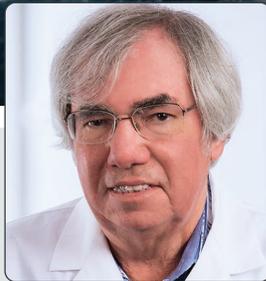# THE COMPLEX CAUSES OF COMPLEMENT-MEDIATED HEMOLYSIS IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA:

SIMPLIFIED BY EXPERTS

Lawrence Rice, M.D.
Chief of Hematology,
Houston Methodist Hospital
Houston, TX

David Dingli, M.D., Ph.D.
Division of Hematology,
Mayo Clinic,
Rochester, MN

In this Expert Perspectives newsletter, Dr Lawrence Rice and Dr David Dingli share their knowledge of the normal and dysregulated complement system, how these defects contribute to disease symptoms, and unmet treatment needs in the management of PNH.

Paroxysmal nocturnal hemoglobinuria (PNH) is caused by an acquired deficiency in complement regulatory proteins that renders red blood cells (RBCs) vulnerable to lysis.[1] At its core, PNH is characterized by the triad of hemolysis, bone marrow failure, and an increased risk for thromboembolism.[2] Significant, and often disruptive, fatigue is one of the most common symptoms of PNH.[3] Anemia in PNH is multifactorial and may result as a combination of both hemolysis and bone marrow failure.[3] Patients also suffer from a broad and variable range of additional signs and symptoms, including an increased risk for thrombosis, renal impairment, and hemoglobinuria, as well as symptoms related to smooth muscle dystonia.[1]

Fundamentally, hemolysis in PNH is driven by inappropriate activation of the complement system on the surface of RBCs that is primarily caused by the alternative pathway of the complement system.[4] PNH is largely characterized by intravascular hemolysis mediated by the terminal complement system, which culminates in the uncontrolled formation of the MAC—an assembly of proteins that forms a pore in target cell membranes.[1,4]

C5 inhibitors marked a significant breakthrough in the management of patients with PNH. However, there is still room to improve outcomes in patients with PNH. In particular, the C5 inhibitors address only the terminal complement system, which is linked to intravascular hemolysis.[4] In some patients treated with these agents, continued activity of the alternative pathway may render RBCs susceptible to ongoing extravascular hemolysis.[4,5] Proximal complement inhibition (Factor B, Factor D, and C3) has been identified as an emerging topic of interest in PNH due to its potential to affect both IVH and EVH.

THE COMPLEMENT SYSTEM

The complement system is a critical component of the immune system that plays a role in both innate and adaptive immune responses.[6] (Figure 1).[7] It is divided into three different pathways: classical, lectin, and alternative. All three converge at the activation of C3 convertase.[7]

The alternative pathway is continuously activated at a low level by spontaneous hydrolysis of C3 into C3a and C3b fragments.[7] The proximal complement system is initiated when Factor B binds C3b and is cleaved by Factor D, forming the C3 convertase. The formation of the C3 convertase results in an amplification loop that dramatically accelerates C3 cleavage into its fragments.[7]
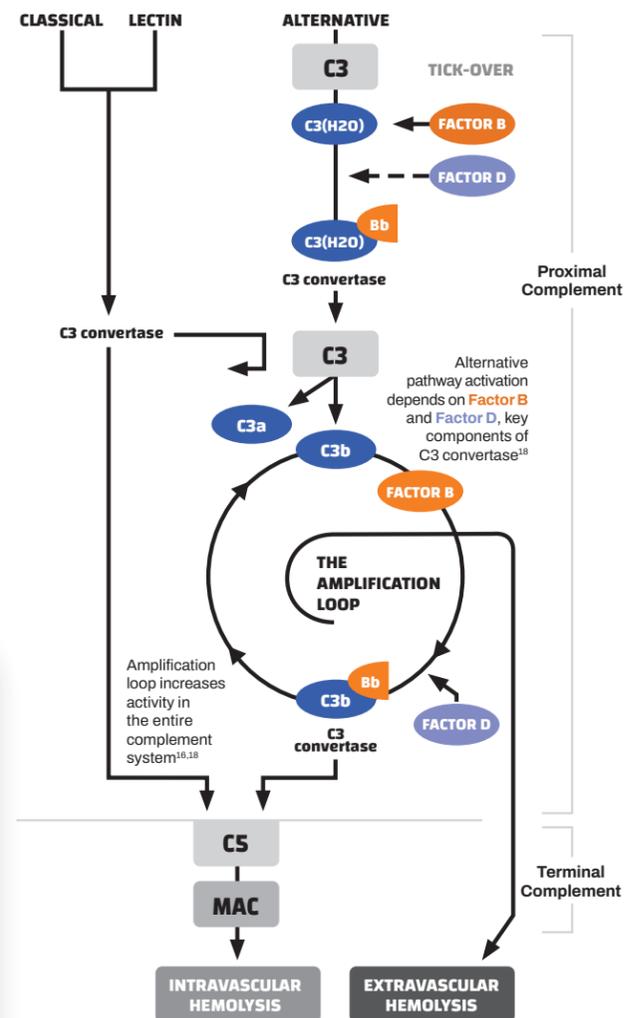
The C3b fragment plays two important roles in the normal complement system[1,7]:

- **It initiates the terminal complement system by forming part of the C5 convertase. The terminal complement system culminates in the formation of the downstream membrane attack complex (MAC) and target cell lysis.**

- **C3b binds to target cells, tagging them for destruction by phagocytes in the liver and spleen.**

Figure 1. The Complement System[7]

> " *The complement system is a critical part of both innate and adoptive immunity that has allowed us to survive and evolve by protecting us against infectious insults that have been constant threats to all living species.* "

\- DR LAWRENCE RICE

WHAT IS THE FUNDAMENTAL DEFECT IN PNH?

PNH is an acquired clonal disorder of hematopoietic stem cells (HSCs) caused by a somatic mutation in the *PIGA* gene that results in RBC progeny that are vulnerable to lysis.[1] These mutations lead to partial or complete inability to synthesize glycosylphosphatidylinositol (GPI) anchors, which are required to tether many proteins, including CD55 and CD59, to the cell surface.[8] These critical cell-surface proteins act like natural brakes on the complement system through inhibition of C3 convertase formation (CD55) and prevent polymerization of the membrane attack complex (CD59), thus preventing inappropriate activation.[9]

> " *The fundamental defect in PNH is in biosynthesis of GPI anchors, which tether many proteins, including CD55 and CD59, to the cell membrane. These two proteins normally act as brakes on the complement system—CD55 blocks C3, and CD59 blocks formation of the MAC.* "

\- DR DAVID DINGLI

PNH HSCs may expand and become a significant fraction of the overall HSC population. Two proposed mechanisms of clonal expansion include clonal selection by extrinsic factors (for example, aplastic anemia) and intrinsic clonal evolution (HSCs acquiring additional mutations).[1] It is thought that PNH HSCs are protected against immune attack, resulting in a relative growth advantage over normal HSCs.[1] Thus, the RBCs derived from HSCs that lack CD55 and CD59 can, ultimately, come to represent a substantial fraction of the overall population of RBCs in patients with PNH. These cells, which are unable to inhibit complement activation on their cell surfaces, are thus vulnerable to attack and destruction by the complement system.

> " *The complement system needs to be kept under control—when it goes awry, serious diseases ensue, one of which is PNH.* "

\- DR LAWRENCE RICE

THE COMPLEX CAUSES OF HEMOLYSIS IN PNH

In patients with PNH, more than 5% of red blood cells lack CD55 and CD59 making them susceptible to complement-mediated hemolysis.

Patients with untreated PNH experience constant, low-level hemolysis. Accelerated hemolysis (hemolytic crises) can occur in the presence of triggers that activate complement, such as infection.[1]

There are two types of hemolysis in PNH **(Figure 2)**:

- *Intravascular* **hemolysis occurs due to the lack of CD55 and CD59, formation of the MAC on PNH RBCs, leading to complement-mediated cell lysis**[4]

- *Extravascular* **hemolysis occurs due to the lack of CD55 and deposition of C3 fragments, tagging PNH RBCs for removal and destruction by phagocytes in the liver and spleen**[10]

Anemia in PNH can result from a combination of both hemolysis and bone marrow failure. Hemolysis in PNH is predominantly intravascular; however, in some patients on terminal complement inhibitors, extravascular hemolysis can contribute to the overall clinical picture.[1,5] The dual pathways by which RBCs are destroyed in PNH also have potential implications for treatment.
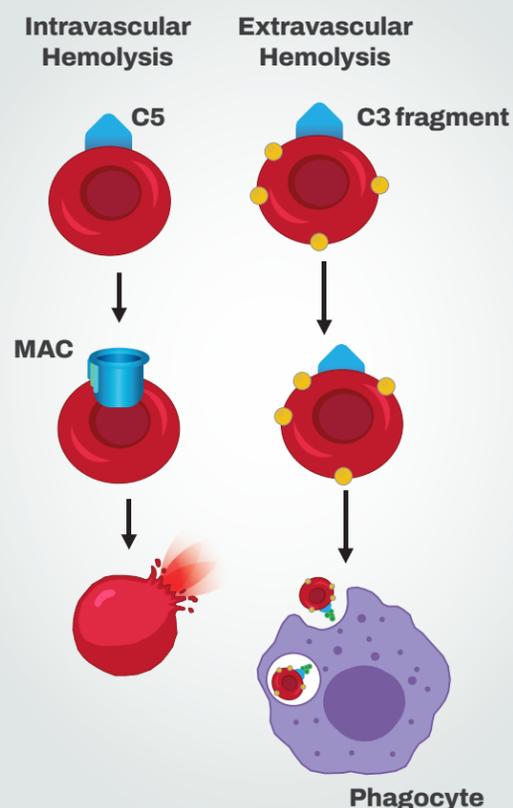
Intravascular Hemolysis **Extravascular Hemolysis**

C5

C3 fragment

MAC

Phagocyte

Figure 2. Intravascular and extravascular hemolysis can contribute to RBC destruction in PNH[10,11]

> " *In untreated patients, intravascular hemolysis is the main way that RBCs are being destroyed. C5 inhibitors block formation of the MAC, and intravascular hemolysis is largely controlled. However, C5 inhibitors compensate for the loss of CD59, but do nothing for CD55 deficiency. The process upstream of C5 is ongoing, resulting in the accumulation of C3b. These cells become targets for monocytes in the liver and spleen.* "

- DR DAVID DINGLI

# EVIDENCE OF ONGOING HEMOLYSIS IN PATIENTS WITH PNH WHO ARE ON TREATMENT

Terminal complement blockade with C5 inhibitors is an FDA-approved treatment modality for patients with PNH.[12,13] While C5 inhibitors are effective in managing PNH in many patients, incomplete blockade in some patients, and events that suddenly increase alternative pathway activity, such as infection, inflammation, and bone marrow failure, may result in episodes of hemolysis.[14]

Clinically, ongoing extravascular hemolysis may manifest as a continued need for RBC transfusion during C5 inhibitor treatment.[15] A study aimed to examine whether residual hemolysis in C5-treated patients with PNH may be explained by extravascular RBC clearance through C3-mediated opsonization. The study tested samples from C5 inhibitor-treated (n=31) and untreated (n=39) patients using the direct antiglobulin test (DAT), an assay used to detect complement on the surface of RBCs. In total, 21 of 31 (68%) C5 inhibitor-treated patients and 3 of 39 (8%) untreated patients were DAT positive, suggesting some degree of complement activation on the surface of these cells.[15] Among treated DAT-positive patients, 16 of 21 (76.2%) required ≥1 transfusion during a median of 11.5 months of treatment vs 1 of 10 (10.0%) patients who were DAT negative who were treated for a median duration of 10.5 months, *P*<0.01. The reduction in transfusion requirements while on C5 inhibitor therapy was significant in both groups relative to pre-treatment requirements, regardless of DAT positivity (*P*<0.01). Mean hemoglobin levels were lower in the DAT-positive vs the DAT-negative group (9.6 ± 0.3 g/dL vs 11.0 ± 0.4 g/dL; *P*=0.02) **(Figure 4)**.[15]

Doctor portrayal.

Figure 4. Transfusion requirements and hemoglobin levels in DAT-positive and DAT-negative patients treated with a C5 inhibitor.[15]
Figure adapted from Hill A et al, 2010.
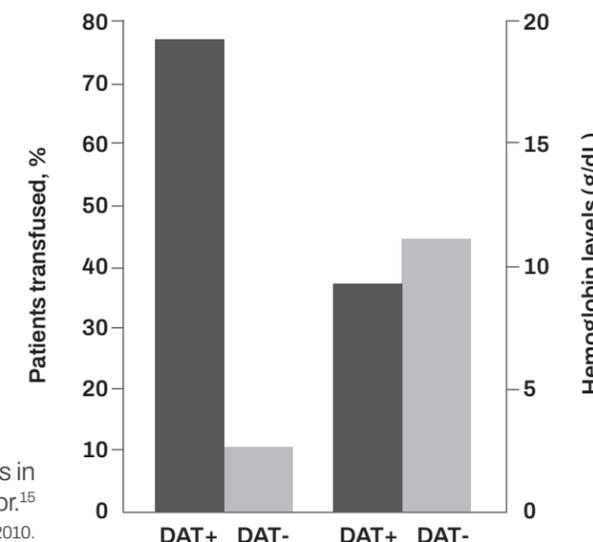
Extravascular hemolysis can persist in some patients on C5 inhibitors due to C3 accumulation on RBCs that are not destroyed by MAC intravascularly.[4] In a study of patients receiving these agents, ongoing deposition of C3 fragments on RBCs was seen (**Figure 5**). Further, when RBCs are radiolabeled, excess radioactivity counts can be detected in the liver and spleen, indicating ongoing RBC destruction in these organs.[5]
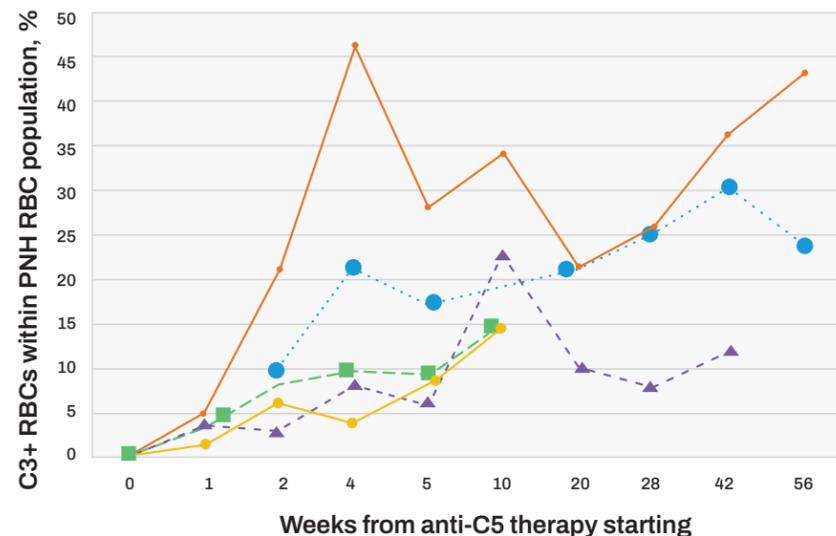

Figure 5. Increasing deposition of C3 fragments on RBCs after initiation of C5 inhibitor therapy. Each line represents an individual patient sample.[5]
Adapted from Risitano A et al, 2009.
Additional studies are needed to further understand the impact of terminal complement inhibition on EVH.

> " *Some patients treated with C5 inhibitors develop extravascular hemolysis. The dynamics of intravascular and extravascular hemolysis is a function of the natural history of the disease and whether patients are on therapy or not.* "

- DR DAVID DINGLI

CONSEQUENCES OF ONGOING HEMOLYSIS IN PATIENTS WITH PNH

While there may be several reasons for suboptimal response to C5 inhibitor therapy, some patients on C5 inhibitors may experience C3-mediated extravascular hemolysis.[2,5]

Incomplete disease control has important clinical consequences for patients with PNH. For example, in one international prospective study, 63% of patients treated with a terminal complement inhibitor (N=41) using a standard dose and schedule showed signs of ongoing hemolysis (as demonstrated by a hemoglobin remaining below 11 g/dL), with 44% demonstrating major response (hemoglobin ≥8 g/dL and transfusion independence), 12% partial (reduction in blood transfusions ≥50%), and 7% minor (no significant change in transfusions or hemoglobin, but a reduction in LDH).[5]

Some patients treated with C5 inhibitors remain transfusion dependent; in one US patient-reported survey (N=122) investigating the symptom burden of PNH among the 54 patients treated with C5 inhibitors for over a year, approximately one-third reported having ≥1 RBC transfusion.[16]

- **Both intravascular and extravascular hemolysis play roles in PNH[1,10]**

- **Extravascular hemolysis is not addressed by terminal complement inhibition[4,5]**

- **Proximal complement inhibition (Factor B, Factor D, and C3) has been identified as an emerging topic of interest in the management of PNH[2]**

> " *Lack of response to C5 inhibitor therapy is rare, but suboptimal responses occur in clinical practice. These patients often continue to require intermittent transfusions.* "

- DR DAVID DINGLI

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