Paroxysmal Nocturnal Hemoglobinuria: Understanding your Disease and Treatments Options

Conference Agenda
Living with Aplastic Anemia, MDS, or PNH
March 24, 2018 • Rockville, MD

Antonio M. Risitano, M.D., Ph.D.
Head of Bone Marrow Transplantation Unit
Federico II University of Naples

CONFLICT OF INTEREST DISCLOSURE
Art. 3.3 sul Conflitto di Interessi, Reg. Applicativo dell’Accordo Stato-Region (5/11/09)
Antonio M. Risitano

<table>
<thead>
<tr>
<th>Company</th>
<th>Type of conflict</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexion</td>
<td>Research funding, member of AB, lecture fee</td>
</tr>
<tr>
<td>Alnylam</td>
<td>Research funding</td>
</tr>
<tr>
<td>Novartis</td>
<td>Research funding, lecture fee, consultant</td>
</tr>
<tr>
<td>RA Pharma</td>
<td>Research funding</td>
</tr>
<tr>
<td>Pfizer</td>
<td>Lecture fee</td>
</tr>
<tr>
<td>Amynnas</td>
<td>Consultant</td>
</tr>
<tr>
<td>Jazz</td>
<td>Lecture fee</td>
</tr>
</tbody>
</table>

PNH association and patients’ days
The Italian experience

GIORNATA PAZIENTI EPN
Napoli, 27 giugno 2009
L’EPN: come si manifesta, come si cura
Dr. Antonio M. Risitano
Ematologia Università Federico II

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA
Epidemiology

Rare disease
• Based on EU definition: prevalence (number of affected patients within the population) < 1/10,000

Paroxysmal nocturnal hemoglobinuria
• Large population studies are lacking
• Prevalence estimated around 2-10 cases per million
• Worldwide distribution, with possible increased rates in Asia (overlapping with increased rates of aplastic anemia)
• No gender difference
• Incidence (number of new cases within the population) estimated around 1-2 new cases per million per year
• No age restriction, even if pediatric cases are rare and the highest incidence is seen in 3rd and 4th decades

Hemoglobinuria: about drinks...
Morosato: a regional wine from Sicily, fortified wine similar to Porto
**Paroxysmal: a condition characterized by an unpredictable clinical course, with some recurrent events (the so-called «crises»)**

**Nocturnal**: these events appear to be particularly frequent in the early morning

**Hemoglobinuria**: the typical event is the change in urine appearance, with a light red-to-black typical color

---

**Hemoglobinuria**

- Hemoglobinuria is not hematuria (in the urine you find only hemoglobin rather than intact blood cells)
- It gives the name to the disease, since it is one of the most evident manifestations (quite impressive especially in old era)

---

**The clinical triad of PNH**

**EPIDEMIOLOGY**: rare disease (1-5 per million/year)

1. **Chronic hemolytic anemia with paroxistic crises**
   - Intravascular hemolysis, complement mediated

2. **Propensity to thromboembolisms**
   - Often at unusual site, especially veins (cerebral veins, hepatic veins, splenic vein)

3. **Variable cytopenia**
   - Stigmata of marrow failure, possible overlapping with aplastic anemia (AA-PNH)

---

**Pathophysiology of PNH**

**Mechanism of protein binding to cell surface**

**GPI-anchor**

---

**Pathophysiology of PNH**

**GPI-anchor**

**Extracellular**

**Transmembrane proteins**

**GPI-linked proteins**

---
**PATHOPHYSIOLOGY OF PNH**

- GPI-anchored proteins
- GPI-linked proteins
- Transmembrane proteins
- Extracellular space
- PHOSPHO-ETHANOLAMINE
- PHOSPHATIDYL-INOSITOL

**THE PIG-A gene**

*Genotype-phenotype correlation*

- **Type I** cells with normal expression of CD59;
- **Type II** cells with partial deficiency of CD59 (Ham test: 3-5 x more sensitive to complement than normal cells);
- **Type III** cells with complete deficiency of CD59 (Ham test: 15-25 x more sensitive to complement than normal cells).

- RBC are labelled with anti CD59 FITC.

Three populations of RBC can be seen:
- Type I cells with normal expression of CD59;
- Type II cells with partial deficiency of CD59 (Ham test: 3-5 x more sensitive to complement than normal cells);
- Type III cells with complete deficiency of CD59 (Ham test: 15-25 x more sensitive to complement than normal cells).

**THE CLINICAL TRIAD OF PNH**

1. **Chronic hemolytic anemia with paroxysmic crises**
   - Intravascular hemolysis, complement mediated

**THE CLINICAL TRIAD OF PNH**

- **Epidemiology**: rare disease (1-5 per million/year)

**EPIDEMIOLOGY**: rare disease (1-5 per million/year)

- **Type**: Hematopoietic stem cell
- **Type**: Platelets
- **Type**: B cells
- **Type**: T cells
- **Type**: NK cells
- **Type**: PMN
- **Type**: Monocytes

**PROTEINS DEFICIENT ON PNH BLOOD CELLS**

- GPI-anchored proteins
- GPI-linked proteins

**THE COMPLEMENT CASCADE**

- Classical pathway
- Lectin pathway
- Alternative pathway

- **Bacteria or other pathogens**

**THE COMPLEMENT CASCADE**

- Classical pathway
- Lectin pathway
- Alternative pathway

- **Erythrocytes**

**Courtesy of Rosario Notaro**
THE COMPLEMENT CASCADE REGULATION IN PNH

THE COMPLEMENT CASCADE REGULATION IN PNH

THE COMPLEMENT CASCADE REGULATION IN PNH

THE COMPLEMENT CASCADE REGULATION IN PNH

THE COMPLEMENT CASCADE REGULATION IN PNH
THE COMPLEMENT CASCADE REGULATION IN PNH

**Chronic intracascular hemolysis with paroxysm**

- Hemoglobinuria
- Anemia
- Abdominal pain
- Dysphagia
- Erectile dysfunction
- Fatigue

**THE CLINICAL TRIAD OF PNH**

1. Chronic hemolytic anemia with paroxistic crises
   - Intravascular hemolysis, complement mediated
2. Propensity to thromboembolisms
   - Often at unusual sites, especially veins (cerebral veins, hepatic veins, splenic vein)
3. Variable cytopenia
   - Stigmata of marrow failure, possible overlapping with aplastic anemia (AA/PNH)

**Epidemiology**: rare disease (1-5 per million/year)

**Natural History of PNH**

Impact of thrombosis on survival and relationship with disease category

De Latour et al., Blood 2008

**Thromboembolism in PNH**

Often multiple events, venous thrombosis, peculiar sites
PATHOPHYSIOLOGY OF THROMBOSIS IN PNH

Red cells

CD55

CD59

Inefficient complement inactivation

Intravascular hemolysis

Propensity to various thromboses

Bone marrow failure

Expansion of PNH clone(s)

Stem cells and hematopoietic progenitors

Bone marrow failure

Expansion of PNH clone(s)

Intrinsic growth advantage?

Extrinsic selective advantage?

Immune privilege?

Platelets

Platelets activation and aggregation

Propensity to venous thrombosis

CD55, CD59?

Other

GPI-AP?

RBC microvesicles

↑ free Hb

NO consumption

Impaired fibrinolysis (uPAR)?

C-mediated platelet consumption

Pleiotropic role of nitric oxide in vascular homeostasis

NO

Vasoconstriction

Platelet aggregation and adhesion

Release of procoagulant factors

Release of growth factors

VCAM-1 dependent inflammatory cell attachment

PATHOPHYSIOLOGY OF THROMBOSIS IN PNH - II

Any hypothesis for the peculiar sites involved in TE of PNH?
PATHOPHYSIOLOGY OF THROMBOSIS IN PNH - II

Endothelial activation (VCAM-1, ICAM-1)

1. Chronic hemolytic anemia with paroxistic crises
   Intravascular hemolysis, complement mediated

2. Propensity to thromboembolisms
   Often at unusual site, especially veins (cerebral veins, hepatic veins, splenic vein)

THE CLINICAL TRIAD OF PNH

1. Variable cytopenia
   Stigmata of marrow failure, possible overlapping with aplastic anemia (AA/PNH)

EPIDEMIOLOGY: rare disease (1-5 per million/year)

STIGMATA OF BONE MARROW FAILURE IN PNH

- Frequent (pan)cytopenia, even severe
- Reduced Erythroid Colony Formation (BFU-E)

Centro Nazionale Ricerche, 1973
Rotoli et al, Blood 1982
A PNH population is present at diagnosis in about 40% of patients (usually <50%, without hemolysis) and subsequent fluctuations are common. Clinical PNH in 5-10%.

Clinical overlap between aplastic anemia and PNH

PNH is... ... still a puzzle!
GPI(-) cells are found in normal people. The prevalence of GPI-deficient cells ranges from 1 to 50 per million in healthy donors. However, a PIG-A mutation is not sufficient for developing PNH.

PNH is related to the expansion of mutated HSCs. PNH HSCs overcome normal hematopoiesis due to the expansion of mutated hematopoietic stem cells. HSCs give rise to various cell lines, including erythrocytes, granulocytes, monocytes, lymphocytes, and platelets.

The dual pathophysiology of PNH involves both persistent (immune) selective pressure and progressive expansion of PNH hematopoiesis. Bruno Rotoli (1937-2009) contributed to the understanding of PNH.

The pathophysiology of PNH includes the relative advantage/immune escape theory. Persistent (immune) selective pressure leads to progressive expansion of PNH hematopoiesis.
### PATHOPHYSIOLOGY OF PNH

#### The dual step hypothesis

- **Normal stem cells**
- **GPI-deficient (PNH) stem cells**

The second step (a "permissive environment"):
- An attack of hematopoiesis sparing PNH HSCs?

### Multiple dimensions of PNH

**PNH is...**
- ... a genetic, not inherited, disorder
  - Inactivating mutations of the PIG-A gene, disabling the synthesis of the GPI-anchor
- ... a clonal, not malignant, disorder
  - Abnormal (PIG-A mutated) blood cells derive from an ancestral hematopoietic stem cell which replaces normal hematopoiesis
- ... an immune-mediated disease, through the adaptive immunity
  - T cell mediated bone marrow failure, accounting for selection of PNH hematopoiesis and possible concomitant aplastic anemia
- ... an immune-mediated disease, through the innate immunity
  - Complement-mediated hemolytic anemia, with associated thrombophilia
- ... a chronic, life-threatening, heterogeneous disease requiring different therapeutic strategies

### PNH is a chronic disease (even in the past)

![Histamine et al., H Exp J Med 1985](image)

### Treatment of PNH

### Paroxysmal Nocturnal Hemoglobinuria

**Therapeutic options before 2007**

- None
- Antiglobulin
- Immunosuppressive
- Low dose corticosteroid
- Danazol
- Bone marrow transplantation
- Reduced cell transplantation
- Other

![Histamine et al., H Exp J Med 1985](image)

### NATURAL HISTORY OF PNH

- **Increased mortality due to:**
  - Thromboembolism
  - Severe marrow failure
  - (Clonal evolution to MDS/leukemia?)

![Histamine et al., H Exp J Med 1985](image)

**De Latour et al., Blood 2008**
ECULIZUMAB AND PNH: EFFECTS ON SURVIVAL

Untreated vs Ecu-treated PNH

Treated PNH vs normal population

Hill et al, Blood 2011

Is there any cure for PNH?

PNH: an EBMT retrospective study

Allogeneic stem cell transplantation in paroxysmal nocturnal hemoglobinuria

In Regos Peffault de Latour, Robert Schreinermitz, Andrea Baccicheto, Enrico Balleo, Carolina A. de Souza, Sthathya Vigneswaran, Rafael Vallettoni, Luis Tonato, Andrea Ticheli, Mohamed Mekki, Sophie de Guilherm, Judith Marsch, Dirk Pasquini, Jean-Yves Marr, and Gerard Natta

211 SCT for PNH from the EBMT database (1978-2007)

PNH patients with AA should receive the same therapy of non-PNH AA patients

Treatment algorithm of aplastic anemia

Updated to 2017

Peffault De Latour, ASH Educational 2016

How to manage... bone marrow failure in PNH?
How to manage... 
...hemolytic anemia in PNH?

**THE COMPLEMENT CASCADE REGULATION IN PNH**

- Classical pathway activation
  - Antibody/antigen complexes
- Alternate pathway activation
  - Microbiological membranes
  - Bacterial LPS
  - Immune complexes
- Lectin pathway activation
  - MBL

** targeting complement inhibitors**

- Eculizumab

**EFFECT OF ECULIZUMAB ON HEMOLYSIS**

- Lactate dehydrogenase (LDH) and transfusion independency

**ECULIZUMAB AND PNH**

- Soliris™ is the first and only approved therapy for the treatment of PNH (USA March 2007, Europe June 2007)
- Terrific efficacy and excellent safety profile (anti-meningococcal vaccination)
- Robust control of intravascular hemolysis (even if hematological benefit is heterogeneous)
- Remarkable effect on thrombophilic risk
- Anticipated effect on survival (to be confirmed with longer follow up)
- Eculizumab is the first choice treatment for all PNH patients to manage both hemolysis and thromboembolic risk of PNH
- Eculizumab has been reported extremely useful in allowing safe pregnancies in PNH women (Kelly et al, NEJM 2015)
Pre-Eculizumab Treatment  
Eculizumab Treatment

**Thrombosis Event Rate**  
(TE per 100 pt-years)

- 92% reduction in event rate with eculizumab

\[ P = 0.0001 \]

\[ (n=195) \]

<table>
<thead>
<tr>
<th>N=41</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 eligible (recent events)</td>
</tr>
<tr>
<td>15 procedures</td>
</tr>
<tr>
<td>Beneficial in all cases (4 CR, 4 PR, 1 IMR)</td>
</tr>
<tr>
<td>Risk of relapse (multiple sites)</td>
</tr>
<tr>
<td>20% hemorrhagic complications (1 fatal)</td>
</tr>
</tbody>
</table>

**Hillmen et al., Blood 2007**

- Normalized by time of observation (pre and post)
- 39 events
- 3 events

**Thrombolytic Therapy with tPA in PNH**

- CT scan
- Magnetic Resonance Venogram

**MANAGEMENT OF THROMBOEMBOLISM IN PNH**

### Secondary prophylaxis
- No absolute contraindication in case of thrombocytopenia
  - Platelet transfusion if required
- Guidelines: all PNH patients experiencing thrombosis should receive indefinite secondary antithrombotic prophylaxis
  - No consensus on the best strategy
  - Low molecular weight heparin
  - High-dose warfarin (INR 3-4)
  - Low-dose warfarin (INR 1.5-3)
  - Anti-platelets agents?
  - Lack of prospective studies
- Complications
  - Recurrence and/or relapse
  - Risk of hemorrhages, especially in patients with thrombocytopenia

**MANAGEMENT OF THROMBOEMBOLISM IN PNH**

### Primary prophylaxis
- No consensus on primary prophylaxis
  - Thromboembolic risk may differ according to ethnicity, genetic predisposition and other disease related features (i.e., PNH WBC clone size)
- Lack of prospective randomized studies
  - International PNH registry project
- Struggles of prophylaxis
  - Low dose warfarin (INR 1.5-3)
  - Low molecular weight heparin
  - Anti-platelets agents (Aspirin)?
  - Novel oral anti-thrombin inhibitors
- Complications
  - Risk of hemorrhage, especially in patients with thrombocytopenia
Long-term effect of eculizumab treatment in PNH
A retrospective comparison (Loschi et al, AJH 2016)

PNH patients with indication to eculizumab (clinically meaningful hemolysis and/or thrombosis)
- Eculizumab: n=123 (>2005)
- Non-eculizumab: n=191

Future goals
- Any room for improvement?
- If yes, how we can do it?

THE CLINICAL RESPONSE TO ECULIZUMAB
The Italian experience

Hematological response

- Hemoglobin ≥11: 26.6%
- Hemoglobin 8 ≤ Hgb < 11: 43.9%
- Hemoglobin ≤50%: 12.2%
- Hemoglobin >50%: 7.3%

- Normal or almost-normal LDH level in all patients
- Persistent reticulocytosis in almost all patients

Risitano et al, Blood 2009

THE COMPLEMENT CASCADE REGULATION IN PNH

Unmet clinical needs in anti-complement therapy
1. Rare intrinsic (genetic) resistance
2. Suboptimal hematological benefit
   - Underlying bone marrow failure
   - Breakthrough (pharmacokinetic and pharmacodynamics)
   - C3-mediated extravascular hemolysis
3. Patient perspective: i.v. therapy, bi-monthly infusion, (hospitalization)
4. Limited access (worldwide) and costs
The perfect complement inhibitor for PNH

1. As safe as first-generation inhibitors (eculizumab)
2. Similar control of intravascular hemolysis, as compared with eculizumab
3. Possible effect on C3-mediated extravascular hemolysis
4. Effective in rare genotypes?
5. Possibly better in terms of patients compliance (administration route, frequency): no hospitalization?
6. Cost: a cheap treatment for everybody, worldwide

“Answers are better than questions”
Neapolitan philosopher, 1973—..................about 2100

---

Second generation complement inhibitors for PNH

Take home messages

1. Alternative anti-C5 agents (or terminal complement inhibitors) may result in limited benefit
   - Possible improvement of treatment compliance: administration route and intervals (reduced/abolished hospitalization?)
   - Reduced costs?
   - Likely no clinical benefit over eculizumab (except for C5 mutations)

2. Second-generation inhibitors must target early complement activation
   - C3-mediated extravascular hemolysis is the main unmet clinical need in PNH
   - C3 inhibitors: compstatin
     - Optimal strategy for PNH, due to deranged regulation along all the three pathways
     - Initial data in PNH very encouraging (mostly add-on therapy)
     - Subcutaneous availability, but need of s.c. INFUSIONS
   - Alternative pathway inhibitors: anti-FB and anti-FD
     - Preliminary data in PNH (anti-FD only) very promising (add-on therapy)
     - Orally available
     - Short half-life; risk concerning “missing doses”

---

ACKNOWLEDGEMENTS

Naples, Federico II University
Serena Marotta
Francesco Grimaldi
Simona Pagliuca

Philadelphia, PA
John Lambert
Daniel Ricklin
Edimara S Reis
Yixin Huang
Zhouer Lin
Hui Chen
Ranillo R. G. Resuello (Manila)
Robert A. DeAngelis

Florence, IT
Rosanna Notaro
Lucio Luzzatto

Paris, Hopital St. Louis
Geordi Socci
Regis Pfeuflit De Latour
London, King’s College
Ghulam Mutti
Judith Marsh
Asthin Kudakarasan
San Paolo University
Rodrigo Calado
Philip Schenborn
NH, NHLBI
Neal S. Young

Cleveland, Cleveland Clinic
Jacolew Maciejewski

---

EMOGLIBINURIA PAROSSISTICA NOTTURNA

Cos’è

- Thanks to charities!
- Also in Italy patient association and patients’ day
- What is the disease: definition starting with the name
- Clinical presentations
- Pathogenesis
- Prognosis
- Therapies
  - Cursived
  - Supportive
- Etiologic, disease-changing
- Perspective
MANAGEMENT OF THROMBOEMBOLISM IN PNH

Secondary prophylaxis

- Once experienced one thromboembolic event, PNH patients are at very high risk of further thromboembolism
  - No absolute contraindication in case of thrombocytopenia
  - Platelet transfusion if required
- Guidelines: all PNH patients experiencing thrombosis should receive indefinite secondary antithrombotic prophylaxis
  - No consensus on the best strategy
    - Low molecular weight heparin
    - High-dose warfarin (INR 3-4)
    - Low-dose warfarin (INR 1.5-3)
    - Anti-platelet agents?
  - Lack of prospective studies

Complications
- Recurrence and/or relapse
- Risk of hemorrhages, especially in patients with thrombocytopenia

Thrombolytic Therapy with tPA in PNH

Recurrences of TE are frequent and standard anticoagulant therapies are largely ineffective in preventing further TE episodes

Primary prophylaxis

- No consensus on primary prophylaxis
  - Thromboembolic risk may differ according to ethnicity, genetic predisposition and other disease related features (i.e., PNH WBC clone size)
- Lack of prospective randomized studies
  - International PNH registry project
    - Retrieve retrospective data
    - Possibly design prospective studies
- Strategies of prophylaxis
  - Low dose warfarin (INR 1.5-3)
  - Low molecular weight heparin
  - Anti-platelet agents (Aspirin)?
  - Novel oral anti-thrombin inhibitors
- Complications
  - Risk of hemorrhage, especially in patients with thrombocytopenia

Hall et al, Blood 2003