Abstract

Objective: to review the indications, main steps and complications of bone marrow transplantation in children.

Sources: Medline-based literature review.

Summary of the findings: we comment about the indications of autologous, allogeneic and syngeneic bone marrow transplantation, donor selections, harvest and infusion of the hematopoietic progenitor cells that will reconstitute the hematopoietic and immune systems. We describe the different conditioning regimens and the new sources of cells, such as cord blood. We also describe the most common events after the procedure, including infections, graft versus host disease, and cardiovascular, pulmonary, hepatic, genitourinary, and gastrointestinal complications. The late effects and their impact on quality of life are also discussed.

Conclusions: bone marrow transplantation does not confer an absolutely normal life span to all the patients; however, it represents the only chance of cure for children with certain neoplastic or immunological diseases. By knowing the steps of the procedure, pediatricians can be a source of information on bone marrow transplantation to the patients and their families.


Introduction

Bone marrow transplantation (BMT) is the intravenous infusion of hematopoietic stem cells in order to reestablish marrow function in patients with damaged or defective bone marrow.1

The first report of intravenous infusion of bone marrow dates back to 1939,2 when a patient received 18 ml of bone marrow from a sibling in an attempt to treat aplastic anemia. However, the current scientific grounds for BMT are based on experiences with mice and guinea-pigs. These animals were submitted to lethal doses of radiation and survived with subsequent intravenous infusion of bone marrow.3 Successful experiments were also carried out with dogs during the 1950s and 60s; the animals were administered myeloablative dosages of whole-body irradiation and, next, bone marrow transplantation from related donors.4,5 The studies with dogs were the main model for the development of BMT in humans.

Identification and understanding of the human histocompatibility system, mapped to the chromosome 6, contributed in a decisive manner to the success of
transplantations. In 1968, three BMT of siblings with identical HLA-extended haplotypes were carried out in children with immunodeficiency; these patients, however, were not administered previous radio- or chemotherapy, but rather BMT alone. In March of 1969, in Seattle (USA), Dr. E. D. Thomas and team carried out the first successful allogeneic BMT with a model that is used to date; the model consisted of a leukemia patient who received lethal doses of whole-body irradiation and BMT from a sibling. In 1990, the same Dr. E. D. Thomas received the Nobel Prize of Medicine for the experimental and clinical work in bone marrow transplantation.

BMT is the procedure that better demonstrates the importance of integrating laboratory and clinical research. Science should not be limited to in-laboratory work, since the whole body of knowledge that constitutes science is knitted together by insights and information from different stages of development and application of technology.

**Methods**

We carried out a review of the literature based on Medline database and on selected references with historical importance; references were assessed according to the study design with preference given to controlled assays including large populations, and to more important periodicals.

The most frequently reviewed periodicals were the New England Journal of Medicine and Blood and Bone Marrow Transplantation. We also reviewed two well-known textbooks:


**Transplantation modalities**

There are three modalities of bone marrow transplantation:

- **Allogeneic transplantation**, in which the patient receives bone marrow transplants from a relative (related donor) or not (nonrelated donor).
- **Syngenic transplantation**, in which the patient receives bone marrow transplants from an identical twin; this modality is rare considering the frequency of identical twins in the general population.
- **Autologous transplantation**, in which the patient receives his own, previously harvested cells. This modality was first employed in the late 1970s in treatment of patients with malignant lymphoma.

**Stem cell sources**

Hematopoietic stem cells can be harvested directly from the iliac crest by multiple spinal tap and bone marrow aspiration procedures; from apheresis of the peripheral blood; and, more recently, from umbilical cord blood. The term bone marrow transplantation remains despite the fact that aspiration of bone marrow is no longer the only procedure for harvesting hematopoietic stem cells.

Aspiration of bone marrow requires hospitalization and general anesthesia of the donor. The donor is positioned prone and several tap procedures of the posterior iliac crests are carried out. These procedures allow for aspiration, using adequate needles, of the amount of bone marrow required for the transplantation; the required amount of bone marrow is usually estimated at 10 ml/kg of weight of the receptor. This estimation usually allows for an adequate number of stem cells for engraftment.

Bone marrow is disposed into an appropriate container or beaker with anticoagulant and, later, filtered for removal of fats and spicules. The rate of severe complications of BMT is low, for approximately 0.4% of cases. The reported complications usually occurred in donors with previous history of diseases and half of them could be attributed to anesthesia. The most frequent complaint in BMT patients is pain on the site of spinal tap, which usually recedes with common analgesics. Most donors are discharged 24 hours after collection. Transfusion of red blood cell concentrate to the donor is necessary only in cases of harvesting large volumes of bone marrow; this may occur if the receptor has a body mass much higher than that of the donor. Oral replacement of iron is recommended for 30 days. Most allogeneic transplants are still carried out with this form of harvest.

Peripheral blood hematopoietic stem cells are harvested with the help of apheresis equipment after mobilization of cells from the bone marrow to the peripheral blood using granulocyte colony-stimulating factor (filgrastim). Patients submitted to autologous transplantation can also be previously submitted to chemotherapy. It was during the 1980s that harvesting of stem cells from peripheral blood became well-known and used in over 90% of autologous transplantations and in approximately 20% of allogeneic transplantations. Proper harvesting requires a large-caliber venous access, in which case most patients require a double-lumen catheter. The most frequent complications involving harvesting of peripheral blood hematopoietic stem cells are related to catheter passage (pneumothorax). In this sense, the procedure should be carried out by well-experienced surgeons. Filgrastim can cause side effects such as bone pain, headaches, and fever. However, it is not common for these events to prompt the discontinuation of harvesting.

Currently, experiences with apheresis of patients weighing less than 10 kg are restricted due to limitations imposed by hemodilution.
Harvesting of peripheral blood stem cells in healthy pediatric donors is a matter of ethical discussion. The long-term risks related to granulocyte colony-stimulating factors has not been widely studied, but is apparently minimal.\textsuperscript{19,20}

The first successful experience with use of umbilical cord blood (UCB) as a source for bone marrow grafts was reported in 1988. At the time, Dr. Elaine Gluckman, in France, successfully treated a patient with Fanconi’s anemia using UCB from a sibling for hematopoietic reconstitution after myeloablative chemotherapy.\textsuperscript{21} Umbilical cord blood is collected right after birth, and later processed and frozen until infusion.\textsuperscript{22}

Table 1 presents the types of BMT and the sources for harvesting of grafts.

**Basic requirements for bone marrow transplantation**

In order to carry out BMT, it is necessary to observe:

- Patients in appropriate clinical conditions without severe organ or system dysfunction;
- Availability of stem cells for the procedure (cryopreserved or compatible donor with bone marrow, UCB, or peripheral blood stem cells);
- Confirmation that BMT is the best treatment for the baseline disease. In cases of neoplastic diseases, it is preferable that diseases be in remission;
- Availability of family, psychological, social, and economic resources for following the recommended posttransplantation follow-up.

The procedures should be carried out by a multidisciplinary team including:

- Doctors and nurses with training and experience on BMT;
- Psychologists, nutritionists, social workers.

The hospital where the procedures will be carried out should have:

- Individual rooms, preferably with air-conditioning and high-efficiency particulate air filtration (HEPA) filters;
- Blood bank with resources to freeze and defrost stem cells, to carry out apheresis, and to provide irradiated and filtered hemoderivatives rapidly and in sufficient amounts;
- Advisors of other specialties, including dermatology, pathology, infectology, gastroenterology, radiology, radiotherapy, surgery, and psychiatry;
- Services of imaging diagnosis;
- Services of clinical pathology with the ability to carry out routine and special examinations such as serum cyclosporin and CMV antigenemia.

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**Table 1** - Types of bone marrow transplantation

<table>
<thead>
<tr>
<th>Type of transplantation</th>
<th>Source of hematopoietic progenitor cells</th>
<th>Donor</th>
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</thead>
<tbody>
<tr>
<td><strong>Autologous</strong></td>
<td>Bone marrow</td>
<td>Patient himself/herself</td>
</tr>
<tr>
<td></td>
<td>Peripheral blood</td>
<td></td>
</tr>
<tr>
<td><strong>Allogeneic</strong></td>
<td>Bone marrow</td>
<td><em>Related:</em> (sibling or other family member)</td>
</tr>
<tr>
<td></td>
<td>Peripheral blood</td>
<td><em>Unrelated:</em> Any person that is not related to the patient</td>
</tr>
<tr>
<td></td>
<td>Umbilical cord blood</td>
<td></td>
</tr>
<tr>
<td><strong>Syngeneic</strong></td>
<td>Bone marrow</td>
<td>Identical twin</td>
</tr>
<tr>
<td></td>
<td>Peripheral blood</td>
<td></td>
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</table>
Screening donors

Differently from what occurs in most cases of solid organ transplantations, the level of compatibility between donor and patient is crucial for successful BMT. This was a surprise to the earlier investigators, considering that in the case of dogs there were no significant complications related to small immune differences. The human leukocyte antigen (HLA) is mapped to the short arm of chromosome 6, and is responsible for our immune identity. The HLA follows simple Mendelian inheritance. In this sense, patients who have siblings have a 25% chance of having an HLA-identical donor.1 Evidently, this probability increases proportionally to the number of siblings. Currently, families have less children and, thus, the chance for finding an HLA-identical related donor also decreases. Other factors for screening of donors are age, avoiding extremely young or old donors; weight, preferably greater than or equal to that of the receptor; medical history; overall clinical status; and sex. Donors should be submitted to full clinical examinations, laboratory examinations, and blood typing. It is preferred to find identical blood type donors, though blood group differences are not a contraindication for donation of bone marrow.

It is important to carry out careful examination of diseases related to genetic inheritance. Fanconi’s anemia, for example, can have very hard-to-notice manifestations in some patients.

Candidates to syngenic transplantation presupposedly have an identical twin as a donor; those to autologous transplantation require previous harvesting and cryopreservation of their own cells.

Table 2 presents the main exams requested before transplantation for patients and donors.

In case there are no HLA-identical related donors, one alternative is to look for relatives who are partially compatible; it is not frequent to find relatives with these characteristics. Another alternative would be to seek nonrelated donors of bone marrow or umbilical cord blood. The details for transplantations with nonrelated donors will be described later.

Indications

Indications for allogeneic and syngenic transplantation

Allogeneic and syngenic BMTs can be used in the treatment of several diseases, including:

- Nonneoplastic diseases:
  - Severe aplastic anemia (SAA); 23
  - Fanconi’s anemia; 24
  - Immunodeficiencies (Chediaki-Higashi syndrome, Wiskott-Aldrich syndrome, combined severe immunodeficiency diseases); 25-26
  - Osteopetrosis; 27
  - Storage diseases (adenoleukodystrophy, childhood metachromatic leukodystrophy); 28
  - Thalassemia major; 29
  - Sickle cell anemia with severe clinical manifestations and available related donor; 30

Neoplastic diseases:

- Chronic myeloid leukemia (CML); 31
- Acute myeloid leukemia (AML) in first remission and with poor prognosis factors, or second remission; 32,33
- Acute lymphocytic leukemia (ALL) in first remission and with poor prognosis factors, or subsequent remissions; 34,35
- Myelodysplastic syndromes including monosomia of chromosome 7 and chronic myelomonocytic leukemia; 36
- Acute (malignant) myelofibrosis; 37
- Non-Hodgkin’s lymphoma in second or third remission. 38

Indications and specifics of autologous transplantation

Autologous BMT has been studied as a form of treatment for several pediatric neoplasias. The purpose of autologous BMT is to render high-dose chemotherapy feasible in cases of diseases sensitive to increase in chemotherapy dosage. The limiting dose toxicity of chemotherapy is based on

<table>
<thead>
<tr>
<th>Exams</th>
<th>Patient</th>
<th>Donor</th>
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<tbody>
<tr>
<td>HLA typing</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Complete blood test including platelet count</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>ABO and Rh typing</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Serological exams for Chagas disease, Syphilis, HIV, HTLV I and HTLV II, Cytomegalovirus, Hepatitis B and C, Herpes, Mononucleosis, Toxoplasmosis</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Chest x-ray</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Electrocardiogram</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>AST, ALT, PT, aPPT, bilirubins</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Qualitative urinalysis</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Parasitological stool examination</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Urea, creatinine</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Spirometry</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Biopsy and bone marrow aspiration</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>X</td>
<td></td>
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<tr>
<td>Echocardiogram</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 - Exams required prior to bone marrow transplantation
myelosuppression; hence, reinfusion of hematopoietic stem cells allows for use of higher doses that could not be administered without this resource. Autologous transplantation offers less complexity and complications in comparison to allogeneic BMT; this does not, however, dismiss the need for a well-trained and specialized multidisciplinary team. Similarly to allogeneic transplantation, there are constant discussions on the matter of indication for autologous BMT. Currently, there is evidence that autologous BMT is effective in the treatment of the following diseases:

- Hodgkin’s lymphoma in second remission;³⁹
- Non-Hodgkin’s lymphoma in second remission;⁴⁰
- High-risk neuroblastoma; in this case, treatment with autologous bone marrow transplantation and 13-cis-retinoic acid presented a positive impact on prognosis;⁴¹
- Ewing’s sarcoma in second remission after complete excision of the tumor;⁴²
- AML; despite the fact that this is a disease involving the bone marrow, others have reported a positive impact of autologous transplantation following remission;³³
- Wilm’s tumor in second remission;⁴³
- Germ-cell tumors in second remission;⁴⁴
- High-risk or second remission medulloblastoma.⁴⁵

**Venous access**

Central venous access with good caliber is fundamental for carrying out BMT successfully. The most frequently used catheter is single- or double-lumen Hickman catheter.⁴⁶ This type of device allows for easy collection of exam samples and infusion of hemoderivatives, antibiotics, and parenteral nutrition.

The catheter is introduced into the subclavian vein or external jugular vein (occasionally, the internal jugular vein is used) and the extremity into the right atrium. The catheter is inserted through a subcutaneous tunnel. Insertion of the catheter should be carried out by an experienced surgeon, and it should only be handled by a well-trained nursing staff.

**Pretransplantation host conditioning**

Pretransplantation host conditioning is aimed at eradicating residual disease of the patient and at inducing immunosuppression in order to allow for engraftment of stem cells. Initially, whole-body irradiation was used separately to condition for BMT; subsequently, cyclophosphamide was used in combination with irradiation. Currently, the combination is the most widely used conditioning technique. In autologous transplantation patients, the only objective of conditioning is eradicating residual diseases.

The choice of the better host conditioning regimen should be made according to the baseline disease. Table 3 presents the current, main regimens for conditioning of hosts.

**Infusion of hematopoietic stem cells**

At most services that carry out allogeneic BMT of related donors and syngenic BMT, bone marrow and peripheral blood stem cells are infused immediately after harvesting, using a central venous catheter. In case of ABO incompatibility between donor and receptor, procedures of removal of red blood cells from the bone marrow and, sometimes, of plasmapheresis on the receptor should be carried out before infusion. These procedures are aimed at avoiding severe reactions to transfusion.⁵¹

### Table 3 - Major regimens for conditioning of hosts

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Diseases commonly treated with this regimen</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBI (12 Gy) + Cy (120 mg/kg)</td>
<td>ALL, AML, CML, SAA</td>
<td>34</td>
</tr>
<tr>
<td>TBI (12 Gy) + VP-16 (60mg/kg)</td>
<td>ALL</td>
<td>35</td>
</tr>
<tr>
<td>Bu (16 mg/kg) + Cy (120 mg/kg)</td>
<td>CML, AML</td>
<td>32</td>
</tr>
<tr>
<td>Bu (4 mg/kg) + Cy (200 mg/kg)</td>
<td>SAA</td>
<td>47</td>
</tr>
<tr>
<td>Bu (16 mg/kg) + Mel (140 mg/kg)</td>
<td>AML, CML, solid tumors</td>
<td>48</td>
</tr>
<tr>
<td>Cy (200 mg/kg)</td>
<td>SAA</td>
<td>49</td>
</tr>
<tr>
<td>TBI (12 Gy) + Ara-C (36g/m²)</td>
<td>ALL</td>
<td>50</td>
</tr>
<tr>
<td>BEAM – BCNU (300mg/m²) + VP-16 (800mg/m²) + Ara-c (800mg/m²) + Mel (140 mg/m²)</td>
<td>Hodgkin’s and non-Hodgkin’s lymphomas</td>
<td>39</td>
</tr>
</tbody>
</table>

Bu: Busulfan - TBI: Total body irradiation – Cy: Cyclophosphamide VP-16: Etoposide – Mel: Melphalan
Ara-C: Cytosine arabinoside
In cases of autologous or UCB transplantation, cells are frozen using cryoprotectants such as dimethyl sulfoxide (DMSO). Prior to infusion, cells are thawed at bedside in double-boilers and then, administered to the patient. Hydrocortisone and prometazine are used in routine protocols and diphenhydramine prior to transplantation, since DMSO can cause anaphylactic reactions.

The use of peripheral blood hematopoietic stem cells in allogeneic transplantations presents a more rapid recovery of leukocytes and platelets and is more cost-effective than use of bone marrow. However, studies have reported that incidence of chronic graft versus host disease (GVHD) was significantly higher after blood stem cell transplantation. In this sense, apparently there are advantages in use of peripheral blood stem cells in patients with high-risk leukemia.

The most common complications during and immediately after infusion of cells are nausea, vomit, hematuria, and abdominal pain, which are all related to DMSO in the infusion.

Patients who received nonfrozen bone marrow are subject to developing transfusion reactions that are also common to other hemoderivatives.

Infused cells are counted according to CD34 cell-surface marking threshold. It is suggested that cell count be higher than 2.5 x 10^6 positive CD34 cells per kg of weight. ACD34 cell count is of little value for UCB transplantations; the best parameter in this case is total nucleated cell count, which should be higher than 2.0 x 10^7 cells per kg of weight.

Cells can be handled prior to infusion, in which case depletion of T-lymphocytes is a way to decrease incidence of GVHD, especially in the presence of some level of HLA incompatibility between donor and patient.

For elimination of the minimal residual disease in autologous bone marrow transplantations, it is possible to use in vitro chemotherapeutics or specific antibodies against the disease, a procedure named purging. A randomized study is being carried out by the Children’s Oncology Group in the United States for assessment of the impact of this procedure in survival of patients submitted to autologous BMT due to neuroblastoma.

**Post-BMT complications**

**Bone marrow aplasia**

The day (D) of bone marrow infusion is defined as day 0. The previous days, when host conditioning is carried out, are defined as negative days (-2, -1), and the following days as positive (+2, +3, ...).

After transplantation, the bone marrow of the host will remain in a state of aplasia for approximately two to three weeks. During this period, there is greater risk for infections, anemia, and bleedings.

Usually, the leukocyte counts fall below 100 cells/mm³ around D+4 depending on the type of conditioning and baseline disease. Engraftment is considered successful when the cell count remains higher than 500 cells/mm³ for three consecutive days; on average, this occurs between D+15 and D+19 after allogeneic BMT from related donor.

The platelet count also usually falls below 10,000 cells/mm³; it is considered that platelet count is recovered when counts of 20,000 cells/mm³ are achieved without need for transfusion for more than seven days. This usually occurs on D+19 to D+25.

Recovery of bone marrow function is influenced by other factors such as type of transplantation, number of cells infused, and infections.

Platelet transfusions are carried out when platelet counts fall below 10,000 cells/mm³ or when there is a sign of active bleeding. Hemoglobin count is also maintained higher than 9 to 10 g/dl with the help of transfusions. Red blood cell and platelet concentrates should be irradiated in order to deactivate lymphocytes, and be filtered in order to decrease leukocyte count. This allows for decrease in incidence of transfusion GVHD, cytomegalovirus (CMV) infection, and anaphylactic reactions.

Granulocyte colony-stimulating factors and macrophages can also be used during the post-BMT period to induce recovery of white cells, though its use is controversial.

**Graft versus host disease (GVHD)**

All patients who received hematopoietic stem cells from allogeneic bone marrow, peripheral blood, or umbilical cord blood are at risk for developing graft versus host disease (GVHD).

GVHD can also occur in cases of syngenic transplantation and, more rarely, in autologous transplantation.

GVHD is mediated by immunocompetent donor cells, especially the T-lymphocytes. Even in cases of total HLA compatibility, the presence of smaller, noncompatible antigens that cannot be detected by conventional screening methods can cause GVHD. The risk for developing GVHD in patients with related donors is of approximately 20%, whereas in patients with nonrelated donors, it is of up to 80%.

Other risk factors for GVHD are female donors with previous gestation, older donors, or inadequate immunoprophylaxis techniques.

Acute GVHD is manifest after engraftment and, by definition, it can occur up to D+100 following transplantation. The most commonly affected areas are the skin, liver, and gastrointestinal tract. Table 4 presents the grades of the most frequent manifestations of acute GVHD.

Prophylaxis is carried out with drugs such as cyclosporin, methotrexate, and tacrolimus. Normally, GVHD grade I is
not treated. Grades II and III are treated with methyl-prednisolone combination. Severe GVHD presents poorer prognosis and can be treated with mycophenolate-mofetil and anti-thymocyte globulin (ATG).58

Chronic GVHD is a clinical and pathological disease involving several organs and systems, and it is expressed as an autoimmune chronic disease.59 Chronic GVHD usually involves the skin, liver, eyes, and oral mucosa; it may also involve the gastrointestinal tract, lungs, and neuromuscular system. Chronic GVHD affects 13% of transplanted children before 10 years of age and 30% of children from 10 to 19 years of age. In transplantations with nonrelated donors, the incidence of the disease can be as high as 40%.

The characteristic of skin lesions in chronic GVHD resemble lichen planus with atrophy of the epidermis and focal skin fibrosis without inflammations. There can also be cases of generalized scleroderma, which, in severe cases, leads to restriction of movements. Kerato-conjunctivitis, photophobia, and dry mouth are other manifestations of the disease. Chronic GVHD is classified as limited and extensive (Table 5).60

Limited GVHD often does not require treatment, whereas extensive GVHD requires prolonged treatment (40 weeks) with corticoids, cyclosporin, and psoralen plus ultraviolet.58

Despite the fact that development of GVHD represents an important cause for morbidity and mortality in transplanted populations, an interesting phenomenon (graft-versus-leukemia) has been reported in transplanted leukemia patients with GVHD. It is suggested that there are T-cell-mediated antitumor effects in the host.61-64 This effect, which is already well-documented in CML and AML is called graft-versus-leukemia and decreases risk for relapse of the disease. Conversely, we also observed that patients who received syngenic BMT or bone marrow handled for T-lymphocyte depletion are at lower risk for developing GVHD but at higher risk for relapse.

Infections

Almost all transplanted patients present fever after conditioning and are highly susceptible to severe infections. Bacterial infections are the most common type and affect the lungs, facial sinuses, and catheter.65-67 Bacterium such as the *coagulase-negative Staphylococcus* and *Staphylococcus aureus* are frequently identified in patients. Gram-negative agents are also frequently reported, such as

| Table 4 | Clinical and laboratory grading of acute graft versus host disease (GVHD) |
|--------|-----------------------------|-----------------------------|-----------------------------|
| Grade  | Skin                        | Liver                       | Intestine                   |
| I      | Maculopapular exanthema     | Bilirubin between 2-3 mg/dl | Diarrhea 500-1,000 ml/day or 280–555 ml/m²/day |
|        | < 25% of body surface       |                             |                             |
| II     | Maculopapular exanthema     | Bilirubin between 3-6 mg/dl | Diarrhea 500-1,000 ml/day or 555-833 ml/m²/day |
|        | between 25-50% of body surface |                         |                             |
| III    | Generalized erythroderma    | Bilirubin between 3-6 mg/dl | Diarrhea > 1,500 ml/day or >833ml/m²/day |
| IV     | Exfoliation and blisters    | Bilirubin > 15 mg/dl        | Intense pain– Paralytic ileus |

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Clinical and laboratory grading of chronic GVHD</th>
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<tbody>
<tr>
<td>Limited chronic GVHD</td>
<td></td>
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<tr>
<td>One or both criteria</td>
<td></td>
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<tr>
<td>1 - Localized skin involvement</td>
<td></td>
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<tr>
<td>2 - Liver dysfunction due to chronic GVHD</td>
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</table>

| Extensive chronic GVHD                  |
| 1 - Generalized skin involvement or     |
| 2 - Localized involvement and/or liver dysfunction due to chronic GVHD |

| Plus |
| A) Hepatic histology showing chronic hepatitis with necrosis and cirrhosis or |
| B) Ocular involvement or               |
| C) Involvement of salivary glands or oral mucosa indicated through biopsy or |
| D) Involvement of other target organs  |
the *Enterobacter* and *Pseudomonas*. Treatment of infections is carried out with wide-spectrum antibiotics and the schemes can vary according to site of transplantation, the most frequent pathogens, and the sensitivity patterns.

Fungi are also included in the group of infectious agents affecting transplanted patients. The prophylactic administration of fluconazole presented a positive impact in these patients, decreasing the number of infections by *Candida albicans*. However, the prevalence of other species of *Candida* resistant to this prophylaxis has increased. Invasive aspergillosis affecting more than one site presents high mortality rates.

The prophylactic treatment of *Pneumocystis carinii* is carried out with three weekly doses of sulfamethoxazole and trimethoprim; this infectious agent is no longer considered an important cause of complications in transplanted patients. Pentamidine can be used in patients with intolerance to sulfamethoxazole and trimethoprim. CMV infections are common in patients submitted to allogeneic transplantations; the higher incidence of CMV infections occurs between D+28 and D+100 and it is secondary to reactivation in latent virus, first infection, or reinfection. This infection, however, is rare in patients submitted to autologous transplantations. The most important manifestation of CMV is interstitial pneumonia, which can be fatal in up to 75% of cases. CMV activity is identified with polymerase chain reaction (PCR) tests or early positive results of virus antigenemia, allowing for preemptive therapy; in other words, before clinical manifestation of the disease. Ganciclovir is administered for approximately 14 days or, in cases of resistance or intolerance, foscarnet therapy. Filtering leukocytes in transfusion of red blood cell and platelet concentrates with the objective of lowering leukocyte count, decreases the risk for CMV infection or reinfection from contaminated donors.

Respiratory syncytial virus can cause severe and often fatal interstitial pneumonitis. Other virus such as the parainfluenza and influenza can also cause pulmonary complications in transplanted patients considering that the antiviral treatments in this case are poorly efficient. Surveillance of viral infections is important in order to avoid dissemination of hospital infections.

The adenovirus is associated with diarrhea and late hemorrhagic cystitis in transplanted patients. Reactivation of herpes simplex and herpes zoster virus can be prevented with prophylactic administration of acyclovir. The risk for infections decreases 100 days after infusions of bone marrow in autologous and syngenic BMT patients and in allogeneic BMT patients who did not develop chronic GVHD. Patients being treated for chronic GVHD are at greater risk for developing infections by encapsulated bacterial agents.

Figure 1 presents the main infectious complications related to bone marrow transplantation.

**Gastrointestinal and hepatic complications**

Vomiting is a frequent complication during and after host-conditioning chemotherapy. These situations are handled with antiemetics such as ondansetron or granisetron. Late vomiting, in turn, can be related to GVHD or CMV infections.

Mucositis affects practically all transplanted patients and involves the gastrointestinal tract with manifestations ranging from hyperemia of the mucosa to ulceration. Mucositis is treated with topical analgesics, oral hygiene, and, in the presence of superinfections, with antiviral and antifungal medication. Presence of diarrhea is frequent in the post-BMT period; this complication should be further studied since it could be related to multiple factors including cell desquamation due to mucositis, acute GVHD, and infections by bacterial or viral enteropathogens.

**Figure 1** - Major complications occurring after bone marrow transplantation.
All BMT patients require nutritional support, which can be provided by means of enteral feeding; parenteral nutrition can be administered to patients who do not tolerate enteral feeding.76,77

The most feared hepatic complication of bone marrow transplantation is veno-occlusive disease of the liver (VOD). This disease is a specific clinical entity with anatomopathological correlation and diagnosed only in the absence of other causes of hepatic disease.78,79 VOD is associated to central lobular congestion of hepatic venulae. The exact physiopathology of this disease is still not well-understood.

Clinical manifestation of VOD occurs in the first 30 days post-BMT with weight gain, ascites, hepatomegaly, jaundice, and right upper quadrant pain.80 Risk factors have been identified for VOD, including patients aged older than 15 years at BMT, as well as elevated liver enzymes during the preconditioning period.78

Continuous infusion of low-dose heparin and ursodeoxycholic acid are used in prophylaxis routines at certain centers; in this sense, prospective randomized studies attest the effectiveness of the earlier.81

Treatment of VOD is basically aimed at patient support and severe forms of VOD are often fatal; however, positive results with the use of certain drugs have been reported in the literature.82

Mild and severe forms of acute VOD of the liver present poor prognosis despite the treatment, which is usually intensive and prolonged, thus increasing the risk for infectious complications.

Table 6 presents causes of hepatic complications.78

**Cardiopulmonary complications**

The lung is another organ that is frequently affected by complications of BMT. It is subject to bacterial, fungal, and viral pneumonia; bronchiolitis, and GVHD, as we described above.

Pulmonary fibrosis can be a case of late morbidity after bone marrow transplantation due to the effects of radiotherapy or busulfan.83

Host conditioning doses of cyclophosphamide are cardiotoxic themselves. However, most patients who develop cardiomyopathies have a history of use of anthracycline prior to BMT; this chemotherapeutic drug has a well-known cardiotoxic potential.

Chest radiotherapy can also induce or exacerbate cardiotoxic effects.84

**Genitourinary complications**

Drugs such as the cyclophosphamide have metabolites that can cause hemorrhagic cystitis. Adequate hydration of patients and use of drugs such as mesna can attenuate this type of toxicity.75

BMT patients can be affected by acute renal insufficiency associated to radiotherapy and to drugs used during BMT, such as cyclosporin, conditioning therapy, and antibiotics. This insufficiency can be reverted or cause severe damage to renal function, with reported cases of chronic renal insufficiency in some patients.85
In general, the prognosis for glomerular function of children submitted to BMT is good. However, a study carried out to assess glomerular function in children and adolescents observed that tubular dysfunction was found in 40% of patients one and two years after hematological stem cell therapy. Further studies with long-term follow-up are warranted for assessment of the clinical impact of this dysfunction.

**Delayed effects**

The delayed effects of BMT are frequently related to a combination of factors such as baseline disease, type of conditioning, type of transplantation, and acute complications. These effects include:

- Transplantation process-related effects;
  - Chronic GVHD;
  - Immunodeficiency;
  - Lymphoproliferative disease;
  - Graft rejection.
- Host-conditioning regimen-related effects;
  - Pulmonary dysfunction (secondary to radiotherapy, chemotherapy, and chronic GVHD);
  - Eye disorders (cataract following radiotherapy);
  - Neuroendocrine dysfunction;
  - Neuropsychological disorders;
  - Secondary neoplasias.

**Immune dysfunction**

After transplantation, T- and B-lymphocyte levels and immunoglobulines fall below normal. It is interesting to note that part of the immunity of the donor can be transferred to the receptor, this is called adoptive immunotherapy. In this sense, anti-hepatitis B surface antigen (anti-HBsAg) negative patients can turn positive when receiving bone marrow from actively immunized donors.

Immunologic recovery of patients is progressive, but can be delayed by chronic GVHD. It is recommended to administer vaccines to receptors one year after the transplantation considering that adoptive immunity usually does not last long.

**Growth and development**

Host conditioning procedures that include whole-body radiotherapy are associated to endocrine dysfunctions such as hyperparathyroidism and growth hormone deficiency. Children receiving conditioning regimens without irradiation presented normal growth.

Gonadal dysfunction is frequent in transplanted patients, thus indicating the need for hormonal replacement and early menopause in girls. Sterility is also common in these patients and can be related to the chemotherapy regimen used prior to BMT. There are patients who can become pregnant after the transplantation, though with a higher incidence of abortions.

There were no anomalies in full term newborn infants born to BMT mothers.

**Graft rejection**

Acute graft rejection as in the case of solid organ transplants is rare in BMT. Usually, the original hematopoiesis of the host is reestablished and the donor cells disappear progressively.

Rejections are more frequent in SAA, but incidence of rejections has decreased following the use of more intense conditioning regimens with busulfan and cyclophosphamide.

**Posttransplantation relapse**

Posttransplantation relapse offers an extremely poor prognosis and is an indicator of resistant diseases.

Patients submitted to autologous BMT normally are not indicated for a second transplantation and are treated with alternative protocols.

One of the alternatives for leukemia patients is cessation of immunosuppressants, in cases of early relapse, in an attempt to induce the GVL effect. Another treatment is infusion of donor lymphocytes in order to stimulate the GVL effect that can induce remission of the disease. This alternative has been used successfully in CML and AML, but with little success in ALL.

A second transplantation can be considered if the relapse occurs six or more months after the first transplantation, and also if the patient was submitted to conditioning with no radiotherapy in the first transplantation.

**Secondary neoplasias**

Patients submitted to BMT present an increased risk for other neoplasias due to the carcinogenic effect of chemotherapy and radiotherapy. There is also the risk for lymphoproliferative diseases related to the Epstein Bar virus.

The probability for onset of secondary neoplasias is approximately 6% for a 15-year period after BMT.

**BMT of nonrelated donors**

Less than 30% of candidates to BMT have a compatible related donor. In order to overcome this problem, there are bone marrow banks with computerized files of HLA-typing of potential donors that can be accessed in the search for transplants.

In case a compatible donor is found, further tests are carried out for confirmation. Next, the hematopoietic stem cells are harvested for the transplantation. Though bone marrow banks do not store bone marrow itself, they are costly to maintain due to the large number of typing
procedures required. There is usually a prolonged waiting period from identification of a compatible donor to harvesting of cells, which can be as long as a few months. During this period, several patients present progression of the disease.

In the United States, the National Marrow Donor Program has over three million registered donors and approximately 70% of patients who refer to this service find a compatible donor. In Brazil, the search for compatible donors is carried out using the REDOME system of bone marrow donor registration; this system searches for compatible donors in and outside Brazil.

The indication for BMT of nonrelated donors should be carefully considered, since the hosts will be at greater risk for complications such as infection, rejection, and GVHD resulting in higher cost of BMT and prolonged hospitalization. BMT of related donors and partial HLA compatibility present risks similar to BMT of nonrelated donors.

The prognosis for children in BMT of nonrelated donors or of partially-compatible related donors is better than that for adults.

### Umbilical cord blood

After the first UCB transplantation, this source of stem cells was further studied with related and, subsequently, nonrelated donors.

UCB presents interesting properties, including lower probability of inducing acute and chronic GVHD, even in cases of partial HLA compatibility; GVL; lower rates of viral infection by EBV and CMV.

In this sense, after 1992, the Placental Blood Program created by Dr. P. Rubenstein of the New York Blood Center gave way to systematic cryopreservation of UCB for transplantations. One of the advantages of this program is that UCB units can be sent worldwide in a relatively rapid manner.

The steps below are for collection, processing, cryopreservation, and use of UCB:

1. Right after birth, the umbilical cord is clamped by the obstetrician and handed over to the head nurse responsible for the collection. The placenta is placed into a sterilized support set higher than the water and, subsequently, the blood will flow with gravity;
2. the water is subsequently sent to the blood bank for processing. Next, the mother is interviewed and blood samples are collected for maternal serum exams;
3. at the blood bank, a blood sample is collected from the water for HLA typing, serum and bacteriological exams, and cell count procedures; next, the water is processed and cryopreserved in liquid nitrogen;
4. after all results are in, the water is available for transplantation; in case of problems indicated by the exams, the blood may be discarded;
5. the database of the UCB bank receives all the information related to the collected water. Currently, transplantations are carried out when the count of HLA-compatible antigens is less than or equal to two, and the cell count of the water is greater than 2 x 10^7 cells per kg of weight of the receptor;
6. if the patient finds an adequate match of UCB, the respective unit is sent to the service that will carry out the transplant in a special liquid nitrogen container.

Over 40,000 units of UCB are currently cryopreserved worldwide and more than 1,000 transplants have been carried out using this source of stem cell. This number has been increasingly progressively over the years.

The complications of UCB transplants are related to limited cell count in the unit, which leads to a delay in engraftment and increasing transplantation risk with more need for antibiotics and hemotherapeutic support. Patients with increased body mass can also encounter difficulties in finding UCB units. Another disadvantage of UCB is that in cases of relapse it does not allow for infusion of donor cells for second remission.

Until this date, the techniques for expansion of cells ex-vivo are not completely satisfactory.

The storage of UCB for personal or family use is a matter of great ethical discussion; in general, these units are only collected in cases of specific application, for example, a sibling who is a candidate for BMT. Only one case of autologous UCB transplantation has been reported until today. The main UCB banks do not store units for private use.

It is not recommended that mothers of patients who are candidates to BMT to get pregnant in an attempt to give birth to a potential UCB donor. There are several risks such as HLA-incompatibility, prolonged waiting period, low cell count, and, moreover, only a few centers have the capability of collecting and cryopreserving UCB safely.

### Survival and quality of life after BMT

The Pediatric Oncology Service at the Hospital de Clínicas de Porto Alegre carried out 16 autologous transplants from August of 1997 to January of 2001 in the treatment of different neoplasias. The median for duration of follow-up was 13 months with an 81% survival rate (13/16). The service carried out 12 allogeneic BMT of related, fully-compatible donors for a survival rate of 75% (9/12) and a median of 20 months. We also carried out three UCB transplantations using New York Blood Center units. The first UCB transplantation patient, a 7-month old male infant, presented severe combined immunodeficiency.
Engraftment was successful and documented, patient was discharged from the hospital but died 71 days after the procedure with interstitial pneumonia. The second patient was indicated for transplantation for second remission of AML. The patient, who is a boy, is currently four years old and still in remission two years after BMT and without medication. The third patient, a seven-year old boy diagnosed for Chediak-Higashi syndrome, died 21 days after infusion of stem cells and before engraftment.

The post-BMT survival depends on several factors such as the baseline disease, previous treatment, duration of evolution, age range, and remission. Carriers of neoplasias who receive transplantation in remission have a better prognosis. The survival rate for patients with SAA is higher in cases of early transplantation, before receiving an elevated number of transfusion of hemoderivatives. A recent publication has shown that CML patients in chronic phase and transplanted up to three months after diagnosis, presented a five-year leukemia-free survival of 91%, whereas patients in blastic phase had their survival estimated at 22%. This rate is even lower than 10% in cases of BMT in patients with relapse of AML.

The posttransplantation quality of life is related chiefly to chronic complications and family structure of the patient; quality of life tends to improve with time. Patients in remission two years after the transplantation have an 89% chance of survival for the following five years. The mortality in this population is related to factors such as second neoplasias; impact of treatment in organs such as the heart, lungs, and kidneys; relapse; and chronic GVHD. Six years after the transplant, patients transplanted due to SAA have the same mortality rate of the general population.

With the assessment of the information above, it is possible to conclude that BMT does not allow all patients to return to an absolutely normal life, but it represents a considerable progress in the treatment of diseases with limited therapeutic alternatives.

**Future perspectives**

There are several lines of research being followed for making BMT a safer and more widely applicable procedure. The progress in understanding of the immune mechanisms involved has allowed for development of new, lower-toxicity conditioning regimens, the recovery of patients with relapse of diseases after BMT, and better understanding and use of the graft versus tumor effect. This effect, which is similar to that of GVL, has been recently assessed in a study with adult patients in which the authors concluded that nonmyeloablatve allogeneic stem-cell transplantation can induce sustained regression of metastatic renal-cell carcinoma in patients who have had no response to conventional immunotherapy, thus clearly indicating an immune reaction to the tumor.

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