Stem cell transplantation for paroxysmal nocturnal haemoglobinuria in childhood

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Summary. Paroxysmal nocturnal haemoglobinuria (PNH) is a clonal haematopoietic disorder characterized by chronic or intermittent intravascular haemolysis, variable cytopenia and an increased risk of thrombosis. Stem cell transplantation (SCT) is a curative therapeutic option, but its risks must be carefully weighed against the natural course of PNH. World-wide experience with SCT for PNH in the paediatric age group is scarce. We report on two adolescents suffering from PNH with life-threatening complications who were successfully transplanted from unrelated donors. Indications and techniques of SCT in childhood PNH are discussed and an overview of the literature is given.

Keywords: paroxysmal nocturnal haemoglobinuria, transplantation, PNH, SCT, BMT.

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired blood disorder with symptoms of haemolysis and thrombotic diathesis. The causative abnormal blood cell clone has a somatic mutation in the PIG-A gene leading to deficient glycosyl phosphatidylinositol (GPI) synthesis. The median survival of patients with PNH is 10–15 years (Hillmen et al., 1995). PNH is closely related to aplastic anaemia and/or myelodysplastic syndromes (MDS) (Tooze et al., 1999; Socie et al., 2000).

The only curative treatment for PNH is allogeneic stem cell transplantation (SCT). However, as PNH is not a malignant condition and spontaneous remissions occur in about 15% of patients, the role of SCT is controversial (Hillmen et al., 1995). Furthermore, experience with SCT for PNH is limited. Successful outcome after human leucocyte antigen (HLA)-identical SCT averages 60%, but results are inferior for grafts from unrelated donors (UD). We present two cases of adolescents with severe PNH complications who were successfully transplanted with UD stem cells.

CASE REPORT 1

Pancytopenia and haemolysis were incidentally noted in a healthy 7-year-old girl when she received medical care for a traffic accident (November, 1993). Blood parameters were:

- haemoglobin 7.7 g/dl, reticulocytes 9.8%, platelets 86 × 10^9/L, white blood cell count (WBC) 1.8 × 10^9/L.
- Mean cell volume (MCV) 85 fl, reticulocytes 2.7%, platelets 40 × 10^9/L, WBC 0.6 × 10^9/L, absolute neutrophil count (ANC) 0.1 × 10^9/L.

Bone marrow cellularity was normal.

Extended work-up (sugar–water test, flow cytometry) confirmed the existence of a PNH clone. The respective percentages of cells deficient in GPI-anchored proteins were:

- Erythrocytes 29%, reticulocytes 54%, granulocytes 36%, monocytes 94%, lymphocytes 12%.
- The proportion of clonal granulocytes increased to 91% within 3 months.

Treatment with cyclosporine (CyA) and granulocyte colony-stimulating factor (G-CSF) was initiated in January 1998. One month later, a thrombosis of the hepatic veins and inferior vena cava (Budd–Chiari syndrome) was diagnosed and a transjugular intrahepatic portosystemic shunt (TIPS) was created. In order to prevent further thrombotic complications, oral anticoagulation with phenprocoumon was started despite considerable thrombocytopenia (platelets 18 × 10^9/L). Over the following 9 months, two episodes of TIPS obstruction, increasing liver failure, pulmonary embolism and splenic vein thrombosis occurred. In the absence of a favourable haematopoietic response to CyA/G-CSF, a decision to undertake
allogeneic SCT was made. The girl was transplanted from a matched UD at age 12 years (in January 1999) (Table I). Complications of SCT were acute graft-versus-host disease (GVHD) grade 2 of the skin and grade 3 of the gut, and cytomegalovirus (CMV) reactivation. Until d 160 after SCT, 29 red cell transfusions and 312 platelet transfusions were necessary. As of January 2002 (3 years after SCT), the now 16-year-old girl is well and attends school regularly. Chimaerism is complete and there is no GPI-deficient blood cell population. The hepatic vein thrombosis itself has remained unchanged, but the TIPS remains in place and functioning. Haematological sequelae are limited to persistent thrombocytopenia (platelets 50·10^9/l) due to a moderately decreased number of megakaryocytes and hypersplenism.

### CASE REPORT 2

The 12-year-old boy was referred because of frequent petechiae, pallor and dizziness in September 1993. Blood parameters were: haemoglobin 4·4 g/dl, MCV 94 fl, reticulocytes 1·8%, platelets 10·1·10^9/l, WBC 3·1·10^9/l, ANC 0·2·10^9/l. After severe aplastic anaemia was diagnosed, immunosuppressive treatment with CyA was initiated. Partial remission, defined as transfusion independence and an ANC greater than 0·5·10^9/l was achieved after 4 months. However, several attempts to reduce the CyA dose resulted in deterioration of the cytopenia. In November 1994, 1·3 years after first presentation, sugar–water test and acid serum test were performed and both were positive. One year later (November 1995), a GPI-deficient clone could be demonstrated by flow cytometry. Another 2 years later (May 1997), haemolysis intensified. At that time, the percentages of GPI-deficient cells were: erythrocytes 14%, reticulocytes 35%, granulocytes 52%, monocytes 34%, lymphocytes 9%. The percentage of GPI-deficient red cells increased over time while the respective percentages of the other lineages decreased. Therefore, the existence of two independent PNH populations was assumed. In an attempt to stimulate normal myeloid progenitor cells, G-CSF was added to CyA. The boy fared rather well without major clinical events and his blood counts were acceptable with haemoglobin 9·2–10·6 g/dl, platelets 70–160·10^9/l and ANC 0·7–3·8·10^9/l. One and a half years later (December 1999), the ANC dropped to below 0·5·10^9/l and the haemoglobin declined to 4·7 g/dl. Bone marrow morphology revealed refractory anaemia with excess blasts (RAEB). The karyotype of bone marrow cells was normal. Because transition into myelodysplasia-related acute myeloid leukaemia (AML) occurred 2 months thereafter, matched UD SCT was carried out at the age of 19 years (March 2000) (Table I). Transplant-related morbidity included moderate veno-occlusive disease, CMV reactivation, gram-negative sepsis, renal failure, acute GVHD grade 2 of the liver, two episodes of generalized seizures and haemorrhagic cystitis. As of January 2002 (22 months after SCT), the patient has full haematological recovery and complete chimaerism. He has recently graduated from secondary school.

### DISCUSSION

Five series of allogeneic SCT in PNH with a total of 92 patients have been reported (Kawahara et al. 1992; Bemba et al. 1999; Sasó et al. 1999; Raiola et al. 2000; Woodard et al. 2001). The results of these reports are summarized in Table II. Of the 77 patients who received a graft from an HLA-identical sibling, 49 (64%) survived. The main causes of transplant-related death were graft failure (seven patients), GVHD (six patients) and interstitial pneumonia (four patients). Nineteen of the 49 surviving patients were reported to suffer from chronic GVHD. Literature on unrelated donor SCT for PNH is limited to 11 cases.

### Table I. Characteristics of stem cell transplantation in patients 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at SCT (years)</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Interval from diagnosis to transplant</td>
<td>5-2 years (aplastic anaemia and haemolysis)</td>
<td>7-0 years (aplastic anaemia)</td>
</tr>
<tr>
<td>Conditioning</td>
<td>Total body irradiation (10 Gy; liver and lungs: 6 Gy)</td>
<td>Busulphan (4·4 mg/kg)</td>
</tr>
<tr>
<td>Graft</td>
<td>6/6-matched unrelated donor</td>
<td>6/6-matched unrelated donor</td>
</tr>
<tr>
<td>GVHD prophylaxis</td>
<td>Cyclosporine</td>
<td>Methotrexate and cyclosporine</td>
</tr>
<tr>
<td>Neutrophil engraftment</td>
<td>d 14</td>
<td>d 14</td>
</tr>
</tbody>
</table>

PNH, paroxysmal nocturnal haemoglobinuria; MDS, myelodysplastic syndrome.
or progression to leukaemia. Regimens were chosen because of normal marrow cellularity. Whether a non-ablative procedure is also safe in cellular marrows is an intense immunosuppression is the key process. Whether a normal PNH clone, but many authors advocate that by what mechanisms non-ablative SCT eliminates the antigen.

Patient 2 developed RAEB, which soon progressed to AML. He was therefore enrolled in a study of the European Working Group on MDS in Childhood and received conditioning therapy according to the protocol (Locatelli et al., 2001).

There is a long-standing discussion of whether a myeloablative conditioning regimen is necessary for SCT in PNH without myelodysplasia or leukaemia (Antin et al., 1985; Raiola et al., 2000). A number of PNH patients have successfully been transplanted after conditioning with cyclophosphamide only (Antin et al., 1985; Kawahara et al., 1992; Saso et al., 1999) or after reduced-intensity conditioning with cladribine, busulphan and anti-thymocyte globulin (Suenaga et al., 2001). It is not fully understood by what mechanisms non-ablative SCT eliminates the abnormal PNH clone, but many authors advocate that intense immunosuppression is the key process. Whether a non-ablative procedure is also safe in cellular marrows is controversial. In the two cases reported here, myeloablative regimens were chosen because of normal marrow cellularity or progression to leukaemia.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Patients</th>
<th>Conditioning</th>
<th>Donor</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kawahara et al</td>
<td>1992</td>
<td>6</td>
<td>Cy (n = 4) or Bu + Cy (n = 2)</td>
<td>HLA-identical sibling</td>
<td>6/6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>or ATG + Cy (n = 1)</td>
<td>syngeneic</td>
<td>2/2*</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>16</td>
<td>TBI + Cy (n = 2) or Bu + Cy (n = 6)</td>
<td>HLA-identical sibling</td>
<td>9/16</td>
</tr>
<tr>
<td>Saso et al</td>
<td>1999</td>
<td>24</td>
<td>Cy (n = 3) or Bu + Cy (n = 25)</td>
<td>HLA-identical sibling</td>
<td>27/48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>or TBI + Cy (n = 20)</td>
<td>syngeneic</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>Bu + Cy (n = 4) or TBI + Cy (n = 2)</td>
<td>matched-unrelated</td>
<td>1/6</td>
</tr>
<tr>
<td>Raiola et al</td>
<td>2000</td>
<td>7</td>
<td>Bu + Cy</td>
<td>HLA-identical sibling</td>
<td>7/7</td>
</tr>
<tr>
<td>Woodard et al</td>
<td>2001</td>
<td>3</td>
<td>TBI + ATG + Cy + Ara-C</td>
<td>matched-unrelated</td>
<td>3/3</td>
</tr>
</tbody>
</table>

*PNH relapse in one patient.

Cy, cyclophosphamide; Bu, busulphan; TBI, total body irradiation; ATG, anti-thymocyte globulin; Ara-C, cytarabine.

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REFERENCES


