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EXPERIMENTAL HEMATOLOGY

Experimental Hematology 32 (2004) 397–407

Factors associated with outcomes of unrelated cord blood transplant: Guidelines for donor choice

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(Received 17 November 2003; revised 6 January 2004; accepted 16 January 2004)

Objective. **Optimizing cord blood donor selection based mainly on cell dose and human leukocyte antigen (HLA) disparities may further improve results of unrelated cord blood transplants (UCBT).**

Materials and Results. **We analyzed 550 UCBTs for hematologic malignancies reported to the Eurocord Registry. Main outcomes and prognostic factors were analyzed in univariable and multivariable analyses incorporating center and period effects and using death and relapse as competitive risks for nonfatal endpoints. Nucleated cell (NC) dose before freezing and number of HLA disparities had a significant influence on outcome. Cumulative incidence (CI) of neutrophil and platelet recovery was associated with the number of HLA mismatches, number of NC before freezing, and use of granulocyte colony-stimulating factor. Coexistence of HLA class I and II disparities and high CD34 cell dose in the graft were associated with graft-vs-host disease grades III–IV. CI of disease relapse was higher in matched transplants showing a graft-vs-leukemia effect increased in HLA-mismatched transplants. Overall 3-year survival was 34.4%. Prognostic factors for survival were recipient age, gender, and disease status.**

Conclusion. **Our results provide indications for a better choice of cord blood units according to cord blood cell content and HLA.** © **2004 International Society for Experimental Hematology. Published by Elsevier Inc.**

Wider application of allogeneic bone marrow transplantation (BMT) is limited by the absence of suitable human leukocyte antigen (HLA)-matched donors and the occurrence of complications related to graft vs-host disease (GvHD), which are more severe with increasing HLA disparity. An important advance in the field of allogeneic hematopoietic stem cell (HSC) transplant is the use of alternative sources of HSC, which has allowed us to extend the indications of allogeneic transplantation to patients lacking an HLA-identical sibling. In the absence of such a donor, mismatched-related or matched-unrelated donors are sought. Results of unrelated donor transplants have improved over time, mainly due to

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0301-472X/04 $–see front matter.
doi: 10.1016/j.exphem.2004.01.002

the introduction of high-resolution molecular HLA typing, although use of this technique has decreased the probability of finding a donor matched for HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ and HLA-DP loci [1–6]. Despite the increasing number of bone marrow donor registries that now contain more than eight million donors worldwide, some patients cannot be transplanted because of the lack of HLA-identical donors. Since the first successful allogeneic umbilical cord blood transplant (UCBT) performed in 1988 to treat a child with Fanconi anemia [7], the development of cord blood banks (CBBs) and the number of transplants performed have been increasing steadily. This first success opened the way to an entire new field in the domain of allogeneic HSC transplantation with the demonstration that a single umbilical cord blood unit could be cryopreserved, thawed, and transplanted in a myeloablated host and could permanently engraft. Compared to adult HSCs, cord blood HSCs have a proliferative advantage, and cord blood immune cells are less reactive than adult lymphocytes, which decreases the risk of severe GvHD. Umbilical CBBs have been established, with more than 100,000 units available. More than 2,500 UCBTs have been performed in children and, increasingly, in adults with malignant and nonmalignant diseases [8–13]. Previous studies have shown that the results of UCBT were comparable to those obtained in BMT recipients. In terms of survival, engraftment was delayed but GvHD was reduced [14–16]. These results are related to cell dose and probably to the number of HLA disparities.

This study was designed to assess the influence of pretransplant donor-related factors, including the cell content of the graft and the number and type of HLA incompatibilities (both factors on which intervention could be easily made), on transplant outcomes in an attempt to delineate guidelines for donor choice. This could help clinicians to choose the best cord blood unit based on these data and other patient-related risk factors.

Patients and methods

Data collection and study population

Eurocord, which operates on behalf of the European Blood and Marrow Transplant Group (EBMT), is an international registry that includes European and non-European centers, all performing either related or unrelated cord blood transplants. It works in close collaboration with Netcord banks (*www.netcord.org*) [10,17,18].

The EBMT Registry is a voluntary working group of more than 450 transplant centers located mainly in Europe. The participants are required to report all consecutive stem cells transplantations and follow-up on an annual basis. The Eurocord and EBMT databases are carefully checked to detect overlaps and discrepancies in reported data and to verify the compliance of the transplant centers. This ensures that all consecutive transplants at the EBMT centers are registered in our database.

Non-EBMT centers report their cord blood transplants unless cord blood units do not come from Netcord banks, which inform our group of all units delivered to these transplant centers. Then, similarly to EBMT centers, we prospectively collected clinical data and outcomes directly from the transplant centers on an annual basis.

For each transplant, data on patient and disease characteristics and transplant outcomes were collected by standardized questionnaires. Submitted data were reviewed by two physicians, and computerized error checks were performed to ensure data quality. Criteria of inclusion in the study were as follows: all consecutive UCBTs with a minimum follow-up of 3 months, receiving a single-unit, nonexpanded, unrelated-donor UCBT together with complete data on HLA typing (A and B by serology typing and DRB1 by allelic typing).

Most often, the thawing procedure followed the technique described by Rubinstein et al. [8,9]. Data on conditioning regimen, GvHD prophylaxis, and supportive care were collected for all patients.

CBBs and HLA typing

Twenty-three banks provided cord blood units (Appendix 2). Cord blood unit characteristics provided by the banks were date of collection, volume, number of cells collected, number of CD34, CFU-GM, ABO groups, sex, infectious markers, and HLA typing. The data were double checked with the transplant centers and any discrepancies clarified. In addition, transplant centers provided information on the number of nucleated cells (NC) and $CD34^+$ cells infused. Donor-recipient histocompatibility was determined by serology for HLA-A and HLA-B antigens and by DNA typing for the DRB1 locus. All HLA data were reviewed, and queries regarding patient and donor HLA typing were verified by the transplant centers. Transplants were classified as HLA-mismatched with 1, 2, 3, or 4 differences if disparities were detected in HLA-A, HLA-B, or HLA-DRB1 loci.

Outcomes

Hematopoietic recovery. Neutrophil recovery (defined as a neutrophil count $>0.5 \times 10^9$/L for 3 consecutive days) and platelet recovery (defined as an untransfused platelet count $>20 \times 10^9$/L for 7 consecutive days) were analyzed separately.

Graft-vs-host disease. Acute and chronic GvHD were diagnosed and graded at each transplant center according to the Seattle criteria [19,20]. All patients were considered at risk for developing GvHD starting from day +1 after transplant, given that this complication has been observed as early as day +4. Patients who survived for more than 100 days posttransplant with sustained donor engraftment were considered at risk for developing chronic GvHD, which, if it occurred, was graded as limited or extensive.

Relapse. Relapse was defined and analyzed as time from transplant to date of evidence of malignant cells in peripheral blood, bone marrow, or extramedullary sites. All patients were analyzed in the same manner, independent of disease status.

One-hundred-day transplant-related mortality. One-hundred-day transplant-related mortality (TRM) was defined taking into account all nonleukemia deaths occurring within 100 days after transplantation.

Overall survival. Overall survival, whatever the cause of death, was computed from the day of transplant.

Statistical analysis

For analysis, July 1, 2002 was used as the reference date, that is, the day on which all centers locked data on patient outcomes. The outcomes after transplantation were all right-censored. For overall survival, Kaplan-Meier estimates provided estimated incidence over time. Cox models were used to evaluate the joint influence of patient-, disease-, and transplant-related variables (Table 1) on outcome. However, the other (nonfatal) endpoints shared a competing-risks setting, that is, patients could develop events that precluded the occurrence of the event of interest; for example, after death or relapse, no GvHD and no recovery could occur. Therefore, these other endpoints (neutrophil recovery, acute and chronic GvHD, relapse, TRM) were analyzed by cumulative incidence curves for estimating incidence over time and by Fine and Gray models [21] to assess prognostic factors. In each case, model selection used the same sequence of steps. Due to the previously reported center effect on outcome of patients who underwent BMT [22], we first tested for center effect on each outcome, using frailty models unadjusted and adjusted for covariates shown as either prognostic or imbalanced between large and small centers. When significant, the center effect was incorporated in subsequent prognostic analyses using marginal Cox models. Moreover, we previously observed that transplants performed after 1998 had a better outcome than transplant preformed earlier. Therefore, we tested for period effect; if period effect was significant, we then used stratified Cox models. To assess the joint influence of patient-, disease-, and transplant-related variables on each outcome (besides possible center and period effects), we first fitted univariable models. Second, all variables with $p < 0.05$ determined by the likelihood ratio test were included in a multivariable model. Cause-specific hazard ratios (HR) were estimated with 95% confidence intervals (95%CI). Finally, first-order interactions between cell dose and HLA disparities were tested. Statistical analysis was performed using the SAS 8.2 (SAS, Inc., Cary, NC, USA) and S-Plus 2000 (MathSoft, Inc., Seattle, WA, USA) software packages.

Results

Patients characteristics

A total of 550 patients transplanted from July 1994 to December 2001 (median follow-up 30.5 months, range 6–90) were analyzed. Four hundred four (73%) were transplanted after January 1998. Questionnaires were provided by 107 transplant centers from 28 countries; 15 (14%) of these centers included more than 10 patients each, 19 (18%) between 5 and 10 patients, and 73 (68%) fewer than 5 patients. Table 1 lists the main characteristics of the patients, donors, and transplant factors. Median recipient age was 9.4 years (range 100 days–62 years); 356 (65%) patients were younger than 15. Most patients (68%) were transplanted for acute leukemia at various stages. Disease status was defined as early (first remission of acute leukemia or first chronic phase for chronic leukemia), intermediate (subsequent remission for acute leukemia, complete remission for other hematologic malignancies, and accelerated phase for chronic leukemia), or advanced (patients not in remission for acute leukemia or other hematologic malignancies, blastic crisis

Table 1. Patient-, disease-, transplant-related characteristics of the patients

Characteristics*	No. of patients (%) or median (Q1–Q3)
Center size (no. of patients)	
≤5	137 (25%)
5–10	107 (19.5%)
≥10	306 (55.5%)
Age (years [n = 550])	9.4 (4.5–21.1)
Children (age ≤15)	356 (65%)
Male gender	308 (56%)
Weight (kg [n = 548])	33.9 (17–55)
Diagnosis	
Acute myeloid leukemia	237 (43%)
Acute lymphoblastic leukemia	118 (21%)
Secondary acute leukemia	20 (4%)
Chronic leukemia	68 (12%)
Lymphoma	25 (5%)
Myelodysplasia	60 (11%)
Other*	22 (4%)
Disease status at transplant	
Early	105 (19%)
Intermediate	210 (38%)
Advanced	232 (43%)
For leukemia	
Favorable (first CR + 2CR + 1CP)	222/443 (50%)
Previous transplant	84 (16%)
Graft after 1998	404 (73%)
Positive CMV serology	309/541 (57.1%)
Donor sex/recipient sex	
Male/male	143 (27%)
Male/female	119 (23%)
Female/male	150 (28%)
Female/male	115 (22%)
ABO compatibility	
Identical/minor/major disparities	211 (39%)
	134 (25%)
	192 (36%)
HLA compatibility	
match/class I/class II/class I+II	53 (10%)
	242 (44%)
	105 (19%)
	150 (27%)
No. of cells at freezing	
Nucleated cells (10^7/kg [n = 532])	4.06 (2.6–7.2)
CD34 (10^5/kg [n = 277])	1.37 (0.77–2.49)
No. of infused cells	
Nucleated cells (10^7/kg [n = 546])	3.11 (1.89–5.20)
CD34 (10^5/kg [n = 383])	1.38 (0.70–2.72)
Cell loss after thawing (% [n = 492])	22.4 (5–37)
GvHD prophylaxis	
CsA alone	44 (8%)
CsA + Prednisone	360 (65%)
CsA + Prednisone + ALG	20 (4%)
CsA + MTX±Prednisone	65 (12%)
Tracolimus + MTX	29 (5%)
Others	32 (6%)
MTX-containing regimens	105 (19%)
Early hematopoietic growth factors+	329 (60%)
Irradiation containing regimens	315 (57%)
Associated with one drug	124
Associated with two or more drugs	191
Busulfan-containing regimens	235 (43%)
Associated with one drug	99
Associated with two or more drugs	136

Q-quadratile; CR = complete remission; CP = chronic phase; CMV = cytomegalovirus; CsA = cyclosporine A; ALG = antilymphocyte globulin; MTX = methotrexate.

*For continuous variables, number of patients available are reported.

+Early means between day 0 to day +7 after unrelated and blood transplantation.

for chronic leukemia, secondary leukemia). Eighty-four patients 84 (16%) underwent UCBT after failure of a first autologous or allogeneic BMT. The conditioning regimen varied according to disease and disease status: 79 (14%) patients were conditioned with busulfan and cyclophosphamide; 93 (17%) with total body irradiation and cyclophosphamide; and the remainder with 1 of these 2 associations plus other drugs. Four hundred forty-four (81%) patients received antilymphocyte antibody during the conditioning regimen. Fifty-three (10%) patients were HLA matched, 243 (44%) patients had 1 HLA difference, 218 (40%) had 2 HLA differences, and 36 (6%) patients had 3 or more HLA differences with the donor. With regard to HLA disparities, 242 (44%) patients had 1 or 2 class I mismatches, 105 (19%) patients had 1 or 2 class II mismatches, and 150 (27%) patients had mismatches for both class I and II loci. The median number of NC infused was 3.11×10^7/kg, and the median number of CD34 cells infused was 1.38×10^5/kg. Methotrexate was given in addition to cyclosporine A in 105 (19%) patients; granulocyte colony-stimulating factor (G-CSF) was administered for accelerating neutrophil recovery in 329 (60%) patients.

Overall, a total of 416 patients had neutrophil recovery (16 after day 60), 196 developed grade II–IV acute GvHD, 120 relapsed (including 30 with acute GvHD), and 330 died. The main outcome measures are listed in Table 2. Of note, a significant center effect—even after adjustment—was observed for neutrophil recovery, acute GvHD, and TRM. Therefore, the center effect was incorporated in further analysis of these endpoints. Nonparametric estimates of cumulative incidence (CI) curves of each outcome are shown in Fig. 1.

Early endpoints

Neutrophil engraftment. Overall, 416 patients had neutrophil engraftment, and 122 either relapsed or died before engraftment (with 60-day CI of 74%; Fig. 1a and Table 2). As reported in Table 2, there was a center effect and a period effect on neutrophil recovery, so subsequent multivariable analyses were based on stratified marginal Cox models. In multivariable analysis, the number of HLA disparities (with 60 day-CI of 83% in case of no HLA disparity down to 53.2% in case of at least 3 HLA disparities; HR = 0.786, 95%CI = 0.681–0.907, $p = 0.0010$) and the number of NC at freezing (with 60 day-CI of 79.6% when $\geq 4 \times 10^7$/kg vs 69% when $< 4 \times 10^7$/kg; HR = 1.004, 95%CI = 1.001–1.006, $p = 0.00077$) were associated with the occurrence of neutrophil recovery, jointly with the early use of G-CSF (with 60 day-CI of 75.9% vs 71.1% without hematopoietic growth factor [mainly G-CSF]; HR = 1.66, 95%CI = 1.34–2.05, $p < 0.0001$). More specifically, the hazard of neutrophil recovery was log linearly related to the number of HLA disparities (the higher the number of disparities, the lower the hazard; Fig. 2a) and to the number of NC at freezing, whatever the number of HLA disparities, although the slope decreased for 3–4 disparities. Finally, there was no statistical evidence of any interaction between cell dose and HLA disparities ($p = 0.81$).

Table 2. Main outcome measures: incidence and evaluation of center effect and period effect

Early outcomes	60-day cumulative incidence (95CI)	100-day cumulative incidence (95CI)	180-day cumulative incidence (95CI)	*P* Value for testing center effect (nonadjusted/adjusted*)	*P* Value for testing period effect†
Neutrophil recovery	74.0% (70.3–77.6%)	76.3% (72.7–79.9%)		0.0019 0.0120	0.040
Platelet recovery	28.6% (24.8–32.4%)	43.3% (39.0–47.5%)	50.5% (46.2–54.8%)	0.0055 0.0740	0.12
Acute GvHD grade ≥II	33.3% (29.3–37.2%)	35.8% (31.8–39.8%)		0.000031 0.000016	0.64
Acute GvHD grade ≥III	19.1% (15.8–22.4%)	20.1% (16.7–23.5%)		0.083 0.054	0.45
Treatment-related mortality	22.5% (19.0–26.0%)	34.2% (30.2–38.2%)		0.000026 0.013	0.0036
Late outcomes	12-month cumulative incidence (95CI)	3-year cumulative incidence (95CI)	5-year cumulative incidence (95CI)	*P* Value for testing center effect (nonadjusted/adjusted*)	
Chronic GvHD	25.9% (20.5–31.3%)			0.026 0.030	0.062
Relapse	21.5% (17.9–25.1%)	25.4% (21.3–29.5%)	26.2% (21.9–30.5%)	0.04 0.11	0.62
Overall survival	40.8% (36.5–45.1%)	34.4% (29.8–39.0%)	30.0% (23.5–36.5%)	0.0038 0.079	0.02

GVHD = graft-vs-host disease.

*Through the use of frailty models, either adjusted or not on period, sex, age, good risk, major ABO incompatibility, HLA mismatch, period, busulfantcyclosphosphamide, and cytomegalovirus serology.

†Using marginal Cox model with period introduced as the single covariate.

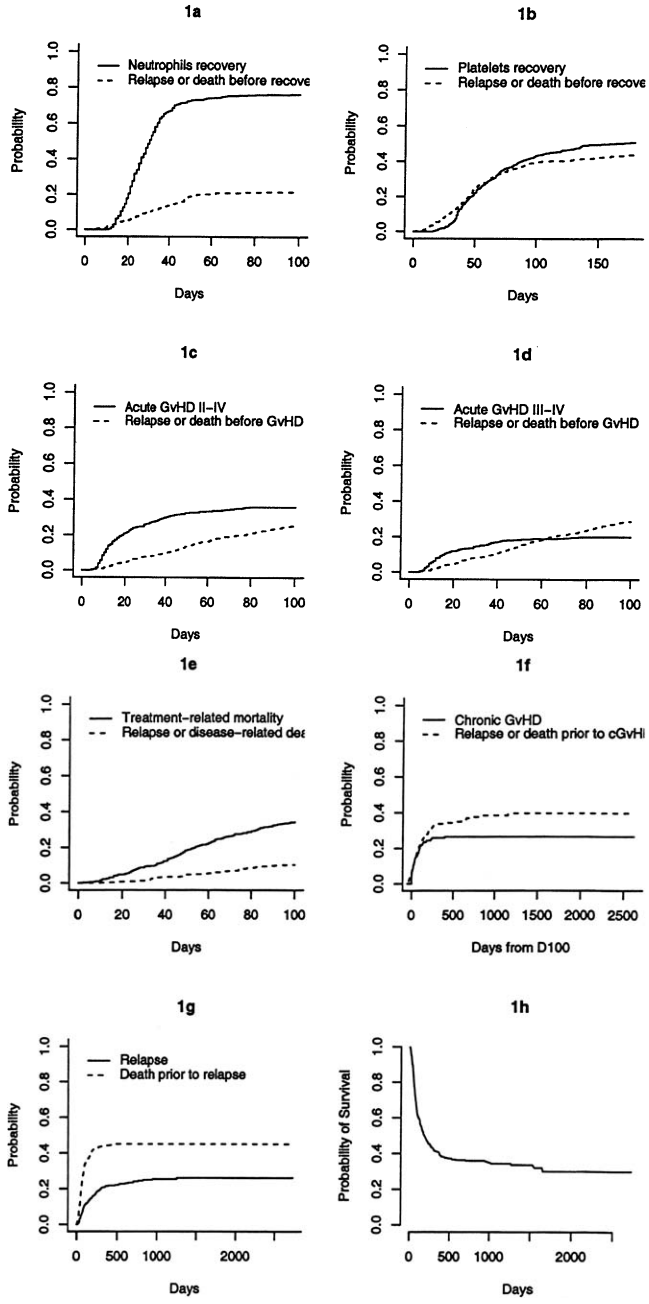

Figure 1. Nonparametric estimates of cumulative incidence curves of each outcome and competing risk events for **(a)** neutrophil recovery ($>0.5 \times 10^9$/L), **(b)** platelet recovery ($>20 \times 10^9$/L), **(c)** acute graft-vs-host disease (GvHD) II–IV, **(d)** acute GvHD III–IV, **(e)** transplant-related mortality, **(f)** chronic GvHD, **(g)** relapse, and **(h)** Kaplan-Meier curve for overall survival. Note that competing risks events refer to events that could arise simultaneously in the patient population, the occurrence of each modifying the subsequent risk of occurrence of the others. In other words, competing risks events refer to events to which any population is simultaneously exposed to, while the occurrence of any of them prevents or modifies the probability of occurrence of the others. In that sense, occurrence of neutrophil or platelet recovery, which actually are "desirable" events, could be considered as events, whereas death prior to recovery defines a competing risks event. In such competing risks analyses, it should be kept in mind that only first events are considered so that, for example, secondary relapses after GvHD are not taken into account.

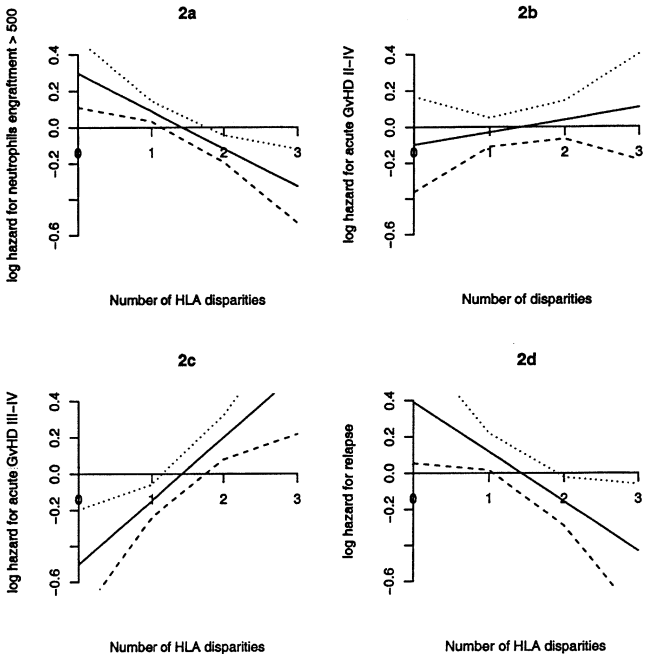

Figure 2. Nonparametric estimation (using splines) of the effect of HLA disparities on the hazard of **(a)** neutrophil recovery, **(b)** acute graft-vs-host disease (GvHD) II–IV, and **(c)** acute GvHD III–IV, and **(d)** relapse. Dotted lines represent the confidence interval. The figure allows clearly depiction of the influence of HLA disparities on each outcome, exhibiting the linearity of the effect (on a log scale). It also points out that the higher the number of HLA disparities, the lower the risk ("chance") of engraftment **(a)**, the higher the risk of acute GvHD grade III–IV **(c)** [whereas it has no impact on the risk of grade II–IV GvHD **(b)**], and the lower the risk of relapse **(d)**.

Platelet recovery. Platelet recovery was observed in 263 patients. Estimated CI of platelet recovery over time (Fig. 2b) reached 50.5% at day 180 (Table 2). As reported in Table 2, there was a center effect for platelet recovery but there was no period effect (with a 180 day-CI of 43.5% before 1998 and 53% thereafter, $p = 0.12$), so prognostic analyses used marginal Cox models. Based on multivariable analysis, a high number of infused NC (with 180 day-CI of 42.4% when $<3.11 \times 10^7$/kg vs 59.9% when $\geq 3.11 \times 10^7$/kg; HR = 1.03, 95%CI = 1.00–1.07, $p = 0.05$) and absence of both class I and II HLA disparities (with 180 day-CI of 53.1% vs 43.3% in case of coexistence of class I and II disparities; HR = 1.46, 95%CI = 1.12–1.92, $p = 0.006$) were jointly selected as predictive of improved platelet recovery, with young age (with 180 day-CI of 58.2% in children vs 36.5% in adults; HR = 0.984, 95%CI = 0.972–0.995, $p = 0.0034$) and early stage of disease (with 180 day-CI of 55.3% vs 41.8% in advanced stage; HR = 1.476, 95%CI = 1.15–1.89, $p = 0.0021$). No statistical evidence of any interaction between cell dose and type ($p = 0.14$) or number ($p = 0.92$) of HLA disparities was found.

Acute GvHD II–IV and III–IV. At day 100, 196 patients had developed grade II–IV acute GvHD, whereas 136 relapsed or

died free of acute GvHD. There were 116 patients in grade I, 86 grade II, 59 grade III, and 51 grade IV. The estimated 100-day CI of grade II–IV acute GvHD was 35.8% and of grade III acute GvHD was 20.1% (Fig. 1c and d, and Table 2). There was no period effect but there was a significant center effect on GvHD II–IV (Table 2), which was taken into account thereafter by using marginal Cox models. Prognostic analyses did not identify any transplant, disease, or donor characteristics associated with increased hazard of acute GvHD grade II–IV. Notably, the number of HLA disparities was not associated with the hazard of acute GvHD of grade II or higher ($p = 0.41$; Fig. 2b), nor was the number of CD34 infused cells ($p = 0.39$). In contrast, the high number of CD34 cells at freezing (HR = 1.01, 95%CI = 1.005–1.019, $p = 0.00041$) and the coexistence of class I and II HLA disparities (HR = 1.876, 95%CI = 1.096–3.21, $p = 0.0093$) were associated with the occurrence of grade III–IV acute GvHD. There was no interaction between HLA disparities and cell dose ($p = 0.19$).

Treatment-related mortality. At 100 days, 56 patients relapsed or died of causes related to the disease, and 186 died of treatment-related causes (Fig. 1e). The principal causes of death during the first 100 days were infection in 38%, toxicity including cardiac failure, liver veno-occlusive disease, respiratory failure, and hemorrhage in 26.63%, acute GvHD in 17.39%, and rejection in 4.89%. As reported in Table 2, there was a center effect and a period effect ($p = 0.0036$) on TRM. In multivariable analysis, TRM was only related to old age (HR = 1.03, 95%CI = 1.02–1.04, $p < 0.0001$), recipient female gender (HR = 1.43, 95%CI = 1.07–1.90, $p = 0.014$), and advanced stage of disease (HR = 1.48, 95%CI = 1.07–2.06, $p = 0.018$). No additive prognostic information was achieved by donor-related factors such as cell dose ($p = 0.40$), and type ($p = 0.83$) and number ($p = 0.58$) of HLA disparities.

Long-term outcomes

Chronic GvHD. Of the 265 patients who had engrafted and still were alive at day 100, 69 developed chronic GvHD and 79 died free of chronic GvHD (Fig. 1f and Table 2). The chronic GvHD was limited in 33 (48%) and extensive in 36 (52%) cases. There was a significant center effect and a trend toward an increased incidence of chronic GvHD after 1998 compared to the previous time period (with an estimated 1-year CI of 25.9% after 1998 and 15.9% before, $p = 0.062$). Using a multivariable model, none of the variables analyzed was significantly associated with outcome.

Relapse. A total of 120 patients relapsed (of whom 94 died), and 236 died before experiencing relapse (Fig. 1g and Table 2). There was a center effect but no period effect on the incidence of relapse (Table 2). The multivariable model retained a low number of HLA disparities (with 1-year CI of relapse of 14.3% in UCBT with 1, 2, or 3 HLA disparities compared to 28.8% with an HLA-matched UCBT; HR = 0.79, 95%CI = 0.63–1.00, $p = 0.05$) as predictive of subsequent relapse, jointly with young age (with 1-year CI of relapse of 25.4% in children vs 14.6% in adults; HR = 0.97, 95%CI = 0.95–0.99, $p = 0.0032$), while the influence of cell dose was erased ($p = 0.09$). Of note, higher the number of HLA disparities, the lower the hazard of relapse (Fig. 2d), suggesting the presence of a graft-vs-leukemia or graft-vs-tumor effect in HLA-mismatched compared to HLA-matched transplants.

Overall survival. Overall, 330 deaths were reported. Estimated 3-year survival for all patients was 34.4 % (Fig. 1h and Table 2). As shown in Table 2, survival was related to the center as well as to the period of transplantation, with a decreased mortality after 1998 (median survival 232 days, HR = 0.72, 95%CI = 0.55–0.95) compared to before 1998 (median survival 100 days, $p = 0.02$). Multivariable model only selected recipient age (HR = 1.017, 95%CI = 1.009–1.025, $p < 0.0001$), recipient female gender (HR = 1.34, 95%CI = 1.06–1.70, $p = 0.015$), and advanced stage of disease (HR = 1.67, 95%CI = 1.37–2.04, $p < 0.0001$) as associated simultaneously with survival. No additional information was reached by cell dose ($p = 0.89$) and type ($p = 0.97$) or number of HLA disparities (with 3-year estimated survival of 33.7% in patients without HLA disparities, 38.5% in those with 1 disparity, 32.1% in those with 2 disparities, and 32.6% in those with 3 or 4 disparities, $p = 0.30$, Fig. 3).

Discussion

This study was designed to identify factors related to cord blood characteristics, mainly the number of cord blood cells

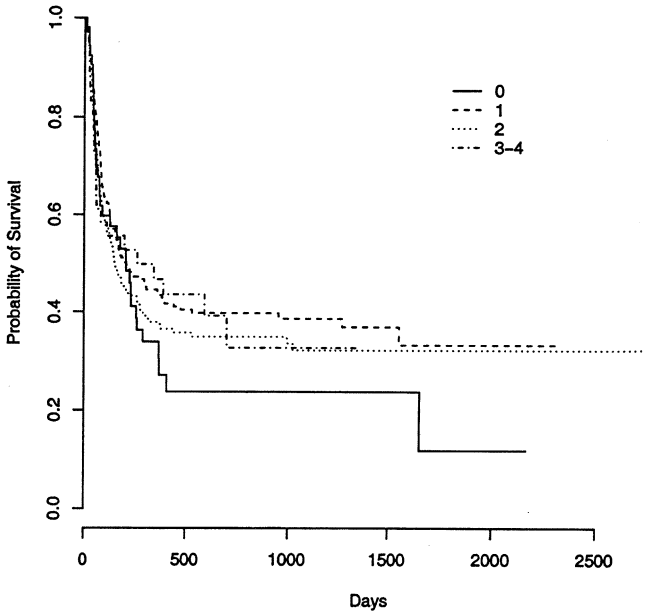

Figure 3. Overall survival after unrelated cord blood transplant in patients with hematologic malignancies according to the number of HLA disparities (HLA-A and HLA-B by serology and allelic typing of HLA-DRB1).

and HLA, which can be used for donor selection before transplant. The study used a registry-based cohort of 550 patients with hematologic malignancies who had undergone UCBT since 1994, when UCBTs began in Europe.

First, we confirmed previous findings on the association of NC dose with the speed and probability of neutrophil and platelet recovery. Almost all series on UCBT have demonstrated the profound impact of cell dose infused, measured as total NC [12,14,23], colony-forming cells [24], $CD34^+$ cells [25], and nucleated red blood cells [26], on engraftment. We also used the NC dose at freezing as a surrogate marker of the number of progenitors in the graft, because this measure is well standardized and is likely to be reproducible between laboratories, and mainly because this information is readily available during the search process. We observed that the higher CD34 cell dose infused, the higher the probability of acute GvHD grade III–IV. This observation must be confirmed in unicentric studies because of interlaboratory variation of measurements of $CD34^+$ cells [27] and grading of GvHD. However, contrary to previous reports [24,26], the prognostic influence of cell dose when predicting survival or TRM disappeared when considered in multivariable analyses. This probably is due to different interacting patient-related factors, such as age, gender, and advanced stage of the disease, which capture all prognostic information.

On the other hand, the influence of HLA disparities on the outcome of UBCT is controversial and not fully established. In our series, we found an association between the number or type of HLA disparities with engraftment and acute GvHD grade III–IV, and, more interestingly, with relapse, suggesting a graft-vs-leukemia effect. However, TRM and survival were not associated with HLA disparities, as previously reported [25]. This could be explained by different interacting factors; for example, HLA disparities had a negative impact on engraftment and a positive impact on preventing relapse. However, these findings appear to contradict a previous report, based on a series of 562 UCBTs (including 404 with hematologic malignancies), in which HLA disparities were not associated with the probability of neutrophil recovery and GvHD but with 1-year TRM [23]. The reasons for the difficulty in establishing, from the available data, consensual guidelines for donor choice based on HLA incompatibilities could be related to the heterogeneity of the patient population, the differences in the distribution of the number of HLA disparities across studies, and the absence of high-resolution molecular typing for classes I and II. In our study, all results of HLA typing were verified in the CBBs, transplant centers, and laboratories where HLA typing was performed by serology for HLA-A and HLA-B and by high-resolution molecular typing for DRB1.

It has been suggested that cell dose and number of HLA mismatches interact mutually on engraftment and on other outcomes [12,13,17,23]. Thus, a higher cell dose in the graft could partially overcome the negative impact of HLA for each level of HLA disparity; however, this hypothesis has not been proved. We found that both NC dose at freezing and HLA disparities were associated with the probability of myeloid engraftment, whereas $CD34^+$ cell dose and HLA disparities were jointly associated with the probability of acute GvHD grade III–IV (but not with acute GvHD grade II–IV; Fig. 2b). A further objective was to delineate an algorithm to help clinicians choose the best cord blood unit based on these data in light of other -patient and disease-related factors. To accomplish this, the thresholds of the number of nucleated cord blood cells before freezing and the maximal number of HLA disparities allowed in cord blood donor selection must be established. Based on our data, we found that cell dose at freezing and the number of HLA mismatches (Fig. 2a), were log linearly related to the hazard of neutrophil engraftment. Thus, we observed that the higher the number of cord blood cells, the lower the number of HLA disparities and the higher the probability of engraftment. Accordingly, no thresholds for cell dose and HLA disparities could be defined, although engraftment appeared to be reduced in the case of three or more HLA disparities. A further question was the choice among different cord blood units. For example, would a cord blood unit with 4×10^7/kg NCs but no HLA disparities be preferable to a cord blood unit with 6×10^7/kg NCs but one HLA disparity? No accurate answer to this point can be determined from our data. This would have required a larger sample given that, for instance, there are only 53 patients with no HLA disparities, including 19 with NC at freezing below 4×10^7/kg, 7 with cells between 4 and 6×10^7/kg, and 24 with cells at or above 6×10^7/kg.

Other limitations of our study can be raised. We used a registry-based analysis, with inclusion of all consecutive UCBTs from EBMT and non-EBMT (using Netcord units) centers. To further examine whether the non-EBMT centers introduced some bias in estimates, we restricted analyses to EBMT centers, without markedly modifying our results (data not shown). Another concern in this registry-based study is the center effect, as centers differ in terms of their practice and expertise with allogeneic transplantation. Thus, when necessary we considered the heterogeneity in baseline risks across centers in defining prognostic factors through mixed effects models. We also incorporated potential period effect, before and after 1998, because we and others have reported the impact of cell dose on outcomes in 1997 and 1998 [14,23]. UCBTs performed before 1998 had a higher TRM and lower probability of neutrophil recovery compared to those performed after 1998.

Finally, in order to overcome the problem of cell dose in cord blood transplants, several approaches currently are being investigated. Other biologic properties of cord blood HSC should be investigated further to determine possible reasons for delay of engraftment of cord blood transplants compared to BMT [16,28,29], such as immaturity of stem cells, which may require more cell divisions before differentiation to

progenitors [30,31], lack of subpopulations facilitating engraftment, or homing defects.

We found that patients who received hematopoietic growth factors (HGF) had earlier engraftment, but the other outcomes were not modified. Use of prophylactic HGF in patients undergoing UCBT should be investigated in prospective trials, because it appears that its use delays immune reconstitution in haploidentical transplants [32]. Another approach to improve short-term engraftment could be to expand cord blood progenitors in vitro. This area of investigation seems particularly interesting, as in vitro studies have shown that expansion was increased in cord blood compared to bone marrow cells [30]. Preclinical studies show that with combined use of early-acting cytokines (such as thrombopoietin, FLT3 ligand, and stem cell factor), it is possible to expand early progenitors capable of secondary and tertiary repopulation of NOD/SCID mice [33,34]. Two clinical phase I studies of transplantation of expanded cord blood cells have demonstrated that CD34 selection and ex vivo expansion of CB prior to UCBT is a feasible procedure; however, additional data are required to assess the clinical efficacy of expanded CB progenitors [35,36]. Another avenue of research is the possibility of using several cord blood units in order to increase the stem cell dose [37,38] or using reduced-intensity preparative regimens [39]. In the small number of multicord transplants reported, it was found that only one unit engrafted whereas the others were rejected. From a practical point of view, these findings reinforce the need for umbilical CBBs to collect as many cells as possible and to improve the technique of processing and volume reduction in order to accumulate units with high cell counts.

All of these strategies, together with identification of risk factors for outcomes in homogenous group of diseases [40,41] and establishment of criteria for cord blood selection, probably will improve outcomes of UCBT. These approaches and the encouraging results of UCBT in adults certainly will extend the use of unrelated cord blood [42–44].

In conclusion, our results show that two major factors—cell dose and HLA—can be used to choose a cord blood unit. The higher the number of cells, the lower the number of HLA disparities and the higher the probability of engraftment; the higher the number of HLA disparities, the higher the incidence of acute GvHD grade III–IV and the lower the risk of relapse. These results are useful for choosing the cord blood donor in situations where a high number of cells is mandatory, as in UCBT for adults or in nonmyeloablative UCBT.

Acknowledgments

Eurocord is supported by European grant QLK3-CT-1999-00380. The authors thank Valerie Bons, R.N., from Eurocord, Dr. Tokiko Nagamura from Tokyo CBB, Dr. Peter Horn from Dusseldorf CBB, Dr. Lucilla Lechi from Milan CBB, Dr. Sergi Querol from Barcelona CBB, Dr. Françoise Beaujean from the French Network, Dr. Cristina Navarette from London CBB, Dr. Evelyne Marry from France Greffe de Moelle, and all the data managers from transplant centers for checking clinical data. The authors also thank bone marrow donor registries worldwide for collaborating with the Eurocord group.

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Appendix 1. List of participating transplant centers per country and number of transplants per center

Centers reporting cord blood transplants	No. of cases
E Ematologia, Univ. La Sapienza, Dr. W. Arcese, Rome, Italy	41
Hôpital Pédiatrique La Timone, Pr. G. Michel, Marseille, France	30
Hospital Universitario "La Fe", Dr. G. Sanz, Valencia, Spain	28
Hôpital Saint Louis, P. E. Gluckman, Paris, France	28
MD Anderson Cancer Center, Dr. K-W. Chan, Houston, Texas, USA	21
Tokyo Cord Blood Bank, Dr. T. Takahashi, Dr. T. Nagamura-Inoue, Tokyo, Japan	19
Hospital M infantil Val d'Hebron, Pr J. Ortega, Barcelona, Spain	19
Children's Hospital Medical Center, Dr. A. Filipovich, Cincinnati, Ohio, USA°	17
University of Pavia, Pediatric, Dr. F. Locatelli, Pavia, Italy	15
IMSUT, University of Tokyo, Dr. T. Iseki, Dr. S. Asano, Tokyo, Japan	13
Ospedale Regine Margherita, Dr. F. Fagioli, Torino, Italy	13
Sydney Children' Hospital, Pr. M. Vowels/C. Oswald, Randwick, Australia	13
Hôpital de I'Archet, Dr. N. Gratecos, Nice, France	12
Hôpital Saint Antoine, Dr. J.P. Laporte, Paris, France	11
Royal Children's Hospital, Dr. K. Tiedemann, Melbourne, Australia	10
Universita Tor Vergata, St. Eugenio Hospital, Dr. A. Picardi, Dr. S. Amadori, Rome, Italy	10

(*continued*)

Appendix 1. Continued

Centers reporting cord blood transplants	No. of cases
Inst Portugues Oncologia, Dr. M. Abecassis/A. Machado, Lisboa, Portugal	9
Hôpital d'Enfants, Pr. P. Bordigoni, Vandoeuvre Nancy, France	8
FLENI, Dr. B. Diez, Buenos Aires, Argentina	8
The New Children's Hospital, Dr. P. Shaw, Sydney, Australia	7
Sheffield Children's Hospital, Dr. A. Vora, Sheffield, UK	7
Clinica Puerta de Hierro, Pr. MN. Fernandez, Madrid, Spain	6
Hospital Nino Jesus of Madrid, Dr. LM. Madero, Madrid, Spain	6
Hospital Ramon y Cajal, Dr. A. Munoz-Villa, Madrid, Spain	6
Inst. Portugues Oncologia, Dr. P. Pimentel, Porto, Portugal	6
Hôpital Claude Huriez, Pr. J.P. Jouet, Lille, France	6
Azienda Ospedaliera S. Giovanni, Dr. M. Falda, Torino, Italy	6
FHCRC Seattle, Dr. E. Sievers/ A. Mellon, Seattle, Washington, USA	6
MD Anderson Cancer Center, Dr. M. de Lima, Houston, Texas, USA	6
Hospital de Clinicas, Dr. R. Pasquini, Curitiba, Brasil	6
Fairview University of Minesota, Dr. J. Miller, Minneapolis, Minnesota, USA	5
Heinrich-Heine-Universitat, Dr. U. Göbel, Düsseldorf, Germany	5
Hospital Infantil La Fe, Dr. A. Verdeguer/Dr. V. Castel, Valencia, Spain	5
BMT Unit Schneider Children's, Dr. I. Yaniv, Dr. J. Stein, Petach-Tikva, Israel	5
Ospedale di Careggi Dr. A. Bosi, Firenze, Italy	4
Clinica Oncoematologia Pediatrica, Dr. Zanesco/Dr. C. Messina, Padova, Italy	4
City of Hope Medical School, Dr, J. Rosenthal, Duarte, California, USA	4
Tokai University, Dr. S. Kato, Tokai, Japan	4
University Medical Center, Dr. M. Graham, Tuscon, Arizona, USA	4
Prince of Wales Hospital, Dr. Chi Kong Li, Hong Kong, China	4
Hospital Israelita A. Einstein, Dr. E. Ferreira, Sao Paulo, Brasil	3
Roswell Park Cancer Institute, Dr. B. Bambach, Buffalo, New York, USA	3
Hckensack University Medical Center, Dr. S. Goldberg, Hackensack, New Jersey, USA	3
Institut Paoli Calmette, Pr. D. Blaise, Marseille, France	3
Hôpital Jean Minjoz, Pr. J-Y. Cahn, Besancon, France	3
Hospital de la Santa Creu i Sant Pau, Dr. I. Badell Serra, Barcelona, Spain	3
Institute G. Gaslini, Dr. S. Dallorso, Genova, Italy	3
University Children's Hospital, Dr. C. Urban, Graz, Austria	3
University Hospital Motol, Dr. J. Stary, Prague, Czech Republic	3
University of Liege, Pr. Y. Beguin, Liege, Belgium	3
Hospital Infantil La Paz, Dr. A.M. Martinez-Rubio, Madrid, Spain	3
St Sophia Children's Hospital, Dr. S. Grafakos/Dr. J. Peristeri, Athens, Greece	3
University of Bologna, Dr. A. Pession, Bologna, Italy	3
University of Pisa, Dr. C. Favre, Pisa, Italy	3
Children's Associated Medical Froup, Dr. W. Spruce, Dr. J. Allen, San Diego, California, USA	2
Medical City Dallas Hospital, M. Hooker, RN, Dallas, Texas, USA	2
Lombardi Cancer Center, Dr. M. Cairo, Washington, DC USA	2
Emory University School of Medecine, Dr. A. Yeager, Atlanta, Georgia, USA	2
Starship Hospital, Dr. Lochie Teague, Auckland, New Zealand	2
Cardinal Glennon Children's Hospital, Dr. D. Wall, St. Louis, Missouri, USA	2
CETRAMOR, Dr. Saslavski, Dr. J. Cozzi, Rosario, Argentina	2
Leiden University Hospital, Dr. W.E. Fibbe, Leiden, The Netherlands	2
Cliniques Universitaires St. Luc, Dr. C. Vermylen, Dr. B. Brichard, Brussels, Belgium	2
Hotel Dieu, Pr. N. Milpied, Nantes, France	2
Hôp/Cantonal Universitaire, Dr. B. Chapuis, Geneva, Switzerland	2
University Hospital, Dr. B. Simonsson, Uppsala, Sweden	2
Ospedale San Gerardo, Dr. D. Longoni, Dr. C. Uderzo, Monza, Italy	2
Ospedale V Cervello, Dr. R. Scime, Palermo, Italy	2
University Hospital Eppendorf, Dr. A. Zander, Hamburg, Germany	2
Hôpital Robert Debre, Pr. E. Vilmer, Paris, France	2
Institut Catala d' Oncologia, Dr. A. Granena, Barcelona, Spain	2
Canterbury Health Laboratory, Dr. N. Patton, Christchurch, New Zealand	2
The Children's Hospital, Dr. R. Quinones, Denver, Colorado, USA	1
University Hospitals of Cleveland, Dr. M. Laughlin, Cleveland, Ohio, USA	1
Saint Joseph's Hospital and Medical Center, Dr. A. Rubin, Paterson, New Jersey, USA	1
Oregon Health Sciences University, Dr. T. Moore, Portland, Oregon, USA	1

(*continued*)

Appendix 1. Continued

Centers reporting cord blood transplants	No. of cases
Washington University School of Medicine, Dr. D. Adkins, St. Louis, Missouri, USA	1
De Vos Children's Hospitals, Dr. D. Pietryga, Grand Rapids, Michigan, USA	1
University of Colorado Hospital, Dr. E. Shpall, Denver, Colorado, USA	1
CEMIC, Dr. B. Koziner, Buenos Aires, Argentina	1
James Whitcomb Riley Hospital for Children, Dr. F. Smith, Indianapollis, Indiana, USA	1
The Cleveland Clinic Foundation, K. Sands, RN, Cleveland, Ohio, USA	1
Hôpital Saint Justine, Dr. M. Champagne, Montreal, Canada	1
Queen Mary Hospital, Dr. Lee Tsz Leung, Hong Kong, China	1
Cardinal Bernardin Cancer Center, Dr. P. Stiff, Maywood, Illinois, USA	1
The Children's Hospital, Dr. E. Guinan, Boston, Massachusetts, USA	1
Children's Memorial Hospital, Dr. N. Snead, Chicago, Illinois, USA	1
Catholic University, Dr. F. Barriga, Santiago, Chile	1
Miami Children's Hospital, Dr. C. August, Miami, Florida, USA	1
Hackensack University Medical Center, Dr. J. Brochstein, Hackensack, New Jersey, USA	1
University of Nebraska Medical Center, Dr. A. Groves, Omaha, Nebraska, USA	1
Hospital Privado Centro Medico de Cordoba, Dr. A. Soria, Cordoba, Argentina	1
Royal Marsden Hospital, Dr. R. Powles, Sutton, UK	1
Children's Hospital, University of Helsinki, Dr. U. Pihkala, Helsinki, Finland	1
Hadassah University Hospital, Dr. A. Nagler, Dr. S. Slavin, Jerusalem, Israel	1
Hôpital La Miletrie, Pr. F. Guilhot, Dr. A. Sadoun, Poitiers, France	1
Ospedale Maggiore di Milano, Dr. G. Lambertenghi Deliliers, Milano, Italy	1
Hôpital Haut Leveque, Pr. J. Reiffers, Pessac, France	1
University Hospital Lund, Dr. A. Bekassy, Lund, Sweden	1
ITMO, Dr. J. Milone, La Plata, Argentina	1
Royal Victoria Infirmary, Dr. A. Dickinson, Newcastle upon Tyne, UK	1
Evangelismos Hospital, Dr. N. Harhalakis, Athens, Greece	1
Martin Luther Univ.-Wittenberg Klinik für Kinder, Dr. A. Wawer, Halle, Germany	1
Hospital Clinico, Dr. D. Caballero, Salamanca, Spain	1
Antartida Hospital Privado, Dr. V. Milovic, Buenos Aires, Argentina	1
Our Lady's Hospital for Sick Children, Dr. A. O'Marcaigh, Dublin, Ireland	1
Hôpital Debrousse, Dr. G. Souillet, Lyon, France	1

Appendix 2. List of cord blood banks and number of units delivered

Cord blood banks	No. of cases
GRACE CBB (Italian Network), Dr. P. Rebulla, Italy	121
New York CBB, Dr. P. Rubinstein, USA	120
Düsseldorf CBB, Dr. P. Wernet, G. Koegler, Germany	85
Barcelona CBB, Dr. J. Garcia, Dr. S. Querol, Spain	55
French CBB, Dr. P Herve, Dr. M. Benbunan, Dr. B Dezay, Besançon, Paris, Bordeaux, France	31
London CBB, Dr. M. Contreras, S. Armitage, UK	26
Tokyo CBB, T. Takahashi, Dr T. Nagamura-Inoue, Japan	25
Saint Louis CBB, Dr. D. Wall, USA	23
University of Colorado CBB, Dr. E. Shpall, USA	17
Belgium CBB, Dr. Y. Beguin, Belgium	10
Sydney CBB, Dr. M. Vowels, C. Oswald, Australia	9
Malaga CBB, Spain	5
CRIR - American Red Cross CBB, USA	5
Tokai CBB, Japan	4
Nokkaido CBB, Japan	4
Leiden CBB, Dr. W.E. Fibbe, The Netherlands	3
Mount Sinai CBB, USA	2
New Castle CBB, UK	1
Madrid CBB, Spain	1