Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal hematopoietic stem cell disorder that manifests with hemolytic anemia, bone marrow failure, and thrombosis.

Current status

- PNH is due to a deficiency of cell surface molecules which inhibit the action of complement on red cells

History

- One of the earliest descriptions of PNH was by Dr Paul Strübing, who in 1882 described a 29-year-old man who presented with fatigue, abdominal pain, and severe nocturnal paroxysms of hemoglobinuria. Strübing deduced that the hemolysis was occurring intravascularly as the patient's plasma turned red following severe attacks of hemoglobinuria. Later reports by Marchiafava and Micheli led to the eponym, Marchiafava-Micheli syndrome, but it was Enneking, in 1925, who introduced the term paroxysmal nocturnal hemoglobinuria.

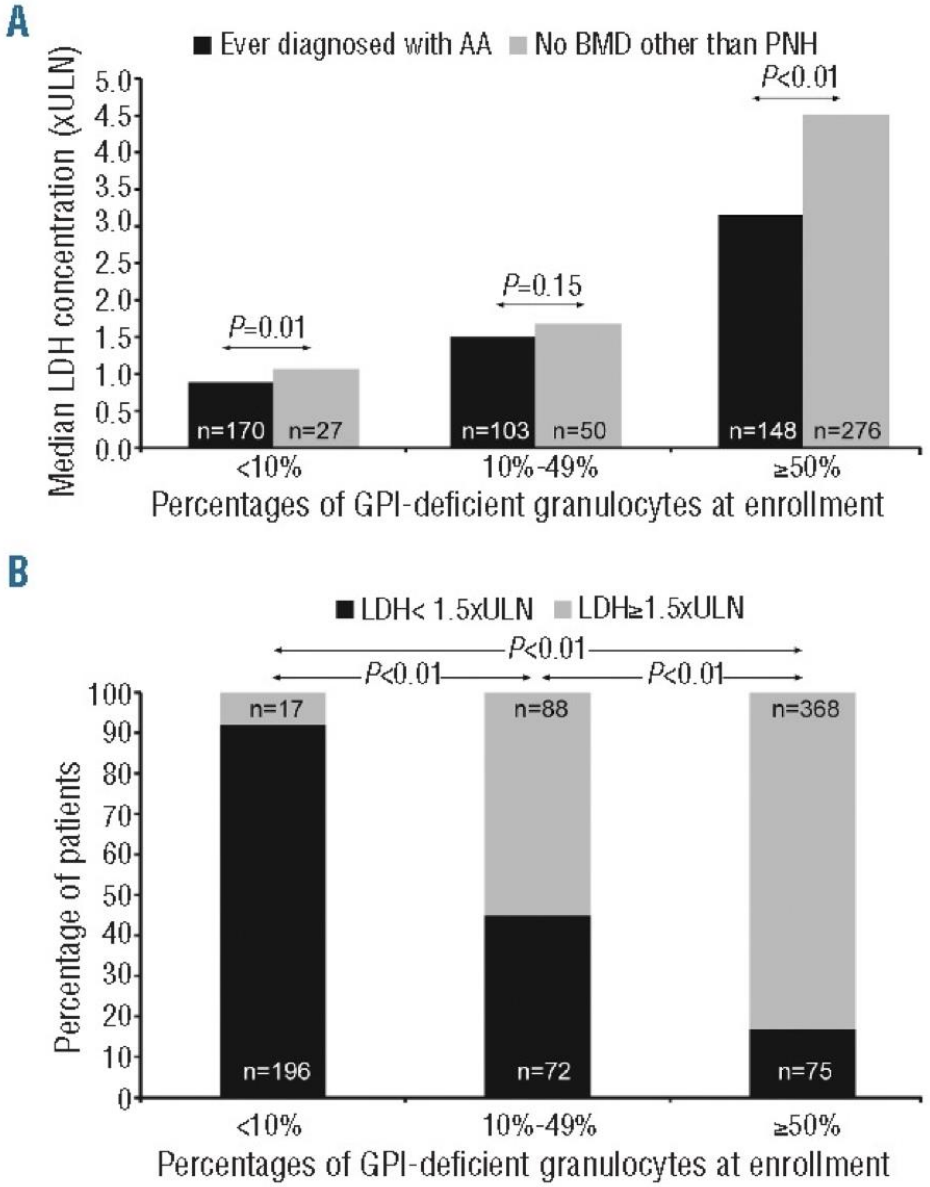
Early diagnostic tests

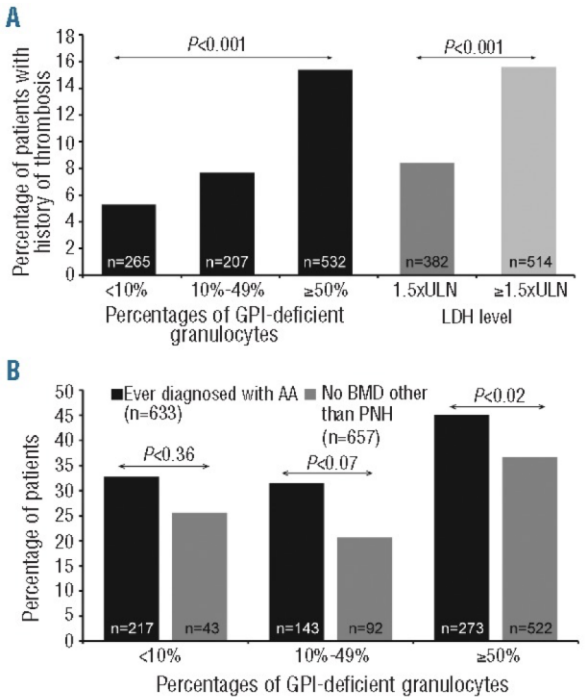
Thomas Hale Ham investigated the possibility that sleep-associated acidosis contributed to hemolysis in PNH. He discovered that lowering the pH of fresh plasma from 7.0 to 6.5 resulted in the lysis of PNH RBCs.

Later, in the 1960s, the sucrose hemolysis test (SLT) was devised. This test is a superior screen for PNH. The SLT uses the principle that low ionic strength isotonic sucrose causes serum globulin aggregates to fix complement on the surface of RBCs.

Parameter	Patients (n=1610)
Age, years, median (range)	42 (3-99)
Females, n (%)	857 (53.2)
Age at disease start, years, median (range)	32 (3-87)
Disease duration, years, median (range)	4.6 (<1-47)
Lactate dehydrogenase, ×ULN, median (5^{th}, 95^{th} percentile)[a]	1.96 (0.65, 10.32)
Hematologic parameters, median (5^{th}, 95^{th}percentile)	
Hemoglobin, g/L (n=1425)	106 (70, 145)
Platelets, $\times 10^9$/L (n=1430)	131 (19, 271)
Absolute neutrophils, $\times 10^9$/L (n=1275)	1.7 (0.00, 5.10)
Absolute reticulocytes, $\times 10^9$/L (n=971)	113 (27, 400)
Reticulocytes, % (n=1024)	3.6 (0.82, 13.06)
Granulocyte clone size, %, median (5^{th}, 95^{th} percentile)	68.1 (0.36, 99.30)
<10%, n (%)	280 (17.4)
10-49%, n (%)	247 (15.3)
≥50%, n (%)	832 (51.7)
Unknown, n (%)	251 (15.6)
History of thrombotic events, n (%)[b]	250 (15.5)
1	169 (10.5)
2	45 (2.8)
3	19 (1.2)
4+	10 (0.6)
Unknown	7 (0.4)
Time (years) since most recent thrombotic event, median (range)	3.8 (<1-45)
History of impaired renal function, n (%)[b]	223 (13.9)
Anticoagulation therapy, n (%)[b,c]	501 (31.1)
Immunosuppressive therapy, n (%)[b,c]	301 (18.7)
Pain medication, n (%)[b,c]	133 (8.3)
Eculizumab, n (%)[c,d]	411 (25.5)

[a]Includes only patients who had not received eculizumab in the year prior to enrollment and who had LDH value available (n=900). [b]Data not available for 60 patients (3.7%). [c]Any use in the 12 months prior to enrollment. [d]An additional 80 patients (5.0%) were reported as having received treatment with eculizumab, though the dates of administration were unknown. PNH: paroxysmal nocturnal hemoglobinuria; ULN: upper limit of normal; BMD: bone marrow disorder; AA: aplastic anemia.


A
Ever diagnosed with AA
No BMD other than PNH
Median LDH concentration (xULN)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P<0.01
P=0.15
P=0.01
n=170
n=27
n=103
n=50
n=148
n=276
<10%
10%-49%
≥50%
Percentages of GPI-deficient granulocytes at enrollment
B
LDH< 1.5xULN
LDH≥1.5xULN
P<0.01
P<0.01
P<0.01
Percentage of patients
100
90
80
70
60
50
40
30
20
10
0
n=17
n=88
n=368
n=196
n=72
n=75
<10%
10%-49%
≥50%
Percentages of GPI-deficient granulocytes at enrollment


A
Percentage of patients with history of thrombosis
P<0.001
P<0.001
n=265
n=207
n=532
n=382
n=514
<10%
10%-49%
≥50%
1.5xULN
≥1.5xULN
Percentages of GPI-deficient granulocytes
LDH level
B
Ever diagnosed with AA (n=633)
No BMD other than PNH (n=657)
P<0.36
P<0.07
P<0.02
Percentage of patients
n=217
n=43
n=143
n=92
n=273
n=522
<10%
10%-49%
≥50%
Percentages of GPI-deficient granulocytes

Treatment	Number (%) of patients	
	Ever diagnosed with AA (n=701)	With no history of BMD (n=776)
Anticoagulation therapy[b]	**147 (21.0)**	**326 (42.0)**
Immunosuppressive therapy[b,c]	**270 (38.5)**	**22 (2.8)**
Eculizumab therapy[b]	**131 (18.7)**	**262 (33.8)**
Red blood cell transfusion[d]	262 (37.4)	266 (34.3)
Immunosuppressive therapy[c] plus:		
Anticoagulation	**35 (5.0)**	**6 (0.8)**
Eculizumab	**37 (5.3)**	**8 (1.0)**
Red blood cells	**123 (17.6)**	**13 (1.7)**
Anticoagulation plus:		
Eculizumab	**53 (7.6)**	**108 (13.9)**
Red blood cells	**63 (9.0)**	**124 (16.0)**
Eculizumab plus red blood cells	60 (8.6)	84 (10.8)

Bold values indicate statistically significant difference between groups (P<0.001) calculated from χ^2 test for categorical variables and Student's t-test for continuous variables. [a]Patients may have received more than 1 type of treatment. [b]In prior 12 months. [c]Immunosuppressive therapy includes cyclosporine and/or anti-thymocyte globulin. [d]In prior 6 months. PNH: paroxysmal nocturnal hemoglobinuria; AA: aplastic anemia; BMD: bone marrow disorder.

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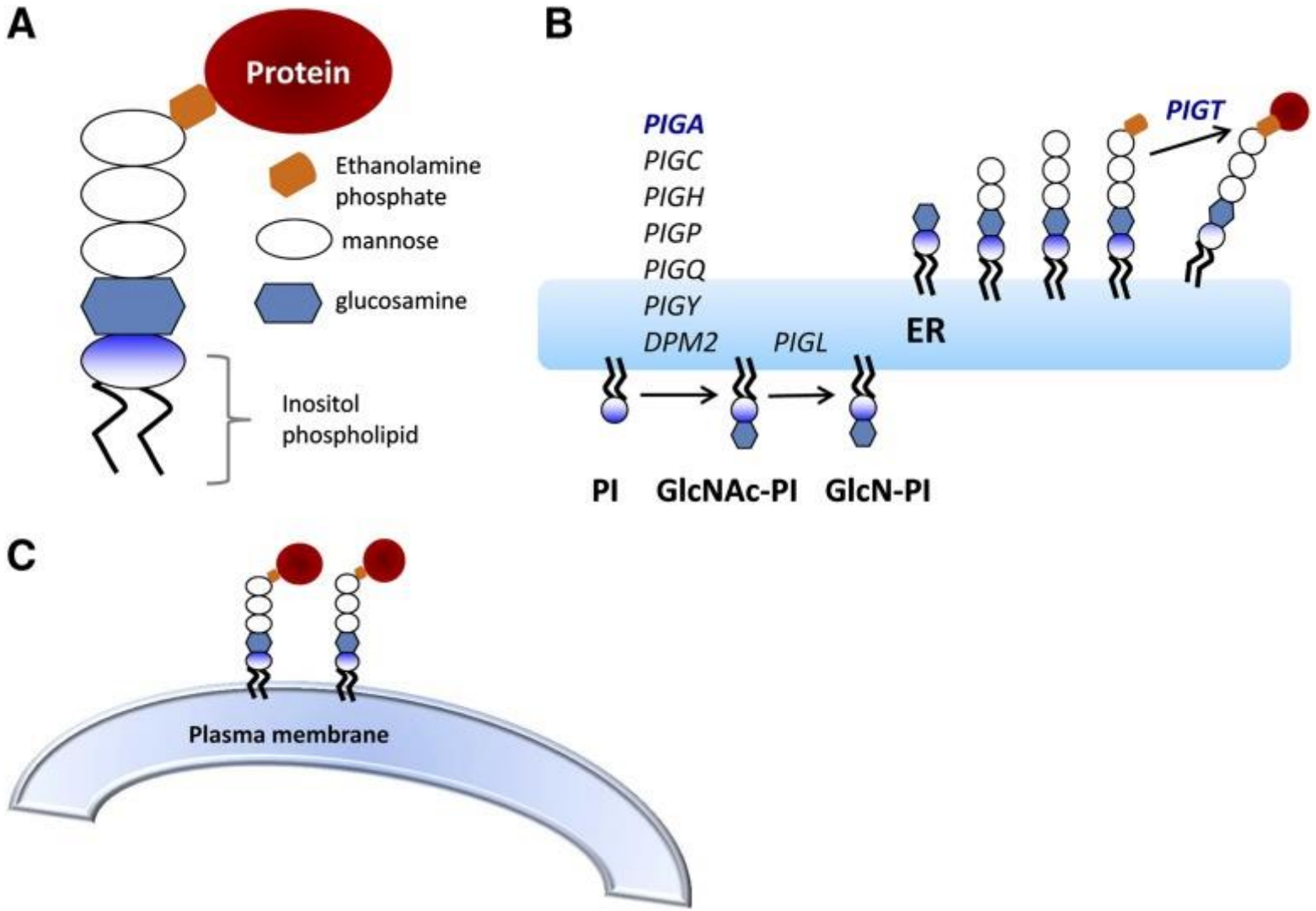
Figure 1

GPI anchor biosynthesis. (A) Core structure of the GPI anchor. The inositol-phospholipid (PI) is anchors into the lipid bilayer of the plasma membrane. The glycan core consists of a molecule of *N*-glucosamine, 3 manose molecules (Man), and a molecule of ethanolamine phosphate. The protein is covalently attached through an amide bond to an ethanolamine on the terminal mannose. (B) GPI anchor biosynthesis takes place in the endoplasmic reticulum. *PIGA* is 1 of 7 subunits involved in the first step of GPI anchor biosynthesis. There are ≥10 additional steps and >25 genes involved. After the protein is attached to the GPI anchor, the mature GPI-anchored protein goes to the Golgi, where fatty acid remodeling occurs and (C) eventually the GPI anchored protein is transported to the plasma membrane.


A
Protein
Ethanolamine phosphate
mannose
glucosamine
Inositol phospholipid
B
PIGA
PIGC
PIGH
PIGP
PIGQ
PIGY
DPM2
PIGL
PIGT
ER
PI
GlcNAc-PI
GlcN-PI
C
Plasma membrane

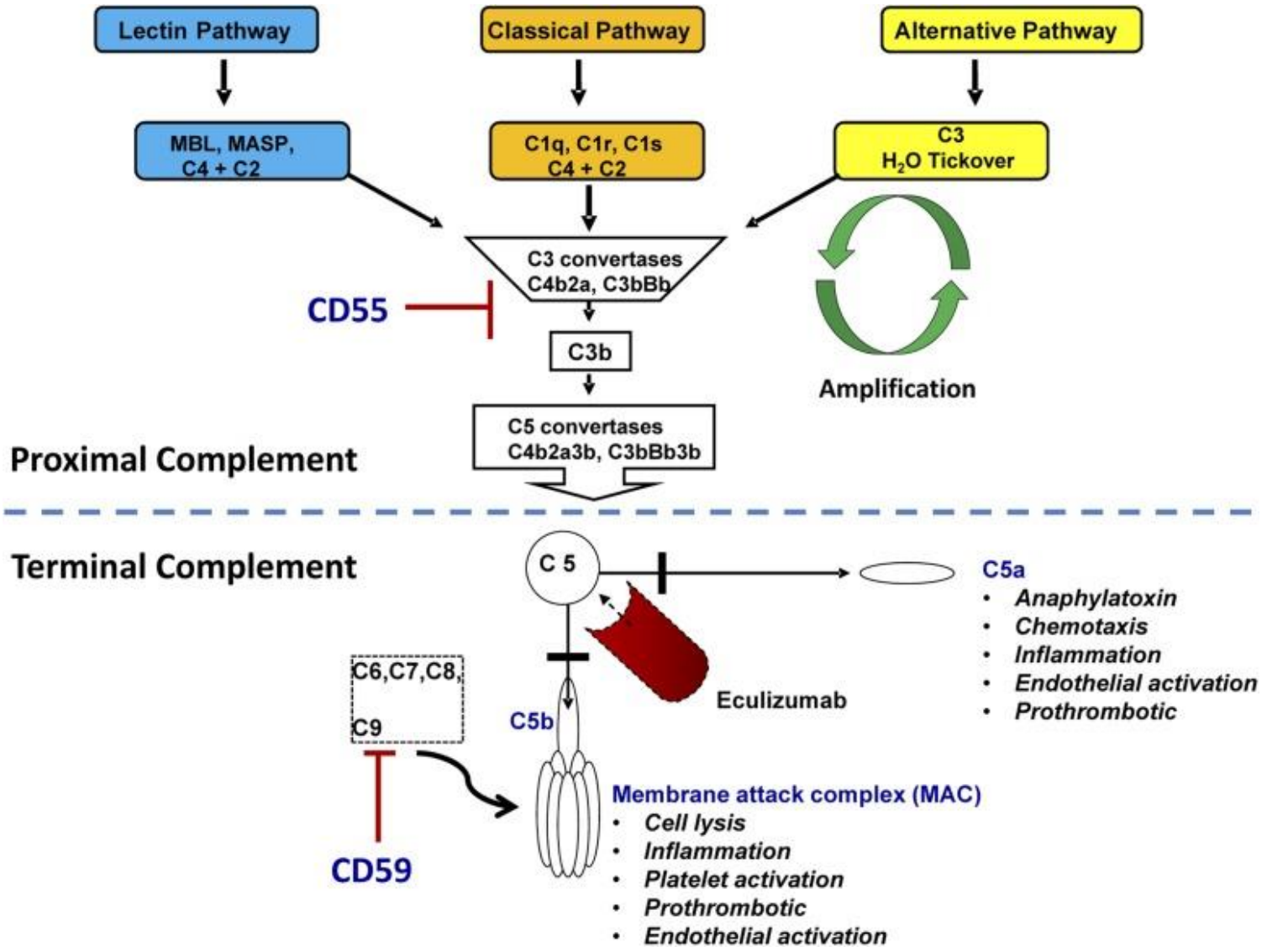

Lectin Pathway
Classical Pathway
Alternative Pathway
MBL, MASP, C4 + C2
C1q, C1r, C1s C4 + C2
C3 H_2O Tickover
C3 convertases C4b2a, C3bBb
CD55
C3b
Amplification
C5 convertases C4b2a3b, C3bBb3b
Proximal Complement
Terminal Complement
C 5
C5a
• Anaphylatoxin
• Chemotaxis
• Inflammation
• Endothelial activation
• Prothrombotic
Eculizumab
C6,C7,C8, C9
C5b
CD59
Membrane attack complex (MAC)
• Cell lysis
• Inflammation
• Platelet activation
• Prothrombotic
• Endothelial activation

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Figure 2

Complement regulation and eculizumab. The lectin, classical, and alternative pathways converge at the point of C3 activation. In PNH, hemolysis is usually chronic because the alternative pathway is always in a low-level activation state through a process known as tick-over. Terminal complement begins with cleavage of C5 to C5a and C5b. C5b oligomerizes with C6, C7, C8, and multiple C9 molecules to form the MAC. CD55 inhibits proximal complement activation by blocking the formation of C3 convertases; CD59 inhibits terminal complement activation by preventing the incorporation of C9 into the MAC. The absence of CD55 and CD59 on PNH cells leads to hemolysis, inflammation, platelet activation, and thrombosis. Eculizumab inhibits terminal complement activation by binding to C5 and preventing generation of C5a and C5b.

Pathophysiology #!

- Hemolysis in PNH is complement driven and is a result of a deficency of complement regulatory proteins.
- An expansion of hematopoietic stem cells with a deficiency in GPI a glycolipid which anchors over 150 proteins to cell surface.
- Somatic mutation inPIGA, an x-linked gene required for GPI biosynthesis.

Pathophysiology #2

- Key is a deficiency in complement inhibitory proteins CD55 and CD59 resulting in complement mediated hemolysis as well as acitivation of paltelets ,monocytes and granulocytes.
- CD59 interacts with membrane attack complex preventing lytic pore foprmation by blocking the aggregation of C9.

Pathophysiology #3

- CD55 a glycoprotein increases rate of destruction of membrane-bound C3 convertase.
- Summary: CD 55 reduces amount of C3 that is cleaved and CF59 reduces the number of MAC.
- C3 hydrolyzes and leads to forkmation of C3 ponvertase . Hemolysis is continuous because of this complement activation but can be increased with stresses such as surgery , infection and inflammation.

Pathophysiology #4

- The mechanism of intravascular hemolysis begions with the increased acitivity of C3 convertases on the surface of PNH red cells as a result of the lack of CD55.
  this leads to activation of C3, C% and terminal complemntcomponents resulting on formation of MAC. The absence of CD59 leads to incontrolled formation of MAC

Pathophysiology #5

- Extraqvscular hemolysis begins with increased opsonization of PNH erythrocyters by complemnt frgments mostly C3d. These red cells are the cleared and destroyed by the RE system.
- Intravascular hemolysis predominates

Genetics of PNH

- GPI biosynthesis – 10 steps and 26 gene products. The PIGA gene product is 1 of 7 proteis ninvolved in the first step. PIGA is on x chromosome and thus a single somatic mutation in a stem cell can cause PNH. Nuerous mutations have been described

PNH stem cells and marrow failure

- Mutation needs to be on self renewing stem cell and must achieve clonal dominance.
- Relationship to aplastic anemia may be due to lack of surface antigen targets.
- Some PIGA mutations do not cause PNH.
- PIGA mutation n MDS give small clinically irrelevant PNH populations.

Diagnosis and Classification of PNH

- PNH is a clionical diagnosis that must ber confirmed with peripheral blood flow cytometry to detect absence of CD33 and CD59 on 2 or more lineages.
- Detection is with monoclonal antibody staoning and a fluorescent aerolysin (FLAER). This latter binds to the glycnapotion of the GPI anchor.

Diagnosis and classification

- Three categories;
- 1.) Classical PNH with hemolytic and thrombotic components
- 2.) In the context of primary marrow disorders such as aplastic anemia and MDS
- 3.)Subclinical PNH with small clones

Anemia

- Hemolysis and marrow failure
- IV hemolysis, increased reticulocyte and elevated LDH.
- Patients may have a high percentage of pNH granulocytes

Thrombosis

- Most common cause of mortality. Can be anywhere but venous most common.
- Common sites include abdomonal)hepatoc, Portal and mesenteric) and cerebral 9sagittal and cavernous sinus) veins.
- Most common site pof thrombosis is hepatic vein (Budd-Chiari syndrome).
- There are protrombotic microparticles, scavenging of NO and complement activation. Also defective fibrinolysis.

Smooth Muscle Dystonia

- Abdominal paon, esophageal spasm , dys – phagia and erectile dysfunction are common symptoms with classical PNH. These are due to intravascular hemolysis and release fo free hemoglobin.
- Depletion of NO key.Tbis functions to maintain smooth muscle relaxation and onhibit platelet acrtivation.

Other

- Chronic kidney disease
- Pulmonary hypertension – the Trifecta

Therapy

- Essentially
- Eculizumab and stem cell transplantation

Eculizumab

- Humanized monoclonal antibody which blocks the terminal compliment by binding to C5.
- Given IV Q daily for first 5 weeks and then biweekly. This inhibits the formation of the MAC and on doing so compensated for the CD59 deficency. It does not compensate for the CD55 deficiency

Eculizumab

- It is highly effective for IV hemolysis but most patients continue with extravascular hemolysis due to C3d activity. Thus many treated patients have positive coombs and mild anemia and increased retics.,
- Common side effect is headache due to acute increase in NO but usually transient.
- Life-threatening infections with Neisseria most serious risk – 5% after 10 years - need vaccination.

Complications

- Headache due to increasing NO – transient
- Neisseria infections – 5% at 10 years – vaccinate.

Efficacy

- Eliminates or reduces need for transfusions.
- Improves quality of life
- Reduces thrombosis ??
- Improves renal function
- Long- term outcome of multinstional studies not yet published (up to 7 years)

Reason to treat

- Not all patients need treatment
- Severe anemia, thrombosis , frequent pain , debilitationg fatigue, worsening renal fiunction of dyspnea are good ondications for therapy.
- Patients with large clones >50 % pNH granulocytes and >10% PNH red cells with high LDH and reticuloyte count most likely to benefit.

Table 1
Monitoring the PNH patient on eculizumab

Monthly (draw tests before drug infusion)	**Yearly**
Complete blood count	PNH flow cytometry
Reticulocyte count	
Serum bilirubin	
Serum LDH	
If evidence of extravascular hemolysis (anemia and elevated reticulocyte count)	
Direct antiglobulin test	

Stem cell tranplantation

- With significant marrow failure a reasonable second line option

New Ideas

- Relationship between pulmomnary hypertension and PNH raises unique therapeutic options
- 1.) low dose radiation
- 2.) RUNX-1 inhibitors
- 3.) mewenchymal stem cell vesicles