Pathogenesis and management of antiphospholipid syndrome

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Abstract
Antiphospholipid antibodies are a heterogeneous group of autoantibodies which have clear associations with thrombosis and pregnancy morbidity, and which together constitute the ‘antiphospholipid syndrome’ (APS). However, the pathophysiology of these complications is not well understood and their heterogeneity suggests that more than one pathogenic process may be involved. Diagnosis remains a combination of laboratory analysis and clinical observation but there have been significant advances in identifying specific pathogenic features such as domain I-specific anti-beta 2GPI antibodies. This in turn has pointed to endothelial and complement activation as important factors in the pathogenesis of APS. Consequently, although anticoagulation remains the standard treatment for thrombotic APS and during pregnancy, the realisation that these additional pathways are involved in the pathogenesis of APS has significant implications for treatment: agents acting outside the coagulation system such as hydroxychloroquine for pregnancy complications and sirolimus as an inhibitor of the mammalian target of rapamycin (mTOR) pathway are now under evaluation and represent a radical change in thinking for haematologists. Conventional anticoagulation is also under challenge from new, direct acting anticoagulants. This review will provide a comprehensive overview of the evolving understanding of APS pathogenesis and how this and novel therapeutics will alter diagnosis and management.
Introduction

Antiphospholipid syndrome (APS) is characterized by vascular thrombosis and/or pregnancy loss or morbidity in association with persistent positivity of autoantibodies known as antiphospholipid antibodies (aPL) (Miyakis et al, 2006) (Table 1). Vascular thrombosis could be arterial, venous, or small vessel, in any tissue or organ and must be confirmed by objectively (i.e. unequivocal findings of appropriate imaging studies or histopathology). Thrombosis in APS should show no signs of inflammation in the vessel wall (Miyakis et al, 2006). Although many patients with APS have an associated autoimmune disorder, thrombotic events in APS are not accompanied by histological evidence of vessel wall inflammation. Nonetheless, inflammatory mediators and inflammatory responses in endothelial cells, monocytes and neutrophils are implicated in APS pathogenesis. The International Consensus Criteria (ICC: Sydney; updated Sapporo) (Miyakis et al, 2006) for APS were designed for scientific clinical studies but have been adapted for the diagnosis of APS in routine clinical practice. The British Committee for Standards in Haematology (BCSH) guidelines (Keeling et al, 2012) adopt similar criteria for the diagnosis of both thrombotic and obstetric APS. Non-criteria features of APS such as heart valve disease, livedo reticularis, thrombocytopenia and nephropathy may present in association with thrombosis and/or pregnancy morbidity or as isolated features. The APS Clinical Features Task Force of the 14th International Congress on aPL analysed the relevance of the most frequent non-criteria manifestations and concluded that available data support the inclusion of thrombocytopenia, heart valve disease, renal microangiopathy (aPL nephropathy), chorea, and longitudinal myelitis as part of APS Classification Criteria (Abreu et al, 2015). However,
these features are not yet included in the ICC. APS can occur in isolation or in association with other autoimmune disorders such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (Miyakis et al, 2006).

aPL are a heterogeneous group of autoantibodies, which include lupus anticoagulant (LA), IgG and IgM anticardiolipin antibodies (aCL) and anti-β₂-glycoprotein-I (anti-β₂GPI) antibodies. Their primary targets are phospholipid-binding proteins, although antibodies directed against phospholipids and other proteins also occur. As early as 1952 Conley and Hartmann wrote a brief report about two patients with SLE and a ‘peculiar haemorrhagic disorder’ with prolonged blood clotting times and clear evidence of an anticoagulant in plasma mixing studies (Conley & Hartmann et al, 1952). As this inhibitor was predominantly found in patients with SLE, the in vitro anticoagulant phenomenon was called LA. The paradoxical correlation between vascular thrombosis and LA was first described in 1963 by Bowie et al (Bowie et al, 1963) and followed by many others. Originally, it was thought that these autoantibodies bound to phospholipid; hence ‘antiphospholipid antibodies’ but in the 1990s it was shown that aPL recognized phospholipids indirectly via phospholipid-binding plasma proteins (Galli et al, 1990). Although many plasma proteins have been found to be antibody ligands in APS, antibodies against β₂GPI have the most significant association with pathogenicity (Bas de et al, 2004). Some authors have challenged the primacy of anti-β₂GPI antibodies in the pathogenesis of APS (Lackner & Muller-Calleja et al, 2016) and shown that some co-factor independent antibodies can induce thrombus formation in a mouse model (Manukyan et al, 2016).
Laboratory diagnosis of APS requires presence of LA in plasma or aCL of IgG and/or IgM isotype in serum or plasma, present in medium or high titre (i.e. >40 GPL or MPL, or >the 99th centile) or anti-β₂GPI of IgG and/or IgM isotype in serum or plasma (in titre >the 99th centile). The positive results must be obtained on two or more occasions, at least 12 weeks apart (Miyakis et al, 2006). Inter-laboratory variability remains a problem in the performance of the aCL and anti-β₂GPI ELISAs due to the lack of uniformity in reference materials for calibration.

Nonetheless, identification of these antibodies has allowed development of diagnostic testing and investigation of pathogenic mechanisms. These in turn offer additional therapeutic options which are currently under investigation. Even conventional anticoagulant therapy is under review while the role of direct acting oral anticoagulants is investigated. The key events in APS are summarised in figure 1. The aim of this review is to examine the evolving understanding of APS pathogenesis and how this and other therapeutic options will change the way we manage this potentially devastating disorder.

**Pathogenesis of Antiphospholipid syndrome**

Despite clear associations between aPL and thrombosis and with pregnancy morbidity, the pathophysiology of these complications is not well understood, with their heterogeneity suggesting that more than one pathogenic process is involved. Moreover, even though aPL are persistently present in the systemic circulation, thrombotic events occur only occasionally, suggesting that the development of aPL is a necessary, but not sufficient step in the development of APS and that other factors play a role. Such ‘second hits’ or ‘triggers’
likely push the thrombotic/haemostatic balance in favour of thrombosis and may include infection, endothelial injury, inflammation, immunological and other non-immunological procoagulant factors such as oestrogen containing contraceptive pills, surgery and immobility (Pengo et al, 2011). The patient’s genetic constitution, in relation to genes for inflammatory mediators, may also be a critical variable in the development of clinical APS manifestations.

**Antibody specificities and evidence for pathogenicity of anti-β2-GPI**

In 1990, McNeil et al. identified that the binding of aPL to cardiolipin requires the presence of β2GPI as a cofactor (McNeil et al, 1990). β2GPI is an apolipoprotein; a member of the complement control family and is considered to be a natural inhibitor of coagulation. In addition, *in vitro* studies have shown that it has anti-angiogenic (Yu et al, 2008) and anti-apoptotic (Maiti et al, 2008) activities. It consists of 326 amino acids, arranged in 5 highly homologous complement-control protein domains, designated I to V from the N- to the C-terminus. β2GPI can adopt two different conformations: a circular conformation in plasma maintained by interaction between the first and fifth domain and an “activated” open conformation (Agar et al, 2010). In the presence of anionic phospholipids, the circular conformation of the protein unfolds, exposing antigenic determinants that are normally shielded from the circulation (van Os et al, 2011)[Figure 2]. One of these “cryptic” antigenic determinants, within domain I (DI), is the epitope for the pathologic autoantibodies (de Laat et al, 2006, Ioannou et al, 2007); which will not bind to the closed circulating form. Unfolding of β2GPI on the endothelial surface may be facilitated by release of molecules such as thioredoxin in response to oxidative stress which can reduce surface thiols on β2GPI. Binding
of β2GPI on endothelial cells (EC) normally has a protective function against oxidative stress induced cell injury (Ioannou et al, 2010; Ioannou et al, 2011) but antibody binding fixes β2GPI in this open conformation on the phospholipid surface and the antibody-β2GPI complexes bind to a variety of receptors (e.g., Toll-like receptors 2 and 4, annexin A2, and glycoprotein 1bα) on different cell types, including ECs, monocytes, trophoblasts and platelets (de Groot & Meijers, 2011). This binding may trigger intracellular signalling and inflammatory responses.

Direct evidence for the pathogenicity of these antibodies comes from infusion of autoantibodies from APS patients to mice with injured blood vessels showing that they potentiate thrombus formation (Arad et al, 2010). Specifically, purified anti-β2GP1 IgG autoantibodies, but not anti-β2GPI depleted IgG or normal human IgG potentiated thrombus size in a dose-dependent manner. Although several clinical studies and systematic reviews suggested that LA is a stronger risk factor for the development of thrombosis (DE Groot et al, 2005; Galli et al, 2003), other studies have shown that the presence of LA alone is not associated with thromboembolic events (Pengo et al, 2005; Pengo et al, 2007). Similar results were obtained in the Leiden Thrombophilia case–control study (de Groot et al, 2005) where positive LA with negative anti-β2GPI or antiprothrombin ELISAs was not a risk for DVT (OR 1.3, 95% CI: 0.3–6.0). However, a recent systematic review and meta-analysis found that LA and aCL antibodies were associated with an increased risk of VTE (OR = 6.14 [CI 2.74; 13.8] and OR = 1.46 [CI 1.06; 2.03] respectively) but there was a non-significant trend for anti-β2GPI (OR = 1.61 [CI 0.76; 3.43]). For arterial thrombosis, all three antibodies showed a significant association: ORs for LA, aCL and anti-β2GPI were 3.58 (CI 1.29–9.92), 2.65 (CI
1.75–4.00) and 3.12 (CI 1.51–6.44) respectively (Reynaud et al, 2014). de Laat and co-workers found that anti-β₂GPI with LA activity are antibodies that are responsible for the thromboembolic complications in APS (de Laat et al, 2004). Moreover, they showed that a subgroup of anti-β₂GPI recognizing epitope Gly40-Arg43 (G40-R43) in domain I (DI) of the molecule cause LA and that their presence correlates strongly with thrombosis (de Laat et al, 2005). This has been confirmed by various other studies (Pengo et al, 2015; de Laat et al, 2009).

Consequently, these pathogenic antibodies will only bind purified β2GPI when coated onto an appropriate negatively charged surface and this has important implications for the design, selection and significance of diagnostic assays (de Laat et al, 2005; de Laat et al, 2006). It has been shown that exposure of the critical DI epitope G40-R43 on β₂GPI is highly variable between commercial anti-β₂GPI assays. As a consequence, false negatives can arise in assays characterized by a reduced exposure of G40-R43 (Pelkmans et al, 2013).

The presence of triple aPL positivity, as defined by the detection of LA and high titers of aCL and anti-β₂GPI antibodies, correlates better with both thrombosis and pregnancy morbidity than any other aPL profile (Pengo et al, 2010): the risk of recurrent thrombosis in triple positive patients was around 30% over a 6-year follow up period (Pengo et al, 2010). Triple positive patients also have high titres of autoantibodies that bind the major B-cell epitope on DI of the β₂GPI molecule (Banzato et al, 2011). Thus DI anti-β₂GPI autoantibodies; confer LA activity associated with the highest risk of thrombosis (de Laat et al, 2009), frequently present in triple aPL-positive patients, are closely associated with both thrombosis and pregnancy loss (de Laat et al, 2009) and increase thrombus size in mouse models (Pericleous
Of note, about 30% of patients with anti-β₂GPI antibodies are negative for DI-anti-β₂GPI antibodies (Pengo et al., 2015) and the clinical significance of autoantibodies reacting with epitopes other than DI was investigated in a multicentre study (Artenjak et al., 2015). In this study serum samples from 201 autoimmune patients with IgG anti-β₂GPI activity (87 APS, 67 APS plus SLE and 47 with SLE only) were analysed for their binding to six β₂GPI peptides corresponding to amino acid clusters on domains I-II, II, III and III-IV. Results showed a diverse clinical association with reactivity to different epitopes on β₂GPI, suggesting all domains were relevant. Therefore, more detailed profiling of domain specificity and avidity of anti-β₂GPI antibodies may be useful as risk stratification for clinical events.

Studies using cell cultures demonstrated that monocytes, ECs and platelets can also be activated by aPL with anti-β₂GPI activity inducing the expression on EC of intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, and of tissue factor (TF) on both EC and monocytes (Pierangeli et al., 2008). Activated platelets increase expression of glycoprotein IIbIIIa and synthesis of thromboxane A₂ (Pierangeli et al., 2008). However, it is possible that cells in culture may not behave as they do in vivo.

Non-criteria Antibodies

The role of so called non-criteria antibodies such as anti-phosphatidylserine/prothrombin, anti-phosphatidic acid, anti-vimentin/cardiolipin complex anti-protein C/S and variety of other autoantibodies with specificities such as factor XII, factor X, Annexin A5 and Annexin
A2 in the pathogenesis and diagnosis APS remains to be established. The role of DI-anti-β2GPI antibodies in pathogenesis of APS is discussed above. In a small cross-sectional study we demonstrated that anti-protein C antibodies are associated with resistance to endogenous protein C activation and a severe thrombotic phenotype in APS (Arachchillage et al, 2014). Several studies have analysed the role of the IgA isotype of aPL. In particular, the pathogenicity of IgA aPL was established by demonstrating that mice, injected with IgA aPL from patients with APS, developed thrombosis (Pierangeli et al, 1995). In a recent systematic review to establish the prevalence of non-criteria antibodies and of resistance to Annexin A5 anticoagulant activity (AnxA5R) in APS and control populations, 16 retrospective studies of 1404 APS patients, (1839 disease control and 797 healthy controls) were examined. It was found that IgA anti-β2GPI antibodies (129/229, 56.3%) were most prevalent, followed by AnxA5R (87/163, 53.4%) and IgG anti-Domain I (241/548, 44.0%) (Rodriguez-Garcia et al, 2015). However, in addition to the retrospective data collection, the results were affected by wide variation in the sample size, discrepancy in assay methodology and the different cut off levels used for assay positivity. Prospective multicentre studies with adequate sample size using more uniform methodology are essential to determine the implications of these antibodies for the management of APS.

**Pathogenic mechanisms involving other pathways**

There is evidence from several animal models that complement activation, with excess C3a and C5a generation, plays a role in thrombotic manifestations in APS. Complement activation contributes to vascular inflammation and complement inhibition may ameliorate aPL-induced thrombosis, offering a potential new approach to therapy (Girardi et al, 2003a).
Mice treated with IgG from APS patients with high levels of aPL and subjected to a femoral vein pinch model of thrombosis, developed larger thrombi and higher soluble TF activity than controls (Romay-Penabad et al, 2014). The co-administration of rEV576 (coversin), a recombinant protein inhibitor of C5 activation, resulted in significantly smaller thrombi and reduced TF activity (Romay-Penabad et al, 2014). Consistent with this hypothesis, low complement levels (C3, C5) have been demonstrated in patients with APS (Oku et al, 2009).

In a study of 186 patients with aPL Breen et al, found significantly increased levels of fragment Bb and C3a compared to normal controls [NC] (Breen et al, 2012). In the RAPS (Rivaroxaban in Antiphospholipid Syndrome) trial of 111 APS patients; we demonstrated that APS patients with previous VTE had significantly increased complement activation compared to NC which was decreased by rivaroxaban compared to warfarin therapy (Arachchillage et al, 2016).

Catastrophic APS (CAPS) is a rare but potentially fatal variant of APS characterized by sudden onset of extensive microvascular thrombosis at multiple sites leading to multi-organ failure (Cervera et al, 2014). Widespread complement activation may contribute to CAPS and this is supported by individual case reports of patients unresponsive to anticoagulation and immunosuppression, but successfully treated with C5 complement inhibitor eculizumab (Shapira et al, 2012). As discussed below, complement activation may also be a central mechanism in aPL-induced pregnancy loss and intrauterine fetal growth restriction (Salmon et al, 2003). Complement pathway activation offers several potential targets for therapeutic complement inhibition as shown in figure 3.
Microvascular thrombosis is one of the major pathologic manifestations of APS and may occur alone or in combination with large-vessel thrombosis. Kidneys represent the most important site of microvascular thrombotic/microangiopathic APS and is associated with renal failure, thrombotic microangiopathy, and hypertension (Griffiths et al, 2000). In patients who undergo renal transplantation for APS, the microvascular thrombotic lesions often recur. In cultured vascular ECs, IgG antibodies from patients with APS stimulated the mammalian target of rapamycin (mTOR) through the phosphatidylinositol 3-kinase (PI3K)–AKT pathway (Canaud et al, 2014) leading to cell proliferation. It has been shown that mTOR pathway activation plays a role in endothelial proliferation and intimal hyperplasia in aPL positive patients, which leads to microthrombosis, peripheral ischemia, skin ulcers, diffuse alveolar haemorrhage or aPL nephropathy (Manning & Cantley, 2007). The vascular endothelium of proliferating intrarenal vessels from patients with APS nephropathy showed evidence of mTORC pathway activation.

Another proposed mechanism of aPL initiated thrombosis is through interference with the anticoagulant activity of activated protein C (APC), resulting in acquired activated protein C resistance (APCr) (Nojima et al, 2005): aPL inhibition of the thrombomodulin mediated activation of protein C (PC), as well as the anticoagulant activity of APC (Malia et al, 1990), have been observed. Upregulation of the TF pathway, (Adams et al, 2001) by down regulation of its principal inhibitor, TF pathway inhibitor (TFPI) (Liestol et al, 2007) may also increase thrombotic risk and studies have reported the presence of auto-antibodies against TFPI (αTFPI) in APS patients (Adams et al, 2001). Many in vitro studies have shown aPL mediated upregulation of TF expression on monocytes (Lambrianides et al, 2010) and
endothelial cells associated with inflammatory cytokines and adhesion molecules (Willis et al, 2015); aβ2GPI has also been shown to suppress TFPI dependent inhibition of the TF pathway of coagulation (Boles & Mackman et al, 2010).

Pathogenesis of pregnancy complications in APS

The presence of aPL probably constitutes the single most recognisable risk factor in the majority of cases of recurrent pregnancy loss and late placenta-mediated obstetric complications. Animal studies have demonstrated that the passive transfer of aPL promotes fetal loss and placental thrombosis and also inhibits trophoblast and decidual cell functions in vitro (Branch et al, 1990). Cross-species investigations have shown that the exposure of pregnant rats to a purified IgG fraction from women with APS directly inhibits embryo growth (Ornoy et al, 2003). Immunopathology of obstetric APS differs from that of thrombotic APS, especially in the case of recurrent early miscarriages, where thrombosis is neither a universal nor a specific feature (Out et al, 1991). Nonetheless, LA may be associated with extensive placental necrosis, infarction and thrombosis in women with recurrent pregnancy loss and extensive villous infarction following first trimester miscarriage in a patient with SLE and positive LA and aCL was first described in 1996 (Nayar & Lage, 1996).

Annexin V is a cationic protein which normally serves an anticoagulant function by aggregating on trophoblast membranes and blocking FXa and prothrombin binding. Thrombosis during the development of the normal materno-placental circulation may arise via interference with (Rand et al, 1997; Rand et al, 2010) this function and women with APS have disrupted and reduced annexin V binding to the surface of trophoblastic cells of the
intervillous space compared with controls (Rand et al, 1997). During differentiation to syncytium, trophoblast membrane anionic phospholipids also bind β2GPI and hence β2GPI-dependent aPL which may then lead to defective placentation via complement activation, inflammatory damage and placental apoptosis (Girardi et al, 2010). Studies of animal and human placenta have demonstrated that complement activation by aPL may play a major role in the pathogenesis of recurrent pregnancy loss (Salmon et al, 2003). Appropriate complement inhibition is an essential requirement for normal pregnancy as evidenced by the finding that deficiency of Crry (a membrane-bound complement regulatory protein that blocks C3 and C4 activation) leads to progressive embryonic loss in mice (Xu et al, 2000). It has been hypothesised that aPL bound to trophoblasts activate complement via the classical pathway, generating split products that mediate placental injury causing fetal loss and growth restriction. Passive transfer of IgG from women with recurrent miscarriage and aPL results in a significant increase in the frequency of fetal resorption and reduced average weight of the surviving fetuses, compared to mice treated with IgG from healthy individuals (Holers et al, 2002). These effects can be blocked by inhibition of the complement cascade using a C3 convertase inhibitor or by antibodies or peptides that block C5a-C5a receptor interactions (Girardi et al, 2003; Pierangeli et al, 1999). Furthermore, mice deficient in C3 are resistant to the fetal injury induced by aPL (Girardi et al, 2003; Holers et al, 2002) and studies in factor B-deficient mice indicate that the alternative complement pathway is also required. Inflammatory tissue injury is likely mediated by tumour necrosis factor (TNF) alpha, which increases in murine decidua after exposure to aPL (Berman et al, 2005) and antibody blocking of TNF-α reduces fetal resorption (Blank et al, 2003). Finally, the therapeutic effect of heparin has been shown to
operate via inhibition of complement rather than inhibition of coagulation (Girardi et al, 2004). The PROMISE study (Predictors of pregnancy outcome: biomarkers In APS and SLE) is a prospective multicentre observational study which will further evaluate the role of complement in SLE and aPL-associated pregnancy complications (https://clinicaltrials.gov/ct2/show/NCT00198068).

Animal and human cell culture studies have demonstrated that binding of aPL (in particular β2GPI-dependent antibodies) to human trophoblasts affects other critical cell functions; inhibition of proliferation and syncitia formation, decreased production of human chorionic gonadotrophin and defective secretion of growth factors, as well as inducing apoptosis (Pierangeli et al, 2008). Moreover aPL may impair the sequential expression of cell adhesion molecules, such as integrins and cadherins, in trophoblastic and decidual cells essential for normal placentation (Di Simone et al, 2002). Mechanisms for aPL-mediated fetal loss and complications are summarised in Table 2.

**Management of thrombosis in antiphospholipid syndrome**

Despite the implication of other mechanisms as described above, anticoagulation rather than immunosuppression remains the mainstay of management in patients with thrombotic APS. Two randomised controlled trials have demonstrated that high-intensity warfarin is not superior to moderate-intensity warfarin (INR 2.0-3.0) for the prevention of recurrent VTE (Crowther et al, 2003; Finazzi et al, 2005). Current recommendation is that patients with APS and otherwise unprecipitated VTE should remain on anticoagulants indefinitely with a target INR of 2.5, which reduces recurrent VTE by 80-90% compared to no treatment (Danowski et
Indefinite anticoagulation for APS patients is supported by evidence that APS patients carry a higher risk of recurrent thrombosis compared to aPL negative subjects and that the thrombotic risk may increase with time (Ruiz-Irastorza et al, 2002). However, in a systematic review, the strength of this association was uncertain because the available evidence was of very low quality (Garcia et al, 2013). Thrombosis after a clear precipitant should not prompt testing for APS and requires only three months of anticoagulation (Keeling et al, 2012).

With regards to the optimal intensity of anticoagulation following arterial thrombosis, an earlier prospective cohort study, the Antiphospholipid Antibodies and Stroke Study (APASS), found no benefit of low intensity warfarin anticoagulation (target INR 1.4-2.8) over aspirin (325mg/day) in stroke prevention (Levine et al, 2004). However, the study had important limitations such as low target INR and aPL testing being performed only on entry to the study raising the possibility that some participants may have had transient antibody positivity. Crowther and Finazzi’s studies demonstrated that moderate-intensity warfarin (INR 2.0-3.0) is effective for arterial thrombosis and APS, although patients with arterial thrombosis were poorly represented in these trials (only, 24% and 32% respectively) (Crowther et al, 2003; Finazzi et al, 2005). Ruiz-Irastorza and colleagues recommended, following a systematic review of cohort studies, that APS with arterial thrombosis and/or recurrent venous events should be treated with warfarin at an INR >3.0 (Ruiz-Irastorza et al, 2007). Although the 13th International Congress on Antiphospholipid Antibodies task force also recommended an INR >3.0 or combined platelet inhibitor-anticoagulant (INR 2.0–3.0) therapy for arterial thrombosis (Ruiz-Irastorza et al, 2011), this was a non-graded
recommendation due to lack of consensus within the panel members. Standard practice currently is for a target INR of 2.5, with escalation to a higher target INR should thrombosis recur (Keeling et al, 2012).

Warfarin’s narrow therapeutic range and multiple interactions are further complicated in APS by the variable responsiveness of thromboplastins to LA, leading to misleading INR results. However, in the majority of patients with APS monitoring of INR is not a major issue and the problem is limited to specific thromboplastins (Tripodi et al, 2001).

**Direct acting oral anticoagulants**

Direct acting oral anticoagulants (DOACs) are fixed dose oral anticoagulants with predictable anticoagulant effect and consequently no need for routine monitoring. DOACs are established as therapeutic alternatives to VKAs for treatment and secondary prevention of VTE in patients without APS. Although aPL are reported in approximately 10% of patients with VTE (Andreoli et al, 2013), phase III clinical trials have not compared warfarin vs DOAC for this group and experience is limited to case reports. A total of 96 APS patients are reported to be treated with DOACs (82/96 [85.5%] rivaroxaban, 13/96 [13.5%] dabigatran and 1/96 [1%] apixaban) (Bachmeyer & Elalamy, 2014; Schaefer et al, 2014; Win & Rodgers, 2014; Betancur et al, 2016; Noel et al, 2015; Sciascia et al, 2015; Signorelli et al, 2016; Son et al, 2015). Only 8/96 (8.3%) of the patients received a DOAC as acute treatment of VTE. 17 patients had recurrent thrombosis. However, those who had recurrent arterial thrombosis had a severe thrombotic phenotype at the time of starting a DOAC and standard
risk APS patients who were on warfarin with target INR of 2.0-3.0 had no recurrent events. Only 2 patients (2/96 [2.1%]) had bleeding events.

Rivaroxaban has been shown to effectively reduce thrombin generation in plasma from patients with thrombotic APS compared to warfarin in a recently published randomised clinical trial (Cohen et al, 2016), but there are no data from intervention trials designed to assess clinical endpoints. Several other clinical trials assessing the use of DOAC is thrombotic APS are still recruiting participants ([https://clinicaltrials.gov/NCT02157272], [https://clinicaltrials.gov/NCT02295475],[https://clinicaltrials.gov/NCT02116036]). However, given their general efficacy, DOAC could be considered as an alternative anticoagulant in patients with APS and VTE that are usually treated with standard-intensity warfarin, when there is known VKA allergy or poor anticoagulant control. DOACs have not been studied in patients with arterial thrombosis even without APS and the comparison has been always with standard intensity warfarin anticoagulation. Therefore, DOACs are not recommended in APS patients with arterial thrombosis or those with recurrent VTE whilst achieving therapeutic anticoagulation with warfarin.

For the small proportion of patients with APS who develop recurrent thrombosis despite apparently adequate anticoagulation an increase in warfarin intensity is recommended (ie: target INR of 3.5). There are limited therapeutic options for patients who have recurrent thrombosis despite high-intensity warfarin. These include addition of an antiplatelet agent or LMWH, including high-intensity LMWH (maintaining peak anti Xa levels 1.6 – 2.0IU/ml for once daily dosing and peak 0.8 – 1.0IU/ml for twice daily dosing) or combined factor Xa and factor IIa inhibitors (Arachchillage et al, 2016). When anticoagulation has failed, other
available therapeutic options include combining anticoagulation with immunosuppression and/or immunomodulation with modalities including rituximab, hydroxychloroquine, statins, rituximab complement inhibitors and mTOR inhibitors such as sirolimus.

**Hydroxychloroquine**

Hydroxychloroquine (HCQ) is a well-established treatment for patients with SLE and rheumatoid arthritis due to its anti-inflammatory effects; inhibiting cytosolic phospholipase A2 and reducing the release of the cytokines interleukin (IL)-6 and TNF (Sperber et al, 1993). It is currently recommended as a baseline therapy in all patients with SLE who have no contraindications (Ruiz-Irastorza et al, 2010). HCQ also inhibits platelet aggregation and arachidonic acid release, reducing thrombus size in mice models of APS (Espinola et al, 2002; Rand et al, 2010) and protects the Annexin V anticoagulant shield from disruption by aPL (Rand et al, 2010). Its antithrombotic activity is demonstrated by its efficacy as thromboprophylaxis after hip surgery (Johansson et al, 1981) and in patients with SLE, without increased bleeding (Schmidt-Tanguy et al, 2013). Recent APS consensus guidelines support its use as an adjuvant to anticoagulation in patients with recurrent thrombosis despite anticoagulation (Ruiz-Irastorza et al, 2011). A phase III multicentre trial exploring the effect of HCQ as primary thrombosis prophylaxis in individuals with persistently positive antiphospholipid antibodies [https://clinicaltrials.gov//NCT01784523](https://clinicaltrials.gov//NCT01784523) has been completed and findings are awaited.
Statins

Statins inhibit the enzyme HMG-CoA reductase which has a central role in hepatic cholesterol production but also have pleiotropic effects including anti-inflammatory and antithrombotic actions on EC and monocytes (Ferrara et al, 2003). Furthermore, there is evidence they increase fibrinolysis and decrease tissue factor mRNA expression; opposite effects to aPL (Ferrara et al, 2004). These observations and the demonstrated VTE reduction in patients receiving statins (Ridker et al, 2009) suggest a possible benefit from their use in APS. In a prospective open-label pilot study of fluvastatin in aPL positive patients, Erkan et al, demonstrated that fluvastatin can reduce pro-inflammatory and pro-thrombotic biomarkers such as interleukin (IL)-6, IL1β, vascular endothelial growth factor, TNF-α and interferon–α (Erkan et al, 2014). Based on available evidence, the 14th International Congress on Antiphospholipid Antibodies Task Force Report on APS Treatment Trends stated that statins cannot be recommended in APS patients in the absence of hyperlipidemia, but may be a useful treatment adjunct in APS patients with recurrent thrombosis despite adequate anticoagulation (Erkan et al, 2014).

Rituximab

Rituximab appears to be an effective treatment for thrombocytopenia and haemolytic anaemia associated with aPL (Erre et al, 2008) and may improve some other manifestations such as skin ulcers (Erkan et al, 2013). Rituximab has been used for the treatment of difficult cases such as refractory thrombocytopenia or other non-criteria aPL manifestations, CAPS, and recurrent VTE in over 50 case reports. In a review by Kumar et al, it was reported that in 19 of 21 published cases; rituximab had a beneficial clinical effect. aPL levels were
significantly decreased in ten of 12 cases (Kumar & Roubey, 2010). Review of the CAPS registry of 20 patients treated with rituximab reported a recovery rate of 75% of acute episodes in combination with multiple treatment strategies including anticoagulation, steroids, plasma exchange and cyclophosphamide (Berman et al., 2013); consequently, the responses cannot be clearly attributed to rituximab alone. Furthermore, there was no consistency in the reduction in aPL level in these patients.

**Complement inhibition**

Experimental evidence discussed above has led to speculation that inhibition of complement may be a useful therapeutic strategy in APS. So far only preliminary and anecdotal data are available. A phase II clinical is evaluating the use of eculizumab to enable renal transplantation and prevent post-transplant thrombotic microangiopathy (TMA) in patients with a history of CAPS (http://clinicaltrials.gov/show/NCT01029587) is ongoing, but not recruiting participants. Preliminary data of this study from 80 patients showed that eculizumab is effective in reducing the incidence of acute antibody-mediated rejection in sensitized deceased donor kidney transplant recipients (KTR). Patient and graft survival and kidney function at 1yr were similar to those expected for non-sensitized KTR (Glotz et al., 2015). Another phase II multicentre clinical trial evaluating the safety and tolerability of intravenous ALXN1007 (C5a inhibitor) in persistently aPL-positive patients with at least one of the non-criteria manifestations of APS is underway https://clinicaltrials.gov/NCT02128269.
**Sirolimus**

Sirolimus is an mTOR inhibitor which is currently used to prevent reactive arterial stenosis after coronary stenting. A cohort of patients with APS nephropathy who required transplantation and were receiving sirolimus had no recurrence of vascular lesions and had decreased vascular proliferation on biopsy as compared to APS patients not receiving sirolimus (Canaud et al., 2014). Graft survival at 144 months was 7/10 (70%) versus 3/27 (11%) in the two groups. Further larger studies are required to establish the routine use of mTOR inhibitor in APS patients.

**Potential future therapeutic options**

TIFI is a 20-amino acid peptide from human cytomegalovirus, which shares similarities with the PL-binding site in the β2GPI molecule, DV. *In vitro* evidence suggests that TIFI inhibits the binding of labelled β2GPI to human ECs and mouse monocytes (Ostertag et al., 2006). In a mouse model of APS, infusion of TIFI reduced the binding of fluoresceinated β2GPI to ECs and reduced thrombosis size after aPL infusion (de la Torre et al., 2012). A dimeric peptide of β2-glycoprotein-DI (DI dimer) has a similar effect (Agostinis et al., 2014). These peptides are not yet in clinical trials but may offer a new paradigm for APS treatment in the future.

**Management of CAPS**

Anticoagulation, intravenous immunoglobulin (IVIG), plasma exchange, immunosuppressive therapy, prostacyclin, fibrinolytics and defibrotide have all been used in the management of CAPS. Case reports described above suggest eculizumab may be effective in CAPS
unresponsive to other treatments (Shapira et al, 2012) and in preventing relapse after kidney transplantation. There are reported cases of use of rituximab in patients with CAPS with variable responses (Erre et al, 2008). Espinosa et al reviewed 9 such patients: two patients died, but notably in three of the surviving patients tests for aPL became negative after rituximab treatment (Espinosa et al, 2011).

**Management of asymptomatic carriers of aPL**

Another unanswered question is whether primary thromboprophylaxis is indicated in subjects with aPL and how risk of a first thrombosis can be stratified in order to personalise treatment. A prospective evaluation of the incidence and risk factors for a first vascular event in 258 asymptomatic individuals with aPL determined that the annual incidence rate was 1.86% compared to 0.1% in the general population with a median follow up of 35 months (range 1-48) (Ruffatti et al, 2011). Hypertension and the presence of LA were identified as independent risk factors for development of thrombosis (Ruffatti et al, 2011). In a study of 104 subjects with triple positivity, there were 25 first thrombotic events (5.3% per year) with a cumulative incidence of 37.1% (95% confidence interval [CI]: 19.9%-54.3%) after 10 years. Aspirin did not significantly reduce the incidence of thromboembolic events (Pengo et al, 2011). The Antiphospholipid Antibody Acetylsalicylic Acid (APLASA) study randomised individuals with asymptomatic, persistently positive aPL to receive 81mg of aspirin or placebo daily (Erkan et al, 2007). There was no significant difference in outcome between the two arms. On current evidence, thromboprophylaxis with aspirin and/or vitamin K antagonist is not recommended for asymptomatic, aPL positive individuals.
Whether subjects who are triple positive aPL should be treated differently remains to be established.

**Treatment of obstetric APS**

Heparin and low dose aspirin (LDA) is the treatment of choice for RPL associated with APS. This is based on several important studies (Kutteh, 1996; Rai *et al.*, 1997). In a meta-analysis of data from five trials that randomly assigned patients to either heparin and aspirin or aspirin alone for the management of APS-related pregnancies (Mak *et al.*, 2010), the overall live birth rates in 334 patients were 74.27 and 55.85% respectively (RR 1.301; 95% CI 1.040, 1.629). The American College of Chest Physicians (ACCP) guidelines (Bates *et al.*, 2012) recommend treating women with obstetric APS, who meet revised Sapporo criteria with heparin and LDA in the antepartum period as soon as pregnancy is confirmed. LMWH is generally favoured due to a lower incidence of heparin induced thrombocytopenia and low risk of osteopenia compared to unfractionated heparin. The key observation made by Girardi *et al.* in 2004 that heparin can inhibit complement mediated apoptosis provides a possible explanation for its efficacy independent of its anticoagulant activity (Girardi *et al.*, 2004). Heparin may also act by inhibiting aPL binding to trophoblastic cell membranes, modulating trophoblast apoptosis, promoting trophoblast cell invasiveness, and reducing complement activation with the ensuing inflammatory response at the decidual-placental interface (Franklin & Kutteh, 2003).

Women with obstetric APS but no prior history of VTE, Post-partum thromboprophylaxis should be considered offered for 6 weeks following delivery based on risk assessment.
However, there is variation in guidelines as well as clinical practice regarding this. The Nimes Obstetricians and Haematologists Antiphospholipid Syndrome (NOH-APS) observational study reported that the annual rates of deep vein thrombosis (1.46%; range 1.15%-1.82%), pulmonary embolism (0.43%; range 0.26%-0.66%), superficial vein thrombosis (0.44%; range 0.28%-0.68%), and cerebrovascular events (0.32%; range 0.18%-0.53%) were significantly higher in women with pure obstetric APS compared with aPL-negative women with obstetric morbidity, despite low dose aspirin primary prophylaxis (Gris et al, 2012). BCSH (Keeling et al, 2012) and RCOG guidelines are in keeping with this observation. The RCOG Green-top Guideline (https://www.rcog.org.uk/globalassets/documents/guidelines/gtg-37a.pdf), states “Persistent antiphospholipid antibodies (LA and/or anticardiolipin and/or anti-β2-GPI 1 antibodies) in women without previous VTE should be considered as a risk factor for thrombosis such that if she has other risk factors she may be considered for antenatal or postnatal thromboprophylaxis (either 10 day or 6 weeks based on risk assessment). If VTE develops during pregnancy, treatment with therapeutic doses of LMWH should be employed during the remainder of the pregnancy and for at least 6 weeks postnatally and until at least 3 months of treatment has been given in total (https://www.rcog.org.uk/globalassets/documents/guidelines/gtg-37b.pdf). However, women with thrombosis during pregnancy and positive aPL should be reassessed after pregnancy in case long-term anticoagulation is indicated.

With standard treatment, approximately 70% of pregnant women with APS have successful pregnancy outcome (Bramham et al, 2010). There are limited therapeutic options and no guidelines for those who do not respond to heparin and LDA. Addition of low-dose
prednisolone to standard treatment in the first-trimester improved the live birth rate in refractory aPL-related first trimester pregnancy loss (Bramham et al, 2011): nearly two-thirds of pregnancies (61%) resulted in live births, of which 8 (57%) were uncomplicated term pregnancies. However, the frequency of some complications remained elevated, mainly preterm delivery (21%). Data on the usefulness of IVIG in obstetric APS are conflicting (Parke et al, 1989; Branch et al, 2001).

In a mouse model of obstetric APS HCQ prevented fetal death and the placental metabolic changes as measured by proton magnetic resonance spectroscopy (Bertolaccini et al, 2016). In the same study, they showed that HCQ prevented complement activation in vivo and in vitro. Complement C5a levels in serum samples from APS patients and APS-mice were lower after treatment with HCQ while the antibodies titres remained unchanged. HCQ prevented not only placental insufficiency but also abnormal fetal brain development in APS (Bertolaccini et al, 2016). A European multicentre retrospective study of 30 patients with APS and 35 pregnancies showed a better outcome of pregnancies that were treated by the addition of HCQ when compared with previous pregnancies under the conventional treatment (Mekinian et al, 2015). Sciascia et al reported HCQ-treatment was associated with a higher rate of live births (67% vs 57%; P = .05) and a lower prevalence of aPL–related pregnancy morbidity (47% vs 63%; P = .004) (Sciascia et al, 2016).

**Conclusion**

Despite the clear association between aPL and the thrombotic and obstetric manifestations of APS, the exact mechanisms by which aPL cause these problems is yet to be fully
established. However, the activation of complement and endothelial cells by DI specific anti-β2GPI antibodies has emerged as an important component. Incorporation of DI specific anti-β2GPI antibodies into diagnostic panels may allow better identification of those at high risk for clinical events. Related but distinct pathways also link aPL to the complications of pregnancy. Warfarin, heparin, and/or antiplatelet drugs are still the standard of care for APS-thrombosis and although attractive, DOAC can at present only be considered as an alternative in patients with APS and VTE that are usually treated with standard-intensity warfarin when there is known VKA allergy or poor anticoagulant control. The realisation that additional pathways are involved in the pathogenesis of APS has significant implications for treatment. Agents acting outside the coagulation system such as hydroxychloroquine for pregnancy complications and sirolimus as an inhibitor of the mammalian target of rapamycin (mTOR) pathway are now under evaluation and represent a potential radical change in thinking for haematologists.

Authorship

DRJ Arachchilage performed the literature search and wrote the first draft. M Laffan critically reviewed the manuscript and both authors approved the final manuscript.

Disclosure of conflict of interest

Authors state that they have no relevant conflict of interest
References


Table 1: The international consensus (revised Sapporo) criteria for diagnosis of obstetric antiphospholipid syndrome

<table>
<thead>
<tr>
<th>Clinical criteria</th>
<th>Laboratory criteria</th>
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<tr>
<td>1. One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation</td>
<td>1. LA present in plasma, on two or more occasions at least 12 weeks apart</td>
</tr>
<tr>
<td>2. One or more pre-term births of a morphologically normal neonate before the 34th week of gestation because of:</td>
<td>2. aCL of immunoglobulin (Ig)G and/or IgM isotype in serum or plasma, present in medium or high titre (i.e. &gt;40GPL units or MPL units, or &gt; the 99th centile), on two or more occasions, at least 12 weeks apart</td>
</tr>
<tr>
<td>(i) eclampsia or severe pre-eclampsia or</td>
<td>3. aβ2GPI of IgG and/or IgM isotype in serum or plasma (in titre &gt;the 99th centile), present on two or more occasions at least 12 weeks apart</td>
</tr>
<tr>
<td>(ii) recognized features of placental insufficiency</td>
<td></td>
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<tr>
<td>3. Three or more unexplained consecutive spontaneous miscarriages before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded</td>
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</table>

OAPS is diagnosed if at least one of the clinical criteria and one of the laboratory criteria are met

OAPS: Obstetric antiphospholipid syndrome; LA: lupus anticoagulants; aCL: anticardiolipin antibodies; aβ2GPI: antiβ2glycoprotein-I antibodies
Table 2. Mechanisms for aPL-mediated fetal loss or complications

<table>
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<tr>
<th>Mechanism</th>
<th>Supporting evidence from studies in humans or animals/comments</th>
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<tr>
<td>Intraplacental thrombosis</td>
<td>Animal studies have demonstrated that the passive transfer of aPL promotes fetal loss and placental thrombosis and also inhibits trophoblast and decidual cell functions in vitro (Branch et al, 1990) Placental thrombosis and infarction shown in APS patients with intra-uterine fetal death (Out et al, 1991; Nayar &amp; Lage, 1996) aPL binding to monocytes, endothelial cells, platelets and plasma components of the coagulation cascade (Zhang &amp; Mccrae, 2005) may lead to placental thrombosis. aPL might induce a procoagulant state at the placental level by disrupting the anticoagulant annexin A5 shield on trophoblast and endothelial cell monolayers (Rand et al, 1997)</td>
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<tr>
<td>Inflammation</td>
<td>β2GPI-dependent aPL are able to react with human stromal decidual cells in vitro, inducing a proinflammatory phenotype (Zhang &amp; Mccrae, 2005)</td>
</tr>
<tr>
<td>Interference with Annexin V function</td>
<td>Thrombosis during the development of the normal materno-placental circulation may arise via interference with function of Annexin V (Rand et al, 1997; Rand et al, 2010) and women with APS have diminished annexin V binding to the surface of trophoblastic cells of the intervillous space compared with controls. (Rand et al, 1997)</td>
</tr>
<tr>
<td>Inhibition of syncytium-trophoblast differentiation and defective placentation/placental apoptosis</td>
<td>aPL (in particular β2GPI-dependent antibodies) bind to human trophoblasts and affect several cell functions (Agostinis et al, 2011; Girardi, 2003a), inducing cell injury and apoptosis, inhibition of proliferation and syncitia formation, decreased production of human chorionic gonadotrophin, defective secretion of growth factors and impaired invasiveness which lead to defective placentation (Pierangeli et al, 1999).</td>
</tr>
<tr>
<td>Complement activation</td>
<td>Passive transfer of IgG from women with recurrent miscarriage and aPL results in a significant increase in the frequency of fetal resorption and IUGR, compared to mice treated with IgG from healthy individuals [(Holers et al, 2002). These effects can be blocked by inhibition of the complement cascade using a C3 convertase inhibitor or by antibodies or peptides that block C5a-C5a receptor interactions (Girardi et al, 2003; Pierangeli et al, 1999). Mice deficient in complement C3 are resistant to fetal injury induced by aPL (Girardi et al, 2003; Holers et al, 2002). Heparin prevents obstetric complications caused by aPL, because it blocks complement activation rather than through its antithrombotic properties (Girardi et al, 2004).</td>
</tr>
</tbody>
</table>

aPL = antiphospholipid antibodies; IUGR = intrauterine growth restriction
Legends to figures

Figure 1. Key events in the history of Antiphospholipid syndrome (Adapted from figure originally done by Prof. Mike Greaves)
Figure 2. Schematic representation of folded (circular) and unfolded conformation of beta2 glycoprotein I and subsequent binding to anti-beta2 glycoprotein I antibodies (Adapted from van Os et al, 2011)

In the presence of anionic phospholipids, the circular conformation of the protein unfolds, exposing antigenic determinants that are normally shielded from the circulation (A-C) which facilitate the binding to antibodies (D).
Figure 3. Complement pathway activation. aPL induced activation of classical pathway leading to thrombosis and pregnancy complications and potential targets for therapeutic complement inhibition (Adapted from Arachchillage & Hillmen, 2015)

The complement cascade is activated by one of the three pathways. Activation leads to the formation of C3 convertase, resulting in the formation of C5 convertase and membrane attack complex (MAC). aPL recognize domain I of β2GPI and stabilizes its open configuration on the cell surface. This is followed by binding of complement factor C1q resulting in the activation of the complement system. The formed anaphylatoxins cause cell damage and induce a prothrombotic phenotype, leading to both thrombosis and pregnancy failure. Potential therapeutic targets of complement inhibition also shown