PNH from the perspective of Paediatric Haematologists

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Fabio Timeus disclosures

- Alexion: travel grants and honoraria for speaking at meetings.
Diagnosis and management of paroxysmal nocturnal hemoglobinuria

Blood. 2005 Dec 1; 106: 3699-3708

Classification of PNH:

- Classic PNH
- PNH in the setting of another specified bone marrow disorder
- Subclinical PNH
## PNH in children

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<th>Reference</th>
<th>N</th>
<th>Age at Diagnosis (years)</th>
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<th>Hemolytic</th>
<th>Thrombosis</th>
<th>Treatment</th>
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Paroxysmal nocturnal hemoglobinuria with onset in childhood and adolescence


- 26 patients, median age 14.3 years (0.8-21.4)
- dark urine in 15%
- moderate-severe bone marrow failure at presentation in 58%
- TE in 31% of patients
- Treatment: prednisone, androgens, ATG, cyclosporine
- in one patient AML treated with BMT
- median survival since diagnosis 13.5 years
van den Heuvel-Eibrink MM, Bredius RG, te Winkel ML, Tamminga R, de Kraker J, Schouten-van Meeteren AY, Bruin M, Korthof ET.

Childhood paroxysmal nocturnal haemoglobinuria (PNH), a report of 11 cases in the Netherlands


- 11 patients, median age 12 years
- 7 AA, 4 MDS
- hemosiderin in urine only in one patient
- PNH clone size in granulocytes 11-90%
- median diagnosis delay 2 months
- TE in 2 patients
- BMT in 5 patients

Paroxysmal nocturnal hemoglobinuria in pediatric patients


- 12 patients, median 13 years
- 6 AA, 5 MDS
- PNH clone size 35-92%
- TE in 6 patients
- HSCT in 5 patients
- Eculizumab therapy in 3 patients
- Diagnosis delay in 7 patients

Paroxysmal nocturnal hemoglobinuria in childhood and adolescence- a retrospective analysis of 18 cases

Indian Journal of Pediatrics, 2008 June

- 18 patients median age 16 years (range 11-18)
- Dark urine in 7 (39%)
- PNH/AA in 9 (50%)
- TE in one patient (6%)
- Response (low quality) to danazol/prednisolone in 66% of classic PNH and in 55% of PNH/AA
Urbano-Ispizua A et al.

Clinical characteristics of classical paroxysmal nocturnal hemoglobinuria (PNH) in pediatric patients: a comparison with classic PNH in adults. An international PNH Registry Study.

ASH annual Meeting 2011 118: abstract 2102

- 49 patients, median age 15 years (5-17) with classic/hemolytic PNH
- No differences in neutrophils, reticulocytes, LDH, GPI-negative clone size, number of transfusions between pediatric and adults patients
- No differences in clinical manifestations, except for less fatigue than in adults.
- TE incidence 12.2% in paediatric patients and 23.9% in adults
PNH in paediatric patients

- Bone marrow failure in the majority of patients at diagnosis
- Dark urine uncommon
- Thrombosis less frequent than in adults
- Diagnosis often delayed
Acquired aplastic anaemia and PNH

- PNH and acquired aplastic anaemia (AA) are closely related with a possible reciprocal evolution.
- Previous studies in adults with AA found a high incidence of PNH clones at diagnosis (Maciejewski et al., 2005; Ishiiyama et al., 2003; Sugimori et al., 2006).
- Selective advantage of PNH+ progenitors in the presence of immune-mediated damage (Karadimitris A and Luzzatto L, 2001).
PNH clones and response to IST in AA

• Positive correlation between the presence of a PNH clone at diagnosis and favourable response to IST (Maciejewski et al, 2005; Sugimori et al, 2006; Afable et al, 2011; Kulagin et al, 2014)

• Not confirmed by other studies (Yoshida et al, 2008; Scheinberg et al, 2009; Timeus et al, 2010; Sutton et al, 2013; Timeus et al 2014)
Clinical impact of HLA-DR15, a minor population of paroxysmal nocturnal haemoglobinuria-type cells, and an aplastic anaemia-associated autoantibody in children with acquired aplastic anaemia


- 103 AA paediatric patients
- PNH clone in 21.4% (range 0.04-0.81%)
- No significant difference in the response to IST between PNH+ and PNH- patients at diagnosis
Phillip Scheinberg, Michael Marte, Olga Nunez and Neal S. Young

Paroxysmal nocturnal hemoglobinuria clones in severe aplastic anemia patients treated with horse anti-thymocyte globulin plus cyclosporine

Haematologica 2010;95 (7):1075-1080

• 47 pediatric patients

• 14 (30%) with a PNH clone>1% at diagnosis

• No difference in response rates to IST between patients with or without a pretreatment PNH clone

• Trend towards a gradual decrease in clone size after IST
Sutton KS, Shereck EB, Nemecek ER, Kurre P

Immune markers of disease severity and treatment response in pediatric acquired aplastic anemia

Pediatr Blood Cancer 2013; 60: 455-460

- 26 patients
- 9/23 SAA and 1/3 MAA were PNH+
- no correlation between PNH status or PNH clone size and response to IST
Therapy of PNH in pediatric patients

- PNH/AA: SCT, IST
- PNH/MSD: SCT, IST
- Classic PNH: supportive, corticosteroids, androgens, eculizumab, SCT

The clinical relevance of minor paroxysmal nocturnal hemoglobinuria clones in refractory cytopenia of childhood: a prospective study by EWOG-MDS

Leukemia 2014; 28: 189-192

- 87 RCC <18 years
- PNH clones in erythrocytes and/or granulocytes in 41%
- PNH clone size 0.01-58%
- PNH-positive patients significantly older than PNH-negative
- 14 PNH-positive and 14 PNH-negative patients treated with rabbit-ATG and CSA
- At day 180, PR in 71% of PNH+ and in 36% of PNH- patients
Allogeneic stem cell transplantation in paroxysmal nocturnal hemoglobinuria

Haematologica 2012 Nov;97(11):1666-73

- 211 patients transplanted for PNH in 83 EBMT centres from 1978 to 2007

- Comparison with a cohort of 402 non-transplanted patients with PNH diagnosed in 92 French centers between 1950 and 2005

- 5-year overall survival rate± SE for patients transplanted for thromboembolism: 54±7; for aplastic anaemia:69±5; 86±6 for recurrent hemolytic anaemia

- Thrombo-embolism as an indication for transplantation was associated with a worse outcome
Efficacy and safety of eculizumab in children and adolescents with paroxysmal nocturnal hemoglobinuria


• an open label, multi-centre phase I/II study.
• seven patients, age 11-17 years
• 3 PNH/AA (PNH clone size 3.9%-18.4%)
• 4 classic PNH (PNH clone size 12.1%-66.7%)
• TE in 3
• 600 mg weekly for 4 weeks, 900 mg in week 5 and 900 mg every 2 weeks thereafter
• Steady state levels at week 4
• Treatment well tolerated, normalisation of LDH levels by week 2 of treatment, one out of 2 transfusion-dependent patients became transfusion-independent

Paroxysmal nocturnal haemoglobinuria clones in children with acquired aplastic anaemia: a prospective single centre study.


- patients followed since 1998
- 24 patients: median age 8.7 years
- IST in 20 patients
- flow cytometry on granulocytes; cut-off CD11b+/CD59- >0.15%
- response rate to IST higher in patients PNH- at diagnosis
- 4 patients PNH- → PNH+ at relapse or CSA tapering
- the appearance of a PNH clone in a previously PNH- patient was associated with an increase of the apoptotic rate of circulating CD34+ cells
Absolute count and apoptotic rate of circulating CD34+ cells: three colour flow cytometry analysis for CD45/CD34/AnnexinV
PNH clone: two-colour flow cytometry analysis for CD11b/CD59
two-colour flow cytometry analysis for CD11b/CD59: normal controls (elective minor surgery)
Percentage of CD11b+/CD59- cells

- n = 87
- Median = 0.001%
- Mean + 3SD=0.14%
Fig 1. Group I: PNH clones in AA patients diagnosed after 1 January 1998 and with flow cytometry surveillance since diagnosis. Patients who were PNH+ at diagnosis: Patients 14, 16: underwent related haemopoietic stem cell transplantation (HSCT); Patient 17: complete response after 1st IST; Patient 11: complete responder, re-occurrence of PNH clone at CSA tapering; Patients 9, 13: partial response after 2nd IST and still on CSA; Patient 15: non responder after 1st and 2nd IST, developed refractory anaemia with excess blasts after 42 months and is alive after haploidentical HSCT (Timeus et al, 2005); Patients 10, 12: diagnosed in 2009 and still transfusion-dependent, PNH clone disappeared after 1st IST. Patients who were PNH- at diagnosis: Patients 5 and 7 underwent related HSCT; all others responded to IST.

Group II: Patients diagnosed before 1998 with flow cytometry surveillance during IST or off-therapy. Patients off therapy: Patients 18, 22: responders after 1st IST; Patient 20: responder after 2nd IST. Patients on CSA: Patient 24: non responder after 1st and 2nd IST, alive after unrelated HSCT; Patient 23: responder after 2nd IST; Patients 21, 19: relapsed after 1st IST with appearance of PNH clone at relapse, no response to 2nd IST and alive after HSCT.

(Timeus et al, Br J Haematol. 2010)
Paroxysmal Nocturnal Hemoglobinuria Clones in Children with Acquired Aplastic Anemia: A Multicentre Study

Fabio Timeus1,2, Nicoletta Crescenzi2, Daniela Longoni3, Alessandra Doria2, Luiselda Foglia2, Sara Pagliano2, Stefano Vallero1, Valentina Decimi3, Johanna Svahn4, Giuseppe Palumbo5, Antonio Ruggiero4, Baldassarre Martire7, Marta Pillon8, Nicoletta Marra9, Carlo Dufour4, Ugo Ramenghi2, Paola Saracco2

1 Pediatric Onco-Hematology, Regina Margherita Children’s Hospital, Turin, Italy, 2 Pediatric Hematology, University of Turin, Turin, Italy, 3 Pediatric Department MBBM Foundation S. Gerardo Hospital, Monza, Italy, 4 Hematology Unit, G. Gaslini Children’s Hospital, Genoa, Italy, 5 Pediatric Onco-Hematology Department, Bambin Gesù Children’s Hospital, Rome, Italy, 6 Pediatric Oncology, Polyclinico Gemelli, Rome, Italy, 7 Department of Pediatrics, University of Bari, Bari, Italy, 8 Pediatric Onco-Hematology Unit, University Hospital of Padua, Padua, Italy, 9 Department of Pediatric Haematology-Oncology, Santobono-Pausilipon Hospital, Naples, Italy
### AIEOP centres participating to the study

<table>
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<tr>
<th>AIEOP CENTRES</th>
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<th>N. PATIENTS IN IST</th>
<th>N. PATIENTS OFF THERAPY</th>
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Materials and Methods

• 85 patients

• 39 at diagnosis (8 BMT as first line therapy)

• 25 entered the study during IST

• 20 followed since off-therapy

• 1 selected case after allogeneic BMT

• peripheral blood PNH cells were detected by a lack of CD59 expression on granulocytes by a two-color FCA for CD59 and CD11b

• cut-off value 0.15% (PNH clone median in normal controls= 0.001%, mean+3SD= 0.14%)

• the results were confirmed by more sensitive techniques (three or six-color sequential gating analysis for CD45/33/66b or CD45/33/15/24/14/FLAER)
Six-colour flow cytometry analysis for CD45, 33, 15, 24, 14, FLAER in normal controls (elective minor surgery)
Percentage of GPI-negative cells

- n = 80
- Mean +3SD=0.006 %
Results

• In 33 patients, PNH clones were sporadic or intermittent.
• In 13 patients, the clones persisted for more than 3 following controls.
• In 8 patients PNH- at diagnosis a PNH clone appeared later during IST (clone size 0.16-1.71).
• In 2 patients, a PNH clone appeared at relapse, or CSA tapering
• In 2 patients, presence of a PNH clone >10% with evidence of hemolysis (AA/PNH)
• No correlation between LDH levels and PNH clone size when <10%
• No thrombosis was observed
PATIENTS AT DIAGNOSIS (n=39)

- PNH – 59%
- PNH + 41% (clone size 0.17-10.4%)
PATIENTS STUDIED DURING IST (n=31+25)

PNH – 47%

PNH + 53%
(clone size 0.16-65.5%)
PATIENTS IN OFF-THERAPY
n=20

PNH – 55%

PNH + 45%
(clone size 0.16-4.0%)
33 SPORADIC PNH + PATIENTS (72%)

13 PERSISTANT PNH + PATIENTS (28%)
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**PNH clone evaluation at diagnosis and outcome of 30 AA patients treated with IST.**

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<th>PNH clone at diagnosis</th>
<th>Number of patients</th>
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<th>Complete responders at 180 days</th>
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hATG = horse ATG; rATG = rabbit ATG; CSA = cyclosporin

In patients treated with hATG, evaluation at 180 days showed 2 complete responders (CR, 14%), 7 partial-responders (PR, 50%) and 5 non-responders (NR, 36%), whereas in patients treated with rATG, evaluation at 180 days showed 1 CR (7%), 2 PR (14%), 11 NR (79%) (2-tailed Fisher test p=0.0542).

Among the 11 patients PNH+ at diagnosis, CR and PR were respectively 1 (9%) and 4 (36%), whereas NR were 6 (55%). Among the 19 patients PNH- at diagnosis CR and PR were respectively 3 (16%) and 5 (26%), whereas NR were 11 (58%) (2-tailed Fisher test p=1). In all the 4 PNH+ patients who were treated with rATG the clone persisted at day +180, whereas it disappeared in 4/7 patients receiving hATG up-front.
Response to IST

- No significant differences between patients who were PNH+ or PNH- at diagnosis

- No significant differences between patients PNH- at diagnosis and the subgroup with a persistent PNH+ population
Follow up of an AA patient treated with HSCT at first line therapy. A GPI-negative population appeared after graft failure (autologous reconstitution, 100% recipient chimerism) at AA relapse and disappeared after starting immune-suppressive therapy with cyclosporine (persistent autologous reconstitution 100% recipient chimerism); complete remission was reached.
An AA patient in IST. Variations of GPI-negative population size. The patient developed a typical PNH without hemoglobinuria.
July 2014, six months after ATG
July 2014, six months after ATG
Follow-up?

Eculizumab?

HSCT?
Thanks to …

Paediatric Department - University of Turin
Regina Margherita Children’s Hospital. - Turin

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Hematology Unit
Ugo Ramenghi
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Loredana Farinasso
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