Consolidation treatment for high risk solid tumors in children with myeloablative chemotherapy and autologous hematopoietic progenitor stem cell transplantation

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Background: In childhood cancer, consolidation treatment with chemotherapy followed by autologous hematopoietic progenitor stem cell transplantation is currently an accepted treatment modality in patients with high-risk solid tumors or in patients who have relapsed after conventional treatment.

Objectives: The objective of this study was to describe the results of transplantation of a group of children who had high-risk solid tumors or relapsed after conventional chemotherapy regimens.

Methods: A retrospective analysis was conducted from January 1998 to October 2004 of all children with pathologic diagnoses of high-risk solid tumors or children that had previously relapsed after conventional chemotherapy and that were subsequently submitted to autologous hematopoietic progenitor stem cell transplantation. The analysis included overall survival rates, event-free survival rates, mortality rates and chemotherapy complications.

Results: Nineteen patients were submitted to this approach. The age range was from 27 to 196 months with a median age of 52 months. The overall survival rate at 100 days was observed in 79%, the three-year event-free survival rate was 63%. The mortality rate secondary to the myeloablative chemotherapy regimen was 21% (n = 4). Only three patients (15.8%) relapsed with tumor progression after transplant.

Conclusion: Autologous hematopoietic progenitor stem cell transplantation is still a successful procedure in patients with solid tumors refractory to conventional chemotherapy.

Keywords: Hematopoietic stem cell transplantation; Neoplasms; Child

Introduction

Recently, disease-free survival (DFS) for children with solid tumors has improved considerably; more than 70% of children achieve 5-year tumor-free survival. However, there is a group of patients who have some clinical and biological characteristics with poor prognostic factors at diagnosis.

Unfavorable factors are associated with a high failure rate to traditional treatment regimens including chemotherapy, surgery and radiotherapy. Therefore, children with solid tumors and adverse risk factors require new therapeutic strategies[1].

Preclinical studies and clinical correlations of the effect of alkylating agents have demonstrated that there is a linear-logarithmic relationship between the chemotherapy dose and cytotoxicity. Therefore, increases in chemotherapy of 3 to 10 times denote multiple-logarithmic increases in the destruction of malignant cells. However, the main limit of this principle is the extended myelotoxicity which puts the patient's life at risk[2-6].

The reconstitution of the hematopoietic system after myeloablative doses of chemotherapy and/or total body irradiation (TBI) can induce severe or even fatal complications if the patient is not rescued with autologous CD34 hematopoietic progenitor cells obtained either from bone marrow or peripheral blood. This approach has been one of the most highly successful strategies to limit secondary myelotoxicity[3,7].

Many investigators have demonstrated the importance of using myeloablative doses with different chemotherapeutic regimens including hematologic support with hematopoietic stem cell transplantation. This approach, widely used as consolidation therapy in high-risk solid tumor pediatric patients, has proved to be an excellent complement to conventional multidisciplinary approaches including surgery and chemotherapy[4,8]. However, there are many questions regarding the best combination of myeloablative chemotherapy for these types of tumors, how to eliminate TBI from myeloablative regimens without any significant change in response rates, as well as which of these strategies is the best to avoid further relapse in this group of children.

The aim of the current study was to evaluate the efficacy and safety of a myeloablative chemotherapy program without TBI using melphalan, cyclophosphamide and etoposide followed by consolidation-rescue with autologous hematopoietic progenitor stem cell transplantation (AHPSCT) as treatment for high-risk solid tumors in children. All these drugs, with demonstrated efficacy, are available in Mexico[6].

Methods

A phase II clinical protocol was carried out according to the best clinical practice guidelines. The protocol was approved by the Ethics Committee of the institution, guaranteed by the *Comisión Federal para la Protección contra Riesgos Sanitarios* (COFEPRIS). Inclusion criteria were an age of under 18 years old with high-risk solid tumors and advanced tumor stage from January 1998 to October 2004 and followed for up to 121 months after transplantation. All patients were registered in our institution. Parents of patients signed an informed consent form in accordance with the Helsinki Declaration as revised in 2008. Within these criteria, this population needed to have a disease-free life survival expectancy of less than 25% with conventional therapy at the time of diagnosis or advance disease after the first relapse.

All patients went through conventional treatment protocols, including surgery, radiotherapy and chemotherapy, prior to being considered eligible for the AHPSCT procedure. Patients who demonstrated a tumor response greater than 90% in diameter determined by image studies before entering the transplantation program were included. Children with partial response (< 90%) or relapse were excluded. All patients included in the study were fully evaluated before entering the AHPSCT program. Those with any evidence of organic dysfunction were excluded from this program.

Cryopreservation of progenitor cells

The hematopoietic progenitor cells were obtained from bone marrow in the operating room under general anesthesia through multiple punctures of both posterior-superior iliac crests or peripheral blood through apheresis[9] using a Baxter cell separator model CS300 through a double lumen central catheter. Mobilization of progenitor cells was obtained using colony stimulating granulocyte factor at 10 mcg/kg/day for 5 days, prior to the collection[5]. Bone marrow or peripheral blood stem cells were preserved by cryopreservation as published elsewhere[10] until they were re-infused intravenously. In both cases the collection of the progenitors was carried out to obtain from 3.5 to 4.5 x 10^8 mononuclear cells per kilogram of patient's weight. Through flow cytology the cell dose was established to guarantee a graft in a range of 3-5 x 10^6 CD34+ cells/kg[11,12].

Cryopreservation was carried out through a programmable cryopreservation unit that allowed a gradual temperature decrease of -1ºC/min over 90 minutes; 10% dimethyl sulfoxide (DMSO) was used as a cryoprotective agent. Thus, the progenitor cells were stored in liquid nitrogen at -190ºC[10] until the day of the infusion. An *in vitro* purge using methylprednisolone and etoposide was carried out for patients who presented bone marrow tumor infiltration using the doses and methodology published elsewhere[13]. Subsequent 0.4% trypan blue exclusion test was performed to assess cell viability.

Myeloablation regimen

After the hematopoietic progenitor cells were obtained, children were initiated with a hydration program at 3000 mL/m^2/day from Day -10 to Day 0. The myeloablation program consisted of oral melphalan 200mg/m^2 split over four days commencing from Day -9 to Day -6, intravenous (IV) cyclophosphamide at 120 mg/kg split over two days (Day -5 and Day -4) and IV etoposide at 1200 mg/m^2 was administered as a 24-hour infusion on Day -3. On Days -2 and -1 the patients received only intravenous hydration.

Infusion of hematopoietic progenitors

The infusion was carried out on Day 0. The bags of thawed units were transported directly to the transplant unit at a temperature of 4°C. They were infused in a closed system, through a central line over an average time of ten minutes. All the patients were treated with a single IV dose of furosemide (1 mg/kg), a single IV dose of ondansetron (5 mg/m^2) and a single IV dose of methylprednisolone (2 mg/m^2) prior to the infusions.

Prophylactic therapy and hematopoietic support

All children had intravenous prophylactic treatment with fluconazole (10 mg/kg/day/dose) and acyclovir (10mg/kg/day/dose). Immunoglobulin was not routinely used in these patients because the cost is very high; it was preferred to monitor immunoglobulin levels with administration only to those with low levels during or after transplant. Granulocyte colony stimulating factor was administered intravenously at 10 mcg/kg every 24 hours from Day +3. These medications were administered when the neutrophil count was under 0.5 x 10^9 cells/L and maintained until the count was above 1.0 x 10^9 cells/L. At that time, children had their treatment changed to oral medications, which included fluconazole (10 mg/kg/day) and prophylaxis with trimethoprim-sulfamethoxazole (10 mg/kg/day) three times per week for 60 days.

Children were transfused packed red blood cells at 10-20 mL/kg when hemoglobin levels were below 8.0 g/dL; if the platelet count was below 20 x 10^3/μL, apheresis platelet transfusion was administered. All blood transfusion products were previously irradiated.

Response assessment

A transplant graft was considered successful when the neutrophil count increased by more than 0.5 x 10^9 cells/L and the reticulocyte count by more than 2% within the first 30 days after transplant.

Data was gathered on forms especially designed for the study. The STATA package version 11 was used for statistical analysis. Data on the overall survival (OS) and event-free survival (EFS) were analyzed as was the toxicity with central trends, spread, frequencies and means. An actuarial Kaplan Meier method was used to determinate the OS and EFS considering the period between the AHPSCT and the time of death, relapse or the last visit to the center.

Similarly, the parameters of efficiency were defined as EFS evaluated at 118 to 121 months. Safety parameters were defined as an OS at Day 100 post-transplant adjusted according to the hematologic and non-hematologic toxicity criteria established by the World Health Organization (WHO).

Results

A total of 19 consecutive patients (ten male - 52.6% and nine female - 47.4%) were enrolled in this study. The ages ranged from 27 to 192 months with a median age of 52 months (Table 1). The commonest diagnosis was neuroblastoma in nine cases (47.4%) of which 89% were stage IV and 11% stage III according to the International Neuroblastoma Staging System; three patients (33.3%) presented infiltration of the bone marrow at the time of diagnosis.

The Ewing sarcoma family of tumors (ES) was the second most common diagnosis in 26.3% of the cases (n = 5); all had metastatic disease in the bone at the time of diagnosis. Similarly, two patients (40%) presented infiltration of the bone marrow and one patient (20%) had metastasis to the liver. Two patients had Wilms tumors (10.5%) after the first relapse. Three patients were found to have germinal tumors-endodermal sinus tumor (15.7%) that, similar to the other patients with Wilms tumors, achieved a second complete remission.

The bone marrow was used as the source of hematopoietic progenitor cells in six patients (31.6%) and peripheral blood in 13 (68.4%). On average, two aphaereses were required to obtain an adequate dose of mononuclear cells.

The median dose of mononuclear cells was 4×10^8/kg of the recipients weight with an interquartile range (IQR) of 0.6 (Range: $3.5\text{-}12.3 \times 10^8$/kg). The median quantity of CD 34^+ cells was 3.16 $\times 10^6$ (IQR 1.5) (Range: $1.2\text{-}6.4 \times 10^6$/kg). *In vitro* purges with methylprednisolone and etoposide were used in only three cases. Viability using the trypan blue exclusion test was higher than 90%. The median time to neutrophil engraftment was 22 days (Range: 19-

26 days) for patients transplanted with bone marrow while for those transplanted with peripheral blood, the median time to neutrophil engraftment was 14 days (Range: 10-19 days). There was a statistically significant difference between the groups (p-value = 0.002). Engraftment was not attained in only one case (5.3%).

The median time to platelet engraftment was 45 days (Range: 32-68 days) for patients transplanted with bone marrow while for those transplanted with peripheral blood the median time was 36 days (Range: 29-58 days). The median time of hospital stay within the transplant unit was 30 days (Range: 22-42 days).

The OS at 100 days was 79% and EFS was 63% in a 3-year follow-up period (Figure 1). Currently 11 patients are alive and without evidence of tumor activity. Eight patients died; for five patients (26%) death was associated to the myeloablative chemotherapy regimen with the secondary cause being septic shock. Only three patients (15%) relapsed with tumor progression after transplant.

The most common toxicity was neutropenia with all 19 patients (100%) presenting grade IV toxicity (WHO criteria). On average toxicity was present on Day +3 (Range: Day -3 to Day +5). As a whole, patients required an average of 5.8 transfusions of packed red blood cells (Range: 2-19). Platelets by aphaeresis transfusions were administered with a median of 11.6. Granulocyte stimulating factor was given for an average of 19 days (Range: 11-32 days; median: 24).

The second cause of toxicity was gastrointestinal with 12 patients presenting with mucositis (63%): two patients (16.7%) level IV, four children (33.3%) level III and six patients (50%) level II. Neutropenic colitis was presented by seven patients (36.8%) all of whom required parenteral nutrition.

Table 1 - Pediatric patients submitted to autologous hematopoietic progenitor stem cell transplantation (AHPSCT)

Patient number	Diagnosis	Gender	Stage	Age (months)	Status of remission prior to AHPSCT	Source of Stem cells	Current status	Last visit	Cause of dead
1	Neuroblastoma	Male	IV	48	2nd	BM	Alive	3/2008	
2	Ewing sarcoma	Female	Metastasis to Bone, bone marrow	192	1st	BM	Dead	10/1998	Sepsis
3	Neuroblastoma	Female	IV	24	1st	PB	Alive	6/2005	
4	Ewing sarcoma	Male	Metastasis to Bone, bone marrow	108	1st	BM	Dead	9/1999	Sepsis
5	Neuroblastoma	Male	IV	52	1st	BM	Alive	3/2011	
6	Germ cell tumor	Female	Metastasis to lung	132	2nd	BM	Dead	1/2001	Relapsed
7	Neuroblastoma	Female	IV	24	1st	PB	Alive	6/2006	
8	Germ cell tumor	Male	Metastasis to mediastinum	96	1st	PB	Alive	1/2004	
9	Neuroblastoma	Female	IV	36	1st	BM	Alive	11/2006	
10	Wilms tumor	Female	IV	52	2nd	PB	Alive	8/2004	
11	Neuroblastoma	Male	IV	32	1st	PB	Dead	5/2001	Sepsis
12	Neuroblastoma	Male	IV	48	1st	PB	Alive	3/2006	
13	Wilms tumor	Male	IV	108	2nd	PB	Alive	11/2006	
14	Neuroblastoma	Male	IV	54	1st	PB	Dead	9/2004	Relapsed
15	Ewing sarcoma	Female	Metastasis to bone, bone marrow, liver	192	1st	PB	Dead	3/2004	Relapsed
16	Germ cell tumor	Male	IV	84	1st	PB	Alive	3/2010	
17	Ewing sarcoma	Female	Metastasis to bone	48	1st	PB	Dead	4/2004	Sepsis
18	Neuroblastoma	Female	III	32	1st	PB	Alive	2/2011	
19	Ewing sarcoma	Male	Metastasis to lung and bone	168	1st	PB	Dead	11/2004	Sepsis

BM: bone marrow; PB: peripheral blood

Infectious complications were identified in 11 patients (57.9%). Four patients (36.3%) had bacteriologically isolated septicemia, three patients (27.3%) had pneumonia, two (18.2%) had rectal abscess and the other two patients had skin infections. The isolated germs were *enterobacteria* in 45.5% (n = 5) of the cases, *Staphylococcus epidermidis* in 27.3% (n = 3) and *Candida albicans* in 27.3% (n = 3). Hepatic toxicity was presented by four patients (21%); all cases were identified by liver function tests. Six patients (31.6%) required intensive care, four of whom died as previously mentioned. None of the patients had veno-occlusive disease.

We observed that the type of tumor histology was important because survival of patients with ES was 20% in a follow-up of between three and 18 months. However, the EFS in the same period was 0% in comparison to those with neuroblastoma, where the OS was 88.8% (p-value = 0.035) in a follow-up of from three to 89 months and the EFS was 77.8% (p-value =0.01). The median survival of all patients was between 118-121 months (Figure 1 & 2).
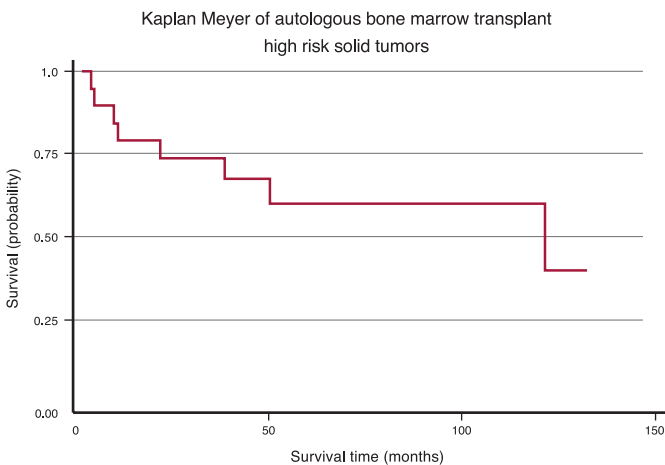

Figure 1 - Event-free survival of 19 pediatric patients with high-risk solid tumors conditioned with melphalan, cyclophosphamide and etoposide and autologous hematopoietic progenitor stem cell transplantation
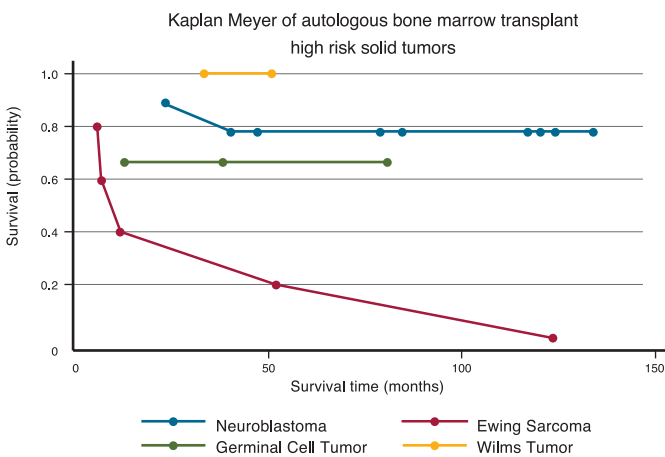

Figure 2 - Event-free survival by tumor type in 19 pediatric patients conditioned with melphalan, cyclophosphamide and etoposide and autologous hematopoietic progenitor stem cell transplantation

Another important factor was the time at onset of severe bone marrow aplasia. The patients were divided into two groups in respect to this variable. Group 1, patients presenting severe aplasia before Day 0 (n = 5) and Group 2, patients presenting aplasia after Day 0 (n = 14). The OS at Day 100 for Group 1 was 20% while for Group 2 it was 100% (p-value =0.02).

The presence of bacterial infections had an influence on the outcome with a survival of 20% at Day 100 compared with 100% for the patients who did not have bacterial infections.

The synergy between early severe myelotoxicity-bone marrow aplasia and infectious complications were the most unfavorable factors and documented in four patients that died.

Discussion

Clinical evidence has demonstrated the effectiveness of increasing chemotherapy doses to enhance the destruction of tumor cells. This is the most solid basis for myeloablative chemotherapy in AHPSCT as consolidation treatment for high-risk solid tumors. It can be achieved by alkylating agents alone or in combination with some other agents depending upon the type of tumor[14,15].

In most cases the benefit has been complemented with the use of TBI[16]. However the short and long-term deleterious effects of radiation in children increase the morbidity and decrease, in many cases, the quality of life without an easily identifiable therapeutic benefit[17].

In a previous work by our group of 15 consecutive children with advanced cancer submitted to three different myeloablative chemotherapy regimens and AHPSCT, the OS was 40% and the mean DFS was 19 months[6]. However the current study shows that single chemotherapy regimens can give more standardized results. Morbidity and a strict patient selection probably would offer better results. The present investigation evaluated the results of a single chemotherapy regimen based on melphalan, cyclophosphamide and etoposide without TBI. The EFS for the entire group was 63% in a three-year follow-up. Initially, these results exceeded the life probability for patients, as they had relapsed to first and/or second line conventional chemotherapy regimens.

A conditioning scheme with melphalan, cyclophosphamide and etoposide was selected, because it has proven to be efficient when treating multiple solid tumors in childhood, besides the fact of being accessible and affordable in Mexico.

The results obtained in this research are similar to those obtained by other groups. In 1998 Ozkaynak[18] reported a similar experience in 27 pediatric patients with high-risk solid tumors using a combination of alkylating agents (cyclophosphamide, carboplatin, melphalan and etoposide) and AHPSCT. DFS at 3 years was 60%, patients presented engraftment by Day +12.5 and presented a 16% transplant-related mortality rate. All deaths were caused by secondary sepsis due to infections by candida. After surgery, patients were examined daily while in the hospital and then at one and six weeks after discharge. Follow-up evaluations were then conducted at 3- to 6-month intervals for the first year and thereafter yearly for the remainder of the patient's life.

Kletzel[19] demonstrated the usefulness of myeloablative doses of chemotherapy followed by AHPSCT in a phase I/

II study of patients with high-risk neuroblastoma. They used the combination of thiotepa (300 mg/m²/day) for three days followed by cyclophosphamide (1500 mg/m²/day) for four days. In addition, an *in vitro* purge of bone marrow was carried out for 16 patients; the bone marrow was not purged for 23 patients. Peripheral blood was used in 13 patients with engraftment by Day 19 in patients with purged bone marrow and by Day 17.5 in patients with non-purged bone marrow and by Day 13 in patients that were given peripheral blood. Twenty-nine out of 51 patients that were included in the study presented progressive disease in a follow-up of 1140 days. There are multiple studies that have demonstrated the usefulness of melphalan in the myeloablation regimen[20,21] for neuroblastoma; however, most of them include TBI. In these studies the OS did not exceed 40%. This was due to the elevated mortality rate secondary to TBI. Even so, these studies outline the usefulness of melphalan to treat neuroblastoma. This observation is substantiated by the current study, in which, analyzing the group of patients with neuroblastoma separately, the OS was 88.l% and the EFS was 77.8%.

The use of high dose chemotherapy followed by AHPSCT in patients with high-risk Wilms tumor and/or after relapse has been established as an efficient therapy as reported by Campbell et al.[22]. These authors treated 13 patients after the first relapse. All patients were given different ablative regimens. The EFS was 60% and there was no transplant-related mortality.

The current study included patients with germ cell tumors after the first relapse. It is important to consider that the experience with high-dose chemotherapy and autologous stem cell transplant in children is still limited. In adult patients the experience is broader. Recently, a study of adult patients showed that half of the patients submitted to this procedure had a favorable outcome[23].

We consider the results with ES in this study are not comparable with those obtained by other groups[24]. The combination of chemotherapeutic agents used in our study did not have an impact on the EFS. Therefore, we must consider the use of a new regimen of myeloablation chemotherapy. Currently some groups have obtained promising results in ES with an OS of 53% to 86%[25]. In these studies a combination of carboplatin, ifosfamide and etoposide was used[26]. Other childhood solid tumors have been evaluated including osteosarcoma[27], medulloblastoma[28] and malignant astrocytoma[29].

The rate of infectious complications in our series, as well as mortality associated with the transplant protocol is higher than what has been reported in international literature. The rate of infectious complications in first transplant patients in our unit has decreased due to an increase in the experience of the group with this type of patients. This has enabled us to continue the autologous transplant program for solid tumors in an outpatient program with favorable results. AHPSCT can be harvested from bone marrow progenitor cells although peripheral blood is a more viable option especially in pediatric patients. However, cord blood is another realistic source given the potential contamination with tumor cells from the same patient and the risk of myelodysplastic syndrome post-transplant.

There is no question that this therapeutic approach is harmless not only in the acute phase of treatment but in the medium and long term follow up especially for children that received TBI as part of their treatment protocol[30,31].

Conclusion

AHPSCT is still a successful procedure in patients with solid tumors refractory to conventional chemotherapy.

Novel conditioning regimens, primarily reduced intensity and non-myeloablative regimens, allow patients with significant co-morbidities to undergo transplantation with reduced morbidity and mortality. Combinations of immune-modulatory cytokines and monoclonal antibodies with autologous transplantation are among the advances being explored in contemporary hematopoietic stem cell transplantation. We should continue selecting children with advanced stage high-risk solid tumors, who may benefit from this treatment modality.

The challenge in the future will be to establish strategies that avoid subsequent relapses after AHPSCT using measures such as immunotherapy or target therapy.

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