Boccaccio C, et al. Intensive chemotherapy with hematopoietic cell transplantation after ESHAP therapy for relapsed or refractory non-Hodgkin's lymphoma. Results of a single-centre study of 65 patients. Leuk Lymphoma 1999;33:543-50.
9. Giesselbrecht C, Schmitz N, Mounier N, Ma D, Trneny M, Hagberg H et al. R-ICE versus R-DHAP in relapsed patients with CD20 difuse large B-cell lymphoma (DLBCL) followed by stem cell transplantation and maintenance treatment with rituximab or not: first interim analysis on 200 patients. CORAL study. Blood (ASH Annual Meeting Abstracts), Nov 2007; 110: 517.
10. Friedberg JW. Unique toxicities and resistance mechanisms associated with monoclonal antibody therapy. Hematology Am Soc Hematol Educ Program 2005:329-34.
11. Zelenetz AD SM, Vose J, Younes A, Kaminski MS. Patients with transformed low grade lymphoma attain durable responses following outpatient radioimmunotherapy with tositumomab and iodine I 131 tositumomab (Bexxar). Blood 2002;100:357a.
12. Morschhauser F, Illidge T, Huglo D, Martinelli G, Paganelli G, Zinzani PL, et al. Efficacy and safety of yttrium-90 ibritumomab tiuxetan in patients with relapsed or refractory diffuse large B-cell lymphoma not appropriate for autologous stem-cell transplantation. Blood 2007;110:54-8.
13. Press OW, Eary JF, Appelbaum FR, Martin PJ, Badger CC, Nelp WB, et al. Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. N Engl J Med 1993;329:1219-24.
14. Vose J, Bierman P, Bociek G, Loberiza F, Enke C, Hankins J, Armitage J. Radioimmunotherapy with 131-I tositumomab enhanced survival in good prognosis relapsed and high-risk diffuse large B-cell lymphoma (DLBCL) patients receiving high-dose chemotheapy and autologous stem cell transplantation [ASCO abstract 8013]. J Clin Oncol 2007:8013.
15. Krishnan A, Nademanee A, Fung HC, Raubitschek AA, Molina A, Yamauchi D, et al. Phase II trial of a transplantation regimen of yttrium-90 ibritumomab tiuxetan and high-dose chemotherapy in patients with non-Hodgkin's lymphoma. J Clin Oncol 2008;26:90-5.
16. Khouri IF, Saliba RM, Hosing C, Okoroji GJ, Acholonu S, Anderlini P, et al. Concurrent administration of high-dose rituximab before and after autologous stem-cell transplantation for relapsed aggressive B-cell non-Hodgkin's lymphomas. J Clin Oncol 2005;23:2240-7.
17. Horwitz SM, Negrin RS, Blume KG, Breslin S, Stuart MJ, Stockerl-Goldstein KE, et al. Rituximab as adjuvant to high-dose therapy and autologous hematopoietic cell transplantation for aggressive non-Hodgkin lymphoma. Blood 2004;103:777-83.
18. Martin P, Furman RR, Ruan J, Elstrom R, Barrientos J, Niesvizky R, et al. Novel and engineered anti-B-cell monoclonal antibodies for non-Hodgkin's lymphoma. Semin Hematol 2008;45:126-32.
19. Robertson MJ, Kahl BS, Vose JM, de Vos S, Laughlin M, Flynn PJ, et al. Phase II study of enzastaurin, a protein kinase C beta inhibitor, in patients with relapsed or refractory diffuse large B-cell lymphoma. J Clin Oncol 2007;25:1741-6.
20. Abramson JS, Shipp MA. Advances in the biology and therapy of diffuse large B-cell lymphoma: moving toward a molecularly targeted approach. Blood 2005;106:1164-74.
21. Chen L, Monti S, Juszczynski P, Daley J, Chen W, Witzig TE, et al. SYK-dependent tonic B-cell receptor signaling is a rational treatment target in diffuse large B-cell lymphoma. Blood 2008;111:2230-7.

Allogeneic stem cell transplantation for thalassemia major

Emanuele Angelucci and Donatella Baronciani

Struttura Complessa Disciplina di Ematologia e Centro Trapianto Cellule Staminali Emopoietiche "Wilma Deplano", Ospedale Oncologico di Riferimento Regionale "Armando Businco", Cagliari, Italy. E-mail: emnang@tin.it.

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Allogeneic hemopoietic stem cell (HSC) transplantation in thalassemia major has been a cornerstone of the development of cell-based therapies[1] and a matter of passionate debate during the '80s and early '90s.[2] The aim of HSC transplantation in thalassemia is to replace the ineffective endogenous thalassemic erythropoiesis with an effective allogeneic substitute and to obtain a lasting, permanent, clinically effective correction of the hemolytic anemia[3,4] and of the transfusion-associated pathologies, including iron overload. The large Pesaro experience demonstrated that this target has been achieved (Figure 1).

The issue of HSC transplantation in genetic disease is much different from that of HSC transplantation in malignancies. In the case of thalassemias, a graft versus leukemia effect in not required but the expansion of the erythroid compartment together with the enhanced immunological activity likely related to repeated transfusions result in the risk of graft rejection and disease recurrence being higher[5] than those observed for malignant diseases.

Application of transplantation world-wide

The HSC transplantation therapeutic approach pioneered by the Pesaro group[5-11] is now applied widely worldwide. The European Group for Blood and Marrow Transplantation (EBMT) has established the hemoglobinopathy registry which now contains detailed epidemiological data on over 3,000 patients. Since the early '90s, between 133 and 197 transplants per year have been registered (Figure 2).

The EBMT registry highlights the pioneering role of the Pesaro group in this field (Figure 3) and shows the wide diffusion of the procedure after 1993 (the year in which Professor Lucarelli gave the Ham-Wasserman lecture at the St. Louis ASH meeting).

Medical therapy for thalassemia major

Medical therapy of thalassemia is one of the most spectacular medical successes of the last two decades. Thalassemia has been transformed from a lethal disease of infancy into a chronic disease of adulthood[12] with a dramatic increase in both survival and life expectancy. An Italian retrospective study clearly demonstrated the enormous progress for patients born in the last two decades compared with patients born in earlier decades (Figure 4).

In recent years we have witnessed spectacular advances in our knowledge of iron overload pathophysiology, which have been accompanied by a substantial improvement in quality and availability of diagnostic capability, and by the development of new,

effective and safe oral chelation regimens.[13] These newly developed oral chelators today offer a reliable alternative to deferoxamine therapy and promise to further increase the successes of medical therapy.

However, the very large majority of patients live today in non-industrialized countries, where availability of these effective therapies is severely curtailed. A recent survey by Bernadette Model indicated that over 330,000 hemoglobinopathy affected infants are born annually (83% sickle cell disorders, 17% thalassemias).[14] While in industrialized countries prenatal screening has dramatically decreased the number of newborns affected by thalassemia major, underdeveloped countries have so far been unable to implement prenatal diagnosis and counseling due to limitations in available funds and expertise.

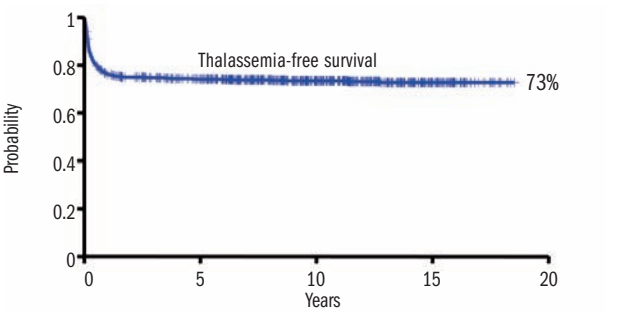

Figure 1. **Results of hemopoietic stem cell transplantation in 900 consecutive patients, aged 1-35 years, transplanted from an HLA identical sibling in Pesaro since December 1981.** ***Courtesy of Guido Lucarelli.***

The dilemma of a choice

No prospective randomized clinical trial will be able to provide a definitive answer to the challenge of choosing between HSC transplantation and medical therapy for each individual patient. In absence of definitive evidence, the choice has to be based on large prospective transplant studies and retrospective medical therapy trials in conjunction with individual preference. This decision process is by definition highly individualized and patient specific, since it must consider age, clinical status, willingness, capability and compliance to adhere to the appropriate transfusional-chelation regimen, quality of life and resources. For pediatric patients, parents face an even more difficult decision.

Availability of resources

In the treatment of thalassemias, availability of resources is a key issue. A modern complete transfusion – chelation regimen is a high technology therapy requiring unique expertise and resources. Medical therapy, without even considering the associated complications, is very expensive and clearly not widely available worldwide. A recent Italian study based on cost/benefit estimation from a societal perspective, quantified tariffs, expenses, and net earnings in 2006 prices, generated an estimated mean direct cost of medical therapy (transfusion + deferoxamine chelation) of approximately 15,000 euros /year/ patient.[15] In addition, proper medical therapy for thalassemias requires an increasing number and array of advanced technologies (like cardiac MRI) which have to be provided by an increasing number of treatment centers.[16] HSC transplantation is also a high-skills/ high-technology therapy, which is becoming increasingly available worldwide: the unpublished EBMT survey indicates that presently more than half of the transplants for thalassemia are performed outside industrialized/developed countries.

Since the largest number of patients with severe thalassemia is now situated in either underdeveloped or still developing countries, the availability of resources

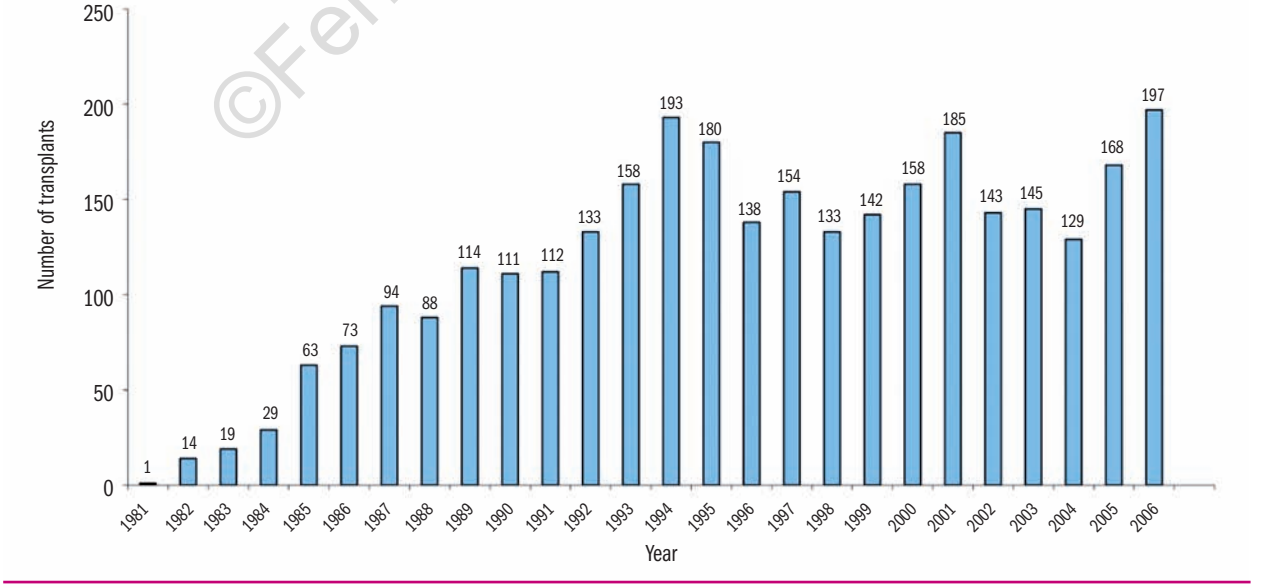

Figure 2. **Numbers of hemopoietic stem cell transplants performed for thalassemia through the years in centers of the European Registry for Blood and Marrow Transplantation (EBMT). Unpublished data from the Hemoglobinopathy Registry of the European Group for Blood and Marrow Transplantation.**

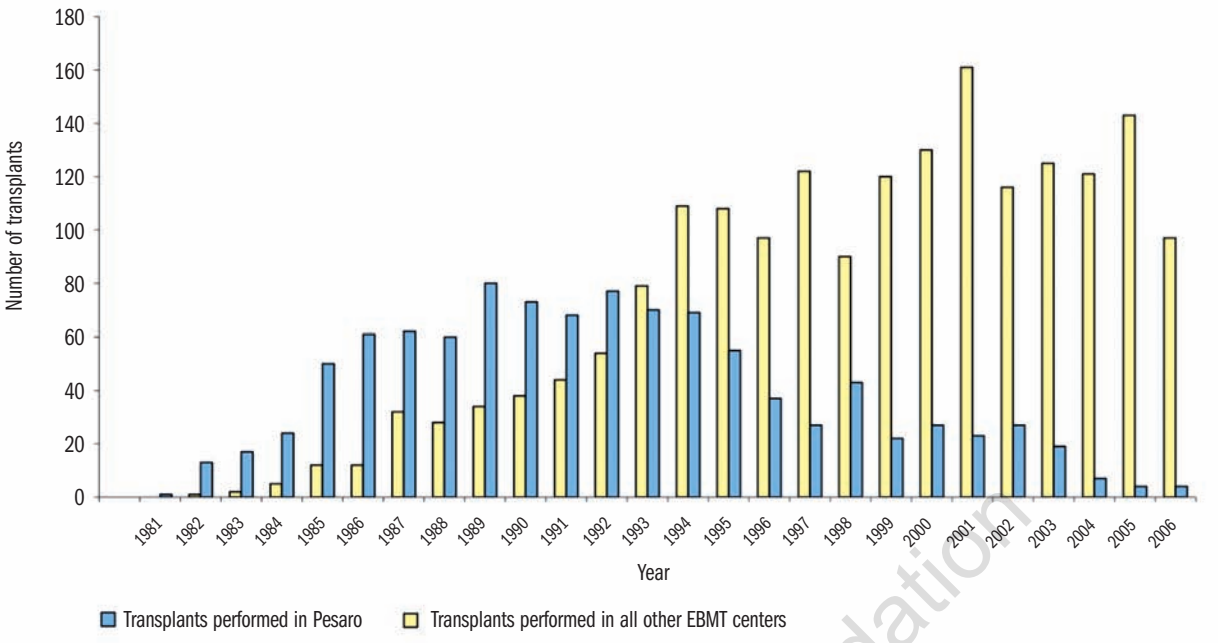

Figure 3. Numbers of hemopoietic stem cell transplantations performed for thalassemia through the years in centers of the European Registry for Blood and Marrow Transplantation (EBMT). The blue bars indicate the transplants performed in Pesaro. The yellow bars indicate the transplants performed in all other centers per year. Unpublished data from the Hemoglobinopathy Registry of the European Group for Blood and Marrow Transplantation.

rather than published literature is the main driver of therapeutic decisions. In this setting, transplant is likely to be the most cost effective approach, but only for a limited number of patients.

Mixed chimerism

In the field of hemopoietic stem cell transplantation mixed chimerism is defined as the concurrent presence of donor and recipient hematopoietic cells. Mixed chimerism is commonly reported after transplantation in thalassemia with a myeloablative conditioning. In a prospective study involving more than 300 consecutive patients, mixed chimerism was observed in 28% of them within the first two months.[17] By the second year after the transplant, 43% of the chimeric patients evolved to complete engraftment (i.e., residual host cells were no longer detectable), 35% showed a progressive loss of donor-engrafted cells and had recurrence of thalassemia, 22% (12% of all survivors) had persistent mixed chimerism. After the second year, the chimeric state remained stable in all patients in long follow-up. During the same period of observation, no patient who had complete engraftment (i.e., 100% donor cells) at the second month of follow-up subsequently developed graft failure or recurrence of thalassemia.[17]

In patients who maintained mixed chimerism after the second year of follow-up, a partial engraftment (up to 20% of marrow cells) was still able to maintain a normal hemoglobin level avoiding blood transfusions. In this population, no signs of increasing iron overload or other clinical complications of thalassemia were detectable, thus rendering this congenital disease completely controlled as in full chimera patients. Therefore, contrary to common expectations and beliefs, complete donor hematopoiesis is not essential for sustained engraftment and thalassemia recurrence prevention. Two factors appear to contribute to this phenomenon: presence of expanded T-cell clonotypes that could be responsible for the induction of donor/recipient tolerance, maintaining at the same time a normal immune status and a selective survival of the non-thalassemic red cells.

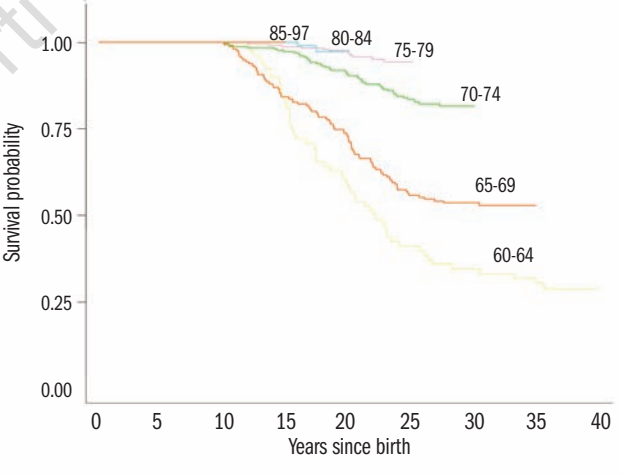

Figure 4. Kaplan-Meier survival curves in 977 medically treated patients with thalassemia in Italy. Survival is calculated after the first decade of life, by birth cohort. Reprinted with permission.[12]

Regardless of the mechanism, correction of anemia

by relatively low degrees of mixed chimerism after HSC transplantation provides the rationale for the development of minimally-ablative preparative regimens to obtain partial engraftment and at the same time avoid or significantly diminish transplant-related morbidity and mortality. The evidence that a partial engraftment of a normal erythropoiesis is able to control thalassemia could also be the basis for a gene therapy program (when safely available) since complete eradication of the thalassemic marrow is not necessary for clinical control of the disease. The objective to reduce transplant related toxicity is particularly indicated for adult and advanced-disease patients in whom a significant transplant related mortality has been observed.[18, 19]

Alternative conditioning regimens

Because it is not necessary to completely eradicate a malignant clone, mixed chimerism and minimally ablative conditioning regimens have been postulated to be the future direction for HSC transplantation in thalassemia and other hemoglobinopathies.

However, limited attempts performed in this direction failed. Recently two sobering experiences have been reported.[20,21] Overall, 11 patients affected by thalassemia or sickle cell disease were given an allogeneic stem cell transplantation following a non-myeloablative regimen. As expected, transplant related toxicity was minimal but after a transient engraftment all patients but one experienced a return of the underlying condition.[20,21] These data indicate that stable donor engraftment after reduced intensity conditioning is difficult to achieve among patients with hemoglobinopathies. The expanded erythroid thalassemic clone and the immunological condition of thalassemia patients are likely to be the reason for this clinical behavior which is also not a trivial problem for the more aggressive myeloablative regimen. Moreover, the addition of donor lymphocyte infusion to sustain the engraftment does not appear to be a rational approach in terms of risk/benefit ratio for thalassemia.

Alternative source of stem cells

The large majority of transplants in thalassemia have been performed using bone marrow derived stem cells. In the EBMT survey, more than 80% of the transplants have been performed using bone marrow derived cells. Bone marrow derived stem cells continue to be the preferred source of stem cells for transplantation in thalassemia even in the most recent years.

In 2003, Locatelli first successfully proposed the use of identical sibling cord blood derived hemopoietic stem cells for transplantation in thalassemia.[22]

Alternative donor

Only approximately one in three of patients has an HLA identical sibling inside the family.[23] Use of alternative donors include mismatched donors and matched unrelated donors. Mismatched donor transplantation has been studied in a very limited number of trials.[24] Because of the experimental role of this procedure and the alternatives available today, it should not be recommended unless in selected clinical cases in which other approaches have been documented to be not practicable.

HSC transplantation from a matched unrelated donor has been applied by a multicentric Italian study which demonstrated satisfactory results[25] similar to those obtained from sibling donors in pediatric and adult patients.[25] However the limited number of cases reported (< 100) and the limitation in donor selection means this procedure should be reserved for specialized centers.

Unrelated cord blood transplantation is standard practice today for HSC therapy of malignancies. Tolerance of 1-2 HLA antigen disparity, fast availability, and low incidence of graft versus host disease make this option attractive, especially for diseases in which a graft versus tumor effect is not requested. Preliminary experience with this approach in the setting of thalassemias is promising but limited (77% overall survival and 65% disease free survival in 36 cases reported)[26] and should therefore be considered an experimental approach.

Mixed chimerism after cord blood translantation

In this issue of the journal, Daniela Lisini and coworkers studied the effect of early chimerism on long-term engraftment after HLA identical sibling cord blood derived hemopoietic stem cell transplantation for thalassemia.[27] Contrary to what had been widely documented[17, 27] after adult hemopoietic stem cell transplantation, Lisini *et al.* demonstrate that early mixed chimerism is not predictive of long-term graft failure and thalassemia recurrence. Sustained engraftment (full chimera or persistent long lasting clinical effective mixed chimera) occurred in all patients without further immunological therapy despite demonstration of early mixed chimerism. The more pronounced tolerance of this kind of hemopoietic stem cell could be the basis of this different behavior. This observation is quite relevant because it provides evidence that the concept of early mixed chimerism as predictor of graft failure is not applicable after cord blood transplantation, and may open the way to further studies of reduced intensity - reduced toxicity conditioning for cord blood transplant. This regimen could reduce mortality and still sustain full or partial engraftment able to control anemia. This hypothesis will need to be tested in controlled experimental pilot trials.

Transplantation for thalassemia in the era of oral iron chelatorrs

The central role of hemopoietic stem cell transplantation in thalassemia has now been fully established, through a long, controversial, hard, but in the end productive debate. Transplantation remains today the *only definitive curative* therapy for thalassemias and other hemoglobinopathies. The development of oral chelators did not change this position. However, this has not settled the debate on how this curative but potentially lethal treatment stands vis-à-vis a medical, non-curative therapy for adult and advanced disease patients. Transplantation technologies have improved substantially during recent years and their outcome is likely to

be much better today than it was in the '80s. Recent data indicated a probability of overall survival and thalassemia free survival of 97% and 89% for patients with no advanced disease and of 87% and 80% for patients with advanced disease.[28] Similar data are coming out of the unpublished EBMT survey with a 90% 5-year overall survival in over 1,000 patients transplanted world wide during recent years.

We should make our best effort to further reduce transplant related mortality and to extend the opportunity of transplant even to patients lacking an HLA identical donor and those without modern healthcare resources. This is the challenge for the definitive cure of thalassemias and hemogobinopathies in the third millennium.

References

1. Appelbaum FR. Hematopoietic-cell transplantation at 50. N Engl J Med 2007;357:1472-5.
2. Lucarelli G, Clift R, Angelucci E. Deferoxamine in thalassemia major. N Engl J Med 1995;332:271; author reply 2-3.
3. Schrier SL, Angelucci E. New strategies in the treatment of the thalassemias. Annu Rev Med 2005;56:157-71.
4. Di Bartolomeo P, Santarone S, Di Bartolomeo E, Olioso P, Bavaro P, Papalinetti G, et al. Long-term results of survival in patients with thalassemia major treated with bone marrow transplantation. Am J Hematol 2008;83:528-30.
5. Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Giardini C, et al. Bone marrow transplantation in patients with thalassemia. N Engl J Med 1990;322: 417-21.
6. Lucarelli G, Polchi P, Izzi T, Manna M, Agostinelli F, Delfini C, et al. Allogeneic marrow transplantation for thalassemia. Exp Hematol 1984;12:676-81.
7. Lucarelli G, Polchi P, Galimberti M, Izzi T, Delfini C, Manna M, et al. Marrow transplantation for thalassaemia following busulphan and cyclophosphamide. Lancet 1985; 1:1355-7.
8. Lucarelli G, Galimberti M, Polchi P, Giardini C, Politi P, Baronciani D, et al. Marrow transplantation in patients with advanced thalassemia. N Engl J Med 1987;316:1050-5.
9. Lucarelli G, Weatherall DJ. For debate: bone marrow transplantation for severe thalassaemia (1). The view from Pesaro (2). To be or not to be. Br J Haematol 1991;78:300-3.
10. Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Durazzi SM, et al. Bone marrow transplantation in adult thalassemia. Blood 1992;80:1603-7.
11. Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Giardini C, et al. Marrow transplantation in patients with thalassemia responsive to iron chelation therapy. N Engl J Med 1993;329:840-4.
12. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 2004;89: 1187-93.
13. Angelucci E, Barosi G, Camaschella C, Cappellini MD, Cazzola M, Galanello R, et al. Italian Society of Hematology practice guidelines for the management of iron overload in thalassemia major and related disorders. Haematologica 2008;93:741-52.
14. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ 2008;86:480-7.
15. Scalone L, Mantovani LG, Krol M, Rofail D, Ravera S, Bisconte MG, et al. Costs, quality of life, treatment satisfaction and compliance in patients with beta-thalassemia major undergoing iron chelation therapy: the ITHACA study. Curr Med Res Opin 2008;24:1905-17.
16. Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2008;10:42.
17. Andreani M, Nesci S, Lucarelli G, Tonucci P, Rapa S, Angelucci E, et al. Long-term survival of ex-thalassemic patients with persistent mixed chimerism after bone marrow transplantation. Bone Marrow Transplant 2000;25: 401-4.
18. Lucarelli G, Clift RA, Galimberti M, Angelucci E, Giardini C, Baronciani D, et al. Bone marrow transplantation in adult thalassemic patients. Blood 1999;93:1164-7.
19. Lucarelli G, Clift RA, Galimberti M, Polchi P, Angelucci E, Baronciani D, et al. Marrow transplantation for patients with thalassemia: results in class 3 patients. Blood 1996; 87:2082-8.
20. Jacobsohn DA, Duerst R, Tse W, Kletzel M. Reduced intensity haemopoietic stem-cell transplantation for treatment of non-malignant diseases in children. Lancet 2004;364: 156-62.
21. Iannone R, Casella JF, Fuchs EJ, Chen AR, Jones RJ, Woolfrey A, et al. Results of minimally toxic nonmyeloablative transplantation in patients with sickle cell anemia and beta-thalassemia. Biol Blood Marrow Transplant 2003; 9: 519-28.
22. Locatelli F, Rocha V, Reed W, Bernaudin F, Ertem M, Grafakos S, et al. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. Blood 2003;101:2137-43.
23. Delfini C, Donati M, Marchionni D, Nesci S, Paradisi O, Valentini M, et al. HLA compatibility for patients with thalassemia: implications for bone marrow transplantation. Int J Cell Cloning 1986;4:274-8.
24. Gaziev D, Galimberti M, Lucarelli G, Polchi P, Giardini C, Angelucci E, et al. Bone marrow transplantation from alternative donors for thalassemia: HLA-phenotypically identical relative and HLA-nonidentical sibling or parent transplants. Bone Marrow Transplant 2000;25:815-21.
25. La Nasa G, Argiolu F, Giardini C, Pession A, Fagioli F, Caocci G, et al. Unrelated bone marrow transplantation for beta-thalassemia patients: The experience of the Italian Bone Marrow Transplant Group. Ann N Y Acad Sci 2005; 1054:186-95.
26. Jaing T-H, Tan P, Rosenthal J, Wang B, Gjertson D, Petz L, Chow R. Unrelated cord blood transplantation (CBT) for thalassemia. Blood (ASH Annual Meeting Abstracts), Nov 2006;108: 554.
27. Lisini D, Zecca M, Giorgiani G, et al. Donor/recipient mixed chimerism does not predict graft failure in children with β-thalassemia given an allogeneic cord blood transplant from an HLA-identical sibling. Haematologica 2008;93:1859-67.
28. Lucarelli G, Gaziev J. Advances in the allogeneic transplantation for thalassemia. Blood Rev 2008;22:53-63.