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Paroxysmal nocturnal hemoglobinuria revisited: news on pathophysiology, clinical course and treatment

Paroxysmale nächtliche Hämoglobinurie: neue Erkenntnisse zur Pathophysiologie, klinischem Verlauf und Behandlung

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Abstract: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare benign clonal disorder of hematopoiesis. Its etiologic basis is the clonal dominance of a *PIGA-mutated* hematopoietic progenitor that gives rise to GPI-deficient blood cells that are unable to express complement inhibitors like CD55 or CD59. These cells are prone to complement-induced hemolysis. In classic hemolytic PNH, granulocyte clone sizes can be up to 90% or more. Much progress has been made during the last years in understanding the interrelations of complement-dependent hemolysis; its consequences like thrombosis, renal failure or pulmonal hypertension; and possible treatment strategies. To gain clinical relevance, the PNH clone has to make a relevant contribution to blood cell generation; however, a minimal clone size as a threshold for the occurrence of symptoms such as thrombosis, dyspnea, chest or abdominal pain cannot be given. Such symptoms can be seen in a relevant proportion of patients with clone size smaller than 10%. The driver for the clonal dominance of the *PIGA*-mutated hematopoiesis is still not clear. Recently, data about coexisting mutations in cancer genes have been published and new mechanisms for autoimmunity have been presented. The success of eculizumab in the treatment of PNH patients has stimulated the development of a variety of new strategies for complement inhibition. This review will focus on the most important findings in pathophysiology, clinical course and treatments during the last years.

Keywords: complement inhibitors; eculizumab; paroxysmal nocturnal hemoglobinuria; pathophysiology; therapy.

Zusammenfassung: Die paroxysmale nächtliche Hämoglobinurie (PNH) ist eine seltene gutartige klonale Erkrankung der Blutbildung. Ursache für die PNH ist eine klonale Dominanz von hämatopoietischen Progenitorzellen aus welchen Blutzellen entstehen, bei denen komplementinhibitorischen Proteine wie CD55 oder CD59 nicht exprimiert werden können. Solche Zellen sind anfällig gegenüber komplementvermittelter Hämolyse. Die klassische hämolytische PNH zeigt Granulozytenklongrößen von 90% und mehr. In den letzten Jahren haben wir viel über den Zusammenhang zwischen komplementvermittelter Hämolyse und ihrer klinischen Konsequenzen wie Thrombose, Niereninsuffizienz oder Pulmonale Hypertension und möglicher Behandlungsstrategien gelernt. Um klinisch relevant zu werden muss der PNH-Klon einen relevanten Beitrag zur Blutbildung leisten. Eine Schwellenwert für die klinische Relevanz eines PNH-Klons kann nicht angegeben werden, da auch kleine Klone Symptome wie Thrombosen, Dyspnoe, thorakale oder abdominelle Schmerzen verursachen können. Solche Symptome treten mit einen relevanten Anteil auch bei Patienten mit Klongrößen, die kleiner als 10% sind auf. Der Auslöser für die Entstehung der klonalen Dominanz ist bislang nicht geklärt. Kürzlich wurde gezeigt, dass unterschiedliche Mutationen von Genen in PNH-Klonen gefunden werden, die auch bei hämatologischen Neoplasien vorkommen.

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Auch neue Autoimmunmechanismen wurden beschrieben. Der Erfolg von Eculizumab in der Behandlung von PNH-Patienten hat die Entwicklung von neuen Komplementinhibitoren angeregt. Dieser Review fokusiert auf die wichtigsten Erkenntnisse der letzten Jahre auf dem Gebiet der Patophysiologie, dem klinischen Verlauf und der Therapie der PNH.

Schlüsselwörter: Eculizumab; komplementinhibitorischen Proteine; paroxysmale nächtliche Hämoglobinurie; Pathophysiologie; Therapie.

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal disorder of hematopoiesis that is characterized by chronic intravascular hemolysis, a severe thromboembolic diathesis and variable bone marrow failure that progress into aplastic anemia in 15% of cases [1, 2]. Despite its benign nature, PNH induces substantial morbidity and mortality [3, 4]. Diagnosis is often delayed due to the high variability of symptoms.

PNH has no sex preference, and, on average, a PNH patient is 32 years at the start of symptoms, has a nearly doubled LDH and a hemoglobin level of 10.6 g/dL, and shows a mild cytopenia that translates into a platelet count of 130,000/μL and a neutrophil count of 1700/μL. The mean granulocyte clone size, i.e., the proportion of cells with the defect of the glycosylphosphatidyl-inositol (GPI) anchor, is about 68%. Around one-tenth of patients have a history of renal failure and are on pain medication, and 16% have a history of thrombotic events (TE) (Table 1) [5].

Hemolysis is caused by a lack of decay accelerating factor (CD55) and membrane inhibitor of reactive lysis (MIRL, CD59) on PNH erythrocytes. These complement inhibitory proteins are expressed on the cell surface by the GPI anchor. The synthesis of the GPI anchor is disrupted in PNH cells because of mutations in the phosphatidylinositol N-acetylglucosaminyltransferase subunit A (*PIGA*-) gene. Its product catalyzes for the first step of GPI-anchor synthesis at the endoplasmatic reticulum. Proteins without an alternative transmembrane form like CD55 and CD59 cannot be expressed on the cell surface in PNH erythrocytes [6, 7].

Table 1: Symptoms of PNH patients in the international PNH registry.

Symptom	(%)
Fatigue	80
Dyspnea	64
Headache	63
Hemoglobinuria	62
Abdominal pain	44
Erectile dysfuncion (males)	38
Chest pain	33
Poor quality of life	23
History of TE	16
History of impaired renal function	14

Because of the absence of complement inhibitory proteins on the cell surface, patients are vulnerable to develop hemolytic crisis in states with physiologic complement activation such as infections, inflammation, surgery or pregnancy. The consequences of intravascular hemolysis may include hemoglobinuria, renal failure, anemia or perilous TEs. Before the introduction of prophylactic anticoagulation or a treatment with complement inhibitors, about 40% of patients have experienced a TE after 14 years of disease and survival was only 48% at 15 years after diagnosis [4].

Allogeneic transplantation is still the only cure for PNH patients, but it is a treatment modality with a high morbidity and mortality, with only limited published data so far. Supportive care with the substitution of folic acid, anticoagulation and transfusion was the only treatment option for many years until the approval of eculizumab in 2007 in Europe and the United States [1, 2]. Eculizumab is a humanized monoclonal antibody that blocks complement C5. Thus the terminal cascade of the complement system is blocked and therefore the GPI-deficient erythrocytes are protected against an otherwise unregulated terminal complement attack. The termination of hemolysis and reduction of symptoms with eculizumab are impressive. Transfusion rates in patients dropped dramatically under eculizumab therapy [8–10].

In this review, we will put a focus on the progress that was achieved during the last years.

Always the same mutated gene that impairs an orchestra of enzymes?

In the early 1990s, acquired mutations in the *PIGA* gene were described as the reason for the impairment of complement inhibition in PNH patients [7, 11–13]. It is localized on the short arm of the X-chromosome and encodes for an enzyme that synthesizes the first step of the generation of the GPI anchor at the cytosolic side of the endoplasmatic reticulum. Mainly insertions or deletions are responsible for a complete impairment of the *PIGA* gene product, leading to a frameshift and thus to a complete failure of the expression of GPI-anchored proteins on the cell surface [6]. Such populations can be defined as type III cells in diagnostic assays using flow cytometry in conjunction

with monoclonal antibodies or GPI-binding toxins that are linked with fluorochromes. Populations with a reduced expression of GPI or GPI linked proteins are called type II cells. Missense mutations are the cause for a suboptimal but not complete disrupted function of the *PIGA*-gene product. Type I cells show a normal phenotype [6].

As mentioned above the mutation is acquired and due to silencing of one X-chromosome in females the incidence of PNH is equal in males and females. There is no mutational hotspot and some patients show GPI-deficient cells that derived from different mutated hematopoietic progenitors.

Recently the exclusive role of *PIGA* mutations in the genesis of PNH has been challenged. Mutations of other parts of the PIG enzyme complex are also capable of impairing the expression of GPI-linked proteins and inducing complement dependent hemolysis. We have described a patient that has a biallelic mutation in *PIGT*. The gene is located on the long arm of chromosome 20 and the product is responsible for the attachment of proteins to the GPI anchor. The patient had a germ line single nucleotide substitution affecting the splice acceptor site of intron in one allel and an acquired deletion of 8 megabases in the other. As a consequence all GPI-linked proteins on the cell surfaces of the cells with the biallelic mutation were lacking and this induced symptomatic PNH [14, 15].

Beyond the mutation of genes involved in the GPI-anchor syntheses defects of complement regulators themselves can induce PNH-like intravascular hemolysis. Recently an inborn autosomal recessive defect of CD59 was described that induced a disease with neurologic complications and severe intravascular hemolysis. The hemolysis could be successfully treated with eculizumab and the neurologic symptoms decreased significantly [16]. Supportive care in these patients with erythrocyte transfusion is challenging because CD59 is a blood group with a high frequency [17].

Complex clonal architecture in PNH

It is thought that the mutation of the phosphatidylinositol N-acetylglucosaminyltransferase subunit A (*PIGA*) gene occurs in a hematopoietic stem cell (HSC) in PNH. Assuming a strict stem cell definition this suggestion has never been proven. Transplantation of GPI deficient hematopoietic progenitors in NOD SCID mice showed colonization of GPI negative cells in bone marrow, but these progenitors failed to establish hematopoiesis as no peripheral blood cells could be detected. Interestingly progenitors from healthy controls showed no such colonization of the bone marrow [18] suggesting an advantage of GPI negative hematopoiesis. On the other hand, GPI negative blood cells remained stable or decreased in mouse models of *PIGA*-mutation with a mosaic of normal and mutated cells [19, 20] and showed no tendency to expand. To the best of our knowledge there are no data of *PIGA*-mutated HSCs that established hematopoiesis after sequential transplantation in an animal model.

For the generation of symptoms the *PIGA*-mutated hematopoietic progenitor has to contribute substantially to the generation of blood cells. The previous view suggested a clonal selection and expansion of a GPI-negative HSC clone. The so-called immuno escape hypothesis explains this process by an immune mediated impairment of the GPI-positive hematopoiesis. The GPI-negative HSCs escape from the attack because of the absence of surface proteins that are central for the autoimmune attack [21]. According to the immune escape hypothesis this explains the common occurrence of PNH in the context of bone marrow failure (and vice versa). Without GPI deficient hematopoiesis the patients develop aplastic anemia (AA). However, about 15% of PNH patients will progress to AA despite GPI negative hematopoiesis and about 43.5% had a history of AA [5].

A recent publication has described reactive T-cells against the GPI anchor itself. The authors supposed that these cells might contribute to the bone marrow failure in PNH patients [22]. However, many data like these are strongly suggestive for the autoimmune escape hypothesis. A definitive proof for this mechanism is still pending.

Intrinsic factors may play a role in the expansion of PIGA mutated stem cells [23].

Upregulation of transcription factors in *PIGA* mutated granulocytes have been described [24]. Some of the literature includes patients with additional mutations like *HMGA2* or *JAK2 V617F* mutations [24–27]. A recent publication has shown additional mutations in about half of PNH patients. Interestingly the group with additional mutations showed a higher proportion of GPI deficient granulocytes in peripheral blood (68.9 vs. 49.8%) but did not differ in their clinical presentation. Around 30% of these acquired mutations were present before the acquisition of the *PIGA* mutation. In about 10% of cases the *PIGA* mutation occurred first. Many cancer genes (e.g., *U2AF1*, *TET2*, *MAGEC1*,*BRPF1*, *NRXN3*, *KDM3B*, *SLC20A1*, *MUC7*, *PEX14*, *FBN1*, *SUZ12*, *ASXL1, BCOR, DHX29, MECOM, RIT1, JAK2 V617F*) were among the mutated genes and some cases showed more than one mutation [25]. Despite the mutation of cancer genes malignant transformation of PNH to AML is a rare event. The view on PNH as a benign hematologic tumor [27] is therefore an interesting approach to the pathogenesis of PNH but it must be emphasized that the role of such mutations as a driver is still speculative.

Different mutations of typical cancer genes can be found in healthy people with increasing age [28]. Recent data showed that many AA patients have mutations in *BCOR*, *AXSL1* or *DNMT3A* without a progression to PNH, myelodysplastic syndrome or acute myeloid leukemia. Patients with *BCOR* mutations had a good response to immunosuppression and overall survival while patients with *AXSL1* or *DNMT3A* mutation had a worse prognosis. On the other hand, some patients with *AXSL1* or *DNMT3A* mutations showed hematologic remissions with expanding clones without evidence of malignant transformation [27, 29].

A new hypothesis of Ratajczak et al. brings hemolysis in the context of clonal dominance. Their model describes PNH as a disease of increased mobility of *PIGA* mutated hematopietic progenitors. HSC have impaired adhesion to bone marrow niches due to impairment of lipid raft formation and show enhanced mobility. Stimulation by sphingosine-1 phosphate might stimulate mobilization into peripheral blood. Sphingosine-1 phosphate is enriched in erythrocytes and is released during lysis [30].

Further research is needed to determine the exact role of mutations, autoimmune reactions and other phenomena in the genesis in PNH and AA to present a consistent pathophysiologic model of PNH.

What drives complement: the induction of spontaneous hemolysis and new approaches of targeting complement

Strong activators of complement such as infections, inflammation, pregnancy and surgery lead patients to an increased risk of hemolytic crises and sometimes lethal TEs. But why does hemolysis take place in an otherwise healthy non pregnant PNH patient. The chronic hemolysis in PNH is mediated by a spontaneous hydrolysis of complement factor 3 (C3), the so-called tick over of the alternative complement pathway. Tick over generates a C3 convertase (C3bBbP) that would normally be inhibited by CD55 [31, 32]. Soluble factor H and factor I are plasma factors that inhibit C3 and should not be impaired in PNH patients, furthermore, it has been shown that factor H can modulate the hemolysis in PNH-erythrocytes in vitro [33]. Further cleavage of C3bBbP results in cleavage and activation of complement factor 5 (C5) and establishment of the membrane attack complex (MAC) (Figure 1). The MAC is normally inhibited by CD59 [6]. The MAC is a huge pore that is conductive for water and electrolytes and disrupts the integrity of the

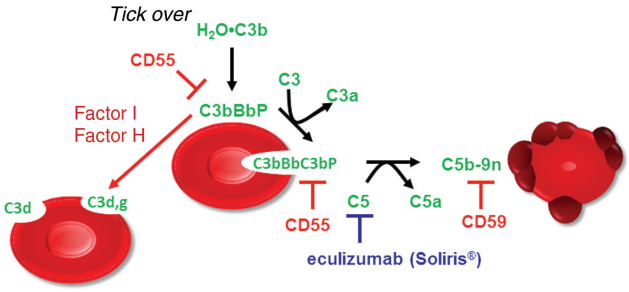

Figure 1: The mechanism of chronic hemolysis.
C3 is activated by spontaneous hydrolysis. This process is called tick over. The tick over generates a C3 convertase, that cleaves C3 and generates thus a C5 convertase that activates C5. Activated C5 induces the membrane attack complex. In physiological states the establishment of the MAC is prevented by CD59 and CD55 prevents binding of activated C3. Factor I and factor H are soluble complement inhibitors that cleave C3b to C3d. C3d might protect erythrocytes from further complement attacks [68–70]. Eculizumab prevents cleavage of C5 but does not prevent binding of C3 fragments that might induce opsonization and thus extravascular hemolysis.

cell membrane and induces thus intravascular hemolysis in PNH. The C5 inhibitor eculizumab prevents the formation of the MAC and protects CD59 deficient erythrocytes against lysis. Alternative strategies of C5 inhibition are currently under investigation. A clinical trial using a RNAi that targets C5 (Aln-CC5) production in the liver was initiated [34]. A trial with a cyclic peptide inhibitor of C5 (RA101348) is in preparation [35]. Coversin is another C5 inhibitor that additionally inhibits leukotriene B4. It is a recombinant protein derived from a tick (*Ornithodros moubata*) where it prevents host immunological reactions during feeding. Such newer strategies might overcome some limitations of terminal complement inhibition by the monoclonal antibody eculizumab. The so-called breakthrough hemolysis presents typically at the end of a 14 day application cycle due to the declining action of the antibody [36]. A polymorphism p.Arg885His of C5 that disrupts the eculizumab epitope is responsible for the poor response in 3.2% of Japanese PNH patients. This polymorphism has a relatively high prevalence in Asians as 3.5% of Japanese showed the mutations and it is present in Han Chinese [37]. The sparing of inhibition of upstream complement activation by eculizumab has clinical relevance as many patients still show signs of hemolysis and need transfusion under therapy [10]. Terminal complement inhibition shifts hemolysis to extracellular due to bound activated C3 fragments. Without therapy opsonization plays no role because the erythrocyte is lysed anyhow by the MAC before it reaches the reticuloendothelial system [38–40].

The last issue is addressed by targeting complement factors that are upstream of C5. An interesting approach

was a fusion protein of factor H and complement receptor 2 named TT30. TT30 inhibits complement on the level of C3 and should therefore prevent the binding of C3 fragments [41]. Unfortunately the clinical trial was stopped because of an inability to reach the planned enrollment. Variants of factor H, an inhibitor of C3 are also under development [42]. The experimental agent H17 is a chimeric deimmunized antibody against the complement fragment C3b with in vitro activity [31]. APL-2 is a C3 inhibiting peptide that is currently under clinical investigation for the indication of PNH. Aurin tricarboxylic acid, a topoisomerase inhibitor also shows activity against different complement factors including C3 [43]. Other C3 targeting agents are the peptide inhibitors Cp40 in PEGylated and non-PEGylated forms [44].

Beside targeting C5 and C3, inhibition of C1 esterase [45] or factor D [46] showed also promising in vitro activity.

The field of complement inhibition is growing rapidly. We are seeing a focus on the development of C3 inhibitors. The first clinical trials are on the way so we are expecting new therapeutic options to be available in the near future.

What we have learned from complement inhibitory therapy: the consequences of intravascular hemolysis

Eculizumab was first approved for the avoidance of transfusion. But from the TRIUMPH study we have learned that patients benefit beyond avoidance of transfusion [10].

The quality of life improved dramatically in these patients. And that was not just explained by the higher hemoglobin level [10]. Hemolysis makes the patient sick by itself, therefore interruption of the hemolytic process solves many problems of PNH patients. This holds true even when it relates to difficult outcome measures such as overall survival.

Clinical consequences of intravascular hemolysis damage include renal failure, secondary pulmonal hypertension, dysphagia, abdominal or chest pain episodes and life threatening TEs [5]. All these manifestations can be linked to nitric oxide (NO)-depletion that is caused by the constituents of the erythrocytes [47–50]. The transformation of hemoglobin into methemoglobin consumes NO and this reduces NO that leads to smooth muscle dystonia and lack of inhibition of platelet aggregation and factor XIII. Arginase which is released from lysed red cells consumes L-arginin, the substrate of the NO-synthase. Thus hemolysis consumes NO and impairs its synthesis [50]. Additional hemolysis dependent mechanisms contribute to the genesis of the diathesis of TEs. The most important mechanisms are the generation of microvesicles that express phosphatidylserine on their “extracellular” site and an interaction of two serine protease systems that can activate each other: the complement and the clotting system [49, 51–55] (Figure 2). For more detailed discussion of this important and complex issue we suggest the excellent review from Hill et al. [49].

With the interruption of intravasal hemolysis NO levels improve and so do the consequences of hemolysis. Most importantly the incidence of thrombosis declines about 85% in retrospective analysis from the international eculizumab studies TRIUMPH, SHEPHARD and EXTENSION [56]. Analysis of the registry data confirmed these findings [57]. TE is the main reason for mortality. In congruence to this finding Kelly et al. have shown that eculizumab treated patients had equal overall survival with age matched controls in the British eculizumab cohort [58]. Again this finding was confirmed by the analysis of the international eculizumab cohort [59]. These findings underline the central role of complement activation in the genesis of morbidity and mortality in PNH patients.

Size still matters. Whom to treat?

The IPIG consensus meeting classified PNH in three categories: classical or hemolytic PNH, the PNH in the context of another marrow failure syndrome such as aplastic anemia and the so-called subclinical PNH [1].

It is difficult to define a clone size from the patients that are prone to developing symptoms. Very small clones lacking GPI-anchored proteins can even be found in healthy people [60].

Baseline data of the international PNH registry showed that even clones smaller than 10% can induce typical symptoms of intravascular hemolysis but were usually more frequent in the group of patients with granulocyte clones >50% [5]. Most of the reported symptoms can be seen in the context of NO depletion as explained above.

The most striking consequence of PNH is the development of a TE. These events tend to be located at unusual sites like abdominal thrombosis (e.g., Budd-Chiari syndrome) or cerebral vein occlusion (Table 2). Pulmonary embolisms or deep veins are involved in <40% of cases and central nervous system or myocardial events occur in <23% [56, 61, 62] (Table 2). Many untreated patients die of a thrombotic event [3, 4]. Prevention of TE is therefore a main goal of therapeutic intervention in every patient.

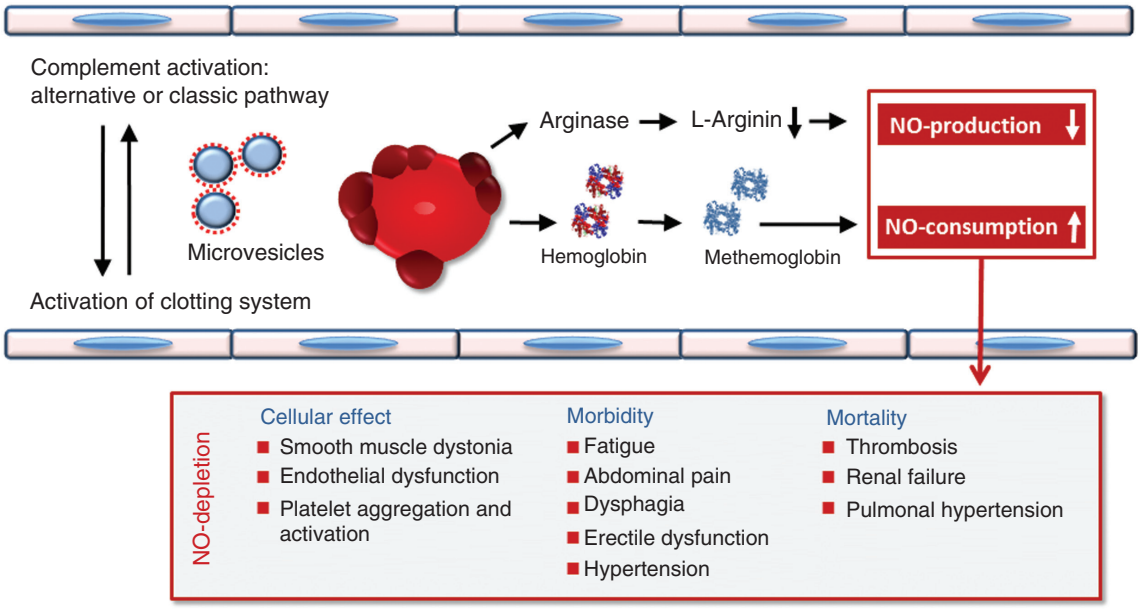

Figure 2: The consequences of intravascular hemolysis (adopted from [49, 50]).
Activation of complement via the alternative of classic pathway induce intravascular hemolysis. The erythrocyte releases hemoglobin. This is transformed into methaemoglobin by oxidation with nitric oxide (NO). This process consumes NO. Another ingredient of the erythrocyte is arginase, that catalyzes the formation of L-ornitin by consumption of L-arginine. Arginine is the substrate for the NO-synthase. Thus formation of NO is inhibited. These two processes induce NO-depletion. Microvesicles are generated from lysing erythrocytes, from platelets and endothelial cells (not shown) by complement activation. They are negatively charged because the expression of phosphatitylserine that is normally located in the intracellular side of the membrane bilayer. The binding of tenase and prothrombinase complex to the negatively charged membrane surface is facilitated by Ca^{2+} (not shown). The activated complement and clotting system can activate each other leading to a circulus vitiosus of thrombotic events in PNH (for more details see the review from Hill et al. [49]).

Table 2: Sites of thrombosis.

	Lee et al. [61]	Hillmen et al. [56]
Number of patients	301	195
Number of TE	81	124
TE site	proportion of events, %	
Arterial events	30.9	15.3
Cerebrovascular event	14.8	13.7
Myocardial infarction/ unstable angina	8.6	1.6
Venous events	69.1	84.6
Deep vein thrombosis	19.8	33.1
lower extremities	19.8	18.5
other	–	14.5[a]
Hepatic and portal vein	8.6	16.9
Mesenteric, visceral veins	11.1	18.5[b]
Renal vein	11.1	–
Pulmonary embolus	12.3	6.5
Cerebral veins or sinuses or jugular thromboses	2.5	5.6

[a]Including inferior vena cava, bilateral lower extremity, pelvic, ureter, axillary, subclavian, and brachiocephalic veins. [b]Including splenic veins. Note definitions of TE site might differ in the two studies. Not all sites of TEs mentioned in the studies are included.

A granulocyte clone of more than 50% was an established risk factor for the development of a TE and application of cumarines has been shown to lower the incidence of thrombosis in this group of patients [63]. A recently published analysis of 301 PNH patients from the South Korean National PNH Registry has not found a statistic significant correlation of clone size and risk of hemolysis but has shown that symptoms like hemoglobinuria, chest pain, abdominal pain, in conjunction with an elevated LDH of about 1.5 fold increases the risk for development of a TE from 10.3 (hematuria and dyspnea) to 17.8 (abdominal pain) up to 19.0 (chest pain) fold. LDH elevation alone increased the risk by a factor of 7.0 and symptoms without LDH elevation increase the risk no more than 2.9 fold [61].

Recent data from the international PNH patient registry of 1610 patients from 273 centers in 25 countries confirmed the increase of TE of about 15.4% of patients having had a TE in the group with granulocyte clone sizes of >50% vs. 5.3% in the group with smaller clones (<10%) ($p<0.05$). Levels of LDH also increased with size of the granulocyte clone. Assuming a strong pathophysiologic interdependence of hemolysis and thrombosis a positive

correlation of clone size and the occurrence of TE is reasonable. However, we have to emphasize that small clone sizes can induce substantial morbidity [5]. A threshold for a "safe" clone size could not be given.

Recently the effectivity of eculizumab was reviewed in a Cochrane review. Only data of randomized trials were considered. Therefore only the TRIUMPH study was included. The authors concluded that prescription of eculizumab for PNH could "neither be supported nor rejected, unless new evidence from a large high quality trial" could be established [64]. We rate the benefit of complement inhibition as more positive than the strict formalistic evaluation of the drug suggests. As mentioned above PNH is a rare disease. Our knowledge was mainly based on publications of single center evaluations over many years [3, 4, 65]. Performing randomized trials in a rare disease is challenging. The authors of the Cochrane review are presume incomplete outcome data of TRIUMPH as the first publication did not report all relevant outcome measures. However, a post hoc analysis of the TRIUMPH collective reported the data of TE [56] and long-term follow-up from the study collective is also available [66]. Malignant transformation, OS and TE are indeed of concern in PNH patients but are infrequent enough so that many more patients than the 87 of the TRIUMPH study are needed to evaluate these primary endpoints. Especially the assessment of malignant transformation also needs much longer follow-up.

The PNH registry enrolls patients in more than 30 states and has actually enrolled 3140 patients with and without eculizumab treatment. Analysis of all these available datasets provides evidence for normalization of OS and significant reduction of TEs. The findings of TRIUMPH were confirmed [57] – a retrospective analysis but on a large number of patients representing the real-world outcome of PNH. Given the effects of eculizumab on serious complications like TE and its impact on overall survival it would be difficult to justify a new clinical trial testing complement inhibition against placebo.

A few recommendations for the management of PNH-patients

Having read all this you might think "oh very interesting but how does this translate into practice?".

First of all consider PNH as a differential diagnosis. Perform flow cytometry of peripheral blood cells to test for GPI-anchor or GPI-linked proteins in states of Coombs negative hemolysis, bone marrow failure, atypical thrombosis and unexplained iron deficiency.

We suggest following the German guidelines to manage patients with PNH [67] (Figure 3). Always keep in mind the high risk arising from TE. Everything that puts a patient at risk of strong complement activation like surgery, infection or pregnancy should alert physician and patient.

Ask patients about hemolysis associated symptoms irrespective of clone size. Perform repeated testing of clone size, especially in patients with bone marrow failure syndromes like AA.

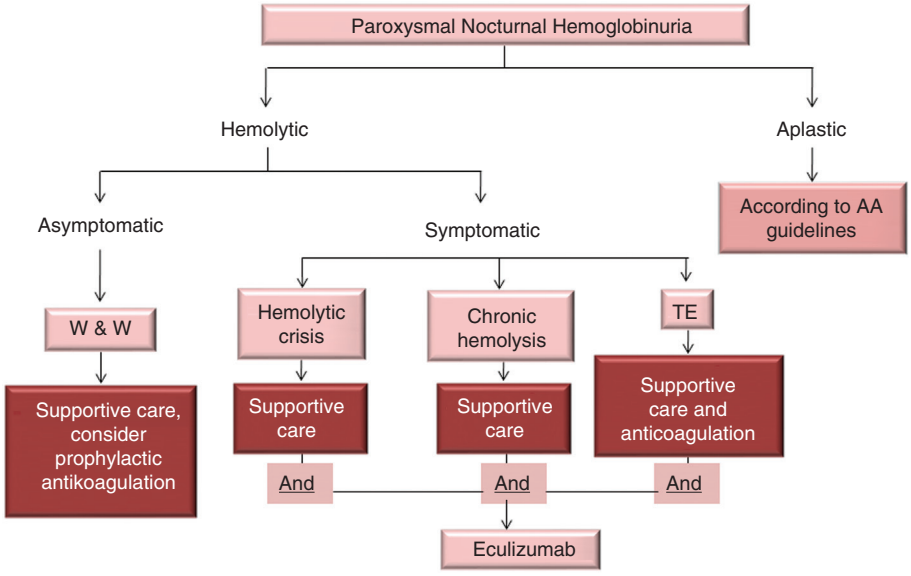

Figure 3: Treatment algorithm adopted from the German PNH guidelines [67].
Treatment differs between patients with hemolytic PNH and patients with pancytopenia. Patients with pancytopenia are treated according to guidelines of aplastic anemia. The decision to treat with eculizumab depends on symptoms regardless of clone size.

For any symptomatic patient we suggest giving eculizumab regardless of clone size. For any asymptomatic patient with clone size of >50%, prophylactic anticoagulation should be considered (cumarine derivate or equivalent) unless treated with eculizumab. There are no trials for new anticoagulants or even heparines. Heparines are often used in PNH, we consider them as equivalent to cumarines. For any patient without eculizumab prior to a significant complement activating stimulus (e.g., pregnancy) eculizumab with or without anticoagulation should be considered. Overlap syndromes with bone marrow failure and hemolysis or other symptoms due to PNH need careful evaluation of the relative contribution of bone marrow failure and PNH to the overall clinical presentation. The plan for treatment of such complex cases should be set up after consultation with an expert center.

In case of life threatening TE consider immediate eculizumab in conjunction with conventional therapy of the thrombotic site.

In cases where eculizumab has no therapeutic effect keep in mind that some ethnic groups like Asians show variants of C5 that lead to poor response. C5 genotyping should be considered in such patients.

Summary

Our view on PNH has changed over the last years. We have to become familiar with a perspective of thinking about PNH as a heterogeneous disease entity with different disease manifestations and different risks for developing complications such as thromboembolic events or aplastic anemia. We have learned a lot about the central role of complement dependent hemolysis and its substantial clinical consequences. The successful use of eculizumab has encouraged developments of new complement inhibitors. Despite these progresses the genesis of PNH is still elusive. Understanding the molecular and cellular processes underlying the clonal dominance of *PIGA* mutated hematopoiesis might lead us to new curative treatments beside allogeneic stem cell transplantation, which is still the only curative treatment in PNH but comes with a high morbidity and mortality rate.

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