Plan

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    - prophylaxis of thrombosis
    - treatment of thrombosis
    - treatment during pregnancy

- Literature
PNH: History

- First description by a German physician Paul Strübing in 1889
- More detailed description by Dr. Ettore Marchiafava and Dr. Alessio Nazari in 1911, then Dr. Ferdinando Micheli in 1931 (Marchiafava-Micheli syndrome)
- Identification of the molecular basis of PNH helped to define the physiology of the complement system in humans. (47)
PNH: Definition

- Rare acquired disorder of hematopoietic stem cells
- Incidence: not really known but estimated 1-2/1,000,000 inhab./year
- Somatic mutation in X-linked gene: the phosphatidylinositol glycan class A (PIG-A)
- PIG-A is required for synthesis of glycosyl phosphatidylinositol (GPI) anchor
- Deficiency in GPI anchored proteins (many)
- Lack of CD59 « MIRL » and CD55 « DAF » (complement regulatory proteins) leads to complement mediated intravascular hemolysis (47)
A Normal, steady state

Alternative pathway
Classical pathway
Lectin pathway

C3 convertase
C5 convertase

C3b
C5b
C6
C7
C8
C9

MAC

A normal (CD55+, CD59+) red cell can withstand the hazard of complement activation.

B PNH, steady state

Alternative pathway
Classical pathway
Lectin pathway

C3 convertase
C5 convertase

C3b
C5b
C6
C7
C8
C9

MAC

An abnormal (CD55−, CD59−) red cell (PNH cell) will be lysed sooner or later by activated complement (intravascular hemolysis).
PNH: Definition

- Non malignant clonal expansion: 2 steps hypothesis (53)

  - step 1: PIG-A mutation
    - no clonal expansion, asymptomatic

  - step 2: injury to the normal bone marrow
    - T cells and NK cells attack
    - normal hematopoietic cells destroyed
      but PNH cells escape
    (absence of GPI anchored targeted peptides or accessory molecules required for T cells attack)

  $\Rightarrow$ bone marrow failure, clonal expansion
PNH: Diagnosis

- (Ham and sucrose tests)

- Flow cytometry with monoclonal antibodies that bind to specific GPI-anchored proteins (percentage of PNH cells measured)\(^{(38)}\)
  - clone size
    - use at least 2 different monoclonal antibodies, directed against 2 different GPI-anchored proteins (rare congenital deficiencies of CD59 or CD55)
    - on at least 2 different cells lineages (risk of falsely negative tests if only red cells are screened – recent hemolytic crisis or recent transfusion)

- phenotypic mosaicism
  - PNH III cells: completely deficient in GPI anchored-proteins
  - PNH II cells: partly deficient
  - « PNH » I cells: express GPI-anchored proteins at normal density
PNH: Diagnosis

- FLAER (fluorescein-labeled proaerolysin)\(^{(34)}\)
  - Aerolysin = the principal virulence factor of the bacterium Aeromonas hydrophila
  - binds selectively and with high affinity to the GPI anchor
  - more accurate assessment of the GPI anchor deficit in PNH (PNH clones <1% can be detected)

- complete blood count, reticulocytes, LDH, bilirubin, haptoglobin, iron stores, BM aspirate and biopsy, cytogenetics
PNH: Clinical aspects

- 3 different forms (clinical polymorphism)\(^{(3, 4, 34)}\)
  - Classic PNH
    - young adults
    - intravascular hemolytic anemia (with crisis)
      - Hb <12 g/dL
      - hemoglobinuria (in the morning)
      - jaundice
      - general symptoms (asthenia, malaise)
      - worsening of hemolysis during infection (complement activation above basal level)
    - no evidence of bone marrow failure (neutro >1500/µL, platelets >120 000/µL)
    - dysphagia, abdominal pain, erectile dysfunction (35%)
    - thrombosis
    - recurrant infectious diseases (ORL, lungs): 40%, 2d cause of death
    - risk of MDS/AML :5% / 2,5% at 10y (x100)
PNH: Clinical aspects

- PNH associated with aplastic anemia
  - small PNH clone (majority of patients: <10%)
  - clinical/biochemical evidence of hemolysis
  - 2 or 3 cytopenias (Hb <10 g/dL, neutro <1000/µL, platelets <80 000/µL)
  - BM failure dominates the clinical picture
PNH: Clinical aspects

- **Subclinical PNH**
  - clone size <1%
  - no hemolysis

- We find PNH clone in 60% of AA and in 20% of low risk MDS\(^{(47)}\)
  - associated to AA: high probability of response to immunosuppressive therapy
  - associated to low risk MDS (refractory anemia)
    - less pronounced morphological abnormalities of the blood cells
    - more severe thrombocytopenia
    - lower rate of karyotypic abnormalities
    - higher incidence of HLA-DR15
    - lower rate of progression to AML
    - higher probability of response to cyclosporine

- Hypothesis that aplastic anemia and a subgroup of low risk MDS are immune-mediated diseases
  - immunity provides the selection pressure that favors the outgrowth of PNH clone
## PNH: Clinical aspects

<table>
<thead>
<tr>
<th></th>
<th>Classic PNH</th>
<th>Aplastic anemia-PNH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40y</td>
<td>30y</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>35%</td>
<td>20%</td>
</tr>
<tr>
<td>Median clone size</td>
<td>91,6%</td>
<td>16,8%</td>
</tr>
<tr>
<td>Median survival</td>
<td>18y</td>
<td>&gt;22y</td>
</tr>
<tr>
<td>Thrombotic events (at 10y)</td>
<td>37,9%</td>
<td>27,8%</td>
</tr>
<tr>
<td>Medullar aplasia</td>
<td>20% at 10y</td>
<td>100%</td>
</tr>
</tbody>
</table>
PNH: Pathophysiology of disease

- Intravascular hemolysis
  - free Hb in the plasma
  - NO fixation to Hb
  - NO depletion
    - smooth muscle dystonias
      - dysphagia
      - abdominal pain
    - erectile dysfunction
  - arterial constriction
    - reduced blood flow to the kidneys
    - arterial hypertension
    - pulmonary hypertension (+undiagnosed thrombosis)
PNH: Thrombotic events

- Occurs in 40% of PNH.
- Cause of death in 40-67% of patients (major cause of death)
- Arterial (15%) and venous (85%) thrombosis
- Involve more than one site (20.5%)

**Usual sites**
- deep veins thrombosis
- pulmonary embolism
- myocardial infarction
- CNS arteries

**and unusual sites**
- hepatic veins (Budd-Chiari syndrome, the most frequent thrombotic complication of PNH: 40.7%)
- cavernous sinus
- CNS veins
- mesenteric veins
- dermal veins
PNH: Thrombotic events

- Age of onset lower than in general population (46y vs 73y)
- Recurrences despite anticoagulant therapy described\(^{(3)}\)
- Thrombosis during pregnancy and post-partum
  - Increased risk of thrombosis
    - Maternal mortality 20%
    - Perinatal mortality 10%
PNH: Pathophysiology of thrombosis

- Unknown - multifactorial...

- Role of hemolysis? controversial...
  - no link between
    - severity of hemolysis and thrombosis
    - severity of anemia and thrombosis
  - NO depletion\(^{(40)}\)
    - causes arterial constriction
    - increases platelets activation and aggregation
    - enhances TF expression?
  - ADP release induce platelets activation\(^{(5)}\)
  - human RBC stroma activate the alternate complement pathway\(^{(7)}\)
    - interaction of activated complement components with platelets
    - release of PF-3
    - activation of coagulation
Mechanisms and clinical implications of thrombosis in paroxysmal nocturnal hemoglobinuria
PNH: Pathophysiology of thrombosis

- CD59 and CD55-deficient platelets
  - complement can induce platelet activation
  - one study showed increased adherence of platelets to the abdominal vessels.\(^{(1)}\)
  - PNH platelets, after MAC (membrane attack complex) stimulation, expose more FVα-binding sites and increase thrombin generation more than normal platelets\(^{(40)}\)
  - BUT other studies showed impaired function of PNH platelets (reactive downregulation in response to chronic hyperstimulation)
PNH: Pathophysiology of thrombosis

- Endothelial cells
  - Direct toxicity of free hemoglobin released
  - Endothelial precursor cells seem to be bone marrow-derived cells
    - Probable same GPI-anchored proteins deficiency
      - More susceptible to complement injury
      - Thrombosis in unusual sites
  - Damage resulting from the uptake of monocyte-derived MVs (released from GPI-deficient monocytes upon complement damage)
    - These MVs contain tissue factor, thus increasing TF expression on endothelial cells
  - Increased number of EMVs with a prothrombotic and proinflammatory phenotype in PNH
  - Increased levels of endothelial cells activation markers (VWF, sVCAM-1)
PNH: Pathophysiology of thrombosis

- Possible role of C5a protein
  - binds to a receptor on granulocytes
  - recruitment and activation of granulocytes and monocytes
    (enhanced in GPI-deficient white blood cells?)
    - release of inflammatory molecules
      - damage the endothelium
      - TF expression
    - release of monocyte-derived MVs, containing TF
  - = link between inflammation and thrombosis?
PNH: Pathophysiology of thrombosis

- Tissue factor pathway inhibitor (TFPI)
  - TFPI limits coagulation initiation by inhibiting TF formation
  - mainly produced by the endothelium of the microvasculature
  - bound to GPI-anchored protein (deficiency in PNH)
  - loss of TFPI expression has been associated with increased risk of thrombosis
PNH: Pathophysiology of thrombosis

- Neutrophil proteinase-3 (PR-3)\(^{(19)}\)

  - Membrane bound protein, co-localized with GPI-anchored neutrophil antigen 2a
  - Absence of neutrophil antigen 2a in PNH leads to loss of expression of neutrophil proteinase-3
  - PAR-1 (the predominant human platelet thrombin receptor) is a substrate for PR-3
  - Impaired down regulation of PAR-1 \(\rightarrow\) increased propensity for platelet activation
  - PR-3 also modulates coagulation in various other ways
PNH: Pathophysiology of thrombosis

- Urokinase-type plasminogen activator receptor (u-PAR)\(^\text{(10,13,15,17)}\)
  - central role in the regulation of haemostatic processes on cell surface
    - binding of u-PA (urokinase plasminogen activator) to u-PAR converts plasminogen into plasmin
      - involved in fibrinolysis, make it localized pericellularly
  - GPI-anchored protein
  - underexpressed on cellular surface in PNH (granulocytes and monocytes)
  - increased concentrations of soluble uPAR (released from PNH hematopoietic cells)
    - impaired and displaced fibrinolytic system?

- Other fibrinolysis parameters
  - conflicting results
PNH: Pathophysiology of thrombosis

- Increased circulating MVs in PNH patients\(^{(1,8)}\)
  - procoagulant properties of MVs (exposing PS, containing TF) have been demonstrated in vitro
  - complement activation may stimulate the release of procoagulant MVs
  - no link between clone size and number of MVs
  - majority of them come from platelets\(^{(8)}\)
  - EMVs levels are also increased in PNH patients, with prothrombotic and proinflammatory phenotypes\(^{(9)}\)
  - similar level of erythrocyte MVs in vivo but PNH erythrocytes release higher amounts of procoagulant MVs upon complement stimulation in vitro (may suggest rapid clearance from the circulation)\(^{(40)}\)
PNH: Pathophysiology of thrombosis

- Increase of procoagulant activity (fact V, fibrinogen, fact VIII, fact IX, fact X and vWF)(10)
  - positive correlation with clone size

- Frequency of congenital thrombophilia factors is not increased in PNH(40)
  - testing for such factors may identify PNH patients at additional risk
  - its value for treatment decision in PNH is unknown
  - authors do not recommend routine testing
PNH: Risk factors for thrombosis

- Large WBC clone
  - clone >50%: 10y cumulative incidence of thrombosis = 34,5%
  - clone <50%: 10y cumulative incidence of thrombosis = 5,3%

- Previous history of thrombosis

- Old age (>55y)

- Use of transfusions

- Other ??? Elevation of D-dimers?

  → Indications of prophylactic anticoagulation?
PNH: Treatment of subclinical PNH and AA/PNH\(^{(47)}\)

- **Subclinical PNH**
  - no symptoms, no treatment
  - close monitoring (every 6-12 months) because expansion of the clone may occur

- **PNH associated with aplastic anemia**
  - treatment of the underlying bone marrow failure syndrome (allogenic transplantation, immunosuppressive therapy)
PNH: Treatment of classic PNH

- Eculizumab (Soliris®; Alexion Pharmaceuticals) \(^{(6, 18, 31)}\)
  - humanized monoclonal antibody
PNH: Treatment of classic PNH/
eculizumab

- blocks the activation of terminal complement C5 and prevents the formation of C5a and C5b-9
PNH: Treatment of classic PNH/eculizumab

- administration schedule
  - 25-45 min IV infusion
  - induction dose of 600 mg every 7 days for 4 doses
  - then 900 mg 7 days later
  - followed by a maintenance dose of 900 mg every 14 days

- vaccination against Neisseria meningitidis at least 14 days prior to receiving eculizumab
  - 0.5%/y risk of neisserial sepsis (even after vaccination)\(^{(34)}\)
  - revaccinate every 3 to 5 years
  - medical attention if patient develops signs of neisserial infection
PNH: Treatment of classic PNH/eculizumab

- TRIUMPH (randomized placebo-controlled phase 3 study)
  - in transfusion-dependant PNH patients with good bone marrow reserve.
  - reduces hemolysis, transfusion requirements,
  - improves anemia, fatigue and measures of quality of life

- SHEPHERD (open-label phase 3 study)\(^{(6)}\)
  - to investigate the long-term safety and efficacy of eculizumab
  - 97 patients enrolled, median age 41 y, follow up 52 weeks
  - 89/97 pat. got a fast response and maintained complete inhibition of hemolysis \(\rightarrow\) efficient
  - most common adverse events: headache, nasopharyngitis, upper respiratory tract infection
  - 8,3% of infections possibly related to treatment
  - 44 SAEs (none was considered probably or definitely related to treatment) \(\rightarrow\) well tolerated
PNH: Treatment of classic PNH/ eculizumab

- Impact on overall survival?
  - Survival for patients on eculizumab appears to be similar to that of the normal population (50)
PNH: Treatment of classic PNH/ eculizumab

- Reasons for suboptimal response (47-48)

- breakthrough intravascular hemolysis near the end of a treatment cycle
  - cycles of 13 or 12 days
  - increase dose of eculizumab

- extravascular hemolysis
  - Frequently, persistent elevation of reticulocytes, bilirubin and LDH levels, persistent reduction of haptoglobin
  - A small group of eculizumab-treated patients: little or no improvement in either anemia or constitutional symptoms
  - inhibition of intravascular lysis by eculizumab
    - increase of PNH red blood cells, loaded with early components of the complement system (C3 opsonins)
      - eculizumab does not block the C3 convertase (not down regulated by CD55)
    - recognized by reticuloendothelial cells
    - hemolysis in the spleen (and the liver)
PNH: Treatment of classic PNH/eculizumab
PNH: Treatment of classic PNH/eculizumab

- What to do?
  - No treatment if no constitutional or anemia symptoms or transfusion dependence
  - Corticosteroids or splenectomy?
PNH: Treatment of classic PNH/eculizumab

- Reduction of thrombosis with eculizumab
  - Number of TE events and incidence rates determined in patients from 3 clinical studies with eculizumab\(^{(31)}\)
    - 87-94% reduction of the thrombotic events rate during eculizumab treatment
  - Decrease in plasma D-dimers levels with eculizumab\(^{(44)}\)
    - Suppression of thrombin generation
  - Study of markers of thrombin generation and fibrinolysis in 23 PNH patients\(^{(18)}\)
    - Before and after 5 and 11 w of eculizumab treatment

- Eculizumab reduces the activation of the plasma hemostatic system and the vascular endothelium
  - Role of the endothelial cell activation in the pathogenesis of thrombosis in PNH?
- No effect of eculizumab on the number of EMVs, on MVs procoagulant activity or on the number of ICAM-1 expressing EMVs.
PNH: Treatment of classic PNH/eculizumab

- Rapid and sustained decrease in the markers of thrombin generation and inflammation during eculizumab therapy\(^{(44)}\)
  - significant reduction in D-dimers, thrombin-antithrombin complex, IL-6, soluble P-selectin (expressed by activated platelets and endothelial cells), TFMP (tissue factor bearing microparticles)
  - no link between hemolysis (LDH) and markers of thrombin generation and inflammation
  - the decrease of TFMP didn’t correlate with changes in markers of thrombin generation and inflammation
PNH: Treatment of classic PNH/eculizumab

- What happens if we stop eculizumab?
  - risk of hemolytic crisis
    - increased number of PNH red blood cells during eculizumab treatment
  - risk of thrombosis?
    - one case described of a first thrombotic event 3 weeks after the last eculizumab dose (women died)
      - during anticoagulant prophylaxy (therapeutically dosed phenprocoumon/Marcoumar®)
      - no hemolysis
      - mechanism?
PNH: Treatment of classic PNH

- Bone marrow transplantation
  - the only curative option
    - important graft-versus-PNH effect
    - lack of suitable donors
    - high rates of treatment-related morbidity/mortality
      - 2-year survival probability: 56% (34)
      - acute and chronic graft versus host disease: 15% and 20%
    → only for patients with life threatening cytopenias or disabling hemolysis or thrombosis that is not controlled with eculizumab

- Supportive care
  - transfusions if needed (efficient, may lessen hemolysis by suppressing erythropoiesis)
  - folate supplements
  - iron supplements if needed (hemoglobinuria, hemosiderinuria), rare during eculizumab therapy (sometimes iron depletion therapy needed)
  - EPO: increases hemolysis symptoms, may be used when patients remain anemic with eculizumab and have low EPO level
  - corticosteroids: long term toxicity, use for acute hemolysis crises
  - androgens (Danazol)? no data
PNH: Prophylaxis of thrombosis

- Primary prophylactic anticoagulation?
  - no randomized trials
  - some data suggest that warfarin effectively reduces thrombotic risk
    - anticoagulation in PNH patients is difficult
      - thrombocytopenia (aplasia, liver failure) → risk of bleeding
      - difficult to maintain a therapeutic INR with warfarin
        - oesophageal spasm
        - mesenteric insufficiency
          → irregular feeding
    - recommendation of the international PNH interest group:
      - consider vitamin K antagonist prophylaxis in patients with PNH granulocyte clone >50% and no contraindications for prophylaxis
  - other authors say:
    - No proven benefit for primary prophylactic anticoagulation (except pregnancy)
PNH: Prophylaxis of thrombosis

- aspirin? glycoprotein IIb-IIIa receptor antagonists?
  - platelets may have a role in the pathophysiology of thrombosis in PNH
  - most thrombotic events occur in the venous system
- LMWH?
- New oral anticoagulants (rivaroxaban, dabigatran, ...)?
- Immunosuppressive therapy?
  ⇒ need to define subgroups of PNH patients who could benefit from anticoagulation (risk factors)
- Eculizumab is the best preventive treatment for thrombosis
PNH: Treatment of thrombosis

- If life-threatening thrombosis → thrombolytic therapy
- Anticoagulation therapy
  - foundation of treatment
  - warfarin? LMWH? NOAC?
  - for how long? lifelong?
PNH: Treatment during pregnancy

- greater need for folate and iron supplementation

- prophylactic full anticoagulation with LMWH if no contraindication
  - example: enoxaparin 1mg/kg/12h SC
  - starting when pregnancy is confirmed
  - monitor anti-factor Xa levels
  - sometimes need to switch to unfractionated heparin if a cesarean section is planned
  - anticoagulation has to be continued during the post-partum period

- eculizumab?
  - listed as category C pharmaceuticals (drug with little or no information available)
  - important potential benefit of eculizumab in reducing thrombosis
  - IgG2 isotypes do not cross the placenta, probably little impact on the fetus.
  - single case report: eculizumab was administered to a pregnant PNH patient in week 30 of her pregnancy (twins). No complications were reported.


