PNH – Current Thinking on the Disease, Diagnosis, and Treatment

Where have we been, where are we going?

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PNH

- Case
- What is PNH?
- What causes PNH?
- Relationship to aplastic anemia
- What are the clinical signs & symptoms of PNH?
- How is PNH diagnosed?
- How is PNH treated?
- What does the future hold?

PNH – A study case

- 32 y/o female who developed aplastic anemia in 1998. She underwent treatment with anti-thymocyte globulin (ATG) and cyclosporine × 6 months.
- In July 2002, her aplastic anemia recurred. She underwent another ATG treatment with cyclosporine continued until 2006.
- 2004 starts becoming more anemic. Erythropoietin initiated.
- 2006 develops episodes of dark urine, requires occasional transfusion of PRBCs.
- Referred to Duke University Medical Center in 2006.
Physical exam unremarkable

WBC 6.4, Hgb 8.7 g/dl, HCT 29, platelet 228K, LDH 3193 U/L, T bilirubin 1.8, Retic 6.9%

What is the differential for her hemolysis?

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PNH – A study case

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What is the differential for her hemolysis?

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PNH – A study case

PNH screen – Red cells: Type I 43%, Type II 10%, Type III 47%

PNH screen – Granulocytes 88% lack CD59

Treated with prednisone. Continues to have flares in hemolysis associated with abdominal pain, fatigue.

2008 Starts eculizumab.
What is PNH?

- Paroxysmal – sudden onset
- Nocturnal – occurring at night (or early in morning upon awakening)
- Hemoglobinuria

Despite the name, most patients do not present this way.

What is PNH?

- A rare and unusual acquired hematologic disorder characterized by
  - Intravascular hemolysis (breaking apart of red cells in the blood vessels)
  - Bone marrow failure (cytopenias= low blood counts)
  - Thrombosis (Blood clots)
- There is an incredible amount of clinical heterogeneity amongst patients with PNH.

1st published case report of PNH - 1866

Case Study

Gull WW. Guys Hospital Reports 12:381-392, 1866.
What is PNH?

In summary, PNH has:
- Been around a long, long time
- Has been complicated and difficult to understand
- Presents in many different ways

What causes PNH?

- PNH is due to a change (mutation) in a single gene in a bone marrow "stem" cell.
  - What is a mutation?
  - What is a stem cell?
- PNH is due to a condition that allows this mutated cell to become the dominant cell in the bone marrow.
What is a mutation?

- A mutation is a "mistake" in a gene that arise when a cell divides and has to copy the DNA. This mistake is not corrected and is passed on to "daughter cells" and all subsequent cells.
- Our dividing cells are always acquiring mutations. Most of the time these are "silent"
- Mutations can cause:
  - No effect
  - Complete absence of the protein produced by that gene
  - An altered protein with decreased or different function

What is a stem cell?

- A stem cell has two properties
  - Can divide to produce daughter cells and more stem cells (self renewal) forever.
  - Can "differentiate" (mature) into many different types of cells.
- Many different types of stem cells
  - Embryonic
  - Induced pluripotent stem cells (IPS)
  - Tissue specific
  - Hematopoietic
- Many different sources of hematopoietic stem cells
  - Bone marrow
  - Peripheral blood
  - Umbilical cord
  - Autologous

Hematopoietic Stem Cells

In PNH, a mutation occurs in a single gene (PIG-A) in a single hematopoietic stem cell.
What causes PNH?

- The mutation in the PIG-A gene in a hematopoietic stem cell leads to a defect in the production of an anchor protein that ties other proteins to the cell surface.
  - Sometimes the mutation leads to a partial decrease in the amount of anchor protein that is made and the cells have a partial deficiency (Type II cells).
  - Some patients have several stem cells with different mutations in PIG-A gene.

What causes PNH?

- The lack of the GPI anchor protein leads to a lack of many proteins on the surface of affected blood cells.
- In PNH, the major two proteins lacking on the surface of the red cells are CD59 and CD55.
- These proteins are important in protecting the red cells from complement.
- Other missing proteins may play a role but this is still unclear.
  - What is complement? (next slide please)

What is Complement?

- Complement is a group of proteins that are part of our immune system.
- Complement circulates in an inactive form.
- A little bit of complement is always being activated spontaneously, especially at night.
- Many different events can activate complement including trauma, infection, stress, etc.
- Complement will attack certain bacteria by making pores in the surface of the bacteria.
- In PNH, activated complement will attack red cells causing them to “lyse” (burst).
Terminal Complement Activation Renders RBCs Susceptible to Lysis

What happens when red cells lyse?

- The red cells are destroyed - anemia
- Hemoglobin is released into the plasma (the fluid part of blood)
- Some of the hemoglobin passes through the kidneys and into the urine leading to the dark color of the urine
  - Loss of iron
  - May lead to kidney damage in the long run
- Free hemoglobin binds nitric oxide
  - What is nitric oxide?

What is nitric oxide?

- A gas produced by the body to regulate smooth muscle cells.
- An increase in free nitric oxide causes smooth muscle cells to relax. A decrease causes smooth cells to contract.
- Smooth muscle cells are in many tissue
  - Blood vessel walls: ischemia, impotence
  - Esophagus and GI tract: esophageal spasm, reflux, abdominal pain
What causes PNH?
How do cells with a mutation take over the bone marrow?

- Normal people may carry cells in their bone marrow with a PIG-A mutation, usually at a very low level and probably not in a true stem cell.
- In PNH, something allows the abnormal cells to become the dominant cells and become the major population in the marrow (anywhere from 1 to over 90% - referred to as the clone size).
  - This "something" may be related to aplastic anemia, a disease of poor production of the bone marrow.

How is PNH related to aplastic anemia?

- Many patients with PNH have or will develop aplastic anemia, or have a history of having had aplastic anemia.
- Many PNH patients have evidence of poor production of cells by their bone marrow (Bone marrow failure) leading to low white cell counts or low platelet counts.
- The cause of aplastic anemia (immune assault?) may also play a role in the development of PNH.

Models of pathogenesis

<table>
<thead>
<tr>
<th>Normal Marrow</th>
<th>Aplastic Anemia</th>
<th>PNH</th>
<th>MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune Assault</td>
<td>Immune Assault</td>
<td>PNH (hemolysis)</td>
<td>PNH (hypoplasia)</td>
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<td>Immune Assault</td>
<td>Immune Assault</td>
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<tr>
<td></td>
<td></td>
<td>PNH (hemolysis)</td>
<td>PNH (hypoplasia)</td>
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</tbody>
</table>
The clinical picture of PNH

- Hemolysis due to complement activation
  - Anemia and fatigue
  - Hemoglobinuria, kidney damage
  - Nitric oxide trapping => Esophageal spasm, abdominal pain, pulmonary hypertension, impotence, fatigue?
- Thrombosis – Cause of blood clots is still unknown
  - Unusual sites of blood clots
- Bone marrow failure
  - Decreased blood counts (cytopenias)

PNH – How is it diagnosed?

Average delay to diagnosis exceeds 3 years; may be greater than 10 years?

- PNH continues to be primarily a clinical diagnosis, which can be confirmed by laboratory analyses
- Signs and symptoms are highly variable and may mirror other conditions
- Most common symptoms at presentation are not unique to PNH
  - Hemolytic anemia, often requiring transfusions
  - Fatigue
  - Dyspnea
  - Abdominal pain or dysphagia

PNH – How is it diagnosed?

Methods of Historical Interest

- Ham Test – acidified serum lysis test
  - Specific but not sensitive
- Sugar Water Test – serum in isotonic sucrose solution
  - Sensitive but not specific
- Complement lysis sensitivity test – lysis by antibody and limiting complement
  - Defined PNH II (moderately abnormal)
  - and PNH III (markedly abnormal red blood cells)
PNH – How is it diagnosed?

Flow Cytometry: Diagnostic Test for PNH

- Perform on peripheral blood
- Test both granulocytes and erythrocytes
  - Erythrocytes alone are not sufficient due to hemolysis and the dilution effect of transfusions
- Use monoclonal antibodies against GPI-anchored proteins, such as CD55 or CD59
- PNH blood cells (PNH clone) are cells missing GPI-anchored proteins
- New guidelines for diagnosis issued by the Clinical Cytometry Society

PNH – How is it diagnosed?

Fluorescent AERolysin (FLAER)

- FLAER binds to the GPI-anchor itself, rather than to a single protein such as CD55 or CD59
- FLAER provides much greater signal noise and better accuracy than an antibody against a single target

PNH – What is “clone size” and why is it important?

- Clone size refers to the percentage of bone marrow stem cells that are abnormal.
- Since PNH red cells are being destroyed faster than normal red cells, the percentage of PNH red cells is usually much less than the true clone size.
- Clone size is much more accurately estimated by the % of PNH granulocytes or monocytes.
PNH – Diagnosis
What is “clone size” and why is it important?

• In general, the higher the clone size, the more symptomatic. This includes hemolysis but also the chance of developing thrombosis.

• However, there are no absolute rules. Patients with low clone size can still develop a thrombosis, and patients with high clone size can be relatively asymptomatic.

• Clone size should not be used to make any current treatment recommendations.

Treatment of PNH

• Who needs to be treated?
• With what?
• Does everybody respond?
• What is the long term outlook?

PNH – Who needs to be treated?

• Patients with blood clots
• Patients with symptomatic anemia from hemolysis
• Patients with severe bone marrow failure

The Treatment “Grey zone”

• Patients with hemolysis who are not symptomatic
  ◦ Can we prevent long term complications such as blood clots, renal failure, or pulmonary hypertension?
How do we treat PNH -hemolysis

- Transfusion
- Iron, folic acid
- Steroids
- Eculizumab (Soliris)

What does Eculizumab do?

- Quickly and effectively blocks complement activation at C5.
- Blocks hemolysis and related effects
- Stops hemoglobinuria
- Markedly reduces transfusion requirements
- Hemoglobin / hematocrit may not return to “normal”

Reduction in LDH During Eculizumab Treatment in TRIUMPH and SHEPHERD

TRIUMPH placebo patients switched to SOLIRIS after week 26.
All TRIUMPH patients entered the long-term extension study.
SHEPHERD: Eculizumab Reduced Transfusions

- **Overall**
  - Pre-Treatment: 8
  - Post-Treatment: 17

- **< 4 Units**
  - Pre-Treatment: 0
  - Post-Treatment: 0

- **4 - 14 Units**
  - Pre-Treatment: 2
  - Post-Treatment: 3

- **15 - 25 Units**
  - Pre-Treatment: 10
  - Post-Treatment: 2

- **> 25 Units**
  - Pre-Treatment: 33
  - Post-Treatment: 3

**Transfusion Requirements 12 Months Prior to Treatment**

**Median Units Packed RBCs**

- Overall: < 4 Units
- 4 - 14 Units
- 15 - 25 Units
- > 25 Units

**Overall (n = 97)**

**12 Months Prior to Treatment**

**Mean Change from Baseline FACIT-Fatigue Score**

- Pre-Treatment: 0
- Post-Treatment: 8

**Improvement**

- **Overall** (n=179)
  - Stage 1 - 2 (n=77)
  - Stage 3 - 5 (n=39)

**Renal Function with Eculizumab in Different Baseline Populations - 12 Months**

- **Segment of PNH Population**
  - Overall (n=176)
  - Stage 1 - 2 (n=77)
  - Stage 3 - 5 (n=39)

**Percentage of Patients (%)**

- No Change
- Improvement
- Worsening

**Results**

- **Overall**
  - No Change: 58.1%
P=0.001
  - Improvement: 15.2%
P=0.001
  - Worsening: 26.7%
P=0.02

- **Stage 1 - 2**
  - No Change: 71.4%
P=0.02
  - Improvement: 5.2%
P=0.001
  - Worsening: 5.2%
P=0.001

- **Stage 3 - 5**
  - No Change: 76.9%
P=0.01
  - Improvement: 20.5%
P=0.02
  - Worsening: 26%
P=0.02

**Functional Assessment of Chronic Illness Therapy-Fatigue instrument.**

**P<0.001.**

**P-value compared with baseline based on signed rank test.**

**Improvement**

- **Overall** (n=179)
  - Stage 1 - 2 (n=77)
  - Stage 3 - 5 (n=39)

**Eligibility**


**Conclusion**

- Eculizumab reduced transfusions.
- Improved fatigue.
- Improved renal function in different baseline populations.
Change in BNP during eculizumab treatment

![Bar chart showing change in BNP during eculizumab treatment compared to placebo.](chart.png)

Eculizumab vs placebo (P<0.001)

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Week 26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>39.4</td>
</tr>
<tr>
<td>Eculizumab</td>
<td>43.8</td>
</tr>
<tr>
<td>Placebo</td>
<td>52.5</td>
</tr>
<tr>
<td>Eculizumab</td>
<td>26.3</td>
</tr>
</tbody>
</table>

PHT with NT-proBNP ≥160 pg/mL


What eculizumab does not do

- Probably does not help bone marrow failure (improve other low blood counts)
- Completely correct anemia
- Teach you how to play the violin if you have never played before

Downside of Eculizumab treatment

- Increased risk of meningococcal infections
  - All patients must be vaccinated
  - All patients educated on signs and symptoms of meningitis and what to do
  - All patients given cards describing this
- Cost
- Possible coating of red cells with C3 complement leading to their destruction (extravascular) and anemia
- Inconvenience
  - Must be given intravenously every 12-14 days
How do we treat or prevent blood clots?
- Coumadin prophylaxis
- Acute treatment with lytic agents (clot busters)
- Anticoagulation therapy
- Bone marrow transplantation
- Eculizumab

Effect of Eculizumab on Thrombosis
- 92% Fewer thrombotic events with SOLIRIS treatment

How do we treat bone marrow failure?
- Stimulating agents such as erythropoietin
- Immunosuppressive agents (ATG, cyclosporine A)
- Bone marrow transplantation

PNH – What do patients die from?

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Duke</th>
<th>Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombosis</td>
<td>16 (42%)</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>Abd site</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Other site</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Arterial</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>4 (10.5%)</td>
<td>9 (24%)</td>
</tr>
<tr>
<td>Severe Infection</td>
<td>14 (36.6%)</td>
<td>14 (36.8%)</td>
</tr>
<tr>
<td>MDS/AML</td>
<td>3 (8%)</td>
<td>6 (16%)</td>
</tr>
<tr>
<td>Renal failure</td>
<td>3 (8%)</td>
<td>7 (18%)</td>
</tr>
<tr>
<td>Other malignancy</td>
<td>2 (5%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (5%)</td>
<td>0</td>
</tr>
</tbody>
</table>


Possible long term effects of Eculizumab

- Improve kidney function
- Prevent pulmonary hypertension
- Increase survival

PNH Survival – Pre-eculizumab
Eculizumab Has a Major Impact on Survival in PNH

Survival is comparable to age and gender-matched control population out to 8 years

- 96% (76/79) patient survival
- There was no difference in mortality between patients on eculizumab and the normal population (P=0.46)

Survival is comparable to age and gender-matched normal population out to 8 years

<table>
<thead>
<tr>
<th>Time (years)</th>
<th>Cumulative surviving (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>89</td>
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<td>3</td>
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<td>6</td>
<td>72</td>
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<tr>
<td>7</td>
<td>62</td>
</tr>
<tr>
<td>8</td>
<td>53</td>
</tr>
<tr>
<td>9</td>
<td>44</td>
</tr>
</tbody>
</table>


Patient Survival in the Eculizumab Study Population

- Overall survival was 97.6% (95%CI 93.7-99.1) at 3 years and was maintained through 5.5 years of ongoing eculizumab treatment (N=195)

<table>
<thead>
<tr>
<th>Time [months]</th>
<th>Cumulative surviving (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>99.5</td>
</tr>
<tr>
<td>2</td>
<td>99.1</td>
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<tr>
<td>3</td>
<td>98.7</td>
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<tr>
<td>4</td>
<td>98.3</td>
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<tr>
<td>5</td>
<td>97.9</td>
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<td>6</td>
<td>97.5</td>
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<td>97.1</td>
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<td>8</td>
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<td>96.3</td>
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<td>10</td>
<td>95.9</td>
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<tr>
<td>11</td>
<td>95.5</td>
</tr>
<tr>
<td>12</td>
<td>95.1</td>
</tr>
</tbody>
</table>


Where are we going?

- Improve current therapy
  - Oral eculizumab
  - Increase treatment intervals
- Find other ways to inhibit complement
- Understand how PNH cells take over the bone marrow so we can reverse this process (Restore normal stem cells)
- Gene therapy
- Stem cell transplants