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Mixed chimerism after bone marrow transplantation for thalassemia major

Thirty-four thalassemia patients were studied for chimerism by fluorescent in situ hybridization or variable number tandem repeats after bone marrow transplantation. Mixed chimerism was detected in 9 patients with host cells ranging from 4 to 56%. One had graft rejection and the others were transfusion independent. Mixed chimerism was common but mostly without deleterious effect.

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After allogeneic bone marrow transplantation (BMT), some patients still have residual recipient hematopoiesis. The co-existence of recipient's and donor's hematopoietic systems is called mixed chimerism. Some patients may remain in stable mixed chimerism while others may convert to complete donor chimerism or graft rejection. The prediction of final outcome of mixed chimerism may help to decide interventions and prevent graft rejection.

Forty-four thalassemia major patients underwent transplantation in the Prince of Wales Hospital. Two patients received umbilical cord blood, four received peripheral blood stem cells and the others received a bone marrow transplantation. Conditioning was busulphan 16 mg/kg, cyclophosphamide 150-200 mg/kg, anti-thymocyte globulin 90 mg/kg.[1] One had primary graft rejection after a cord blood transplant. Chimerism state was monitored using peripheral blood leukocytes at 1, 3, and 6 months and then annually. For sex-mismatched donor-recipient pairs , fluorescent in situ hybridization (FISH) for X and Y probes was adopted. For sex-matched pairs, the variable number tandem repeat (VNTR) was utilized to test for DNA polymorphism. Five patients died early. Four patients did not have informative DNA markers for chimerism study and one had graft rejection at 71 days.

Thirty-four patients were serially followed up for chimerism and 21 developed complete donor chimerism. Thirteen patients (35.1%) were detected to have residual host cells during follow-up. Four had a low level of recipient cells (<10%) and all converted to complete donor chimerism. Nine patients with > 10% recipient cells were considered as having definite mixed chimerism. One patient (PT40) rejected the donor's graft at 9 months and PT17 achieved complete donor chimerism at 2 years. Seven patients (20.5%) had persistent mixed chimerism with 4 to 56% recipient cells at the follow-ups between 3 to 8.5 years (Table 1). All patients were transfusion independent. PT42 had an increasing percentage of recipient cells (60%) and severe anemia (Hb 5.9 g/dL). Donor leukocyte infusion with 3×10^8/kg CD3 cells was given at 8 months. The latest study at 34 months showed 47% recipient cells and Hb 7.9 g/dL. Age at BMT, sex matching, splenectomy, donor's thalassemia trait status, serum ferritin, liver enzymes, number of nucleated cells and CD34 cells infused did not have an impact on development of mixed chimersm. Four patients had a major mismatch for ABO blood group with their donors but none had mixed chimerism. Busulphan levels were measured in 18 patients. The areas under the curve of busulphan levels in patients with mixed chimerism were lower than those of patients with complete donor chimersim, on day 1, 2, 3 or 4 of the measurements. The mean area under the curve of the busulphan levels of the 4 days was 774 and 908 μmol × min/L for patients with mixed and complete chimerism, respectively (p=0.067) (Table 2). Hb F level was increased in 23 patients (67.6%) and was more common in patients with donors who were thalassemia trait carriers, 91% versus 27.3% (p<0.001).

Table 1. Mixed chimerism: recipient cell percentage after BMT.

	1 m.	*2 m.*	*3 m.*	*6 m.*	*1 y.*	*2 y.*	*3 y.*	*4 y.*	*5 y.*	*6 y.*	*7 y.*	*8 y.*
PT1	6	–	–	50	–	31.1	30	20	49.7	35	31	36
PT17	4.3	–	11	14	6.7	0.5	0	0	0	0	–	–
PT21	1.3	5.2	25	20.5	12.3	17.5	–	21.5	18.2	–	–	–
PT27	16	24	32	47	51	50	58	–	–	–	–	–
PT29	12	10	6	14	14	11	18	16	–	–	–	–
PT32	10	7.5	5	22.7	61.2	77	77	–	56	–	–	–
PT39	0	0	–	–	4	11.2	4.8	4	–	–	–	–
PT40	2.8	–	1.3	5	38.4	–	–	–	–	–	–	–
PT42	6.5	–	18.8	60.3	75.8	48.7	46.5	–	–	–	–	–

Abbreviations: m. = months; y. = years.

Table 2. Median busulphan area under curve level (μmol x min/L).

	Mixed chimerism (n = 6)	*Complete chimerism (n = 12)*
Day 1	596	697 (*p* = 0.177)
Day 2	844	918 (*p* = 0.083)
Day 3	848	876 (*p* = 0.864)
Day 4	539	947 (*p* = 0.060)
Average day 1-4	774	908 (*p* = 0.067)

In malignant diseases, persistent mixed chimerism is always associated with relapse of leukemia. In non-malignant diseases, residual recipient cells may stay in harmony with the donor's system and rejection does not occur.[2,3] The mechanism of maintaining stable mixed chimerism is unknown. In this study, 26% of patients were found to have mixed chimerism and this is similar to the 36% previously reported.[4] Thalassemia patients have a higher incidence of mixed chimerism. The hypercellular bone marrow may be more resistant to ablative conditioning. Repeated blood transfusion before BMT may stimulate patients' immune system, thus rendering them more resistant to immunoablation.[2]

Intensity of conditioning regimen was reported to have an impact on mixed chimerism.[5] Manna reported that thalassemia patients who received lower doses of cyclophosphamide had a higher incidence of mixed chimerism.[6] One study showed that busulphan levels did not affect transplant outcome.[7] In this study, however, there was a trend towards patients with mixed chimerism having had lower levels of busulphan and this deserves further study. The incidence of graft rejection in thalassemia patients with mixed chimerism was reported to be high, 20 out of 55 patients.[8] More than >25% residual host cells early after transplant is highly predictive of graft rejection.[3] In this study, only one patient with mixed chimerism developed graft rejection. We used a more intensive conditioning and this may account for the low rejection rate. We observed a high percentage of elevated Hb F production and this is in concordance with a previous report that

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erythropoiesis is increased under stress, especially in heterozygous thalassemia donors.[9] Studying BFU-E may be more useful to detect the erythroid chimerism. In conclusion, mixed chimerism is common after transplantation. It is difficult to predict the outcome of mixed chimerism and graft rejection may be reduced with a more intensive preparative regimen.

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Increasing risk of relapse after allogeneic stem cell transplant for adult acute lymphoblastic leukemia in ≥ 2nd complete remission induced by highly intensive chemotherapy

From this retrospective single center analysis adults with acute lymphobalstic leukemia transplanted in ≥2nd CR from an HLA-identical sibling later than 1993 had a worse outcome. As the transplanted-related mortality improved by time, this result was essentially due to the increased relapse rate. The intensity of the pre-transplant salvage chemotherapy was identified as the main factor influencing the post-transplant relapse-risk.

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We report the outcome of 32 adults with acute lymphoblastic leukemia (ALL) in ≥2nd complete remission (CR) transplanted from an HLA-identical sibling over a consecutive 14-year period, aiming at evaluating whether the long-term results from a single Center have improved over the years.

Seventeen patients were transplanted between 1987 and 1993 (cohort 1) and 15 between 1994 and 2000 (cohort 2). Isolated extramedullary relapse occurring in 4 patients (CNS=2; CNS + testis=1; jaw=1) was considered when determining the remission number (cohort 1=2; cohort 2=2). Patient, donor and graft characteristics are reported in Table 1. Most patients received induction and rescue chemotherapy according to the GIMEMA protocols,[1-5] but the intensity of chemotherapy significantly changed among the patients over the years. If an HLA-identical sibling was available, patients with unfavorable cytogenetics [i.e. translocations t(9;22) or t(4;11)] were given stem cell transplantation (SCT) in 1st CR according to the policy of the GIMEMA Group. Therefore, in our study there was no patient with a diagnosis of ALL Ph^+ or MLL^+. Monitoring of minimal residual disease and its evaluation at transplant was not routinely performed. Nine of 17 patients transplanted before 1994 were prepared with a standard regimen consisting of 12 Gy fractionated total body irradiation (TBI) or 16 mg/Kg b.w. busulphan associated with 120 or 200 mg/kg b.w. cyclophosphamide, while all 15 patients transplanted ≥ 1994 received an alternative regimen consisting of an intensified three-drug combination chemotherapy or high-dose VP-16 associated with TBI. The stem cell source was bone marrow in 29 patients and peripheral blood in 3. As graft-versus-host disease (GVHD) prophylaxis, all patients received cyclosporine, associated with a short course of methotrexate in 14 patients. The close-out date for analyses was August 31, 2001. Baseline patient, donor and graft characteristics were compared using the χ^2 and Fisher's test when appropriate for categorical and the Mann-Whitney test for continuous variables. The end-points were overall survival (OS), leukemia-free survival (LFS), relapse and transplant-related mortality (TRM). Actuarial probabilities were estimated by the method of Kaplan and Meier and comparison of survival curves was performed by the log-rank test. All the variables reported in Table 1 were tested in univariate analysis and those found to be significantly associated with each end-point ($p<0.05$) entered multivariate analysis using the Cox proportional hazards regression model. A significance level of $p=0.05$ was used for the multivariate analysis.

Table 2 summarizes the results of the univariate analysis: only variables significantly associated with either of the end-points are reported. The incidence and severity of acute and chronic GVHD were not significantly different between the 1st and 2nd cohort (grade 2-4 acute GVHD: 47% both; chronic GVHD: 46% vs. 54%). In multivariate analysis, the only prognostic factor