Intrabone Cord Blood Hematopoietic Stem Cell Transplantation in a Subset of Very High-risk Pediatric Patients

A Safety and Feasibility Pilot Study

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Summary: The main limit of umbilical cord blood hematopoietic stem cell transplantation is a more difficult engraftment related to the number of cells infused per kilogram of recipient body weight. This limit makes the cord blood a suboptimal source of hematopoietic stem cells for transplantation in case of difficult engraftment situations. Direct intrabone cord blood (CB) injection has been recently investigated as a solution to cell dose problem in the adults population, but there is a lack of data concerning this approach in pediatric patients. Here, we describe 5 pediatric patients undergoing intrabone cord blood transplantation (IBCBT) for different diseases characterized by a high risk of posttransplant graft failure. The conditioning regimen differed according to the disease, whereas the GvHD prophylaxis consisted of cyclosporine, mycophenolate, and ATG. The median numbers of total nucleated cells infused and $CD34^+$ cells were 3.3×10^7/kg, 2×10^5/kg. All the patients showed complete hematological recovery and complete donor engraftment. No patient had secondary graft failure, whereas 1 patient relapsed 6 months after IBCBT. No patient died of transplant-related complications. Our results show that IBCBT is safe and feasible in pediatrics as well, and suggest that IBCBT might be an attractive option to overcome some limits of umbilical cord blood hematopoietic stem cell transplantation.

Key Words: cord blood hematopoietic stem cells transplantation, intrabone, pediatrics

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Umbilical cord blood hematopoietic stem cell transplantation (UCBT) is one of the most interesting therapeutic options for patients who need hematopoietic stem cell transplantation (HSCT), but lack an HLA-identical familiar donor. Since the first UCBT was successfully performed in 1988,[1] a number of studies have been carried out on this kind of HSCT in both pediatric and adult populations and, as a meta-analysis published in 2007 summarizes, there are no differences in a 2-year overall survival between children given unrelated UCBT or bone marrow transplantation for both malignant and nonmalignant diseases.[2]

All previous studies, aimed at highlighting factors influencing the outcome of patients undergoing UCBT, have shown that the risk of early adverse transplant-related events was inversely correlated with both the number of cells infused per kilogram of recipient body weight and HLA mismatches in the donor-recipient pairs.[3,4] In the light of these findings in cases of HLA compatibility of 5 of 6 antigens, it is recommended to select cord blood units (CBUs) containing at least 2.5 to 3×10^7 nucleated cells per kilogram of recipient body weight. If an HLA compatibility of 4 of 6 antigens is present, the cell dose should be increased to 3.5×10^7 nucleated cells per kilogram of recipient body weight, especially in settings of nonmalignant diseases.[5]

As it can be estimated that only 20% of CBUs collected and stored in cord blood banks worldwide contain a sufficient number of cells for transplantation in an adult patient of standard weight according to these recommendations, different strategies, such as double cord blood transplantation,[6] cord blood hematopoietic stem cell ex vivo expansion,[7] and direct intrabone CB injection,[8] have been proposed to overcome this restriction.

A phase I/II study was carried out to establish the safety and efficacy of the intrabone injection of cord blood hematopoietic stem cells in adult patients affected by malignancies.[9] This study has shown that intrabone cord blood transplantation (IBCBT) is a suitable and safe option to overcome the problem of cell dose in UBCT.

At present, there are no data available on this new administration route of CB HSC for pediatric patients.

Here, for the first time, we report on a selected pediatric population undergoing IBCBT.

MATERIALS AND METHODS

Patients

The main aim of this study was to test the feasibility and safety of IBCBT in a pediatric population as measured by possible procedure-related complications and donor-derived neutrophil and platelet engraftment.

During the period between December 2007 and March 2009 five children (3 female and 2 male) from 1 to 14 years of age were enrolled in this experimental study.

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Data on the patient characteristics are summarized in Table 1.

Eligibility for this experimental protocol was determined by a high risk of standard procedure failure related either to the primary disease (patients 521, 441, and 575) or to some features of CBUs such as an HLA compatibility of less than 5 out of 6 antigens associated with a sub-optimal total nucleated cell (TNC) content (patients 551 and 583).

However, as there is a lack of previous data on this approach in pediatric population, only CBUs with TNC content over the safety threshold reported by the literature were selected for the intrabone injection.

This study was approved by the Institutional Review Board of the Regina Margherita Children's Hospital (Turin, Italy) (protocol CB01 no. 382/CEI AA OO OIRM/ S.Anna- Ordine Mauriziano di Torino Comitato Etico Interaziendale) and written informed consent was obtained from the parents of patients or their legal guardians.

Conditioning Regimen and Graft Versus Host Disease Prophylaxis

Considering their different characteristics and pretransplant therapy, the patients were prepared for transplantation with different conditioning regimens (summarized in Table 1).

Antithymocyte globuline (Genzime, UK) (3.5 mg/kg/day intravenously on days −4, −3, and −2), cyclosporine A (3 mg/kg/day intravenously from day 7, then adjusted to maintain a serum trough concentration between 100 to 250 μg/L), and mycophenolate mofetil (30 mg/kg/day in 2 doses from day +1 to day +27) were administered as the GvHD prophylaxis in all 5 cases.

All patients received 5 mcg/kg/day granulocyte colony stimulating factor and antiinfective prophylaxis according to our center's guidelines.

UCB Unit Characteristics and Cell Preparation

Umbilical CBUs were selected, shipped, and stored according to the Italian Bone Marrow Donor Registry's standard cord blood protocol. Before transplantation, confirmatory high-resolution HLA class I and class II typing of the CBU and the recipient were performed. HLA compatibilities between the recipient and the CBU are summarized in Table 2.

At the collection time, the TNC dose per kilogram of recipient body weight ranged from 3.6×10^7 to 11.6×10^7.

On day 0 the cord blood units were thawed in a 37°C water bath, according to the Rubinstein method.[10] After washing, the supernatant was removed and the cells were resuspended in a reduced volume (ranging from 12 to 20 mL) of saline solution plus dextran and albumin, and aliquoted in 4 to 5 syringes of 5 mL each.

TABLE 1. Patients' Characteristics

UPN	Age	Sex	Disease	Previous alloHSCT (HSC source)	Status at HSCT	Months From Diagnosis* to IBCBT	Conditioning
521	1	F	JMML	1 (haplo)	Primary Graft Failure	5	TT 5 mg/kg twice daily intravenously for 1 day (total dose 10 mg/kg) FLU 40 mg/m^2 once daily intravenously for 4 days (total dose 160 mg/m^2) L-PAM 140 mg/m^2 once daily intravenously for 1 day (total dose 140 mg/m^2)
551	8	F	ALL t(9;22)	0	1 RC	6	TBI 1200 cGy in 6 fractions for 3 days TT 5 mg/kg twice daily intravenously for 1 day (total dose 10 mg/kg) CTX 60 mg/kg once daily intravenously for 2 days (total dose 120 mg/kg)
441	3	F	HLH	3 (CB) (MMUD) (MMUD)	AD	8	TREO 14 g/m^2 once daily intravenouslyfor 3 days (total dose 42 g/m^2) TT 4 mg/kg twice daily intravenously on day 1 (total dose 8 mg/kg) CTX 50 mg/kg once daily intravenously for 2 days (total dose 100 mg/kg)
575	4	M	JMML in NF1	0	1 CP	6	BUSULPHAN 4 mg/kg intravenously in 4 doses daily for 4 days (total dose 16 mg/kg) CTX 60 mg/kg once daily intravenously for 2 days (total dose 120 mg/kg) L-PAM 140 mg/m^2 once daily intravenously for 1 day (total dose 140 mg/m^2)
583	14	M	ALL	0	2 CR	6	TBI 1200 cGy in 6 fractions for 3 days TT 5 mg/kg twice daily intravenously for 1 day (total dose 10 mg/kg) CTX 60 mg/kg once daily intravenously for 2 days (total dose 120 mg/kg)

*Either primary or of disease recurrence.

AD indicates active disease; ALL, acute lymphoblastic leukemia; CB, intravenous cord-blood transplantation; CP, chronic phase; CR, complete remission; CTX, cyclophosphamide; F, female; FLU, fludarabine; haplo, haploidentical hematopoietic stem cell transplantation; HLH, hemophagocytic Lymphohystocytosis; HSCT, hematopoietic stem cell transplantation; IBCT, intrabone cord blood transplantation; JMML, Juvenile Myelomonocytic Leukemia; L-PAM, melphalan; M, male, MMUD, mismatched unrelated peripheral blood stem cell transplantation; NF1, type 1 neurofibromatosis; TBI, total body irradiation; TREO, treosulfan; TT, thiotepa; UPN, unique patient number.

TABLE 2. Infused Products Data

UPN	No. HLA Mismatches (Mismatch Locus)	TNC $\times 10^7$/kg Prethawing	TNC $\times 10^7$/kg Postthawing CD34$^+$ $\times 10^5$/kg Postthawing	TNC $\times 10^7$/kg Lost	CFU-GM $\times 10^4$/kg LTC-IC $\times 10^2$/kg
521	1 (HLA-B)	11.6	4.6	0.6	56.1
			2		4.4
551	2 (HLA-A and B)	3.6	3.3	0.3	1
			3		3.7
441	1 (HLA-B)	9.6	7	0.06	2.4
			8		0.5
575	1 (HLA-A)	4	3	0	4.65
			1		12.52
583	2 (HLA-A and B)	4.1	2	0.1	0.4
			2		0

CFU-GM indicates colony forming unit-granulocyte macrophage; HLA, HLA compatibility; LTC-IC, long-term culture initiating cell; TNC, total nucleated cell; UPN, unique patient number.

The supernatant removed was evaluated for cell loss. An aliquot of the sample was tested for the cell content, viability, CD34$^+$ enumeration, sterility, and clonogenic potential.

Viable CD34$^+$ cells were evaluated by the dual-platform International Society of Hematotherapy and Graft Engineering ISHAGE protocol[11] on Facs Canto II (Beckton Dickinson).

The sterility was tested at the end of the procedure, using the standard colorimetric system Bact/Alert (Biomerieux INC, Durham, NC) on an aliquot of supernatant medium. Short-term and long-term clonogenic potential was evaluated according to the previously described methods.[12,13]

Cell Injection

The first intrabone cord blood cell injection was performed under general anesthesia in an operating theatre to test the feasibility and safety of the procedure, and was easily carried out without any problems; all the other cases were performed in the Bone Marrow Transplantation Ward after a short-term deep sedation. Inducing short-term deep sedation consisted of intravenous administration of midazolam and ketamine or propofol, and did not require intubation or assisted mechanical ventilation. Four or 5 bilateral injections were easily performed in the upper iliac crests using a 14-gauge bone marrow aspiration needle according to the previously described technique.[9]

Definitions

The neutrophil engraftment was defined as the first of three consecutive days after transplantation when the absolute neutrophil count was over 0.5×10^9/L and 1×10^9/L. The platelet engraftment was defined as the first of three consecutive days after transplantation when the absolute platelet count was over 20×10^9/L, 50×10^9/L, and 100×10^9/L, or higher without transfusion support.

Donor chimerism was determined on days +30 and +60 after UCBT on bone marrow cells and on days +180 and +365 on whole peripheral blood mononuclear cells by quantitative polymerase chain reaction of informative short tandem repeats in the recipient and the donor, according to the previously described method.[14]

The GvHD represents the scored combined data emerging from serial physical exams and laboratory findings, according to the current criteria.[15,16]

The patient had 2 bone marrow aspirates on days +30 and +60 after IBCBT to assess the disease response to treatment. If other sites were involved, specific diagnostic examinations were planned.

Hematological complete remission (CR) was defined as less than 5% of blasts in the bone marrow and the absence of circulating blasts with normal leukocyte and platelet counts in peripheral blood.

Central nervous system CR was defined as an absence of either clinical symptoms or radiological imaging features due to the disease and no blasts in the cerebrospinal fluid.

Extramedullary CR was defined as the absence of radiological or histological features due to the disease.

RESULTS

Conditioning Regimen

The conditioning regimen was well tolerated in all cases. We did not observe any cases of grade III extrahematological toxicity according to the Bearman criteria.[17]

CBUs Preparation

All CBUs were washed and their volume was then reduced with very little cell loss. In only one case (patient 521), we observed a large discrepancy between the TNC number at the time of collection and the TNC count performed at our lab. After thawing, the median TNC dose/kg infused and the median CD34$^+$ cell dose/kg infused were 3.3×10^7 (range, 2 to 7) and 2×10^5 (range, 1 to 8), respectively. The CFU-GM median number was 2.4×10^4/kg (range, 0.4 to 56.1). The median long-term culture initiating cell content was 3.7×10^2/kg (range 0 to 12.52). No bacterial or fungal contaminations were observed. Final cell suspension volume ranged from 12 to 20 mL.

Cell Injection

The injection was well tolerated by all patients. They only mentioned moderate pain that did not interfere with their usual inpatient activities. No severe side effects, such as hemorrhages or infections, were observed.

Patients' Follow-up

The course during the aplasia was regular for all the patients and no documented bacterial or fungal infections were recorded.

We observed cytomegalovirus reactivation in 3 patients who were treated with either foscarnet or ganciclovir and none of them developed cytomegalovirus disease.

We did not observe any EBV replication increase in this cohort of patients.

All the patients achieved complete hematological recovery: absolute neutrophil count greater than $0 \cdot 5 \times 10^9$/L and 1×10^9/L occurred at a median of day +21 (range, 13 to 32) and day + 28 (range, 14 to 63), respectively. Platelets above 20×10^9/L occurred at a median of day +40 (range, 28 to 52) from IBCBT and they were stably maintained since all patients achieved a platelet count over 50×10^9/L and 100×10^9/L at a median of day +49 (range 28 to 63) and at a median of day +73 (range, 63 to 138).

On days +30 and +60 after IBCBT, all patients showed complete donor chimerism on bone marrow cells which, except for the relapsing patient, was maintained at the following controls on peripheral blood.

Four patients experienced grade I acute GvHD and one had grade II acute GvHD, twhich resolved after metilprednisolone 2 mg/kg/die treatment.

None of the patients developed chronic GvHD.

Four patients are alive in CR with a median follow-up of 29 months (range, 19 to 34).

One of them (the Juvenile Myelomonocytic Leukemia patient in neurofibromatosis type 1) relapsed 6 months after the transplantation and died. None of the patients died of transplant-related complications (Table 3).

DISCUSSION

The main suggestion emerging from our small series of pediatric patients described in this report is that IBCBT is also feasible and safe for pediatric patients, and that this approach is suitable for further investigations.

The early endpoints of the previous study[9] carried out on an adult population were the safety and efficacy of this new administration route of CB HSCs, as measured by the donor-derived neutrophil and platelet engraftment. As compared with published findings of cordblood transplants[18,19] in which an intravenous route of administration was used, the sustained recovery of platelets was reported to be more rapid, irrespective of cell dose and HLA matching, showing that IBCBT is a potential effective option for patients who need an HSCT but lack a related/unrelated adult hematopoietic stem cell donor.

As the prognosis of pediatric patients undergoing UCBT has been shown to be strictly connected with the number of cells infused and with the HLA mismatches in the donor-recipient pair,[20] IBCBT might reduce the transplant-related mortality, particularly for children engrafted with CBUs with a lower cell content and more than 2 HLA mismatches.

TABLE 3. Patients' Outcomes

UPN	Follow-up (mo)	Outcome
521	34	Alive and disease free
551	31	Alive and disease free
441	29	Alive and disease free
575	27	Disease recurrence at +6 mo from IBCB, dead
583	19	Alive and disease free

UPN indicates unique patient number.

Moreover, the observation that all but one of our patients had stable engraftment, even though they were at high risk of treatment failure, suggests that IBCBT might also be a suitable option to treat difficult engraftment situations.

The case of patient 441 is emblematic in this sense. She was diagnosed with hemophagocytic lymphohistiocytosis at the age of 4 months. After completing the induction therapy according to protocol hemophagocytic lymphohistiocytosis 04 (Treatment Protocol for Haemophagocytic Lymphohistiocytosis 2004), she underwent 3 HSCTs (1 from a CBU and 2 from the same mismatched unrelated peripheral blood hematopoietic stem cell donor), but she always experienced autologous reconstitution. After the third transplant, she also experienced central nervous system disease recurrence. No other matched bone marrow or peripheral blood stem cell donors were available. The only source of hematopoietic stem cells available for a further transplant was a CBU. The intrabone procedure was chosen as there was a very high risk of further graft failure. Now, at 29 months from IBCT she is alive, disease free and the chimerism on peripheral blood shows 100% donor cells.

The main controversies related to the IBCBT procedure are the risks related to the manipulation of CBUs. Our data, which we obtained by comparing the CBU cell number at the time of collection and the cell count performed at our lab after the procedure, show that the cell loss is very low. We only observed 1 case (patient 521) with a discrepancy between the total cell number before and after the whole procedure. This difference may have been due to an overestimated cell count at the collection as, by computing the number of cells in the supernatant removed and by subtracting the percentage of cell death during the freezing procedure, we did not find any number of cells that could explain it. Data emerging from this report show that this approach is also safe and feasible in pediatrics and make it suitable for further investigations aimed at representing possible advantages making IBCBT a valid option to overcome some of the limits of intravenous CBT in specific settings of patients.

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