

Overview of the current issues and advances in haemopoietic stem cell transplantation for β -thalassemia major

S.S. Anak

Istanbul University, Istanbul School of Medicine, Dept. of Pediatric Hematology/Oncology/BMT, Turkey

Abstract

Bone marrow transplantation (BMT) is the only possible curative treatment for β -thalassemia major. The largest experience occurred in Pesaro, Italy, where the BMT was applied after a standard risk assessment. The patients were divided into 3 risk classes based on liver size by physical examination, the presence or absence of fibrosis by liver biopsy, and adherence to regular iron chelation. Outcomes were mainly affected by the risk status. After modifications to the conditioning regimens, the risk of transplantation-related complications in high-risk recipients reduced considerably. As a result, outcomes after transplantation have become more similar across risk categories. For BMT, most centers use bone marrow instead of peripheral blood in thalassemia. Some studies showed that peripheral blood stem cell transplantation (PBSCT) is better than BMT with regard to hematologic recovery, hospitalization period, leukemia-free survival, overall survival (OS), and transplant-related mortality (TRM). No significant differences were seen in grade II to IV acute GVHD (aGVHD); but the incidence of chronic GVHD (cGVHD) was significantly higher in the PBSCT group. BMT from unrelated donors may offer similar results to those obtained using HLA-identical family donors, at least for patients who are not fully compliant with conventional treatment and do not yet show severe complications of iron overload. All studies conclude that MUD BMT might be a good alternative for patients with less risk factors. Another study concluded that, at present, due to high graft failure and GVHD rates, BMT from alternative donors should be restricted to patients who have poor life expectancies because they cannot receive adequate conventional treatment or because of alloimmunization to minor blood antigens. In another study unrelated cord blood transplantation (CBT) was compared to related donor transplantation for children with β -thalassemia. The results were comparable to the survival

rates of related-donor BMT for thalassemia. It has always been a dream for parents to have a new baby who might be a donor for his/her sibling and save his/her life. Today some families tried to learn the HLA group of the fetus using prenatal diagnosis. The last step in this development was preimplantation genetic diagnosis (PGD). PGD has become available as an alternative to prenatal diagnosis in order to avoid the risk for pregnancy termination, because PGD allows selection of unaffected embryos before a pregnancy is established. Gene therapies, the ultimate idea, involves replacing allogeneic stem cell transplantation with the transfer of normal globin genes into patient-derived autologous haematopoietic stem cells, bypassing the need for allogeneic donors and the immunosuppression required to achieve engraftment of the transplanted cells and to eliminate the risk of donor-related graft-versus-host disease. The successful preclinical studies in thalassaemia mouse models, the accumulating data on lentiviral vector-mediated HSC transduction and the anticipated increased safety of lineage-restricted globin SIN lentiviral vectors strongly support the initiation of Phase I gene therapy clinical trials in β -thalassaemia.

Introduction

β -thalassemia major is a genetic defect that causes an ineffective erythropoiesis with hemolysis. The main treatment principles are regular red blood cell (RBC) transfusions with iron chelation therapy for transfusion-related iron overload and supportive care. The disease and treatment-related complications such as liver, cardiac, endocrine, and posttransfusion viral infections result in poor quality of life and increased mortality. Bone marrow transplantation (BMT) is the only possible curative treatment for β -thalassemia major. The defective gene is replaced by the normal hematopoietic cells after BMT.¹

For many countries, especially around the Mediterranean region, β -thalassemia major is an important health problem, requiring life-long supportive care. Turkey is one of the countries where the prevalence of β -thalassemia major is still very high and causes an important burden for health facilities.

During the last 25 years, BMT as a curative therapeutic approach for thalassemia has undergone considerable change and development. The largest experience occurred in Pesaro, Italy where the BMT was applied after a standard risk assessment. The patients were divided into 3 risk classes based on liver size by physical examination, the presence or absence of fibrosis by liver biopsy, and adherence to regular iron chelation. Outcomes were mainly affected by the risk status. After modifications to the conditioning regimens, the risk of transplantation-related complications in high-risk recipients reduced considerably. As a result, outcomes after transplantation have become more similar across risk categories. The most recent results after human leukocyte antigen (HLA)-matched sibling bone marrow transplantation (BMT) for Pesaro class I or II and class III recipients show thalassemia-free survival probabilities of 87%, 85%, and 80%, respec-

Correspondence: S.S. Anak, Istanbul University, Istanbul School of Medicine, Dept. of Pediatric Hematology/Oncology/BMT, Turkey.

Key words: HSCT, thalassemia major.

©Copyright S.S. Anak, 2011

Licensee PAGEPress, Italy

Thalassemia Reports 2011; 1(s2):e8

doi:10.4081/thal.2011.s2.e8

This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (by-nc 3.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Parts of this work were presented at the "12th International Conference on Thalassemia and Hemoglobinopathies", Antalya (Turkey), 11-14 May 2011.

tively. Despite these improvements, long-term transfusions and organ damage from iron overload still appear to impact the long-term outcomes negatively. In the most recent update, the transplantation-related mortality in class I or II and class III patients was 3% and 10%, respectively, and 8% to 12% of pediatric recipients experienced graft rejection after BMT.^{2,3}

The results of HLA-matched sibling BMT for thalassemia major of the patient series outside Italy were not as successful, but the reasons for this were obscure when compared.⁴⁻⁷ In a recent report, 179 patients with β -thalassemia major who received BMT from HLA-matched sibling were reviewed. The distribution of Pesaro risk class I, II, and III categories was 2%, 42%, and 36%, respectively. The 5-year probabilities of overall- and disease-free survival were 91% and 88%, respectively, for patients with Pesaro risk class II, and 64% and 62%, respectively, for Pesaro risk class III.¹

New perspectives

For BMT, most centers use bone marrow instead of peripheral blood in thalassemia. In Pesaro, Italy, >1500 patients have received BMT, with the 20-year probability of thalassemia-free survival (TFS) around 80%. Studies performed by the European Group for Blood and Marrow Transplantation (EBMT) and International Bone Marrow Transplantation Registry (IBMTR) in hematologic malignancies showed that peripheral blood stem cell transplantation (PBSCT) is better than BMT with regard to hematologic recovery, hospitalization period, leukemia-free survival, overall survival (OS), and transplant-related mortality (TRM). No significant differences were seen in grade II to IV acute GVHD (aGVHD); but the incidence of chronic GVHD (cGVHD) was significantly higher in the PBSCT group.⁸ In a meta analysis involving 15 studies (9 cohorts, 5 randomized trials, and 1 database review) a significant increase in the rate of aGVHD and cGVHD was seen in PBSCT compared with BMT.⁹ In a study where 87 β thalassemia patients treated with PBSCT compared to 96 BMT were evaluated, the 2-year disease-free survival was 76% in both groups, but the median time to neutrophil and platelet recovery in PBSCT patients was significantly lower than BMT patients. Grade II-IV acute GVHD and chronic GVHD were more frequent in PBSCT versus BMT group.⁸

Another important issue for the application of BMT is finding a suitable donor. Most of the experience in the last two decades was based on BMT using HLA-matched sibling donors. But for patients without a healthy sibling donor, new possibilities like matched-unrelated donors or preimplantation genetics were evaluated.

In Italy, to evaluate whether BMT from an HLA-matched unrelated volunteer donor can offer a probability of cure comparable to that obtained when the donor is a compatible sibling, a study was carried out involving 68 thalassemia patients transplanted in six BMT Centers. Fourteen patients were classified in risk class 1; 16 in risk class 2; and 38 in risk class III of the Pesaro classification system. Nine patients (13%) had either primary or secondary graft failure. Fourteen patients (20%) died from transplant-related causes. Grade II-IV acute graft-versus-host disease (GVHD) developed in 24 cases (40%), and chronic GVHD in 10 cases (18%). Overall survival (OS) in the cohort of 68 patients was 79.3%, whereas the Kaplan-Meier estimates of disease-free survival (DFS) with transfusion independence was 65.8%. In the group of 30 thalassemic patients in risk classes 1 and 2, the probability of OS and DFS were 96.7%. BMT from unrelated donors may offer similar results to those obtained using HLA-identical family donors, at least for patients who are not fully compliant with conventional treatment and do not yet show severe

complications of iron overload.¹⁰ In another study from China, in only 9 patients, full engraftment was achieved in eight patients. 7 patients had acute GVHD, while one patient developed chronic GVHD. One patient died of pulmonary hemorrhage.¹¹ In Thailand experience, BMT from MUD was carried out in 11 children with severe thalassaemia. Grade 2-4 acute GVHD developed in 7 patients and chronic GVHD in 3 patients. All 11 patients are alive without thalassaemia after a median follow-up time of 397 days.¹² All studies conclude that MUD BMT might be a good alternative for patients with less risk factors. In another study, evaluating alternative donors, twenty-nine patients with thalassemia were given a BMT from an alternative donor. Six of the 29 donors were HLA-phenotypically identical and two were mismatched relatives, 13 were mismatched siblings and eight were mismatched parents. Twenty-three patients were in class 2 or class 3, whereas six patients were in class 1. Thirteen of 29 patients (44.8%) had sustained engraftment. The probability of graft failure or rejection was 55%.

The incidence of grade II-IV acute GVHD was 47.3% and chronic GVHD was 37.5%. The probability of overall and event-free survival was 65% and 21%, respectively, with median follow-up of 7.5 years (range 0.6-17 years) for surviving patients. The degree of HLA disparity between patient and donor did not have a significant effect on survival. Transplant-related mortality was 34%. It is concluded that, at present, due to high graft failure and GVHD rates, BMT from alternative donors should be restricted to patients who have poor life expectancies because they cannot receive adequate conventional treatment or because of alloimmunization to minor blood antigens.¹³ In another study from China, unrelated cord blood transplantation (CBT) was compared to related donor transplantation for children with β -thalassemia. In 35 patients, 40 transplants were performed between October 2003 and September 2009. HLA matching at enrolment was 6/6 (n. 8), 5/6 (n. 16), 4/6 (n. 27), or 3/6 (n. 1) by low-resolution HLA-A, -B, and high-resolution DRB1. The 5-year OS and thalassaemia-free survival after the first transplant were 88.3 and 73.9%, respectively. The cumulative incidence of TRM at 2 years was 11.7%. These results were comparable to the survival rates of related-donor BMT for thalassemia.¹⁴

It has always been a dream for parents to have a new baby who might be a donor for his/her sibling and save his/her life. Before prenatal HLA typing was possible, families tried to have more children with the hope of finding a matching donor (Burgio *et al.*, 1987, 1997). Later some families tried to learn the HLA group of the fetus using prenatal diagnosis. Kearney & Caplan (1992) approached the problem of curettage if the fetus was not HLA-matching. The last step in this development was preimplantation genetic diagnosis (PGD). PGD has become available as an alternative to prenatal diagnosis in order to avoid the risk for pregnancy termination, because PGD allows selection of unaffected embryos before a pregnancy is established. Despite the need for ovarian stimulation and in vitro fertilization (IVF) to be part of the procedure, PGD has become an acceptable method for avoiding the birth of children with genetic disorders.¹⁵ The first application of PGD together with HLA matching was for a child with Fanconi's Anemia.¹⁶ The most discussed reality was the instrumentalization of the child. The future child would be used as an instrument to cure another child.¹⁷ These ethical issues must be discussed for new concepts.

The strategy optimized for preimplantation genetic diagnosis (PGD) of haemoglobinopathies combined with HLA matching involves a minisequencing based genotyping of HLA regions A, B, C and DRB combined with mutation analysis of the gene regions involved by mutation. Analysis of at least eight polymorphic short tandem repeat (STR) markers scattered through the HLA complex has also been included to detect potential contamination and crossing-

over occurrences between HLA genes. The strategy was clinically applied for HLA matching in 17 cycles (14 for β -thalassaemia, one for Wiscott-Aldrich syndrome and two for leukaemia). In total, 22 (14.8%) embryos were obtained that were HLA-matched with the affected siblings, 14 (9.4%) of which were unaffected and transferred back to the patients. Four clinical pregnancies were obtained, three of which (one twin, two singletons) are ongoing and were confirmed as healthy and HLA-identical with the affected children.¹⁸

PGA with HLA matching is widely used for hemoglobinopathies. In some countries, this procedure is forbidden because of religious or ethical issues. In Turkey this procedure is applied successfully. In a survey done by Turkish Pediatric BMT group in May 2009, among 1250 BMT cases, 263 were for Thalassemia Major. Among these 9 were from donors born by PGA/IVF procedure in 5 centers. 6 were A&W, 2 were very recent to evaluate and one rejected.

Gene therapies has always been the ultimate idea for the solution of genetic diseases. This approach involves replacing allogeneic stem cell transplantation with the transfer of normal globin genes into patient-derived, autologous haematopoietic stem cells. This highly attractive strategy offers several advantages, including bypassing the need for allogeneic donors and the immunosuppression required to achieve engraftment of the transplanted cells and to eliminate the risk of donor-related graft-versus-host disease. But effective gene therapy for hemoglobinopathies will require high numbers of autologous gene-engineered hematopoietic stem cells to be reintroduced into the patients.

Because high numbers of corrected cells will be required to physically compete for niche space against the large pool of unmodified stem cells and the engrafted gene-corrected stem cells will not have any selective advantage compared with the endogenous stem cells. Stem cell mobilization using G-CSF is the most convenient and effective approach to achieve this goal, but it can have severe side effects like splenic rupture in the case of severe thalassemia.^{19,20} The successful preclinical studies in thalassaemia mousemodels, the accumulating data on lentiviral vector-mediated HSC transduction and the anticipated increased safety of lineage-restricted globin SIN lentiviral vectors strongly support the initiation of Phase I gene therapy clinical trials in β -thalassaemia.

However, questions remain regarding the optimal vector design, the optimal means of acquiring and engrafting HSCs in patients with β -thalassaemia and potential complications associated with an adult thalassaemia patient population. These questions must be addressed before the enormous potential for gene therapy in the treatment of the thalassaemias can be realised.^{19,20}

A. Yesilipek, *et al.* recently presented the results of HSCT for β -thalassaemia patients on behalf of Turkish Pediatric Stem Cell Transplantation Group. Between Jan 1991-June 2009, 245 children with β -thalassaemia major underwent first allo HSCT in 9 centers in Turkey. M/F ratio was 129/116 and the median age was 6.6 (range1-22 years).Forty-one patients were in Class I, 137 in Class II, 63 in Class III and class is not known in 11 patients. Stem cell sources were bone marrow in 88, peripheral blood in 137 and cord blood in 20. All donors were HLA matched related donors. Conditioning regimens consisted of BU + CY in 95, BU+CY+ATG in 100, Pesaro Protocol 26 in 40, BU+CY+ Tio-Thepa (TT) in 3. CsA alone or in combination with MTX or metylprednisolon is used for graft versus host disease (GvHD) prophylaxis. Median follow-up period after HSCT was 61 months, (14-231 months). Acute GvHD was observed in 42 children, 31 of which had Grade II-IV.

Chronic GvHD have occurred in 29 patients, 8 with extended form. Thalassaemic reconstitution has been observed in 43 transplantations. Nineteen patients expired in the first 100 days and transplantation related mortality (TRM) was 7.75%. EFS (thalassaemia free and alive) and OS were 68.0% and 85.0%, respectively.²¹

Conclusions

In conclusion, the role of transplantation for β -thalassaemia major, which is a chronic, nonmalignant condition, depends on the available supportive care measures in that country. Still, with best conditions, most persons die of thalassaemia or of complications related to its treatment in the fourth or fifth decades of life. So, the successful transplantation outcomes reported should encourage the staff and families for the possibility of HLA-matched sibling BMT, when there are limited resources.

References

1. Sabloff M, Chandy M, Wang Z, et al. HLA-matched sibling bone marrow transplantation for β -thalassaemia major. *Blood*. 2011; 117:1745-50.
2. Sodani P, Gaziev D, Polchi P, et al. New approach for bone marrow transplantation in patients with class 3 thalassaemia aged younger than 17 years. *Blood*. 2004; 104:1201-3.
3. Lucarelli G, Galimberti M, Giardini C, et al. Bone marrow transplantation in thalassaemia: the experience of Pesaro. *Ann N Y Acad Sci*. 1998;850:270-5.
4. Ullah K, Khan B, Raza S, et al. Bone marrow transplant cure for β -thalassaemia major: initial experience from a developing country. *Ann Hematol*. 2008;87:655-61.
5. Walters MC, Sullivan KM, O'Reilly RJ, et al. Bone marrow transplantation for thalassaemia: the USA experience. *Am J Pediatr Hematol Oncol*. 1994;16:11-7.
6. Chern JP, Su S, Lin KH, et al. Survival, mortality, and complications in patients with β -thalassaemia major in northern Taiwan. *Pediatr Blood Cancer*. 2007;48:550-4.
7. Cappellini MD, Cohen A, Piga A, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with β -thalassaemia. *Blood*. 2006;107:3455-62.
8. Ghavamzadeh A, Irvani M, Ashouri A, et al. Peripheral blood versus bone marrow as a source of hematopoietic stem cells for allogeneic transplantation in children with class I and II β thalassaemia major. *Biology of Blood and Marrow Transplantation*. 2008;14:301-8.
9. Cutler C, Giri S, Jeyapalan S, et al. Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. *J Clin Oncol*. 2001;19:3685-91.9a.
10. Nasa GL, Argioli F, Giardini C, et al. Unrelated bone marrow transplantation for β -thalassaemia patients, the experience of the italian bone marrow transplant group. *Ann.N.Y.Acad.Sci*. 2005;1054:186-95.
11. Feng Z, Sun E, Lan H, et al. Unrelated donor bone marrow transplantation for β -thalassaemia major:an experience from China. *Bone Marrow Transplantation*. 2006;37:171-4.
12. Hongeng S, Pakakasama S, Chairipoomkere W, et al. Outcome of transplantation with unrelated donor bone marrow in children with severe thalassaemia. *Bone Marrow Transplantation*. 2004;33:377-9.
13. Gaziev D, Galimberti M, Lucarelli G, et al. Bone marrow transplantation from alternative donors for thalassaemia: HLA-phenotypically identical relative and HLA-nonidentical sibling or parent transplants. *Bone Marrow Transplantation*. 2000;25:815-21.
14. Jaing TH, Hung IJ, Yang CP, et al. Unrelated cord blood transplantation for thalassaemia: a single-institution experience of 35 patients. *Bone Marrow Transplantation*. 2011;1-7.
15. Verlinsky Y, Rechitsky S, Sharapova T, et al. Preimplantation HLA Testing. *JAMA*. 2004;291:2079-85.

16. Verlinsky Y, Rechitsky S, Schoolcraft W, et al. Preimplantation diagnosis for Fanconi anemia combined with HLA matching. *JAMA*. 2001;285:3130-3.
17. Pennings G, de Wert G. Evolving ethics in medically assisted reproduction. *Human reproduction update*. 2003;9:397-404.
18. Fiorentino F, Biricik A, Karadayi H, et al. Development and clinical application of a strategy for preimplantation genetic diagnosis of single gene disorders combined with HLA matching. *Molecular human reproduction*. 2004;10: 445-60.
19. Yannaki E, Stamatoyannopoulos G. Hematopoietic stem cell mobilization strategies for gene therapy of β thalassemia and sickle cell disease. *Ann. N.Y. Acad. Sci.* 2010;1202:59-63.
20. Yannaki E, Emery DW, Stamatoyannopoulos G, et al. Gene therapy for β -thalassaemia: the continuing challenge. *Expert reviews in molecular medicine*. 2010;12:1-20.
21. Yesilipek A, Ertem M, Cetin M, et al. On behalf of the turkish pediatric stem cell transplantation group. Haematopoetic stem cell transplantation in β -thalassaemia: Turkish experience. *Bone marrow transplantation*. 2011;46:616.

Non-commercial use only