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## **Immunotherapeutic strategies in antiphospholipid syndrome**

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## **Abstract**

Antiphospholipid syndrome (APS) is an autoimmune condition, characterized by the persistent presence of antiphospholipid antibodies (aPL) and either thrombosis or obstetric morbidity. The cornerstone of therapy is long-term anticoagulation to reduce morbidity and mortality; however, better understanding of the immunological pathways may direct us to develop future therapeutic strategies. We provide an overview of the current understanding of the immunopathogenesis of this perplexing condition and its associated morbidities and current evidence for some of the immunotherapeutic strategies.

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## Introduction

Antiphospholipid syndrome (APS) is a complex clinicopathological condition, characterised by the persistent presence of antiphospholipid antibodies (aPL) and either thrombosis or obstetric morbidity. The classification criteria have evolved since Hughes' original description<sup>1</sup> of clusters of systemic lupus erythematosus (SLE) patients with recurrent spontaneous abortions, thrombosis and neurological abnormalities associated with certain serological findings, into a revised set of criteria that place importance on the persistence of antiphospholipid antibodies in relation to either thrombotic or pregnancy morbidity (See Table 1).<sup>2</sup>

The levels and types of aPL are important, in the definition of the latest classification criteria in APS patients.<sup>3,4</sup> The threshold levels of aPL have been specified to exclude low titre antibodies. However, there is no standard across different laboratories to define precisely what moderate to high levels of aPL means. Each laboratory can have their own reference ranges for their IgG phospholipid unit (GPL) or IgM phospholipid unit (MPL), and moderate to high levels of aPL are defined as levels greater than the 99<sup>th</sup> percentile of the normal population. Earlier studies examining the magnitude of risk determined by the levels and types of aPL, suggested that lupus anticoagulant is a strong predictor of thrombosis with odds ratio between 4.09 to 16.2 for stroke and deep vein thrombosis.<sup>5</sup> Furthermore the risk significantly magnifies when patients have a

dual or triple positivity to other aPL (eg anti- $\beta$ 2-glycoprotein I and anti-cardiolipin) in addition to their lupus anticoagulant positivity.<sup>6,7</sup> Anti- $\beta$ 2-glycoprotein I antibodies, on their own, are independent predictor for thrombosis, with odds ratio up to 36 times.<sup>{DeCraemer:2016fi}</sup> Beside the different antigenic targets of aPL, isotype variants have also been described, but the non-IgG isotypes do not appear to confer a stronger association with risk of thrombosis.<sup>11</sup>

A distinction has historically been made between “primary” antiphospholipid syndrome and “secondary” antiphospholipid syndrome, which is APS in association with a connective tissue disease (CTD), most commonly SLE. Other associations have been described, for example with chronic viral infections such as HIV or hepatitis C, or with medications such as hydralazine and phenytoin, but these associations often only result in the development of antiphospholipid antibodies without necessarily the clinical syndrome. Considerable overlap occurs with some of the so-called “non-criteria manifestations” of antiphospholipid syndrome and SLE, further adding to the complexity to the syndrome. Some of these manifestations such as thrombocytopenia, immune haemolytic anaemia, livedo reticularis, sterile endocarditis, APS nephropathy, chorea and myelitis to list a few, remain the more challenging aspects in the management of APS.

Catastrophic APS (CAPS) is a rare presentation of APS, and is defined as finding of widespread small vessel occlusion occurring within a short time frame.<sup>12</sup> It is often a complex interplay between a heightened inflammatory state with an activated endothelium and hypercoagulation. Often the histopathology of the end organ reveals intravascular microthrombosis, platelet aggregation, concentric cellular and fibrous intimal hyperplasia.<sup>13</sup> In fact, it shares many common clinicopathological features with other thrombotic microangiopathies such as atypical haemolytic uraemia (aHUS) and thrombotic thrombocytopenic purpura (TTP). The distinction has generally been placed on the finding of markedly elevated aPL, although in some cases it can be difficult to differentiate due to overlapping clinicopathological features.<sup>14</sup> Severe non-immune haemolytic anaemia and thrombocytopenia can be present. Other system involvement includes pulmonary, neurological and renal, as suggested by an European registry of CAPS patients.<sup>14</sup>

### **Immunological basis of APS**

The understanding of the immunopathogenesis of APS has evolved over the years and plays an important part in the future direction of therapeutic development for this condition.<sup>15,16</sup> It began with the description of antiphospholipid antibodies, and recognition that it is indeed a misnomer in the sense that aPL do not target phospholipids, but against a number of plasma

proteins that have affinity for the anionic phospholipids. Among these,  $\beta$ 2-glycoprotein I ( $\beta$ 2GPI) appear to be the common epitope target that mediates pathogenicity.<sup>17</sup> Binding of aPL to  $\beta$ 2GPI results in dimerization of  $\beta$ 2GPI, which increases their affinity to the negatively charged phospholipids and other cell surfaces.<sup>18</sup> Presence of  $\beta$ 2GPI antibodies is strongly associated with thrombosis.<sup>{DEGroot:2005kn}{ZoghiamiRintelen:2005jr}{DeCraemer:2016fi}</sup>

There is ongoing research into the specific component of  $\beta$ 2GPI, for example Domain I, and other non-classical antigenic targets, for example phospholipid-binding plasma protein cofactors (such as prothrombin, protein C, protein S and annexin V), phospholipid-protein complexes (such as vimentin-cardiolipin complexes), and other anionic phospholipids (such as phosphatidylserine, phosphatidylinositol and phosphatidic acid).<sup>19-21</sup> Antibodies to phosphatidylserine-prothrombin complex have been said to be strongly associated with APS and lupus anticoagulant, and it may also be found in some cases of the so-called seronegative APS.<sup>22,23</sup> High titres antibodies to Domain 1 (aD1) identify patients who are traditionally termed triple positive (to lupus anticoagulant, anti-cardiolipin and anti- $\beta$ 2-glycoprotein 1 antibodies) and strongly predict clinical events.<sup>8</sup>

Thrombosis is the end result of a complex interplay between platelets, endothelial cells, monocytes and the coagulation pathways. In APS, it is generally

considered as a diffuse hypercoagulable state, because thrombosis can occur in any vascular bed.<sup>24</sup> Normally tissue factor (TF) becomes exposed where there is injury to the vessel wall, and it serves as a key initiator of the coagulation cascade, required for the activation of factor VIIa that ultimately results in fibrin formation. During an inflammatory state, monocytes and endothelial cells can interact and upregulate expression of TF.<sup>25</sup> In APS, one of the key mechanisms by which aPL promotes thrombosis is via an up-regulation of TF expression and function, demonstrated in the sera of APS patients and their isolated monocytes.

<sup>26,27</sup>

In contrast to tissue factor, annexin A5 is a key anticoagulant protein that normally has a high affinity for the anionic phospholipid bilayer, and forms a protective crystalline shield, preventing activation of the phospholipid-dependent coagulation pathway. The binding of aPL to  $\beta$ 2GPI, disrupts the annexin A5 protective shield, and therefore its thrombomodulatory function. Annexin A5 is also highly expressed on the apical membranes of the placental villous syncytiotrophoblasts and aPL-  $\beta$ 2GPI complexes can disrupt the annexin A5 shield, thereby activating the coagulation pathway.

The binding of aPL-  $\beta$ 2GPI complexes to phospholipid surfaces triggers complement activation via the classical pathway and this is thought to be the main mechanism by which fetal morbidity and mortality results in APS.<sup>28</sup> The

alternate pathway, initiated by activated complement components, also serves as an amplification system. The common final pathway of complement activation is the generation of C3a/C3b, which in turn moves to assemble with C5 convertase to cleave C5 into C5a and C5b. C5a is a potent inflammatory molecule which is a key target in the pathogenesis of obstetric and microangiopathic complications in APS.<sup>29</sup>

C5a contributes to the coagulation cascade by upregulation of neutrophil derived TF expression. In animal models of obstetric APS, C5a is absolutely required for the development of fetal loss. Treatment with non-heparin anticoagulants, such as the indirect anti-factor Xa inhibitor Fondaparinux, have been ineffective because it does not interfere with complement.<sup>30</sup> C5a induced recruitment and activation of neutrophils can lead to trophoblast injury. C5b binds to cell surface and initiates a non-enzymatic assembly of multiple complement proteins called the membrane attack complex (MAC) that can cause direct damage to cell membranes.

Complement regulatory proteins play an important role in counteracting the activation of complement proteins. The best studied ones include Decay Accelerating Factor (DAF/ CD55), Crry, and Factor H, which normally serve to promote resistance to complement attack.  $\beta$ 2GPI is also recognized as a complement regulator, and inhibits complement activation by causing a

conformational change to C3.<sup>31</sup> Embryos deficient in Crry do not survive due to severe placental inflammation from spontaneous complement deposition, suggesting a critical role for complement regulation in fetomaternal tolerance.<sup>32</sup> In one study, expression of DAF/CD55, another complement regulatory protein, was markedly reduced in the endometrial biopsies of aPL positive patients.<sup>33</sup> Mutations of Complement Factor H Related Genes were shown to be associated with aHUS, which can share similar clinicopathological features as the catastrophic form of APS, with widespread microvascular endothelial cell activation, injury and microthrombosis.

The immunological pathways that lead to the overall proinflammatory state in APS have been studied mostly at the level of the endothelial cells and monocytes. The upregulation of tissue factor and other procoagulant molecules are mediated by toll-like receptors and intracellular signaling pathway of p38 mitogen-activated protein kinase and Nuclear factor  $\kappa$ B. Cytokines such as TNF- $\alpha$  and reactive oxygen species can further perpetuate the inflammatory state.<sup>34</sup> More recently the intracellular signaling kinase called mammalian target of rapamycin complex (mTORC) was shown to be upregulated in vascular lesions of APS patients, and together with its upstream pathway involving phosphatidylinositol 3-kinase (PI3k) and AKT it is responsible for cellular cellular and proliferation.<sup>35</sup>

### **Immunotherapeutic strategies**

With this current understanding in the immunopathogenesis of APS, a number of new and existing immunotherapeutic strategies are being investigated to broaden the treatment options for patients with APS. At present, many patients with APS still have significant unmet needs, such as those with refractory disease (either recurrent thrombosis or fetal loss) despite adequate levels of anticoagulation, and others with debilitating non-criteria manifestations that require treatment consideration.

The conventional immunosuppressive approach has limited therapeutic success, but is still used for refractory cases, especially for obstetric complications and in CAPS. Corticosteroids are broad-spectrum immunomodulatory therapy that can inhibit many of the known pathways implicated in APS, however its use is generally reserved for cases of CAPS, or in some cases of non-criteria manifestations. A few other therapeutic strategies are currently under active consideration, specifically targeting the immunomodulatory aspect of the disease pathogenesis of APS.

#### Heparin and low molecular weight heparins

It has long been appreciated that heparin exerts pharmacological effects beyond its anticoagulant effect. Heparin can inhibit complement activation by binding to the anionic phospholipid layer, and blocks C3 cleavage.<sup>30,36</sup> It also plays an important role in promoting trophoblast differentiation at the fetal-placental

interface. Heparin can regulate endometrial decidualization by enhancing protease activity and prevent villous trophoblast cell apoptosis.<sup>37</sup> While the vitamin K antagonist warfarin is an effective treatment in the secondary prevention of thrombosis in APS, treatment with heparin or LMWH may have additional immunomodulatory effects that may be particularly relevant in CAPS, where there is widespread small vessel microthrombosis and thrombotic microangiopathy.<sup>38</sup>

Novel oral anticoagulants such as the direct thrombin inhibitors (eg dabigatran) and direct Xa inhibitors (eg rivaroxaban and apixaban) have been shown to be as effective as warfarin in atrial fibrillation and acute venous thromboembolism, but their efficacy in APS is to be confirmed. Currently a randomized controlled trial called RAPS (Rivaroxaban in AntiPhospholipid Syndrome) is recruiting patients with venous thrombotic APS with or without SLE. There is insufficient experience to evaluate the risks and benefits of direct thrombin inhibitors in pregnancy.

#### Hydroxychloroquine

Hydroxychloroquine (HCQ) has many pharmacological properties including anti-inflammatory, anti-aggregant and immune regulatory effects. In animal studies, HCQ reduces the extent of thrombosis and platelet activation in aPL treated mice. In SLE patients with or without aPL, there have been a number of retrospective

and prospective studies that show a lower prevalence of thrombosis in the cohort treated with HCQ.<sup>39-41</sup> In APS patients, its efficacy is yet to be confirmed with a larger prospective study.<sup>42,43</sup>

Mechanistically HCQ has been shown to reverse the binding of aPL to  $\beta$ 2GPI and restore the annexin A5 shield in human endothelial cells and syncytiotrophoblasts.<sup>44,45</sup> HCQ can also modulate production of proinflammatory cytokines in response to toll like receptor stimulation. The effect of HCQ on the titre of aPL antibodies has not been consistently demonstrated.<sup>46</sup>

### Rituximab

There have been a few case series of rituximab use in APS, with variable efficacy reported. Rituximab (RTX) treatment appears to reduce the titre of aPL which theoretically should reduce thrombotic risk.<sup>47,48</sup> An open label study (Rituximab in Antiphospholipid Syndrome "RITAP") examined the role of rituximab for non-criteria manifestations.<sup>49</sup> Many of the non-criteria manifestations are thought to be most likely immune-mediated, such as the presence of thrombocytopenia, autoimmune haemolytic anaemia, chorea, and myelitis. In an open label study, it was indeed these manifestations that appear most likely to respond to rituximab.<sup>49,50</sup> Furthermore, RTX has also been used in the setting of catastrophic APS with some promising results.<sup>51</sup>

### Eculizumab

The development of eculizumab, a recombinant, fully humanized monoclonal antibody against C5, has substantially improved the high mortality associated with rare diseases such as atypical haemolytic uraemic syndrome, paroxysmal nocturnal haemoglobinuria and C3 glomerulonephropathy.<sup>52,53</sup> There have been a few cases of the use of eculizumab in refractory catastrophic APS, based on experience with complement inhibition in animal models.<sup>54,55</sup> The binding of eculizumab to C5 prevents its cleavage and thereby reduce the production of terminal complement components C5a and membrane attack complex (MAC) C5b-9. The primary organ of effect is at the kidneys where complement mediated endothelial damage and thrombosis, and hence renal injury, is prevented. The optimal duration of treatment is unknown, and monitoring for complement blockade is not straight forward.<sup>56,57</sup> Generally markers of active haemolysis can be used as a surrogate to assess for any ongoing thrombotic microangiopathy.

Other strategies for complement inhibition have been considered in APS, especially in murine models. C5a Receptor antagonist peptides have shown promising results.<sup>58</sup>

### Plasma therapy

In catastrophic APS, plasmapheresis has been advocated as one of modalities to rapidly remove pathogenic autoantibodies. This additionally can help with

management of volume overload and hypertension in patients with acute renal failure. Plasma infusion, in the form of intravenous immunoglobulins, has been used but the reports are largely anecdotal. One controlled study reported benefit in obstetric complications in comparison with prednisolone, when given in addition to low dose aspirin.<sup>59</sup> However, lessons from case series of atypical HUS have shown that more patients respond to eculizumab than plasma therapy.

### Other therapies

Targeted immunosuppressive therapy, such as TNF- $\alpha$  inhibitors have been used in patients with recurrent miscarriages but not specifically in APS patients.<sup>60</sup> Statins, like heparin and hydroxychloroquine, have a number of pleiotropic effects on the immune system. In animal models of APS, statins were shown to abrogate the upregulation of TF on endothelial cells.<sup>61</sup> Some interest has developed around methods to inhibit TF, but currently this is mainly done indirectly. Drugs such as pentoxifylline or dipyridamole are phosphodiesterase inhibitors that have broad-spectrum immunomodulatory actions, and can be considered in refractory cases.<sup>62</sup>

### **Conclusion**

Long-term anticoagulation has been the mainstay of treatment for antiphospholipid syndrome, but there remains many challenging situations in the management of this disease. There are limited other options for those who

may have refractory disease or developed complications to conventional anticoagulation, or for those affected by non-criteria manifestations that traditionally have not always responded to treatment with anticoagulation. Improved understanding of the pathogenesis allows us to consider a number of immunomodulatory pathways that may potentially be amenable to treatment. Of these, B cell and complement targeted therapy have some early promising results and good quality controlled studies are on the horizon in the attempt to answer more questions.

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Table 1 Classification criteria of antiphospholipid syndrome

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*Clinical criteria*

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Vascular thrombosis

- One or more clinical episodes of arterial, venous or small vessel thrombosis in any tissue or organ
- Thrombosis confirmed by unequivocal findings on appropriate imaging or histopathology
- Histopathological confirmation requires presence of thrombosis without significant evidence of inflammation
- Superficial venous thrombosis does not satisfy the criteria for thrombosis for APS

Pregnancy morbidity

- One or more unexplained deaths of morphologically normal foetus at or beyond 10 weeks gestation, or
- One or more premature births of a morphologically normal neonate before 34<sup>th</sup> week of gestation because of pre-eclampsia, eclampsia, or placental insufficiency, or
- Three or more unexplained consecutive spontaneous abortions before the 10<sup>th</sup> week of gestation with other causes excluded

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*Laboratory criteria*

Laboratory testing must be confirmed on two or more occasions at least 12 weeks apart and no more than 5 years from clinical event

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- Lupus anticoagulant (LA) present in plasma
  - Anticardiolipin (aCL) antibody of IgG and/or IgM isotype, greater than 99<sup>th</sup> percentile for the testing laboratory
  - Anti- $\beta_2$  glycoprotein-I antibody of IgG and/or IgM isotype, greater than 99<sup>th</sup> percentile for the testing laboratory
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Table 1. Revised classification criteria for definite APS requires the combination of at least one clinical and one laboratory feature. Greater emphasis has been placed on the persistent positivity of laboratory tests in this revised version, compared to the old Sapporo criteria.

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