Abstract

Introduction

Umbilical cord blood is an important source of hematopoietic stem cells for transplantation (HSCT) as alternative to bone marrow. In this report we discuss the role of Cord Blood Transplant in pediatric hematology-oncology, pointing out three cases with different hematological and oncologic diseases.

Materials and Methods

We report about three cases underwent a HSCT at the Pediatric HSCT Units in Catania, Azienda Policlinico – OVE. We focused our attention to three children treated with an alloengenic HSCT using umbilical cord blood, as stem cell source.

Results

We collected data from a child with acute lymphoblastic leukemia (ALL), in second remission; one child with homozygous sickle cell disease; one case with severe aplastic anemia (SAA). All of these children were addressed to alloengenic umbilical cord blood transplantation. Two of them presented a complete remission of the original disease. The child with SAA recently experienced a bone marrow failure at day +100.

Conclusion

Although it is a limited source of stem cells, umbilical cord blood transplantation is more than a reasonable alternative. It would be preferred in terms of slow but robust engraftment, lower risk of graft vs host disease (GVHD) and of infections. Moreover, in Sicily, the Cord Blood Bank in Sciacca needs to be supported by a tight collaboration with gynecologic clinicians and onco-hematological units.

Introduction

Today Cord blood is seen as a valuable source of hematopoietic stem cells for transplantation as an alternative to stem cells from bone marrow[1]. The transplantation of stem cells from cord blood can be from sibling or from an unrelated donor.

In comparison to bone marrow transplantation, the use of placental blood has some important advantages such as: immediate availability of units; large availability for a higher probability to find donors even among ethnic minorities; absence of risks for donors; risk of transmission of some infectious agents such as cytomegalovirus and Epstein-Barr virus, lower risk of Graft Versus Host Disease or GVHD, therefore less toxicity, and also a lower degree of compatibility needed for the engraftment of the transplant [1-7].

The first transplantation of hematopoietic stem cells obtained from cord blood was performed in France in 1988 on a patient with Fanconi’s Anemia[8]. To date more than 700 in Italy [8].

This therapeutic method is largely used among pediatric patients, representing a viable alternative to bone marrow or peripheral blood transplantation, especially for patients who do not have a suitable donor and cannot wait for the overlong periods associated with the search of a compatible donor [9-10].

Unfortunately, the advantages coexist with factors that discourage the use, for example the limited content of hematopoietic stem cells present in placental blood, for this reason the majority of transplants are carried out in pediatric patients.

To guarantee good conservation and quicker availability banks of cord have been set up, and all the data is managed by the International Registry of Bone Marrow Donors.

The cord blood unit, after the collection in the delivery room, is sent to the bank, where it undergoes a series of specific checks to verify its eligibility in regards to its storage and to define the immunological features in order to analyze the compatibility between donor and recipient. In these structures placental blood is available for the community or in some cases for the same infant or for one of his family member. In this study, done in the Department of Pediatric Oncology and Hematology of Catania, we took into consideration three cases where placental blood was used for transplantation in pediatric patients from 2000 to 2011 and the outcome of transplants. Moreover, this study is to encourage the donation of umbilical cord, particularly in Sicily, due to the presence of a cord blood bank in the town of Sciacca. The fact that our structure belongs to the same region facilitates both donation and transplantation.

Material and Methods

Storage of Cord Blood

The cord blood used for the four transplants was kept at the Umbilical Cord Bank of Sciacca. When the sample arrives at the bank the storage of placental blood provides a phase of control and a phase of processing. If the sample results suitable for future therapeutic purposes, it is filed with a barcode of 12 digits. A fraction of the stored blood is analyzed for the virological control, for HLA typing, for CD34 cell count and Total number of Nucleated Cells, their vitality and the blood-type. The study of the antigen CD34 is performed using a flow cytometry protocol lyse no-wash and the cell vitality with the study of the Actin[11].

After collecting this information essential for the cataloging we can proceed to the storage of the stem cell. The cryoprotective solution is prepared from DMSO and dextran 40 in 10% saline solution or in human albumin solution [12]. An equal volume of a solution of 20% DMSO is added in a controlled manner. A small fraction of the cells process is sent to the bacteriological control, another small fraction will be left outside of the bag for the confirmation of the HLA typing, the remaining amount will be frozen in a bag said “cryocyte” maintained at less than -150°C and continuously monitored [3]. The unit is then inserted into the world database and the transplant center that needs it can require it at any time.

The Transplant

The cryopreserved cord blood units have been transported from the Cord Bank Of Sciacca to our Center where the unit was thawed. At the moment of the transplantation the unit can be infused directly into the patient or it can be diluted or washed to remove the small amount of cryoprotectant (DMSO) used for the freezing.

A nucleated cell count, ABO and Rh typing, a test of cell viability, bacterial and fungal cultures, an assay for hematopoietic progenitor cells, and a CD34+ cell count were performed on a sample from each thawed unit at the time of infusion [13].

In order to complete the study, data from three patients hospitalized and transplanted at the Center of Pediatric Oncology and Hematology were collected from 2000 to 2011. We have developed a database in which we have included: sex, diagnosis, date at diagnosis, patient age at diagnosis, therapy pre-HSCT, disease status pre-HSCT, sex of donor, conditioning regimen, transplant date, quantity of cord blood cells infused, post-transplant complications, date of the engraftment, Outcome of the transplantation (table 1); and a table summarizing the patient’s and the donor’s virological status and before transplantation (table 2).

Among all the patients that we could recruit for this study we have chosen pediatric patients who underwent alloengenic HLA-compatible transplant from a family member donor. The three children who underwent HSCT from cord blood were hospitalized for the following diagnosis: Acute Lymphoblastic Leukemia, Sickle Cell Disease and Severe Aplastic Anemia.

For the child with Acute Lymphoblastic Leukemia diagnosis HSC were collected from the brother’s HLA-compatible cord blood, for the child with diagnosis of sickle cell disease HSC were collected from twin HLA-compatible cord blood (brother and sister) and for the child with SAA diagnosis HSC were collected from the sister’s HLA-compatible cord blood.

Table 1. Clinical Characteristics of the patients.

<table>
<thead>
<tr>
<th>Clinical Parameters</th>
<th>R.V.</th>
<th>S.M.</th>
<th>I.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>6</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Acute Lymphoblastic Leukemia</td>
<td>Sickle Cell Disease</td>
<td>Severe Aplastic Anemia</td>
</tr>
<tr>
<td>Date of transplant</td>
<td>09/11/2001</td>
<td>02/2011</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Virological status of the patient and donor before HSCT.

<table>
<thead>
<tr>
<th>Virological Parameters</th>
<th>R.V.</th>
<th>S.M.</th>
<th>I.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>HBV</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CMV</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Results
CBT at the Regional Reference Center of Pediatric Hematology and Oncology of the Hospital Polyclinico of Catania
At the Regional Reference Center of Pediatric Hematology and Oncology of the Hospital Polyclinico of Catania three transplant from family allologenic cord have been performed from 2000 to 2010.
Of the three patients subjected to familiar allologenic HLA matched cord transplantation, R. V. whose diagnosis of the AAL diagnosed 27 April 1999, underwent the process of infusion of hematopoietic stem cells taken from the cord of her HLA-matched brother. Before the transplantation the little girl did a first treatment AEOF 95 and a second conditioning program. The patient went to second complete remission. The 4th of August 2003 after deciding for a transplant the patient starts a conditioning regimen consisting of high dose chemotherapy with Thiopeta and cyclophosphamide associated with total irradiation. At the moment of the transplant the patient’s body weight was 3 x 10^9 kg Nucleated Cells from the donor’s bone marrow and 7.3 x 10^9 kg of CD34 + cells taken from the donor’s cord blood. Fifteen days after the transplant engraftment of both polymorphonuclear cells and platelet has occurred. After more than thirty days from the transplant the young patient presented a skin and intestinal chronic Graft versus Host Disease (GVHD). The last lasted for three month. In this period of time, the patient is in complete remission with walking difficulties from antineoplastic radiotherapy and chemotherapy.
S.M., the patient with a diagnosis of sickle cell disease dated 3 November 2003, underwent the process of infusion of hematopoietic stem cells taken from twins umbilical cord HLA-matched. From 2003 the child was treated with a splenectomy. In 2009 since the disease persisted a conditioning regimen of Bisulfan, Thiopeta and Fludarabine was started. At the moment of the transplantation 3 x 10^7 kg Nucleated cells from the donor’s cord blood were infused. Immediately the little patient had post-CBT toxicity to oral and gastrointestinal mucosa, which was overcome in ten days. Nine days after the transplantation polymorphonuclear cells engraftment occurred and after twenty-three days of the transplantation the platelet engraftment was detected. To date the data’s drawn from cord blood brought the possibility of treatment for those patients in a terminal phase of the disease but in reality with different kind of leukemia including children with Leukemia acute of the myeloid line with rare megakaryocytes and modest representation of the erythroid line. In relation to this framework it is suspected that the patient has established an ITP (idiopathic Thrombocytopenic Purpura) post-CBT. Therefore there is a strong belief that it could be appropriate for this patient to evaluate the possibility of a new stem cells transplant from his sister’s bone marrow. None of the three patients had infections like CMV (Cytomegalovirus) or EBV (Epstein-Barr Virus) post-transplantation (Table 3).
Discussion
The aim of this study was to evaluate the role of the Cord Blood Stem Cell Transplantation in the treatment of the child with hematopoietic disease, considering the different Regional Referral Centers for Pediatric Hematology and Oncology of the Hospital Polyclinico of Catania. As it has already been seen in other studies the cord blood stem transplantation offers advantages in proliferative terms, including the ability to form a larger number of colonies in cell culture, a more rapid cell cycle, homogeneous growth and longer telomeres [14]. All these properties would favor the engraftment and the growth of hematopoietic stem cells of the cord blood. In addition to this the interval between the time of transplantation and the time of complete remission is shorter and in some cases transplantation is performed only one month after the transplant.
Earlier studies have shown that CBT is an efficient therapeutic method in many onco-hematological pathologies and not only. Among the most treated: Acute, Chronic and Bone Marrow Transplantation. Studies of various cell lines and bone marrow in children with acute leukemia: a comparison study. Lancet, 369; 321:1174, 1989.