Haematologica 2000; 85:59-62
original paper

Transplantation and Cell therapy

Bone marrow transplantation for paroxysmal nocturnal hemoglobinuria

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ABSTRACT

Background and Objectives. **Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disease of the hemopoietic stem cell (HSC) characterized by intravascular hemolysis and increased risk of venous thrombosis. There are different therapeutic approaches for PNH which do not cure the disease, but can decrease its complications. Allogeneic bone marrow transplantation (BMT) may cure PNH. We reports here our experience of seven PNH patients who underwent allogeneic BMT.**

Design and Methods. **Between January 1991 and January 1999 seven patients with PNH, aged 23 to 37, were transplanted with unmanipulated bone marrow from HLA identical siblings. Median time from diagnosis to BMT was 2.5 years (range: 1-16). All patients were transfusion-dependent and had received various treatments before BMT: steroids, vitamins, cyclosporin A (CyA), growth factors. One patient had also been treated with anti-thymocyte globulin. One patient was HbsAg positive and one anti-HCV positive. At the time of BMT the median value of hemoglobin (Hb) was 9 g/dL (range 6.5-11), white blood cells 5×10^9/L (range: 2.9-7.7), platelets 97×10^9/L (range: 31-355), LDH: 2726 U/L. The conditioning regimen was cyclophosphamide (160 mg/kg) and busulfan (10-14 mg/kg), followed by unmanipulated bone marrow (median of 5×10^8 cells/kg) and CyA (+MTX in two patients) for prophylaxis of graft-versus-host disease (GvHD).**

Results. **All seven patients are alive, full chimeras, with complete hematologic recovery and no evidence of PNH, at a median follow up of 51 months post-BMT (6-103). Time to achieve a granulocyte count of $\geq 0.5\times10^9$/L, platelets $\geq 30\times10^9$/L and Hb $\geq$ 10 g/dL was respectively 16, 19 and 22 days. Acute GvHD was limited or mild in six patients, and severe in one. Chronic GvHD was extensive in two patients.**

Interpretation and Conclusions. **This study confirms that HLA identical sibling BMT is an effective therapeutic option for PNH, also in the hemolytic phase of the disease: it also suggests that HBV and HCV infections are not an absolute contraindication.**

Key words: paroxysmal nocturnal hemoglobinuria, aBMT

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disease of the hematopoietic stem cell characterized by intravascular hemolysis, often associated with recurrent nocturnal exacerbations, pancytopenia, and venous thrombosis. Rarely the disease may be complicated by myelodysplastic syndrome (MDS) and leukemic conversion.[1,2]

PNH is caused by a somatic mutation of the PIG-A gene, that synthesizes for glycosyl-phosphtidyl-inositol (GPI), a membrane anchor for various cell surface proteins: GPI-anchored proteins (GPI-AP). Among the GPI-AP there are important regulators of the complement system, and their deficiency is the cause of the intravascular hemolysis in patients with PNH.[3,4]

A close association between PNH and aplastic anemia (AA) is well recognized: patients with AA often show laboratory evidence of PNH. The proportion of patients with AA with PNH clones varies from 13% to 57% in different studies.[3-6] About 15% of previous PNH show a loss of hematopoietic progenitor cells and then marrow hypoplasia.[5] Stem cells from patients with PNH are separated into GPI–AP$^-$ and GPI–AP$^+$ populations. The rate of expansion of a PNH clone might be influenced by the existence of an additional marrow injury: PIG-A mutation could be necessary but not sufficient for development of PNH[3] and the relationship between PNH and AA might be explained by a *conditional growth or survival advantage – of PNH cells – in an environment which is injurious to hematopoietic stem cells through a GPI-mediated mechanism.*[7]

The clinical course of PNH is highly variable, but usually chronic.[8] At diagnosis, most patients (90%) have peripheral blood abnormalities. Thrombotic complications, progression to pancytopenia, myelodysplastic syndrome, and acute leukemia occur respectively in 30%, 15%, 5%, and 1% of PNH patients within 8 years from diagnosis. Other complications are often treatment-related (hemochromatosis, hepatitis).[9]

The survival at 10 years, reported by several authors, ranges from 50% to 71%.[10-12] The prognostic factors for shorter survival are: thrombosis as a complication, development of pancytopenia, development of MDS or acute leukemia, age over 55 years, need for additional treatment, thrombocytopenia at diagnosis.[9] Thrombosis, infections and hemorrhage are the primary causes of death of PNH patients.[10-12] There are different therapeutic approaches for PNH: corticosteroids, androgens, red blood transfusions, immunosuppressive drugs (antilymphocyte globulin, cyclosporin), and anticoagulation which do not cure the

disease, but can decrease its complications.[13-17] Allogeneic bone marrow transplantation (BMT) may cure PNH.[18-23] We report here our experience of seven PNH patients who underwent allogeneic BMT.

Design and Methods

Between January 1991 and January 1999 seven patients with PNH, aged 23 to 37 years, were transplanted with unmanipulated bone marrow from HLA identical siblings. Median age at diagnosis was 25 years and median time from diagnosis to BMT was 2.5 years (range: 1-16). PNH was diagnosed by Ham's test and by flow cytometry analysis that showed absent or reduced expression of GPI–AP. All patients were transfusion-dependent with a median value of ferritin at BMT of 340 ng/mL (range: 14-3,120) and had received various therapies before BMT: steroids, vitamins, cyclosporin (CyA), and growth factors. One patient had been treated with anti-thymocyte globulin (with only platelets showing a minor response); one patient had been splenectomized for thromboembolic complications. One patient was HbsAg positive, one antiHCV positive, and five were cytomegalovirus (CMV) seropositive. At the time of BMT, pancytopenia with marrow hypocellularity was present in four patients, hemolytic anemia with normal platelets and leukocyte count in three; in all patients flow cytometry analysis showed GPI–AP deficiency (mean of granulocytes positive for $CD16^+$ was 5% and mean of monocytes positive for $CD14^+$ 5.7%). At the same time the median value of hemoglobin (Hb) was 9 g/dL (range 6.5–11), leukocytes 5×10^9/L (range 2-7.7), platelets 97×10^9/L (range 31-355), LDH 2,726 U/L (range 2,043-6,800) (Table 1).

The conditioning regimen was busulfan (10-14 mg/kg) followed by cyclophosphamide 40 mg/kg/day on 4 consecutive days (160 mg/kg). Median number of nucleated bone marrow cells transplanted was 5×10^8/kg body weight. Graft-versus-host disease (GvHD) prophylaxis was given with CyA at a dose of 2 mg/kg or CyA+methotrexate (MTX) for two patients (Table 2).

Results

Engraftment

All patients achieved engraftment as documented by sex markers and DNA polymorphism. Median time to achieve a neutrophil count of 0.5×10^9/L, platelets 30×10^9/L and Hb 10 g/dL was respectively 16, 19 and 22 days. Acute GvHD was limited or mild in six patients and severe in one. Chronic GvHD was limited in five patients and extensive in two. The patient UPN 726 (Rh D negative), who had received a large number of transfusions pre-BMT (interval PNH diagnosis; BMT: 16 years), and had a Rh D positive donor, developed a pure red cells aplasia (PRCA) with a high titer of anti-D antibodies; she was treated with plasma exchange and erythropoietin. The same patient had a CMV infection and acute hepatitis (she was positive for HBV and HCV markers).

Follow-up

Median time of follow up from BMT is 51 months (range: 6-103): all seven patients are alive and full chimeras, with complete hematologic recovery, disappearance of symptomatology and of signs of intravascular hemolysis, and normal serum LDH level. Analysis by flow cytometry of peripheral blood cells shows normal expression of GPI-AP: a high percentage of granulocytes expressing $CD16^+$ (range: 90-99%) and of monocytes expressing $CD14^+$ (range: 75-95%) (Table 3).

Quality of life

At the latest follow-up two patients had extensive GvHD, and one of them had recurrent infections. Limited GvHD was present in five patients. Five patients were still on immunosuppressive therapy (one CyA, steroid and azathioprine, two CyA and steroid, three CyA only). All patients are able to work. Karnovski's score is 100% for four patients and 90% for three.

Hepatitis virus infections

One patient was HBsAg, anti-HBe and anti-HBc positive at transplantation, with ongoing chronic hepatitis (ALT 190 IU); his donor was negative for all HBV markers: ALT normalized during the conditioning regimen, and increased starting from day +31; the patient showed a biochemical exacerbation on day +180 with an ALT peak of 1,000 IU, without clinical signs of hepatitis. The acute episode resolved completely and the patient has slightly abnormal ALT two years post-transplantation and is negative for HBsAg.

A second patient was anti-HCV positive before BMT. Interestingly, she was also anti-HBc and anti-HBe positive without detectable antigenemia. She had no major hepatic complications until two years post-transplant, when she developed HBsAg positivity together with signs of acute hepatitis (ALT 420 IU), which lasted 3 months and resolved completely.

Table 1. Patients: data at transplant.

UPN	*Interval dx–BMT (years)*	*Hb g %*	*WBC x10⁹/L*	*Plts x10⁹/L*	*LDH U/L*	*% of granulocytes positive for CD16*	*% of monocytes positive for CD14*
590	3	10.2	5	303	2726	ND	ND
726	16	9	2.9	31	2043	0.4	1
753	1	11	3.4	64	2245	17	19
868	2	8.7	7.7	355	6800	1	3
997	5	6.5	4.9	97	3479	5.9	5.3
1104	1	9	6.9	138	5858	1	0.6
1146	5	8.5	2	80	2102	2	1

Abbreviations: UPN= unique progressive number, dx-BMT= interval in years between diagnosis and bone marrow transplantation, ND = not determined.

Table 2. Transplant data.

UPN	Sex D/R	Age D/R	Conditioning regimen (mg/kg)	GvHD prophylaxis	NC infused x10⁹/kg	Engraftment PMN >500 x10⁹/L	Plts >30 x10⁹/L	Hb >10 g %
590	M/M	43/37	Bu 14/Cy 160	CS 2 mg/kg	3,2	+16	+19	+17
726	M/F	22/27	Bu 10/Cy 160	CS 2 mg/kg	5	+23	+23	+31
753	M/M	38/28	Bu 14/Cy 160	CS 1 mg/kg	7.5	+10	+16	+14
868	M/M	31/37	Bu 14/Cy 160	CS 2 mg/kg	3.5	+13	+12	+22
997	F/M	31/23	Bu 14/Cy 160	CS 2 mg/kg	6	+12	+12.	+26
1104	F/F	33/24	Bu 14/Cy 160	CS 2 mg/kg + MTX	4.4	+16	+19	+17
1146	F/F	29/23	Bu 14/Cy 160	CS 2 mg/kg + MTX	5	+19	+19	+22

Abbreviations: UPN= unique progressive number, D= donor, R= recipient, NC= nucleated cells, M= male, F= female, Bu= busulfan, Cy = cyclophosphamide, CS= cyclosporin, MTX= methotrexate.

Hepatitis reactivations following immune reconstitution have been described.[24]

Discussion

This report confirms that allogeneic BMT can produce long-term disease-free survival in patients with PNH, also in the hemolytic phase of the disease, and should be considered before the occurrence of disease or transfusion-related complications. The PNH clone was eradicated from all 7 patients and complete lympho-hematopoietic engraftment was complete in all of them. One patient had significant problems post-BMT, including prolonged PRCA and CMV infection: this patient had received a large number of transfusions and there had been a long interval between diagnosis and transplantation.

The number of allotransplants for PNH reported in the literature is small: we have identified and tabulated 35 cases, which added to our 7 give a total of 42 from single institutions. Of these, three patients are alive with PNH, 8 are dead and 30 are alive without disease, which gives a figure of 73% disease-free survival (Table 4). There may be bias in published cases, since there is a tendency to report cases with a favorable outcome. Indeed in a recent analysis the *EBMT Aplastic Anaemia Working Party Registry* has identified 46 transplants for PNH from HLA identical siblings, with a median age of 29 (range 10- 46) and a median interval PNH-diagnosis BMT of 794 days (range 30-8680 days): for these patients, the actuarial survival at 5 years is 52% (*unpublished*). The principal cause of death was GvHD. Patients receiving total body irradiation in the conditioning regimen (21%) had a greater incidence of GvHD (50% vs 13%). These results are similar to those in a recent publication from the IBMTR.

These results on larger number of patients are less encouraging than our personal series and do not seem to have changed significantly with time: data were collected on patients transplanted from 1979 to 1997, and suggest that allografts still pose a significant risk of morbidity and mortality. This is particularly true for patients prepared with radiation (*EBMT data, unpublished*), although small numbers preclude multivariate analysis. Transplants from alternative donors may also expose patients to a significantly greater risk.

The unresolved questions are therefore: to which patients and when should the procedure be offered?

Table 3. Outcome.

UPN	Full chimeras	aGVHDc	GvHD	CMV	% of GR CD16⁺	% of MO CD14⁺	Survival (months)
590	+	II	Limited	+	92	82	108
726	+	II	Extensive	+	99	93	78
753	+	II	Limited	–	96	75	72
868	+	I	Limited	+	94	87	48
997	+	I	Extensive	–	90	95	33
1104	+	I	Limited	–	98	89	17
1146	+	III	Limited	+	98	86	11

UPN = unique progressive number, aGvHD = acute graft versus host disease, cGvHD = chronic graft-versus-host disease, CMV = cytomegalovirus, GR = granulocytes, MO = monocytes.

Table 4. Review of the literature.

Author	No.of pts	AA preBMT	Donor	Cond. regimen	Outcome
Bemba *et al.*	16	6	HLA-id sibling	+	9 alive
Antin *et al.*	4	4	HLA-id sibling	+	All alive. No PNH
Szer *et al.*	4	4	3 HLA-id sibling 1 twin	+ +	All alive. No PNH
Kawahara *et al.*	9	6	6 HLA-id sibling 2 twins 1 parent non HLA-id	+ – +	All alive. 1 PNH All alive. 1 PNH Dead. Graft rejection.
Kolb *et al.*	2		1 twin 1 HLA-id sibling	+ -	Alive. PNH Alive.
Saso *et al.*	57	18	48 HLA-id sibling 2 twins 1 parent 6 unrelated donors	+ –/+ + +	27 alive 2 alive Dead 1 alive

Abbreviations: AA= aplastic anemia.

It is relatively easy to proceed with a transplant when patients have significant risk factors, such as thrombosis, pancytopenia, MDS or acute leukemia, requirement for additional treatment, thrombocytopenia at diagnosis or a marked need for transfusions. Our present report suggests that BMT can also be offered to patients without risk factors, and with low transfusion requirement.

Although most patients have been prepared with the combination of cyclophosphamide and either radiation or an alkylating agent such as busulfan, the use of cyclophosphamide alone is a possibility which should be tested. Indeed cyclophopshamide alone may be sufficient to allow engraftment of allogeneic HLA identical cells: this is especially true after the demonstration that clonality in PNH patients does not necessarily translate into malignancy, and that normal individuals do have granulocytes with the point mutation at the PIG-A gene level.[23] In conclusion, although the number of patients is small, we believe these results further support the use of allogeneic hemopoietic stem cells for the management of PNH.

Contributions and Acknowledgments

AMR is a recipient of an educational grant from the Department of Hematology University "Federico II" of Naples. AMR was responsible for data analysis and writing the manuscript. MTVL, TL, FG, NM, GB, SB, and FF were responsible for the patients' care; FB and OF carried out the flow cytometry analyses. AB was responsible for the analysis of the results and writing the paper.

Funding

This work was supported by the Associazione Italiana Ricerca contro il Cancro (A.I.R.C.) Milano and the Associazione Ricerca Trapianto Midollo Osseo (A.RI.T.M.O.), Genoa, Italy.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received July 26, 1999; accepted November 10, 1999.

Potential implications for clinical practice

- HLA identical sibling bone marrow transplantation is an effective therapeutic option for PNH, also in the hemolytic phase of the disease.
- HBV and HCV infections are not an absolute contraindication for BMT.

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