Thrombosis in the antiphospholipid syndrome

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INTRODUCTION

Antiphospholipid antibodies (aPLA) are heterogenous antibodies directed against phospholipid–protein complexes. Antiphospholipid syndrome (APS) is diagnosed when arterial and/or venous thrombosis or recurrent fetal loss occurs in a patient in whom screening for aPLA are positive. Because both thrombosis and fetal loss are common in the population, persistent positivity of aPLA is important. This syndrome is predominant in females (female to male ratio is 5 to 1), especially during the childbearing years[1-7].

As in the other autoimmune disorders, aPLA and APS may accompany other autoimmune diseases and certain situations. APS is referred to as "primary" when it occurs alone or "secondary" when it is associated with ot-her autoimmune disorders, especially with systemic lupus erythematosus (SLE)[8]. Besides these autoimmune conditions, aPLA may be present in healthy individuals, in patients with hematologic and solid malignancies, in patients with certain infections [syphilis, leprosy, human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), etc.], and in patients being treated with some drugs (phenothiazines, procainamide, phenytoin etc.). Those antibodies are defined as "alloimmune aPLA", and they are generally transient and not associated with the clinical findings of APS[9]. A minority of APS patients may acutely present with multiple simultaneous vascular occlusions affecting small vessels predominantly, and this is termed as "catastrophic APS (CAPS)"[1-7].

Milestones in the Antiphospholipid Syndrome History

The first antiphospholipid antibodies were discovered by Wasserman et al.[10] in 1906. Wasserman developed a complement-fixation test by incubating the serum of patients with a saline extract of liver obtained from fetuses with congenital syphilis. This

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Antifosfolipid sendromu

Anahtar Kelimeler: Antifosfolipid sendromu, Antifosfolipid antikorlar, Tromboz.

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test became the basis of the VDRL (Venereal Disease Research Laboratory) test which is now also used for non-specific aPLA screening. Mass screening of Wasserman test showed that many patients with autoimmune diseases had "false-positive" results. Mary Pangborn[11] in 1946 isolated "cardiolipin" from the bovine heart as an antigenic target the for Wasserman test. In 1972, Feinstein and Rapaport[12] showed an association between false-positive syphilis screening tests and the presence of a circulating anticoagulant, termed as "lupus anticoagulant (LA)". Actually it is a misnomer, as more than 50% of the patients with LA do not have SLE, and although LA acts as an anticoagulant in vitro, in vivo it is paradoxically associated with thrombotic events. In 1980, Perumal Thiagarajan[13] demonstrated that an Immunoglobuline M (IgM) antibody with LA activity cross-reacted with negatively charged phospholipids. In 1983 Harris and colleagues[14] described a radioimmunoassay for anticardiolipin antibodies (ACLA) that was considerably more sensitive than previous tests. Subsequently an enzyme-linked immunosorbent assay (ELISA) test for ACLA was developed[15]. The clinical associations of thrombosis and fetal loss were found to be similar in patients with ACLA and LA. In 1985 Graham Hughes et al.[16] introduced the term "anticardiolipin syndrome", which was soon changed to "antiphospholipid syndrome" in 1987[17]. In the first APS classification arterial thrombosis, venous thrombosis, recurrent fetal loss and thrombocytopenia were regarded as clinical criteria; LA positivity and moderate or high ACLA IgG/IgM positivity were regarded as laboratory criteria.

In 1990, two groups independently demonstrated that ACLA required protein cofactors for binding to the phospholipids[18,19]. The most common of these proteins was β2-glycoprotein (GP)-I which binds to the anionic phospholipids and also possesses an anticoagulant activity[20]. Although β2-glycoprotein-I is the predominant target of the "autoimmune" aPLA, other phospholipid-binding proteins including prothrombin, protein C, protein S, and annexin V have been described as playing a similar role.

In 1998, preliminary classification criteria for the classification of "definite" APS were described at an international meeting in Sapporo, Japan[1]. Clinical criteria included vascular thrombosis and pregnancy morbidity. Laboratory criteria were established as the detection of LAs, and presence of anticardiolipin IgG and IgM antibodies (Table 1). According to these criteria, "definite" APS is considered if a patient has at least one clinical and one laboratory criteria[1]. Validation studies showed that Sapporo criteria have 71% sensitivity and 98% specificity in the diagnosis of "definite" APS patients[21].

Antiphospolipid Antibodies (aPLA)

Antiphospholipid antibodies are a heterogeneous family of immunoglobulins. Despite their name, aPLA do not bind to phospholipids alone, but are directed to plasma proteins which are complexed with negatively charged phospholipids. Although several anti-phospholipid antibodies are defined, in two of those (ACLA and LA) clinical studies confirmed an association with the clinical complications of APS.

Anticardiolipin antibodies are measured by ELISA, and reported by GPL ad MPL units for IgG and IgM ACLA, respectively[22]. In patients with APS, ACLA are not simply directed to the cardiolipin antigen, it recognizes β2-GPI –cardiolipin complexes in the ELISA microplates. Since low titer ACLA may be present in the normal population, moderate (20-80 GPL, 20-50 MPL) to high positive (more than 80 GPL or 50 MPL) results are needed for the diagnosis of APS[23].

Lupus anticoagulants are screened by phospholipid-dependent coagulation tests (e.g. activated partial thromboplastin time, kaolin clotting time, Russell's viper venom clotting time, dilute prothrombin time, or textarin time). Prolonged screening test sho-

Table 1. Sapporo criteria for the classification of the APS[1]*

A. 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 imaging studies or histopathology.

2. Pregnancy morbidity:

a. One or more unexplained deaths of a morphologically normal fetus at or beyond 10th week of gestation, or

b. One or more premature births of a morphologically normal neonate at or before 34th week of gestation because of severe pre-eclampsia, or severe placental insufficiency, or

c. Three or more unexplained consecutive abortions before the 10th week of gestation.

B. Laboratory criteria:

1. Anticardiolipin IgG or IgM antibodies present at moderate or high levels in the blood on two or more occasions at least six weeks apart,

2. Lupus anticoagulants present in plasma on two or more occasions at least six weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis.

* Definite antiphospholipid syndrome (APS) is considered to be present if at least one of the clinical and one of the laboratory criteria are met.

uld not be corrected by mixing of normal plasma (mixing studies), but rather by addition of phospholipid (phospholipid neutralization procedure). For the diagnosis of LA, other coagulopathies including specific factor inhibitors should be excluded[24].

β2-GPI is a plasma protein with high affinity for negatively charged phospholipids. It has been shown that most of the ACLA are β2-GPI-dependent[18,19]. Since β2-GPI neutralizes negatively charged phospholipids and inhibits coagulation cascade, the presence of anti-β2-GPI antibodies may predispose to thrombosis.

Anti-prothrombin antibodies may be present in 50-90% of APS patients[4-6]. Anti-prothrombin antibodies may cause hypothrombinemia and may be associated with bleeding symptoms in patients with APS. Bajaj et al.[25] showed that anti-prothrombin antibodies are non-neutralizing antibodies, which bound prothrombin without inhibiting its conversion to thrombin. They suggested that hypoprothrombinemia resulted from the rapid clearance of prothrombin/anti-prothrombin antibody complexes from the circulation.

Although it has been shown that both anti-β2-GPI and anti-prothrombin antibodies might be associated with clinical features of APS, most of these studies are retrospective, and these antibodies are not included in the Sapporo criteria[1].

Other aPLA (antibodies directed to zwitterionic phospholipids, anti-phosphatidylserine, anti-phosphatidylinositol, anti-phosphatidylglycerol, etc) may be detected in patients with APS. The clinical importance of other aPLA are unclear in the absence of a positive LA and/or ACLA test[1].

There are also some aPLA that can bind directly to negatively charged phospholipids themselves. These aPLA do not require a phospholipid binding protein (i.e. β2-GPI). They may be seen in patients with infectious diseases such as syphilis, HIV and EBV infections, and usually do not cause a thrombotic complication. Routine screening tests may not distinguish infection-induced (so-called "non-pathogenic") anti-cardiolipin antibodies[3]. Infection-induced anti-cardiolipin antibodies are usually temporary.

Pathophysiology of Thrombosis in the APS

Antiphospholipid antibodies, which are obtained from patients with APS have been shown to play a direct role in the development of thrombotic complications in animal models[26]. APS is regarded as an antibody-mediated prothrombotic disorder. Although several hypotheses have been proposed, the exact pathophysiologic mechanisms causing thrombotic complications of APS have not been clarified.

In vitro studies showed that aPLA may activate endothelial cells, inhibit protein C and protein S activity, activate platelets, increase tissue factor expression, and impair the fibrinolytic activity[2,3,6]. In the last decade, more studies have been focused on interaction between aPLA and Endothelial Cells (EC). It has been shown that binding of aPLA activates EC, which results in the expression of P-selectin, Intercellular Cell Adhesion Molecule-1 (ICAM-1), and vascular cell adhesion molecule-1, and induces thrombosis in animal models[26-29]. Combes et al.[30] showed that in vitro generation of endothelial microparticles was increased in patients with lupus anticoagulants.

Besides these in vitro studies, we have recently demonstrated that endothelial functions determined by brachial artery ultrasound were impaired in patients with primary APS[31]. In this study we evaluated brachial artery responses to the ischemia (endothelium-dependent dilatation) and to sublingual nitroglycerin administration (endothelium-independent dilatation) in primary APS patients (n= 31) and matched healthy controls (n= 27). There was no significant difference between primary APS patients and healthy controls with respect to basal brachial artery diameter. Although endothelium-independent dilatation responses are similar, endothelium-dependent dilatation responses were significantly lower in primary APS patients than in controls. Impaired endothelium-dependent responses were more prominent in the subgroup of patients with arterial involvement compared to the patients with venous involvement[31].

Since APS is an extremely heterogeneous disorder with a broad spectrum of clinical presentation, determination of the patients who have high risk of thrombosis is very important. Although many epidemiological studies showed an increased risk of thrombosis in patients positive for aPLA, some patients with high titers of aPLA did not develop thrombosis, even in long-term follow-up. These facts supported the hypothesis that there were additional inherited or acquired thrombogenic factors, that influenced the development of thrombosis in those patients ("double or multiple hit" hypothesis)[2].

In the last decades, several genetic disorders have been identified in patients with thrombosis, especially with venous thromboembolism[32]. Natural anticoagulant (protein C, protein S, antithrombin) deficiencies, factor V G1691A (FV Leiden) mutation and prothrombin G20210A (PT G20210A) (prothrombin G20210A mutation were the most common causes of hereditary thrombophilia. It has been shown that natural anticoagulant deficiencies are quite rare in patients with APS. Several studies investigating the role of factor V Leiden mutation and prothrombin G20210A mutation in patients with APS gave conflicting results[33-40]. It is unclear whether the presence of these mutations increased the thrombotic risk in APS patients.

Factor XIII Val34Leu polymorphism is a newly described polymorphism which is located in the three amino acids away from the thrombin activation site of the factor XIII-A subunit. It has been reported that the presence of Leu allele may decrease both arterial and venous thrombosis risk, and increase bleeding tendency. In a cohort of 60 APS patients with thrombosis, 22 aPLA-positive patients without thrombosis and 126 healthy controls, we could not find any effect of factor XIII Val34Leu polymorphism on the thrombosis in patients with APS[41].

Clinical Manifestations of APS

Thrombotic complications are the major causes of morbidity and mortality in patients with APS. APS may affect any system and organ in the body including heart, brain, kidney, skin, lung, and placenta (Table 2)[2-7]. Although the thrombosis may occur in any site of the vascular tree venous thrombosis is encountered most commonly. About 2/3 of the thrombotic events are venous, mainly deep and superficial veins in the lower extremity; the remaining 1/3 are seen in the arterial system. In patients with APS, the risk of the thrombotic recurrence is high compared to patients without APS, especially after the discontinuation of oral anticoagulant therapy[2-6]. APS patients may acutely present with multiple simultaneous vascular occlusions affecting small vessels predominantly, and this is termed as "catastrophic APS (CAPS)". Precipitating factors have been identified in the majority of the CAPS patients, such as infections, surgery, pregnancy, SLE flares, withdrawal of anticoagulation therapy, and trauma. CAPS definition requires thrombotic involvement of at least three different organ systems over a period of days or weeks. Clinical manifestations of CAPS are related to the extent of organ involvement and cytokine release of affected tissues. Renal dysfunction (70%), pulmonary involvement such as adult respiratory distress syndrome (ARDS) and pulmonary embolism (66%), skin involvement such as skin necrosis and livedo reticularis (66%), central nervous system manifestati-

Table 2. Clinical manifestations of the APS

System	Findings
Cardiovascular system	Acute coronary syndromes, angina pectoris, cardiac valve involvement, non-bacterial thrombotic endocarditis, atherosclerosis, peripheral arterial disease, myocarditis intermittent claudication
Arterial system	Thrombosis in the large, medium or small arteries
Venous system	Thrombosis in the large, medium or small veins
Cerebrovascular	Transient ischemic attack, cerebral arterial occlusion, chorea, convulsions, dementia, transverse myelitis encephalopathy, migraine, Sneddon's syndrome, pseudo-tumor cerebri, cerebral venous thrombosis, mononeuritis multiplex
Obstetrical	Fetal loss, pre-eclampsia, eclampsia, HELLP syndrome, fetal growth retardation, utero-placental insufficiency, infertility
Hematological	Thrombocytopenia, autoimmune hemolytic anemia, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome
Dermatological	Livedo reticularis, Reynaud's phenomenon, leg ulcers, purpura, acrocyanosis, cutaneous infarcts, digital gangrenes, Degos's disease, anetoderma
Ophthalmological	Retinal, arterial and venous thrombosis, amaurosis fugax, transient or persistent visual loss, photophobia
Gastrointestinal	Budd-Chiari's syndrome, mesentery embolism, nodular regenerative hyperplasia of the liver, intestinal vaso-occlusive disease, ischemic colitis, pancreatitis, hepatic or splenic infarct
Pulmonary	Pulmonary embolism, pulmonary hypertension, alveolar hemorrhage, fibrosing alveolitis, respiratory distress syndrome
Renal	Renal vein thrombosis, renal arterial thrombosis, acute or chronic renal failure, hypertension, hematuria, nephrotic syndrome
Psychiatric	Psychosis, cognitive dysfunction

ons such as cerebral, arterial and venous thrombosis, convulsions, and encephalopathy (60%), and cardiac manifestations such as valve involvements and myocardial infarctions (53%) are the major clinical findings. Thrombocytopenia, usually severe thrombocytopenia is present (60%). Hemolysis and disseminated intravascular coagulation (DIC) may occur. Recommendations for the treatment of CAPS are intravenous heparin, plasmapheresis, and steroids. Treatment of CAPS should include the precipitating conditions such as the treatment of infections with appropriate antibiotics and the treatment of SLE flares. The mortality rate is quite high (more than 50%) even in presumably properly treated patients[42,43].

Although in the general population pregnancy loss is most frequent during the first nine weeks of gestation, the risk of pregnancy loss in patients with APS is greatest after the 10th week of gestation. Besides the fetal losses, it is now clear that aPLA may cause pre-eclampsia, eclampsia, fetal growth retardation, utero-placental insufficiency, preterm birth, and reproductive failure. Pregnancy morbidity in women with APS may result from impaired placental circulation due to placental thrombosis, impaired throphoblastic invasion, and hormone production[1,2,8].

Thrombocytopenia is reported in about 20-40% of patients with APS, is usually mild (70.000-120.000/mm^3), and does not require any clinical intervention. Severe thrombocytopenia (lower than 50.000/mm^3) is seen in only 5-10% of the patients[44-46]. Although thrombocytopenia has been defined as a clinical criterion in the first classification of APS, it is not included in the preliminary classification of definite APS recently proposed in Sapporo[1]. The patients who had aPLA and thrombocytopenia as the only clinical manifestation in the absence of other APS findings were defined as "probable" or "possible" APS. In a recent prospective study, however, we have demonstrated that the immune thrombocytopenic purpura (ITP) patients who presented with thrombocytopenia and had positive tests for aPLA had increased risk of thrombosis. We have proposed that measurement of aPLA, especially LA in patients with initial diagnosis of ITP may identify a subgroup of patients with higher risk of developing APS[47].

The severity of thrombocytopenia may affect the development of thrombosis in patients with APS. When the APS patients were divided into three groups according to platelet counts as non-thrombocytopenic, moderately (50.000-100.000/mm^3), and severely thrombocytopenic (below 50.000/mm^3), the rates of thrombosis development were found as 40%, 32%, and 9%, respectively[44,45]. On the other hand, this data shows that moderate thrombocytopenia does not prevent development of thrombosis in patients with APS, and anti-thrombotic prophylaxis should be considered in those patients[44-47].

Although thrombocytopenia is a common finding in patients with APS, bleeding complications are very rare, even in severe thrombocytopenia. The presence of bleeding symptoms in an APS patient with moderate thrombocytopenia should alert to consideration of the presence of anti-prothrombin antibodies and other diseases which may affect hemostasis such as DIC, uremia etc. Severe thrombocyopenia may require therapy. Treatment strategies in those patients are similar to those in patients with ITP.

Interestingly there are a few case reports describing the correction of thrombocytopenia with aspirin[48,49], warfarin[50,51], and anti-malarial drugs[52]. It has been suggested that inhibition of platelet activation, aggregation, and platelet consumption may help to increase platelet count in those patients.

Management of Thrombotic Complications in APS

Management of patients with aPLA mainly depends on the presence of clinical symptoms and findings:

a. aPLA-positive individuals with no APS symptoms or findings: It is known that aPLA may be present in 1-5% of healthy individuals[53]. If aPLA-positive individuals have no history or findings of APS, treatment should not be considered[3,4,54,55]. Although some investigators recommend prophylactic therapy for aPLA-positive individuals if they face acquired thrombotic risk (puerperal period, surgery, immobilization etc.)[7,54], there are no prospective or controlled studies investigating the effectiveness of anti-thrombotic prophylaxis in those individuals.

b. Primary prophylaxis of thrombosis in aPLA-positive patients: Although many experts recommend using anti-thrombotic prophylaxis in aPLA-positive patients who fullfill Sapporo APS criteria and have no history of thrombosis, there are only a few studies addressing this issue. The difficult point is to define thrombotic risk in aPLA-positive patients who had no thrombosis. Erkan et al.[56] showed that APS patients with fetal losses were also at high risk of thrombosis, and they recommended prophylactic aspirin therapy, 325 mg/day. Petri et al.[57] reported that hydroxychloroquine might have a protective effect against thrombosis in secondary APS (SLE-APS) patients. In a prospective study, 82 ITP patients were evaluated for aPLA and it was found that a significant portion of patients who had aPLA positivity developed APS in 5 years of follow-up. It has been reported that LA was an important risk factor for the development of thrombosis in ITP patients[47].

c. Treatment of venous thrombosis in APS: Treatment of the first venous thrombotic attack is similar to that in idiopathic venous thrombosis. Heparin and oral anticoagulation therapy are routinely used for this purpose. Recurrency of thrombosis is higher in patients with APS compared to patients with no aPLA and venous thrombosis[58], especially in the first six months of thrombosis and after the cessation of the therapy[58-60]. However, the duration and the intensity of the treatment are not clear. In the first studies, it has been suggested that high intensity (INR > 3) oral anticoagulant therapy should be used in APS[58,60]. Recent studies however, showed that high intensity oral anticoagulant therapy is not superior to conventional dose oral anticoagulant therapy (INR 2-3) for the prevention of thrombosis in those patients[2,3,7,61-64]. Ames et al.[63] also showed that high intensity oral anticoagulant therapy might increase bleeding complications in patients with APS. Coexistence of thrombocytopenia is the major cause for bleeding. The duration of oral anticoagulant therapy is another debated issue. Although life-long therapy was recommended in the first studies recent prospective studies have yielded no firm conclusions. The current recommendation from the American College of Chest Physicians for anticoagulant therapy for APS patients who sustain a venous thrombo-embolic event is warfarin with target INR of 2.5 (Grade 1A) for 12 months[65]. The thrombotic risk modification including cessation of smoking, discontinuation of hormone replacement therapy, correction of body mass index etc. is also very important in the management of thrombosis in those patients.

d. Treatment of arterial thrombosis in APS: Acute coronary syndromes, transient ischemic attacks, and cerebral arterial thrombosis are the most common causes of arterial involvement in patients with APS. Morbidity and mortality are high in arterial involvement. In APS patients with acute coronary syndromes, high intensity oral anticoagulant therapy is recommended. The effectiveness of additional aspirin therapy is debated[7]. In the (APASS) Antiphospholipid Antibody in Stroke Study Group study[64], APS patients with stroke were randomized to either aspirin (325 mg daily) or warfarin (targeted INR 2.2), and were compared for the risk of recurrent stroke. The APASS study found no significant difference between the two arms. It is important to determine other systemic findings to decide the treatment mo-

dalities in APS patients with stroke. In stroke patients who have atrial fibrillation and cardiac valve disorders use of warfarin therapy with moderate to high intensity is recommended.

Conclusion

Anti-phospholipid syndrome is an antibody-mediated disorder with thrombotic tendency, obstetric complications and multisystemic symptoms. Although many studies have showh the association between aPLA and clinical findings of APS the exact pathophysiologic mechanisms are still unclear. A heterogeneous clinical picture and lack of prospective controlled studies had resulted in different treatment modalities in patients with APS. It is clear that current anticoagulant therapy is ineffective in APS patients. In the future, elucidation of the pathogenetic mechanisms of APS may allow use of new treatment modalities such as new anticoagulant drugs, statins, immunoadsorption procedures, vaccinations, IL-3, peptide competitors, and complement inhibitors.

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