

Influence of Nucleated Cell Dose on Overall Survival of Unrelated Cord Blood Transplantation for Patients with Severe Acquired Aplastic Anemia: A Study by Eurocord and the Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation

Regis Peffault de Latour,¹ Duncan Purtill,² Annalisa Ruggeri,² Guillermo Sanz,³
 Gerard Michel,⁴ Virginie Gandemer,⁵ Sebastien Maury,⁶ Joanne Kurtzberg,⁷
 Carmen Bonfim,⁸ Mahmoud Aljurf,⁹ Eliane Gluckman,² Gerard Socié,¹
 Jakob Passweg,¹⁰ Vanderson Rocha^{1,2}

Information is scarce on outcomes after unrelated cord blood transplantation (UCBT) for patients with severe aplastic anemia (SAA). We retrospectively analyzed 71 patients (median age, 13 years; 28 adults) with SAA (9 with paroxysmal nocturnal hemoglobinuria [PNH]) who received a single-unit ($n = 57$; 79%) or double-unit UCBT ($n = 14$; 19%) in 32 centers between 1996 and 2009. A reduced-intensity conditioning regimen was provided in 68% of the patients. The cumulative incidence (CI) of neutrophil recovery was $51\% \pm 6\%$ at day 60, with significantly better engraftment seen in recipients of higher prefreezing total nucleated cell (TNC) dose ($>3.9 \times 10^7/\text{kg}$; hazard ratio [HR], 1.5; $P = .05$). The CI of platelet engraftment at day 180 posttransplantation was $37\% \pm 7\%$, that of grade II-IV acute GVHD was $20\% \pm 5\%$, and that of chronic GVHD at 3 years was $18\% \pm 5\%$. At a median follow-up of 35 months (range, 3-83 months), the estimated probability of 3-year overall survival (OS) was $38\% \pm 6\%$. Significantly improved OS was seen in recipients of $>3.9 \times 10^7$ TNCs/kg prefreezing (45%, compared with 18% for recipients of $\leq 3.9 \times 10^7$ TNC/kg; HR, 0.4; $P = .007$). These results highlight the fundamental role of cell dose for both engraftment and OS in patients with SAA undergoing UCBT.

Biol Blood Marrow Transplant 17: 78-85 (2011) © 2011 American Society for Blood and Marrow Transplantation

KEY WORDS: Acquired bone marrow failure, Alternative donor transplant

From the ¹Bone Marrow Transplant Unit, Saint Louis Hospital, Paris, France; ²Eurocord, Saint Louis Hospital, Paris, France; ³Department of Hematology, Hospital Universitario La Fe, Valencia, Spain; ⁴Department of Hematology, La Timone Hospital, Marseille; ⁵Department of OncoPediatrics, Rennes, France; ⁶Department of Hematology, Henri Mondor Hospital, Creteil, France; ⁷Division of Blood and Marrow transplantation, Department of pediatrics, Duke University Medical Center, Durham, NC; ⁸Bone Marrow Transplant, Federal University of Parana, Curitiba, Brazil; ⁹King Faisal Specialist, Riyadh, Saudi Arabia; and ¹⁰Geneve university Hospital, Geneva, Switzerland.

Financial disclosure: See Acknowledgments on page 83.

The first 3 authors contributed equally to this work.

Correspondence and reprint requests: Dr Regis Peffault de Latour, Service d'Hématologie Greffe, Hôpital Saint-Louis 1 Av Vellefaux, 75010 Paris, France (e-mail: regis.peffaultdelatour@sls.aphp.fr).

Received April 5, 2010; accepted June 9, 2010

© 2011 American Society for Blood and Marrow Transplantation
 1083-8791/\$36.00

doi:10.1016/j.bbmt.2010.06.011

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) from an HLA-matched related donor is the treatment of choice for patients with severe aplastic anemia (SAA) [1]. In the absence of an HLA-identical sibling, the current standard treatment for SAA is the combination of antithymocyte globulin (ATG) and cyclosporine (CsA) [2]. After immunosuppressive therapy (IST), overall response is achieved in about two-thirds of patients, the cumulative incidence (CI) of relapse among responders is approximately 20%-30%, and clonal evolution occurs in about 10%-15% of cases [3-5]. Both refractory and relapsed patients are treated with repeated courses of IST. Patients refractory to initial IST historically have a dire outcome, with long-term survival rates of 20%-30% [4], whereas the response rate is about 40%-50% in relapsed patients [6]. The management of patients

with refractory or recurrent SAA is challenging. HSCT from an unrelated donor (UD) represents an alternative salvage therapy for those patients.

The outcome of HSCT with UD transplants for patients with SAA has improved in the last decade [7,8]. Better donor-recipient HLA matching has played a major role in this improvement, but significant changes in the conditioning regimen and supportive measures have contributed as well [9,10]. The recent study on UD-HSCT from the SAA Working Party of the European Group for Blood and Marrow Transplantation (SAA WP-EBMT), included a total of 100 patients with SAA. The 5-year overall survival (OS) rate was 75%, with low incidences of both acute and chronic graft-versus-host disease (aGVHD, cGVHD) [11]. Results of UD-HSCT have improved to such an extent as to possibly affect treatment strategies. In children without a matched sibling donor, current guidelines recommend proceeding to HSCT after a failure of one course of IST, provided that a fully matched donor at the allele level for major histocompatibility complex class I and II antigen is available [12]. In adults, an alternative donor transplant is an option for second-line treatment in patients failing one or two courses of IST [8,13].

Unfortunately, many patients, especially those from ethnic minority groups or less-homogeneous populations, do not have a suitable UD. The percentage of such patients will vary between 5% and 40%, according to the patient's ethnic origin [13]. Unrelated cord blood transplantation (UCBT) is an alternative option that has been successfully used in patients with hematologic malignancies [14,15]. To date, there have been only a few reports on UCBT in patients with SAA. Primary reports showed poor outcomes and a high incidence of graft failure [14,16], whereas some later small series and case reports have reported successful UCBT for SAA [17-20]. The largest cohort reported to date, 31 patients in a Japanese study, had a 2-year OS of 41%, suggesting that UCBT can be an alternative treatment for SAA patients who fail IST and have no suitable donor [21]. We conducted a retrospective analysis on 71 patients diagnosed with SAA (9 with paroxysmal nocturnal hemoglobinuria [PNH]) who underwent UCBT in 32 centers (including 23 EBMT centers).

PATIENTS AND METHODS

Data Collection

Eurocord is a registry of related and UCBT that works in collaboration with the EBMT and Netcord banks. Eurocord and EBMT databases provided data on UCBT. Centers not associated with EBMT were asked to complete reports if UCB units were obtained from Netcord banks. All data were verified and updated

by the institution's physicians and data managers. All patients or legal guardians provided informed consent for the UCBT according to the Declaration of Helsinki.

Inclusion Criteria

This retrospective study included patients with SAA (based on the criteria of the International Agranulocytosis and Aplastic Anemia Study) (1) who received an unrelated and unmanipulated single-unit or double-unit UCBT; (2) who were older than 1 year at the time of diagnosis of SAA; and (3) for whom there were adequate and sufficient data to perform the analysis. Patients with PNH were included in case of SAA and a subclinical PNH clone. In the Eurocord database, there was no patient with classic PNH (including hemolysis and thrombosis). Patients with constitutional bone marrow (BM) failure were excluded from the study because most of them have been previously reported [22] as well as patients who had received a previous allograft. In June 2009, 71 out of 439 patients transplanted for BM failure syndrome (BMFS) and registered on the Eurocord registry were eligible for this study.

Endpoint Definitions

The primary endpoint was OS at 3 years. Other endpoints included incidence of neutrophil recovery, defined as the first of 3 consecutive days with a neutrophil count of at least $0.5 \times 10^9/L$, and the incidence of platelet recovery as the first of 7 consecutive days with an unsupported platelet count of at least $20 \times 10^9/L$. Graft failure was defined as no sign of neutrophil recovery, as well as transient engraftment of donor cells up to 60 days posttransplantation. Reduced-intensity conditioning (RIC) was defined according to published criteria [23]. aGVHD and cGVHD were diagnosed and graded according to published criteria [24], with histopathologic confirmation when possible. Chimerism data was evaluated in the first 3 months posttransplantation. Full donor chimerism was defined as the presence of $>95\%$ of the cells of donor origin, mixed chimerism if $>5\%$ and $<95\%$ of donor cells, and autologous recovery if $<5\%$ of donor cells. Data on the method of chimerism detection were not reported.

Statistical Analysis

Data were analyzed through June 2009. The CI function (CIF), using death as a competing event, was used to estimate neutrophil and platelet engraftment and aGVHD and cGVHD. The Kaplan-Meier method was used to estimate OS. The variables evaluated included recipient age, donor sex, recipient-donor sex mismatch, number of pre-UCBT transfusions for red blood cells (RBCs) and platelets, recipient cytomegalovirus (CMV) serologic status, disease duration before UCBT, single-unit versus double-unit transplant,

Table 1. Patient and Disease Characteristics (n = 71)

| Characteristic | n/N (%) or Median (Range) |
|--|---------------------------|
| Age at transplantation, years | 13 (2-68) |
| Children (age <18 years) | 43 (61%) |
| Weight at transplantation, kg* | 47 (9-100) |
| Males/females | 38/33 |
| Recipient CMV-positive | 43/68 (63%) |
| Diseases | |
| SAA† | 62/71 (87%) |
| PNH | 9/71 (13%) |
| Previous immunosuppressive treatment | 55/62 (89%) |
| Time interval from diagnosis of SAA to transplantation, months | 14 (2-140) |
| More than 20 red blood cell units infused | 35/63 (56%) |
| More than 20 platelet transfusions | 42/63 (56%) |

CMV indicates cytomegalovirus; SAA, severe aplastic anemia; PNH, paroxysmal nocturnal hemoglobinuria.

*Twenty-nine 29 patients weighed >50 kg.

†Including 1 patient with posthepatitis SAA.

ABO compatibility, HLA matching by serology and high-resolution DNA typing, number of mononuclear cells and CD34⁺ cells of the cord blood units at the time of freezing and infusion, conditioning regimen, and year of UCBT. Continuous variables were categorized as follows. Each variable was first divided into 3 categories of percentiles; if the relative event rates (ratio of the observed number of events to the expected number of events in a category, assuming no variation across categories) in 2 or more adjacent categories (and the mean times to event) were not substantially different, these categories were then grouped. The best cutoff point of total nucleated cell (TNC) dose was the 33rd percentile for survival and neutrophil recovery; for the other variables, no clear pattern was observed for the primary outcome, and thus the median was taken as a cutoff point [25]. For assessment of prognostic factors using the CIF, univariate and multivariate analyses were performed using Gray's test [26] and the proportional subdistribution hazard regression model of Fine and Gray [27]. For OS, the log-rank test and the Cox proportional hazards model were used in univariate and multivariate analyses. Each potential risk factor was tested independently. All factors that reached $P \leq .10$ in the univariate analysis were included in the multivariate model. All models were built using a forward stepwise method. Only factors that reached $P \leq .05$ were retained in the final model. Statistical analyses were performed with SPSS (SPSS Inc, Chicago, IL), and S-PLUS (Insightful Corp, Seattle, WA) software packages.

RESULTS

Patient and Disease Characteristics

A total of 71 patients from 23 EBMT transplant centers and 9 non-EBMT centers who underwent UCBT between January 1996 and June 2009 met the criteria for inclusion into the study. Patient and disease

Table 2. Graft- and Transplantation-Related Characteristics

| Characteristic | n/N (%) or Median (Range) |
|--|---------------------------|
| Number of unrelated CB units | |
| 1 | 57 (79%) |
| 2 | 14 (19%) |
| Number of HLA disparities* | |
| 6/6 match | 5 (10%) |
| 5/6 match | 17 (33%) |
| 4/6 match | 26 (51%) |
| 3/6 match | 3 (6%) |
| Number of HLA disparities† | |
| 2 units 5/6 match | 2 (14%) |
| 2 units 4/6 match | 6 (43%) |
| 2 units 3/6 match | 1 (7%) |
| 1 unit 5/6 and 1 unit 4/6 match | 2 (14%) |
| Number of nucleated cells collected, $\times 10^7/\text{kg}$ | |
| Single CB | 4.3 (2-35) |
| Double-unit CB | 7.4 (5-15) |
| Number of CD34 ⁺ cells collected, $\times 10^5/\text{kg}$ | |
| Single CB | 2.1 (0.4-19) |
| Double-unit CB | 3 (1-87) |
| Number of nucleated cells infused, $\times 10^7/\text{kg}$ | |
| Single CB | 3.2 (1.5-16) |
| Double-unit CB | 6.2 (3.2-13.2) |
| Number of CD34 ⁺ cells infused, $\times 10^5/\text{kg}$ | |
| Single CB | 1.8 (0.3-7.8) |
| Double-unit CB | 2.9 (1.1-7.1) |
| Conditioning regimen‡ | |
| RIC | 48 (68%) |
| Flu-based regimen | 31 (46%) |
| Myeloablative | 22 (31%) |
| Cy + Bu | 10 (15%) |
| Cy + TBI 12-14 Gy | 8 (12%) |
| Use of TBI | |
| Low-dose | 14 (21%) |
| High-dose | 11 (16%) |
| GVHD prophylaxis§ | |
| CsA + prednisone | 38 (55%) |
| CsA + mycophenolate mofetil | 13 (19%) |
| Other | 18 (26%) |
| Use of ATG | 53/67 (79%) |

CB indicates cord blood; RIC, reduced-intensity conditioning; Flu, fludarabine; Cy, cyclophosphamide; Bu, busulfan; TBI, total body irradiation; CsA, cyclosporine A; ATG, antithymocyte globulin.

*One unit, antigen-level HLA-A and -B and allele-level HLA-RDBI typing.

†Two units, antigen-level HLA-A and -B and allele-level HLA-RDBI typing; 3 missing data on the second unit.

‡Four missing data on conditioning regimen.

§Two missing data on GVHD prophylaxis.

characteristics are summarized in Table 1. As shown, most patients had failed a first course of IST (n = 55; 89%) and were heavily transfused before transplantation; 35 patients received >20 RBC units (56%), and 42 patients received >20 platelet transfusions (67%). The median time from diagnosis of SAA to transplantation was 14 months (range, 2-140 months).

Graft and Transplant Characteristics

Graft and transplant-related characteristics are summarized in Table 2. A total of 57 patients received a single-unit UCBT, and 14 received a double-unit UCBT. The conditioning regimen varied according to the transplant center. A total of 48 patients received an RIC regimen, and 22 received a myeloablative (MA) conditioning regimen; information on condition reducing

regimen was missing in one patient. The most commonly used RIC regimens were cyclophosphamide (Cy; 50-200 mg/kg) + fludarabine (Flu; 90-200mg/m²) + ATG (n = 15), Cy (50-200 mg/kg) + ATG (n = 10), and Cy (50-100 mg/kg) + Flu (120-200 mg/m²) + TBI (2 Gy) + ATG (n = 7). The most commonly used MA regimens were Cy (90-200 mg/kg) + busulfan (Bu; 4.8-20mg/kg) + ATG (n = 10) and Cy (120-200 mg/kg) + TBI (12-14 Gy) + ATG (n = 8). GVHD prophylaxis varied among the individual centers (Table 2).

Engraftment and Chimerism Studies

The CI (\pm standard deviation) of neutrophil recovery (without evidence of rejection or autologous recovery) was 51% \pm 6% by day 60. Neutrophil recovery occurred in 37 patients, at a median of 25 days (range, 6-91 days). Thirty-four patients showed no sign of neutrophil recovery; 8 of these patients died early (within 30 days post-UCBT), 4 had autologous reconstitution (still alive at the time of the analysis), 6 died in aplasia, and 12 underwent a second transplantation (4 with a single unit, 4 with a double UCBT and 4 with a transplant from a related mismatched donor), of whom 2 are alive. Two out of 4 patients with missing data regarding the treatment of primary graft failure were still alive at the time of the last analysis. In univariate analysis, the following variables were associated with a higher incidence of neutrophil recovery (Table 3): recipient sex (58% for female vs 39% for male; $P = .05$), prefreezing TNC dose $>3.9 \times 10^7$ /kg (58% vs 33%; $P = .03$), and infused TNC dose $>3.4 \times 10^7$ /kg (63% vs 39%; $P = .03$). In multivariate analysis, the only factor associated with shorter time and higher probability of engraftment was prefreezing TNC dose $>3.9 \times 10^7$ /kg (HR, 1.5; 95% confidence interval, 1-2.2; $P = .05$). The CI of neutrophil recovery according to TNC dose before freezing is shown in Figure 1.

The CI of platelet engraftment was 37% \pm 7% by day 180. Platelet recovery occurred in 29 patients (46%) at a median of 45 days (range, 15-127 days). In univariate analysis, the only factor associated with higher incidence of platelet engraftment was prefreezing TNC dose $>3.9 \times 10^7$ /kg; $P = .05$). No factor was associated with platelet engraftment in multivariate analysis.

Chimerism was available for 32 of 37 patients who engrafted (26 of 30 recipients of single-unit and 6 of 7 recipients of double-unit transplants). Among single-unit recipients, 22 (85%) had complete chimerism and 4 (15%) had mixed chimerism (2 became complete chimeras during follow-up). In recipients of double-unit UCBT, 5 (86%) had complete chimerism and 1 (14%) had mixed chimerism. In 5 evaluable patients, engraftment was derived from one unit in 4 and from both units in 1.

Table 3. Univariate Analysis for Outcomes after UD UCBT in Patients with SAA (n = 71)

| Covariate | Neutrophil Engraftment at Day 60 | | OS at 3 Years | |
|---|----------------------------------|---------------|---------------|--------------|
| | n | (% \pm SD) | n | (% \pm SD) |
| Age, years | | | | |
| >13 | 36 | 53 \pm 8.5 | 36 | 45 \pm 9 |
| ≤ 13 | 35 | 46 \pm 8.5 | 35 | 32 \pm 8 |
| P | | .72 | | .44 |
| Recipient sex | | | | |
| Male | 33 | 39 \pm 8.5 | 33 | 14 \pm 7 |
| Female | 38 | 58 \pm 8 | 38 | 59 \pm 8 |
| P | | .049 | | .001 |
| Sex match | | | | |
| Match | 32 | 53 \pm 8.5 | 32 | 26 \pm 8 |
| Mismatch | 36 | 44 \pm 9 | 36 | 46 \pm 9 |
| P | | .54 | | .36 |
| Time from diagnosis to UCBT, months | | | | |
| >14 (median) | 30 | 50 \pm 9.5 | 30 | 43 \pm 10 |
| ≤ 14 | 30 | 37 \pm 9 | 30 | 32 \pm 9 |
| P | | .52 | | .37 |
| RBC transfusions | | | | |
| >20 | 35 | 54 \pm 8 | 35 | 38 \pm 9 |
| ≤ 20 | 28 | 46 \pm 11.5 | 28 | 41 \pm 10 |
| P | | .71 | | .83 |
| Platelet transfusions | | | | |
| >20 | 42 | 52 \pm 8 | 42 | 38 \pm 8 |
| ≤ 20 | 21 | 48 \pm 11.5 | 21 | 43 \pm 11 |
| P | | .65 | | .87 |
| CMV status | | | | |
| Positive | 43 | 49 \pm 8 | 43 | 42 \pm 8 |
| Negative | 24 | 54 \pm 10.5 | 24 | 38 \pm 10 |
| P | | .69 | | .52 |
| ABO | | | | |
| Match or minor mismatch | 41 | 51 \pm 8 | 41 | 38 \pm 8 |
| Major mismatch | 27 | 53 \pm 10 | 27 | 36 \pm 10 |
| P | | 1.0 | | .64 |
| HLA disparity | | | | |
| 0-1 | 24 | 54 \pm 10.5 | 24 | 44 \pm 10 |
| >1 | 41 | 59 \pm 8.5 | 41 | 38 \pm 10 |
| P | | .6 | | .61 |
| Transplant | | | | |
| Single | 57 | 49 \pm 6.5 | 57 | 37 \pm 7 |
| Double | 14 | 50 \pm 10.5 | 14 | 43 \pm 14 |
| P | | .84 | | .55 |
| Conditioning regimen | | | | |
| RIC | 46 | 48 \pm 7.5 | 46 | 47 \pm 8 |
| Myeloablative | 21 | 62 \pm 11 | 21 | 24 \pm 9 |
| P | | .43 | | .11 |
| TNCs $\times 10^7$ /kg | | | | |
| Prefreezing > 3.9 | 43 | 58 \pm 7.5 | 43 | 45 \pm 8 |
| Prefreezing ≤ 3.9 | 21 | 33 \pm 11 | 21 | 18 \pm 9 |
| P | | .03 | | .004 |
| Infused > 2.6 | 43 | 56 \pm 8 | 43 | 44 \pm 8 |
| Infused ≤ 2.6 | 21 | 38 \pm 11 | 21 | 20 \pm 10 |
| P | | .14 | | .04 |
| CD34 ⁺ cells $\times 10^5$ /kg | | | | |
| Prefreezing > 1.6 | 40 | 53 \pm 8 | 40 | 46 \pm 8 |
| Prefreezing ≤ 1.6 | 21 | 48 \pm 11.5 | 21 | 24 \pm 9 |
| P | | .52 | | .05 |
| Infused > 1.3 | 36 | 56 \pm 8.5 | 36 | 45 \pm 9 |
| Infused ≤ 1.3 | 18 | 39 \pm 12 | 18 | 39 \pm 12 |
| P | | .5 | | .48 |

UD indicates unrelated donor; UCBT unrelated cord blood transplantation; SAA, severe aplastic anemia; OS, overall survival; RIC, reduced-intensity conditioning; TNCs, total nucleated cells.

Graft-versus-Host Disease

The CI of grade II-IV aGVHD was 20% \pm 5%. Fourteen of 57 single-unit UCBT recipients

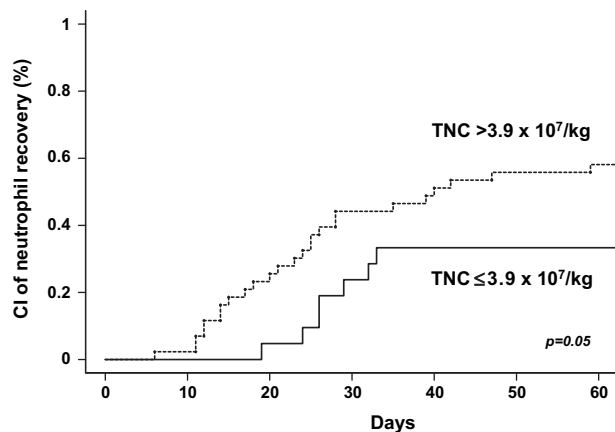


Figure 1. CI of neutrophil recovery according to TNC dose before freezing.

developed aGVHD grade II-IV (CI at 3 years, $21\% \pm 2\%$) compared with 3 of 14 double-unit UCBT recipients (CI at 3 years, $17\% \pm 3\%$). Thirty-four patients were assessable for cGVHD; the CI at 3 years was $18\% \pm 5\%$. Five patients (15%) developed limited and 6 (18%) developed extensive cGVHD. Eight of 57 single UCBT recipients presented cGVHD (CI at 3 years, $17\% \pm 5\%$) compared with 3 of 14 double-unit UCBT recipients (CI at 3 years, $25\% \pm 4\%$). Because of the low number of events, a prognostic factor analysis for the incidence of aGVHD or cGVHD was not performed.

OS

After a median follow-up time for survivors of 35 months (range, 8-83 months), the probability of OS was $38\% \pm 6\%$ at 3 years. The following factors were associated with an improved OS rate (Table 3): recipient sex (59% for female vs 14% for male; $P = .001$), prefreezing NC dose $>3.9 \times 10^7/\text{kg}$ (45% vs 18%; $P = .004$), and infused NC dose $>2.6 \times 10^7/\text{kg}$ (44% vs 20%; $P = .04$). In multivariate analysis, the only factor associated with better OS was prefreezing NC dose ($>3.9 \times 10^7/\text{kg}$; HR, 0.39; 95% confidence interval, 0.2-0.78; $P = .007$) (Figure 2). Of note, 16 of 21 patients (76%) who received MA conditioning died compared with 23 of 46 patients (50%) who received an RIC regimen ($P = .11$). Forty-two patients died during the study. As depicted in Table 4, infections ($n = 16$; 38%) and graft failure ($n = 14$; 32%) were the main causes of death.

DISCUSSION

To the best of our knowledge, the current series is the largest multicenter study reporting the outcomes after UCBT for patients with SAA. We were able to analyze 71 patients who underwent transplantation over a 13-year period. The retrospective settings, the

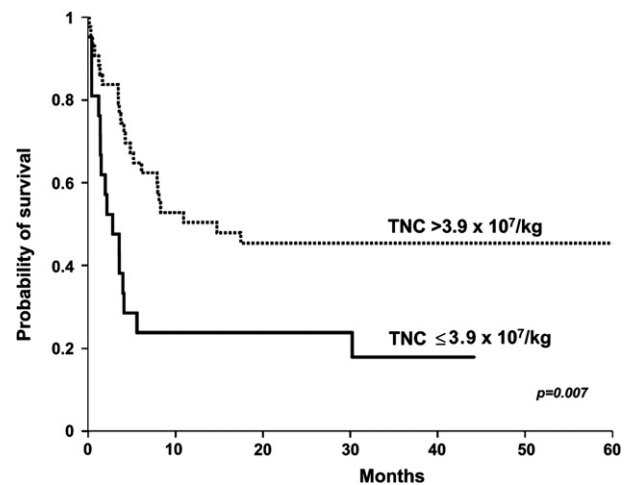


Figure 2. Estimated 3-year OS according to TNC dose.

heterogeneity of conditioning regimens and supportive care, as well as changes in HSCT procedures during the study period, are definitive drawbacks to this type of study. However, we confirm that the main problems encountered with UCBT in SAA are the high incidence of graft failure and infections. The 38% OS at 3 years is quite similar to the 41% probability of OS at 2 years reported in a previous Japanese cooperative study that included 31 patients [21], and is clearly lower than the one recently observed after transplants from an adult unrelated donor (UD) [11]. The main finding of the present study is the demonstration of the strong impact of TNC dose ($>3.9 \times 10^7/\text{kg}$) on outcome, both on engraftment and OS.

Graft rejection has been a major concern for many years in SAA, and cell dose is a recognized key factor for engraftment [28-30]. The low number of hematopoietic progenitor cells and hematopoietic stem cells in umbilical cord blood (UCB) translates into an increased risk of graft failure and delayed hematopoietic engraftment, which may be especially

Table 4. Causes of Death (n = 42)

| Cause | n (%) |
|----------------------------|----------|
| Infections | 16 (38%) |
| Bacterial | 5 (12%) |
| Viral | 3 (7%) |
| Fungal | 3 (7%) |
| Unknown | 2 (5%) |
| EBV-associated LPD | 3 (7%) |
| Rejection or graft failure | 14 (32%) |
| GVHD | 3 (7%) |
| Hemorrhage* | 3 (7%) |
| Multiorgan failure | 3 (7%) |
| Other† | 3 (7%) |

EBV indicates Epstein-Barr virus; LPD, lymphoproliferative disease; GVHD, graft-versus-host disease.

*Includes lung (empyema; $n = 1$); central nervous system, unknown origin ($n = 1$); and gut (dysplasia; $n = 1$).

†Includes interstitial pneumonitis ($n = 1$), veno-occlusive disease ($n = 1$), and missing data ($n = 1$).

relevant for patients with SAA. In the current study, the rate of engraftment was poor (51% and 37% CI for myelogenous and platelet engraftment, respectively) but improved to 58% and 49%, respectively, for patients receiving UCBT with prefreezing TNC dose $>3.9 \times 10^7/\text{kg}$. These data indicate that a patient with a nonmalignant disease should receive a higher cell dose than patients with malignant disease to obtain engraftment. The current recommendations are 4.9×10^7 TNC/kg at collection and 3.5×10^7 TNCs/kg at infusion [29]. Although this finding could suggest a benefit from using two CB units, the small number of patients who received two CB units in this study precludes drawing meaningful conclusions regarding the benefit of double-unit UCBT as an approach to increasing cell dose in this setting. The current study also failed to show any relationship between HLA match and outcomes as has been suggested recently [31]. The huge impact of cell dose and small sample size are possible explanations for this result.

Significantly improved transplantation outcomes have been achieved recently by the use of less immunogenic blood products, such as leukocyte-depleted erythrocytes and platelets collected by cytopheresis [32,33]. Along with advances in supportive care and better HLA matching, this could help explain the improved outcomes of UD-HSCT in patients with SAA in the last decade [7,8]. The long time from diagnosis to transplantation (median, 14 months; range, 2-140 months) indicates that UCBT was the last option for those patients. Furthermore, the majority of patients were heavily transfused before transplantation. Thus, increased risk of rejection because of the potential alloimmunization induced by multiple transfusion exposure and the long duration of disease before transplantation could explain, at least in part, the high rejection rate observed [34]. Patients' pretransplantation anti-HLA antibodies seem to have an impact on neutrophil and platelet engraftment after UCBT [35]. Unfortunately, we were not able to collect data on the presence of HLA antibodies in this highly transfused population and cannot speculate on their potential role on engraftment. Progressive mixed chimerism is known to lead to poor transplantation outcome in HSCT for aplastic anemia [36]. The low proportion of mixed chimerism in our study is congruent with the low incidence of late rejection ($n = 2$) and suggests that once achieved, CB engraftment is durable.

The optimal conditioning regimen for UD-HSCT remains uncertain [7,10,11,37-39]. In our cohort, patients treated with a RIC regimen (mainly Flu-based) showed a trend toward better OS compared with patients who received a standard MA regimen. Moreover, all patients who received TBI 12 Gy died ($n = 10$; data not shown). Thus, if confirmed by others, our findings suggest that an RIC regimen is preferred over an MA regimen in patients with SAA undergoing

UCBT to ensure engraftment and avoid regimen-related morbidity and mortality. Among the few studies published with a reasonable number of patients with SAA who underwent UCBT, the best results were obtained from the combination of Flu + Cy + TBI with ATG [20] or without ATG [21]. The high heterogeneity of conditioning regimens and the high frequency of ATG use in the current study precluded a meaningful analysis of the potential relationship on outcome.

The global incidence of GVHD in our cohort was low despite the degree of HLA mismatch (the majority of our patients received a 4/6 graft). The high frequency of ATG use might have contributed to this finding. Nonetheless, the literature reports a relatively low incidence of severe GVHD after UCBT in SAA [20,21,40]. Thus, a single agent (ie, CsA or tacrolimus) could provide effective GVHD prophylaxis after UCBT in SAA, as has been suggested previously [21].

Infectious complications were a major concern in our population and represented the primary cause of mortality. Previous studies have reported a high rate of severe or fatal infections in UCBT recipients [41,42], especially in adult patients [43], concordant with the delay in immune reconstitution [44]. This is not unexpected in our cohort considering the median disease duration (14 months) before UCBT. Once again, the high rate of ATG use in the current study might have contributed to the burden of infectious mortality. Moreover, 3 patients (7%) died from an Epstein-Barr virus (EBV)-associated lymphoproliferative disorder (LPD). Thus, weekly monitoring of EBV DNA levels in this setting is essential [45]. Early use of anti-CD20 in patients with increasing viral load also could be beneficial [45-47].

In conclusion, this study demonstrates the pivotal role of TNC dose ($>3.9 \times 10^7/\text{kg}$ TNCs/kg) on both engraftment and OS using unrelated CB as the stem cell source in patients with SAA. Graft failure and infection remain major issues in this particularly high-risk population. The result of well-designed prospective trials, like one currently underway in France, which incorporate the requirement of a large cell dose and hopefully demonstrate better OS, are needed before including UCBT in the treatment strategy for SAA can be recommended.

ACKNOWLEDGMENTS

The authors would like to thank the following investigators for their contribution to this study: Claudio Brunstein, Fairview University of Minnesota, Minneapolis, USA; Jean François Rossi, Lapeyronie Hospital, Montpellier, France; Judith Marsh, Kings College London School of Medicine, London, UK; Christian Urban, Medical University Graz, Graz, Austria;

François Guilhot, La Miletie Hospital, Poitiers, France; Bernard Rio, Hotel Dieu Hospital, Paris, France; Alois Gratwohl, University Hospital Basel, Basel, Switzerland; Nalini Janakiraman, Henry Ford Hospital, Detroit, USA; Andrea Soria, Hospital Privado Centro Médico de Cordoba, Argentina; Mitchel Cairo, Children's Hospital of Columbus, New York, USA; Ann Woolfrey, Fred Hutchinson Cancer Research Center, Seattle USA; Marcos de Lima, MD Anderson Cancer Center, Houston, USA; Karen Ballen, Dana Farber Cancer Institute, Boston, USA; Jerry Stein, Schneider Children's Medical Center of Israel, Petach Tikvah, Israel; Yves Benoit, Paediatric Clinic C. Hooft, Gent, Belgium; Ana Maria Martínez-Rubio, Hospital Infantil, Universitario La Paz, Madrid, Spain; Alessandro Rambaldi, Ospedale Bergamo, Bergamo, Italy; Franco Locatelli, Policlinico San Matteo, Pavia, Italy; Jean Pierre Jouet, Hopital Claude Huriez, Lille, France; Paolo Di Bartolomeo, Civile Hospital, Pesaro, Italy; Jan Cornelissen, Erasmus MC-Daniel den Hoed Cancer Centre, Rotterdam, Netherlands; Arturo Iriando Atienza, Hospital U. Marqués de Valdecilla, Spain; Anna Paola Iori, Univ. 'La Sapienza', Rome, Italy; Ulla Pihkala, University of Helsinki, Helsinki, Finland; Alain Fisher, Necker Hospital, Paris, France.

Financial disclosure: Regis Peffault de Latour was supported by a bursary award from the Aplastic Anemia and Myelodysplastic Syndrome International Foundation and a grant from France Hemoglobinurie Paroxystique Nocturne.

AUTHORSHIP STATEMENT

Regis Peffault de Latour designed the study, analyzed data, and wrote the manuscript. Duncan Purtill and Annalisa Ruggeri collected and analyzed data and edited the manuscript. Guillermo Sanz, Gerard Michel, Virginie Gandemer, Sebastien Maury, Joanne Kurtzberg, Carmen Bonfim, Mahmoud Aljurf, Eliane Gluckman, Gerard Socié, and Jakob Passweg collected data and edited the manuscript. Vanderson Rocha designed the study, analyzed data, and wrote the manuscript.

REFERENCES

- Horowitz MM. Current status of allogeneic bone marrow transplantation in acquired aplastic anemia. *Semin Hematol.* 2000;37:30-42.
- Frickhofen N, Rosenfeld SJ. Immunosuppressive treatment of aplastic anemia with antithymocyte globulin and cyclosporine. *Semin Hematol.* 2000;37:56-68.
- Kojima S, Hibi S, Kosaka Y, et al. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. *Blood.* 2000;96:2049-2054.
- Rosenfeld S, Follmann D, Nunez O, et al. Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome. *JAMA.* 2003;289:1130-1135.
- Frickhofen N, Heimpel H, Kaltwasser JP, et al. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. *Blood.* 2003;101:1236-1242.
- Young NS, Scheinberg P, Calado RT. Aplastic anemia. *Curr Opin Hematol.* 2008;15:162-168.
- Deeg HJ, O'Donnell M, Tolar J, et al. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. *Blood.* 2006;108:1485-1491.
- Maury S, Balere-Appert ML, Chir Z, et al. Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. *Haematologica.* 2007;92:589-596.
- Deeg HJ, Amlyon ID, Harris RE, et al. Marrow transplants from unrelated donors for patients with aplastic anemia: minimum effective dose of total body irradiation. *Biol Blood Marrow Transplant.* 2001;7:208-215.
- Kojima S, Matsuyama T, Kato S, et al. Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: the Japan Marrow Donor Program. *Blood.* 2002;100:799-803.
- Bacigalupo A, Socie G, Lanino E, et al. Fludarabine, Cyclophosphamide, Antithymocyte Globulin, with or without 2 Gy TBI, for alternative donor transplants in Acquired Aplastic Anemia (SAA): A report from the EBMT-SAA Working Party, *Haematologica.* 2010;95:976-982.
- Kennedy-Nasser AA, Leung KS, Mahajan A, et al. Comparable outcomes of matched-related and alternative donor stem cell transplantation for pediatric severe aplastic anemia. *Biol Blood Marrow Transplant.* 2006;12:1277-1284.
- Young N, Bacigalupo A, Marsh J. Aplastic Anemia: Pathophysiology and Treatment. *Biol Blood Marrow Transplant.* 2009;15:119-25.
- Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med.* 1998;339:1565-1577.
- Sauter C, Barker JN. Unrelated donor umbilical cord blood transplantation for the treatment of hematologic malignancies. *Curr Opin Hematol.* 2008;15:568-575.
- Neudorf SM, Blatt J, Corey S, et al. Graft failure after an umbilical cord blood transplant in a patient with severe aplastic anemia. *Blood.* 1995;85:2991-2992.
- Lau FY, Wong R, Chui CH, et al. Successful engraftment in two adult patients with severe aplastic anemia using nonmyeloablative conditioning followed by unrelated HLA-mismatched cord blood transplantation. *J Hematother Stem Cell Res.* 2001;10:309-311.
- Mao P, Zhu Z, Wang H, et al. Sustained and stable hematopoietic donor-recipient mixed chimerism after unrelated cord blood transplantation for adult patients with severe aplastic anemia. *Eur J Haematol.* 2005;75:430-435.
- Ohga S, Ichino K, Goto K, et al. Unrelated donor cord blood transplantation for childhood severe aplastic anemia after a modified conditioning. *Pediatr Transplant.* 2006;10:497-500.
- Chan KW, McDonald L, Lim D, et al. Unrelated cord blood transplantation in children with idiopathic severe aplastic anemia. *Bone Marrow Transplant.* 2008;42:589-595.
- Yoshimi A, Kojima S, Taniguchi S, et al. Unrelated cord blood transplantation for severe aplastic anemia. *Biol Blood Marrow Transplant.* 2008;14:1057-1063.
- Gluckman E, Rocha V, Ionescu I, et al. Results of unrelated cord blood transplant in Fanconi anemia patients: risk factor analysis for engraftment and survival. *Biol Blood Marrow Transplant.* 2007;13:1073-1082.
- Bacigalupo A, Ballen K, Rizzo D, et al. Defining the intensity of conditioning regimens: working definitions. *Biol Blood Marrow Transplant.* 2009;15:1628-1633.
- Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995;15:825-828.
- Byar DP. *Cancer Clinical Trials: Methods and Practice.* Oxford, UK: Oxford Medical Publications; 1988.

26. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.
27. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competitive risk. *Ann Stat*. 1988;16:1141-1154.
28. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med*. 2004;351:2276-2285.
29. Rocha V, Gluckman E. Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. *Br J Haematol*. 2009;147:262-274.
30. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med*. 2004;351:2265-2275.
31. Barker JN, Scaradavou A, Stevens CE. Combined effect of total nucleated cell dose and HLA-match on transplant outcome in 1061 cord blood recipients with hematological malignancies. *Blood*. 2009;115:1843-1849.
32. Stroncek DF, Rebulla P. Platelet transfusions. *Lancet*. 2007;370:427-438.
33. Klein HG, Spahn DR, Carson JL. Red blood cell transfusion in clinical practice. *Lancet*. 2007;370:415-426.
34. Ades L, Mary JY, Robin M, et al. Long-term outcome after bone marrow transplantation for severe aplastic anemia. *Blood*. 2004;103:2490-2497.
35. Takanashi M, Fujiwara K, Atsuta Y, et al. Anti-HLA antibodies in unrelated cord blood transplantation, Bone Marrow Transplantation. 2009;43, Sup 1 abstract 143.
36. Lawler M, McCann SR, Marsh JC, et al. Serial chimerism analyses indicate that mixed haemopoietic chimerism influences the probability of graft rejection and disease recurrence following allogeneic stem cell transplantation (SCT) for severe aplastic anaemia (SAA): indication for routine assessment of chimerism post-SCT for SAA. *Br J Haematol*. 2009;144:933-945.
37. Bacigalupo A, Locatelli F, Lanino E, et al. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. *Bone Marrow Transplant*. 2005;36:947-950.
38. Georges GE, Storb R. *Allogeneic Hematopoietic Cell Transplantation for Aplastic Anemia*. Oxford, UK: Blackwell; 2004.
39. Khouri IF, Keating M, Korbly M, et al. Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. *J Clin Oncol*. 1998;16:2817-2824.
40. Kang HJ, Shin HY, Choi HS, et al. Fludarabine, cyclophosphamide plus thymoglobulin conditioning regimen for unrelated bone marrow transplantation in severe aplastic anemia. *Bone Marrow Transplant*. 2004;34:939-943.
41. Saavedra S, Sanz GF, Jarque I, et al. Early infections in adult patients undergoing unrelated donor cord blood transplantation. *Bone Marrow Transplant*. 2002;30:937-943.
42. Long GD, Laughlin M, Madan B, et al. Unrelated umbilical cord blood transplantation in adult patients. *Biol Blood Marrow Transplant*. 2003;9:772-780.
43. Safdar A, Rodriguez GH, De Lima MJ, et al. Infections in 100 cord blood transplantations: spectrum of early and late post-transplant infections in adult and pediatric patients 1996-2005. *Medicine (Baltimore)*. 2007;86:324-333.
44. Komanduri KV, St John LS, de Lima M, et al. Delayed immune reconstitution after cord blood transplantation is characterized by impaired thymopoiesis and late memory T-cell skewing. *Blood*. 2007;110:4543-4551.
45. van Esser JW, Niesters HG, van der Holt B, et al. Prevention of Epstein-Barr virus lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. *Blood*. 2002;99:4364-4369.
46. Gartner BC, Schafer H, Marggraff K, et al. Evaluation of use of Epstein-Barr viral load in patients after allogeneic stem cell transplantation to diagnose and monitor posttransplant lymphoproliferative disease. *J Clin Microbiol*. 2002;40:351-358.
47. Blaes AH, Cao Q, Wagner JE, et al. Monitoring and preemptive rituximab therapy for Epstein-Barr virus reactivation after anti-thymocyte globulin containing nonmyeloablative conditioning for umbilical cord blood transplantation, Biol Blood Marrow Transplant. 2010;16:287-291.