Management of the antiphospholipid syndrome: new approaches

The antiphospholipid syndrome (APS) is an autoimmune, multisystemic disorder associated with recurrent thrombosis (arterial and/or venous) and pregnancy loss, among other clinical manifestations. The hallmark of the disease is the presence of antiphospholipid (aPL) antibodies (i.e., anticardiolipin [aCL] antibodies, lupus anticoagulant and anti-β₂glycoprotein I [anti-β₂GPI] antibodies). This review addresses current modalities used for primary prevention and treatment of clinical manifestations in patients with APS. Furthermore, based on new knowledge regarding the pathogenic and molecular mechanisms that are triggered and mediated by aPL antibodies, new targeted modalities for treatment in APS are discussed. These new treatments – potentially more effective and with fewer side effects – might be used in the future to treat clinical manifestations of this disease that are associated with devastating consequences, and are pending adequate clinical trials.

KEYWORDS: anti-β₂glycoprotein I antibodies, antiphospholipid antibodies, antiphospholipid syndrome, lupus anticoagulant, treatments for antiphospholipid syndrome

Antiphospholipid syndrome (APS) is an autoimmune and multisystemic disorder of thrombosis and pregnancy loss, associated with the persistent presence of antiphospholipid (aPL) antibodies [1–3]. These antibodies are directed against protein antigens that bind to anionic phospholipids, such as β₂glycoprotein I (β₂GPI) and prothrombin. Thrombosis is the major manifestation in patients with APS, but the spectrum of symptoms and signs associated with aPL antibodies has considerably broadened, and other manifestations such as thrombocytopenia, nonthrombotic neurological syndromes, livedo reticularis, skin ulcers, hemolytic anemia, pulmonary hypertension, cardiac valve abnormality and atherosclerosis have also been related to the presence of these antibodies [1–3].

Antiphospholipid syndrome was first coined more than 25 years ago, and has often been referred to as ‘the syndrome of the black swan’, given its unusual presentation [1]. APS was first described in a subset of patients with systemic lupus erythematosus (SLE) that had abnormal lupus anticoagulant (LAC) test results. APS can occur in the presence of other autoimmune disorders, particularly SLE, or in the absence of SLE or other autoimmune disorders (primary APS). APS is now recognized as the most common cause of acquired hypercoagulability in the general population [4], and as the most important treatable cause of recurrent miscarriage [5]. aPL antibodies can be detected in up to 40% of SLE patients and affect disease morbidity since they are associated with recurrent thrombosis, pregnancy loss, thrombocytopenia, and worse lupus nephritis and kidney transplantation outcomes [6]. Cardiovascular morbidity and mortality is a frequent complication in SLE, where the risk of myocardial infarction is raised 50-fold. In addition to traditional risk factors such as hypertension or diabetes, several factors more specifically related to lupus are proposed to be of importance, including inflammation and aPL antibodies. A study of patients with SLE demonstrated that anticardiolipin (aCL) positivity preceded the onset of a more severe form of SLE, as well as SLE complicated with thrombosis, pregnancy loss and thrombocytopenia [7].

The preliminary classification criteria for APS were revised in 2006, and include the presence of both clinical and laboratory criteria [2,3]. Patients must have vascular thrombosis (arterial or venous) and/or pregnancy morbidity. With respect to vascular thrombosis, any tissue or organ must be confirmed with imaging or histopathology, for which histology excludes inflammation in the vessel wall. Regarding pregnancy morbidity, one of the following must be present:

- One or more unexplained death of a normal fetus at or beyond the 10th week of gestation;
- One or more premature birth of a normal neonate at or beyond the 34th week of
gestation due to severe pre-eclampsia, eclampsia or placental insufficiency;

- Three or more unexplained, consecutive, spontaneous abortions before the 10th week of gestation that excludes maternal, anatomic, hormonal or parental chromosomal anomalies.

Laboratory criteria include the presence of either LAC in plasma of medium to high titer IgG or IgM aCL isotypes, and or IgG or IgM anti-β2GPI, on two or more occasions, at least 12 weeks apart [5].

Antiphospholipid syndrome can be described as a spectrum of multiorgan involvement. At one end, healthy individual and elderly patients may develop aPL antibodies in the absence of clinical manifestations, and this is called asymptomatic APS. This may be due to an infection, malignancy, may be drug induced, or a result of the aging process, and the titers of aPL antibodies are usually low [8–14]. On the other end, catastrophic APS (CAPS) comprises 1% of APS cases and is associated with multiple, widespread vascular occlusions, high titer aPL antibody and significant mortality [15]. The majority of patients present somewhat inbetween, with either primary or secondary APS.

Antiphospholipid antibodies are heterogeneous and bind to various protein targets, including the plasma protein β2GPI, prothrombin, tissue plasminogen activator, plasmin, annexin A2 and thrombin [16–20]. There are many mechanisms involved in APS, and many in vivo animal model studies have described the pathogenesis of thrombosis, endothelial cells and pregnancy loss [21–24]. Investigators have demonstrated that endothelial cells express significantly higher amounts of cellular adhesion molecules (CAMs), such as ICAM-1, VCAM-1 and E-selectin, when incubated with aPL antibodies in vitro. Our group has demonstrated that aPL antibodies activated endothelium in vitro and in mouse models, and this correlated with the enhancement of thrombus formation in vivo [23,25].

However, there have been limitations, and our understanding of the pathophysiology of APS is incomplete owing to unknown mechanisms of thrombosis or to heterogeneity of the aPL antibodies.

In this article, we will first review the role of current therapies, along with concomitant side effects, and with what is currently known on the pathophysiology of this rare but serious condition. Importantly, we discuss possible new targeted treatments to ameliorate APS-related clinical manifestations.

### Management of thrombosis in antiphospholipid syndrome

#### Anticoagulation

The goals in the management of persistently aPL-positive APS patients is primary prophylaxis in treatment of the acute thrombus, and secondary prevention for further clot. Patients commonly present with a thrombus as the first symptom of APS, and treatment of the acute thrombotic event is similar in the general population; this consists of both pharmacologic therapy as well as minimizing reversible risk factors (immobility, smoking, taking an oral contraceptive pill and so on).

Patients with venous thrombosis are initially treated with heparin (most commonly low molecular-weight heparin) followed by coumadin. Retrospective studies have reported that the recurrence of thrombosis is high, and ranges from 22 to 69%, and more than 70% of patients with venous thrombus and 90% of arterial clots recur [26–29]. If the arterial circulation is involved, patients are prone to stroke and transient ischemic attacks. Therefore, patients need to be on prolonged, lifelong anticoagulation; however, the duration and intensity is questionable. Trials comparing moderate-intensity (international normalized ratio [INR] 2–3) and high-intensity (INR 3–4) anticoagulation offer comparable clot protection, and it is acceptable to use a targeted INR between 2 and 3 in venous thrombosis and an INR of 3 in arterial events [26–30]. If patients experience recurrent thrombosis, INR should be raised to 3–4, and low-dose 81 mg aspirin is often added. However, the risk of bleeding must be carefully monitored. Based on an Italian prospective study, the rate of life-threatening bleeding in patients taking warfarin is 0.25% annually and rises steeply when the INR exceeds 4 [29]. The risk of bleeding is no greater with anticoagulation and APS compared with other disease processes. In fact, Ruiz-Irastorza et al. have demonstrated that it is quite rare to have serious hemorrhagic events while taking high-intensity oral anticoagulation with a targeted INR of 3.5 [31]. Limitations of the study included a young population group with primary or SLE-associated APS, as older patients on anticoagulation are more at risk of bleeding [31].

It is well-known that some patients may have subclinical or asymptomatic APS and have a 0–3.8% annual risk of thrombosis [32]. These patients are at increased risk of clotting, owing to prothrombotic factors; these include smoking, estrogen-containing oral contraceptive
pills, recent surgery within the last 3 months, immobility, and illicit drug use such as cocaine. Therefore, it recommended that these factors should be minimized, and prophylaxis with anti-coagulation should be considered during high-risk periods. Erkan et al. compared aspirin versus placebo for primary prevention in APS and found no difference in thrombotic events. Therefore, patients must be risk stratified according to both cardiovascular and noncardiovascular risk factors before prescribing aspirin.

Lastly, patients may develop multiple vascular occlusions in a short period of time, with associated high-titer aPL antibodies (CAPS). Approximately 250 cases of CAPS have been described in the literature and in 2000, an international registry in Europe was created. CAPS comprises 1% of APS cases, and patients must meet the following diagnostic criteria:

- Have at least three or more organs involved
- Develop manifestations within 1 week or less
- Establish confirmation by histopathology of small vessel occlusion in at least one organ
- Laboratory confirmation for the presence of aPL (LAC or aCL)

Women are more prone than men (2.5:1 ratio), and patients usually have a thrombotic event that often precedes the multiple occlusions. Patients clinically present with multiorgan involvement and the kidneys (70% of patients in the CAPS registry), pulmonary (65%), CNS (55%), cardiac (50%) and gastrointestinal (44%) system are involved. Thrombocytopenia was reported in 46% of the CAPS patients, LAC and aCL were found in 79 and 86%, respectively, and antinuclear antibodies were positive in 48% of cases. Mortality is quite high in CAPS, and occurred in 47% of patients, of which cardiac events predominated.

Several pathogenic mechanisms have been described in CAPS, and endothelial cells have been demonstrated to be involved in the pathogenesis. It is believed that cytokines, activated complement products and autoantibodies interact with endothelial cells and upregulate procoagulation and endothelial adhesiveness. Cytokines such as IL-1, IFN-α and TNF-α appear to be important mediators of endothelial activation. Endothelial cells can also produce cytokines, such as IL-1, IL-6, IL-8 and TNF-α, which upregulate adhesion molecule expression. In addition, complement is involved and activates adhesion molecules in the endothelium, which may predispose patients to CAPS. Finally, autoantibodies, such as aPL, antiendothelial cells, and anti-dsDNA, have been demonstrated to react with endothelial cells in vitro and to upregulate adhesion molecules and tissue factor (TF).

Consensus for the treatment of CAPS is lacking in terms of large multicentered studies. Current therapies are based on small case series. The goals of treatment are to prevent the progression of thrombosis and limit the production and circulation of inflammatory mediators. Thus, treatment consists of multimodal therapy, from anticoagulation to immunosuppressant agents like corticosteroids, or to cytotoxic agents such as cyclophosphamide, and to plasmapheresis and intravenous immunoglobulin. However, with immunosuppression, patients are at increased risk of infection and possible increased risk for malignancy.

Antiplatelet agents

Antiplatelet therapy like aspirin, clopidrogel and dipyridamole have been used for secondary prevention in patients with stroke and transient ischemic attacks. Kaul et al. published a small study of eight warfarin-naive patients treated with antiplatelet therapy and found only one recurrence of stroke while on clopidrogel after 17 patient-years. Once again, the risk–reward must be carefully addressed when planning to use antiplatelet therapy.

Management of obstetric complications in antiphospholipid syndrome

Pregnancy losses in the general population are quite common. However, the challenge that physicians and researchers face is the patient with pregnancy complications and an existing history or new diagnosis of APS. As discussed earlier, there are specific criteria for pregnancy-related APS manifestations, including pregnancy loss, pre-eclampsia, eclampsia and so on. Significant efforts have been devoted to understanding the pathophysiology of obstetric complications in APS. The general consensus is that they are due to abnormal placental function, resulting from abnormalities in uteroplacental circulation. Early reports have shown thrombosis, infarction and necrosis in the placenta of failed pregnancies. Out et al. has published a large case–control study showing placental thrombosis/infarction in 82% of woman with aPL and fetal death. Research has shown alterations in the prostacyclin and thromboxane pathway, increased expression of...
tissue factor and protein C, and decreased levels of annexin A5 induced by aPL antibodies that may be involved with placental thrombosis and fetal loss [45–50]. Furthermore, murine models have demonstrated complement activation and blockade of C3 convertase, C5–C5a receptor interactions and protective effects against aPL-induced pregnancy complications in mice deficient in C3, C4 or C5 [51,52]. Finally, oxidative stress and concomitant placental damage has also been proposed [40–42,53].

There have been inconsistencies in standardized clinical trials detailing recurrent pregnancy loss and APS. The issues include selection bias, definition and type of pregnancy loss, and variability in patients, including those with low titer levels of IgG and IgM aCL. In one study, women with a low titer of IgG or IgM aCL antibodies had no greater chance of having an aPL-related pregnancy event than women who were aCL negative [54]. It is also important to understand that patients who are pregnant and have APS may not only suffer pregnancy complications, but may also have strokes, transient ischemic attacks, thrombosis and so on. Hence, it is recommended that patients with APS should undergo counseling before becoming pregnant. If patients have underlying SLE, they must understand that their SLE may flare during pregnancy. Anemia and thrombocytopenia must be minimized as this may be a complication of APS. Women should seek care with an obstetrician who is familiar with APS, and closely follow up with their rheumatologist, hematologist and primary care physician.

The current mainstay treatment for pregnant APS patients is anticoagulation. Experts in the field, and the American College of Chest Physicians (ACCP), recommend that all pregnant women with APS be treated with low-dose aspirin and subcutaneous, unfractionated heparin or low molecular-weight heparin [40,41]. Pregnant woman with APS and a prior history of thrombus are recommended to receive adjusted weight-based anticoagulation, while those with APS and solely obstetric complications are recommended to receive prophylactic dosed anticoagulation. Some experts recommend warfarin for patients with recurrent thrombosis during pregnancy; however, the risk–reward benefit must be carefully weighed up due to potential birth defects associated with warfarin. The use of warfarin should be avoided during the first trimester of pregnancy owing to its teratogenic effects. However, warfarin can be used in the second and third trimester of pregnancy. On the other hand, pregnant women who are otherwise healthy with recurrent pregnancy and low-titer aPL antibodies do not require anticoagulation. In fact, one study by Pattison et al. found no difference in live birth rates comparing low-dose aspirin with placebo [42].

Postpartum anticoagulation has been determined to be very important in woman with APS, and often, warfarin is added as prophylaxis following delivery. The goal INR for these patients is 2.5. Postpartum thromboprophylaxis using heparin is recommended up to 6 weeks after delivery. In patients with a diagnosis of APS based on obstetric complications and who do not have prior thrombus, guidelines for the postpartum thromboprophylaxis regimen are lacking. However, their risk of thrombosis is higher than the normal population and a candid discussion between the doctor and patient is warranted. As with all anticoagulants, the risk–reward benefit must be carefully weighed.

**New treatments for APS**

The treatment of thrombosis as well as the prevention of recurrent thrombosis in APS has been focused on utilizing antithrombotic medications. Recurrences, in spite of treatment, have been reported and the use of oral anticoagulation at a relatively high INR for a long period of time has been associated with a high risk of bleeding, with the need for frequent monitoring and patient compliance with diet and lifestyle modifications are essential for optimal therapy. Moreover, the approach for patients with aPL antibodies without a previous thrombotic event is still debated. Some physicians would recommend prophylaxis with low-dose aspirin, although there are no evidence-based data supporting that low-dose aspirin alone is sufficient for primary thrombosis prophylaxis. It is well-known that aPL antibodies might be persistently present in the serum of APS patients for long periods of time, but thrombotic events only occur occasionally. It has been suggested that aPL antibodies (‘first hit’) increase the thrombophilic threshold (i.e., induce a prothrombotic/proinflammatory phenotype in endothelial cells), but that clotting only takes place in the presence of a ‘second hit’ or trigger event (i.e., an infection, a surgical procedure, use of estrogens, prolonged immobilization and so on) [54]. Current treatments of thrombosis in APS are directed to modulate the final event or second hit. Treatments that modulate early effects of aPL antibodies on target cells—that is monocytes or endothelial cells—(first hit) would be more beneficial and potentially less harmful than current treatments.
Barriers to the development of new drugs for APS include the multifactorial nature of thrombosis, controversies regarding the strength of association between aPL antibodies and thrombotic events and the fact that the mechanisms of aPL-induced thrombosis are not well understood. In the long-term management of APS patients, controlled studies with warfarin alternatives and the new anticoagulant agents (i.e., oral direct and indirect thrombin inhibitors) as well as newer therapeutic agents are vital. However, it is possible that the current anti-thrombotic approach to aPL-positive patients will be replaced by an immunomodulatory approach in the future, as our understanding of the mechanisms of aPL-mediated thrombosis increases. Understanding the molecular mechanisms triggered by aPL antibodies and identifying biomarkers released as a consequence of cellular activation may help to design new ways to treat clinical manifestations in APS.

### Tissue factor: a biomarker of disease in APS?

Tissue factor upregulation has been advocated as an important mechanism to explain the pro-thrombotic effects of aPL antibodies (Abs). Our group and others have demonstrated the upregulation of TF expression and function in endothelial cells and monocytes treated with aPL Abs, which is accompanied by an increase in IL-6 and IL-8 secreted by those cells. Several studies have shown that monocytes isolated from patients with APS exhibit increased expression of TF mRNA and antigen. Enhanced TF expression and procoagulant activity were observed in monocytes isolated from healthy individuals incubated with serum, plasma and purified total IgG from patients with APS, demonstrating the causative role of autoantibodies of patients with APS in monocyte TF expression. In particular, anti-β₂GPI human monoclonal antibodies derived from patients with APS enhanced monocyte TF mRNA and TF activity [55–62]. Hence, procoagulant cell activation, accompanied with TF expression, and TF pathway upregulation is one of the key events considered, explaining the pathophysiology of thrombosis in patients with APS. In addition, previous studies showed elevated plasma levels of soluble (s) TF in APS patients [63]. Furthermore, the presence of antibodies against tissue factor pathway inhibitor (TFPI) in certain APS patients suggests that negative regulation of TF activity might also be impaired in these patients [64–66].

Previous reports indicate a close relationship between TF and vascular permeability factor/VEGF, a family of proteins involved in normal vascular development and in important pathologies including cancer, wound healing and inflammation. The vascular permeability factor/VEGF proteins are widely regarded as the most relevant proteins involved in the development of the vasculature. Previous studies reported increased plasma levels of VEGF in APS patients. In one study, Cuadrado et al. analyzed the VEGF and Flt-1 expression levels in monocytes of APS patients, the molecular mechanisms involved in their aPL-induced expression and their association with the elevated TF expression found in these patients. Their data primarily showed that monocytes from APS patients expressed increased levels of both VEGF and Flt-1 in comparison with monocytes from healthy donors [57]. Thus, VEGF might act as a regulatory factor in aPL-mediated monocyte activation and TF expression, thereby contributing to the proinflammatory–prothrombotic phenotype of APS patients. Some investigators have demonstrated increased levels of sVCAM-1 in patients with aPL antibodies and thrombosis [67].

Dilazep, an antiplatelet agent, inhibits in vitro aPL-induced monocyte and endothelial cell TF expression at a post-transcriptional level, probably by way of its effects as an adenosine uptake inhibitor, since an increased extracellular concentration of adenosine inhibits TF expression [56]. Other possible therapies are the antiplatelet agent dipyridamole, an adenosine uptake inhibitor similar to dilazep and pentoxifylline, that inhibit its lipopolysaccharide (LPS)-induced monocyte TF expression [68–70]. In addition, angiotensin converting enzyme inhibitors, such as captopril, have been demonstrated to significantly inhibit LPS-induced monocyte TF activity, antigen expression and gene transcription [71,72].

Hence, TF is emerging as a potential biomarker of disease in APS. Given the relationship between increased TF activity, expression of CAMs and upregulation of proinflammatory cytokines with thrombosis in APS, pharmacological agents that block TF activity or expression of CAMs or inflammatory cytokines may be a novel and attractive therapeutic approach in APS.

### Blocking the binding of aPL antibodies on target cells

As shown in Figure 1, β₂GPI, the main target recognized by aPL antibodies, binds to endothelial cells and monocytes through its fifth
domain. aPL/anti-β₃GPI antibodies then bind to domain I of β₃GPI, and upon clustering and formation of complexes, they trigger cell activation [73–75]. Hence, blocking the binding of aPL antibodies or inhibiting the binding of β₃GPI to target cells may be the most specific approach to ameliorate their pathogenic effects without interrupting any important physiologic mechanisms. Ostertag et al. demonstrated that TIFI, a 20 amino acid peptide that mimics the phospholipid-binding region in domain five of β₂GPI, significantly decreased thrombus size in mice injected with IgG-APS by displacing the binding of β₂GPI to target cells [75]. TIFI also inhibited (in a dose-dependent fashion) the binding of fluorescinated β₂GPI to endothelial cells and murine macrophages in vitro [75]. In a recent study, Ioannou et al. demonstrated that soluble recombinant domain I of β₂GPI abrogates, in a dose-dependent fashion, the in vitro and in vivo effects of anti-β₂GPI antibodies, underscoring the possibility of utilizing decoy peptides that are part of β₂GPI to abrogate the binding of pathogenic aPL antibodies to target cells in the treatment of patients with APS. Human studies are needed to establish the safety and efficacy of such a treatment [76,77].

Statins in APS
Statins are potent inhibitors of cholesterol synthesis in the mevalonate pathway. In the general population, clinical trials of statin therapy have demonstrated beneficial effects in the primary and secondary prevention of coronary heart disease as well as ischemic stroke [78]. However, their beneficial effects are only partially explained by their ability to lower cholesterol levels. Pleiotropic effects of statins have been reported, which include decreasing the expression of CAMs in monocytes and affecting leukocyte–endothelial interactions, and down-regulating inflammatory cytokines in endothelial cells or increasing fibrinolytic activity. Studies have demonstrated that simvastatin and fluvastatin decrease TF mRNA expression and activity in cultured human monocytes obtained from healthy individuals [79]. Treatment with cerivastatin, simvastatin, pravastatin and fluvastatin substantially reduced TF expression in atherosclerotic lesions along with suppression of inflammation in atheroma, independently of lipid lowering, in animal models [80]. These findings have been corroborated in humans by the findings of the double-blind, placebo-controlled Atorvastatin and Thrombogenicity of the Carotid Atherosclerotic Plaque (ATROCAP) study, in which 4–6 months of treatment with atorvastatin (20 mg/day) was associated with 29% lower TF antigen levels and 56% lower TF activity in atherosclerotic plaques compared with the data obtained from subjects receiving placebo [81]. Hence, cardiovascular benefit is provided by lowering raised cholesterol levels and by modulation of the inflammatory component of this disease. Statins also directly inhibit IFN-γ-induced MHC-II expression in vitro, thus preventing subsequent T-cell activation [82]. Lastly, in a recent study, simvastatin induced a rapid and significant reduction in the proteinuria level and in the expression of lymphocyte activation markers in a small series of SLE patients [83]. Statins, including simvastatin and fluvastatin, have been demonstrated to reverse TF upregulation induced by TNF-α and LPS in a dose-dependent manner [84,85].

In the medical community, there have recently been many publications concerning the benefit of statins following the recent results from the justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin (JUPITER) study, in which patients with normal low-density lipoprotein levels of less than 130 mg/dl and elevated C-reactive protein (CRP) levels of greater than 2.0 mg/dl receiving 20 mg rosuvastatin daily experienced a significant reduction in cardiovascular events, nonfatal myocardial infarction and nonfatal stroke [86]. Studies have suggested that fluvastatin has beneficial effects on aPL-mediated pathogenic effects. First, one study showed that fluvastatin prevented the expression of CAMs and IL-6 in endothelial cells (EC) treated with aPL Abs [87]. Subsequently, we demonstrated that the thrombogenic and proinflammatory effects of aPL antibodies in vitro could be abrogated in mice fed with fluvastatin for 15 days [88], and this effect was independent of the cholesterol-lowering effects of the drug. We then demonstrated that fluvastatin inhibited the effects of aPL antibodies on TF expression on EC in vitro at doses utilized to reduce cholesterol levels in patients [89]. Subsequently, we examined whether proinflammatory/prothrombotic markers are elevated in patients with aPL antibodies and whether treatment with fluvastatin has an effect on them. VEGF, sTF, sTNF-α, sICAM-1, sE-selectin, CRP and sVCAM-1 were determined by ELISA in the sera of 93 patients with APS and from 60 healthy controls. Proinflammatory markers were also determined in the sera of nine patients diagnosed with APS (eight with primary APS and one with concomitant SLE).
before (baseline) and 30 days after daily treatment with 40 mg fluvastatin. (Clinical Trials. gov Identifier: NCT00674297 [201]). Mean levels of TNF-α, VEGF and sTF were significantly elevated in the sera aPL-positive patients when compared with controls (p-values: 0.0413, 0.027 and 0.0209, respectively). No significant differences were observed in the mean values of sICAM-1, sE-sel, sVCAM-1 or CRP between APS patients and controls. All APS patients were medium–high positive for aCL and anti-β2 GPI antibodies. After 30 days of treatment with fluvastatin, seven out of nine, three out of nine and six out of nine patients showed a variable but significant decrease in the titers of VEGF, sTF and TNF-α (Table 1). These findings may justify the use of VEGF, sTF and TNF-α as biomarkers of APS and of fluvastatin in the treatment of the disease [90]. Utilizing proteomic analysis, Lopez Pedrera et al. demonstrated that inflammatory proteins can be reversed following 1 month of treatment with fluvastatin [91]. Furthermore, Martinez et al. demonstrated that rosuvastatin decreases

Figure 1. Events triggered by aPL antibodies on endothelial cells. aPL antibodies bind to β2 GPI that in turn binds to endothelial cell membrane receptors (i.e., TLR and annexin A2) and induces phosphorylation of p38 MAPK, NF-κB activation and translocation and induction of iNOS, leading to a proinflammatory and prothrombotic effect (i.e., expression of VCAM-1, ICAM-1, E-selectin, TF, IL-1, IL-6 and IL-8). aPL antibodies may also enhance effects on EC by activating complement and releasing complement split products (C3a and C5a).

aPL: Antiphospholipid; Ab: Antibody; β2 GPI: β2 glycoprotein I; ICAM-1: Intercellular cell adhesion molecule-1; IL: Interleukin; NOS: Nitric oxide synthase; NF-κB: Nuclear factor-κ B; p38 MAPK: p38 mitogen activated protein kinase; TF: Tissue factor; TLR: Toll-like receptors; VCAM-1: Vascular cell adhesion molecule-1.
VCAM-1 expression by human umbilical vein endothelial cells exposed to APS serum in an in vitro model [92].

In summary, although statins have been used in primary and secondary cardiovascular disease prevention, no conclusive data exist for thrombosis prevention in aPL-positive patients. Experimental evidence in APS models and the recent randomized clinical trial demonstrating rosuvastatin’s protective effect against the first major cardiovascular events in the general population without hyperlipidemia but with elevated high-sensitivity CRP levels [93] justifies clinical studies of statins in nonpregnant aPL-positive patients. Based on the data available, it is conceivable that statins may be beneficial in reversing upregulation of TF, CAMs and inflammatory cytokines in EC and monocytes. Upon successful completion of clinical trials, statins might be useful in the treatment of patients with APS. Finally, statins would be an appealing prophylactic therapy in patients with high levels of aPL antibodies and without a history of thrombosis. Statins are teratogenic and therefore, their use in pregnancy is contraindicated. Side effects must be closely monitored, including elevated liver function tests and potential hyperglycemia and diabetes mellitus. The use of statins in the management of patients with APS needs to be further delineated in well-designed mechanistic and clinical studies.

p38 MAPK & NF-κB inhibitors in APS

Several published studies have shown that aPL-mediated activation of endothelial cells and monocytes is mediated by p38 MAPK and NF-κB, leading to the upregulation of TF, pro-inflammatory cytokines and adhesion molecules (VCAM-1 and so on). Importantly, these effects can be abrogated in vitro and in vivo by specific p38 MAPK and NF-κB inhibitors (SB203580 and MG132) (Figure 1 & 2). Platelet activation induced by aPL antibodies is also mediated by p38 MAPK and inhibited in vitro and in vivo by SB203580) [94–99].

Because of the broad proinflammatory role of p38 MAPK and NF-κB in several in vitro systems, inhibition of this pathway has been advocated as a novel therapeutic strategy for inflammatory diseases. Some p38 MAPK inhibitors are currently being tested in clinical trials for the potential use in endotoxic shock [100–102].

GPIIb/IIIa inhibitors

Espinola et al. demonstrated that prothrombotic properties of aPL antibodies can be explained in part by their ability to enhance the activation of platelets pretreated with low doses of ADP or thrombin. In their study, aPL antibodies enhanced the expression of platelet membrane glycoproteins, particularly GPIIb/IIIa and GPIIIa, when platelets were pretreated with suboptimal doses of a thrombin receptor agonist peptide (TRAP) [103]. The authors also demonstrated that aPL-enhanced thrombosis in vivo can be abrogated by infusions of a GPIIb/IIIa antagonist (1B5) monoclonal antibody, and aPL-mediated thrombophilia is not observed in GPIIb/IIIa-deficient mice. Recently, Jimenez et al. reported that double heterozygosity polymorphisms for platelet glycoproteins Ia/IIa and IIb/IIIa increases arterial thrombosis in patients with APS [104]. These data indicate that GPIIb/IIIa antagonists or platelet membrane glycoprotein IIb/IIIa receptor inhibitors may prove to be useful in the treatment of an acute thrombotic event.

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<th>Patient no./diagnosis</th>
<th>VEGF decrease %</th>
<th>sTF decrease %</th>
<th>TNF-β decrease %</th>
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<td>83</td>
<td>42</td>
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<tr>
<td>B (PAPS)</td>
<td>51</td>
<td>NS</td>
<td>62</td>
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Seven out of nine, three out of nine, and six out of nine patients showed a variable but significant decrease in VEGF, sTF and TNF-α titers, respectively.

NS: No significant decrease; ND: Not determined; PAPS: Primary antiphospholipid syndrome; SAPS: Antiphospholipid syndrome with concomitant systemic lupus erythematosus; sTF: Soluble tissue factor.
particularly an arterial event, in patients with APS (Figure 2). In addition, the combination of GPIIb/IIIa antagonists and an ADP receptor antagonist, such as ticlopidine, is an attractive therapeutic strategy that provides a fast and continuous platelet inhibition since prestimulation of platelets by agonists leads to the exposure of phosphatidylserine (PS) on the cell outer membrane forming an anti-β,GP/β,GPI complex on the exposed PS before interacting with a specific platelet receptor to potentiate activation. No data exist in APS patients regarding GPIIa/IIIb receptor inhibitors as of yet.

**Hydroxychloroquine**
Hydroxychloroquine (HCQ) is an antimalarial drug, although the precise mechanism of its anti-inflammatory action is not known. In addition to its anti-inflammatory effects, immunomodulatory effects of HCQ include increasing the pH of intracellular vacuoles, interfering with antigen processing and inhibiting T-cell-receptor- and B-cell-receptor-induced calcium signaling. HCQ also has antithrombotic effects by inhibiting platelet aggregation and arachidonic acid release from stimulated platelets (Figure 2). In the general population, HCQ has been historically

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**Figure 2. Events triggered by aPL antibodies on platelets.** APL antibodies bind to dimerized β,GPI that in turn binds to platelets receptor(s) (i.e., apoER2); and induce cell activation/aggregation through phosphorylation of p38 MAPK, cPLA2 and release of TXA2 and expression of GPIIb/IIIa.

aPL: Antiphospholipid; apoER2: Apolipoprotein ER2; β,GPI: β,glycoprotein I; cPLA2: Phospholipase A2; p38 MAPK: p38 mitogen activated protein kinase; TXA2: Thromboxane A2.
used as a prophylactic agent against deep vein thrombosis and pulmonary embolism after hip surgeries [113].

Hydroxychloroquine is now considered an essential therapeutic choice in the management of lupus. HCQ has been demonstrated to decrease the probability of lupus flares, the accrual of damage and to possibly protect SLE patients from vascular and thrombotic events [114]. Furthermore, HCQ may facilitate the response to other agents in SLE patients with renal involvement [115]. More recently, chloroquine and HCQ have been demonstrated to improve survival in a cohort of 232 SLE patients after adjusting for patients’ characteristics and disease activity [116]. It has recently been suggested that HCQ may affect the TLR9- and IFN-α-mediated activation of dendritic cells and antigen presentation in SLE [117].

In aPL-injected mice, HCQ decreased the thrombus size and the aPL-enhanced thrombus formation in a dose-dependent manner in experiments carried out at the principal investigators laboratory (Silvia S Pierangeli’s laboratory, University of Texas, TX, USA) [118]. Furthermore, HCQ inhibits the aPL-induced expression of platelet GPIIb/IIIa receptor (platelet activation) in a dose-dependent fashion (Figure 2) [105]. Recently, using 3D atomic microscopy force height images, Rand et al. showed that HCQ also reverses the binding of aPL–β3/GPI complexes to phospholipid bilayers [119]. In SLE patients, those receiving HCQ experienced fewer thrombotic events and in the Baltimore Lupus Cohort, investigators demonstrated a decreased risk of arterial thrombosis [116]. Other investigators demonstrated that HCQ decreases the risk of thrombosis in patients with SLE (OR: 0.67). In a Cox multiple failure time analysis, HCQ protected against thrombosis and increased survival in patients with SLE. In a cross-sectional study in which Erkan et al. compared 77 APS patients who had previous vascular events (65% had no other systemic autoimmune diseases) with 56 asymptomatic (no history of thrombosis or fetal loss) aPL-positive patients (18% had no other systemic autoimmune diseases), logistic regression analysis suggested that HCQ protects against thrombosis in asymptomatic aPL-positive individuals [120]. In summary, although there is experimental and clinical evidence that HCQ might decrease the incidence of thrombosis in patients with SLE, both detailed mechanistic and controlled studies are needed to determine the effectiveness of HCQ for primary and secondary thrombosis prevention in patients with APS. At this time, even though there are insufficient data to recommend HCQ for primary and secondary prevention, it might be reasonable to add HCQ to anticoagulation agents in APS patients who develop recurrent thrombosis despite optimum anticoagulation.

Multiple studies have demonstrated a reduction in thrombotic events in SLE patients receiving HCQ [120,121]. However, some studies demonstrate a sharp contrast to the protective effects on thrombus and it appears reasonable that HCQ can be used as a second-line agent, of HCQ as well as anticoagulation, in patients with APS and thrombus. In addition, before starting therapy, it is important to screen for macular toxicity with visual field and fundoscopic examination every 6–12 months.

Complement activation in APS

Complement activation has emerged as a common event in the pathogenesis of many diseases, many of them being associated with endothelial activation owing to the presence of complement receptors on endothelial cells. Investigators have shown that complement activation is a critical early mediator in APS by linking aPL antibodies to thrombosis and fetal injury. Recently, studies have demonstrated the involvement of C3, C5 and the membrane attack complex in aPL-mediated thrombosis, endothelial cell activation and fetal loss (in mice) [48,49]. Specifically, complement activation by aPL antibodies can significantly contribute to thrombosis by increasing TF expression in various cell types, in particular through C5 and membrane attack complex binding to specific receptors in endothelial cells and upregulation of TF. Pierangeli et al. demonstrated that inhibition of C5 activation using anti-C5 monoclonal antibodies prevented thrombophilia induced by aPL in aPL-treated mice, suggesting that C5a–C5a receptor interactions are critical mediators of aPL Ab-induced pregnancy complications [122,123].

Hypocomplementemia can be found in patients with primary APS, according to several publications, reflecting complement activation and consumption [124–126]. It has been hypothesized that pathogenic aPL antibodies bind to target cells and that they then activate the complement cascade as a necessary intermediate step, generating potent mediators of platelet and endothelial cell activation including C3, C5, and C5b-9 membrane attack complexes [127]. In turn, the complement system damages the endothelial cells leading to a procoagulant state and undesirable
thrombosis. A growing body of evidence shows that this pathway acts upstream of other important effector mechanisms in aPL-associated thrombosis and it is possible to speculate that the complement system could be a potential therapeutic target in patients with APS.

■ **aPL antibodies & TNF-β**

Some studies have suggested that TNF-α DNA vaccination prevents clinical manifestations of experimental APS [128]. Others have demonstrated that TNF-α is a mediator of pregnancy loss in APS animal models and that it is produced in response to complement activation; both TNF deficiency and blockade provides fetal protection in aPL-treated mice. Berman et al. found that aPL antibodies cause a rapid increase in decidual and systemic TNF-α levels and identified the release of TNF-α as a critical intermediate that acts downstream of C5 activation [129].

Although there are no reports of the use of anti-TNF therapy in APS patients, patients treated with TNF antagonists might have significantly increased IgM and IgG titers [130]. The development of aCL positivity in this circumstance is generally not associated with an increased number of thromboembolic events; however, this risk cannot be excluded until otherwise proven.

Thus, inhibitors of TNF-α may provide effective treatment for some patients with APS and recurrent pregnancy loss.

■ **IL-6 inhibitors in APS?**

IL-6 is an important cytokine in atherothrombotic disorders, and levels of this cytokine have been demonstrated to predict atherosclerosis and cardiovascular risk. In addition to inducing a proinflammatory and prothrombotic state, IL-6 also inhibits endothelial vasodilation and is associated with arterial stiffness. Studies have demonstrated increased levels of IL-6 levels in APS patients and an association between IL-6 and anti-β2GPI titers in aPL-positive patients who were on oral contraceptives at the time of thrombosis [131]. However, the potential effect of IL-6 inhibition in APS patients is unknown. Given that endothelial cell activation and widespread inflammation of the endothelium with elevated TNF-α, IL-1 and IL-6 levels have been demonstrated in patients with APS, anticytokine agents might, therefore, have a role in the management of aPL-positive patients, but no human data is currently available to support this.

■ **Autoantibody production in APS**

Price et al. demonstrated that β2GPI binds selectively to the surface of apoptotic thymocytes to generate an epitope for aPL autoantibodies, and impaired clearance of apoptotic cells may predispose a genetically susceptible individual to mount an immune response against the molecules bound to the apoptotic blebs, leading to autoantibody production against these antigens (i.e., β2GPI, prothrombin and so on) [132–134]. Subang et al. showed that apoptotic cell-bound β2GPI complexed with native cardiolipin is structurally altered, highly immunogenic and induces the production of IgG aPL antibodies [135]. Studies have demonstrated that autoreactive T cells against β2GPI, via oxidized β2GPI, may contribute to the production of antibody generation via toll-like receptors [136]. Arai et al. demonstrated that β2GPI-specific autoreactive CD4+ T cells in patients with APS preferentially recognize the antigenic peptide containing the major phospholipid-binding site and have the capacity to stimulate B cells to produce anti-β2GPI antibodies through IL-6 expression and CD40–CD40 ligand engagement [137,138]. Recently, Kahn et al. showed that pathogenetic B cells, in a murine model of spontaneous APS, arise in a CD4+ T-cell- and CD28-dependent manner, and aCL are generated in the germinal center, which is relatively independent of BAFF. They also found that blockade of BAFF alone is sufficient to prevent APS onset and to prolong survival without any effect on aCL development [139].

■ **Molecular mimicry in APS**

Immunization of mice with peptides from viral and bacterial origin that share similarity with various domains of β2GPI leads to the production of pathogenic antibodies [140–142]. Importantly, using animal models, bacterial or viral antigens with regions of β2GPI inhibited the thrombogenic properties of aPL antibodies and prevented fetal loss in mice [75,143,144]. These findings strongly suggest a molecular mimicry mechanism in APS. Importantly, the use of peptides that mimic regions of β2GPI that have shown beneficial effects in animal models may be an attractive new modality to treat clinical manifestations of APS in affected patients.

The molecular mimicry hypothesis creates new directions for therapy modalities, namely specific peptide toleragens and antimicrobial treatment. Cicconi et al. reported disappearance of APS following *Helicobacter pylori* eradication [145]. Blank et al. demonstrated a decrease
in the incidence of pregnancy loss and improvements in the clinical manifestations of APS were noted in the mice treated with ciprofloxacin compared with those given ceftazidime [146]. From our own group, studies have demonstrated significant reduction of aPL-mediated pregnancy loss and thrombosis by TIFI, a synthetic peptide from cytomegalovirus that closely resembles the phospholipid-binding domain of β2GPI (located in domain V and discussed in a previous section) [79].

Furthermore, there is evidence that infectious agents may play a role, not only in the initial production of aPL antibodies, but also as a potential second (inflammatory) hit, particularly in CAPS [15]. Hence, it may be possible to speculate about the possibility of antibiotic treatment or a more aggressive vaccination schedule to modulate infectious events in patients with aPL antibodies.

**Rituximab**

There is no doubt that APS is an antibody-mediated autoimmune disease. Therefore, it is reasonable to consider therapies that lower specific pathogenic antibody levels in patients with established APS. Rituximab, an IgG1/k mouse/human chimeric monoclonal antibody directed against the B-cell surface receptor trans-membrane surface antigen (CD20) expressed by mature B cells, is used in patients with rheumatoid arthritis and for the treatment of B-cell non-Hodgkin’s lymphoma. Rituximab kills B cells in vitro and depletes B cells from the peripheral blood by either activation of complement, induction of cell-mediated lysis, apoptosis or by increased sensitization to cytotoxic agents and/or corticosteroid [147,148].

Recently, rituximab has been demonstrated to be a good treatment for life-threatening CAPS in a small number of patients [149–151]. Rituximab has been successfully used in case reports of patients with aPL, autoimmune-mediated thrombocytopenia and hemolytic anemia. Statkute et al. demonstrated normalization of aCL antibody titers after autologous hematopoietic stem-cell transplantation in patients with APS secondary to SLE [152]. A systematic review of the off-label use of rituximab in APS revealed the higher rate of therapeutic response in patients with APS (92%) [153], and an increasing number of case reports clearly suggests the need for clinical trials to evaluate the effect of rituximab in the treatment of resistant APS. Currently, Erkan et al. are conducting a pilot study of rituximab for the anticoagulation-resistant manifestations of antiphospholipid syndrome (RITAPS) open-label Phase II trial using rituximab to study patients who are aPL positive and resistant to conventional anticoagulation (Clinical trials.gov Identifier: NCT00537290 [202]). This trial will provide systematic data on the clinical, laboratory and serological parameters of rituximab-treated aPL-positive patients.

**Conclusion & future perspective**

Significant knowledge has been gained in the last 25 years with respect to the pathogenesis of APS. It is now clear that the disease is multisystemic and heterogeneous in presentation and prognosis. Various target cells and intracellular mechanisms have been identified including: platelets, endothelial cells, monocytes, trophoblasts, placenta, decidual cells. Current treatments for the clinical manifestations of APS include life-long anticoagulation and immunosuppression. None of these treatments have proven to be efficacious in all affected patients and they are associated with significant side effects. With the understanding of the molecular interactions triggered by aPL antibodies, date from in vitro and in vivo experiments, new targeted treatments are now becoming available. The safety and efficacy of these new therapeutic approaches will need to be evaluated in well-designed clinical trials in the next 5–10 years.

<table>
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<th>Executive summary</th>
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<tr>
<td>- Antiphospholipid (aPL) antibodies activate platelet, endothelial cells and monocytes involving p38 MAPK.</td>
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<td>- Tissue factor is an important procoagulant molecule upregulated by aPL antibodies in monocytes and endothelial cells.</td>
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<td>- There are no consensus guidelines to treat patients with antiphospholipid syndrome (APS)-related pregnancy losses.</td>
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<td>- Statins and hydroxychloroquine seem promising as new treatments for APS. Statins would not be a viable option during pregnancy.</td>
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<td>- GPIIb/IIIa inhibitors to treat APS-induced thrombosis?</td>
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<td>- Are cytokines such as IL-6 and TNF-α biomarkers of disease?</td>
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<tr>
<td>- Is molecular mimicry involved in the generation of aPL antibodies? Is it possible to use tolerogenic peptides to prevent production of aPL antibodies?</td>
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No writing assistance was utilized in the production of this manuscript.

Bibliography

Papers of special note have been highlighted as:
* of interest
** of considerable interest


** Classification criteria for definitive antiphospholipid syndrome (APS) is proposed in this paper.


* Showed for the first time that antiphospholipid (aPL) antibodies activate endothelial cells in vitro.


Management of the antiphospholipid syndrome: new approaches


75 Showed that a peptide that mimics a region of B2GPI reverses thrombogenic properties of aPL antibodies in vitro by displacing B2GPI from target cells.


90 First time that fluvastatin was shown to reduce tissue factor upregulation induced by aPL antibodies in vitro.


* Showed the involvement of p38 MAPK in the up-regulation of tissue factor by aPL antibodies.


* Showed that an inhibitor of NF-κ B reverses thrombogenic properties of aPL antibodies in vivo.


** Demonstrates the involvement of GP Ib-IX.V as a receptor for aPL antibodies in platelets.

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** Showed that hydroxychloroquine reduces thrombosis induced by aPL antibodies in vivo in mice.


* Involvement of C3 and C5 complement components in aPL-mediated thrombosis in vivo.


Management of the antiphospholipid syndrome: new approaches

139 Shows that endothelial cells are a direct target recognized by aPL antibodies.

Websites

201 www.ClinicalTrials.gov Identifier: NCT00674297
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