Immunotherapeutic strategies in antiphospholipid syndrome

Authors:

Alberta Y Hoi[1,2,3], Laura Ross[1], Jessica Day[1], Russell RC Buchanan[1]

1. Department of Rheumatology, Austin Health, Melbourne Australia
2. Department of Rheumatology, Monash Health, Melbourne Australia
3. School of Clinical Sciences, Monash University

Correspondence:

Dr Alberta Y Hoi, MBBS, FRACP, PhD

alberta.hoi@austin.org.au

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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.

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[1] 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).[2]

The levels and types of aPL are important, in the definition of the latest classification criteria in APS patients.[3,4] 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 99th 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.[5] Furthermore the risk significantly magnifies when patients have a

dual or triple positivity to other aPL (eg anti-β2-glycoprotein I and anti-cardiolipin) in addition to their lupus anticoagulant positivity.[6,7] Anti-β2-glycoprotein I antibodies, on their own, are independent predictor for thrombosis, with odds ratio up to 36 times.{DeCraemer:2016fi} 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.[11]

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.[12] 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.[13] 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.[14] 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.[14]

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.[15,16] 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, β2-glycoprotein I (β2GPI) appear to be the common epitope target that mediates pathogenicity.[17] Binding of aPL to β2GPI results in dimerization of β2GPI, which increases their affinity to the negatively charged phospholipids and other cell surfaces.[18] Presence of β2GPI antibodies is strongly associated with thrombosis.{DEGroot:2005kn}{ZoghlamiRintelen:2005jr}{DeCraemer:2016fi}

There is ongoing research into the specific component of β2GPI, 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).[19-21] 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. [22,23] High titres antibodies to Domain 1 (aD1) identify patients who are traditionally termed triple positive (to lupus anticoagulant, anti-cardiolipin and anti-β2-glycoprotein 1 antibodies) and strongly predict clinical events.[8]

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.[24] 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.[25] 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.[26,27]

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 β2GPI, 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- β2GPI complexes can disrupt the annexin A5 shield, thereby activating the coagulation pathway.

The binding of aPL- β2GPI 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.[28] 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.[29]

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 Fondaprinux, have been ineffective because it does not interfere with complement.[30] 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. β2GPI is also recognized as a complement regulator, and inhibits complement activation by causing a

conformational change to C3.[31] 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.[32] In one study, expression of DAF/CD55, another complement regulatory protein, was markedly reduced in the endometrial biopsies of aPL positive patients.[33] 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 κB. Cytokines such as TNF-α and reactive oxygen species can further perpetuate the inflammatory state.[34] 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.[35]

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. [30,36] 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.[37] 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.[38]

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. [39-41] In APS patients, its efficacy is yet to be confirmed with a larger prospective study. [42,43]

Mechanistically HCQ has been shown to reverse the binding of aPL to β2GPI and restore the annexin A5 shield in human endothelial cells and syncytiotrophoblasts.[44,45] 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. [46]

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. [47,48] An open label study (Rituximab in Antiphospholipid Syndrome "RITAP") examined the role of rituximab for non-criteria manifestations.[49] 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.[49,50] Furthermore, RTX has also been used in the setting of catastrophic APS with some promising results.[51]

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 haemoglobulinuria and C3 glomerulonephropathy.[52,53] There have been a few cases of the use of eculizumab in refractory catastrophic APS, based on experience with complement inhibition in animal models. [54,55] 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.[56,57] 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.[58]

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.[59] 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-α inhibitors have been used in patients with recurrent miscarriages but not specifically in APS patients.[60] 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.[61] 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.[62]

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.

Table 1 Classification criteria of antiphospholipid syndrome
Clinical criteria
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 34th week of gestation because of pre-eclampsia, eclampsia, or placental insufficiency, or • Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation with other causes excluded
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
· Lupus anticoagulant (LA) present in plasma · Anticardiolipin (aCL) antibody of IgG and/or IgM isotype, greater than 99th percentile for the testing laboratory · Anti-β_2 glycoprotein-I antibody of IgG and/or IgM isotype, greater than 99th percentile for the testing laboratory

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.

References

1. Hughes GRV. Hughes syndrome/APS. 30 years on, what have we learnt? Opening talk at the 14th International Congress on antiphospholipid antibodies Rio de Janiero, October 2013. lupus. 2014 Apr;23(4):400–6.

2. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). 2006. pp. 295–306.

3. Ruiz-Irastorza G, Hunt BJ, Khamashta MA. A systematic review of secondary thromboprophylaxis in patients with antiphospholipid antibodies. Arthritis & Rheumatism. 2007;57(8):1487–95.

4. Pengo V, Banzato A, Denas G, Jose SP, Bison E, Hoxha A, et al. Correct laboratory approach to APS diagnosis and monitoring. Autoimmunity Reviews. 2013 Jun;12(8):832–4.

5. Galli M, Luciani D, Bertolini G, Barbui T. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. Blood. American Society of Hematology; 2003 Mar 1;101(5):1827–32.

6. Zoghlami-Rintelen C, Vormittag R, Sailer T, Lehr S, Quehenberger P, Rumpold H, et al. The presence of IgG antibodies against beta2-glycoprotein I predicts the risk of thrombosis in patients with the lupus anticoagulant. J Thromb Haemost. 2005 Jun;3(6):1160–5.

7. DE Groot PG, Lutters B, Derksen RHWM, Lisman T, Meijers JCM, Rosendaal FR. Lupus anticoagulants and the risk of a first episode of deep venous thrombosis. J Thromb Haemost. Blackwell Publishing Ltd; 2005 Sep;3(9):1993–7.

8. De Craemer A-S, Musial J, Devreese KMJ. Role of anti-domain 1-β2 glycoprotein I antibodies in the diagnosis and risk stratification of antiphospholipid syndrome. J Thromb Haemost. 2016 Jun 17.

9. Galli M, Comfurius P, Maassen C, Hemker HC, de Baets MH, van Breda-Vriesman PJ, et al. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. The Lancet. 1990 Jun 30;335(8705):1544–7.

10. de Laat HB, Derksen RHWM, Urbanus RT, Roest M, de Groot PG. beta2-glycoprotein I-dependent lupus anticoagulant highly correlates with thrombosis in the antiphospholipid syndrome. Blood. 2004 Dec 1;104(12):3598–602.

11. Galli M, Luciani D, Bertolini G, Barbui T. Anti-beta 2-glycoprotein I, antiprothrombin antibodies, and the risk of thrombosis in the antiphospholipid syndrome. Blood. 2003 Oct 15;102(8):2717–23.

12. Erkan D, Espinosa G, Cervera R. Catastrophic antiphospholipid syndrome: updated diagnostic algorithms. Autoimmunity Reviews. 2010 Dec;10(2):74–9.

13. Reyes E, Alarcón-Segovia D. Pathology of the Antiphospholipid Syndrome. In: The Antiphospholipid Syndrome II. Elsevier; 2002. pp. 131–6.

14. Cervera R, CAPS Registry Project Group. Catastrophic antiphospholipid syndrome (CAPS): update from the 'CAPS Registry'. lupus. 2010 Apr;19(4):412–8.

15. Matsuura E, SHEN L, Matsunami Y, Quan N, Makarova M, Geske FJ, et al. Pathophysiology of beta2-glycoprotein I in antiphospholipid syndrome. lupus. 2010 Apr;19(4):379–84.

16. Wijetilleka S, Scoble T, Khamashta M. Novel insights into pathogenesis, diagnosis and treatment of antiphospholipid syndrome. Current Opinion in Rheumatology. 2012 Sep;24(5):473–81.

17. McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta 2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci USA. National Academy of Sciences; 1990 Jun;87(11):4120–4.

18. Lutters BC, Meijers JC, Derksen RH, Arnout J, DE Groot PG. Dimers of beta 2-glycoprotein I mimic the in vitro effects of beta 2-glycoprotein I-anti-beta 2-glycoprotein I antibody complexes. J Biol Chem. 2001 Feb 2;276(5):3060–7.

19. Iverson GM, Victoria EJ, Marquis DM. Anti-beta2 glycoprotein I (beta2GPI) autoantibodies recognize an epitope on the first domain of beta2GPI. Proc Natl Acad Sci USA. 1998 Dec 22;95(26):15542–6.

20. de Laat B, de Groot PG. Autoantibodies directed against domain I of beta2-glycoprotein I. Curr Rheumatol Rep. Current Science Inc; 2011 Feb;13(1):70–6.

21. Bertolaccini ML, Amengual O, Andreoli L, Atsumi T, Chighizola CB, Forastiero R, et al. 14th International Congress on Antiphospholipid Antibodies Task Force. Report on antiphospholipid syndrome laboratory diagnostics and trends. Autoimmunity Reviews. 2014 Sep;13(9):917–30.

22. Atsumi T, Ieko M, Bertolaccini ML, Ichikawa K, Tsutsumi A, Matsuura E, et al. Association of autoantibodies against the phosphatidylserine–prothrombin complex with manifestations of the antiphospholipid syndrome and with the presence of lupus anticoagulant. Arthritis & Rheumatism. John Wiley & Sons, Inc; 2000 Sep;43(9):1982–93.

23. Sanfelippo MJ, Joshi A, Schwartz S, Meister JA, Goldberg JW. Antibodies to phosphatidylserine/prothrombin complex in suspected antiphospholipid syndrome in the absence of antibodies to cardiolipin or Beta-2-glycoprotein I. lupus. SAGE Publications; 2013 Nov;22(13):1349–52.

24. Arnout J, Vermylen J. Current status and implications of autoimmune antiphospholipid antibodies in relation to thrombotic disease. J Thromb Haemost. 2003 May;1(5):931–42.

25. Napoleone E, Di Santo A, Lorenzet R. Monocytes upregulate endothelial cell expression of tissue factor: a role for cell-cell contact and cross-talk. Blood. 1997 Jan 15;89(2):541–9.

26. Amengual O, Atsumi T, Khamashta MA, Hughes GR. The role of the tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid syndrome. Thromb Haemost. 1998 Feb;79(2):276–81.

27. Dobado-Berrios PM, López-Pedrera C, Velasco F, Aguirre MA, Torres A, Cuadrado MJ. Increased levels of tissue factor mRNA in mononuclear blood cells of patients with primary antiphospholipid syndrome. Thromb Haemost. 1999 Dec;82(6):1578–82.

28. Samarkos M, Mylona E, Kapsimali V. The role of complement in the antiphospholipid syndrome: a novel mechanism for pregnancy morbidity. Seminars in Arthritis and Rheumatism. 2012 Aug;42(1):66–9.

29. Lim W. Complement and the antiphospholipid syndrome. Curr Opin Hematol. 2011 Sep;18(5):361–5.

30. Girardi G, Redecha P, Salmon JE. Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. Nat Med. 2004 Nov;10(11):1222–6.

31. Gropp K, Weber N, Reuter M, Micklisch S, Kopka I, Hallström T, et al. β_2-glycoprotein I, the major target in antiphospholipid syndrome, is a special human complement regulator. Blood. 2011 Sep 8;118(10):2774–83.

32. Xu C, Mao D, Holers VM, Palanca B, Cheng AM, Molina H. A critical role for murine complement regulator crry in fetomaternal tolerance. Science. 2000 Jan 21;287(5452):498–501.

33. Francis J, Rai R, Sebire NJ, El-Gaddal S, Fernandes MS, Jindal P, et al. Impaired expression of endometrial differentiation markers and complement regulatory proteins in patients with recurrent pregnancy loss associated with antiphospholipid syndrome. Mol Hum Reprod. Oxford University Press; 2006 Jul;12(7):435–42.

34. Giannakopoulos B, Krilis SA. The Pathogenesis of the Antiphospholipid Syndrome. New England Journal of Medicine. 2013 Mar 14;368(11):1033–44.

35. Canaud G, Bienaimé F, Tabarin F, Bataillon G, Seilhean D, Noël L-H, et al. Inhibition of the mTORC pathway in the antiphospholipid syndrome. N Engl J Med. 2014 Jul 24;371(4):303–12.

36. Weiler JM, Edens RE, Linhardt RJ, Kapelanski DP. Heparin and modified heparin inhibit complement activation in vivo. J Immunol. 1992 May 15;148(10):3210–5.

37. Hills FA, Abrahams VM, González-Timón B, Francis J, Cloke B, Hinkson L, et al. Heparin prevents programmed cell death in human trophoblast. Mol Hum Reprod. 2006 Apr;12(4):237–43.

38. Cervera R, Espinosa G. Update on the catastrophic antiphospholipid syndrome and the "CAPS Registry". Semin Thromb Hemost. 2012 Jun;38(4):333–8.

39. Tektonidou MG, Laskari K, Panagiotakos DB, Moutsopoulos HM. Risk factors for thrombosis and primary thrombosis prevention in patients with systemic lupus erythematosus with or without antiphospholipid antibodies. Arthritis & Rheumatism. 2008 Dec 30;61(1):29–36.

40. Kaiser R, Cleveland CM, Criswell LA. Risk and protective factors for thrombosis in systemic lupus erythematosus: results from a large, multi-ethnic cohort. Annals of the Rheumatic Diseases. 2009 Feb;68(2):238–41.

41. Jung H, Bobba R, Su J, Shariati-Sarabi Z, Gladman DD, Urowitz M, et al. The protective effect of antimalarial drugs on thrombovascular events in systemic lupus erythematosus. Arthritis & Rheumatism. 2010 Mar;62(3):863–8.

42. Erkan D, Yazici Y, Peterson MG, Sammaritano L, Lockshin MD. A cross-sectional study of clinical thrombotic risk factors and preventive treatments in antiphospholipid syndrome. Rheumatology (Oxford). 2002 Aug;41(8):924–9.

43. Schmidt-Tanguy A, Voswinkel J, Henrion D, Subra JF, Loufrani L, Rohmer V, et al. Anti-thrombotic effects of hydroxychloroquine in primary antiphospholipid syndrome patients. J Thromb Haemost. 2013 Aug;:n/a–n/a.

44. Rand JH, Wu XX, Quinn AS, Ashton AW, Chen PP, Hathcock JJ, et al. Hydroxychloroquine protects the annexin A5 anticoagulant shield from disruption by antiphospholipid antibodies: evidence for a novel effect for an old antimalarial drug. Blood. 2010 Mar 18;115(11):2292–9.

45. Wu X-X, Guller S, Rand JH. Hydroxychloroquine reduces binding of antiphospholipid antibodies to syncytiotrophoblasts and restores annexin A5 expression. American Journal of Obstetrics and Gynecology. 2011 Dec;205(6):576.e7–576.e14.

46. Erkan D, Derksen WJM, Kaplan V, Sammaritano L, Pierangeli SS, Roubey R, et al. Real world experience with antiphospholipid antibody tests: how stable are results over time? Annals of the Rheumatic Diseases. 2005 Sep;64(9):1321–5.

47. Erre GL, Pardini S, Faedda R, Passiu G. Effect of rituximab on clinical and laboratory features of antiphospholipid syndrome: a case report and a review of literature. lupus. 2008 Jan;17(1):50–5.

48. Erkan D, Aguiar CL, Andrade D, Cohen H, Cuadrado MJ, Danowski A, et al. 14th International Congress on Antiphospholipid Antibodies: task force report on antiphospholipid syndrome treatment trends. Autoimmunity Reviews. 2014 Jun;13(6):685–96.

49. Erkan D, Vega J, Ramón G, Kozora E, Lockshin MD. A pilot open-label phase II trial of rituximab for non-criteria manifestations of antiphospholipid syndrome. Arthritis & Rheumatism. 2013 Feb;65(2):464–71.

50. Comellas-Kirkerup L, Hernández-Molina G, Cabral AR. Antiphospholipid-associated thrombocytopenia or autoimmune hemolytic anemia in patients with or without definite primary antiphospholipid syndrome according to the Sapporo revised classification criteria: a 6-year follow-up study. Blood. 2010 Oct 21;116(16):3058–63.

51. Berman H, Rodríguez-Pintó I, Cervera R, Morel N, Costedoat-Chalumeau N, Erkan D, et al. Rituximab use in the catastrophic antiphospholipid syndrome: descriptive analysis of the CAPS registry patients receiving rituximab. Autoimmunity Reviews. 2013 Sep;12(11):1085–90.

52. Zuber J, Fakhouri F, Roumenina LT, Loirat C, Frémeaux-Bacchi V. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. Nat Rev Nephrol. Nature Publishing Group; 2012 Oct 2;8(11):643–57.

53. Rathbone J, Kaltenthaler E, Richards A, Tappenden P, Bessey A, Cantrell A. A systematic review of eculizumab for atypical haemolytic uraemic syndrome (aHUS). BMJ Open. 2013;3(11):e003573–3.

54. Zapantis E, Furie R, Horowitz D. Eculizumab in recalcitrant antiphospholipid antibody syndrome. Arthritis Res Ther. BioMed Central Ltd; 2014 Sep 17;16(Suppl 1):A50.

55. Zikos TA, Sokolove J, Ahuja N, Berube C. Eculizumab Induces Sustained Remission in a Patient With Refractory Primary Catastrophic Antiphospholipid Syndrome. J Clin Rheumatol. 2015 Sep;21(6):311–3.

56. Chatelet V, Frémeaux-Bacchi V, Lobbedez T, Ficheux M, Hurault de Ligny B. Safety and long-term efficacy of eculizumab in a renal transplant patient with recurrent atypical hemolytic-uremic syndrome. Am J Transplant. Blackwell Publishing Inc; 2009 Nov;9(11):2644–5.

57. Le Quintrec M, Zuber J, Moulin B, Kamar N, Jablonski M, Lionet A, et al. Complement genes strongly predict recurrence and graft outcome in adult renal transplant recipients with atypical hemolytic and uremic syndrome. Am J Transplant. 2013 Mar;13(3):663–75.

58. Girardi G, Berman J, Redecha P, Spruce L, Thurman JM, Kraus D, et al. Complement C5a receptors and neutrophils mediate fetal injury in the

antiphospholipid syndrome. J Clin Invest. American Society for Clinical Investigation; 2003 Dec;112(11):1644–54.

59. Vaquero E, Lazzarin N, Valensise H, Menghini S, Di Pierro G, Cesa F, et al. Pregnancy outcome in recurrent spontaneous abortion associated with antiphospholipid antibodies: a comparative study of intravenous immunoglobulin versus prednisone plus low-dose aspirin. Am J Reprod Immunol. 2001 Mar;45(3):174–9.

60. Alijotas-Reig J. Treatment of refractory obstetric antiphospholipid syndrome: the state of the art and new trends in the therapeutic management. lupus. 2013 Jan;22(1):6–17.

61. Ferrara DE, Swerlick R, Casper K, Meroni PL, Vega-Ostertag ME, Harris EN, et al. Fluvastatin inhibits up-regulation of tissue factor expression by antiphospholipid antibodies on endothelial cells. J Thromb Haemost. Blackwell Science Inc; 2004 Sep;2(9):1558–63.

62. Pierangeli SS, Erkan D. Antiphospholipid syndrome treatment beyond anticoagulation: are we there yet? - ProQuest. lupus [Internet]. 2010 Apr;19(4):475–85. Available from: http://search.proquest.com.ezproxy.lib.monash.edu.au/docview/222633234/fulltextPDF/201CC783F87240E8PQ/2?accountid=12528