Current Thinking on the Diagnosis and Management of PNH

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PNH

- Pathophysiology
- Diagnosis
- Management

What Is PNH?

(more than a hemolytic anemia)

- A disorder of the hematopoietic stem cell
- PNH is a consequence of *nonmalignant* clonal expansion of *one or several* hematopoietic stem cells that have acquired a somatic mutation of *PIGA*.
- Progeny of affected stem cells are deficient in all glycosyl phosphatidylinositol-anchored proteins (GPI-APs) that are normally expressed on HSCs.
- Clinical manifestations: hemolytic anemia, thrombophilia, bone marrow insufficiency or failure

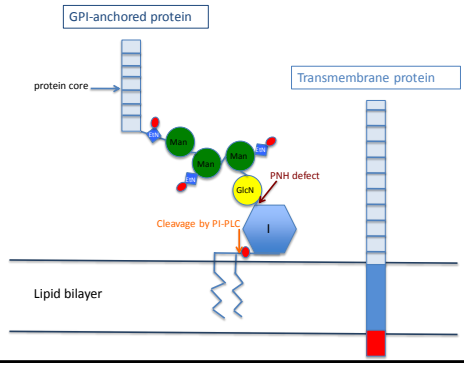


Table 1. ***Glycosyl Phosphytidylinositol Anchored Proteins Deficient in PNH****

Complement Regulatory Proteins†
- CD55 (decay accelerating factor, DAF)
- CD59 (membrane inhibitor of reactive lysis, MIRL)

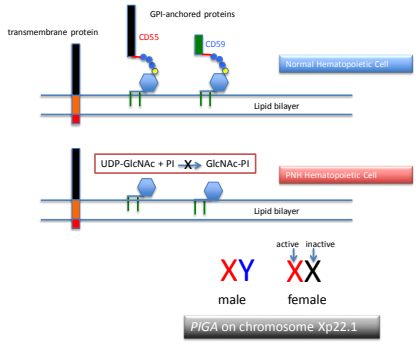
Proteins with Immunological Significance
- CD58 (lymphocyte function antigen-3, LFA-3)
- CD16b (Fc receptor gamma IIIb, FcRgIIIb, CD16b)
- CD14 (endotoxin binding protein)

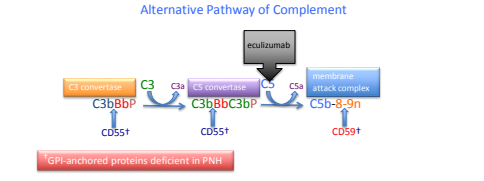
Receptors
- CD87 (urokinase plasminogen activator receptor, uPAR)
- Folate receptor
- Cellular prion protein (*on resting platelets*)

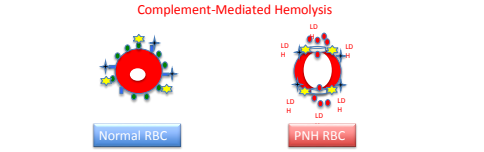
Enzymes
- Leukocyte alkaline phosphatase
- Acetylcholinesterase
- 5′-ectonucleotidase

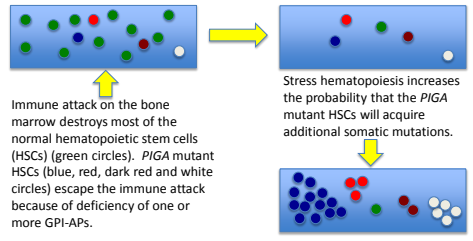
Miscellaneous Proteins
- CD24
- CD48
- CD52 (Campath-1)
- CD66c
- CD66b (formerly CD67)
- CD90 (Thy-1)
- CD108 (JMH-bearing protein)
- gp50-80, GP109, GP157, GP175, GP500

* Partial list
† Deficiency of complement regulatory proteins underlies the hemolytic anemia of PNH









John V. Dacie (1912-2005)

Professor Dacie at 87

Photograph provide by Dr. Wendell Rosse

"I saw my first case of PNH over 25 years ago now, and I must confess I still look upon it as *the* blood disease, unique in its pathology and remarkable in its clinical diversity and haematological interrelationships."

–1963 Address to the Royal Society as President of the Pathology Section

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Table 2. *Signs and Symptoms of PNH*

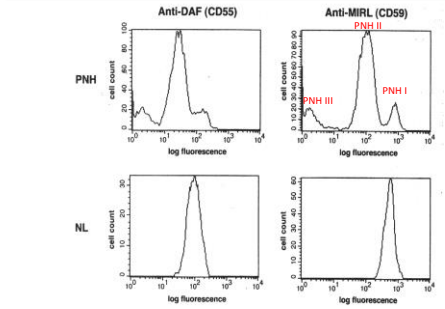
Subjective • Fatigue, lethargy, asthenia, loss of sense of well-being • Abdominal pain • Dyspnea • Chest pain • Odynophagia • Male impotence • Headache
Objective • Hemoglobinuria* • Scleral icterus, jaundice • Thromboembolic events†
Laboratory Abnormalities • Cytopenias (anemia, thrombocytopenia, leukopenia, pancytopenia) • Evidence of intravascular hemolysis (elevated LDH, elevated indirect bilirubin, low haptoglobin) • Flow cytometry demonstrates deficiency of GPI-AP on a portion of red cells, granulocyte and monocytes (lymphocytes are also usually involved but less so than myeloid cells) • Iron deficiency • Hemosiderinuria
* A presenting symptom in approximately 25% of cases † May involve an unusual site (skin, splanchnic veins [Budd-Chiari syndrome], cerebral veins)

Characteristics of PNH

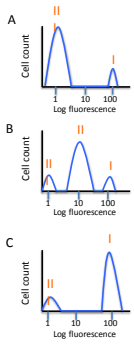
- PNH is not a binary disease as the peripheral blood of patients is a mosaic of normal and abnormal cells
- The percentage of PNH cells varies greatly among patients
- PNH erythrocytes are either completely or partially deficient in GPI-AP's
  - Type III cells are completely deficient
  - Type II cells are partially deficient
  - Type I cells are residual normal cells

Depends on PIGA genotype

Phenotypic Mosaicism Based on Flow Cytometry



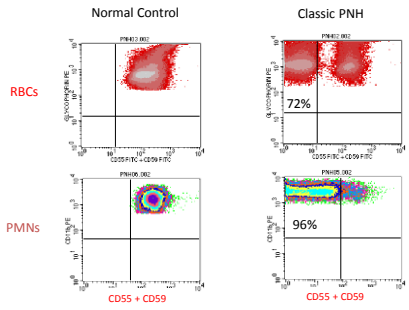
Endo et al. Blood 1996;87:2546-2557



Patients with a high proportion of PNH III cells have disease characterized by brisk intravascular hemolysis

Patients with a high proportion of PNH II cells have low-level chronic hemolysis

Patients with a small proportion of PNH III cells have little chronic hemolysis



Parker & Wae, Fig. 7

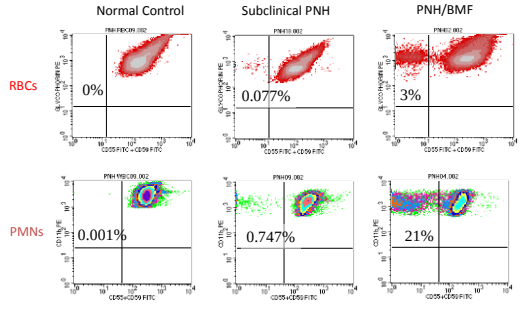


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Table 3. ***Basic Studies for Evaluation and Management of PNH***

- Complete blood count
- Reticulocyte count
- Serum concentration of lactate dehydrogenase (LDH), bilirubin (fractionated), haptoglobin
- Flow cytometric analysis of erythrocytes and granulocytes for expression of GPI-Aps
- Bone marrow aspirate & biopsy and cytogenetic analysis
- Serum erythropoietin concentration
- Serum concentration of blood urea nitrogen (BUN) and creatinine
- Serum iron studies (iron concentration, total iron binding capacity, transferrin saturation, ferritin concentration)
- Urine hemosiderin*

* Urine hemosiderin is indicative of chronic hemolysis but provides no quantitative information

Classification of PNH Guides Management

Table 4. *Classification of PNH

	Category	Rate of Intravascular Hemolysis†	Bone Marrow	Flow Cytometry	Benefit from Eculizumab
Clinical PNH	Classic	Florid (markedly abnormal LDH often with episodic macroscopic hemoglobinuria)	Cellular marrow due to erythroid hyperplasia and normal or near-normal morphology††	Large population (>50%) of GPI-AP deficient PMNs¶	Yes
	PNH in the setting of another bone marrow failure syndrome§	Mild (often with minimal abnormalities of biochemical markers of hemolysis)	Evidence of a concomitant bone marrow failure syndrome§	Although variable, the percentage of GPI-AP deficient PMNs is usually relatively small (<50%)	Typically no, but some patients have relatively large clones and clinically significant hemolysis and may benefit from treatment
	Subclinical	No clinical or biochemical evidence of intravascular hemolysis	Evidence of a concomitant bone marrow failure syndrome§	Small (<1%) population of GPI-AP deficient PMNs detected by high-resolution flow cytometry	No

* Based on recommendations of the International PNH Interest Group (*Blood* 2005;106:3699-3709)
† Based on macroscopic hemoglobinuria, serum LDH concentration and reticulocyte count
†† Karyotypic abnormalities are uncommon
§ Aplastic anemia or low risk myelodysplastic syndrome
¶ Analysis of PMNs is more informative than analysis of RBCs due to selective destruction GPI-AP deficient RBCs

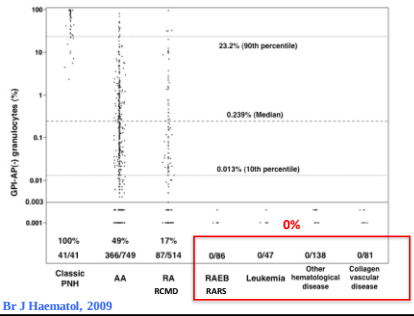
PNH/AA, PNH/MDS Subclinical PNH

- PNH in the setting of another BMF syndrome
  - The association between PNH and aplastic anemia has been appreciated for nearly 60 years and was refined using high-sensitivity flow cytometry
  - The association between PNH and low risk MDS was characterized using high-sensitivity flow cytometry
- Subclinical PNH
  - A product of screening peripheral blood from patients with BMF using high-sensitivity flow cytometry

Basic Evaluation for PNH
Flow cytometric evidence of a population of peripheral blood erythrocytes and granulocytes partially or completely deficient in multiple glycosyl phosphatidylinositol-anchored proteins (GPI-APs)*
Complete blood count; reticulocyte count; biochemical markers of hemolysis [serum concentration of lactate dehydrogenase (LDH)†, bilirubin (fractionated) and haptoglobin]; determination of iron stores
Bone marrow aspirate, biopsy, and cytogenetics§

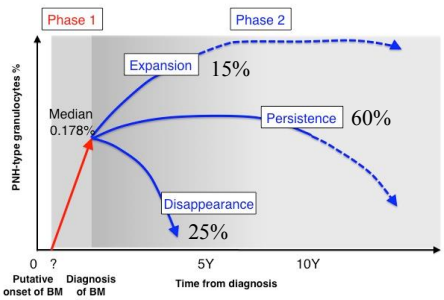
*PNH clone size is determined by the percentage of GPI-AP deficient PMNs
†The most important surrogate marker for intravascular hemolysis
§Bone marrow aspirate and biopsy are used to distinguish classic PNH from PNH in the setting of another bone marrow failure syndrome. Nonrandom karyotypic abnormalities are rare in PNH

PNH-type cells are detectable only in patients with AA and MDS-RA or RCMD



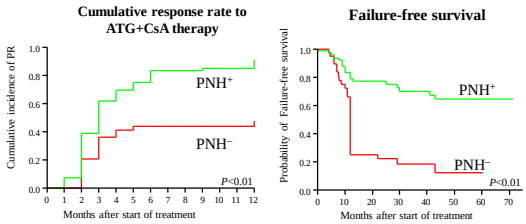
Sugimori, et al. Br J Haematol, 2009

Fate of PNH-type cells



Sugimori, et al. Br J Haematol, 2009

Response to IST and failure-free survival in patients with aplastic anemia



PNH⁺: AA patients showing small populations of CD55⁻CD59⁻ blood cells
PNH⁻: AA patients not showing small populations of CD55⁻CD59⁻ blood cells

Sugimori C, et al. Blood 2006

PNH/Aplastic Anemia and Low risk MDS

- The presence of a PNH clone appears to be a surrogate marker for immune pathology in patients with aplastic anemia and low risk MDS
- Finding of a PNH clone in the setting of aplastic anemia and MDS predicts a favorable response to immunosuppressive therapy

Management of PNH Based on Disease Classification

Classify PNH based on flow cytometric characteristics, reticulocyte count, serum LDH concentration, bone marrow analysis

Subclinical PNH

No specific PNH therapy—focus on underlying bone marrow failure syndrome*

*Some, but not all, studies suggest a favorable response to immunosuppressive therapy (IST)

Management of PNH Based on Disease Classification

Classify PNH based on flow cytometric characteristics, reticulocyte count, serum LDH concentration, bone marrow analysis

Subclinical PNH

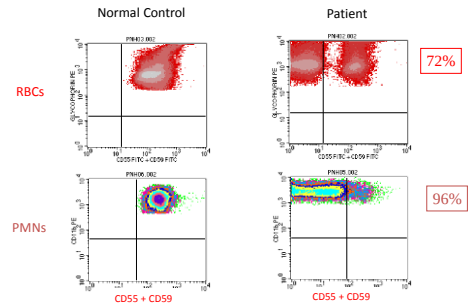
No specific PNH therapy—focus on underlying bone marrow failure syndrome*

PNH/BMF

Focus on bone marrow failure†
Patients with large PNH clones may benefit from eculizumab¶

BMF, bone marrow failure (aplastic anemia and low risk MDS); BMT, bone marrow transplant
*Some, but not all, studies suggest a favorable response to immunosuppressive therapy (IST)
†BMT eradicates the PNH clone. Treatment with IST does not affect PNH clone size
¶<10% of patients with PNH/BMF have PNH clone size >50%

Flow Cytometric Diagnosis of Classic PNH



Management of PNH Based on Disease Classification

Classify PNH based on flow cytometric characteristics, reticulocyte count, serum LDH concentration, bone marrow analysis

Subclinical PNH

No specific PNH therapy—focus on underlying BMF syndrome*

PNH/BMF syndrome

Focus on BMF†
Patients with large PNH clones may benefit from eculizumab¶

Classic PNH

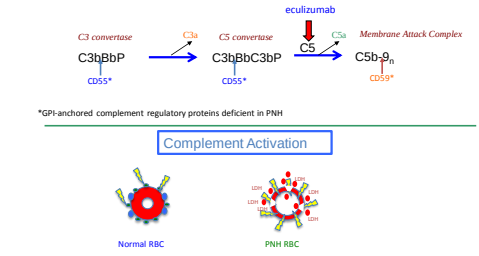
Treat with eculizumab§

BMF, bone marrow failure (aplastic anemia and low risk MDS); BMT, bone marrow transplant
*Some, but not all, studies suggest a favorable response to immunosuppressive therapy (IST)
†BMT eradicates the PNH clone, and typically, treatment with IST does not affect PNH clone size
¶<10% of patients with PNH/BMF have PNH clone size >50%
§Some patients respond to Danazol as first line therapy

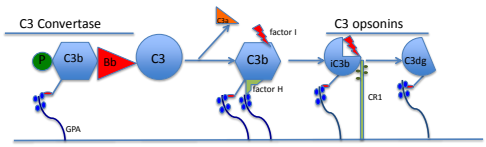
Suboptimal Response to Eculizumab

- A small minority of patients with classic PNH experience only modest improvement in constitutional symptoms
- Although serum LDH concentration returns to normal or near normal in all PNH patients treated with eculizumab, anemia and reticulocytosis persists in most patients with classic PNH and some remain transfusion dependent

Alternative Pathway of Complement Activation on Erythrocytes

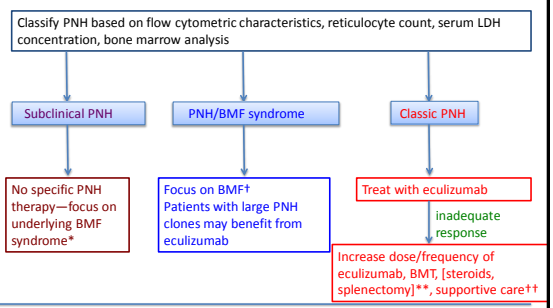


Generation of C3 Opsonins* on Erythrocytes



*C3 opsonins, iC3b and C3dg, target RBCs for destruction by reticuloendothelial cells expressing complement receptors:
CR2 ⟹ C3dg
CR3 ⟹ iC3b

Management of PNH Based on Disease Classification



BMF, bone marrow failure (aplastic anemia and low risk MDS); BMT, bone marrow transplant
*Some, but not all, studies suggest a favorable response to immunosuppressive therapy (IST)
†BMT eradicates the PNH clone, and typically, treatment with IST does not affect PNH clone size
** Carefully consider for patients with clinically significant extravascular hemolysis
†† Transfusion

Allogeneic SCT for PNH

- The PNH clone can be eradicated by allogeneic hematopoietic stem cell transplant
- In the era of complement inhibitory therapy, is there an indication for allogeneic BMT?

Indications for Transplant Before Eculizumab

- **Bone marrow failure**
  - Decision on transplant based on aplastic anemia or less commonly MDS
- **Major complication of PNH**
  - Refractory, transfusion-dependent hemolytic anemia
  - Recurrent, life-threatening thromboembolic disease

Parker *et al*, Blood 2005

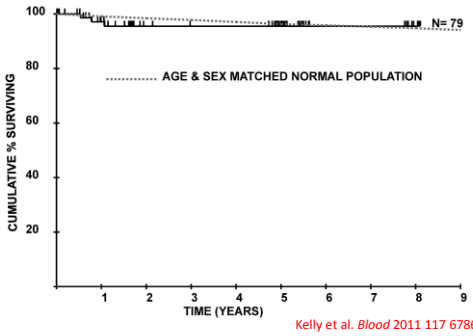
Indications After Eculizumab

- **Bone marrow failure**
  - Decision on transplant based on aplastic anemia or less commonly MDS
- **Major complication of PNH**
  - ~~Refractory, transfusion-dependent hemolytic anemia~~
  - ~~Recurrent, life-threatening thrombo-embolic disease?~~
  - Patient Circumstances, Including Preference?

Allogeneic SCT for PNH

- Transplant related mortality is in the range of 20%-35%
- Chronic GvHD ranges from 25-75% depending on transplant regimen
- However, patients who survive transplant usually have a good to excellent performance status
- Myeloablative and non-myeloablative regimens are effective in eradicating the PNH clone

Survival of Patients with PNH Treated with Eculizumab



Kelly et al. *Blood* 2011 117 6786-6792

"Ah, just the person I was looking for."

P. C. Vey, *The New Yorker*