Paroxysmal Nocturnal Hemoglobinuria in 2013

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PNH

- Introduction
- History
- Clinical Manifestations
- Pathogenesis
- Diagnosis
- Treatment
- PNH network and PNH registry
- Conclusions
Hallmark features of PNH

- Nocturnal hemoglobinuria related to intermittent hemolytic anemia
- Bone marrow failure resulting in pancytopenia
- Thrombophilia resulting in increased risk for thrombosis
Paul Struebing. Paroxysmale Haemoglobinurie. Deutsche Medizinische Wochenschrift 1882:8:1

• 1st patient reported
• 29 year old cart maker with dark urine after sleep.
• Patients plasma was red and urine contained a yellowish brown pigment
• Dr Strubing suggested that red cells destroyed within the blood stream.
## History

<table>
<thead>
<tr>
<th>Year</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1882</td>
<td>Description of first PNH case (P.Strubing)</td>
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<tr>
<td>1939</td>
<td>Complement mediated lysis and development of Hams test (T.Ham)</td>
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<tr>
<td>1966</td>
<td>Description sugar lysis test (R Hartmann)</td>
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<tr>
<td>1969</td>
<td>Characterisation of Decay Accelerating Factor (DAF) (E. Hoffman)</td>
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<tr>
<td>1973</td>
<td>First allogeneic BMT for PNH (R.Storb)</td>
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<tr>
<td>1989</td>
<td>Characterisation of MIRL (CD59) and GPI anchor (C Parker)</td>
</tr>
<tr>
<td>2003</td>
<td>Cloning of PIG-A gene (T Kinoshita)</td>
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<tr>
<td>2007</td>
<td>FDA approval of eculizumab</td>
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</tbody>
</table>
Demographics and Survival

• Median age at presentation: 34 years
• Incidence 1-10 cases per million population
• Overall survival at 10 years 76%, at 20 years approx 50% (de Latour et al Blood 2008) (before eculizumab)
• Often begins with anemia
• Episodic hemolysis at night or hemolysis precipitated by infection, surgery and transfusion
Clinical Manifestations

• **Due to intravascular hemolysis:**
  – Anemia, hemoglobinuria, fatigue, renal failure, recurrent urinary tract infection, Abdominal pain, bloating, back pain, headache, Esophagospasms, erectile dysfunction, cholelithiasis

• **Due to thrombosis:**
  – Venous thrombosis: Abdominal vein thrombosis, portal hypertension, esophageal varices, cerebral vein thrombosis, retinal vein thrombosis, deep vein thrombosis, pulmonary emboli
  – *Rare:* Arterial thrombosis

• **Due to bone marrow failure:**
  – Anemia, infections, bleeding, Myelodysplastic syndrome
  *Rare:* Transformation to acute myeloid leukemia (AML)

Adapted from Bessler M, Hematology 2008
Pathophysiology of PNH

- Acquired hemolytic anemia with venous thrombosis, pancytopenia and risk of transformation to MDS and AML suggests mutation of hemopoietic stem cells
- Glycosyl phosphatidyl inositol (GPI) anchor deficiency on hemopoietic cells
- Related to PIG-A gene mutation (phosphatidyl inositol glycan complementation class A)
- PNH cells have cell surface deficiency in all proteins that use the GPI anchor
Schematic representation of the structure and mutations in the PIGA gene

Short arm of chromosome X
17kb long
6 exons
Only 1 mutation sufficient to abolish GPI linked protein expression
To date over 180 mutations identified
Majority of mutations lead to loss of glycosyltransferase activity and inactive PIGA protein

Hematology 2008;2008:491-506
GPI-anchored surface proteins on human hematopoietic cells
Complement system

- Biochemical cascade which helps antibodies to eliminate pathogens
- Part of the innate immune system (non adaptable)
- Proteases cleave specific proteins and initiate a cascade of cleavages
- End result of cascade is amplification of immune response and activation of cell killing membrane attack complex (or terminal complement complex)
To summarize

- PNH phenotype is caused by PIG-A gene mutation
- This leads to a lack of GPI anchor bound proteins on cell surface
- This leads to increased susceptibility of red cells to complement mediated hemolysis (lack of MIRL and DAF)
- This leads to in vivo activation of clotting by increased platelet aggregation (? Related to nitric oxide depletion), thrombin generation and expression of tissue factor by endothelial cells
- This leads to pancytopenia presumably due to deficient production of hemopoietic stem cells
- Hallmark of PNH are hemolysis, pancytopenia and thrombosis
Diagnosis of PNH
Bone Marrow biopsy from PNH patient
Laboratory tests for diagnosis (traditionally)

- **Ham Test**: complement activation by acidification of serum leads to lysis of patients red cells

- **Sucrose lysis test**: complement activation by isotonic sucrose solution
Laboratory tests (today)

• Flow cytometric analysis
  – CD59 and CD55 on red cells
  – CD59 and CD55 on granulocytes
  – FLAER (fluorescently labeled aerolysin)

• PIG-A gene mutation analysis
Flow cytometric demonstration of the absence of CD59 on erythroid cells
FLAER (fluorescently labeled aerolysin)

- Bacterial toxin (Aerolysin) binds to GPI linked cell surface structures
- secreted by Aeromonas hydrophilia
- Fluorochrome labeled form of aerolysin (=FLAER)
- Highly sensitive to detect GPI anchor deficient granulocytes (0.5%)
Test 2

Quantification des GPI-AP par la fixation de FLAER (Toxine bactérienne inactivée) sur les leucocytes seulement

Sensibilité: Avec ce test on parle de déficience en GPI-AP à partir de 0.5% de cellules négatives pour le FLAER et on rapporte dans ce cas la présence d’un clone PNH dans les leucocytes.
Pour le patient ci-dessus il y a présence d’un clone PNH de 20% de déficience sur les monocytes et les granulocytes (On ne tient pas compte des lymphos pour l’analyse)
Treatment of PNH

- Observation (ie PNH clone <10%)
- Supportive care (transfusions, folic acid, iron)
- Glucocorticoids or androgens
- Anticoagulation and antibiotics
- ATG/cyclosporine
- Eculizumab
- Allogeneic bone marrow transplantation
Supportive Care

- Red cell transfusions
- Iron and folic acid supplementation
- Prophylactic anticoagulation
  - Suggested in patients with large PNH clone (granulocytes >50%) and platelet counts > 100 x 10^9/l
  - Suggested in high risk periods ie perioperative, immobilisation
Glucocorticoids

- Non proven
- Mechanism of action unclear, possibly decreasing complement activation
- Variable dosing schedules (prednisone 0.25-1mg/kg daily or every 2nd)
- Appears particularly useful to decrease severity of acute hemolytic crisis
- Androgens (danazol) may decrease anemia
ATG/cyclosporine

- Cytopenias caused by immune mediated suppression of normal cells (GPI positive) producing growth advantage to PNH cells.
- Patients with severe AA and minor populations of CD55-/CD59- cells appear to respond well to ATG treatment (Sugimori C et al, Blood 2006;15:1308-14, N=83, Response rate 91%)
- Immunosuppressive therapy should be considered in all patients with severe aplastic anemia and PNH features (except if allo transplant considered)
- Immunosuppressive therapy appear to improve cytopenias but not other characteristics of PNH
Eculizumab

- Humanized monoclonal IgG kappa antibody
- Binds to human C5 complement protein
- Prevents cleavage to C5a, formation of lytic complement cascade and membrane attack complex (MAC)
- Hybrid of IgG2 and IgG4 (unable to activate complement)
- Remains bound to target until removed from circulation

Brodsky, R. A. Blood 2009;113:6522-6527
Eculizumab Experience in PNH Clinical Trials

**Pilot Study** – Hillmen et al. *NEJM*, 2004
N = 11

**TRIUMPH** – Hillmen et al. *NEJM*, 2006
Pivotal Phase III, Double-Blind, Placebo-Controlled Trial, N = 87

**SHEPHERD** – Brodsky et al. *Blood*, 2008
Broader patient population, including those receiving minimal transfusions or with thrombocytopenia, N = 97

**Long-Term Extension Trial**

*Hillmen Blood*, 2007
Evaluated long-term safety, efficacy and effect on thrombosis; Placebo patients switched to Soliris
N = 187

Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria

**195 Patients With >250 Patient Years of Eculizumab Exposure**
Reduction in intravascular hemolysis (LDH) during treatment with eculizumab

Hillmen P et al. NEJM 2006;355:1233-1243
Mean (SE) units packed red blood cells transfused by pre-treatment transfusion strata during the TRIUMPH and SHEPHERD studies.
Analysis of thrombosis before and during eculizumab therapy

**A. Matched time periods per patient prior to and during eculizumab therapy**

- **39 events**
- **3 events**
- **P = .0001**

**B. Patients receiving concomitant anticoagulation therapy**

- **54 events**
- **1 event**
- **P < .0001**

Hillmen, P. Hematology 2008;2008:116-123
Eculizumab

• Treat patients with
  – Moderate to severe symptoms of PNH
  – Patients who have developed or are at high risk of thrombotic events and/or renal dysfunction

• Improvement of quality of life at a cost of twice monthly infusions

• Increased risk of infections with encapsulated bacteria (Neisseria species) therefore vaccination mandatory
• 79 patients treated with eculizumab in Leeds between 2002-2010, mean duration of 39 months

• Assessment every 3 months in Leeds

• Control group for comparison: 30 patients fulfilling criteria for eculizumab treatment, treated in Leeds, 7 years prior to availability of eculizumab

• Comparison of survival data of eculizumab treated patients with age and sex matched control averages obtained from 2001 UK census data

Overall survival of patients before and after eculizumab

Overall Survival on eculizumab compared with normal population
Survival after 10 years is slightly inferior to controls with causes of death related to bone marrow failure and not hemolysis or thrombosis.
Mean duration of eculizumab treatment 42 months.
Conclusions Leeds

• Eculizumab alters dramatically the natural course of PNH
• Overall survival is improved to a similar level than the general population
• Mean duration of eculizumab treatment of 39 months (1-98)
Allogeneic bone marrow transplantation

• Currently only curative treatment option for PNH
• Appealing as clonal diseases can be cured by allogeneic bone marrow transplantation
• Associated with significant morbidity and mortality
• Difficulty lies in patient selection and because of small numbers of published patients in choice of preparative regimen and graft
Summary. A patient with pancytopenia and paroxysmal nocturnal haemoglobinuria (PNH) following exposure to insecticide spray developed complete marrow failure after inhalation of vapours containing benzol. There was no sign of spontaneous recovery after more than 6 mth of conventional and supportive therapy. The patient was treated with the immunosuppressive agent cyclophosphamide, 50 mg/kg on each of four days, followed in 36 hr by transplantation of marrow from a sibling compatible at the major human histocompatibility locus (HL-A). Intermittent methotrexate therapy was given for 102 days after grafting to prevent graft-versushost disease. The patient showed prompt haemopoietic engraftment indicated by restoration of marrow cellularity and a rise in peripheral blood cell counts beginning on day 11 after the graft. The patient is alive and well with normal haemopoietic function and continued absence of PNH more than 1 yr and 4 mth after transplantation.
BMT and PNH publications

- Vast majority are case reports with 1-5 patients
- Variable preparative regimens
- Variable graft source (syngeneic, related or unrelated)
- Only few studies with more than 10 patients
• 57 patients reported to the International Bone Marrow Transplant registry, 48 with HLA identical sibling donors
• Transplanted between 1978-1995
• Median age 28 (10-47)
• Severe aplastic anemia pretransplant in 32%
• Conditioning regimen:
  • Bu-Cy 30 (53%)
  • Cy-TBI +/- other 12 (21%)
  • Limited field radiation – Cy 11 (19%)
Figure 1. Survival after 48 HLA-identical sibling bone marrow transplants for PNH
Retrospective study of 26 patients

- Median age 32 years
- Between 1988-2006
- HLA matched donor for 22, 1 MUD, 3 MM donors
- Classic and AA/PNH phenotypes (AA alone in 4 patients)
- Bu-CY in 15 patients, RICT in 11
- Cumulative TRM 26% for myeloablative and 63% for RICT approach

Figure 1. Kaplan-Meier probability of disease-free survival for 23 patients transplanted from HLA-identical sibling donor (65%, dotted line) and for all 26 patients (57%, continuous line).
Allogeneic stem cell transplantation in paroxysmal nocturnal hemoglobinuria


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On behalf of the Severe Aplastic Anemia Working Party (SAA WP) of the European Group for Blood and Marrow Transplantation (EBMT) and the French Society of Hematology (SFH)

Study Design

• Retrospective multicenter study conducted by SAA working party of EBMT
• Evaluation of outcomes and risk factors affecting survival in 211 patients receiving allogeneic stem cell transplantation
• Reported to the EBMT between 1978-2007 by 83 centers
• Previously reported data on 402 non transplanted patients reported to the SFH and diagnosed between 1950-2005 used as comparison
• Update of previously published abstracts (ASH 2008 and 2011)
Table 1. Characteristics of patients and their transplants (n=211).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n/N (%) or median (IQR*), N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, female</td>
<td>106/211 (50%)</td>
</tr>
<tr>
<td>Age at transplantation, years</td>
<td>30 (23-39)</td>
</tr>
<tr>
<td>PNH natural history before SCT, months</td>
<td>20 (7-59), 192</td>
</tr>
<tr>
<td>Clone size at transplantation (&lt;3 months before SCT)</td>
<td>56 (32-90), 56</td>
</tr>
<tr>
<td>Classification of PNH at transplantation</td>
<td></td>
</tr>
<tr>
<td>Classical PNH</td>
<td>85/191 (45%)</td>
</tr>
<tr>
<td>PNH in the setting of another bone marrow disorder</td>
<td>103/191 (54%)*</td>
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<tr>
<td>Indications for SCT†</td>
<td></td>
</tr>
<tr>
<td>Severe aplastic anemia</td>
<td>118/191 (62%)</td>
</tr>
<tr>
<td>Recurrent severe hemolytic crises</td>
<td>64/191 (70%)</td>
</tr>
<tr>
<td>Thrombosis†</td>
<td>47/191 (25%)</td>
</tr>
<tr>
<td>Mesenteric veins</td>
<td>17</td>
</tr>
<tr>
<td>Budd Chiari</td>
<td>14</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>6</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>3</td>
</tr>
<tr>
<td>Deep vein thrombosis</td>
<td>2</td>
</tr>
<tr>
<td>Myelodysplastic syndrome/acute myeloid leukemia</td>
<td>13/191 (7%)</td>
</tr>
<tr>
<td>Donor type</td>
<td></td>
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<tr>
<td>HLA-identical sibling</td>
<td>136/210 (65%)</td>
</tr>
<tr>
<td>Source of stem cells*</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>135/210 (64%)</td>
</tr>
<tr>
<td>Peripheral blood stem cells</td>
<td>71/210 (34%)</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide + busulfan</td>
<td>47/144 (33%)</td>
</tr>
<tr>
<td>Cyclophosphamide + total body irradiation (≥ 8 Gray)</td>
<td>22/144 (15%)</td>
</tr>
<tr>
<td>Cyclophosphamide + anti-thymocyte globulin</td>
<td>32/144 (22%)</td>
</tr>
<tr>
<td>Fludarabine-based regimen</td>
<td>42/144 (29%)</td>
</tr>
<tr>
<td>GvHD prophylaxis</td>
<td></td>
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<tr>
<td>Cyclosporine ± methotrexate</td>
<td>154/211 (73%)</td>
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</tbody>
</table>

*IQ: Interquartile range; †Three cases of subclinical PNH; ‡More than one indication for stem cell transplantation (SCT) was possible; §Nine patients were transplanted for renal failure and 18 for other reason; ‡Site was lacking for five cases; ‡Four patients received cord blood as the source of stem cells.
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<tr>
<th>Characteristics</th>
<th>n/N (%) or median (IQR*), N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender, female</strong></td>
<td>222/402 (55%)</td>
</tr>
<tr>
<td><strong>Age at diagnosis, years</strong></td>
<td>36 (25-51)</td>
</tr>
<tr>
<td><strong>Clone size</strong></td>
<td>30 (15-52), 132</td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td></td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>59/402</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>106/402</td>
</tr>
<tr>
<td>Budd Chiari</td>
<td>44</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>33</td>
</tr>
<tr>
<td>Deep vein thrombosis</td>
<td>31</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>7</td>
</tr>
<tr>
<td>Myelodysplastic syndrome/acute leukemia</td>
<td>21/402</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
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<tr>
<td>Immunosuppressive treatment (≥1)*</td>
<td>96/402 (24%)</td>
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*IQR: Interquartile range; 408 patients were eligible but five did not have follow-up and one was not evaluated for complications; Immunosuppressive therapy means one course of antithymoglobulin and/or cyclosporine.
Results

• Median follow up 61 months
• Engraftment failure in 14 (7%)
• Acute GVHD in 85 (40%)
• Chronic GVHD in 57 (27%), extensive in 24 (11%)
• PNH relapse in 1 patient
• Overall survival probability at 5 years 68%
Matching between non SCT patients (SFH registry) and SCT patients (EBMT promise)

- Complications (Severity of thrombosis)
- Age at thrombosis and decade
- Delay between PNH diagnosis and complication

Overall Survival (OS)

Median follow-up: 61 months

68% at 5 years

Deaths: n=64

Factor associated with OS

Recurrent hemolytic crisis

-Thrombosis

p=0.04
Matching between non SCT patients (SFH registry) and SCT patients (EBMT registry) has been analyzed for complications (severity of thrombosis), age at thrombosis, and delay between PNH diagnosis and complication.

The analysis included 24 matched pairs:
- SFH: 122 patients, 121 not confirmed.
- EBMT: 47 patients, 27 with follow-up <6 months post thrombosis.
- Median time since thrombosis: 2 years.

Survival analysis using Log Rank test shows:
- p Log Rank = 0.01
- p Cox stratified on pairs = 0.007
- HR\textsubscript{SCT/non grafted} = 10.0 (1.3-78.1)

Overall Survival (OS) graph indicates significantly better survival for SCT patients compared to non grafted patients.
Differences between IST alone & sib/no IST:

SFH (non SCT)

Period (< vs. ≥ 2000)  
0.17  
0.05
Conclusions BMT

- Allogeneic BMT can cure PNH
- Overall survival rates up to 70%
- SCT is not standard of care in PNH patients with thromboembolic events
- Severe aplastic anemia - PNH patients should be considered for transplant
- PNH patients with hemolytic crisis +/- moderate aplastic anemia should be treated with eculizumab
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§
    - Inadequate response
      - BMT, [steroids, splenectomy]**, supportive care

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
**Consider for patients with clinically significant extravascular hemolysis

Parker et al Hematology 2011
CANADIAN
PNH NETWORK
Excellence in PNH Education and Care
Who Are We?

Hematologists with established benign hematology programs

Oncologists with established programs in bone marrow failure

All with experience in:
PNH diagnosis • PNH assessment • Treatment and follow-up
(including experience with eculizumab (Soliris®))
PNH Referral Centre Locations

- Montreal
- Toronto
- London
- Edmonton
- Hamilton
- Calgary
- Quebec City
- Halifax
- Vancouver
- Montreal
- Toronto
- Hamilton
Our Shared Care Approach

Shared care allows the patient to remain with the referring physician, while benefitting from access to specialized PNH services.
What we stand for

Our Vision
“Patients in Canada must receive world-leading care that extends over case recognition, diagnosis and treatment follow-up.”

Our Mission
1. To maintain the highest standard of clinical practice in the management of PNH
2. To provide continuing health education on the evolving management of PNH patients
3. To participate in PNH registry to gain further understanding of the natural history of PNH
A global, observational, noninterventional study collecting safety, effectiveness, and quality of life data on PNH

Analyzed by a collaborative scientific board, chaired by Professor Peter Hillmen (Hematologist, Leeds Teaching Hospitals, England)

Benefits of your participation:
• Enhance understanding of PNH disease and treatment
• Capture the long-term outcomes of patients in order to better guide and assess treatments and the safety of Soliris®
• Expand robust, international database on PNH for scientific exchange and publications
Enrolled to date:

- Over **1800** patients globally
- Patients from: the United States, Canada, Argentina, Denmark, the Netherlands, the United Kingdom, Belgium, France, Germany, Spain, Switzerland, Finland, Sweden, Australia, New Zealand, and Taiwan

Who can contribute?

- ALL physicians managing patients with PNH, *regardless of therapy*

Who is eligible?

- All patients who have been diagnosed with PNH or have evidence of a positive PNH clone
- All patients with PNH should consider enrolment!
Conclusions

• PNH is a ultrarare clonal disorder resulting in hemolysis, thrombosis and pancytopenia

• Diagnosis by flow cytometry (FLAER)

• Treatment either observation, supportive care, complement blockade with eculizumab or in certain cases allogeneic stem cell transplantation

• Referral to a PNH network center and participation in the PNH registry is encouraged
Thank you for your attention