Combined cord blood and bone marrow transplantation from the same human leucocyte antigen-identical sibling donor for children with malignant and non-malignant diseases

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Outcomes after combined umbilical cord blood and bone marrow hematopoietic stem cell transplantation from the same HLA-identical sibling donor for children with malignant and non-malignant diseases

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- Combined cord blood and bone marrow from the same HLA identical sibling donor gives good results and overcomes the problem of a low cell dose in cord blood.
- Addition of cord blood to bone marrow may have the advantage to decrease GVHD

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Abstract

An umbilical cord blood (UCB) from an HLA identical sibling can be used for transplantation of patients with malignant and non-malignant diseases. However, the low cellular content of UCB units can limit its use. An option to enhance cell dose is to harvest bone marrow (BM) cells from the same donor and infuse them along with the UCB. We studied 156 children receiving a combined graft from 1992 to 2011 in EBMT centers. Median age was 7 years and 78% of patients (n=122) were transplanted for non-malignant diseases, mainly hemoglobinopathies. Acute leukemia (n=26) was the most frequent malignant diagnosis. Most patients (91%) received a myeloablative conditioning. Donor's median age was 1.7 years. Median infused total nucleated cell dose was $24.4x10^7$/kg. Median follow-up was 41 months. Sixty-day neutrophil recovery was 96% within a median of 17 days. The probabilities of acute II-IV and chronic GVHD were 19% and 10%, respectively. Four-year overall survival was 90% (68% in malignant; 97% in non-malignant diseases) with 3% probability of mortality. In conclusion, combined CB with BM transplantation from an HLA identical sibling donor is suitable for patients with malignant and non-malignant disorders with high overall and disease free survival and low incidence of GVHD.

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Introduction

Umbilical cord blood (UCB) was first used to treat a child with Fanconi anemia.[1] Since then, several studies have shown that umbilical cord blood transplantation (UCBT) is as effective as other hematopoietic stem cell transplantation (HSCT) modalities to treat both malignant and non-malignant hematological disorders.[2-11]

Since the institution of cord blood banks, the majority of UCBT are performed using unrelated UCB units.[12-14] However, in selected cases, a family-directed HLA identical UCB can be used for the treatment of a patient in need of transplantation.[15] One of the main limitations to UCBT is the low cellular content of a single CB unit;[16-20] and the association between cell dose and outcomes after CBT has already been demonstrated. Public unrelated cord blood banks have set stringent quality criteria,[20] however, UCB units for related use are often stored in private facilities or local cell therapy units that do not always follow the same rules, especially concerning the minimum number of stem cells collected as very often they can be stored regardless of the cellular content.
The number of related cord blood transplants is very limited despite very good outcomes in both malignant and non-malignant diseases mostly because of cost of UCBT and the lack of information from the families and the physicians. [4,15,21-23] In the related donor setting, an option to enhance the cellular content of a graft is to harvest bone marrow (BM) cells from the same sibling donor and to infuse them in addition to the CBU. To date, there are very few published reports on this approach.[24-26]

The aim of this study is to describe transplantation outcomes in children with hematologic malignant or non-malignant diseases who received a combined graft composed of CB and BM from the same HLA identical sibling donor.

Methods

Data collection

Data on patient, CB unit characteristics and outcomes were collected through the Eurocord and the European Group for Blood and Marrow Transplantation (EBMT) databases. Clinical and outcomes data of each patient were validated and checked for errors and discrepancies at Eurocord. A questionnaire for transplant data verification and specific information request about the donor and BM graft, including side effects of BM harvest was sent to all participating centers. Data managers and physicians in each EBMT center were requested to check and complete missing data and update patient's follow-up. All participating transplantation centers received the synopsis of the study and gave their approval. According to EBMT rules, patients gave informed consent for data entry into the EBMT and Eurocord registries database and for its use for research in accordance with the Declaration of Helsinki. The institution review board of Eurocord scientific committee approved this study.

Selection criteria

The selection criteria for this study were: age less than 21 years; transplantation performed between 1992 and 2011 in EBMT centers; graft source comprised of combined CB and BM from the same HLA identical sibling donor; and no history of previous allogeneic transplantation. All consecutive transplants meeting the study criteria (n=156) were included in the study. Transplantation was performed in 57 centers. Median follow-up of survivors was 41 months (range 3-213).

Definitions and endpoints

HLA compatibility was determined by serology for HLA-A and -B loci and by DNA typing for HLA-DRB1 locus. Neutrophil engraftment was defined as achieving absolute neutrophil count $\geq 0.5 \times 10^9$/L for 3 consecutive days, but excluding patients with evidence of autologous reconstitution. Full donor chimerism was defined as ≥95% leukocytes of donor origin in peripheral blood or marrow samples, measured by different techniques according to transplantation centers.

Autologous reconstitution was defined as ≥95% leukocytes of recipient origin. Mixed chimerism was defined by the presence of >5% but <95% of leukocytes of donor origin. Primary graft failure was defined as the absence of hematopoietic reconstitution of donor origin on day+60 after transplantation. The diagnosis and grading of acute and chronic graft *versus* host disease (GVHD) was assigned by the transplantation center using standard criteria.[27] Relapse (for malignant diseases), graft failure and death from any cause were considered events. Transplantation-related mortality (TRM) was defined as all causes of death related to the transplantation procedure. Overall survival (OS) was calculated from the date of UCBT until death or last observation for surviving patients. The primary outcome was OS.

Statistical analysis

Analysis was performed as of February 2014. Median values and ranges were used for continuous variables and percentages for categorical variables. The probabilities of OS and LFS were calculated using the Kaplan–Meier[28] method and were expressed as percentage ± standard error (SE). Due to the small number of competing events, the probabilities of neutrophil engraftment, grade II–IV acute and chronic GVHD, graft failure, relapse and TRM were calculated using the Kaplan-Meier method. Univariate analysis were performed using the log-rank test and included clinically relevant variables related to the patient (gender, age at transplantation and CMV serostatus), to the disease (malignant versus non-malignant), to transplantation technique (year of transplantation, type of conditioning, use of methotrexate and anti-thymocyte globulin as GVHD prophylaxis) and to the graft (number of infused total nucleated cells and sex compatibility between donor and recipient). For the outcomes with more than 20 observed events, multivariate analyses were performed using Cox proportional hazards regression model.[29] Variables that reached a p-value of 0.15 in the univariate analysis were included in the initial models and variables were eliminated once at a time in a stepwise fashion in order to only keep variables that reached a p-value of 0.05 in the final model. P-values were two-sided. Statistical analyses were performed with SPSS version 19 (SPSS Inc., Chicago, IL, USA).

Results

Patients

Table 1 shows patients and transplantation characteristics (n=156). Median age at HSCT was 7 years (range 1-20).The vast majority of patients (n=122, 78%) had non-malignant diseases and the commonest diagnosis was hemoglobinopathies (n=89, 57%), with 82 patients transplanted for thalassemia and 7 for sickle cell disease. Other frequent diagnosis was bone marrow failure (BMF) (n=24, 15%), with 8 Fanconi anemia, 5 Blackfan-Diamond disease, 7 other hereditary BMF and 4 idiopathic aplastic anemia. Among the 34 patients transplanted for malignant diseases, 26 had acute leukemia and only two of them were transplanted in first complete remission.

Transplantation

Conditioning was myeloablative in 142 (91%) patients and it was busulfan (or treosulfan)-based in 117 (82%) cases. A TBI-based regimen was given to 19 patients. Graft versus host disease (GVHD) prophylaxis consisted of cyclosporine monotherapy in 71 (45%) patients and cyclosporine in combination to methotrexate (MTX) with or without prednisone in 50 (32%) of them. Anti-thymocyte globulin (ATG) was used in 60 (38%) patients, all of them with non-malignant diseases.

Graft composition

Complete information on BM grafts was available for 114 cases. Median collected bone marrow volume was 198 milliliters (range 15-580). Table 2 shows the number of infused CB total nucleated cell (TNC) dose per quartiles, as well as BM TNC dose. Adding BM cells to the CB increased the cellularity of the grafts, resulting in a median TNC dose of 24.4×10^7/kg (range 4.2-455 $\times 10^7$ TNC/kg). Among the 119 patients for whom the number of TNC in UCB was available, 66 patients (55%) had UCB units with cellular content inferior to the accepted dose of 2.5×10^7TNC/kg prior to the addition of the BM cells.

Donors

Complete information regarding donors was available in 104 cases. There were no serious adverse effects reported after BM harvest. Blood transfusions were given to 38 donors (36%) - 31 received one unit, six received two or more units of packed red blood cells. Median donor age at the time of bone marrow harvest was 1.7 years (range 0.3-14.3 years).

Engraftment and graft failure

Overall 60-day probability of neutrophil engraftment was 96±2%, in a median time of 17 days (range 11-105) after transplantation.

Six patients failed to achieve neutrophil engraftment. All of them had non-malignant diseases (5 thalassemia and one immunodeficiency). Chimerism information was available for 5 out of these 6 patients with primary graft failure; 3 had autologous recovery and 2 mixed chimerism within 100 days after transplantation. Five patients are alive; two of them received subsequent allografts.

Acute and chronic GVHD

Probability of grade II-IV acute GVHD (aGVHD) at day 100 was 19±3% (Figure 1) and the median time of onset was 24 days (range 4-123). On univariate analysis, diagnosis of malignant disease (47±9% versus 12±3%, $p<0.001$) and patient negative CMV serology (29±6% versus 13±4%, $p=0.013$) were associated with increased risk of aGVDH, but only the diagnosis was confirmed as an independent factor in multivariate analysis (HR 4.6 95%CI 1.8-11,4; $p=0.001$ for malignant diseases). Of 151 patients at risk, 12 presented grade III-IVaGVHD; 6 of them had non-malignant and 6 malignant diseases.

Probability of chronic GVHD (cGVHD) at 4 years was 10±3% (Figure 2) and the median time of onset was 110 days after HSCT (range 74-884). Out of15 patients who presented cGVHD, 3 had extensive disease. The only risk factor associated with increased risk of cGVHD was the diagnosis of malignant disease (18±7% versus 6±2%, $p=0.012$).

Transplantation-related mortality and causes of death

The 4-year TRM was 3±2%. Out of 34 patients with malignant diseases, 12 relapsed in a median time of 11 months after HSCT (range 3-32). Overall, 16 patients died in a median time of 16 months after HSCT (range 1-132). Among them, 11 had malignant diseases (8 acute leukemia and 3 solid tumor); 9 patients died from disease progression and 2 from GVHD. Of the 5 patients with non-malignant diseases who died, 2 had hemoglobinopathy, 2 immunodeficiency and 1 BMF. Two of these patients died of rejection and the remaining 3 of infections.

Survival

Overall survival at 4 years was 90±3%; it was 97±2% for non-malignant and 68±9% for malignant diseases ($p<0.001$) (Figure 3). There were no other risk factors, besides diagnosis, associated with survival, even when analyzing patients with malignant and non-malignant diseases separately.

Discussion

The use of related cord blood transplant has been limited by the availability and cost of good quality product. In the Eurocord database, 782 related UCBT have been reported since 1988, with an even distribution across the years, except for the first few years. The main indication for related UCBT is hemoglobinopathies.[21,23] An advantage of using related cord blood instead of bone marrow from the same HLA identical sibling is the absence of risk to the donor and the decrease rate of GVH.[30]

One of the main concerns is the cellular content of the stored unit because in directed donations UCB may be stored even when the criteria for minimum cellular content is not met. In related cord blood transplantation, the use of the same HLA identical bone marrow was utilized to increase the number of cells infused. Alternatively, in order to facilitate engraftment, several authors have reported the addition of third party stem cells with some success.[31,32] Our study shows that cord blood and bone marrow transplantation is associated with high neutrophil engraftment and very low mortality, translating into excellent

survival both for non-malignant and malignant hematological diseases. Of note, we observed a low probability of both acute and chronic GVHD, particularly in patients transplanted for non-malignant diseases.

The benefit of a combined graft is rather evident, given the important increase in cellularity brought by the addition of BM to the CB graft, but our results shine light to another important issue, which is the possibility that combining CB and BM in the same graft may have an impact in the incidence of GVHD. Rocha and colleagues have previously reported that, in the related donor setting, UCBT is associated with a decreased risk of both acute and chronic GVHD, compared to BM transplantation[30]. Also, a recent publication by Locatelli *et al* showing similar outcomes of children with hemoglobinopathies given either a CB (n=96) or BM (n=389) HLA-identical sibling graft confirms a decreased risk of GVHD associated with CB grafts (21% versus 11% for aGVH and 12% versus 5% for cGVHD in BM and CB recipients, respectively)[21]. In a study on 149 thalassemia patients transplanted with HLA identical sibling BM grafts, the incidence of acute GVHD was 38% and 5-year incidence of chronic GVHD was 13%[22]; both superior to the ones we found in our study (12% and 6% for cGVHD and aGVHD, respectively) for patients with non-malignant diseases. Considering our results, we can speculate that the addition of CB cells to an HLA-identical sibling BM graft might have an immunomodulatory effect leading to decreased GVHD risk.

Another fundamental issue regards the donors. Although harvesting BM from young children seems to be safe, with no serious adverse effects reported in this study, we observed a somewhat high rate of red blood cell transfusions; therefore the risk associated with these transfusions and general anesthesia needs to be considered.

One may wonder why give CB in combination with BM instead of BM alone. Besides the possible immunomodulatory characteristics of CB discussed previously, having the CB cells available may allow for a smaller BM harvest in a rather young donor.

In conclusion, combined cord blood and bone marrow transplantation is associated with excellent outcomes, both for malignant and non-malignant

diseases. The possibility of decreasing acute and chronic GVHD when associating CB with BM grafts deserves further investigation. Furthermore, when the related CB has low pre-freezing TNC dose, harvesting BM from the same donor to use in combination with CB in the same transplantation procedure is a suitable option.

Authorship and Disclosures: EG and VR conceived the study; VR, LT and AR designed the study; FL, MZ, AY, MC , TG, SA, FF, YB, CA, JF, PL, AB, HS, IB, MB, CDH, PS, AV, provided cases for the study; FV collected and treated the data; LT and FV performed the statistical analysis; LT, FV and EG wrote the manuscript. All authors edited and approved the manuscript. The authors have no conflict of interest to disclose.

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Table 1. Patient and transplant characteristics

Median follow-up (range)	41 (3-213) months
Median age (range)	7 (1-20) years
Median year of transplant (range)	2007 (1992-2012)
Previous ASCT	4
Diagnosis n (%)	
Malignant	34 (22%)
ALL	22 (14%)
AML	4 (3%)
MDS/MPD	3 (2%)
Non-Hodgkin lymphoma	2 (1%)
Solid tumor	3 (2%)
Non-malignant	122 (78%)
Bone marrow failure syndrome	24 (15%)
Hemoglobinopathy	89 (57%)
Immune deficiency	7 (5%)
Other	1 (1%)
Disease status -malignant disease only	32 (94%) ≥CR2
Type of conditioning, n (%)	
RIC	10 (7%)
CY ±FLU	8 (80%)
Other	2 (20%)
MAC	142 (93%)
BU+CY	65 (48%)
Treo+Other	22 (12%)
BU+FLU+Thio	16 (12%)
BU±Other	14 (10%)
TBI	
No	135 (88%)
Yes	19 (12%)
GVHD prophylaxis	
CsA	71 (49%)
CsA+MTX±Other	52 (36%)
CsA±Other	16 (11%)
Other	5 (4%)
Serotherapy (ATG or MoAb)	
No	80 (57%)
Yes	60* (43%)

Abbreviations: ASCT means: autologous transplant;; ALL: acute lymphoblastic leukemia ; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; MPD : myeloproliferative syndrome ; RIC : reduced intensity conditioning ; CY: cyclophosphamide; Fluda: fludarabine; MAC : myeloablative conditioning ; BU: busulfan; Treo : treosulfan ; Thio, thiothepa; TBI, total body irradiation; CsA: cyclosporine; MTX : methotrexate; ATG: antithymocyte globulin ; MoAb : monoclonal antibody CR: complete remission.

Missing data: type of conditioning (RIC vs.MAC), n=4; specific MAC conditioning, n=3; TBI, n=2; GVHD Prophylaxis n=12; ATG, n=16

*All 60 patients who received ATG had non-malignant diseases.

Table 2. Donor and graft characteristics

Donor and graft characteristics	Median	Range	Quartile
Donor's age; years	1.65	0.3-14.3	
BM volume collected; mL	198	15-580	
BM TNC infused x10^7/Kg	21.90	2.5-451	
CB TNC infused x10^7/Kg	2.1	0.1-17.5	1^{st} 1.36
			2^{nd} 2.10
			3^{rd} 3.50
Combined (CB+BM)TNC infused x10^7/Kg	24.4	4.20-455.1	

Abbreviations: mL means milliliter; BM: bone marrow; TNC: total nucleated cells ; Kg : kilogram ; CB : cord blood.

Figure 1. 100-day incidence of acute graft-versus-host disease

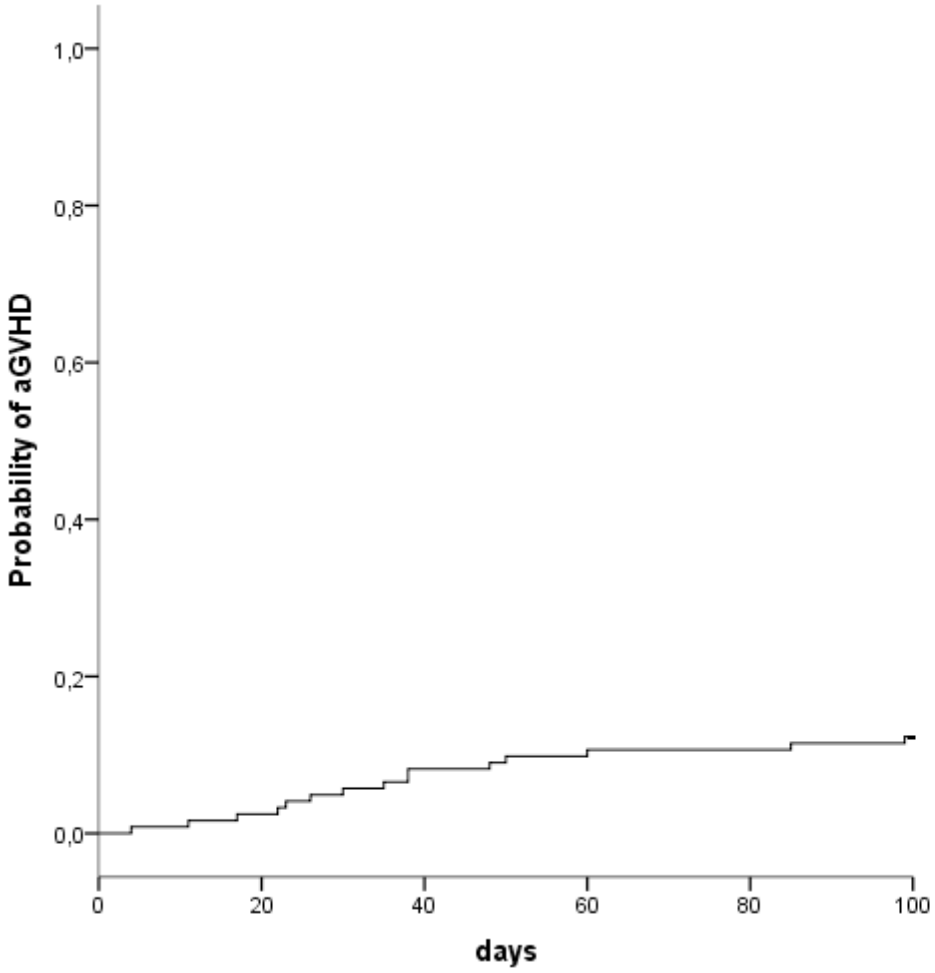

Figure 2. Four-year incidence of chronic graft-versus-host disease

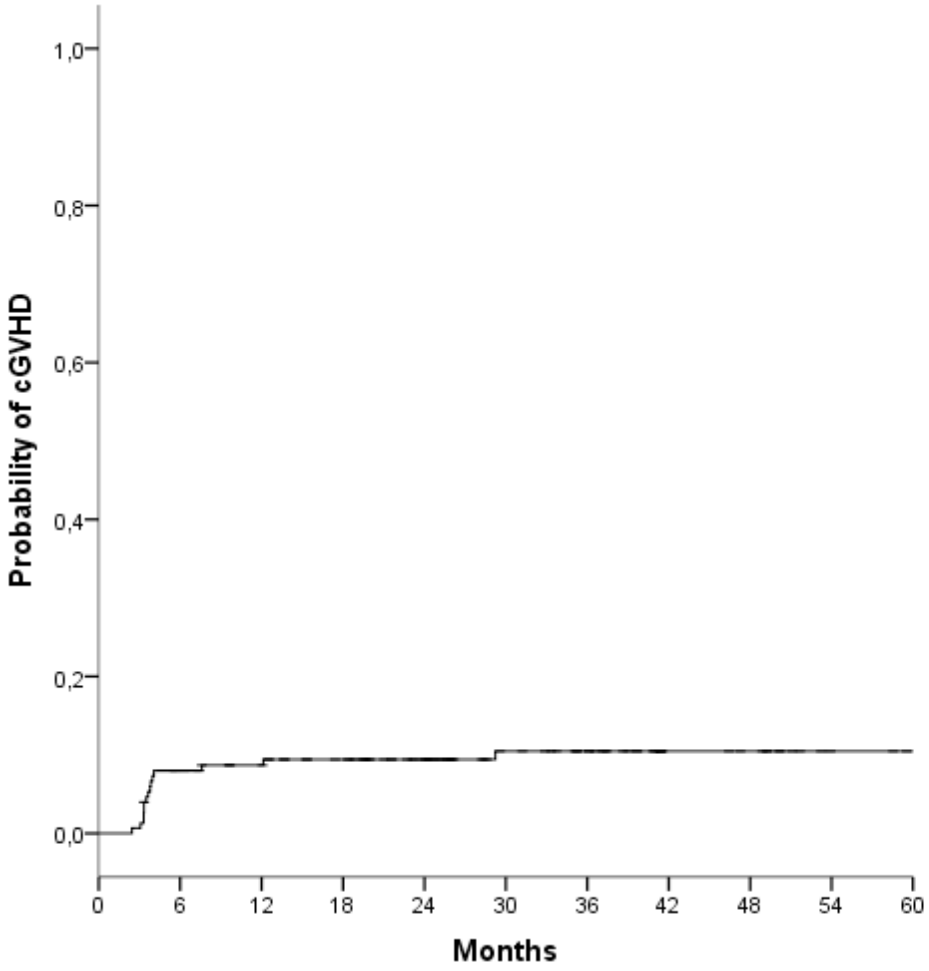

Figure 3. Four-year overall survival according to disease