Paroxysmal Nocturnal Hemoglobinuria (PNH)

Introduction to PNH

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## Disclosures

<table>
<thead>
<tr>
<th>Company name</th>
<th>Research support</th>
<th>Employee</th>
<th>Consultant</th>
<th>Stockholder</th>
<th>Speakers bureau</th>
<th>Advisory board</th>
<th>Other</th>
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No conflicts to declare
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<th></th>
<th>Definite</th>
<th>Probable</th>
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<tbody>
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<td>Thalassemia’s</td>
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<td>Sickle cell disease</td>
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<tr>
<td>Membrane disorders</td>
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<td></td>
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<tr>
<td>(e.g. sferocytosis)</td>
<td></td>
<td></td>
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<tr>
<td>Enzym defects (e.g. PKD, G6PD)</td>
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<td></td>
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<tr>
<td>PNH</td>
<td></td>
<td><em>Yes</em></td>
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<tr>
<td>Other forms of hemolytic disease</td>
<td></td>
<td><em>Yes</em></td>
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</table>
Paroxysmal Nocturnal Hemoglobinuria

Acquired disease (*it is not transmitted to offspring*)

Prevalence: 5-10 cases per million

Geographic distribution: worldwide

Age: any

Gender: *same frequency in men and in women*
Paroxysmal Nocturnal Hemoglobinuria

PNH is a rare clonal acquired blood disorder characterized by the clinical triads:

**Chronic intravascular hemolysis:** with crisis

**Thrombosis:** Often Multiple, Venous, Abdominal

**Cytopenias** *(Bone marrow failure):* Common, sometimes severe
Transmembrane Proteins and GPI-linked Proteins

glycophosphatidylinositol-GPI Anchor

Proteins

Cytoplasm

Cell Membrane
GPI-linked Proteins Deficient on PNH Blood Cells

- CD55
- CD58*
- CD59
- PrPC
- AChE
- JMH Ag
- Dombroch
- HG Ag

- CD55
- CD58*
- CD59
- CD109
- PrPC
- GP500
- Gova/b

- CD55
- CD58*
- CD59
- CD109
- PrPC
- GPI-80
- A2
- ADP-RT

- CD14
- CD55
- CD58*
- CD59
- CD48
- CDw52
- CDw108
- PrPC
- CD16*

- CD73
- CD90
- CD109

- CD16*

- CD55
- CD58*
- CD59
- CD48
- CDw52
- ADP-RT

- CD73
- CD90
- CD109
- CD16*
Paroxysmal Nocturnal Hemoglobinuria (PNH) Pathogenesis

Hematopoietic Stem Cell

Normal cell

PNH cell
Paroxysmal Nocturnal Hemoglobinuria

- Intravascular hemolysis
- Thrombosis
- Bone marrow failure
Paroxysmal Nocturnal Hemoglobinuria

- Intravascular hemolysis
- Thrombosis
- Bone marrow failure
Complement System in PNH

Antigen-Antibody | Classical pathway
---|---
| Lectin pathway
---|---
| Alternative pathway
---|---
Microorganisms | Complement Inhibitors
---|---
Constitutive/ Microorganisms | Membrane
---|---
Complement Inhibitors | Soluble
---|---

Effects of intravascular hemolysis

Intravascular hemolysis

Hemoglobinemia
  - High LDH
  - High Retics

Hemoglobinuria
  - Iron loss

Hemosiderinuria

ANEMIA
  - (low Hgb)

Nitric oxide depletion

smooth muscle dystonia
  - Abdominal pain
  - Fatigue
  - Dysphagia
  - Erectile disfunction
  - (Renal failure)
  - (Pulmonar hypertension)

Iron loss
Paroxysmal Nocturnal Hemoglobinuria

- Intravascular hemolysis
- Thrombosis
- Bone marrow failure
PNH is a severe acquired thrombophilic state - thrombosis in PNH -

• 30-40% of PNH patients suffer from at least a thrombotic event

• 40–67% of deaths in PNH results from thromboembolisms

• More frequent in hemolytic than in aplastic PNH patients

• There is a rough correlation with the size of PNH population - but thrombosis can occur also in patients with small PNH population

• More frequent in patients with congenital and acquired thrombophilic conditions

• Substantial increased risk after the first thrombotic event
### Sites of thrombosis IN PNH

#### Venous Thrombosis

<table>
<thead>
<tr>
<th>Type of Thrombosis</th>
<th>% of Total</th>
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<tbody>
<tr>
<td>Deep vein thrombosis</td>
<td>33.1</td>
</tr>
<tr>
<td>- lower extremity</td>
<td>18.5</td>
</tr>
<tr>
<td>- other</td>
<td>14.5</td>
</tr>
<tr>
<td>Mesenteric/splenic vein thrombosis</td>
<td>18.5</td>
</tr>
<tr>
<td>Hepatic/portal vein thrombosis</td>
<td>16.9</td>
</tr>
<tr>
<td>Pulmonary embolus</td>
<td>6.5</td>
</tr>
<tr>
<td>Cerebral/internal jugular thrombosis</td>
<td>5.6</td>
</tr>
<tr>
<td>Superficial vein thrombosis</td>
<td>4.0</td>
</tr>
</tbody>
</table>

#### Arterial Thrombosis

<table>
<thead>
<tr>
<th>Type of Thrombosis</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ictus/transient ischaemic attack (TIA)</td>
<td>13.7</td>
</tr>
<tr>
<td>Myocardial infarction/unstable angina</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Coagulation, Complement and PNH

Partners in Crime?

Modified from Markiewski et al, Trends Immunol 2007

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Paroxysmal Nocturnal Hemoglobinuria

- Intravascular hemolysis
- Thrombosis
- Bone marrow failure
Bone Marrow Failure and PNH

Dual Pathogenesis of PNH

Complement Susceptibility targeting GPI– cells

Hemolysis

PNH

PNH/AA

Cytopenia

AA

Bone Marrow Failure targeting GPI+ cells (?)

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Paradoxical Expansion of PNH Clone

(Escape Theory)

- PIG-A+ cells
- PIG-A null cells
- crippled PIG-A+ cells
- Noxious agent

Rotoli & Luzzatto, 1989
ID of Lymphocytes that damage normal cells and spare PNH cells
Which is the target?

Skewed TCRβ repertoire (Karadimitris et al, Blood 2000)
Similar TCRβ sequences in oligoclonal T cells (Gargiulo et al, Blood 2007)
GPI specific and CD1d restricted T cells (Gargiulo et al, Blood 2013)
A Unified Pathogenetic Model for PNH and AA

CD1d restricted autoreactive T cells target the GPI anchor
A Unified Pathogenetic Model for PNH and AA

GPI-targeted noxious agent → Damaged Hematopoietic stem cells

PNH clone(s) (GPI-deficient) → Selective expansion of PNH clone(s)

Healthy Hematopoietic System → No clinical consequences

CD59(-) red cells → PNH

Abnormal platelets → PNH

Intravascular haemolysis → PNH

Thrombosis → PNH

Aplastic Anaemia → PNH

Pancytopenia → PNH

A Unified Pathogenetic Model for PNH and AA

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Paroxysmal Nocturnal Hemoglobinuria

Clinical Management
Support treatments

- Folic Acid
- Iron supply
- Red blood cell transfusions
- Anti-coagulant Profilaxis
  - (Thrombolysis)

Phatogenetic treatments

- Allogeneic HSC Transplantation
- Immuno-suppression (ALG/CSA)
  - (Androgens)
Paroxysmal Nocturnal Hemoglobinuria
therapeutic options in pre-eculizumab era

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Paroxysmal Nocturnal Hemoglobinuria

Complement Blockade
- eculizumab -
Eculizumab

Anti-C5 humanized monoclonal antibody

Rother et al, Nature Biotech 2007
Regulation of Complement Cascade in PNH Patients

Complement Inhibitors

- Factor H
- Factor I
- C4bp
- CR1
- CD46

Alternative pathway

Lectin pathway

Classical pathway

C3 → C5 → C5b-C9
Regulation of Complement Cascade in PNH Patients Treated with Eculizumab

Complement Inhibitors

- Factor H
- Factor I
- C4bp
- CR1
- CD46

Eculizumab

- Alternative pathway
- Lectin pathway
- Classical pathway

C5

C3

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Effect of eculizumab on intravascular haemolysis

**LDH levels**

![Graph showing the effect of eculizumab on LDH levels. The graph includes data from the TRIUMPH and SHEPHERD trials.](chart)

**Key**
- Eculizumab
- Placebo
- TRIUMPH – placebo
- TRIUMPH – eculizumab
- SHEPHERD

**References**
Eculizumab abrogates the need of blood transfusion in most PNH patients

Patients without blood transfusion during the previous 6 months

<table>
<thead>
<tr>
<th>Time interval (months)</th>
<th>Patients (n)</th>
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<tr>
<td>0-6</td>
<td>195</td>
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<tr>
<td>6-12</td>
<td>192</td>
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<td>12-18</td>
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<td>18-24</td>
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<td>24-30</td>
<td>134</td>
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<td>30-36</td>
<td>78</td>
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PRE Eculizumab

Hillmen et al, Brit J Haematol 2013
Eculizumab and Thrombosis

*thromboembolism in patients on prophylaxis*

Hillmen *et al*, *Blood* 2007
Overall Survival of PNH Patients in eculizumab era

Survival (%)

Years since diagnosis

Mortality by:
Thrombosis
Bone marrow failure (MDS/leukemia?)

PNH (n 424; De Latour 2008)
PNH (n 80; Hillmen 1995)

Hillmen et al, Brit J Haematol 2013
Paroxysmal Nocturnal Hemoglobinuria

Extravascular Hemolysis
- a pattern change in PNH -
C5 blockade is associated with C3-binding on GPI-RBC

Hgb ≥ 12
8.5 ≤ Hgb < 10

Risitano AM, Notaro R et al, Blood 2009

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Eculizumab treatment is associated with C3-binding on PNH red cells

Patient not on eculizumab

CD59-PE

C3-FITC

Alternative pathway

C3 → C5

Risitano, Notaro et al, Blood 2009

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Paroxysmal Nocturnal Hemoglobinuria

A clinical case
Eculizumab starts and the story changes ..............

- no more blood transfusions and no more thrombosis (despite the discontinuation of anti-coagulant prophylaxis)
Eculizumab stops intravascular hemolysis and related symptoms abrogates or reduce transfusion need reduces thrombotic risk is safe (also during the pregnancy)

However, it is not curative extravascular hemolysis may result in transfusional need it is not available to all patients (even in EU)
Paroxysmal Nocturnal Hemoglobinuria

Future Developments
New complement inhibitors

Classical pathway
- C1
- C4
- C2
- C3
- C5

Lectin pathway
- MBL
- MASP

Alternative pathway
- C3a
- C3b
- C5a
- C5b

MoAb AntiC3
Compstatin
TT30 (FH-CR2 hybrid)
CR1-like (no EPN)
Fattore H-like

Eculizumab
MoAb AntiC5 (no EPN)
Cyclic Peptids
siRNA

Complement Inhibitors

Membrane
- CR1
- Factor H
- CD46
- C4bp

Soluble
- Factor I
- C3
- C5

MoAb AntiCFD (no EPN)
MoAb AntiCFB
siRNA anti CFB
Small molecules
Aptamers
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Lucio Luzzatto

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Nurse Staff Careggi Hospital
Dank aan de Patiënten en hun Families
Merci aux patients et à leurs familles
Thanks to Patients and their Families
Grazie ai Pazienti ed alle loro Famiglie
There is a soul of goodness in things evil, Would men observingly distil it out.

William Shakespeare, King Henry V, IV, i