New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years

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When prepared for transplantation with busulfan (BU) 14 mg/kg and cyclophosphamide (CY) 120 to 160 mg/kg, patients with thalassemia in risk class 3, aged younger than 17 years, who receive transplants from HLA-identical donors, had a 30% incidence of transplant rejection with recurrence of thalassemia. This, relatively poor, outcome was ascribed to insufficient immune suppression or to inadequate eradication of the thalassemic marrow, or both. In an attempt to enhance both immune suppression and eradication of the thalassemic clones, hydroxyurea, azathioprine, and fludarabine were added to the BU and CY. This regimen, called protocol 26, was applied to 33 consecutive patients with class 3 thalassemia aged younger than 17 years and was well tolerated with 93% survival. The incidence of recurrent thalassemia after the transplantation decreased from 30% to 8%. (Blood. 2004;104:1201-1203)

Introduction

Beta thalassemia is a genetic disorder resulting in absent or reduced beta globin chain synthesis producing hemolytic anemia. Patients are treated with red blood cell transfusions and iron chelation to prevent organ iron overload.[1] This therapy averts the worst consequences of fulminant hemolytic anemia and complications resulting from extension of the marrow space, but it still results in iron overload. Currently, the only cure for thalassemia is allogeneic bone marrow transplantation which corrects the genetic defect in the hematopoietic system[2] by the use of allogeneic stem cells, the origin of which must be immunologically acceptable.[3,4]

Patients with thalassemia can be categorized into 3 classes of risk for marrow transplantation,[5,6] and the category of greatest risk is class 3.[7] The outcome of bone marrow transplantation was studied for 122 consecutive patients with class 3 thalassemia aged younger than 17 years prepared with a regimen of busulfan (BU) 14 mg/kg followed by cyclophosphamide (CY) 120 or 160 mg/kg. For these patients, Kaplan and Meier probabilities of survival and thalassemia-free survival, and cumulative incidences of rejection and nonrejection mortality were, respectively, 79%, 58%, 30%, and 18%.[6,8] The main problem was rejection, characterized as return of the thalassemic clone with no erythropoiesis of donor origin, which usually can also be defined as relapse. One approach to reducing this hazard is to increase the power of the preparative regimen to eradicate thalassemic erythropoiesis without adding to nonmarrow toxicity.

From March 1997 all patients with class 3 thalassemia aged younger than 17 years were registered on a new protocol identified as protocol 26. This report describes the results obtained in a group of 33 consecutive patients registered on protocol 26.

Patients and methods

Patient categorization

A system has been described for assigning patients undergoing marrow transplantation for thalassemia to prognostically useful categories. This system categorizes risk on the basis of hepatomegaly, the presence of portal fibrosis in pretransplantation liver biopsies, and the quality of chelation during the years before transplantation.[5] This quality of chelation was characterized as regular when deferoxamine treatment was initiated within 18 months of the first transfusion and administered subcutaneously for 8 to 12 hours continuously daily for at least 5 days each week. The chelation variable was defined as irregular for any deviation from this requirement. The age in months when the patient first received regular chelation was recorded. A chelation index was calculated that described the number of months each patient did not receive regular chelation as a percentage of the number of months the patient should have received chelation by the definition above. With this index, a completely satisfactory chelation history is represented by 0% and a completely unsatisfactory one by 100%.[6] The study protocol was approved by the Institutional Review Boards of the Hemoglobinopathy Unit and Transplant Center of the Ospedale San Salvatore of Pesaro and the Mediterranean Institute of Hematology. Informed consent was provided according to the Declaration of Helsinki.

Patient characteristics

Thirty-three patients with the risk factors for class 3 and aged younger than 17 years received bone marrow transplants from matched related donors between March 1997 and November 2002. Characteristics potentially influential on the outcome of transplantation are presented in Table 1. All patients received marrow from HLA-identical siblings, except for patient unique patient number (UPN) 1230 for whom the donor was an HLA phenotypically identical cousin and patients UPN 1444, UPN 1454, and UPN 1457 for whom the donors were phenotypically identical mothers.

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Table 1. Patient characteristics at transplantation for 33 patients younger than 17 years

UPN	Sex	Country	Age, y	No. of Tx	CI	Ferritin, ng/mL	GOT, U/L	GPT, U/L	LIC, mg/g dry weight	FS	CAH
1211	F	Albania	16	230	100	2591	49	60	30.9	Mo	No
1230	F	Italy	16	290	100	3221	42	60	13.6	Se	Yes
1231	F	Kuwait	11	75	100	1900	16	32	8.2	Mi	No
1236	M	Saudi Arabia	16	200	100	2557	31	29	28.3	Ci	Yes
1262	F	Albania	12	200	100	4533	77	55	30.1	Se	No
1288	M	Saudi Arabia	12	150	37	968	41	43	15.8	Mi	No
1292	M	Kuwait	9	80	100	1624	34	31	24.5	Mo	Yes
1319	M	Syria	16	200	100	1087	27	20	7.8	Ci	No
1324	F	Pakistan	10	125	13	1391	35	24	20.5	Mo	Yes
1331	F	Kuwait	12	118	100	3558	47	72	38.1	Ci	Yes
1339	F	Lebanon	12	100	100	3158	34	45	26.4	Mo	Yes
1342	F	Palestine	6	90	54	1967	37	57	28.7	Mo	No
1347	F	Kuwait	4	18	100	4016	220	260	15.5	Se	No
1357	F	Saudi Arabia	9	80	10	1140	31	28	7.5	Mi	No
1362	M	Libya	9	18	100	1226	30	51	0.9	Mo	No
1363	M	Nepal	4	6	100	1925	67	65	15.7	Mi	No
1371	F	Albania	14	130	100	2067	48	69	30.5	Se	No
1372	F	Kuwait	15	200	100	6300	13	13	30.2	Mo	No
1374	M	Jordan	7	30	100	4463	66	88	40.1	Se	No
1375	M	Italy	10	130	5	2721	27	52	14.4	Se	No
1389	F	Iran	14	150	49	2835	29	16	19	Mi	No
1409	M	Iraq	5	70	100	2338	55	93	20.3	Se	No
1413	F	Kuwait	9	106	100	2449	32	27	9.3	Mo	Yes
1414	F	Saudi Arabia	15	120	100	5306	12	18	14.4	Mo	Yes
1427	F	Italy	14	280	100	3386	32	30	17.4	Mo	No
1429	M	Romania	11	10	100	1000	39	37	4.9	Mo	Yes
1435	M	Pakistan	10	60	100	3492	16	23	15.8	Mo	Yes
1444	M	Palestine	8	90	100	3231	51	48	20.1	Se	Yes
1445	F	Palestine	11	120	100	953	38	26	2.7	Mo	Yes
1448	M	Palestine	16	130	100	4578	47	38	38.1	Ci	Yes
1449	M	Palestine	15	80	100	2120	31	43	4.2	Mi	No
1454	M	Palestine	5	60	100	1619	31	42	18.8	Mo	Yes
1457	M	Albania	12	140	100	1257	79	45	14.1	Mo	Yes

UPN indicates unique patient number; No. of Tx, number of red cell transfusions before transplantation; CI, chelation index; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; LIC, liver iron concentration; FS, portal fibrosis (Mi, mild; Mo, moderate; Se, severe; Ci, cirrhotic); CAH, chronic active hepatitis (yes, present; no, absent).

Transplantation regimen

We attempted to suppress erythropoiesis by intensive hypertransfusion and chelation. Between day −45 and day −11 before the transplantation, 40 mg/kg deferoxamine was continuously infused through a central venous catheter each 24 hours. Red cells were transfused every 3 days to maintain the hemoglobin level between 140 and 150 g/L (14 and 15 g/dL). During this time interval hydroxyurea 30 mg/kg daily and azathioprine 3 mg/kg daily were administered to eradicate marrow, and growth factors, granulocyte colony-stimulating factor (G-CSF; Neupogen, Filgrastim; Dompè-Biotec, Milan, Italy) and erythropoietin (Globuren; Dompè-Biotec), were given twice weekly to maintain stem cell proliferation in the face of hypertransfusion, thereby facilitating the effect of the hydroxyurea. Fludarabine was administered at a dosage of 20 mg/m^2/d from day −17 through day −13. Starting on day −10, 14 doses of busulfan (BU) 1 mg/kg were administered orally 3 times daily over 4 days (total dose 14 mg/kg over 4 days), followed by intravenous cyclophosphamide (CY) 40 mg/kg daily on each of the next 4 days (total dose 160 mg/kg).

Marrow (median nucleated cell dose 2.9×10^8/kg body weight; range, 1.2-7.8 $\times$ 10^8/kg body weight) was infused 36 hours after the last dose of CY. The date of marrow infusion was designated as day 0. Prophylaxis against graft-versus-host disease (GVHD) consisted of CY 7.5 mg/kg intravenously on day 1, methotrexate 10 mg/m^2 intravenously on days 3 and 6, and cyclosporine (CSA) 5 mg/kg intravenously daily from day −2 through day 5 reduced to 3 mg/kg/d intravenously until oral administration of 12.5 mg/kg/d could be tolerated. The dose of CSA was tapered from day 60 until discontinuation at 1 year. Additional GVHD prophylaxis consisted of prednisolone 0.5 mg/kg from day −1 until day 25. All patients were treated in positive-pressure isolation rooms and received antibiotics and acyclovir starting on day −1 and amphotericin starting on day 8, continued through day 25. Trimethoprim 5 mg/kg/d was administered from day −9 through day −2 and restarted from day 35 twice weekly. All blood products administered after transplantation were irradiated with 30 Gy. Acute and chronic GVHD were graded according to the Seattle criteria.[9,10] The first-choice drug for treatment of acute GVHD was prednisolone at escalating dosages up to 10 mg/kg/d, depending on the degree and duration of GVHD.

Tests of chimerism

Fluorescent in situ hybridization. Fluorescent in situ hybridization (FISH) was performed on peripheral blood (PB) and bone marrow (BM) to detect marrow engraftment when host and donor were sex mismatched. A biotinylated pY3.4 probe complementary to a part of the Y chromosome was used as reported in accordance with the manufacturer's instructions (Oncor, Gaithersburg, MD). Positive and negative controls were included with each test.

DNA extraction. High–molecular weight DNA was extracted from PB or BM by using a commercial DNA blood mini kit, in accordance with the manufacturer's instructions (Qiagen, Hilden, Germany).

Polymerase chain reaction. For chimerism analysis polymerase chain reaction (PCR) amplification of 4 different minisatellite loci was used (33.6, SE33, APOB, and D1S80). PCR-amplified products were run on precast 10% nondenaturanting polyacrylamide gels (NOVEX, San Diego, CA) followed by silver staining procedure. A semiquantitative estimation of mixed chimerism (MC) was used, in which recipient-band intensity and

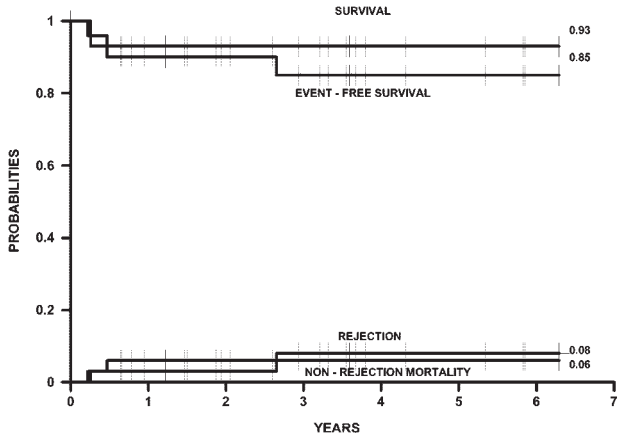

Figure 1. Kaplan-Meier probabilities of survival, thalassemia-free survival, and cumulative incidences of rejection and nonrejection mortality in 33 thalassemic patients aged younger than 17 years, prepared for transplantation with protocol 26.

donor-band intensity were compared with dilution experiments in the patient and donor.[11]

Statistical analyses

Estimates of overall survival and event-free survival were calculated by the Kaplan-Meier method.[12] Rejection and nonrejection mortality were calculated as cumulative incidences.[8] In estimating event-free survival, rejection, recurrence of thalassemia, and death were recorded as events. Rejection was defined as the development of complete marrow aplasia or recurrence of thalassemia (a return to the pretransplantation pattern of globin-chain synthesis).

Results

Figure 1 presents the Kaplan-Meier probabilities of survival and thalassemia-free survival, as well as cumulative incidences of rejection and nonrejection mortality for the 33 patients in this study.

The median duration of follow-up was 36 months (range, 8-67 months). Two patients rejected their grafts, one at day 82 (surviving with thalassemia at nearly 1 year) and one at 2.7 years (surviving with thalassemia at 3.2 years). Two patients died, one of interstitial pneumonia of unknown etiology on day 97 and the other of aspergillosis on day 174. All 30 surviving patients without recurrence showed full chimerism with functioning grafts in the tests performed on days 21 to 28 and days 50 to 60 and annually thereafter until censored by duration of follow-up or (in the one case with late rejection) detection of rejection. GVHD grade I or II developed in 3 patients and resolved without any additional treatment. Two patients showed cytomegalovirus infection without disease, which resolved after ganciclovir treatment. All 29 surviving patients without rejection are well with a Karnofsky score of 100.

Discussion

Patients with homozygous beta-thalassemia can be cured by transplantation of normal or heterozygous stem cells after eradication of the thalassemic hematopoietic system. In an early study of transplantation for the treatment of thalassemia, we demonstrated that patients could be categorized into 3 classes on the basis of liver damage and chelation history before transplantation.[5,6] Subsequent studies suggested that the mortality associated with being in the worst prognostic class (class 3) could be reduced from 47% to 18% by using regimens containing less cyclophosphamide. However, the rejection rate with these regimens increased from 12% to 30% in patients with class 3 thalassemia aged younger than 17 years. Such patients have a greatly amplified hematopoietic marrow compartment because of a history of poor compliance with transfusion and chelation therapy. We assumed that the high rate of autologous hematopoietic reconstitution was not only because of immunologic rejection but also related to this major expansion of the hematopoietic system and consequent failure to eradicate the thalassemic marrow. For this reason we devised a regimen intended to reduce the expansion pressure on the erythron by 35 days of hypertransfusion to hemoglobin levels not less than 140 g/L (14 g/dL) accompanied by appropriate chelation through a central venous catheter. During this period we added azathioprine and hydroxyurea to reduce hematopoiesis without intolerable toxicity. Growth factors were used because we were concerned that the hypertransfusion might suppress nucleic acid synthesis to the extent that stem cells were not susceptible to the hydroxyurea. Fludarabine was added because of its well-demonstrated marrow suppressive and high immunosuppressive activities. We did not attempt to evaluate the influence of various components of the regimen. The supportive care and the prophylaxis or treatment of GVHD was the same as in the previous studies of transplantation in class 3 thalassemia. In a group of patients with characteristics similar to those reported in the previous studies,[6] this regimen improved the Kaplan-Meier probability of thalassemia-free survival for patients with class 3 thalassemia aged younger than 17 years at the time of the transplantation from 58% to 85% together with a reduction from 30% to 8% of the probability of the return of the thalassemic hematopoietic clone after transplantation. The immunosuppressive qualities of fludarabine may have contributed to the effectiveness of this regimen, but this study was not designed to isolate this effect.

The protocol presented in this report, identified as protocol 26, appears to be a well-tolerated and effective association of drugs for eradication of the hematopoietic system of thalassemic patients.

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