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Hematopoietic cell transplantation in acute lymphoblastic leukemia: better long-term event-free survival with conditioning regimens containing total body irradiation

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ABSTRACT

Background and Objectives. **There is only limited experience with conditioning regimens based on busulfan for patients with acute lymphoblastic leukemia (ALL). Therefore, the aim of this study was to compare the event-free survival (EFS), transplant-related mortality (TRM) and the probability of relapse (PR) of patients undergoing hematopoietic cell transplantation (HCT) for ALL conditioned with or without total body irradiation (TBI).**

Design and Methods. **The study sample consisted of 156 patients conditioned with regimens based on TBI (n=114) or on high doses of oral busulfan (BU) (n=42). Most of the BU group received phenytoin as prophylaxis for seizures. The median follow-up was 6 years.**

Results. **EFS at 6 years was 43% (95% CI 35%-51%) versus 22% (95% CI 10%-34%) in the TBI and BU subsets, respectively (p=0.01). TRM at 18 months was 22% and 17% in the BU and TBI groups (p=0.24), respectively. At 3 years actuarial PR was 71% in the BU group and 47% in the TBI group (p=0.01). In the multivariable analysis, a worse EFS was associated with BU, relative risk (RR) 1.7; advanced disease versus 1st and 2nd complete remission (CR) at HCT, RR 2.5; absence of chronic graft-versus-host disease, RR 1.8; development of veno-occlusive disease RR 2.2 and shorter CR duration before transplant.**

Interpretation and Conclusions. **TBI was associated with a lower relapse rate and better EFS, even in patients in 1st and 2nd CR, than schemes based on high doses of busulfan. This suggests that conditioning regimens based on TBI should remain the standard method of preparative regimen for patients with ALL.**

Key words: acute lymphoblastic leukemia, hematopoietic cell transplantation, total body irradiation, conditioning regimen, busulfan

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Patients with acute lymphoblastic leukemia (ALL) undergoing hematopoietic cell transplantation (HCT) have usually received total body irradiation (TBI) as part of their conditioning regimen. The experience with chemotherapy-based regimens is limited and has usually developed due to the shortage of local TBI facilities and their inability to provide as many sessions of TBI as increased transplantation activity required.[1,2] Although randomized trials comparing different conditioning regimens have shown similar results for chronic myeloid leukemia (CML) and acute myeloblastic leukemia (AML),[3-5] too few patients with ALL have been included to draw any proper conclusion. The commonest chemotherapy-based regimens have been based on the utilization of high doses of busulfan like the scheme proposed by Tuschka *et al.* more than one decade ago.[6] The pharmacokinetics of high doses of oral busulfan is quite unpredictable for each individual and is influenced by many factors.[2,7] This has led some teams to measure drug levels and make consequent adjustments, and to the recent development of an intravenous busulfan formulation. Our aim was to compare the long-term outcome of patients with ALL who received TBI in the conditioning regimen with those treated with high doses of oral busulfan without drug adjustments. Our results show better long-term outcomes, in terms of relapse incidence and survival in patients receiving TBI.

Design and Methods

Patients

Medical records of 156 consecutive patients with ALL who underwent an allogeneic (ALLO) or autologous (AUTO) HCT were reviewed. HCTs were performed between 1983 and 1997 at two institutions: Hospital del Niño Jesús which treats pediatric patients and Hospital Universitario de La Princesa, which mainly admits adults. Patients were classified into two

Table 1. Distribution of main ALL features and details of transplant with regard to conditioning regimen.

	TBI n=114	*BU n=42*	*p*
Sex: M/F (n)	64/50	29/13	0.20
Age at HCT (yrs), median (range)	18 (2-59)	15 (10 mos-49)	0.33
Karyotype (n) (available data n= 89) Normal/Adverse/Other	33/16/14	20/5/1	0.72
Type of HCT (n) Allogeneic/Autologous	69/45	21/21	0.31
Source of progenitors (n) Marrow/Peripheral blood	98/16	34/8	0.60
Conditioning regimen (n)	Cy-TBI: 105 Cy-TBI+VP16: 8 Ara C-TBI: 1	BU-CY2: 39 BU-CY+VP16: 2 BUME: 1	
Seizures prophylaxis (n) Phenytoin/Clonazepam/None		36/4/2	
Status at HCT (n) 1st CR/2nd CR/More advanced	50/38/26	21/13/8	0.77
Complete remission/Active disease	92/22	39/3	0.11
Dates of HCT (%) 83-87/88-92/93-97	18/42/40	0/38/62	**0.004**
Duration of complete remission, median of days (range)			
1st CR	226 (20-633)	160 (21-654)	0.15
2nd CR	146 (16-863)	109 (30-231)	0.21
Toxicity (n)			
II-IV acute GVHD (Yes/No)	20/46	5/16	0.76
Extensive chronic GVHD (Yes/No)	5/58	0/14	0.62
VOD (Yes/No)	11/103	7/35	0.25
Fatal VOD(Yes/No)	2/112	5/37	**0.01**
Follow-up (years), median (range)	6.6 (1.3-15.2)	4.2 (1-9.5)	0.13

groups according to the administration or not of total body irradiation as part of the conditioning regimen: A) a TBI group (n=114) and B) a busulfan-based group (BU) (n=42). The main features of ALL at the time of diagnosis and transplant characteristics are displayed in Table 1. Nine patients (n=8 in the TBI group and n=1 in the BU group) out of 156 received a second HCT later. This second HCT was performed due to malignancy relapse after first HCT in all cases except one which was due to graft failure. All the cryopreservation procedures were carried out at the Hospital Universitario de La Princesa.

Transplant details

Patients (Table 1) were scheduled to receive TBI except in the event of lack of local availability of TBI at the time of HCT (n=35) or if any medical condition precluded the use of TBI, i.e. age less than 4 years or prior radiotherapy which would presuppose a high risk of toxicity (n=7). During the first 5 years of the study, all patients were conditioned with TBI, and afterwards, when transplant activity increased, some patients received busulfan-based regimens. Patients were scheduled to receive an allogeneic transplant whenever a suitable familiar donor was available. Otherwise, they underwent an autologous HCT. Only one patient who was 59 years old at the time of transplant directly received an autologous HCT.

Table 2. Distribution of main ALL features and transplant details with regard to type of transplant.

	ALLO n=90	*AUTO n=66*	*p*
Sex: M/F (n)	54/36	27/39	0.52
Age at HCT (years), median (range)	19.5 (10 mos-49)	16 (2-59)	0.78
Karyotype (n) (available in n=89) Normal/Adverse/Other/Unknown	26 /17/10	27/4 /5	**0.03**
Conditioning regimen (n) BU/TBI	21/69	21/45	0.31
Status at HCT (n) 1st CR/2nd CR/More advanced	39/27/24	32/24/10	0.22
Complete remission/Active disease	69/21	62/4	**0.007**
Dates of HCT (%) 83-87/88-92/93-97	22/30/48	2/56/42	**<0.001**
Duration of complete remission, median of days (range)	144 (16-485)	211 (16-863)	**0.001**

TBI based regimens were comprised mainly of cyclophosphamide (120 mg/kg) plus TBI (CY-TBI). TBI consisted of 1200 cGy, fractionated in either 4 (n=29) or 6 doses (n=85). The lungs were shielded at 900 cGy. The majority of BU group patients were conditioned with the Tuschka *et al.* scheme of oral busulfan 16 mg/kg and cyclophosphamide 120 mg/kg (BUCY-2).[6] Busulfan was dosed at 600 mg/m^2 in 9 children under 6 years old. Prophylaxis of busulfan-related seizures was done in 36 out of 42 cases with phenytoin[8] and in 4 cases with clonazepam. Busulfan levels were not measured, so individual adjustments were not performed.

All allogeneic HCTs were performed with non-T-depleted grafts and sibling donors. All except one in the TBI group were HLA identical. Graft-versus-host disease (GVHD) prophylaxis consisted of a combination of cyclosporine and methotrexate in the majority of cases.

Five autologous grafts (n=4 in the TBI group and n=1 in the BU group) were purged with anti CD19, anti CD20 and complement.

Definitions

Only data from first transplants were considered. Status at HCT was categorized into three groups: first complete remission (CR), second CR, and more advanced disease. Patients were considered evaluable for acute GVHD if they survived at least 10 days after an allogeneic HCT or if they developed acute GVHD whenever it start-

ed. Patients were considered evaluable for chronic GVHD if they survived at least 100 days after an allogeneic HCT or if they developed chronic GVHD whenever it begun.

Patients who presented with active disease at the time of transplant and never achieved CR after it, were excluded from the analysis of the probability of relapse (PR) post-HCT. Patients who remained in CR after HCT were censored at the time of last contact.

Transplant-related mortality (TRM) (those deaths which occurred while remaining in continuous complete remission) was considered only for first HCTs. Otherwise they were considered as leukemia-related. Those patients who received a second transplant were censored at the time of this later transplant.

Event-free survival (EFS) was calculated as the time between the date of HCT and the first of the following events: date of relapse, date of second HCT or date of death. Patients with none of these events were censored at the time of last contact.

Statistics

Mean values were compared with Student's t test. The distribution was compared with the chi-squared test and corrected with Yates's method or Fisher's exact test. Differences in actuarial probability of TRM, PR, EFS and the probability of developing acute GVHD or chronic GVHD between subgroups were analyzed with the log-rank test and log-rank test adjusted for status at HCT. The type of preparing regimen, age at HCT, type of transplant (allogeneic versus autologous), status at transplant and all the variables that, when compared in the univariate analysis, reached a $p<0.10$ were also included in the Cox regression analysis for TRM, PR and EFS.

Results

Age, sex, CNS infiltration, number of leukocytes, and prior holocranial radiotherapy were equally distributed in both groups. Cytogenetic data were available in 57% of the cases, (55% in the TBI group and 62% in the BU group) with no differences in the percentage of adverse karyotype [t(9;22), t(4;11), trisomy 8 or multiple associations]: 25% in the TBI group versus 19% in the BU group of known cases. The TBI group had a higher percentage of active disease at the time of transplant than the BU group, although the difference was not statistically significant. There was no difference either in the time from status to transplant, the distribution of type of transplants (allogeneic versus autologous), source of hematopoietic progenitors or type of GVHD prophylaxis scheme.

With regard to the year of transplantation, there was a statistically significant difference between the TBI and BU groups: BU cases were carried out more recently ($p<0.001$). Thus, the follow-up period of living patients was longer in the TBI group (median 6.6 years, range: 1.3-15.2) than in the BU group (median 4.2 years, range: 1-9.5).

Outcome of allogeneic versus autologous cases

The main transplant features are shown in Table 2. Compared to allogeneic cases, patients who received an autologous transplant had a lower percentage of adverse karyotypes, a lower proportion of cases with active disease at transplant and were performed more recently. Time from status to transplant was shorter for allogeneic cases. Actuarial transplant-related mortality was higher in allogeneic than in autologous cases, 25% (95% CI 17%-33%) and 10% (95% CI 4%-16%) at 18 months respectively, $p=0.05$. There was no difference concerning the actuarial probability of relapse and event-free survival, even when the analysis was done for subgroups with the same status at HCT (data not shown).

Comparisons between TBI and BU groups (Tables 1 and 3)

Toxicity and transplant-related mortality

Liver veno-occlusive disease (VOD) developed in 9% and 16% of TBI and busulfan-treated patients respectively (p=N.S.). Fourteen out of eighteen patients who developed VOD died. Death in this subgroup was due to VOD (7 cases), relapse (4 cases), cytomegalovirus pneumonia (2 cases) and pulmonary hemorrhage in one case. VOD was the direct cause of death in 2% of the 114 patients of the TBI group and in 12% of the 42 cases of the BU group (χ^2 $p=0.01$). There were no deaths due to VOD in children younger than 6 years. Grade II to IV acute GVHD developed in 20 out of 66 of the evaluable allogeneic TBI group and in 5 out of 21 patients of the BU group (p=N.S.). Chronic GVHD was extensive in 5 out of 63 and in none out of 14 cases in the TBI and BU groups, respectively (p=N.S.). The analysis of the actuarial probability of developing acute or chronic GVHD did not reveal differences between groups. Taking into account both subsets of patients below and above 6 years old, we did not find differences in the incidence of acute GVHD, chronic GVHD or VOD. Overall, there were no differences in TRM at 18 months between the TBI [17% (95% CI 11%-23%)] and BU groups [22% (95% CI 10%-34%)].

Probability of relapse

One hundred and fifty patients were considered evaluable for the probability of relapse after HCT. Actuarial probability of relapse was 71%

Table 3. Probability of outcome (Kaplan Meier method): transplant-related mortality, relapse and event-free survival at different times after transplant. Comparisons between TBI and BU cases were made with the log-rank tests stratifying for status at transplant.

	Transplant-related mortality (%)		*Probability of relapse (%)*		*Event-free survival (%)*		
	6 mos	*18 mos*	*1 yr*	*3 yrs*	*1 yr*	*3 yrs*	*6 yrs*
Overall	*p*=0.24 n=156		*p*=0.01 n=150		*p*=0.01 n=156		
TBI	13 (3)*	17 (3)	34 (4)	47 (5)	54 (4)	43 (4)	43 (4)
BU	22 (6)	22 (6)	57 (8)	71 (8)	33 (7)	22 (6)	22 (6)
1st CR	*p*=0.02 n=71		*p*=0.47 n=71		*p*=0.04 n=71		
TBI	8 (3)	13 (4)	31 (6)	38 (7)	62 (6)	55 (7)	55 (7)
BU	34 (10)	34 (10)	42 (13)	49 (13)	38 (10)	33 (10)	33 (10)
2nd CR	*p*=0.64 n=51		*p*=0.007 n=51		*p*=0.02 n=51		
TBI	12 (5)	15 (6)	29 (7)	46 (8)	60 (7)	45 (8)	45 (8)
BU	8 (7)	8 (7)	67 (13)	84 (10)	30 (12)	15 (10)	15 (10)
Advanced	*p*=0.53 n=34		*p*=0.24 n=28		*p*=0.81 n=34		
TBI	25 (8)	25 (8)	52 (12)	72 (11)	30 (9)	17 (7)	17 (7)
BU	13 (11)	13 (11)	72 (17)	100 (0)	25 (15)	0 (0)	0 (0)

**Standard error; mos: months; yr: year.*

(95% confidence interval (CI) 55%-87%) and 47% (95% CI 37-57%) at three years in the BU group and TBI group, respectively (*p*=0.01) (Figure 1B). When the analysis was done for subgroups with the same status at TPH, only the subset of patients presented in second CR showed a statistically significant difference in the probability of relapse at three years between the TBI group, 46% (95% CI 30-62%) and the BU group, 84% (95% CI 64-100%), *p*=0.007. Patients in 1st CR and advanced disease also showed a higher trend of relapse incidence in the BU group. There were no differences in the time from HCT to relapse between the TBI group, median: 6 months (range: 1.4-34.6) and the BU group, median: 7.6 months (range: 2.4-27.8).

Event-free survival

With a median follow-up of more than 6 years and more than 4 years in the TBI and BU groups respectively, actuarial event-free survival was 43% (95% CI, 35-51%) and 22% (95% CI, 10-34%) at 3 years (*p*=0.01). No events were recorded later than 3 years post-transplant in any of the treatment groups. The EFS of those patients who were grafted while in first CR was 55% (95% CI, 41-69%) and 33% (95% CI, 13-53%) in the TBI and BU groups, respectively (*p*=0.04). EFS of patients in second CR was 45% (95% CI, 29-61%) and 15% (95% CI, 0-35%), respectively (*p*=0.02). The 34 patients with more advanced disease had a very bad outcome: only 17% in the TBI group and 0% in the BU group (*p*=N.S.) remained alive and relapse-free at three years (Figure 1A).

Multivariable analysis (Table 4)

Transplant-related mortality was significantly associated with the development of VOD, hazard ratio (HR) 5.3 and age at transplant, HR 1.05. Allogeneic cases exhibited higher TRM in the univariate analysis, but the difference was not statistically significant in the multivariate analysis. There was a trend towards higher TRM in the busulfan group, *p*=0.08. The probability of relapse was associated with: BU group, HR 1.7; advanced disease versus 1st and 2nd CR, HR 1.5; shorter duration of complete remission, HR 1.005; and absence of chronic GVHD, HR 3.9. A worse event-free survival was statistically related to: BU group, HR 1.7; advanced disease versus1st and2nd CR, HR 2.5; absence of chronic GVHD, HR 1.8; shorter duration of complete remission, HR 1.002; and the development of VOD, HR 2.2; and was independent of the type of transplant and age.

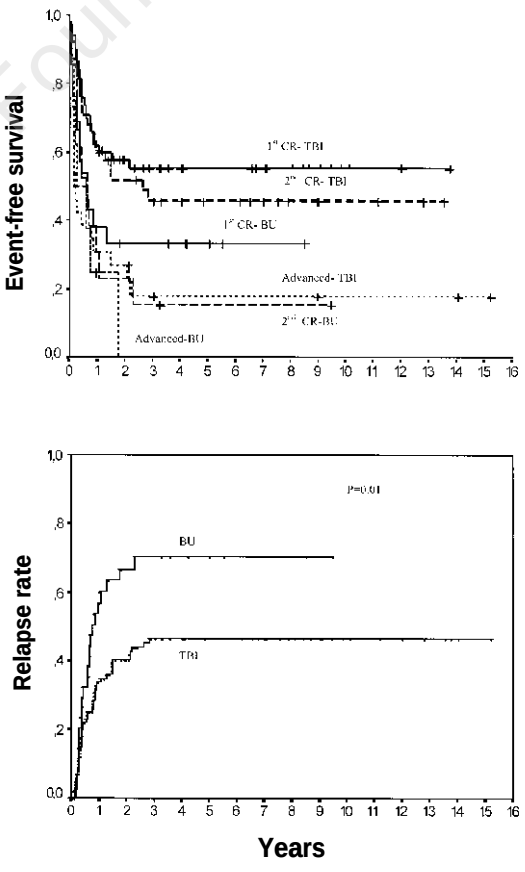

Figure 1. Kaplan-Meier curves representing the data of: A) comparison of the event-free survival between conditioning regimen (TBI versus BU) for the different status at transplant; B) comparison of the relapse rate between conditioning regimen (TBI versus BU) in the whole group. Comparisons were made with the log-rank test.

Table 4. Univariate analysis with log-rank test and multivariate regression analysis with the Cox model for TRM, PR and EFS. N= number of evaluable patients. j: Age at transplant was analyzed with groups younger versus older than 15 years in the univariate analysis and as a continuous variable per year in the Cox method. q: Duration of complete remission was analyzed with groups below and above the median value in the univariate analysis and as a continuous variable per day in the Cox method. τ: Dates of transplant were grouped into 3 periods: years 83-87, 88-92 and 93-97 for the univariate analysis.

	Baseline value	*Compared value*	*N*	*Univariate analysis*	*Multivariate analysis*		
				p	*Hazard ratio*	*95% C I*	*p*
Transplant related mortality							
Age φ	<15 years	≥ 16 years	156	0.02	1.05	1.02-1.08	0.001
Conditioning	TBI	Busulfan	156	0.24	2.1	0.7-5.3	0.08
Type	Autologous	Allogeneic	156	0.05	2	0.9-4.9	0.15
Status	1st and 2nd CR	Advanced	156	0.25	-		-
Dates τ			156	0.81	-		-
Chronic GVHD	Absence	Presence	76	0.21	-		-
Duration of CR θ	> Median	< Median	131	0.87	-		-
Sex	Male	Female	156	0.71	-		-
Source	Bone marrow	Peripheral blood	156	0.23	-		-
Acute GVHD	Absence	Presence	90	0.29			
VOD	Absence	Presence	156	0.0001	5.3	2.2-12.5	0.0001
Probability of relapse							
Conditioning	TBI	Busulfan	150	0.01	1.7	1.03-2.9	0.03
Type	Allogeneic	Autologous	150	0.10	1.5	0.9-2.4	0.10
Status	1st and 2nd CR	Advanced	150	0.001	2.5	1.4-4.3	0.001
Duration of CR θ	> Median	< Median	131	0.006	1.005	1.002-1.007	0.001
Chronic GVHD	Presence	Absence	74	0.0005	3.9	1.4-10.8	0.006
Dates τ			150	0.72	-		-
Age φ	<15 years	≥ 16 years	150	0.57	-		-
Sex	Male	Female	150	0.55	-		-
Karyotype	Non adverse	Adverse	87	0.25	-		-
Source	Bone marrow	Peripheral blood	150	0.92	-		-
Acute GVHD	Presence	Absence	85	0.49	-		-
VOD	Absence	Presence	150	0.46	-		-
Event free survival							
Conditioning	TBI	Busulfan	150	0.01	1.7	1.1-2.7	0.01
Type	Allogeneic	Autologous	156	0.88	1.1	0.7-1.6	0.67
Status	1st and 2nd CR	Advanced	156	0.0001	2.5	1.5-3.9	0.0001
Duration of CR θ	> Median	< Median	131	0.01	1.002	1.003-1.004	0.02
Chronic GVHD	Presence	Absence	76	0.007	1.8	1.1-3	0.008
Dates τ			156	0.94	-		-
Age φ	<15 years	≥ 16 years	156	0.57	1.01	0.99-1.03	0.09
Sex	Male	Female	156	0.88	-		-
Karyotype	Non adverse	Adverse	89	0.20	-		-
Source	Bone marrow	Peripheral blood	156	0.39	-		-
Acute GVHD	Absence	Presence	90	0.65	-		-
VOD	Absence	Presence	156	0.004	2.2	1.2-3.9	0.006

CR=complete response; GVHD=graft-versus-host-disease; VOD=veno-occlusive disease.

Discussion

Total body irradiation constituted the central part of conditioning regimens for HCT in both the first experimental animal assays and human trials. Regimens based on chemotherapy alone developed in response to practical limitations, since transplantation activity increased faster than the availability of TBI.[1,2] Since then, the most widely used radiation-free regimen for acute and chronic leukemias has been the combination of high doses of busulfan and cyclophosphamide (BUCY). However, there is concern about the equality of both regimens in terms of anti-malignancy effectiveness and toxicity. Up to now, six randomized trials comparing regimens with and without TBI have been reported.[3-5,9-11] These have included patients with CML and acute leukemia. Two of these trials,[3,5] focusing on CML in 1st chronic phase, did not reveal any apparent differences in terms of disease-free survival. With regard to AML, Blaise *et al*.[4] reported more relapses, more transplant-related mortality and a worse leukemia-free survival in the busulfan-treated patients. Dusenbery[11] treated a few patients with AML and found a trend toward better disease-free survival with TBI in patients with advanced disease. Recently, the *Nordic Bone Marrow Transplantation Group* have reported their long-term toxicity and survival data[12] of a previously published trial,[10] which involved subjects with ALL, AML and CML. They concluded that BUCY-2 regimens produced more VOD, chronic GVHD, alopecia and obstructive bronchiolitis than the CY-TBI regimen. This last regimen achieved better leukemia-free survival and less TRM in the subset of patients with advanced diseases. A meta-analysis[13] that compiled the data of five of these trials[3-5,9,10] concluded that TBI-based regimens cause less VOD than BUCY-based regimens and that BUCY is unlikely to have a clinically rel-

evant disease-free survival advantage over TBI. However they could neither confirm nor rule out such an advantage for TBI. The long-term follow-up of four of these randomized trials has been reported more recently: 10-year survival seems to be similar for CML patients treated with either Cy-TBI or BUCY-2, but it is lower in AML patients treated with BUCY-2.[14]

Here, we present our experience with HCT in ALL using TBI or high doses of busulfan as conditioning regimen. The allocation of patients to either regimen was mainly based on the availability of TBI at the time of transplantion, except for seven patients who received a chemotherapy-based scheme due to prior radiotherapy or age younger than 4 years. Inadvertent bias could have been introduced because of the retrospective nature of the study. Both treatment groups presented a similar distribution of adverse factors. However, as TBI cases were transplanted earlier, a hypothetical learning period would have worked against the TBI group. We found a better long-term event-free survival with TBI-based preparative regimens: 43% (95% CI, 35-51%) and 22% (95% CI, 10-34%) in the TBI and BU group, respectively at three years (p=0.01). The probability of survival free of leukemia in the TBI group was almost twice that in the BU group, HR 1.7 (95% CI 1.1-2.7), p=0.01. EFS was also adversely influenced by advanced status at transplant, shorter interval from status to transplant, absence of chronic GVHD and the development of VOD and was independent of age at transplant and type of HCT (allogeneic versus autologous). A higher probability of relapse in the BU group accounted for the superiority of results in the TBI group. Thus, the actuarial probability of relapse was 71% (95% CI 55-86%) in the BU group, while it was 47% (95% CI 37-57%) in the TBI one (p=0.01). In accordance with this finding, we have previously reported that children with ALL after an allogeneic transplant achieved better post-graft donor chimerism if prepared with radiation containing regimens and that the state of complete donor chimerism was associated with a lower relapse rate.[15] The probability of relapse in our series was also statistically higher in advanced stages, in the absence of chronic GVHD and in patients with a shorter duration of complete remission. There was a trend towards higher transplantation-related mortality in the BU group, HR 2.1 (95% CI 0.7-5.3), p=0.08 and TRM was also negatively affected by older age at HCT and the development of VOD. VOD was the direct cause of death in 12% of the BU group compared to in 2% of the TBI group (p=0.01). There were no differences in fatal acute GVHD, chronic GVHD or interstitial pneumonitis between groups. We did not find any differences between patients younger and older than 6 years, although this may be due to the low number of children under 6 years old in our series.

The aforementioned randomized prospective trials[3-5,9-11] included too few patients with ALL to draw any valid conclusion about the role of the non-TBI conditioning regimens in this disease; only 23 and 18 patients reported by Blume[9] and Ringden,[12] respectively, were conditioned without TBI. The *European Group for Blood and Marrow Transplantation* (EBMT) retrospectively reviewed the data of 241 patients with ALL treated without TBI.[16] The CY-TBI group showed better outcomes than the BU-CY cohort only in the autologous setting in patients in 2nd CR or more advanced stages, with more than 2 years from diagnosis to transplantation, in terms of a lower probability of relapse and higher EFS. More recently, the *International Bone Marrow Transplantation Registry* has reported a multicenter study comprised of 176 children with ALL treated with BU-CY, who achieved worse leukemia free survival than those prepared with the CY-TBI scheme.[17] However, an interim analysis of an ongoing randomized trial for children with ALL with a short accrual, has not yet revealed any differences in terms of relapse or transplant-related mortality.[18] Two other non-controlled trials of allogeneic HCT conditioned with busulfan for ALL in complete remission, resulted in figures of about 40% of EFS at three years.[19,20] Nevertheless, the subset of 50 patients of our series undergoing allogeneic HCT in complete remission and prepared with TBI reached an EFS of 57% (95% CI, 43-71%) versus 19% (95% CI 1-37%) in the 19 patients conditioned with busulfan regimens (p=0.0003).

Busulfan pharmacokinetics exhibits great inter- and intra-patient variability[2,7,21] and is influenced by age, type of disease, obesity, food intake, circadian rhythm, alterations in liver function and busulfan bioavailability. Clearance is higher in children than in adults, leading to less therapeutic effectiveness and lower toxicities in the former.[22] Concomitant use of other drugs commonly administered in the transplant setting for example acetaminophen due to its glutathione depleting properties, phenytoin,[23] and itraconazole,[24] may also affect the pharmacology of busulfan. In animal models, high doses of busulfan were found to produce severe and protracted granulocytopenia, thrombocytopenia and anemia without remarkable changes in survival of skin allografts or humoral antibody responses.[1] Although the combination of high doses of busulfan and cyclophosphamide produces enough immunosuppression to succeed with engraftment, the sensitivity of lymphocytic and myelocytic precursors may not be similar.[25] Also, more long-term toxicity to lymphocytes is suggested as more prolonged immunossupressive

effects are achieved with TBI than with busulfan in terms of delayed recovery of contact hypersensivity and alloantigen responses.[26] Moreover, the distribution of busulfan appears to be different depending on the underlying disease. Thus, children with lysosomal storage diseases have longer elimination half-lives[27] and busulfan clearance was found to be lower in non-Hodgkin's lymphomas than in patients with CML.[28] Gibbs *et al*. found that busulfan clearance was higher in obese subjects, and that expressing clearance based on body surface area or adjusted ideal body weight could abrogate differences depending on weight. However, this adjustment does not abolish differences due to the underlying disease.[28] Moreover, patients with CML seem to tolerate higher busulfan levels with less severe toxicities.[29] Busulfan clearance in ALL patients has not yet been studied specifically.

Seizures develop in about 10% of patients receiving high doses of busulfan.[8] Phenytoin has been the most widely used prophylactic against busulfan-related seizures.[30] Most of our patients in the BU group received phenytoin as prophylaxis against busulfan-related seizures from the day before they started busulfan until the day after busulfan was withdrawn. Phenytoin, due to its properties as an inducer of liver detoxifying systems such as cytochrome P450 or glutathione S-transferases, may alter busulfan pharmacokinetics. It has been shown that phenytoin increases busulfan clearance[23] and leads to less myelotoxicity in murine models.[31] Low busulfan concentrations might lead to a higher relapse rate as has already been shown in some diseases such as CML[29] or higher graft failure incidence,[32] while higher busulfan levels are associated with increased risk of VOD.[33] Moreover, survival has been shown to be higher in patients with myelodysplastic syndromes undergoing HCT with targeted busulfan levels.[34] It is worth emphasizing that previous randomized trials with busulfan-based regimens did not use adjusted busulfan levels. Nor did they mention busulfan-related seizure prophylaxis[3-5,9,11,19,20] except in one study,[10,12] which used benzodiazepines that do not seem to alter busulfan distribution.[23] In spite of the lack of published evidence of equal clinical effectiveness of chemotherapy-based regimens, as many as 35% of HCTs in ALL patients are performed without TBI in Spain[35] and about 24% in Europe (*EBMT Data Office, personal communication*).

In summary, our experience in ALL suggests that conditioning regimens with combinations of oral busulfan and prophylactic phenytoin without adjusting drug levels, offer worse results than TBI containing regimens even in favorable cases in 1st and 2nd CR. Whether these differences are due to busulfan distribution variations produced by interactions with phenytoin, warrants further research. Until clinical trials with either intravenous busulfan or adjusted busulfan levels offer more favorable results, it seems safer to use TBI for hematopoietic cell transplantation in ALL patients, unless medical contraindications exist.

Contributions and Acknowledgments

EG and RC were the main investigators. They designed the study, collected part of the data, did the statistical analysis, interpreted the results and wrote the manuscript. LM and MAD collected part of the data and were responsible for the clinical care of the pediatric patients. PM collaborated in collecting the data. JL, RA and AF were responsible for the clinical care of the patients. JF contributed to the design of clinical protocols and as the Senior Author is cited last. All the authors approved the final manuscript. The criteria for the order in which the names of the author appear are based on their contribution to the design, development of the study, analysis and interpretation of the data.

Disclosures

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Potential implications for clinical practice

- Conditioning regimens based on TBI should remain the standard preparative regimen for patients with ALL until clinical trials with either intravenous busulfan or adjusted busulfan levels offer more favorable results in ALL patients.

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