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PROGRESS IN HEMATOLOGY

Recent Progress in Paroxysmal Nocturnal Hemoglobinuria

Thrombosis in paroxysmal nocturnal hemoglobinuria in the complement inhibitor era: mechanisms, risk stratification, and clinical management

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Abstract

With the advent of complement inhibition therapy, the severe thrombotic complications of paroxysmal nocturnal hemoglobinuria (PNH)—once described as the most vicious acquired thrombophilic state—no longer pose the same imminent clinical threat. Nevertheless, thrombotic events still occur, albeit at much lower frequency, raising both clinical and mechanistic questions. Among the most pressing are: Under what circumstances does anti-complement therapy fail to prevent thrombosis, and which patient- or therapy-specific factors contribute to this risk? When and where should anticoagulation, anti-platelet therapy, or even prophylaxis be considered? At a mechanistic level, what are the drivers of complement-mediated thrombosis in PNH, and how do they differ from those in other thrombotic conditions involving complement activation? This review addresses these questions by summarizing current evidence on complement-induced thrombosis, integrating clinical and experimental findings. It highlights unresolved issues, including when complement blockade is insufficient and explores the distinction between complement-driven thrombotic states, such as PNH, and intrinsic complement-related diseases without thrombotic complications, such as C3 glomerulopathy. Finally, it proposes a pragmatic framework for anticoagulation and prophylaxis and provides an outlook on critical directions for basic and clinical research, with the goal of further elucidating the complex interplay between complement activation and thrombosis.

Keywords Paroxysmal nocturnal hemoglobinuria · Thrombosis · Complement inhibitors · Anticoagulation · Breakthrough hemolysis

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare condition due to an acquired somatic mutation at the level of the hematopoietic stem cell, involving *PIGA* gene [1]. The latter encodes for a glycosyl-phosphatidyl-inositol (GPI) glycoprotein that acts like an anchor for several molecules at the cell's surface. Among such GPI-anchored molecules, the natural complement inhibitors CD55 and CD59 are lost at the surface of PNH blood cells that are therefore susceptible to complement-mediated lysis. Clinically, the disease is characterized by the triad of intravascular hemolysis (IVH), bone marrow failure, and thrombotic risk [1–5]. The latter represents the main cause of morbidity and mortality in PNH and may be the presenting clinical features at diagnosis or may complicate the course of the disease later on. It has been stated by Luzzatto et al., that "PNH is the most vicious thrombophilic condition."

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This is largely due to the unpredictability of thrombosis in these patients, to the possible occurrence of venous as well as of arterial events, to the localization at both typical and atypical sites, such as cerebral veins and splanchnic vessels, and to the multiple physio-pathologic mechanisms underlying the development and worsening of thrombotic episodes [4–6]. The amount of evidence produced about PNH in the last 20 years clearly showed that anti-aggregation and anticoagulation are not enough to prevent PNH-related thrombosis, largely due to the incomplete control of the several mechanisms involved [1, 4–6]. Conversely, the advent of complement inhibition revolutionized the management of PNH and did significantly abate thrombotic risk thus improving patients' survival [1, 4–6]. In particular, the first anti-C5 inhibitor eculizumab was able to control IVH, by preventing the formation of the membrane attack complex (MAC) and was associated with a near normalization of lactate dehydrogenase (LDH) levels with a parallel improvement of anemia and quality of life. Importantly, the drug significantly reduced the incidence of thrombosis and was also shown to aid the recovery of prior thrombi [1]. Still, the question is open as whether a PNH patient with a prior thrombosis should be discontinued from anticoagulation after starting eculizumab. Additionally, there is no clear-cut evidence about the utility of primary anti-thrombotic prophylaxis in patients with PNH without thrombotic history. Furthermore, today, several novel complement inhibitors are available, including long-acting anti-C5 compounds, ravulizumab and crovalimab, upstream inhibitors of C3, factor B, factor D and MASP, as well as dual inhibitors of the terminal and proximal complement cascade. All these drugs are aimed at improving hemolysis and anemia and are expected to provide at least a similar mitigation of thrombotic risk as that described for eculizumab. However, data coming from clinical trials still have a shorter follow-up and patients with recent major adverse vascular events (MAVEs) were generally excluded, thus making it difficult to draw definite conclusions. Finally, the possible reappearance of hemolysis in patients treated with complement inhibitors (namely breakthrough hemolysis, BTH), during complement amplifying conditions ("pharmacodynamic" BTH during infections, traumas, surgery, etc.) or because of insufficient drug concentrations (i.e., "pharmacokinetic" BTH), may increase thrombotic risk [1]. In this review, we will recapitulate the physiopathology of thrombosis in PNH and will make the point about its possible predictors and mitigation strategies in the current era.

Pathophysiology and mechanistic aspects of complement-mediated thrombosis

Lactate dehydrogenase levels of 1.5 × the upper limit of normal (ULN) were found a significant predictor of thrombotic disease and death in complement inhibitor-naïve patients with PNH [2, 3]. Thus, clinically the occurrence of thrombosis in PNH has been linked to elevated LDH levels, which reflect complement-mediated erythrocyte lysis [4, 5]. This raises the question of whether complement activation itself, erythrocyte lysis, or a combination of both represents the principal trigger of thrombosis—through varying contributions of prothrombotic cell activation and activation of the coagulation cascade. Numerous in vitro and ex vivo studies, supported by compelling findings in some specialized rodent models, demonstrate a bidirectional relationship between complement activation and the prothrombotic state, irrespective of whether this involves cellular activation or stimulation of the plasmatic clotting cascade (reviewed in [6]).

Evidence from animal models and in vitro studies

Data from mechanistic in vitro/ex vivo assays and key animal studies have not translated to the human setting. For example, complement-activating agents accelerate coagulation in normal rabbit blood but not in C6-deficient rabbits; reconstitution with purified C6 restores this response, establishing C6 as essential for complement-driven acceleration of coagulation in rabbits [7]. In contrast, experiments in blood from C6- and C7-deficient human patients failed to reproduce these findings [8, 9]. Similarly, in C3-deficient mice, platelet aggregation in response to certain agonists is reduced, accompanied by prolonged tail-bleeding time, delayed microvascular occlusion, and unstable thrombi [10]. Although these findings indicate a role for complement in coagulation in animal models, they appear not to translate to human physiology (see below).

Focusing on the reverse direction—whether activation of the coagulation cascade triggers complement activation—mechanistic in vitro studies have observed some cross-activation [11, 12]. However, in a non-human primate study, Keshari et al. demonstrated that robust in vivo generation of thrombin and plasmin in baboons does not activate complement, whereas bacterial sepsis induces strong complement responses [13, 14]. These results argue against direct protease-driven complement activation under physiological conditions in vivo. Given these conflicting findings from in vitro, ex vivo, and animal studies, clinical data are particularly valuable for understanding the true physiological and pathophysiological roles of complement in humans.

Clinical experience with complement inhibition

The clinical experience of systemic terminal pathway blockade supports the notion that complement inhibition does not impair hemostasis. Inhibition of C5 with eculizumab, ravulizumab, crovalimab, pozelimab, or zilucoplan in various diseases does not cause bleeding complications. This applies not only to thrombotic conditions such as PNH and atypical

hemolytic uremic syndrome (aHUS), where treatment corrects a prothrombotic phenotype, but also to non-thrombotic disorders such as generalized myasthenia gravis (gMG) and neuromyelitis optica spectrum disorder (NMOSD) [15–22].

Similarly, in rare cases of complete C3 deficiency in humans, the clinical phenotype is dominated by recurrent severe infections (especially by encapsulated bacteria) and immune complex disease (*e.g.,* glomerulonephritis), without evidence of spontaneous or excessive bleeding [23–25]. Therapeutic C3 inhibition in human disease mirrors this observation: No bleeding phenotype is observed [26].

Taken together, clinical trial evidence and years of practice with C5 and C3 inhibitors—now complemented by emerging data from inhibitors of factor B, factor D, the C5a receptor, and C1s—support the conclusion that therapeutic complement inhibition does not cause major pathological alterations in the hemostatic system.

Complement-mediated thrombosis versus classical thrombosis

In diseases characterized by complement-mediated thrombosis, such as PNH and aHUS, complement inhibition dramatically reduces severe or fatal thrombotic events that were largely resistant to anticoagulation and/or anti-platelet therapy. This highlights that complement-mediated thrombosis arises from mechanisms distinct from those driving classical venous thromboembolism, e.g., as in atrial fibrillation-related thrombosis (Figs. 1, 2).

While complement may modulate or enhance hemostatic responses to vessel injury or stasis [27, 28], it is not the primary driver in these settings, in contrast to PNH and aHUS. Consequently, in primarily complement-driven, prothrombotic diseases, prevention of thrombotic events can only be reliably achieved by complement inhibition rather than conventional anticoagulant therapy. Secondary complement activation after primary vascular injury is subordinate (and probably dispensable) to coagulation and platelet activation, explaining why complement blockade does not impair hemostasis.

C3 glomerulopathy as a non-thrombotic complementopathy

This distinction becomes especially clear when considering whether primary complement activation inherently induces a prothrombotic phenotype. Notably, the prototypical complement-mediated glomerulopathy, C3 glomerulopathy (C3G), is characterized by widespread, often consumptive, complement activation but typically does not associate with thrombotic complications (with rare exceptions in C3G/aHUS overlap syndromes [29]). In

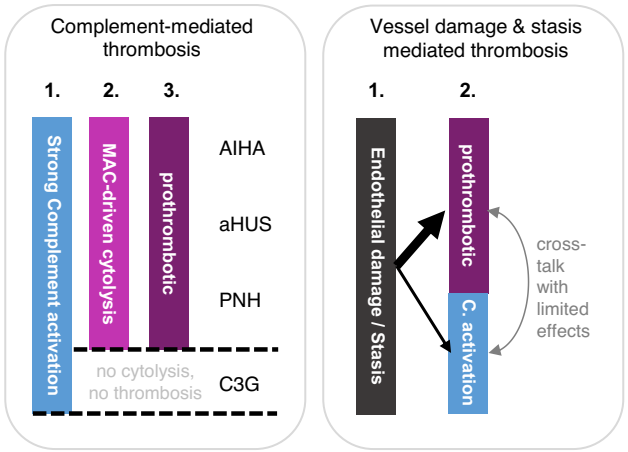

Fig. 1 Sequence of events in complement-mediated versus endothelial damage or stasis mediated thrombosis. *Left panel*: Strong complement activation in primarily complement-mediated diseases can result in membrane attack complex (MAC)-mediated cytolysis. When erythrocytes and/or endothelial cells are affected, this can trigger the release of intracellular danger signals, which in turn promote robust prothrombotic cell activation and thrombosis. By contrast, in C3 glomerulopathy (C3G), strong complement activation occurs predominantly in the fluid phase, and terminal components deposited in the glomerular glycocalyx remain non-cytolytic due to inhibition by soluble MAC regulators such as vitronectin (S protein) and clusterin. The absence of cytolysis in C3G correlates with the rarity of thrombotic complications, despite (partially) consumptive complement activation. *Right panel*: Stasis can trigger the coagulation cascade, which may secondarily interact with the complement system, although conclusive in vivo evidence in humans is lacking. Endothelial injury, by contrast, leads to immediate prothrombotic cell activation and coagulation, accompanied by complement activation at the site of damage. In these contexts, complement activation is secondary both in timing and in effect size. While interactions between coagulation, prothrombotic cells, and complement exist, their quantitative impact under physiological conditions appears limited

archetypical C3G, complement activation occurs primarily in the fluid phase and deposits in the glomerular glycocalyx. Terminal complement components are detected mainly as soluble sC5b-9 complexes bound by S protein (now better known as vitronectin), which prevents membrane insertion and cytolytic activity [30]. Consequently, even robust complement activation in C3G does not induce cytolysis or trigger a prothrombotic cellular response. In contrast, in aHUS and PNH, complement activation is cell-surface oriented, leading directly to cytolysis and release of intracellular danger signals such as ADP, thereby driving thrombotic complications. Taken together, indirect clinical evidence suggests that complement activation alone does not typically induce thrombotic phenotypes in humans. Instead, a prothrombotic state appears to arise primarily when complement activation leads to cell-surface-mediated cytolysis, as seen in PNH, aHUS or autoimmune hemolytic anemia among other diseases (reviewed in [6]).

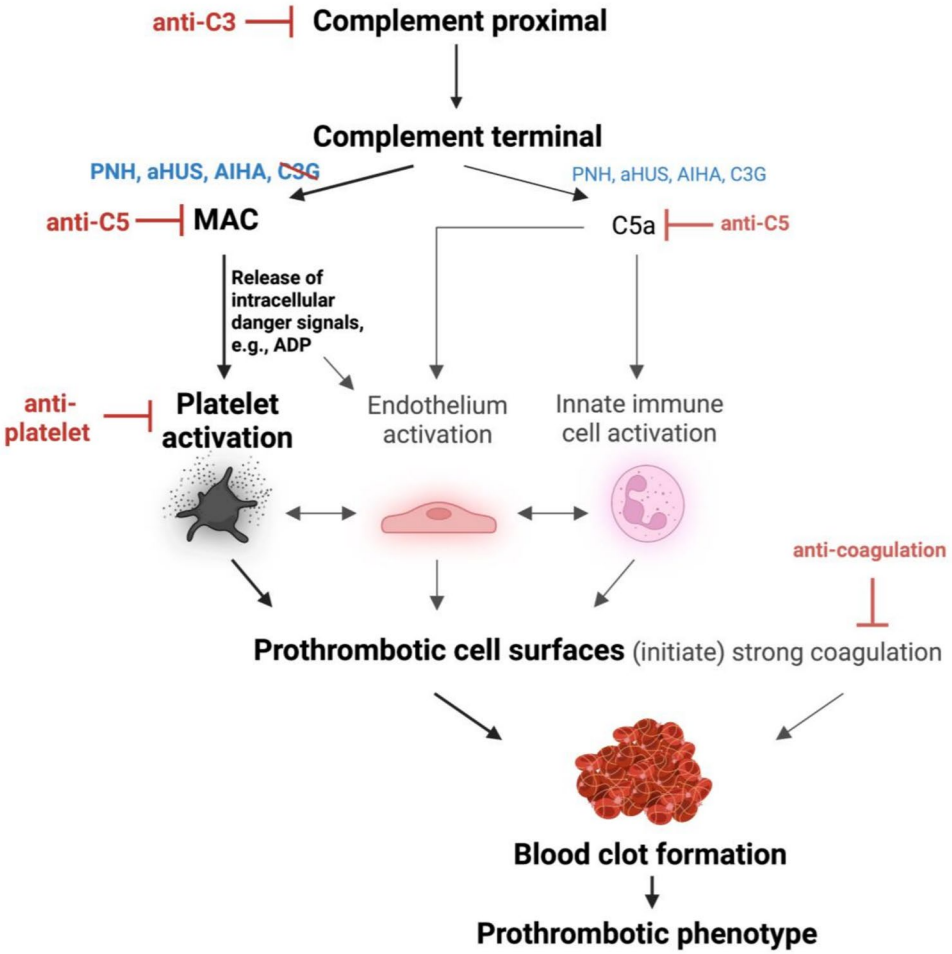

Fig. 2 Schematic diagram of complement-induced prothrombotic phenotype. Figure reproduced from Mannes et al. (with permission). Strong proximal complement activation culminates in terminal pathway activation liberating C5a and inducing MAC assembly. Although C5a is liberated in C3G, PNH, aHUS, and autoimmune hemolytic anemia (AIHA), cytolytic MAC complexes are reported for the latter 3 but not C3G. MAC-mediated release of the intracellular danger signal ADP and cell-lysis fragments induces prothrombotic activation states of platelets and endothelial cells, culminating in thrombosis. Potential therapeutic intervention strategies are shown in red. Elements in bold font depict concepts derived from data of the study by Mannes et al. The illustration was created with BioRender.com

Experimental insights into complement-mediated thrombosis mechanisms

A pivotal study by Mannes et al. experimentally investigated the mechanistic basis of complement-mediated thrombosis, focusing primarily on platelets, using human in vitro and ex vivo assays as well as an in vivo acute mismatched rat transfusion model [31, 32]. In the human whole blood experiments, complement-mediated hemolysis robustly induced platelet activation; however, ADP antagonists alone were sufficient to completely prevent this activation, despite full-blown complement activation. The in vitro studies were complemented by an in vivo model naturally including all relevant cell types like endothelial cells, platelets, and leukocytes, addressing some limitations of the in vitro system. However, this acute transfusion model lasted only two hours, limiting the assessment of longer-term thrombotic processes. Nonetheless, these findings provide direct experimental support for the concept that complement activation per se does not trigger prothrombotic platelet activation unless it leads to membrane attack complex-mediated cytolysis. In fact, erythrocyte lysis typical of PNH is associated with elevated levels of circulating procoagulant microparticles, and with the release of free Hb and NO depletion that may lead to endothelial dysfunction and procoagulant state. The loss of other GPI-linked proteins such as urokinase-type plasminogen activator receptor may further contribute to thrombosis in the presence of erythrocyte microparticles [33].

However, these lines of evidence do not fully account for all clinical thrombotic events in PNH, including cases in patients with small red cell clones and minimal hemolysis (defined as LDH being smaller than twice the ULN), highlighting the likely contribution of non-hemolytic mechanisms to thrombosis [34]. Localized hemolysis and release of intracellular danger molecules may still contribute to prothrombotic cell activation in this setting. Additionally, alternative mechanisms, such as C5a-mediated prothrombotic activation of endothelial cells and leukocytes (in contrast to human platelets which lack C5a receptor 1) [35–37] and their subsequent interactions with platelets—may also play a significant role, highlighting the need for further research in this area. C5 inhibitors significantly lower thrombotic risk,

emphasizing their pivotal role in preventing thrombotic complications in PNH patients, regardless of erythrocyte clone size.

In summary, these findings suggest that complement-driven thrombosis in humans typically is associated with cytolytic activity, whereas fluid-phase or non-cytolytic complement activation alone is insufficient to induce a dominant prothrombotic phenotype, offering a mechanistic explanation for the variable thrombotic risk across complement-mediated diseases.

Clinical epidemiology—thrombosis frequency and characteristics

Up to 10% of PNH patients will present thrombosis at diagnosis, and the rate significantly increases during the disease course [38]. Pre-eculizumab, the rate of thrombosis was about 30% in a 10-year period [39–42]. In a more recent analysis from the international PNH interest group (IPIG) registry, among 4439 patients, 18.8% had a history of MAVEs at diagnosis [43]. Similarly, in the Korean experience from a nationwide registry of 301 patients, the frequency of thromboembolism was 17.9%. [44, 45]. Additionally, about 20% of PNH patients may experience multiple thrombosis, increasing the morbidity and mortality risk [46]. In particular, patients presenting thrombosis pre-eculizumab had an estimated 4-year survival of only 40% [47]. Thrombosis incidence significantly decreased by more than 80% with the introduction of eculizumab, from 7.37 to 1.07 per 100 patient-years, as shown in pivotal clinical trials [15, 16, 48].

Thrombosis can occur at any site (Table 1), more commonly in the venous district (80–85%) [15, 16] and interesting hepatic veins in 40% of cases in retrospective series [39, 40, 42, 46]. Prior to eculizumab, patients often required transjugular intrahepatic portosystemic shunt [49], and liver transplant was contraindicated for the high risk of subsequent Budd–Chiari recurrence [50, 51]. Cerebral thrombosis is also frequent and may present with headache, vomiting, seizures and altered conscious level. In pooled analysis of prospective clinical trials, deep vein thrombosis was the most common (33%), followed by mesenteric (18%), and hepatic ones (16%) [15, 16].

In a recent large real-world study including a total of 2043 PNH patient-years, 56 (21%) developed thrombosis, 43% at disease onset, typically Budd–Chiari syndrome. The frequency was almost halved in patients receiving complement inhibitors (21 vs. 40 thrombotic events per 1000 patient-years in untreated cases, with a 2-year cumulative incidence of thrombosis of 3.9 vs. 18.3%, respectively) [47].

Given the multifaceted clinical presentation, physicians should always be vigilant about thrombotic diathesis and educate the patients to recognize signs and symptoms. Physical examination findings of thrombosis may include hepatomegaly and ascites in case of Budd–Chiari syndrome, splenomegaly in the presence of splenic vein thrombosis, absent bowel sounds in the presence of bowel necrosis, papilledema

Table 1 Frequency and clinical features of PNH-related thrombosis

Type of thrombosis	Frequency among PNH related thrombosis (%)	Clinical features
Venous thrombosis	60–80	Depending on site, more frequently deep venous thrombosis of lower limbs, splanchnic, and cerebral thrombosis
Arterial thrombosis	20–40	Depending on the site, both myocardial infarction and stroke have been reported, as well as mesenteric arterial thrombosis and renal artery obstruction
Specific sites of thrombosis by frequency		
Deep venous thrombosis of lower limbs and pulmonary embolism	10–30	Swelling, painful, warm part of the lower limb, difficulties in movement and walking. Dyspnoea and shortness of breath, tachycardia, etc. in case of pulmonary embolism
Splanchnic thrombosis	7.5–30	In case of Budd-Chiari syndrome and splenic vein thrombosis, abdominal pain, ascites, palpable hepatomegaly, splenomegaly In case of bowel necrosis, absent bowel sounds, fever, obstruction, and rectal bleeding Duodenal venous thrombosis is associated with papillary endothelial hyperplasia, ulceration, and a circumferential mass in the third portion of the duodenum In case of renal vein/artery thrombosis alteration of kidney function
Cerebral vein thrombosis	2–7	Headache, vomiting, seizures, altered level of consciousness, papilloedema, VI and VII cranial nerve palsies, central retinal vein thrombosis, and cerebellar or lower cranial nerve signs for sigmoid sinus thrombosis
Dermal vein thrombosis	1–5	Painful discolored skin lesions, rarely ulcerated; rarely purpura fulminans

in patients with cerebral vein thrombosis, skin nodules that are red and painful in case of dermal vein thrombosis. Any patient with known PNH presenting signs/symptoms of deep venous thrombosis, abdominal or neurological symptoms should have urgent imaging, such as Doppler, abdomen ultrasound, and magnetic resonance of the brain.

Predictors of thrombotic risk

Generally, all patients with PNH should be considered to be at increased thrombotic risk; however, some clinical and laboratory predictors have been identified in large retrospective analyses. Specifically, the granulocyte clone size and the hemolytic activity of the disease, as shown by increased LDH levels, have been consistently reported to correlate with higher thrombosis frequency. Although providing a "cut off" value for PNH clone size or LDH levels would be arbitrary, patients with a granulocyte clone > 50% and/or LDH levels > 1.5 × ULN have a cumulative incidence of thrombosis of about 7 folds compared with the others [41, 52].

Concerning clone size, thrombotic risk appeared increased by 1.64 fold for each 10% increase in PNH affected cells [47]. However, IPIG registry data clearly showed that even patients with clones < 50% remain at increased thrombotic risk. In a recent publication, even patients with granulocyte clone size < 10% had MAVEs in 10.2% of cases, rendering education and vigilance essential [43].

Regarding LDH, the Korean data reported that patients with LDH > 1.5 × ULN have 4.8 folds the risk of mortality as compared to "less hemolytic" PNH patients and reported that thrombosis was one of the significant risk co-factors for mortality in multivariable analysis [45].

Additional risk factor for thrombosis, including cardiovascular ones, inherited (i.e., V Leiden factor, factor II mutation, proteins C or S deficiencies) or acquired thrombophilia (i.e., anti-phospholipid antibodies), as well as acquired iatrogenic risk factors such as hormonal therapies should also be taken into account. In a French series of 15 PNH patients 6/15 thrombotic episodes were linked to oral contraceptive use or being pregnant/postpartum [50], suggesting that PNH patients should avoid estrogen based oral contraception, opting for progesterone only or barrier methods use.

Cleveland experience described higher odds ratios of MAVEs for patients presenting with classic hemolytic phenotype, including erythrocyte clone > 20% (OR 2.5), granulocyte clone > 70% (OR 3.3), *PIGA* VAF > 15% (OR 3.36), reticulocytes > 80 × 10^9/L (OR 2.18), and LDH > 400 U/L (OR 3.5). Additionally, they observed increased risk for thrombosis in patients with type II–dominant erythrocyte clones (OR, 3.7), confirmed in multivariable analysis (OR, 4.09). The latter is a possible marker of "white PNH" displaying a high thrombotic risk despite showing limited hemolytic activity and near normal LDH levels, as already reported by the UK group [47]. Finally, other risk factors such as reduced albumin (OR 3.1) and increased transaminases (OR 2.3) could be linked to prior liver vein thromboses.

To summarize, IVH severity remains the main predictor of thrombosis; LDH ≥ 1.5 × ULN is the proposed practical threshold for high disease activity in routine practice, while levels ≥ 2 × ULN is the cutoff reserved for grading severe breakthrough hemolysis (see next paragraph). The continuous effect of granulocyte clone size (with the < 10 / 10–50 / > 50% categories considered as pragmatic bands), and, more recently, the VAF of *PIGA* mutation are further indicators of thrombotic risk. Finally, non-complement risk modifiers such as general thrombosis/cardiovascular risk factors, low albumin, and elevated transaminases may increase the risk of thrombosis in PNH.

Mitigation strategies

Historically, primary or secondary anticoagulation with heparin or vitamin K antagonist was part of the supportive treatment of PNH and the only available mitigation strategy for thrombotic risk. This was, however, clearly shown to be inadequate for the control of thrombophilia in PNH and the true revolution came in the early 2000s with the introduction of eculizumab that markedly reduced thrombosis incidence by more than 80% in clinical trials [16, 48]. Novel complement inhibitors, either anti-C5 or upstream, showed a similar benefit to eculizumab, although in a shorter follow-up time and with the caveat of excluding patients with recent MAVEs from the clinical trials (Table 2). Across phase 3 trials, only isolated thrombotic events were reported, though with a trend to higher frequency by prolonging follow-up. In fact, notwithstanding complement inhibition, complement amplifying conditions, such as infections, traumas, or surgery, possibly leading to BTH may also trigger breakthrough thrombosis, claiming for anticoagulant prophylaxis in these circumstances.

Mitigation strategies' goals include (1) effectively treating thrombosis, (2) preventing recurrent episodes, and (3) preventing the first episodes in patients who had never experienced MAVEs at PNH diagnosis (Fig. 3).

(1) Those patients presenting with thrombosis should be immediately treated with anticoagulants as per current indications for the management of thromboembolism itself according to thrombotic site and severity (heparin, vitamin K antagonists, locoregional or systemic thrombolysis, anti-platelet agents for arterial events, etc.). Whether novel oral anticoagulants such as direct

thrombin inhibitors and factor Xa inhibitors are more, less, or equally efficacious has not been determined. In fact, despite the noninferiority, safety, and ease of administration of DOACs in the treatment of acute MAVEs, recent data on anti-phospholipid syndrome showed that they are inferior to vitamin K antagonists in some thrombophilic conditions. In any case, no thrombotic recurrence was observed in 19 patients treated with DOACs at a median observation of 17.1 months (IQR, 8.9–45) in the Cleveland experience, possibly suggesting that they may be a feasible choice in this patient population. Notably, thrombocytopenia often complicates PNH, and this issue must be addressed when formulating an anticoagulation management plan. Importantly, given the reduction of thrombotic risk observed with eculizumab, thrombosis is one of the indications for starting complement inhibition as soon as possible. Antibiotic coverage for the risk of capsulated bacterial infections should be considered while programming vaccinations against Meningococcus, Pneumococcus, and Haemophilus. The quick start of anti-complement therapy is supported by the evidence that eculizumab may shape clot structure by reducing fibrinogen levels, thrombin generation and clot density [53]. Furthermore, complement inhibitors have shown quick control of IVH in PNH patients, supporting their use in case of emergency to prevent re-thrombosis or clot progression.

(2) The way of preventing recurrent episodes is to establish a long-term, and possibly stable, control of IVH. This is one of the reasons why complement inhibitors are administered continuously and on a life-long basis. Concerning concomitant anticoagulant treatment, there are no clear guidelines. However, it might be proposed to continue it indefinitely in those patients with additional, non-modifiable risk factors for thrombosis (prosthetic valves, inherited thrombophilia, antiphospholipid antibodies, etc.) or in those with severe episode at atypical site (Budd–Chiari syndrome, cerebral vein thrombosis, etc.). In those patients were PNH was identified as the "sole virtual" trigger for thrombosis, anticoagulation withdrawal might be considered for less severe forms (i.e., deep vein thrombosis of the lower limb), once IVH is under control (i.e., LDH < 1.5 × ULN) and the clot has been completely re-canalized. In the Cleveland experience, no recurrence was registered in 14 patients who discontinued anticoagulation at a median follow-up of 4 years. This

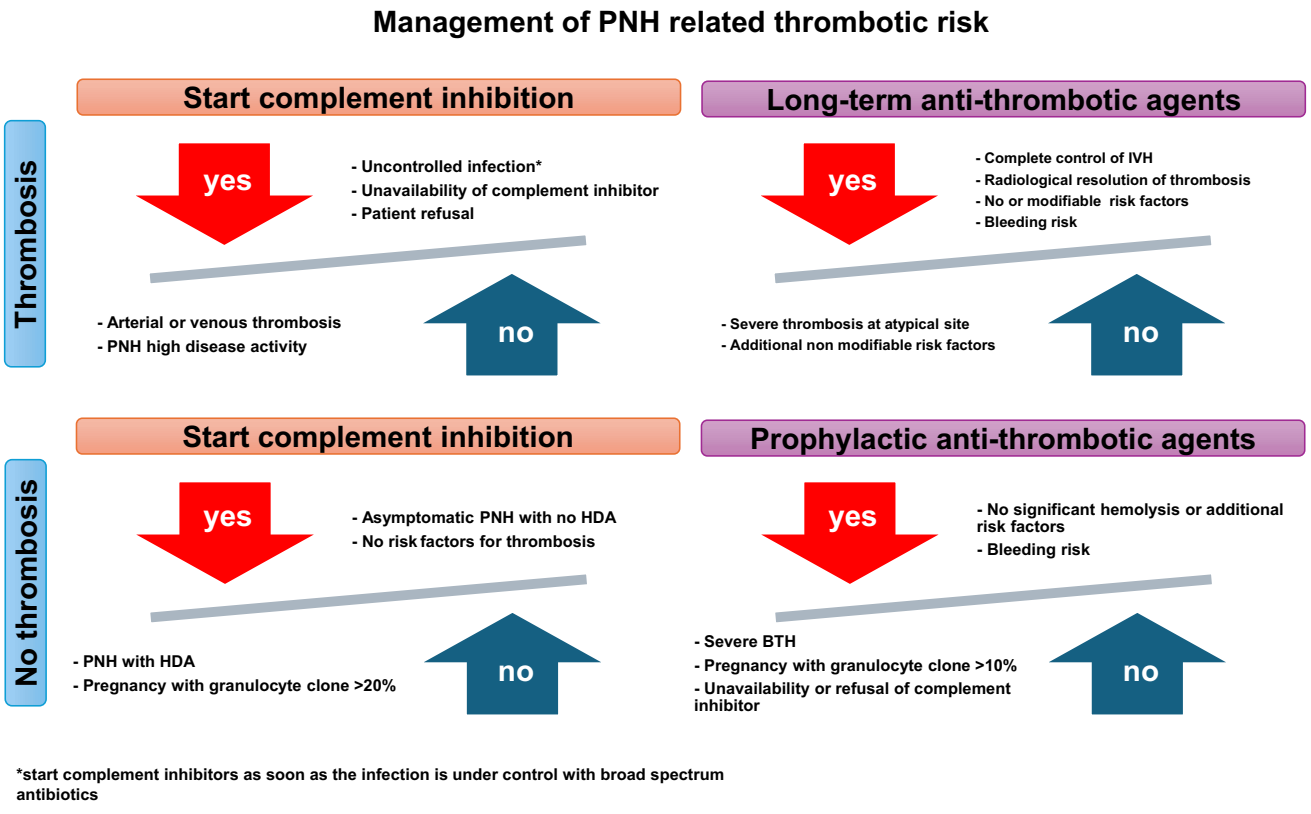

Fig. 3 Mitigation strategies for thrombotic risk in paroxysmal nocturnal hemoglobinuria (PNH). Indications for complement inhibitors and anticoagulant treatment or prophylaxis in patients with or without PNH-related thrombosis

would also limit hospital accesses and risk of bleeding, particularly in patients with thrombocytopenia due to associated bone marrow failure. Importantly, this decision should be shared between the treating physician and the patient, discussing the lack of available data and the possible risks.

(3) Risk of thrombosis may be predicted by PNH high disease activity (i.e., LDH > 1.5 × ULN and PNH-related signs and symptoms) and clone size > 50% on granulocytes. The former is a clear indication to start anti-complement therapy, which is all in all "the best anticoagulant for PNH." Thus, terminal or complement inhibitors used to treat PNH with HDA represents a "primary prophylactic" measure. The role for primary anticoagulant prophylaxis remains for patients with large PNH clones on granulocytes while awaiting anti-complement treatment start, for those living in countries were complement inhibitors are not available, and for those refusing anti-complement treatment. Generally, low molecular weight heparin is offered for temporary prophylaxis. Concerning vitamin K antagonists, in the pre-eculizumab era [54], the UK group showed a 10-year thrombotic rate of 36.5% among 56 patients not taking warfarin versus 0% among the 39 patients on warfarin. Although only 2 serious bleeding events were observed in over 100 patient-years of warfarin therapy, cautious and education is warranted in such cases. Additionally, in PNH patients on anti-complement therapy as well as in those without HDA, not candidate to anti-complement therapy, transient conditions increasing thrombotic risk represent further indications for primary anticoagulant prophylaxis: surgery, traumas, pregnancy and hemolytic crises. In particular, pregnancy and puerperium are generally put on prophylaxis in cases of PNH clone > 10%, and on anti-complement therapy if clone size is > 20%. Eculizumab has clearly demonstrated to decrease maternal and fetal complications, including thrombotic ones, in this patient population [55]. Concerning hemolytic crises, they may occur even in patients on anti-complement therapy (i.e., BTH events). Since BTH may occur with all complement inhibitors, and is often associated with complement amplifying conditions that may increase thrombotic risk per se, primary prophylaxis has been proposed for all severe forms (i.e., those with LDH > 2 × ULN or Hb drop < 8 g/dL for those with baseline Hb < 10 g/dL or drop > 2 g/dL from baseline for those living at higher Hb levels) [56].

Future directions in basic research and clinical practice

Thrombosis remains the leading cause of morbidity and mortality in PNH, making its prevention a central therapeutic goal. Yet, when, where, and how complement activation drives thrombotic complications is not fully understood. Key unresolved questions include why some patients with high LDH develop thrombosis while others do not, why thrombosis occurs at both typical and atypical venous and arterial sites, and which blood cell lineages are most responsible for initiating the prothrombotic cascade. It is likely that one or more lineages derived from the PNH hematopoietic stem cell carry the greatest responsibility. Erythrocytes are strongly implicated, as their complement-mediated lysis and consequent LDH elevation correlate with thrombotic risk. However, the contribution of PNH leukocytes and platelets remains less well defined. Leukocytes, being nucleated, are more resistant to lysis even without CD55 and CD59, but sublytic MAC formation may still induce prothrombotic signaling [57–59]. Platelets are even more enigmatic: small, anucleate, and mechanically sensitive, and they are difficult to study yet may play a pivotal role. PNH platelets could lyse as readily as erythrocytes, releasing danger signals such as ADP, and loss of CD55/CD59 might predispose them to complement-mediated activation. Surprisingly, rare ex vivo studies suggest that type III PNH platelets respond less robustly to agonists than type I platelets, raising the possibility of an "exhausted" phenotype [60, 61]. However, counterintuitively, this blunted reactivity of type III PNH platelets might represent an underappreciated protective mechanism, raising the possibility that thrombotic risk in PNH could be even greater if these platelets were as responsive as their type I counterparts.

Looking ahead, more precise risk prediction in PNH will require a comprehensive assessment of clone sizes across erythrocytes, granulocytes, monocytes, lymphocytes, and platelets, and their relative contributions to prothrombotic cell activation.

To summarize the current clinical approach, all patients with thrombosis should start complement inhibition as soon as possible, upon coverage with broad spectrum antibiotics while programming vaccines against capsulated bacteria. Prompt complement inhibition may be lifesaving and prevents recurrence and progression of the thrombus. Anti-thrombotic agents should be administered as per the specific thrombosis type, balancing against the risk of bleeding in patients with PNH-associated thrombocytopenia. The use of DOACs, though broadly employed, is still supported by limited evidence, as it is the possibility to discontinue anti-thrombotic agents once hemolysis is

under control and clot resolution has occurred. This should be discussed on a case-by-case basis by evaluating the type, site, and severity of thrombosis and always balancing against bleeding risk. For patients without thrombosis, primary prophylaxis with anticoagulant agents may be considered in case of large PNH clone and high disease activity, while complement inhibitor is started and intravascular hemolysis under control, and discontinued thereafter. Additionally, in case of recurrence of hemolysis, particularly during severe BTH, and conditions that increase thrombotic risk, such as pregnancy and puerperium, anticoagulant prophylaxis has been recommended.

While terminal complement blockade with C5 inhibitors remains the gold standard, with the most robust evidence for preventing thrombotic complications, data on upstream complement inhibition are accumulating and show promise. Still, long-term studies will be essential to determine whether these agents provide equivalent protection against thrombosis in addition to their hematologic benefits.

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Data availability statement All data were included in the article manuscript and supplementary material. Additional information may be obtained upon reasonable request to the Corresponding Author.

Declarations

Conflict of interest B.F. received consultancy and speaker honoraria from Alexion, AstraZeneca, Novartis, Roche, Sanofi, and Sobi. BF received research funds from Agios Pharmaceuticals and Zenas Biopharma. C.Q.S. is a named inventor of patent applications that describe the use of complement inhibitors for therapeutic applications. C.Q.S. has received research funding from pharmaceutical companies, honoraria for speaking at symposia organized by Alexion Pharmaceuticals, Sobi and Sanofi, and serving on an advisory committee for Sobi.

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