraine) to severe (ischemic infarctions, recurrent miscarriages, valvular insufficiency) or destructive [multiple organ
The clinical spectrum associated with the presence of aPL is extensive. The events included in the classification criteria of APS are described in (Table 3). Some clinical manifestations, such as valvular disease, livedo reticularis, thrombocytopenia and neurological manifestations, although not included in the diagnostic criteria of the syndrome, are frequently observed in patients with APS [11]. The thrombotic etiology for these events does not seem very likely, as they usually do not subside with antithrombotic therapy. The simultaneous presence of thrombotic and non-thrombotic events in patients with APS leads to the logical conclusion that most of the aPL (and especially anti-β2GPI antibodies) interfere with many different biological mechanisms. Moreover, there is a subset of asymptomatic patients with permanently high titers aPL.

### Antiphospholipid antibody syndrome (APS)

Antiphospholipid antibody syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met:

#### Clinical criteria

1. **Vascular thrombosis**
   - One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.

2. **Pregnancy morbidity**
   - a. One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or
   - b. One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe preeclampsia defined according to standard definitions [11], or (ii) recognized features of placental insufficiency, or
   - c. Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

#### Laboratory criteria

1. **Lupus anticoagulant (LA)** present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies)

2. **Anticardiolipin (aCL)** antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 GPL or MPL, or >the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA

3. **Anti-b2 glycoprotein-I** antibody of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures

*Modified from ref 11*

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**Table 2. Classification criteria for antiphospholipid syndrome**
The literature on the clinical manifestations of APS, although extensive, mostly includes a large number of case reports. Fortunately, since the beginning of the 21st century, significant multicenter studies on large series of patients provide reliable information as to the relative frequency of different clinical manifestations of the syndrome [21,23,24]. In a multicenter study of 1000 patients with APS, 53.1% of patients had primary APS while 36.2% had secondary APS (in association with SLE). The most common manifestations were deep venous thrombosis (38.9%), thrombocytopenia (29.6%), livedo reticularis (24.1%), stroke (19.8%) and pulmonary embolism (14.1%). Less frequent events included superficial thrombophlebitis (11.7%), transient ischemic attacks (11.1%), hemolytic anemia (9.7%) and epilepsy (7%). The fetal abortion was the primary event in 14% of female patients.

4.1 Venous thrombosis
As mentioned above, deep venous thrombosis is the most frequent clinical manifestation of APS. Other vascular sites which may be affected are the larger veins, such as the subclavian, the iliofemoral, the upper abdomen, portal vein, the axillary etc. Venous thrombosis has been described, with much less frequency, in almost every organ of the body, causing related clinical manifestations. Superficial thrombophlebitis, superior vena cava syndrome, renal vein thrombosis, adrenal infarction, Addison’s syndrome, Budd Chiari syndrome, pulmonary hypertension, due to recurrent pulmonary embolism and diffuse pulmonary hemorrhage, due to micro-thromboses are some of the unusual venous thrombotic manifestations of the syndrome. Recent meta-analysis regarding the relationship (odds ratio, OR) of LA and IgG/IgM anti-CL antibodies with venous thrombosis, concludes that all studies report a significant correlation between LA and VTE, with an OR up to 16.2. The correlation of anti-CL with VTE is not confirmed [25]. Titers of anti-β2GPI antibodies seem to increase the OR for venous thrombosis up to 5 times [26].

4.2 Arterial thrombosis
Arterial thrombosis consists a main clinical feature of APS, but appears less frequently than venous [27]. The most common site of arterial thrombosis is the cerebral circulation, usually in the form of stroke or transient ischemic attack [21]. Thrombosis of coronary, renal artery or the mesentery has also been observed. A multicenter population-based study, which examined the risk of arterial thrombosis in patients who were treated with oral contraceptives (RATIO study, Risk of Arterial Thrombosis In relation to Oral contraceptives), showed that the presence of LA is a major risk factor for arterial thrombosis in women under 50 years, while anti-CL and anti-prothrombin antibodies did not increase the risk for ischemic stroke or myocardial infarction [28].

4.3 CNS involvement
The CNS involvement is co-responsible for high morbidity and mortality of the syndrome, with strokes and transient ischemic attack being the most common manifestations [29,30]. The neurological manifestations cover a fairly broad clinical spectrum and may include, apart from stroke and transient ischemic attack, also Sneddon’s Syndrome, epilepsy, dementia, cognitive dysfunction, headaches/migraine, chorea, transverse and spotty myelitis, ocular symptoms, Guillain-Barré, psychosis and depression [31]. More specifically, the presence of anti-CL antibodies in SLE patients has been associated with cognitive disorders. Similarly,
mild cognitive impairment has been recorded in more than 40% of patients with APS and focal white matter lesions \[32\].

4.4 Renal involvement
APS nephropathy is now recognized as a distinct entity from lupus nephritis \[33\]. The most common manifestations of renal involvement are thrombosis or stenosis of renal artery, kidney infarction, thrombosis of the renal vein and end-stage renal disease/renal failure \[34\]. Clinically, the so-called APS nephropathy is characterized by positive aPL in conjunction with vascular nephropathy, which presents with hypertension, low-grade proteinuria, and acute and/or chronic renal failure. The clinical suspicion of this clinical entity should always be placed in young patients with renal artery stenosis, high blood pressure and unexplained deterioration of renal function. The main histopathological findings are: a) thrombotic microangiopathy, as an expression of acute thrombosis, and b) fibrous intimal hyperplasia, arteriosclerosis, focal cortical atrophy, arterial and fibrous infarcts, as for chronic vascular lesions \[34\]. The biopsy proven renal damage is significantly correlated with LA, but not with anti-CL antibodies. A study from our department showed that patients with APS and kidney involvement develop hypertension, elevated serum creatinine levels, characteristic findings in renal biopsy as mentioned previously, and factors associated with poor renal prognosis \[35\]. Moreover, a large series of patients demonstrated that APS nephropathy is presented in 39.5% of patients with SLE and positive aPL, while only in 4.3% of SLE patients without aPL \[36\]. The APS nephropathy is strongly correlated with LA, anti-CL antibodies and livedo reticularis. The prognosis of nephritis in patients with SLE is rather poor in the presence of coexisting APS nephropathy.

4.5 Cardiovascular manifestations
Cardiac manifestations in APS include valvular disease, acute myocardial infarction (AMI), intracardial thrombi, myocardial microthrombosis and valvular lesions similar to Libman-Sacks endocarditis of SLE \[37\]. The latter represent the commonest manifestation in APS with prevalence up to 38% \[38\]. The valves of the left ventricle are most frequently involved, with mitral impairment observed in the majority of cases. It is well known that patients with valvular disease are at higher risk of arterial thromboembolic events \[39\]. In a multicenter prospective study, AMI was the presenting manifestation in 2.8% and reached up to 5.5% during the follow-up period \[40\]. A large prospective study found that high titers of anti-CL antibodies are an independent risk factor for AMI or sudden cardiac death \[41\]. Although some studies do not show a correlation between anti-CL and AMI, there should be a thorough check for aPL in patients aged under 45 with coronary artery disease or AMI without other risk factors.

4.6 Skin manifestations
Skin events in APS vary both in form and severity, with livedo reticularis and leg ulcers being the commonest \[42\]. Livedo reticularis is caused by stagnation of blood in dilated superficial capillary venules and its prevalence in APS is calculated at 24%, according to the study of Cervera et al \[43,44\]. This lesion represents the first manifestation of the syndrome in 17-40% of patients. The clinical features of such lesions, although nonspecific, can lead to the diagnosis of APS. Typically, the histopathological findings of skin biopsies do not reveal evidence of thrombosis (except in the case of CAPS). Livedo reticularis has been
characterized as a major risk factor for arterial thrombosis and it is correlated with high titers of anti-CL antibodies [45]. Other cutaneous manifestations of the syndrome are leg ulcers, superficial thrombophlebitis, necrotizing vasculitis and gangrene.

4.7 Hematological manifestations
Several hematological manifestations such as thrombocytopenia, autoimmune hemolytic anemia, Evan’s syndrome, bone marrow necrosis and thrombotic microangiopathy have been described in APS [46]. Thrombocytopenia, defined by platelet count less than 100-150x10⁹/L, is found in approximately 20% of patients with primary APS and more than 40% of patients with secondary APS in SLE [21,47]. It is usually moderate and does not require any medical intervention. The mechanisms that may lead to thrombocytopenia in patients with APS are not clear. However, the characteristic combination of thrombocytopenia and thrombosis in APS patients suggests that aPL interact with platelets resulting in platelet aggregation and thrombosis. The prevalence of autoimmune hemolytic anemia in APS patients was calculated at 6.6% to 9.7% [23]. On the other hand, studies confirm the presence of aPL in patients with autoimmune hemolytic anemia at a rate ranging from 55 to 72% [48]. The pathogenesis of autoimmune hemolytic anemia is unclear. It has been assumed that anti-CL antibodies bind directly to the surface of red blood cells of patients with APS and hemolytic anemia, although the antigen recognized by aPL has not been identified.

5. Pathogenic mechanisms of thrombosis
Significant in vitro and in vivo studies confirm that aPL are pathogenic [49-51]. The exact mechanism by which these antibodies participate in the prothrombotic tendency of APS, remain to be clearly defined. However, it has been illustrated that the heterogeneity of antibodies is associated with multiple mechanisms of action [52]. These include briefly: the activation of cellular components (endothelial cells, platelets and monocytes), activation of the coagulation cascade, inhibition of the fibrinolytic system, inhibition of natural anticoagulant pathways and activation of the complement system.

5.1 Activation of cellular components
5.1.1 Activation of endothelial cells
The interaction of aPL with endothelial cells has until now been in frequent disputes and misunderstandings. Regarding the study of endothelium in the pathogenesis of APS, major questions have arisen: a) Under what conditions the aPL bind to the surface of endothelial cells in vitro and in vivo; b) What molecular structures on the surface of endothelial cells are responsible for the binding d) What signaling pathways are activated; Table 4 summarizes the effects of aPL on endothelial cells.
In vitro studies have shown that β2GPI binds to immobilized endothelial cells, and allows the anti-β2GPI antibodies to bind to cells and induce a proinflammatory and procoagulant phenotype [53]. A clinical demonstration of endothelial dysfunction in APS patients is the increased level of circulating endothelial microparticles and endothelial cells in peripheral blood [54,55]. In vitro studies have shown that incubation of endothelial cells with aPL and β2GPI, induces the expression of significantly higher levels of adhesion molecules ICAM-1, VCAM-1 and E-selectin. In addition, production of IL-6 and altered metabolism of arachidonic acid, have been demonstrated [56-58]. Animal models suggest that human
### Clinical and laboratory characteristics

<table>
<thead>
<tr>
<th>Condition</th>
<th>Presence at the disease onset n (%)</th>
<th>Cumulative prevalence n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombophlebitis</td>
<td>28 (20.72)</td>
<td>57 (42.18)</td>
</tr>
<tr>
<td>Peripheral Arterial Thrombotic Event</td>
<td>6 (4.44)</td>
<td>12 (8.88)</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>5 (3.7)</td>
<td>11 (8.14)</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>1 (0.74)</td>
<td>1 (0.74)</td>
</tr>
<tr>
<td>Stroke (arterial)</td>
<td>10 (7.4)</td>
<td>32 (23.68)</td>
</tr>
<tr>
<td>Brain Microinfarcts/TIA</td>
<td>1 (0.74)</td>
<td>14 (10.36)</td>
</tr>
<tr>
<td>Brain Venous Thrombosis</td>
<td>1 (0.74)</td>
<td>1 (0.74)</td>
</tr>
<tr>
<td>Epilepsy/Seizures</td>
<td>2 (1.48)</td>
<td>19 (14.06)</td>
</tr>
<tr>
<td>Diplopia/Ocular disorders</td>
<td>1 (0.74)</td>
<td>23 (17.02)</td>
</tr>
<tr>
<td>Peripheral Neuritis</td>
<td>1 (0.74)</td>
<td>30 (22.2)</td>
</tr>
<tr>
<td>Lower Back Pain</td>
<td>1 (0.74)</td>
<td>15 (11.1)</td>
</tr>
<tr>
<td>Headache/Migraine</td>
<td>6 (4.44)</td>
<td>34 (25.16)</td>
</tr>
<tr>
<td>Pleurisy</td>
<td>3 (2.22)</td>
<td>12 (8.88)</td>
</tr>
<tr>
<td>Pulmonary Emboli</td>
<td>7 (5.18)</td>
<td>16 (11.84)</td>
</tr>
<tr>
<td>Pulmonary Arterial Hypertension</td>
<td>1 (0.74)</td>
<td>9 (6.66)</td>
</tr>
<tr>
<td>Nephritis</td>
<td>2 (1.48)</td>
<td>17 (12.58)</td>
</tr>
<tr>
<td>APS associated Nephropathy</td>
<td>1 (0.74)</td>
<td>6 (4.44)</td>
</tr>
<tr>
<td>Budd-Chiari Syndrome</td>
<td>1 (0.74)</td>
<td>1 (0.74)</td>
</tr>
<tr>
<td>Ascites</td>
<td>1 (0.74)</td>
<td>1 (0.74)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2 (1.48)</td>
<td>9 (6.66)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>25 (18.5)</td>
<td>67 (49.58)</td>
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<tr>
<td>Arthritis</td>
<td>9 (6.66)</td>
<td>21 (15.54)</td>
</tr>
<tr>
<td>Livedo Reticularis</td>
<td>2 (1.48)</td>
<td>48 (35.52)</td>
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<tr>
<td>Ischemic Leg Ulcers</td>
<td>2 (1.48)</td>
<td>5 (3.7)</td>
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<tr>
<td>Digital Gangrene</td>
<td>1 (0.74)</td>
<td>1 (0.74)</td>
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<tr>
<td>Maculopapular Rash</td>
<td>3 (2.22)</td>
<td>13 (9.62)</td>
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<tr>
<td>Malar Rash (Butterfly)</td>
<td>8 (5.92)</td>
<td>21 (15.54)</td>
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<tr>
<td>Mucosal Ulcers</td>
<td>6 (4.44)</td>
<td>15 (11.1)</td>
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<td>Vasculitis Rash</td>
<td>1 (0.74)</td>
<td>5 (3.7)</td>
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<tr>
<td>Raynaud</td>
<td>10 (7.4)</td>
<td>34 (25.16)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>8 (5.92)</td>
<td>20 (14.8)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>4 (2.96)</td>
<td>22 (16.28)</td>
</tr>
<tr>
<td>Foetal Loss</td>
<td>23 (23) *</td>
<td>40 (40) *</td>
</tr>
<tr>
<td>Chronic Disease Anemia</td>
<td>1 (0.74)</td>
<td>23 (17.02)</td>
</tr>
<tr>
<td>Hemolytic Anemia</td>
<td>2 (1.48)</td>
<td>22 (16.28)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>5 (3.7)</td>
<td>20 (14.8)</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>1 (0.74)</td>
<td>40 (29.6)</td>
</tr>
</tbody>
</table>

*percentage (%) out of the total of female patients (N= 100)

Grika E. – Vlachoyiannopoulos P. submitted

Table 3. Prevalence of clinical and laboratory characteristics present on the onset of the disease as well as during the follow-up of 135 APS patients (89 PAPS, 46 SAPS). The mean (SD) disease duration was 8.8 years (5.3) and the mean (SD) duration of follow up 7.55 (4.89) years.
Pathogenic Mechanisms of Thrombosis in Antiphospholipid Syndrome (APS)

β2GPI-dependent increased expression of adhesion molecules (ICAM-1, VCAM-1, E-selectin)

Increase of synthesis and secretion of pro-inflammatory cytokines (IL-1, IL-6)

Increased tissue factor expression/upregulation of tissue factor mRNA

Increase of endothelin-1 levels

Induction of apoptosis

Table 4. The effects of aPL antibodies on endothelial cells

Polyclonal and monoclonal aPL activate endothelium and enhance clot formation in vivo [50,59]. Studying the effect of aPL in mice with genetic deletion of ICAM-1, E-selectin and P-selectin, it was demonstrated that aPL-mediated endothelial activation depends on a considerable extent by these molecules [60-62]. Some studies suggest the presence of elevated levels of soluble adhesion molecules and soluble cytokines in patients with aPL and history of thrombosis [63]. Pierangeli et al. examined the pathogenic properties of human anti-CL antibodies in vivo and in vitro. The researchers used an in vivo model of thrombosis in mouse femoral vein. It turned out that antibodies caused leukocyte adhesion to endothelial cells of the microvasculature and increased the expression of adhesion molecules (e.g. VCAM-1). Among the proposed mechanisms, which explain the prothrombotic and proinflammatory properties of aPL, an increased expression of tissue factor (TF) is included. Data on the increase in TF expression in patients with APS is supported by several researchers. Typically, Zhou et al. demonstrated that IgG from patients with APS increases the expression and function of TF in monocytes [64]. Moreover, they described increased levels of soluble TF (sTF) in the peripheral blood of patients with a history of thrombosis and aPL.

The study of the endothelium clearly indicates the potentially beneficial effect of statins in the therapeutic approach of patients. Meroni et al. demonstrated that fluvastatin inhibits the expression of adhesion molecules and IL-6 by endothelial cells [65]. Very recently, proteomic study of monocytes and endothelial cells in patients with APS who received fluvastatin for one month, confirmed a significant reduction in the expression of TF, PAR-1, 2 and VEGF, which is associated with inhibition of the p38MAPK and NF-kB pathway [66]. Furthermore, Shoenfeld et al., based on the immunoregulatory and possibly antithrombotic effect of vitamin D, studied the serum levels of vitamin in patients with APS and its in vitro effects in HUVEC. It was shown that ~50% of patients had vitamin D deficiency (<15ng/ml) which correlates with thrombotic complications and other clinical manifestations of the syndrome (neurological, ocular, livedo reticularis and skin ulcers). In vitro, vitamin D seems to suspend the anti-β2GPI-induced expression of TF [67].

The role of anti-endothelial antibodies, especially of anti-HSP60, in the mechanism of thrombosis was studied in LA positive patients with secondary APS [68]. These antibodies induce endothelial apoptosis and seem to be associated with increased incidence of thrombosis. The researchers suggest that anti-HSP60 bind to the surface of EC and induce expression of anionic phosphatidylinerine, thus providing a target for aPL binding and promotion of a thrombotic phenotype.

The exact nature of aPL-receptors on the surface of endothelial cells remains obscure and is a key subject of current research. Initially, several studies have suggested the simple recognition of β2GPI, bound to the surface of endothelial cells (by the domain V), by anti-β2GPI antibodies. This observation emerged from the crystallographic study of β2GPI. This glycoprotein is a single-chain polypeptide chain composed of five domains (I-V), comprising patterns of the complement control protein family (sushi domains). All domains

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resemble each other, except for domain V that contains an additional carboxy-loop with positively charged amino acids lysine. From the crystal structure of human β2GPI, it was showed that the positively charged region interacts electrostatically with negatively charged phospholipids. Furthermore, a partially hydrophobic loop of the molecule has the ability to enter the phospholipid bilayer and is involved in cell binding of β2GPI [69]. Later, Zhang et al. demonstrated that β2GPI binding to endothelial annexin A2 promotes the activation of endothelium by anti-β2GPI antibodies, possibly through a mechanism of multiple connections (cross linking), thus inducing a procoagulant phenotype [70]. To note that annexin A2 lacks intracellular region and possibly a second adaptor protein participates in intracellular signal transduction. There is evidence that specific antibodies against annexin A2, regardless of aPL, have been detected in plasma from APS patients and seem to correlate with thrombotic events. These antibodies are able to activate EC in a manner similar to anti-β2GPI antibodies [71]. Besides annexin A2, various members of Toll-like receptors (TLRs) family have been proposed as potential receptors of β2GPI/anti-β2GPI complexes on the surface of endothelial cells. Several data suggest the involvement of TLR-4 in aPL-mediated activation of endothelial cells. An important study shows the activation of the molecular pathway of MyD88 (Myeloid Differentiation factor 88), which is associated with TLR-4 - a receptor of bacterial endotoxin or lipopolysacharide (LPS), by anti-β2GPI antibodies [72]. Binding of LPS with TLR-4 triggers the nuclear translocation of NF-kB, the p38MAPK phosphorylation and other intracellular events that lead to the expression of pro-inflammatory cytokines, adhesion molecules and TF [73]. Fischetti et al. showed that the in vivo formation of complexes β2GPI/anti-β2GPI on the endothelial surface induces the development of thrombus, only under the influence of LPS [74]. In similar results, concerning the requirement of a priming factor, conclude several in vivo animal models of thrombosis, which require either photochemical or mechanical injury of the vessel so that the aPL can exert their thrombotic properties. It is not clear why the formation of anti-β2GPI/β2GPI complexes on the endothelial surface cannot induce thrombosis without a priming factor [75]. It is generally accepted that other molecules (not specified so far) could act as protein receptors for β2GPI, since the glycoprotein lacks a recognized specific cell receptor. It is assumed that β2GPI binds to endothelial surface via a "multi-receptor" and the molecular signaling is initiated immediately upon binding of specific antibodies. The study of signal transduction in aPL-mediated endothelial activation continues to be an important area of research. As it was originally shown, there is an increased expression of adhesion molecules by endothelium in vitro. Nowadays, several studies confirm the involvement of NF-kB nuclear translocation and phosphorylation of p38MAPK in, aPL-mediated, induction of transcription, expression and function of TF in endothelial cells and the production of proinflammatory cytokines (IL-6 and IL-8).

5.1.2 Activation of platelets
The thrombophilic tendency in combination with the observation that thrombocytopenia is a frequent manifestation in APS, led early on the assumption that platelet activation is an important factor in the pathogenesis of the disease [52]. It should be noted that the study of platelet as "experimental model" has some peculiarities: platelets as anucleated cell fragments cannot be cultivated in vitro, or studied by techniques of recombinant DNA and on the other hand, unlike endothelial cells, platelet samples can be purified in the absence heparin, leaving unaffected the β2GPI interaction with platelet membranes [76]. Several studies demonstrate platelet activation and aggregation by aPL, both in vitro and in vivo [77,78]. Figure 1 summarizes the main pathogenic effects of aPL in platelets. It has been
Fig. 1. A synopsis of the major and well characterized thrombogenic mechanisms of antiphospholipid (anti-β2GPI) antibodies: Pre-existing anti-β2GPI, probably under a second hit, activate components of innate immunity such as complement, endothelial cells, monocytes (not depicted) and platelets, as well as the coagulation system. More specifically, as shown by in vitro experiments, dimmers of β2GPI anchored to negatively charged phospholipids and TLR-4 are recognized by anti-β2GPI antibodies; then, P38-MAPK mediated endothelial cell activation takes place as depicted by ICAM-1, VCAM-1, E-selectin IL-1,-6,-8 and TF expression which change the phenotype of endothelial cells to a pro-coagulant form. On the other hand, dimmers of β2GPI induced, either, through β2GPI interaction with apo-ER2', or with GPIba, or with PF4 tetramers, activate platelets towards aggregation and release of PF4 and thromboxan B2. Coagulation-fibrinolysis imbalance is induced by the mechanisms depicted in the upper left panel.
demonstrated that aPL cannot bind to the surface of "intact" platelets, while they have the ability to bind to platelets with exposed negatively charged phospholipids in their membranes. Subactivating priming by known agonists, leading to phosphatidylserine exposure on the outer membrane seems to be a prerequisite for aPL to exert their pathogenic effects. One significant indication of platelet activation was the observation that patients with aPL and positive LA had increased urinary excretion of Thromboxane B2 (TXB2), a platelet metabolite secreted upon activation. Furthermore, purified F(ab')2 antibody fragments from the same patients, caused increased platelet aggregation and release of serotonin in the presence of low doses of thrombin.

A second indication of impaired platelet function in APS, is the observation that approximately 40% of patients show prolonged bleeding time, without accompanying bleeding tendency [79]. This assay depends only on primary hemostasis and thus is considered the classic test for the assessment of platelet function. Strong evidence of aPL-induced platelet activation is the enhanced expression of platelet membrane glycoproteins, particularly GPIIb-IIIa (fibrinogen receptor, critical in platelet aggregation) and GPIIa [80]. More recently, Pierangeli et al. showed that in mice with genetic deletion of GPIIb-IIIa, passive immunization with aPL did not increase clot formation [81]. It has been also shown that blood clots are reduced in vivo, in mice that had been injected with monoclonal anti-GPIIb-IIIa antibodies. The possibility to include this type of inhibitors for the treatment of thrombotic events in patients with APS has not yet been confirmed.

A recent suggestion regarding platelet activation derived from the observation that patients with SLE and anti-2GPI antibodies showed increased plasma levels of active von Willebrand factor (vWF), leading to platelet aggregation. It is assumed that under normal circumstances, β2GPI binds vWF, inhibiting its ability to promote adhesion and platelet aggregation, whereas in the presence of anti-β2GPI antibodies this anticoagulant effect is inhibited [82].

The activation of platelets by aPL, in vivo, has been supported by several groups. Joseph et al. studied in vivo the extent of platelet activation from patients with primary or secondary APS and SLE, using flow cytometry (FACS) and ELISA. They showed evidence of platelet activation as a significant increase in CD63 (a lysosomal membrane protein, which moves to the cell membrane after activation), increased binding of PAC-1 (a monoclonal IgM antibody against GPIIa-IIIb), the formation of platelet-leukocytes complexes and increased soluble p-selectin in patients' plasma [83]. Jankowski et al., studying a photochemically-induced thrombosis rat model, demonstrated that anti-β2GPI monoclonal antibodies with LA activity induced the formation of stable thrombi and large aggregates [83].

So far, two receptors have been proposed as mediators of platelet activation by aPL: a) the receptor of apolipoprotein E2' (apoER2') and b) glycoprotein GPIba (subunit of the platelet receptor GPIb/V/IX), which binds multiple ligands, including vWF. Involvement of apoER2' emerged from the research group of De Groot. They constructed chimeric dimers of β2GPI, which mimic the properties of anti-β2GPI/β2GPI complexes and showed an increase in the degree of platelet aggregation and adhesion to collagen [84]. The dimerization of the protein is of major importance, as monomeric β2GPI does not induce the same result. The researchers demonstrated by co-immunoprecipitation that dimeric β2GPI interacts with apoER2', which mediates the activation of platelets and the production of thromboxane. The specific inhibition of apoER2' by a specific monoclonal antibody resulted in reduced adhesion of platelets to collagen [85]. The role of apoER2' was recently investigated in an
animal model of thrombosis. Passive immunization of apoER2'/-/- mice with aPL purified from patients or monoclonal anti-β2GPI or β2GPI chimeric dimers, caused significantly reduced clots and reduced TF expression of vascular cells and macrophages, compared with immunization of control mice [86]. The role of GPIba as a β2GPI-receptor and potential mediator of platelet activation has been studied by in vitro binding assays and selective inhibition using specific antibodies [87]. It appeared that GPIba was able to bind directly β2GPI, suggesting a new mediator in platelet activation and thromboxane production by anti-β2GPI antibodies. It is well established that the intracellular signaling triggered by β2GPI/antiβ2GPI complexes in platelets involve the phosphorylation of p38MAPK which leads to activation of cytoplasmic phospholipase A2 (cPLA2), and ultimately the production of TXB2. However, these events are quite downstream in the intracellular cascade and several efforts have been undertaken to reveal more detailed information regarding the molecular intracellular events [88].

5.1.3 Activation of monocytes
Activation of monocytes by aPL has been extensively studied. The main findings include an increase of TF expression [89] and activity as well as the increased production of proinflammatory cytokines. The proposed β2GPI-receptors mediating monocyte activation are the members of toll-like receptors, TLR-2 and TLR-4, ApoER2' and annexin A2 [90-92]. Although annexin A2 lacks intracellular domain, its involvement is strongly supported by genetic modification experiments (knockdown). Therefore, it has been suggested that an additional adaptor protein should participate in the interaction.

5.2 Disruption of coagulation–fibrinolysis balance
5.2.1 Enhanced coagulation
It is assumed that although aPL cause delayed clotting times in vitro, these antibodies exert procoagulant effects in vivo, with accelerated thrombin formation. The exact pathogenic involvement of aPL in the coagulation cascade remains obscure. Apart from the aPL-mediated increase of tissue factor (TF) expression and activity by endothelial cells and monocytes, which has been proven by in vivo models, the direct role of aPL on coagulation events is controversial. Autoantibodies against thrombin have been detected in a subgroup (up to 43%) of patients with APS [93]. However, after the description by Krilis et al. of the possible interaction between β2GPI and thrombin, the results which support the presence of distinct antibodies against thrombin must be critically re-evaluated [94]. As mentioned above, the effects of LA are caused by either anti-β2GPI/β2GPI complexes, by competitively inhibiting the formation of prothrombinase complex in vitro, or by anti-prothrombin (PT)/prothrombin complexes [15]. It has been supported by several studies that although anti-PT antibodies are involved in the activity of LA, these antibodies by themselves are not associated with thrombosis. A recent multicenter study, completed in 2010, which included patients with APS and LA, concludes that 26% of patients were positive for anti-PT antibodies without any apparent correlation with clinical features of the syndrome [95]. Although initially coagulation factor XII was seen as a component of intrinsic coagulation pathway, it is now accepted that this factor has a more significant role in intrinsic fibrinolysis. Low plasma levels of factor XII in conjunction with presence of aPL has been described. Under current circumstances, the deficiency of factor XII does not cause bleeding but only prolonged aPTT, which can be taken as an expression of LA. This deficiency can
participate in thrombotic events due to its role in fibrinolysis. It has been shown that anti-
β2GPI antibodies inhibit the activation of factor XII, but at high concentrations of antibody the opposite effect was observed. On the other hand, when the plasma is enriched with β2GPI and excess of negatively charged phospholipids, there is again a reduction in the rate of production of activated XIIa [96]. Presence of autoantibodies to factor XII is associated with LA and appears to be distinct from anti-PT antibodies [97,98]. The presence of these antibodies leads to acquired deficiency of factor XII and presents a statistically significant association with recurrent fetal abortions in APS.

5.2.2 Impaired fibrinolysis
Research data suggest that impaired fibrinolysis may contribute to the thrombophilic tendency in APS [97,99-103]. Independent studies have shown an increased activity of plasminogen activator inhibitor-1 (PAI-1) and reduced tissue plasminogen activator (tPA) following venous obstruction in patients with APS. Another possible mechanism suggested that elevated levels of lipoprotein (a) in plasma of patients, which shares structural homology with plasminogen, could compete for binding to fibrin and interfere with plasmin-mediated degradation of fibrin [104]. Recent studies revealed plasmin as a potential antigen in APS and observed that one out of four patients had antibodies to plasmin, which potentially interfere with fibrinolysis. Furthermore, binding of tPA by some aPL with subsequent inhibition of plasminogen activation has been reported [105-107].

β2GPI has also been suggested to have a direct role in fibrinolysis, through direct interaction with components of plasminogen activation. It has been shown that β2GPI blocks the PAI-1-mediated inhibition of tPA in a dose dependent manner and that aPL prevent this inhibitory effect of β2GPI. In addition, monoclonal anti-β2GPI antibodies in the presence of β2GPI cause reduced endogenous fibrinolytic activity, even in the presence of excess clotting factor XIIa. Overall, these data indicate that aPL inhibit both endogenous and exogenous fibrinolysis. It has been supported that nicked β2GPI by plasmin, in the lysine-rich amino acid chain of domain V, has the ability to bind plasminogen and inhibit the formation of plasmin [108,109]. Recent data also give a role to β2GPI and regulation of the fibrinolytic system, showing that β2GPI interacts with tPA and causes tPA-dependent activation of plasminogen and that this process is inhibited by the presence of anti-β2GPI antibodies [110]. The role of annexin A2 in the mechanism of fibrinolysis is also interesting. Apart from this role as endothelial receptor of β2GPI/anti-β2GPI complexes, it is known that annexin A2 induces fibrinolysis by binding tPA and plasminogen. Autoantibodies against annexin A2 have been reported in patients with APS and appear to correlate with a history of thrombosis. These antibodies inhibit the ability of annexin A2 to induce tPA-mediated plasminogen activation and to inhibit fibrinolysis on the surfaces of the EC.

5.2.3 Resistance to natural anticoagulants
The protein C pathway is the most important natural anticoagulant pathway, activated in the presence of low concentrations of thrombin. The activated protein C (activated protein C, APC) exerts its anticoagulant effect through proteolytic inactivation of coagulation factors V and VIII. Apart from the mutation FV Leiden, the resistance in activated protein C activity (APC resistance, APCR) is associated with the occurrence of thrombosis in patients with APS and LA. The percentage of patients with aPL and APCR ranges between 17-75%. In a recent study, APCR was found in 44% of anti-CL/anti-β2GPI positive patients and in 55% of
anti-PS/anti-PT positive patients \([111-114]\). Indeed, both anti-PT and anti-\(\beta\)2GPI antibodies assert to cause APCR \([115]\). These antibodies reduce the APC activity through competition with the latter for binding to phospholipid surfaces. Another possible mechanism through which aPL could cause APCR is the direct interaction between \(\beta\)2GPI and APC \([116]\). It has been shown that \(\beta\)2GPI interact directly with the APC, especially in the presence of anti-\(\beta\)2GPI antibodies, and therefore inhibits its activity. Furthermore, aPL with an affinity for proteins C and S have been described in APS patients with protein C or/and S deficiency. Cross-reactivity between \(\beta\)2GPI and APC, indicating that several anti-\(\beta\)2GPI antibodies recognized APC and vice versa, has also been proposed \([117]\). Protein C and/or S is associated with about five times increased risk of thrombosis, depending on severity of deficiency.

Annexin A5 is a plasma protein with high affinity to anionic phospholipids, thereby creating an anticoagulant "shield" in vascular cells and platelets. Rand et al. suggested that aPL could induce thrombosis and pregnancy morbidity, by competing with annexin A5 for binding to cell surfaces, thereby reducing its anticoagulant properties. However, it must be clarified whether this annexin A5 "shield" exists in vivo, as annexin A5 \(-/-\) mice showed no specific phenotype \([118,119]\).

5.3 Activation of innate immunity/ complement activation

Complement activation appears to have a key role in the thrombophilic diathesis of APS. The involvement of complement was first suggested by the observation of increased activated complement components in plasma of patients with APS and history of thrombosis \([120]\). As mentioned above, aPL induce a proinflammatory and procoagulant phenotype in endothelial cells, monocytes and platelets. It is also well documented that activated complement components are able to bind and activate inflammatory cells and endothelial cells, either directly through C5b-9 membrane attack complex complex (MAC), or through effects mediated by C5a receptor. Furthermore, endothelial cells release TF upon activation by anaphylatoxin C5a. In an in vivo model of surgically induced thrombosis in mice prior to aPL injections, Pierangeli et al. demonstrated that complement activation plays an important role in the induction and stabilization of thrombosis and adhesion of leukocytes to endothelial cells. Mice with genetic deletion of the complement components C3, C5, or C5a receptor were resistant to aPL-induced thrombophilia \([75,121,122]\). Another in vivo study, with rats pretreated by intraperitoneal injection of bacterial LPS and later (after a few hours) injected with polyclonal IgG antibodies from patients with APS, concludes that thrombotic manifestations were mainly depended on the activation of factors C5 and C6 \([74]\). Therefore, it turns out that complement activation significantly participates in the pathophysiology of thrombosis in APS.

It remains unclear why only some people with aPL develop clinical manifestations of disease. It has been argued by several researchers that the activation of innate immunity plays a critical role in two separate stages: a) the "immune" phase, critical for aPL production and b) the "pathological "phase, in which autoantibodies become involved in the induction of thrombosis \([120]\). According to this model, aPL alone is insufficient to cause thrombosis and thus requires the concomitant triggering of innate immunity (e.g., a ligand for TLRs). During those two phases, the innate immunity can be triggered by various stimuli such as trauma, infection, inflammation or ischemia.
5.4 Controversial issues in the thrombogenic properties of aPL- A novel mechanism for platelet activation involving β2GPI/PF4 complexes

Despite the clinical diversity (several manifestations with thrombotic or non-thrombotic etiology) and laboratory heterogeneity (autoantibodies recognizing many different proteins) of APS, it is accepted that only anti-β2GPI antibodies are responsible for the clinical presentation of the syndrome [16]. More specifically, a subpopulation of anti-β2GPI antibody against a cryptic epitope (Arg39-Arg43) in domain I of β2GPI, which is highly correlated with clinical symptoms and prognosis of the syndrome, has been identified [17]. Undoubtedly, β2GPI has an important role in the pathophysiology of APS, yet not entirely clear even after 30 years of intensive research [124]. So far, the best explanation for the role of β2GPI is that anti-β2GPI in APS induce new functions to the glycoprotein.

The newest trend in the study of β2GPI, oriented to reveal conformational changes and / or post-translational modifications of the molecule, which are induced by specific conditions and appear to be associated with the pathogenesis of APS. Therefore, while monomeric β2GPI (due to lack of specific cellular receptor) binds with low affinity to cell membranes, in the presence of anti-β2GPI autoantibodies and upon induction of conformational changes, becomes a "sticky" protein that can easily bind to different cellular receptors. So far, two responsible factors for such tertiary variations have been suggested: the anionic phospholipids and anti-β2GPI antibodies. Very recently, it has been demonstrated that β2GPI can exist in two different conformations, a circular and a stretched one. In circulation, the glycoprotein lies with the circular conformation and adopts a stretched structure after interaction with autoantibodies [125]. The researchers propose that this change could lead to the exposure of a "recognition pattern", which explains why the protein interacts with many different protein receptors. On post-translational modifications of β2GPI, a large multicenter retrospective study was recently published, which concludes that in plasma of APS patients the proportion of oxidized form of β2GPI predominates [126]. It becomes clear that β2GPI is an enigmatic protein with significant "reserve" of new structures and functions that are induced after specific stimuli.

An important indication about the biology of β2GPI and the mechanisms of action of anti-β2GPI is the observation that the dimerization of the molecule on the surface of cell membranes is a critical step to initiate cellular activation. It was shown that the chimeric dimers of β2GPI have the ability to bind and activate platelets, even in the absence of anti-β2GPI antibodies [84]. Of course, the dimerization of ligands occurs in many known interactions with cellular transmembrane receptors. In most cases however, the dimerization of the ligand is induced by the transmembrane receptor, which has not been proven in the case of β2GPI.

In vivo models of passive or active immunization with purified antibodies or β2GPI from APS patients have demonstrated a clear direct relationship between aPL and the increased risk of thrombosis and fetal abortion [51,127,128]. Although the exact pathogenetic mechanisms have not been clarified, the involvement of cellular activation (EC, platelets and monocytes) is now well-documented. One of the most critical questions in the literature is to identify the main cellular receptors through which anti-β2GPI/β2GPI complexes exert their pathogenic effects. As presented in this review, several possible receptors have been proposed and nowadays, the research is evolving towards the identification of important ones and their exact role.

Our research towards this objective was based to a null hypothesis: platelet membrane proteins, which selectively bind to a β2GPI-affinity column, will be those that potentially
interact with β2GPI in vivo. Attempt was made to minimize nonspecific interactions: on the one hand, by the effect of neuraminidase on platelets, to remove large sialic groups from surface glycoproteins and on the other hand, by the purification of platelet membranes and subsequent extraction of membrane proteins. The study highlighted platelet factor 4 (PF4) as the dominant β2GPI-binding protein, after protein identification by mass spectroscopy. The interaction was studied by analytical methodology and confirmed by size exclusion chromatography, which illustrates the composition and stoichiometry of complexes. The important point of interaction is that PF4 contributes to the natural dimerization of β2GPI, enhancing the recognition of low-affinity anti-β2GPI antibodies, which in turn further stabilize the entire complex. With in silico approach, molecular dynamics interface suggested that the interaction consists of electrostatic interactions between the positively charged surface of the PF4 tetramers and negative amino acids in domains III-V of β2GPI, enhanced by the formation of hydrogen bonds. It is worth noting that in the proposed structure of the complex, the critical epitopes in subunit I of β2GPI are arranged in such a geometric conformation that fits perfectly with the geometry of two F(ab') fragments of an antibody.

The functional significance of the formed β2GPI/PF4 complexes studied both at the level of recognition by anti-β2GPI antibodies, and in the induction of platelet activation. The study of the antigenicity of β2GPI indicates that the sera of patients with APS recognize β2GPI/PF4 complexes more powerful than β2GPI alone. Moreover, the results of this study support the phosphorylation of platelet p38MAPK and production of thromboxane B2 (TXB2) by anti-β2GPI/β2GPI/PF4 complexes, after pretreatment of platelets with suboptimal doses of thrombin. The coexistence of anti-β2GPI and β2GPI seemed to be a prerequisite for p38MAPK phosphorylation, while the anti-β2GPI/β2GPI/PF4 complexes significantly enhance this effect.

These experimental data combined with literature information, on the importance of conformational changes of β2GPI and on the necessity of β2GPI dimerization, leads to the following logical model: the binding of β2GPI by PF4 tetramers result to the natural dimerization of β2GPI, which is crucial for interaction with phospholipid surfaces and could also contribute to the induction of conformational changes, thereby revealing cryptic epitopes of domain I. The latter is consistent with the finding of the stronger recognition of complexes from patients’ antibodies.

An interesting observation that arose while guided the study was the known involvement of PF4 in the pathogenesis of heparin-induced thrombocytopenia and thrombosis syndrome (HITT) \[129,130]\]. Given the clinical similarities of two immunologically-mediated thrombophilic conditions, APS and HITT, we recommend PF4 as a "common denominator" of their pathogenesis. One distinguishing point that emerged from this work is that while in HITT, platelet activation induced by heparin/PF4/HITT-antibodies complexes is mediated primarily by Fc fragments of antibodies and stimulation of FcγRIIa receptor, unlike in APS the effects of β2GPI/PF4/anti-β2GPI complexes on platelet activation is independent of the Fc fragments of the antibodies \[35]\.

In summary, our study describes for the first time a new interaction between β2GPI and PF4. The main significance of this interaction is the stabilization of dimeric structures of β2GPI upon interaction with PF4, which facilitates the recognition by specific antibodies. The formation of anti-β2GPI/β2GPI/PF4 complexes induces platelet activation, mainly through the F (ab') 2 fragments of specific antibodies.
5.5 Conclusions

APS is an autoantibody mediated thrombophilia induced by antibodies recognizing phospholipid binding proteins, mainly β2GPI and prothrombin. The mechanisms by which these antibodies induce thrombosis are now beginning to be understood and involve imbalance of coagulation-fibrinolysis, as well as platelet, endothelial cell and monocyte activation towards coagulation. The cellular activation requires dimerization of β2GPI as well as intracellular domains possessing adaptor proteins on the cell surface. The most well characterized ones are the apoER2' and the GPIba (subunit of the platelet receptor GPIb/V/IX), while Annexin A2 and the receptors TLR-2 and -4 have also been implicated in the procoagulant process by some researchers. PF4 is a CXC-chemokine produced by platelets and monocytes; PF4 spontaneously forms tetramers in solution and these tetramers possess sites available for binding of 2 molecules of β2GPI in a way that PF4 favors a natural dimerization of β2GPI, which is then rather accessible for binding by the anti-β2GPI antibodies. The anti-β2GPI/β2GPI/PF4 complexes are immunogenic and thrombogenic, at least by activating platelets.

6. References


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Pathogenic Mechanisms of Thrombosis in Antiphospholipid Syndrome (APS)


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Thrombophilia(s) is a condition of increased tendency to form blood clots. This condition may be inherited or acquired, and this is why the term is often used in plural. People who have thrombophilia are at greater risk of having thromboembolic complications, such as deep venous thrombosis, pulmonary embolism or cardiovascular complications, like stroke or myocardial infarction, nevertheless those complications are rare and it is possible that those individuals will never encounter clotting problems in their whole life. The enhanced blood coagulability is exacerbated under conditions of prolonged immobility, surgical interventions and most of all during pregnancy and puerperium, and the use of estrogen contraception. This is the reason why many obstetricians-gynecologists became involved in this field aside the hematologists: women are more frequently at risk. The availability of new lab tests for hereditary thrombophilia(s) has opened a new era with reflections on epidemiology, primary healthcare, prevention and prophylaxis, so that thrombophilia is one of the hottest topics in contemporary medicine.

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