Bone Marrow Transplantation in Thalassemia (Part 2)

Maryam Zakerinia

Department of Internal Medicine, BMT Unit, Hematology Research Center, and Organ Transplant Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran.

Correspondence:
Maryam Zakerinia MD, Department of Internal Medicine, BMT Unit, Hematology Research Center, and Organ Transplant Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran.
Tel: +98 711 6279611
Fax: +98 711 6474301
Email: transbon@sums.ac.ir
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Abstract
During the last two decades conventional therapy has improved the prognosis of thalassemia. However, despite such improvement it still remains a progressive disease with treatment-related complications such as hepatitis, liver fibrosis, and cardiac disease. Bone marrow transplantation (BMT) can prevent or delay progression of the aforementioned complications. The importance of clinical research in the field of BMT was recognized with the award of the 1990 Nobel Prize in Physiology and Medicine to E. Donnell Thomas, one of the pioneers of BMT in humans. George Mathe' was a pioneer in the early development of clinical BMT. Mathe' and co-workers were the first to describe graft-versus-host-disease and its treatment, and the graft-versus-leukemia effect in human. The first BMT for β-thalassemia major was performed successfully by Thomas and colleagues in Seattle, in 1981. In the same year another patient with β-thalassemia major underwent BMT in Pesaro, Italy, by Lucarelli and others. Since then, several hundred transplantations have been performed worldwide, mostly in Italy. From 1991 through 2007 BMT have been performed on 497 (Tehran=342, Shiraz=155) blood transfusion dependent patients with thalassemia major in Iran, with disease-free survival of 71-77% respectively. Because of high graft failure and high rates of graft-versus-host-disease 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. Beginning in the early 1980s, it was shown that umbilical cord blood contained high levels of hematopoietic progenitor cells.


Keywords • Bone marrow transplantation • thalassemia • graft-versus-host-disease

Donors
The ideal donor is an HLA-identical sibling. A patient in need of bone marrow transplantation (BMT) has an approximately 30% chance of having an HLA match in another sibling. Thus, relying on HLA-identical siblings as the sole donor source leaves seven out of 10 potential transplant patients without the possibility of curative therapy.

As a partial remedy to this problem, HLA-matched unrelated donor transplantations were first attempted in 1973, and many successful transplantations have been done.25 Established in 1986, the National Marrow Donor Program
Patients were Lucarrelli Class 2 or Class 3, between 1969 and 1996. It is likely that most individuals from minority ethnic groups in the registry and their greater potential for HLA polymorphisms, the likelihoods of matching for Hispanic, Asian/Pacific, and black individuals are, respectively, 65%, 45%, and 24%. Meanwhile, worldwide efforts continue to coordinate computer searches. When combined with other international registries, millions of unrelated donors are available to patients in need of a donor.

A second alternative to HLA identical sibling transplants have been the use of T-cell-depleted bone marrow transplants. T-cells are responsible for graft versus host disease (GVHD), and depleting them has been shown to decrease the incidence of GVHD. This has allowed HLA-mismatched BMT (both related and unrelated) to be successfully done. Unfortunately, since T-cells are also responsible for overcoming host resistance to allogeneic engraftment and for the graft-versus-leukemia (GVL) effect, which decreases relapse after allogeneic transplantation, T-cell depletion has also been associated with an increase in graft failure (and subsequent death from marrow aplasia) and relapse. In addition, T-cell depletion has also been associated with delayed recovery in T-cell function, and therefore greater mortality from infections caused by viruses such as cytomegalovirus.

Alternative donor transplants for thalassemia has been reported from 22 teams worldwide between 1969 and 1996. It is likely that most patients were Lucarelli Class 2 or Class 3, with hepatomegaly and history of irregular iron chelation therapy. Forty eight (75%) of the 64 patients survived, with 18 of the survivors experiencing recurrence of thalassemia (disease-free-survival: 53%). Of the 60 patients with genotypically HLA-no identical related donors, 39 have information provided concerning the degree of HLA mismatching; 24 had phenotypically matched parental donors, 11 had one-antigen and four had two-antigen mismatched related donors. The number of surviving patients in the three groups were 19 (79%), seven (64%), and two (50%), respectively.

A study was done involving 68 patients with thalassemia transplanted in six Italian BMT centers. Thirty three male and 35 female patients (age range: 2-37 years; median age: 15) were transplanted from unrelated volunteer donors, all selected by using high-resolution molecular typing of both HLA class I and II loci. 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 GVHD developed in 24 patients (40%), and chronic GVHD in 10 patients (18%). Overall survival in the cohort of 68 patients was 79.3% (CI 67-88%), whereas the Kaplan-Meier estimates of disease-free-survival with transfusion independence was 65.8% (CI 54-77%). In the group of 30 patients with thalassemia in risk Classes 1 and 2, the probability of overall survival and disease-free-survival were 96.7% (CI 90-100%) and 80.0% (CI 65-94%), respectively, whereas in the 38 patients in Class 3, overall survival was 65.2% (CI 49-80%) and disease-free-survival was 54.5% (CI 42-70%). These data show that when donor selections are based on stringent compatibility criteria, the results of unrelated transplantation in patients with thalassemia are comparable to those obtained when the donor is a compatible sibling.

The Rome and Florence group in Italy studied the use of the haploidentical mothers as the donor of hematopoietic stem cells assuming that the immuno-tolerance established during pregnancy will help to bypass the HLA disparity and allow hemopoietic allogeneic reconstitution. They have employed a new preparative regimen for the transplant in 12 children with thalassemia aged 3 to 8 years; using T-cell depleted peripheral blood stem cell transplant plus BM stem cells. All patients received hydroxyurea 60 mg/kg, azathioprine 3 mg/kg from day -59 until day -13, fludarabine 30 mg/m² from day -17 to day -13, busulphan 14 mg/kg, cyclophosphamide 200 mg/kg, thiopeta 10 mg/kg and anti-thymocyte globulin sangost, USA 2.5 mg/kg, followed by a CD34+, T-cell depleted (ClinImacs system), from their haploidentical mothers. The average number of infused CD 34+ cells was 15.4 ×10⁶/kg and the average number of infused T lymphocyte from BM was 1.8 ×10⁶/kg. The results showed that five patients rejected the transplant and were alive with thalassemia, seven patients were disease free with a median follow up of 21 months (range 15-38). None of the seven patients showed acute GVHD.

Umbilical Cord Blood Transplantation

Over the past decade, a new source of hematopoietic stem cells has become available, with the potential to significantly alleviate the shortage of donors that has plagued BMT since its inception. Beginning in the early 1980s, it was...
shown that umbilical cord blood contained high levels of hematopoietic progenitor cells.\textsuperscript{9} In 1989, Broxmeyer and co-workers found that the numbers of colony-forming units contained in umbilical cord blood collections were similar to the numbers obtained from marrow collections in which sustained hematopoietic engraftment was achieved.\textsuperscript{92} These data followed two case reports from the late 1960s and early 1970s suggesting that cord blood infusions caused transient changes in RBC phenotype, not related to the infusion itself, when administered in the clinical setting of conventional dose chemotherapy.\textsuperscript{93}

The first umbilical cord blood transplantation with sustained engraftment was performed in 1988 on a child with Fanconi's anemia who received umbilical cord blood from his HLA-identical sibling. The patient continues to do well to this day.\textsuperscript{94} This initial demonstration of the effectiveness of umbilical cord blood in providing hematopoietic engraftment, in a short time, has generated tremendous activity centered on determining the proper uses of this virtually unlimited supply of hematopoietic stem cells.

Early results indicate that umbilical cord blood transplantation is associated with a lower incidence and less severity of GVHD than other sources of stem cells, potentially decreasing the morbidity and mortality of BMT.\textsuperscript{95} Because of a relatively weaker ability to induce severe GVHD, mismatched cord blood transplants can be used effectively, although HLA-matched grafts engraft faster and reduce post-transplant morbidity and mortality.\textsuperscript{95}

An analysis revealed that cord blood grafts, either matched (6/6 loci of-HLA) or mismatched (5/6 loci of-HLA) with good cell doses, provide outcomes equal to or superior to those of (6/6) matched bone marrow grafts.\textsuperscript{96} The frequency of hematopoietic stem cells in umbilical cord blood is 1 of $1 \times 10^6$ mononuclear cells, in bone marrow is 1 of $3 \times 10^6$ mononuclear cells, and in peripheral blood is 1 of $6 \times 10^6$ mononuclear cells.\textsuperscript{97}

With regard to related allogeneic umbilical cord blood donor; median time to neutrophil recovery is 26 days for umbilical cord blood versus 18 days for bone marrow, cumulative incidence of neutrophil recovery by day 60 is 0.89 (0.82-0.94) in umbilical cord blood transplantation versus 0.98 (0.97-0.99) in BMT (p<0.001). Cumulative incidences of acute GVHD grade II to IV are 0.14 (0.08-0.22) in umbilical cord blood transplantation versus 0.24 (0.22-0.26) in BMT recipients (p=0.02). No different 3-year survival of 0.64 (0.53-0.74) in umbilical cord blood transplantation versus 0.66 (0.64-0.68) for BMT, and no difference in relapse-related death were seen. In conclusion, umbilical cord blood transplantation is associated with relatively slower myeloid recovery but also lower incidence of acute GVHD than BMT.\textsuperscript{98}

**Double Umbilical Cord Blood Transplantation**

Limited cell dose of total nucleated cells and CD34+ cells of umbilical cord blood are the most important barrier to its more widespread use in adults. Patients who receive umbilical cord blood transplantation using two umbilical cord blood units, because of the unavailability of a single unit that has a satisfactory total nucleated cells dose, do as well as patients transplanted with a single adequately sized umbilical cord blood unit. Sustained hematopoiesis after double umbilical cord blood transplantation is usually derived from a single donor. It is not clear how to predict which of the two units will predominate.\textsuperscript{99}

In a study conducted in Shiraz, Iran, BM progenitor cells, peripheral blood leukocytes, and plasma samples were collected from 30 allogeneic BM donors. Umbilical cord blood hematopoietic stem cells, and plasma samples were also collected from 34 umbilical cord blood donors. Viral DNA extracted and purified from collected specimens was processed using nested polymerase chain reactions (PCR) to detect human parvovirus B19 (HPV B19), human herpesvirus-6 (HHV-6), varicella-zoster virus (VZV), human cytomegalovirus (HCMV), and Epstein-Barr virus (EBV). The prevalence of HCMV DNA in collected BM progenitor cells versus umbilical cord blood hematopoietic stem cells were 73% and 23%, respectively. Conversely, HHV-6 DNA, as evaluated by using simple PCR, was not detected in collected specimens. Distribution of the other investigated virus DNAs except EBV DNA was similar in specimens collected from both groups. EBV DNA was not determined in umbilical cord blood hematopoietic stem cells. The results indicated that the risk of viral transmission to BM transplant recipients via umbilical cord blood hematopoietic stem cells is less than that with BM progenitor cells.\textsuperscript{100}

The median age of patients with thalassemia who have undergone umbilical cord blood transplantation has been 3.8 $\pm$ 2.3 years in a study.\textsuperscript{101} Since August 1999 cord blood stem cell bank has been developed in Iran.\textsuperscript{102}

**In Utero Transplantation for Thalassemia**

The early gestational fetal environment is unique and may offer advantages for the transplantation
and engraftment of foreign hematopoietic stem cell. These potential advantages are based on events of normal hematopoietic and immunologic ontogeny. The second major advantage provided by normal ontogeny is immunologic tolerance. CD 34-enriched parental BM cells were transplanted into the peritoneal cavity in few cases. Although the approach has been successful for immunodeficiency syndromes, attempts so far to treat the hemoglobinopathies have failed.\textsuperscript{103}

**Genetic Treatment of the B-Thalassemias**

The transfer of a regulated \( \beta \)-globin gene in autologous hematopoietic stem cells is a highly attractive alternative treatment. This strategy, which is simple in principle, raises major challenges in terms of controlling expression of the globin transgene, which ideally should be erythroid specific, differentiation-and stage-restricted, elevated, position independent, and sustained over time. Using lentiviral vectors, May and colleagues, demonstrated in 2000 that an optimized combination of proximal and distal transcriptional control elements permits lineage-specific and elevated \( \beta \)-globin expression, resulting in the therapeutic hemoglobin production and correction of anemia in \( \beta \)-thalassemic mice. Several groups have by now replicated and extended these findings to various mouse models of severe hemoglobinopathies, thus fueling enthusiasm for a potential treatment of \( \beta \)-thalassemia based on globin gene transfer. Current investigations focus on safety issues and the need for improved vector production methodologies. The safe implementation of stem cell-based gene therapy requires the prevention of the formation of replication-competent viral genomes and minimization of the risk of insertionional oncogenesis. Importantly, globin vectors, in which transcriptional activity is highly restricted, have a lesser risk of activating oncogenes in hematopoietic progenitors than non-tissue-specific vectors, by virtue of their late-stage erythroid specificity. As such, they proved a general paradigm for improving vector safety in stem cell-based gene therapy.\textsuperscript{104}

The success in the long-term correction of mouse models of human beta-thalassemia and sickle cell anemia by lentiviral vectors and evidence of high gene transfer and expression in transduced human hematopoietic cells have led to a first clinical trial of gene therapy for the disease. A lentiglobin vector containing a beta globin gene (G\( \beta ^{A-T87C} \)), which produces hemoglobin (Hb\( \beta ^{A-T87C} \)) that can be distinguished from normal hemoglobin will be used. The Lentiglobin vector is self-inactivating and contains large elements of the beta-globin locus control region, as well as chromatin insulators and other features that should prevent untoward events. The study will be done in Paris with Eliane Gluckman as the principal investigator and Philippe Leboulch as scientific director.\textsuperscript{105}

**Preparative (Conditioning) Regimens**

Three separate objectives must be achieved by the preparative regimen. The defective stem cell population must be damaged sufficiently to be incapable of regeneration, and there must be adequate immunosuppression to achieve successful allogeneic engraftment. Cyclophosphamide can be used successfully to achieve the second objective, but dose not eliminate myeloid stem cells. Busulfan and its dimethylated derivative are highly effective against myeloid stem cells but are not strongly immunosuppressive. The third objective is to provide space/niches for donor stem cells.

Different doses of busulfan (12-24 mg/kg) or dimethylmyleran (5 mg/kg), and cyclophosphamide (120-240 mg/kg) have been given to patients with thalassemia; and a subset of patients has received total body irradiation at a dose of 720 cGy followed by cyclophosphamide 120 mg/kg.\textsuperscript{106}

To reduce transplant related-mortality, Lucarelli and co-workers used busulfan 14 mg/kg, cyclophosphamide 120 mg/kg, and antithymocyte globulin 100 mg/kg in Class 3 patients with 91% survival, 60% event-free survival and a thalassemia recurrence rate of 37%.\textsuperscript{62}

Other centers are using modified protocols, however, the most used regimen is busulfan (3.5-4 mg/kg) administered orally in 3 to 4 divided doses from day -9 to -6 and cyclophosphamide (30-50 mg/kg) from day -5 to -2, antithymocyte globulin (10 mg/kg) from day -5, or -2 to day +2, or +5 (table 3). Reduced intensity regimens (non-myeloablative hematopoietic stem cell transplant) using fludarabine (180 mg/m\(^2\)), antithymocyte globulin (40 mg/kg), and low dose busulfan (8 mg/kg) have resulted in graft rejection.\textsuperscript{107,108}

The Rome, Italy group 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 every 24 hours. Red cells were transfused every 3 days to maintain 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) and erythropoietin, were administered twice weekly to maintain stem cell proliferation in the face of hyper transfusion, thereby facilitating the effect of the hydroxyurea. Fludarabine was administered at a dosage of 20 mg/m²/day 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 40 mg/kg daily in each of the next 4 days (total dose 160 mg/kg). This regimen was applied to 33 consecutive patients aged younger than 17 years with Class 3 thalassemia and well tolerated with 93% survival. The incidence of recurrent thalassemia after the transplantation decreased from 30% to 8%.  

Graft-Versus-Host Disease

Despite the use of sibling donors matched at the major histocompatibility complex and despite post-grafting immunosuppression, GVHD occurs in approximately 70% of patients with successful marrow grafts. The initial organ involved in almost all cases is the skin. Hepatic and intestinal involvement usually appears several days after the rash. Intestinal involvement is mainly in the form of diarrhea but may progress to abdominal pain and ileus. Liver disease is manifested...
by rise in bilirubin (mainly conjugated). Serum glutamic oxaloacetic transaminase is usually in the range of 150 to 750 IU. Rises in alkaline phosphatase are also observed especially in chronic GVHD.\textsuperscript{110}

Onset of acute GVHD occurs between 7 and 60 days after transplantation. The clinical stages according to organ system and overall clinical grading of severity of acute GVHD are shown in table 6.\textsuperscript{110,111}

**Chronic Graft-Versus-Host Disease**

Chronic GVHD occurs in at least 30% to 50% of recipients of transplants from HLA–matched siblings and at least 60% to 70% of recipients of transplants from unrelated donors.\textsuperscript{112}

Onset of chronic GVHD is after day 100 after BMT. The classification of chronic GVHD is shown in table 7.\textsuperscript{113,114}

Prophylaxis for GVHD is with cyclosporine (3 mg/kg/day, in two doses intravenously, from day -2 and will be changed to 10 mg/kg/day per oral as soon as possible (from day +6 on), plus methylprednisolone 0.5 mg/kg/day intravenously (from day -1 until day +21), ± methotrexate (15 mg/m² on day +1, 10 mg/m² on days +3, +6, and +11).

Cyclosporine is gradually reduced (20% per month) until complete cessation at day +365 from transplantation. Methylprednisolone is stopped at day +30 if no active GVHD is present. The first drug of choice for treatment of acute GVHD is methylprednisolone at escalating doses up to 10 mg/kg, depending on the degree and duration of acute GVHD.\textsuperscript{115}

The alternative choices for GVHD treatment are antithymocyte globulin, mycophenolate mofetil (CellCept), thalidomide, tacrolimus (FK 506), campath IH,\textsuperscript{115,116} rapamycin, anti-T-cell or cytokine antibodies, 2-deoxycoformycin, psoralen and ultraviolet A, extracorporeal phopheresis,\textsuperscript{115} and daclizumab, (anti-CD25).\textsuperscript{117}

In the past 3 years by adding mycophenolate mofetil to cyclosporine and methylprednisolone or prednisolone for treatment of acute GVHD (grades II-IV) and chronic GVHD (both limited and extensive) mortality has been reduced in our center (unpublished data).

The use of intravenous immunoglobin (IV

| Table 6: Clinical staging and grading of acute graft-versus-host disease |
|------------------|------------------|------------------|
| Skin             | Liver            | Gut              |
| Stage            | Maculopapular rash <25% of body surface | Bilirubin 2-3 mg/dl | Diarrhea >500 ml/day or persistent nausea |
|                  | Maculopapular rash 25-50% of body surface | Bilirubin 3.1-6 mg/dl | Diarrhea >1000 ml/day |
|                  | Generalized erythroderma | Bilirubin 6.1-15 mg/dl | Diarrhea >1500 ml/day |
| Grade            | Generalized erythroderma with bullae and desquamation | Bilirubin >15 mg/dl | Severe abdominal pain with or without ileus |
| I                | Stage 1-2        | None             | None                |
| II               | Stage 3 or Stage 1 | Stage 4 or Stage 1 | Mild decrease in clinical performance |
| III              | Stage 2-3 or Stage 2-4 | Marked decrease in clinical performance |
| IV               | Stage 4          | Stage 4          | Extreme decrease in clinical performance |


| Table 7: Classification of chronic graft- versus- host disease |
|------------------|------------------|------------------|
| Model            | Type of chronic GVHD | Characteristics |
| Pattern of onset | De novo          | No antecedent acute GVHD |
|                  | Quiescent        | Onset after complete resolution of acute GVHD |
|                  | Progressive      | Direct evolution form acute GVHD |
|                  | Explosive        | Acute and chronic GVHD features are concomitant at the time of onset and very aggressive |
| Extent of disease| Limited          | Skin (localized) associated/nx to mild liver involvement |
|                  | Extensive        | Skin (generalized); or skin (localized) and/or liver (severe), plus eye, mouth, or any other target organ involvement |
| Clinical/ histologic pattern | Lichenoid | Maculopapular rash. Skin is thick and rough. Common oral and ocular involvement. It can evolve to scleroderma. |
|                  | Sclerodermatous  | Thickening and tautness of the skin, usually patchy, occasional blisters and ulcers. Associated or isolated facial involvement causes severe limitation in the range of motion. |

IgG) is approved for prevention of GVHD and infection.\textsuperscript{118} IV IgG is administered in days -1 (500 mg/kg), +7 (250 mg/kg), and +21 (250 mg/kg). If GVHD is present, IV IgG will be continued every 4 weeks.

**Supportive Care**

The patients are treated in HEPA-filtered, positive-pressure isolation rooms and receive trimethoprim/ sulfamethoxazole twice daily from day -9 till day -2, and from day +34 to day +180 (as prophylaxis for pneumocystis carinii, 2 days per week), or ciprofloxacin in patients with glucose-6–phosphate dehydrogenase deficiency, acyclovir (15mg/kg/day from day -1 until day +21), intravenous antibiotics with coverage of G-and G+ bacteria, (amikacin and ceftazidime from day -1), and amphotericin B from day +8 with a prophylactic dosage (0.3 mg/kg). If fever develops, vancomycin or other antibiotics is needed. Patients with suspicious or proven cytomegalovirus infection are treated with ganciclovir and IV IgG.

For major ABO-mismatched transplants, gravity sedimentation with hydroxyethyl starch (HES) at a ratio of 1:8 HES to marrow volume for about 2 hours is performed. For minor ABO-mismatched transplants, centrifugation of BM and separation of plasma may be needed. Antihistamines and intravenous hydrocortisone are given before and after infusion of BM or peripheral stem cells. In the case of ABO incompatibility; for the first 2 weeks after transplantation, transfusion support consist of the administration of recipient type or O type red blood cells or platelets, after 2 weeks, donor type transfusions are used.\textsuperscript{119}

To prevent transfusion-associated GVHD, all blood products (cellular components and fresh plasma, but not fresh frozen plasma (FFP) or cryoprecipitate) are irradiated with a minimum of 2,500 cGy before transfusion to HSC recipients.\textsuperscript{120}

**Mesenchymal Stem Cells**

Mesenchymal stem cells are pluripotent stem cell capable of generating osteoblasts, myoblasts, chondroblasts, tenoblasts, adipocytes and stromal cells. There have been several reports of the immunosuppressive effect of mesenchymal stem cells, both in vitro and in vivo. Co-infusion of a large number of osteoblasts together with HSCs, in a mismatched mouse model, results in successful engraftment and immune reconstitution, whereas control animals died of GVHD or rejection. A trial has been completed in 40 patients with hematologic malignancies, using expanded mesenchymal stem cells from an HLA identical sibling, and co-infusing these cells with a conventional un-manipulated BM or peripheral blood transplant; hemopoietic reconstitution was improved and GVHD was virtually absent both in its acute and chronic form. The protective effect of mesenchymal stem cells on GVHD is being tested in a prospective randomized trial. A study will also start on the use of Mesenchymal stem cells in the unrelated transplant setting.\textsuperscript{121}

**Evaluation of Graft Status**

Engraftment is documented by in situ Y chromosome hybridization of BM or blood samples in sex-mismatched donor-recipient pairs and by PCR analysis of variable number tandem repeat (VNTR) polymorphism and short tandem repeats (STR) in BM and / or blood samples in the case of sex-matched pairs. Globin chain synthesis of marrow and peripheral blood reticulocytes is examined by incorporation of H\textsuperscript{3}-Leucine followed by column or high-pressure liquid chromatography (HPLC).\textsuperscript{109}

**Transplant Related Mortality**

The First cause of transplant related mortality is GVHD. The second one is infection and the third one is hepatic veno-occlusive disease. Veno-occlusive disease is a major regimen related toxicity (more with total body irradiation) after BMT. Endothelial injury, leading to deposition of coagulation factors within the terminal hepatic venules,\textsuperscript{122} and resulting in liver dysfunction occurring within the first 3 weeks after marrow infusion. This is characterized by hyperbilirubinemia and at least 2 of 3 other findings including hepatomegaly, ascites, and 5% or greater weight gain.\textsuperscript{123} The incidences of veno-occlusive disease in patients with thalassemia have been 1-3%.\textsuperscript{57,109}

**Human Cytomegalovirus Infection in BMT Patients**

Severe human cytomegalovirus (HCMV) disease is usually observed in immunodeficient individuals such as BMT patients. In these patients, proof of viral presence is not enough for making clinical decisions; one must report the quantity of virus or viral load in appropriate clinical specimens to demonstrate the relationship between disease severity and HCMV infection. Peripheral blood was collected weekly for 100 days from 26 BMT recipients in Shiraz, Iran. Qualitative and quantitative competitive PCRs on 10\textsuperscript{5} mononuclear
cells were performed for each patient. Results demonstrated that in 14 of 26 patients viral copy number per 10^5 cells had increased, pointing toward HCMV reactivation. This method helped physicians begin pre-emptive therapy with ganciclovir and IV IgG. Of 26 BMT donors and recipients, 25 and 26 were IgG positive, and two and six had HCMV specific IgM antibodies, respectively.

**Hemorrhagic Cystitis**

Hemorrhagic cystitis is considered a major cause of morbidity and mortality after allogeneic BMT, and sometimes even invasive procedures, such as suprapavesical urinary diversion (ileal conduit) and cystectomy may be needed. It occurs after using high doses of cyclophosphamide as a condition regimen in 4-5% of patients who undergo BMT.

Continuous bladder irrigation, recombinant factor VIIa (NovoSeven) infusion, formalin instillation, suprapubic cystostomy, and internal iliac artery ligation are the treatment options.

**Mixed Chimerism after Bone Marrow Transplantation for Thalassemia**

The presence of mixed chimerism is a frequent event after BMT. From 800 patients in Pesaro group, 28% had mixed chimerism within the first 2 months after BMT, while it was 14% and 11% at 1 or 2 years before the transplantation. Rejection occurred in 13% with residual host cells <25%, and 90% in residual host cells >25%. None of the patients who showed presence of residual host cells for a period longer than 2 years rejected the transplant.

Of 88 patients of Shiraz group, 8% developed stable mixed chimerism with a median follow up of 7 years. Treatment with hydroxyurea prevented rejection in two patients. Patients with mixed chimerism have maintained hemoglobin levels between 9 g/dl and 13.5 g/dl without the need for transfusions.

**General Appearance after Bone Marrow Transplantation**

The facial appearance of patients with thalassemia change to normal after 1-3 years of successful BMT depending on the skeletal changes before BMT (personal observation).

Depending on ferritin level and gonadal function after BMT, their height may remain shorter than normal.

Two years after successful BMT, patients with high serum ferritin levels (>2000 µg/dl), should receive Desferal therapy or phlebotomy to reduce the serum ferritin level to below 500 µg/dl.

**Vaccination**

Patients with BMT should be vaccinated based on the suggested schedule.

- Diphtheria, tetanus toxoid: 12, 14, and 24 months after transplantation.
- Haemophilus influenza type b conjugate: 12, 14, and 24 months after transplantation.
- Hepatitis B virus: 12, 14, and 24 months after transplantation.
- 23-valent pneumococcal polysaccharide: 12 and 24 months after transplantation.
- Influenza virus: lifelong, seasonal administration beginning 6 months after transplantation.
- Inactivated poliovirus: 12, 14, and 24 months after transplantation.
- Measles, mumps, and rubella: 24 months after transplantation if receiving no immunosuppression and without chronic GVHD.

**Conflict of Interest:** None declared

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