## Literature review

# Immunothrombosis

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### Introduction

Coagulation and innate immunity are linked processes that orchestrate a host's defense against invading pathogens and injury. The complement system is integral to innate immunity and shares numerous interactions with components of the hemostatic pathway, helping to maintain physiological equilibrium. The term 'immunothrombosis' was introduced to embrace this process, and has become an area of much recent interest.[1] Immunothrombosis designates an innate immune response induced by the formation of thrombi inside blood vessels, particularly in microvessels. The response is supported by immune cells and by specific thrombosis-related molecules and generates an intravascular scaffold that facilitates the recognition, containment and destruction of pathogens; thereby, protecting host integrity without inducing major collateral damage to the host. However, if uncontrolled, it constitutes a major biological process fostering pathologies associated with thrombosis.

The clinical manifestations of the coagulation-complement interaction and the consequences of dysregulation of either system is increasingly appreciated, as seen in many inflammatory and thrombotic disease states, such as sepsis, trauma, atherosclerosis, antiphospholipid syndrome (APS), paroxysmal nocturnal hemoglobinuria (PNH) and some thrombotic microangiopathies.[2,3] This review aims to explore the interactions between hemostatic and complement systems and their potential clinical impact.

### Innate immunity

The innate immune system is the host's immediate nonspecific defense against invading pathogens and infections. These range from physical barriers, such as the skin and mucous membranes, to macrophages, immunoglobulins and varied cellular and cytokine responses. An important constituent of the innate immune defense is the complement system. This process opsonizes bacteria and danger-associated molecular patterns (DAMPs) of invading pathogens by antibodies and soluble plasma proteins, facilitating enhanced pathogen killing by host cellular responses.[4]

The complement system, consisting of over 50 plasma proteins involved in host defense, is organized in three pathways: the immune complex mediated classical pathway, the lectin pathway and the alternative pathway. These pathways converge at the level of complement component C3 and the terminal complement pathway that leads to generating C5a, a potent pro-inflammatory molecule and C5b-9, the membrane attack complex or MAC (Figure 1). To highlight the close interaction between innate immunity and coagulation, a fourth pathway has also been described, where thrombin cleaves and activates C5 to C5a independently of C3 convertase.[5,6]

Key properties of immunothrombosis involve cell-specific prothrombotic pathways inherent to innate immune cells operating within intact vessels to protect the host from nonself pathogens or antigens. In response to certain agonists, monocytes and their microvesicles express activated intravascular tissue factor that then initiates the extrinsic pathway of coagulation. Another important aspect of immunothrombosis is the increasingly recognized entity of neutrophil extracellular traps (NETs). These are complexes of neutrophil DNA and histones causing a multitude of immunothrombotic sequelae. NETs can directly activate FXII of the contact pathway of coagulation as well as tissue factor, to initiate

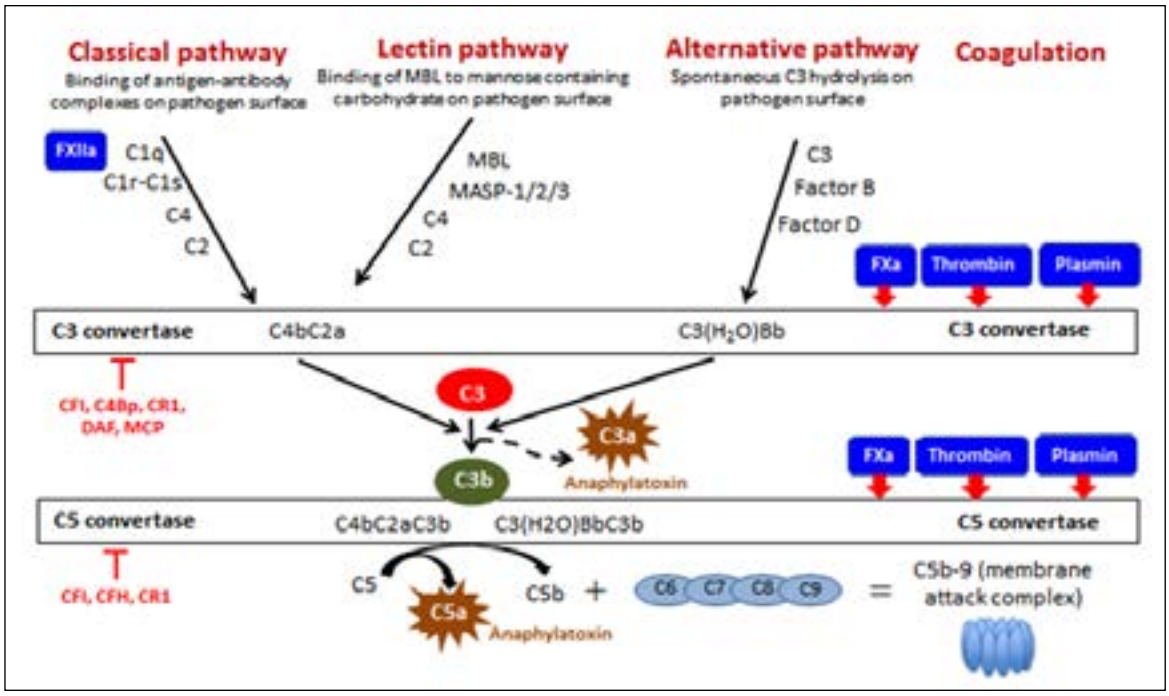


**Figure 1** The complement cascade, its activators and regulatory factors. The complement system can be activated via three traditionally recognized pathways; the classical, lectin and alternative. The classical pathway is initiated by the binding of antigen: antibody complexes on pathogen surfaces, the lectin pathway by the binding of mannose binding lectin (MBL) to mannose containing carbohydrates on the surface of bacteria or viruses, and the constitutively active alternative pathway is initiated when a spontaneously activated complement component binds to the pathogen surface. Each pathway converges to form C3 convertases (i.e. C4bC2a, C3(H2O)Bb) that cleave C3 and subsequently cause C5 cleavage. This process also generates the potent anaphylatoxins C3a and C5a. C5b combines with C6-C9 to produce membrane attack complexes, creating transmembrane channels within infected cellular membranes leading to cell lysis and death. Certain coagulation factors (factor Xa, thrombin, plasmin) also have C3 and C5 convertase activity, contributing to an additional pathway of complement activation. Regulatory factors including decay accelerating factor (DAF, CD55), CD59, factor H (CFH), factor I (CFI), membrane cofactor protein (MCP) and C3b/C4b receptor 1 (CR1) act at various stages of the cascade to control complement activation. FXa, activated factor X; FXIIa, activated factor XII; MASP-1/2/3, mannose-associated serine protease 1/2/3. (Modified from Keragala CB, et al. Br J Haematol. 2018;180:782-98)

the extrinsic coagulation cascade. NETs also bind to von Willebrand factor (VWF), a long multimeric glycoprotein produced and secreted by the Weibel-Palade bodies in endothelial cells, platelets and subendothelial connective tissue, serving to ensnare platelets leading to their aggregation and platelet plug formation. Specific histones present in NETs, namely, H3 and H4, not only directly trigger platelet activation, but also behave as DAMPs, activating complement pathways.[7,8] NETs can also locally concentrate enzymes, such as neutrophil elastase, cathepsin G and myeloperoxidase, which not only promote intravascular thrombus growth but can also cleave endogenous anticoagulants, tissue factor pathway inhibitor and thrombomodulin, thus resulting in a hypercoagulable state (Figure 2).[9-11]

**Effects of coagulation on complement activation and regulation**

The role of the complement system in coagulation, immunity and immunothrombosis is becoming increasingly appreciated, with an expanding body of literature exploring the links between coagulation and complement

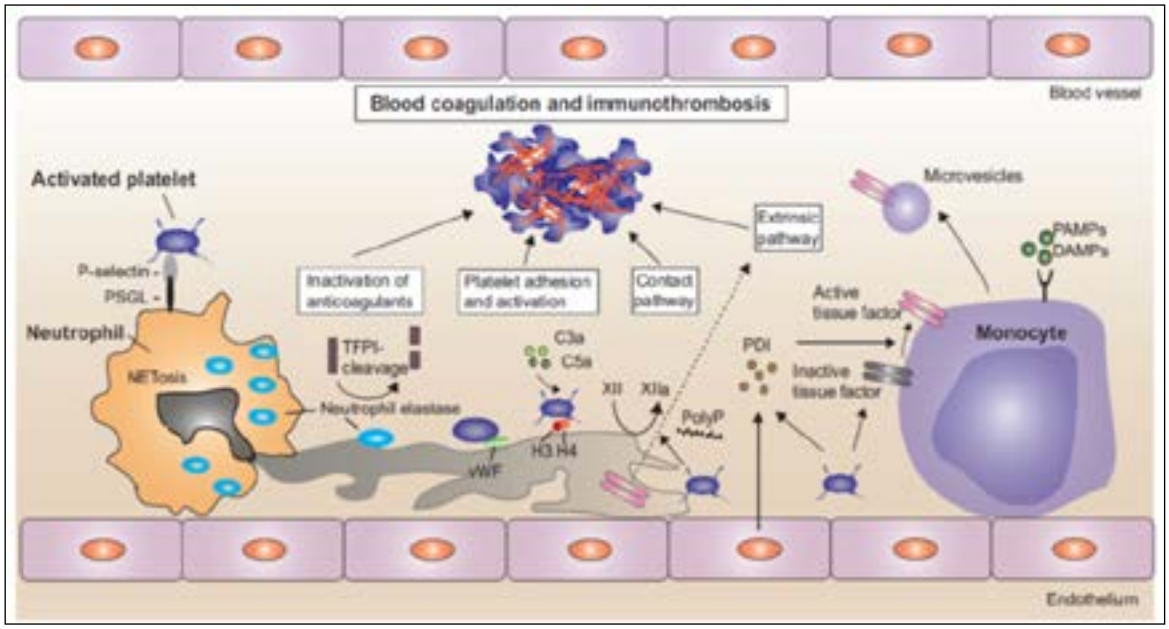


**Figure 2** Platelets, neutrophils and monocytes cooperatively trigger blood coagulation and immunothrombosis in intact blood vessels during the innate immune response. Monocytes are activated by pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) and initiate the extrinsic pathway of coagulation by expressing activated tissue factor and releasing tissue factor bearing microvesicles. Activated neutrophils expel neutrophil extracellular traps (NETs), lattices consisting of DNA and histones that support blood coagulation by several mechanisms. NETs bind activated tissue factor thereby localizing the extrinsic pathway to sites of infection and inflammation. NETs also provide a surface for factor XII activation; thereby, initiating the contact pathway of coagulation. In addition, coagulation is indirectly fueled by neutrophil enzymes that becomes immobilized and concentrated on NETs. Accordingly, neutrophil elastase cleaves natural anticoagulants, including tissue factor pathway inhibitor (TFPI); thereby, further propagating coagulation. NET-bound VWF and histones H3, H4 foster the recruitment and activation of platelets. Platelets in turn facilitate NETosis by interacting with neutrophils via P-selectin and P-selectin glycoprotein ligand (PSGL). Platelets have additional supportive functions during immunothrombosis. Correspondingly, platelet-secreted polyphosphates (polyP) activate factor XIIa and initiate the contact phase of coagulation. In addition, platelet- and endothelial cell-derived protein disulphide isomerase (PDI) likely supports the activation of leukocyte- and microvesicle-derived intravascular tissue factor. The complement system (including C3a and C5a) also supports immunothrombosis, for example, by triggering platelet activation.[11]

activation. A number of thrombosis-related potential complement activators have been studied, including thrombin, plasmin, damaged endothelium, DNA and neutrophil elastase.[12,13] Thrombin directly activates C3 and C5, independently of C3 convertase; thus, introducing the concept of an additional pathway of complement activation.[14] However, thrombin's cleavage of C5 appears suboptimal compared with its robustness in cleaving its classical coagulation substrates, i.e., fibrinogen, factors V, VIII, etc. This is evident in the supraphysiological concentrations of thrombin often used in these studies to achieve detectable amounts of complement cleavage products.[5,15] In addition to thrombin, activated factors XI (FXIa), X (FXa), IX (FIXa) and plasmin have also been found to effectively cleave C3 and C5 to its active components in in vitro and in vivo murine models. FXa and plasmin were identified as more potent drivers of complement cleavage in this context.[5,13]

The complement cleavage products following activation by coagulation factors have also been shown to exhibit robust chemo-attractive properties to human mast cells and neutrophils, highlighting the pro-inflammatory effects of complement cleavage products despite the different methods of activation. Interestingly, one study found that FXa-induced cleavage of C3 was significantly suppressed in the presence of a selective FXa inhibitor, fondaparinux and enoxaparin, in a concentration-dependent manner, highlighting possible anti-inflammatory effects of such agents in these settings.[5]

Factor XII is also recognized as playing an important role in immunothrombosis. Although traditionally perceived as a dispensable coagulation protein, activated factor XII (FXIIa) initiates several pro-inflammatory effects via contact pathway activation, showcasing another unique link between coagulation and inflammation. Some elements known to activate factor XII contact pathway include mast cell-derived heparin, misfolded proteins and collagen and nucleic acids (RNA and DNA).[16] The role of platelet polyphosphates potentially activating the factor XII contact pathway has recently gained significant attention.[17-19] Contact pathway activation then contributes to inflammation by activating the bradykinin-producing kallikrein-kinin system.

Several notable links have been identified between the contact pathway and complement, including kallikrein, gC1qR protein and C1 esterase inhibitor (C1-INH). FXIIa causes conversion of prekallikrein to kallikrein, which in turn drives further factor XII activation and also cleaves high molecular weight kininogen (HMWK), generating bradykinin. Kallikrein is also involved in the cleavage of several complement proteins including C3, C5 and factor B.[20] The multi-ligand binding protein gC1qR was first identified as a cell surface receptor for C1q, and also found to be a major high affinity receptor for HMWK and factor XII.[21] The ability of gC1qR to directly activate the classical complement pathway by binding C1q and also the kallikrein-kinin-bradykinin system by binding HMWK and FXII, enables it to effectively drive thrombo-inflammation and generate vasoactive molecules. The two binding ligands of gC1qR (HMWK and C1q) are co-localized on activated endothelial cells and inflammatory tissue, thus showcasing the potential inflammatory and thrombogenic properties of several disease states including atherosclerosis, malignancy and angioedema.[22] In contrast, C1-INH is a potent inhibitor of the classical complement pathway and a major regulator of the kinin system, neutralizing the effects of FXIIa, FXIa, kallikrein and HMWK-prekallikrein complexes.[20]

Platelets also play an integral role in the cross-talk between coagulation and complement in addition to their vital role in primary clot formation and propagation. Platelets have specially evolved antimicrobial properties that can directly interact with innate immunity while also harboring many pattern recognition receptors. This includes components of the lipopolysaccharide receptor complex that can bind bacterial pathogens and present these to appropriate cells of the innate immune system.[1,23] This has been shown in recent studies describing mechanisms of platelet glycoprotein (GP)1b and complement C3 cooperation to transport systemic bacteria to splenic CD8a+ dendritic cells in mice.[23] This process appears essential to induce the adaptive immune response as well as attempt to maintain sterility of the circulation in the setting of bacteremia.[23]

In addition to roles in antigen presentation and processing, platelet components, similar to coagulation factor proteases, can also directly activate the complement cascade. Chondroitin sulfate A (CS-A), released from platelet alpha granules during platelet activation, is a potent activator of complement in the fluid phase, generating anaphylatoxins, inflammatory mediators and leukocyte activation. Similarly, platelet-bound C3 appears to act as a ligand for leukocyte CR1 (CD35), potentially encouraging platelet-leukocyte interactions.[24,25]

**Effects of complement cleavage products on coagulation**

As mentioned earlier regarding the concept of immunothrombosis, several cells of innate immunity

including monocytes and neutrophils are potent effectors of thrombosis.[1] Similarly, complement proteins play a notable role in this framework of cross-activation. C3a and C5 cleavage products (C5a and C5b) not only form terminal MAC leading to cell lysis, but collectively influence many aspects of coagulation. This includes activating platelets, inducing tissue factor expression and activating endothelial cells resulting in the secretion of VWF as well as enhancing exposure of P-selectin, found on activated endothelial cells and platelets.[1,26] Some early studies also demonstrated that C5b-9 (MAC) is capable of enhancing the exposure of prothrombinase assembly sites on the platelet surface by promoting the secretion of platelet factor V and assembling the functional FXa/FVa complex; thus, providing sites for thrombin propagation.[27,28] Many of these procoagulant effects are clinically apparent in disorders of complement dysregulation, such as PNH and atypical hemolytic uremic syndrome (aHUS), where macrovascular and microvascular thrombi cause significant morbidity and mortality.[29-31] C5 inhibition in these disorders with the monoclonal antibody, eculizumab, significantly ameliorates thrombotic disease, further reinforcing the potent procoagulant effects of C5. This link is also seen in models of APS, discussed later in this review.

Important components of the lectin pathway (LP) of complement activation are the serine protease pattern recognition molecules: mannose-associated serine protease 1 (MASP-1) and 2 (MASP-2). MASP-1 is the key initiator protease of the LP while MASP-2 is the main protease cleaving C4, and some C2, to generate C3 convertase. Further to their role in complement activation, indications in vitro revealed that both enzymes also play a significant role in coagulation.[32] MASP-2 cleaves prothrombin to thrombin while MASP-1 appears to possess a thrombin-like property, cleaving FXIII, HMWK, fibrinogen and activating thrombin activatable fibrinolysis inhibitor.[33] In vivo, MASP-1 appears to be involved in thrombogenesis both in animal models and in human plasma.[34,35] MASP-1 resembles thrombin both structurally and in terms of substrate specificity and is able to induce coagulation only via the cleavage of prothrombin.[35] Conversely, Kozarcanin et al. also demonstrated that platelet activation followed by fibrin formation during clot formation, leads to the activation of both MASP-1 and MASP-2, further amplifying coagulation as well as inflammation in thrombotic disorders.[32]

**Clinical consequences of coagulation and complement coactivation**

Clinical conditions with associated coagulation and complement dysregulation are shown in Table 1. This review will focus on APS.

**Antiphospholipid syndrome (APS)**

APS is an acquired thrombophilia characterized by venous or arterial thrombosis and/or obstetrical morbidity with persistently positive antiphospholipid antibodies (aPL), including lupus anticoagulant (LA), anticardiolipin antibody (aCL), and anti- beta-2-glycoprotein-I (β2GPI).[36,37] Anti-β2GPI antibodies are considered the primary pathogenic antibody in APS.[38-40] The mechanisms by which aPL induce thrombosis remain unclear. Multiple mechanisms have been proposed including inhibition of the natural anticoagulant and fibrinolytic systems.[41-44] Activation of vascular cells including endothelial cells,[45] platelets[46] and monocytes,[47] procoagulant effects of extracellular vesicles,[48] disruption of the annexin A5 shield on cellular surfaces[49] and complement activation.[50-56] The lack of a unifying mechanism likely reflects heterogeneity in pathogenic antibodies and disease biology.

The presence of a LA[57,58] and triple-positivity (presence of LA, aCL and anti-β2GPI antibody)[59, 60] are strong predictors of thrombotic risk in APS.[61] Long term anticoagulation with a vitamin K antagonist remains the standard care for thrombotic APS.[62] A severe form of APS characterized by widespread thrombosis and multi-organ failure developing over less than a week, termed catastrophic APS (CAPS) (Table 2), affects a subset (~1%) of patients with APS.[63] CAPS often presents

**Table 1** Clinical conditions with associated coagulation and complement dysregulation

| Clinical condition | Coagulation dysfunction | Complement dysfunction |
|---|---|---|
| Sepsis | Potent activator of coagulation via tissue factor, PAI-1, fibrin degradation products, endothelial dysfunction | Activation of multiple complement pathways including LP where microbial organisms are involved |
| Trauma induced coagulopathy | Potent activator of coagulation<br>Hyperfibrinolysis<br>Consumptive coagulopathy<br>Interference with platelet activation and function | Increased C3a and C4d on surface of platelets |
| Systemic lupus erythematosus | Complement activation products on platelets may promote platelet activation and thrombosis | Complement activation by nuclear autoantibodies<br>C1q deficiency results in impaired classical pathway activation<br>Deficiencies and mutations in other classical pathway proteins (C1r, C1s, C4, C2)<br>Reduced expression of complement inhibitors (factor H, CD46) increased sensitivity to complement activation<br>Opsonization of cellular debris and microparticles with complement |
| Antiphospholipid syndrome | C5a engages C5aR on neutrophils resulting in up regulation of tissue factor which then activates coagulation and inflammation<br>Tissue factor signaling through PAR-2 is critical in neutrophil activation, trophoblast injury and fetal death<br>Antiphospholipid antibodies can activate complement and also thrombosis | aPL antibodies activate complement on trophoblasts leading to C5a generation<br>Rare germline variants in complement regulatory genes |
| Autoimmune and alloimmune hemolytic anemia | Complement-mediated red cell lysis causes activation of coagulation via<br>- Exposure of phospatidylserine<br>- Release of tissue factor bearing microparticles<br>- Endothelial cell injury<br>- Altered vasodynamics<br>- Release of reactive oxygen species | Activation of the classical complement pathway by IgM antibody-bound agglutinated erythrocytes binding C1s<br>Extravascular hemolysis of C3b-coated erythrocytes<br>Activation of complement by circulating free hemoglobin |
| Paroxysmal nocturnal hemoglobinuria | Platelet activation<br>Absence of u-PA (GPI-linked) receptor with impaired fibrinolysis<br>Vascular toxicity and endothelial dysfunction from free hemoglobin and nitric oxide depletion<br>MAC and C5a generation may induce expression of tissue factor and PAI-1, thus promoting thrombosis and anti-fibrinolysis<br>IL6 produced can promote thrombin generation and inhibit ADAMTS13 activity | Complement-mediated hemolysis by unregulated production of MAC on cell surfaces<br>C5a generated can up regulate IL6, IL8, TNF-$\alpha$ |

**Table 1** Clinical conditions with associated coagulation and complement dysregulation (continue)

| | | |
|---|---|---|
| Atypical hemolytic uremic syndrome | Endothelial cell damage and disruption of microvasculature contributes to thrombosis<br>Platelets are activated by MAC or C5a<br>Unopposed complement-mediated destruction of platelets (due to lack of Factor H and other membrane regulators) | Dysregulation of alternate CP and C3 convertase activity due to loss of inhibitory complement regulators (factors H, B and I; membrane co-factor proteins; CD46) |
| Hereditary angioedema | Unregulated activation of prekallikrein-kallikrein-HMWK-bradykinin system due to C1-INH deficiency or dysfunction | Deficiency/dysfunction of C1- inhibitor results in loss of neutralizing C1s, C1r and MASPs thus dysregulating CP and LP |
| Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis | Neutrophil degranulation by ANCA results in release of tissue factor and NETs contributing to thrombosis | Excessive AP activation<br>C5a can prime neutrophils for ANCA induced degranulation<br>Activated platelets can drive complement activation C3a, C3b, C5b-9 |

ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; AP, alternative pathway of complement activation; Cl-INH, Cl esterase inhibitor; CP, classical pathway of complement activation; GPI, glycosylphosphatidylinositol; HMWK, high molecular weight kininogen; IL, interleukin; LP, lectin pathway of complement activation; MCAC, membrane attack complex; MASPs, mannose-associated serine proteases; NETs, neutrophil extracellular traps; PAI-I, plasminogen activator inhibitor-1; TNF$\alpha$, tumor necrosis factor $\alpha$; u-PA, urokinase type plasminogen activator (Modified from Keragala CB, et al. Br J Haematol. 2018;180:782–98)

**Table 2** Preliminary criteria to classify the catastrophic antiphospholipid syndrome[63]

| **Criteria** |
|---|
| Evidence of involvement of three or more organs, systems and or tissues* |
| Development of manifestations simultaneously or in less than a week |
| Confirmation by histopathology of small vessel occlusion in the least one organ or tissue† |
| Laboratory confirmation of the presence of antiphospholipid antibodies (lupus anticoagulant and/or anticardiolipin antibodies)‡ |
| Definite catastrophic APS |
| All four criteria |
| Probable catastrophic APS |
| All four criteria, except for only two organs, systems and/or tissue involved |
| All four criteria, except for the absence of laboratory confirmation at least 6 weeks apart because of the early death of a patient never tested for aPL before the catastrophic APS |
| 1, 2 and 4 |
| 1, 3 and 4 and the development of a third event in more than one week but less than one month, despite anticoagulation |

*Usually, clinical evidence of vessel occlusions, is confirmed by imaging techniques when appropriate. Renal involvement is defined by a 50% rise in serum creatinine, severe systemic hypertension (> 180/100 mmHg) and/or proteinuria (> 500 mg per 24 h).

†For histopathological confirmation, significant evidence of thrombosis must be present, although vasculitis may coexist occasionally.

‡When a patient has not received a diagnosis of APS, the laboratory confirmation requires that presence of antiphospholipid antibodies must be detected on two or more occasions at least 6 weeks apart (not necessarily at the time of the event), according to the proposed preliminary criteria for the classification of definite APS.

as a thrombotic microangiopathy and has a fulminant course with > 40% mortality despite constituting the best available therapy indicating a need for therapies beyond anticoagulation.[62,63] Complement inhibition has emerged as an attractive therapeutic strategy based on evidence of complement activity among patients with APS, murine models indicating a critical role of complement in aPL-mediated thrombotic complications,[50-53] and reports of the efficacy of terminal complement inhibition with eculizumab among patients with refractory thrombotic APS[64] and CAPS.[65-68] Increased complement activation products including C5b-9,[69] fragment Bb and C3a[70,71] have been observed in sera of patients with APS.[71,72]

Several murine models have also demonstrated aPL-mediated fetal loss to be a complement-driven inflammatory process. Antiphospholipid antibodies appear to bind and activate complement on trophoblasts, leading to C5a generation. C5a then engages the complement receptor C5aR on neutrophils, consequently upregulating tissue factor expression resulting in activation of coagulation as well as inflammation in the decidua with the release of reactive oxygen species.[54,73] It appears that tissue factor's inflammatory signaling through the protease-activated receptor 2 (PAR-2) is a critical event, resulting in neutrophil activation, trophoblast injury and fetal death.[74] This is supported by the observation of PAR-2 knockout mice treated with antiphospholipid exhibiting less neutrophil activation and normal pregnancies. At present, the mainstay of APS treatment during pregnancy is anticoagulation in an attempt to reduce fetal loss. The effectiveness of heparin-based therapy in this context lies in its dual antithrombotic and anti-complement properties.[75] Heparin can bind and inactivate C1, inhibit MAC assembly and stop the formation of C3 convertase.[76,77] Redecha, et al. have also demonstrated the ability of simvastatin and pravastatin (hydroxymethylglutaryl-CoA reductase inhibitors used as anti-hyperlipidemic therapy) to reduce tissue factor and PAR-2 expression on neutrophils and prevent pregnancy loss in this setting; thus, blunting the inflammatory as well as the procoagulant effects of this disorder.[74]

Recently, anti$\beta$2GPI antibodies were shown to activate complement and contribute to thrombosis among patients with APS.[78] Complement activation was investigated via complement-dependent cell killing (using the modified Ham assay) and cell surface deposition of C5b-9 (by flow cytometry) using patient sera. The modified Ham assay uses a line of TF-1 cells that are engineered to lack PIGA and consequently lack membrane proteins to protect cells from activated complement. These PIGA null cells bind terminal complement pathway proteins and are killed in the presence of activated complement. A positive modified Ham was present in 85.7% of CAPS, 35.6% of thrombotic APS and only 6.8% of systemic lupus erythematosus (SLE) sera. A positive modified Ham assay was associated with triple-positivity and recurrent thrombosis. These findings suggest that complement activation may be a marker of more clinically important APS. The authors confirmed by flow cytometry that patient-derived anti-$\beta$2GPI antibodies also induced C5b-9 deposition on PIGA null TF-1 cells, which could be blocked completely by an antiC5 monoclonal antibody, but not by a factor D inhibitor, indicating that complement activation by anti-$\beta$2GPI antibodies occurs primarily through the classical pathway of complement. In addition, the authors sequenced 15 known genes related to complement function and found that patients with CAPS have high rates of rare germline variants in complement regulatory genes (60%) versus patients with APS (21.8%), SLE (28.6%) or normal controls (23.3%) and similar to the rate of mutations in aHUS (51.5%), the prototypical complement-mediated microangiopathy.

A pathogenic model of CAPS is proposed in which aPL are the 'first-hit' that can induce complement activation and cause thrombosis, while patients, who also have a pathogenic complement regulatory gene mutation ("second-hit"), are predisposed to uncontrolled complement activation, leading to CAPS in the setting of a complement amplifying trigger such as infection, surgery, pregnancy or autoimmune disease. These mutations can result in proteins with either a loss of function of

complement-inhibitory proteins or a gain of function in complement activating proteins.

These findings raise several questions and suggest new areas for investigation. Should patients with APS undergo mutational analysis to assess risk for CAPS? Given the significant morbidity and mortality associated with CAPS, should patients receive ongoing complement inhibitor therapy in addition to anticoagulation when the modified Ham test suggests increased complement activity or when flow cytometry shows increased terminal complement C5b-9 deposition?

Several reports describe the successful use of eculizumab to treat CAPS,[65-68] and thrombotic APS refractory to anticoagulation;[64] however, mechanistic data and disease biomarkers are lacking. These findings provide a rationale for studying complement inhibition as a therapeutic strategy for CAPS and anticoagulation-refractory APS.

**Potential immunomodulatory effects of anticoagulation**

Given the complex interplay between coagulation, innate immunity and inflammation, the potential impact of anticoagulation on these physiological systems has gained significant interest.

Warfarin is an oral vitamin K antagonist inhibiting vitamin K-dependent activation of factors II, VII, IX, X and the natural anticoagulants, protein C and protein S. Despite being one of the oldest anticoagulants in use today, literature exploring its effects on immunity is limited. In vitro studies from the late 1980s showed that warfarin inhibits T-lymphocyte activation in a dose-dependent nature. Warfarin was also found to prevent IL2 production although often with concentrations in excess of therapeutic serum levels.[79]

Heparin is a naturally occurring glycosaminoglycan of variable polymer length, inhibiting coagulation by binding to and potentiating the action of antithrombin. It also possesses several anti-inflammatory properties, some of which are independent of its role as an anticoagulant. These include the inhibition of function and expression of adhesion molecules, cytokines, angiogenic factors and complement proteins.[80-82] In fact, studies on the effect of heparin among patients with sepsis, severe sepsis, septic shock or DIC associated with infection consider it a safe and feasible intervention. However, evidence to support reduced mortality in this context requires further investigation.[82,83]

Bobek and Kovarik extensively reviewed the antitumor and antimetastatic effects of warfarin and heparins.[84] Anticoagulation-linked inhibition varied from apoptosis induction, inhibition of differentiation, interference with tumor angiogenesis, metastatic processes and the immune system. The immune system effects of these anticoagulants include activating macrophages and natural killer cells, increasing TNF and interferon , increasing the activity of neutrophils and inhibiting the extravasation of leukocytes from vessel walls. Heparins can also interfere with immune reactions by inhibiting the complement system or by enhancing the susceptibility of malignant cells to immunological attacks.[84]

The last decade has seen the increasing clinical use of new targeted oral anticoagulants, which include direct thrombin inhibitors (dabigatran) and antifactor Xa agents (rivaroxaban, apixaban and edoxaban). These have been clinically proven to provide noninferior antithrombotic therapy compared with warfarin with often, a more favorable bleeding profile.[85] The potential immunomodulatory effects of these agents continue to be explored. A study with a murine stroke model showed that pretreatment of rats with rivaroxaban attenuated stroke severity by a dual antithrombotic and anti-inflammatory mechanism. Rivaroxaban appeared to dampen the inflammatory response in the ischemic brain by down-regulating intercellular adhesion molecule 1 expression and activating CD68+ cells of the immune system.[86]

Rivaroxaban may also be effective in limiting complement activation among patients with APS. A cohort of patients with APS with previous venous thromboembolic disease on therapeutic warfarin was randomized to either switch to rivaroxaban or continue on warfarin. Markers of complement activation were measured at baseline and day 42 and compared with a group of normal controls. Unsurprisingly, patients with APS exhibited significantly higher C3a, C5a and terminal attack complexes compared with normal controls at both time points irrespective of the anticoagulation. Among patients randomized to switch to rivaroxaban, complement activation markers were lower at day 42 compared to those remaining on warfarin.[87] Although the authors noted no correlations between serum rivaroxaban levels and complement activation markers, it remains clear that rivaroxaban may provide immunomodulatory benefits to patients with APS in addition to its primary anticoagulation role.

Until recently, data on the role of direct acting oral anticoagulants (DOACs) in treating thrombotic APS have been limited and derived from case reports, cohort studies and small prospective, open-labelled, randomized controlled studies. In a meta-analysis of 47 studies comprising a total of 447 patients with APS receiving DOACs, 73 patients (16%) developed a recurrent thrombosis after a mean period of 125 months.[88] The recurrent thrombosis occurred in 16.9% and 15% of patients receiving a factor Xa inhibitor (rivaroxaban or apixaban) or a factor IIa inhibitor (dabigatran), respectively. The presence of all three aPL antibodies (triple positivity) was associated with a four fold increased risk of recurrent thrombosis compared with patients with single or dual positive positivity [56% vs. 23%; OR = 4.3 (95%CI: 2.3-7.7), $p < 00001$]. Patients treated for arterial thrombosis with rivaroxaban or apixaban had a higher risk of recurrent thrombosis compared with those with venous thrombosis (32% vs. 14%; OR = 2.8 [95%CI: 1.4-5.7], $p = 0006$).[88]

The results of a randomized controlled trial in which rivaroxaban was compared with warfarin in triple positive patients with APS with a history of thrombosis (TRAPS trial) have recently been reported.[89] The trial was terminated prematurely after the enrolment of 120 patients due to an excess of events among patients in the rivaroxaban arm. Thrombo-embolic events occurred in 12% of patients randomized to rivaroxaban while no thrombotic events were found in the warfarin arm at mean follow-up of 19 months. Major bleeding occurred among four patients (7%) in the rivaroxaban arm and two patients (3%) in the warfarin arm. A study from Japan also suggested that patients with APS treated with factor Xa inhibitors had higher thrombotic events than those treated with warfarin. The ASTRO-APS study compared apixaban and warfarin among patients previously anticoagulated for APS and venous thromboembolism for more than six months. However, this trial has stopped recruiting.

Given that initial anticoagulation choices are often made without knowledge of aPL antibody test results, whether a patient receiving DOAC therapy who is subsequently found to have a positive aPL antibody test should be switched to warfarin when they seem to be clinically stable on their current therapy remains unclear. Could the modified Ham test be used to determine which patients must be treated with warfarin and which patients may be safely treated with a DOAC? At present, the findings recommended that DOACs should not be used for secondary prevention among patients with APS.[90] It would also be interesting to see whether the modified Ham test and mutational testing will identify a subset of patients having a low risk for recurrence and may be able to stop long term anticoagulation.

**Role of complement inhibitors**

The growing appreciation of the molecular links between coagulation and complement pathways has invigorated the development of targeted complement inhibition in specific disease states. At the forefront is eculizumab, a humanized monoclonal antibody against

C5 and the cornerstone of therapy for PNH and aHUS.[91] However, the efficacy of eculizumab in inhibiting MAC formation sees its therapeutic potential expand to other disorders of unregulated complement activation. Diseases complicated by thrombotic microangiopathy, glomerulonephritis (including lupus nephritis) and severe cold agglutinin disease (CAD) are some examples where eculizumab has been studied.[92,93] Eculizumab's role in reducing thrombosis is evident in PNH and aHUS and further research is required to elucidate whether this also holds true in other pathologies where it may have therapeutic potential.[30,94]

Another drug in preclinical trials is compstatin, a cyclic peptide inhibiting complement activation by binding C3 and preventing subsequent C3 cleavage.[95] Other small molecule monoclonal antibodies in development include IFX-1 (InflaRx), a C5a inhibitor designed to treat septic shock; TNT-009, an inhibitor of C1s and the classical pathway to treat CAD; lampalizumab, a factor D inhibitor, and bikaciomab, a factor B inhibitor, both being trialed to treat age-related macular degeneration.[92,96,97] Moreover, several agents operating at the gene expression level are also being developed including antisense for factor B, RNA interference for C5 and preventing C6 expression using locked nucleic acid technology.[97]

## Conclusion

Our understanding and appreciation of the complex relationship between coagulation, innate immunity and inflammation has clearly advanced. The clinical consequences of these interactions are evident in a variety of pathologies where coagulation and inflammation often appear inseparable. Complement inhibitors are gaining significant attention as their therapeutic role expands to include other disorders with complement dysregulation. Additional clinical studies using complement inhibition as a therapeutic strategy for CAPS and anticoagulation-refractory APS are needed. Anticoagulation and its potential for immune augmentation is an exciting prospect requiring further delineation.

## References


1. *Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. Nat Rev Immunol. 2013;13:34-45.*
2. *Jackson SP, Darbousset R, Schoenwaelder SM. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. Blood. 2019;133:906-18.*
3. *Connell NT. Taken the wrong way, a complement becomes catastrophic. Blood. 2020;135:233-4.*
4. *Delvaeye M, Conway EM. Coagulation and innate immune responses: can we view them separately? Blood. 2009;114:2367-74.*
5. *Amara U, Flierl MA, Rittirsch D, Klos A, Chen H, Acker B, et al. Molecular intercommunication between the complement and coagulation systems. J Immunol. 2010;185:5628-36.*
6. *Conway EM. Thrombin: Coagulation's master regulator of innate immunity. J Thromb Haemost. 2019;17:1785-9.*
7. *Semeraro F, Ammollo CT, Morrissey JH, Dale GL, Friese P, Esmon NL, et al. Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. Blood. 2011;118:1952-61.*
8. *Silk E, Zhao H, Weng H, Ma D. The role of extracellular histone in organ injury. Cell Death Dis. 2017;8:e2812.*
9. *Massberg S, Grahl L, Von Bruehl ML, Manukyan D, Pfeiler S, Goosmann C, et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. Nature Med. 2010;16:887-96.*
10. *Esmon CT. Molecular circuits in thrombosis and inflammation. Thromb Haemost. 2013;109:416-20.*
11. *Gaertner F, Massberg S. Blood coagulation in immunothrombosis - at the frontline of intravascular immunity. Semin Immunol. 2016;28:561-9.*
12. *Foley JH. Examining coagulation-complement crosstalk: complement activation and thrombosis. Thromb Res. 2016;141:S50-4.*
13. *Foley J H, Walton BL, Aleman MM, O'Byrne AM, Lei V, Harrasser M, et al. Complement activation in arterial and venous thrombosis is mediated by plasmin. E Bio Medicine. 2016;5:175-82.*
14. *Huber-Lang M, Sarma JV, Zetoune FS, Rittirsch D, Neff TA, McGuire SR, et al. Generation of C5a in the absence of C3: a new complement activation pathway. Nat Med. 2006;12:682-7.*
15. *Krisinger MJ, Goebeler V, Lu Z, Meixner SC, Myles T, Pryzdial ELG, et al. Thrombin generates previously unidentified C5 products that support the terminal complement activation pathway. Blood. 2012;120:1717-25.*
16. *Nickel KF, Long AT, Fuchs TA, Butler LM, Renne T. Factor XII as a therapeutic target in thromboembolic and inflammatory diseases. Arterioscler Thromb Vasc Biol. 2017;37:13-20.*
17. *Faxalv L, Boknas N, Strom JO, Tengvall P, Theodorsson E, Ramström S, et al. Putting polyphosphates to the test: evidence against platelet-induced activation of factor XII. Blood. 2013;122:3818-24.*

18. *Mitchell JL, Lionikiene AS, Georgiev G, Klemmer A, Brain C, Kim PY, et al. Polyphosphate colocalizes with factor XII on platelet-bound fibrin and augments its plasminogen activator activity. Blood. 2016;128:2834-45.*
19. *Gajsiewicz JM, Smith SA, Morrissey JH. Polyphosphate and RNA differentially modulate the contact pathway of blood clotting. J Biol Chem. 2017;292:1808-14.*
20. *Conway EM. Reincarnation of ancient links between coagulation and complement. J Thromb Haemost. 2015;13:S121-32.*
21. *Herwald H, Dedio J, Kellner R, Loos M, Muller-Esterl W. Isolation and characterization of the kininogen-binding protein p33 from endothelial cells. Identity with the gC1q receptor. J Biol Chem. 1996;271:13040-7.*
22. *Peerschke EI, Minta JO, Zhou SZ, Bini A, Gotlieb A, Colman RW, et al. Expression of gC1q-R/p33 and its major ligands in human atherosclerotic lesions. Mol Immunol. 2004;41:759-66.*
23. *Verschoor A, Neuenhahn M, Navarini AA, Graef P, Plaumann A, Seidlmeier A, et al. A platelet-mediated system for shuttling blood-borne bacteria to CD8alpha+ dendritic cells depends on glycoprotein GPIb and complement C3. Nat Immunol. 2011;12:1194-201.*
24. *Hamad OA, Ekdahl KN, Nilsson PH, Andersson J, Magotti P, Lambris JD, et al. Complement activation triggered by chondroitin sulfate released by thrombin receptor-activated platelets. J Thromb Haemost. 2008;6:1413-21.*
25. *Hamad OA, Back J, Nilsson PH, Nilsson B, Ekdahl KN. Platelets, complement, and contact activation: partners in inflammation and thrombosis. Adv Exp Med Bio. 2012;946:185-205.*
26. *Foreman KE, Vaporciyan AA, Bonish BK, Jones ML, Johnson KJ, Glovsky MM, et al. C5a-induced expression of P-selectin in endothelial cells. J Clin Invest. 1994;94:1147-55.*
27. *Wiedmer T, Esmon CT, Sims PJ. Complement proteins C5b-9 stimulate procoagulant activity through platelet prothrombinase. Blood. 1986;68:875-80.*
28. *Hamilton KK, Hattori R, Esmon CT, Sims PJ. Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. J Bio Chem. 1990;265:3809-14.*
29. *Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361:1676-87.*
30. *Hill A, Kelly RJ, Hillmen P. Thrombosis in paroxysmal nocturnal hemoglobinuria. Blood. 2013;121:4985-96.*
31. *Jokiranta TS. HUS and atypical HUS. Blood. 2017;129:2847-56.*
32. *Kozarcanin H, Lood C, Munthe-Fog L, Sandholm K, Hamad OA, Bengtsson AA, et al. The lectin complement pathway serine proteases (MASPs) represent a possible crossroad between the coagulation and complement systems in thromboinflammation. J Thromb Haemost. 2016;14:531-45.*
33. *Krarup A, Gulla KC, Gal P, Hajela K, Sim RB. The action of MBL-associated serine protease 1 (MASP1) on factor XIII and fibrinogen. Biochim Biophysic Acta. 2008;1784:1294-300.*
34. *La Bonte LR, Pavlov VI, Tan YS, Takahashi K, Takahashi M, Banda NK, et al. Mannose-binding lectin associated serine protease-1 is a significant contributor to coagulation in a murine model of occlusive thrombosis. J Immunol. 2012;188:885-91.*
35. *Jenny L, Dobo J, Gal P, Schroeder V. MASP-1 of the complement system promotes clotting via prothrombin activation. Mol Immunol. 2015;65:398-405.*
36. *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). J Thromb Haemost. 2006;4:295-306.*
37. *Garcia D, Erkan D. Diagnosis and management of the antiphospholipid syndrome. N Engl J Med. 2018;378:2010-21.*
38. *Galli M, Borrelli G, Jacobsen EM, Marfisi RM, Finazzi G, Marchioli R, et al. Clinical significance of different antiphospholipid antibodies in the WAPS (warfarin in the antiphospholipid syndrome) study. Blood. 2007;110:1178-83.*
39. *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 U S A. 1990;87:4120-4.*
40. *Arad A, Proulle V, Furie RA, Furie BC, Furie B. Beta(2)-Glycoprotein-1 autoantibodies from patients with antiphospholipid syndrome are sufficient to potentiate arterial thrombus formation in a mouse model. Blood. 2011;117:3453-9.*
41. *Marciniak E, Romond EH. Impaired catalytic function of activated protein C: a new in vitro manifestation of lupus anticoagulant. Blood. 1989;74:2426-32.*
42. *Urbanus RT, de Laat B. Antiphospholipid antibodies and the protein C pathway. Lupus. 2010;19:394-9.*
43. *Liestol S, Sandset PM, Jacobsen EM, Mowinckel MC, Wisloff F. Decreased anticoagulant response to tissue factor pathway inhibitor type 1 in plasmas from patients with lupus anticoagulants. Br J Haematol. 2007;136:131-7.*
44. *Bu C, Gao L, Xie W, Zhang J, He Y, Cai G, et al. Beta2-glycoprotein I is a cofactor for tissue plasminogen activator mediated plasminogen activation. Arthritis Rheum. 2009;60:559-68.*
45. *Zhang J, McCrae KR. Annexin A2 mediates endothelial cell activation by antiphospholipid/antibeta2 glycoprotein I antibodies. Blood. 2005;105:1964-9.*
46. *Shi T, Giannakopoulos B, Yan X, Yu P, Berndt MC, Andrews RK, et al. Anti-beta2-glycoprotein I antibodies in complex with beta2-glycoprotein I can activate platelets in a dysregulated manner via glycoprotein Ib-IX-V. Arthritis Rheum. 2006;54:2558-67.*
47. *Sorice M, Longo A, Capozzi A, Garofalo T, Misasi R, Alessandri C, et al. Anti-beta2-glycoprotein I antibodies induce monocyte release of tumor necrosis factor alpha and tissue factor by signal transduction pathways involving lipid rafts. Arthritis Rheum. 2007;56:2687-97.*

48. *Chaturvedi S, Alluri R, McCrae KR. Extracellular vesicles in the antiphospholipid syndrome. Semin Thromb Hemost. 2018;44:493-504.*
49. *Rand JH, Wu XX, Guller S, Gil J, Guha A, Scher J, et al. Reduction of annexin-V (placental anticoagulant protein-I) on placental villi of women with antiphospholipid antibodies and recurrent spontaneous abortion. Am J Obstet Gynecol. 1994;171:1566-72.*
50. *Pierangeli SS, Girardi G, Vega-Ostertag M, Liu X, Espinola RG, Salmon J. Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia. Arthritis Rheum. 2005;52:2120-4.*
51. *Carrera-Marin A, Romay-Penabad Z, Papalardo E, Reyes-Maldonado E, Garcia-Latorre E, Vargas G, et al. C6 knock-out mice are protected from thrombophilia mediated by antiphospholipid antibodies. Lupus. 2012;21:1497-505.*
52. *Fischetti F, Durigutto P, Pellis V, Debeus A, Macor P, Bulla R, et al. Thrombus formation induced by antibodies to beta2-glycoprotein I is complement dependent and requires a priming factor. Blood. 2005;106:2340-6.*
53. *Agostinis C, Durigutto P, Sblattero D, Borghi MO, Grossi C, Guida F, et al. A non-complement-fixing antibody to beta2 glycoprotein I as a novel therapy for antiphospholipid syndrome. Blood. 2014;123:3478-87.*
54. *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. 2003;112:1644-54.*
55. *Girardi G, Redecha P, Salmon JE. Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. Nat Med. 2004;10:1222-6.*
56. *Redecha P, Tilley R, Tencati M, Salmon JE, Kirchhofer D, Mackman N, et al. Tissue factor: a link between C5a and neutrophil activation in antiphospholipid antibody induced fetal injury. Blood. 2007;110:2423-31.*
57. *de Groot PG, Lutters B, Derksen RH, Lisman T, Meijers JC, Rosendaal FR. Lupus anticoagulants and the risk of a first episode of deep venous thrombosis. J Thromb Haemost. 2005;3:1993-7.*
58. *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. 2003;101:1827-32.*
59. *Pengo V, Ruffatti A, Legnani C, Testa S, Fierro T, Marongiu F, et al. Incidence of a first thromboembolic event in asymptomatic carriers of high-risk antiphospholipid antibody profile: a multicenter prospective study. Blood. 2011;118:4714-8.*
60. *Lee EY, Lee CK, Lee TH, Chung SM, Kim SH, Cho YS, et al. Does the anti-beta2-glycoprotein I antibody provide additional information in patients with thrombosis? Thromb Res. 2003;111:29-32.*
61. *Chaturvedi S, McCrae KR. Clinical risk assessment in the antiphospholipid syndrome: Current landscape and emerging biomarkers. Curr Rheumatol Rep. 2017;19:43.*
62. *Cervera R, Serrano R, Pons-Estel GJ, Ceberio-Hualde L, Shoenfeld Y, de Ramón E, et al. Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. Ann Rheum Dis. 2015;74:1011-8.*
63. *Legault K, Schunemann H, Hillis C, Yeung C, Akl EA, Carrier M, et al. McMaster RARE-Bestpractices clinical practice guideline on diagnosis and management of the catastrophic antiphospholipid syndrome. J Thromb Haemost. 2018;16:1-9.*
64. *Meroni PL, Macor P, Durigutto P, Maso LD, Gerosa M, Ferraresso M, et al. Complement activation in antiphospholipid syndrome and its inhibition to prevent rethrombosis after arterial surgery. Blood. 2016;127:365-7.*
65. *Shapira I, Andrade D, Allen SL, Salmon JE. Brief report: induction of sustained remission in recurrent catastrophic antiphospholipid syndrome via inhibition of terminal complement with eculizumab. Arthritis Rheum. 2012;64:2719-23.*
66. *Wig S, Chan M, Thachil J, Bruce I, Barnes T. A case of relapsing and refractory catastrophic antiphospholipid syndrome successfully managed with eculizumab, a complement 5 inhibitor. Rheumatology (Oxford). 2016;55:382-4.*
67. *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;21:311-3.*
68. *Strakhan M, Hurtado-Sbordoni M, Galeas N, Bakirhan K, Alexis K, Elrafei T. 36-year-old female with catastrophic antiphospholipid syndrome treated with eculizumab: a case report and review of literature. Case Rep Hematol. 2014;2014:704371.*
69. *Davis WD, Brey RL. Antiphospholipid antibodies and complement activation in patients with cerebral ischemia. Clin Exp Rheumatol. 1992;10:455-60.*
70. *Breen KA, Seed P, Parmar K, Moore GW, Stuart-Smith SE, Hunt BJ. Complement activation in patients with isolated antiphospholipid antibodies or primary antiphospholipid syndrome. Thromb Haemost. 2012;107:423-9.*
71. *Devreese KM, Hoylaerts MF. Is there an association between complement activation and antiphospholipid antibody-related thrombosis? Thromb Haemost. 2010;104:1279-81.*
72. *Oku K, Atsumi T, Bohgaki M, Amengual O, Kataoka H, Horita T, et al. Complement activation in patients with primary antiphospholipid syndrome. Ann Rheum Dis. 2009;68:1030-5.*
73. *Weiler H. Tracing the molecular pathogenesis of antiphospholipid syndrome. J Clin Invest. 2008;118:3276-8.*
74. *Redecha P, Franzke CW, Ruf W, Mackman N, Girardi G. Neutrophil activation by the tissue factor/Factor VIIa/PAR2 axis mediates fetal death in a mouse model of antiphospholipid syndrome. J Clin Invest. 2008;118,3453-61.*
75. *Weiler JM, Yurt RW, Fearon DT, Austen KF. Modulation of the formation of the amplification convertase of complement, C3b, Bb, by native and commercial heparin. J Exp Med. 1978;147:409-21.*

76. *Almeda S, Rosenberg R, Bing DH. The binding properties of human complement component C1q. Interaction with mucopolysaccharides. The Journal of Biological Chemistry. 1983;258:785-91.*
77. *Kulkarni PA, Afshar-Kharghan V. Anticomplement therapy. Biologics 2008;2:671-85.*
78. *Chaturvedi S, Braunstein EM, Yuan X, et al. Complement activity and complement regulatory gene mutations are associated with thrombosis in APS and CAPS. Blood 2020;135:239-51.*
79. *Bruserud O, Lundin K. The effect of drugs used in anticoagulation therapy on T lymphocyte activation in vitro. II. Warfarin inhibits T lymphocyte activation. J Clin Lab Immunol. 1987;23:169-73.*
80. *Lever R, Hoult JR, Page CP. The effects of heparin and related molecules upon the adhesion of human polymorphonuclear leucocytes to vascular endothelium in vitro. Brit J Pharmacol. 2000;129:533-40.*
81. *Ludwig RJ. Therapeutic use of heparin beyond anticoagulation. Curr Drug Discov Technol. 2009;6:281-9.*
82. *Zarychanski R, Abou-Setta AM, Kanji S, Turgeon AF, Kumar A, Houston DS, et al. The efficacy and safety of heparin in patients with sepsis: a systematic review and metaanalysis. Crit Care Med. 2015;43:511-8.*
83. *Jaimes F, De La Rosa G, Morales C, Fortich F, Arango C, Aguirre D, et al. Unfractioned heparin for treatment of sepsis: a randomized clinical trial (The HETRASE Study). Crit Care Med. 2009; 37:1185-96.*
84. *Bobek V, Kovarik J. Antitumor and antimetastatic effect of warfarin and heparins. Biomed Pharmacother. 2004;58:213-9.*
85. *Madan S, Shah S, Dale P, Partovi S, Parikh SA. Use of novel oral anticoagulant agents in venous thromboembolism. Cardiovasc Diag Therapy. 2016;6:570-81.*
86. *Dittmeier M, Kraft P, Schuhmann MK, Fluri F, Kleinschnitz C. Pretreatment with rivaroxaban attenuates stroke severity in rats by a dual antithrombotic and anti-inflammatory mechanism. Thromb Haemost. 2016;115:835-43.*
87. *Arachchillage DR, Mackie IJ, Efthymiou M, Chitolie A, Hunt BJ, Isenberg DA, et al. Rivaroxaban limits complement activation compared with warfarin in antiphospholipid syndrome patients with venous thromboembolism. J Thromb Haemost. 2016;14:2177-86.*
88. *Dufrost V, Risse J, Reshetnyak T, Satybaldyeva M, Du Y, Yan XX, et al. Increased risk of thrombosis in antiphospholipid syndrome patients treated with direct oral anticoagulants. Results from an international patient-level data meta-analysis. Autoimmun Rev 2018;17:1011-21.*
89. *Pengo V, Denas G, Zoppellaro G, Jose SP, Hoxha A, Ruffatti A, et al. Rivaroxaban vs warfarin in high-risk patients with antiphospholipid syndrome. Blood. 2018;132:1365-71.*
90. *Arachchillage DRJ, Gomez K, Alikhan R, Anderson JAM, Lester W, Laffan M. Addendum to British Society for Haematology Guidelines on Investigation and Management of Antiphospholipid syndrome, 2012 (Br J Haematol. 2012;157:47-58): use of direct acting oral anticoagulants. Br J Haematol 2020 Jan 13. doi:10.1111/bjh.16308. [Epub ahead of print].*
91. *Hill A, Dezern AE, Kinoshita T, Brodsky RA. Paroxysmal nocturnal haemoglobinuria. Nat Rev Dis Primers. 2017;3:17028.*
92. *Melis JP, Strumane K, Ruuls SR, Beurskens FJ, Schuurman J, Parren PW. Complement in therapy and disease: regulating the complement system with antibody-based therapeutics. Mol Immunol. 2015;67:117-30.*
93. *Sciascia S, Radin M, Yazdany J, Tektonidou M, Cecchi I, Roccatello D, et al. Expanding the therapeutic options for renal involvement in lupus: eculizumab, available evidence. Rheumatol Int. 2017;37:1249-55.*
94. *Weitz IC, Razavi P, Rochanda L, Zwicker J, Furie B, Manly D, et al. Eculizumab therapy results in rapid and sustained decreases in markers of thrombin generation and inflammation in patients with PNH independent of its effects on hemolysis and microparticle formation. Thromb Res. 2012;130:361-8.*
95. *Mastellos DC, Yancopoulou D, Kokkinos P, Huber-Lang M, Hajishengallis G, Biglarnia AR, et al. Compstatin: a C3-targeted complement inhibitor reaching its prime for bedside intervention. Eur J Clin Invest. 2015;45:423-40.*
96. *Ghebrehiwet B. The complement system: an evolution in progress. F1000Res. 2016;5:2840.*
97. *Harris CL. Expanding horizons in complement drug discovery: challenges and emerging strategies. Semin Immunopathol 2018;40:125-40.*