Biol Blood Marrow Transplant 25 (2019) 100–106

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Pediatric

Kinetics and Risk Factors of Relapse after Allogeneic Stem Cell Transplantation in Children with Leukemia: A Long-Term Follow-Up Single-Center Study

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Article history:
Received 28 April 2018
Accepted 7 August 2018

Key Words:
Allogeneic stem cell transplantation
Leukemia
Relapse
Chronic graft-versus-host disease (GVHD)
Children
Risk score

A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an established treatment for high-risk hematological malignancies in the pediatric population, but relapse remains the leading cause of death. We analyzed risk factors associated with relapse.

Data from 353 allo-HSCTs from 1989 to 2015 in our center were studied retrospectively. We performed a multivariate analysis of pre- and postransplantation variables and developed a predictive risk score for relapse using the significant factors in this training cohort. The results were confirmed in a validation cohort of 90 allo-HSCTs done in our institution from 2016 to the present.

A total of 104 patients relapsed after allo-HSCT, with a relapse cumulative incidence of 31 ± 2%. In multivariate analysis, only 2 variables influenced relapse: disease phase (advanced versus early, HR, 2.84; 95% CI, 1.76 to 4.57; $P = .001$) and presence of chronic graft-versus-host disease (GVHD) (acute GVHD versus chronic GVHD [HR, 4.27; 95% CI, 1.99 to 9.15; $P = .0001$] and no GVHD versus chronic GVHD [HR, 6.86; 95% CI, 3.63 to 12.97] $P = .0001$]. Applying the personalized risk score (0 to 3), the relapse cumulative incidence was 70 ± 5% in patients with a score of 3 (without GVHD and in the advanced phase) compared with 6 ± 4% in patients with a score of 0 (with chronic GVHD and in an early phase). This score has been verified in the validation set. With a median follow-up of 54 months, the disease-free survival (DFS) and overall survival rate were 37 ± 3% and 45 ± 4%, respectively.

The association of GVHD with the graft-versus-leukemia effect is clearly established in our study, and the form of GVHD associated with less relapse and the best DFS is the classical form of chronic GVHD according to the National Institutes of Health classification. The proposed relapse risk score was validated in an independent cohort and allows personalization of the prognosis.

INTRODUCTION

Today, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered an established treatment for pediatric patients with high-risk hematological malignancies. Relapse after transplantation remains the leading cause of death after transplantation [1,2], especially in recent years considering that transplant-related mortality has lessened thanks to improved supportive care.

Relapse incidence (RI) following allo-HSCT varies from 10% to 60% depending on patient, disease, and transplant characteristics [3]. However, most of the knowledge regarding risk factors and the outcome of relapsed disease after transplant comes from studies conducted on adult patients [3,4].In the pediatric setting, hematological malignancies are biologically different, and indications for allo-HSCT differ as well. Few studies have analyzed risk factors and patterns of relapse in pediatric patients with hematological malignancies [4]. Our study aimed to (1) analyze risk factors, pattern, timing, and outcome of relapsing disease after allo-HSCT in a pediatric population and (2) develop and validate a score to predict relapse risk.

METHODS

Patients and Disease Characteristics

Retrospective chart review of 294 patients with high-risk hematological malignancies was performed. A total of 353 allo-HSCTs were performed from January 1989 to December 2015, which included 212 boys and 141 girls with a median age of 8 years (range, 1 to 20). The median Lansky (for patients <16 years) or Karnofsky score (for patients ≥16 years) was 90%. A total of 213 patients had acute lymphoblastic leukemia (ALL), 108 had acute myeloid

Financial disclosure: See Acknowledgments on page 106.

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https://doi.org/10.1016/j.bbmt.2018.08.012
1083-8791/

leukemia (AML), and 32 patients had a chronic leukemia including chronic myeloid leukemia (CML) and juvenile myelomonocytic leukemia. A total of 120 patients were in first complete remission (CR), 126 were in second CR, 44 patients were in more than second CR, and 63 patients were considered to have refractory disease at the time of transplantation. Refractory disease was defined as the failure to achieve a morphological remission response at the time of transplantation. The indications for stem cell transplantation in first CR for children with ALL included induction failure (defined as no remission at 1 month following induction treatment), poor cytogenetics, and persistent minimal residual disease (MRD) tested by PCR before transplant (1 month) with a cutoff point of 10^{-3} if disposable. Intermediate or poor risk was the criterion for transplant in children with AML.

In 266 cases, allo-HSCT was the first transplant procedure; in 87 cases, 1 or 2 previous HSCTs had been performed. The indications for autologous transplantation have been early extramedullary relapse in 9 patients with ALL and intermediate risk cytogenetics in 15 patients with AML. Since 2005, autologous transplants in children with leukemia have not been performed. We have included a validation cohort of 90 allo-HSCTs with similar baseline characteristics performed from 2016 to the present (Table 1 provides patients and diseases characteristics). The study protocol was approved by the local ethics committees, and informed consent was obtained from patients or their legal guardians.

Transplantation Procedures

A myeloablative conditioning regimen was used in 188 patients (53%), and the remaining 165 patients (47%) received a reduced-intensity conditioning regimen. Total body irradiation as part of conditioning in hematological malignancies has not been used since 2002. Since 2005, most of the conditioning regimens have been based on the use of fludarabine. We only used serotherapy (antithymocyte globulin or alemtuzumab) in second transplants and umbilical cord blood transplants.

Grafts were obtained from matched related donors in 121 cases (34%), from haploidentical related donors in 105 cases (30%), and from unrelated donors in 127 cases (36%), which included matched unrelated donors, mismatched unrelated donors, and unrelated umbilical cord blood transplants. The stem cell source included peripheral blood stem cells in 270 cases (76%), bone marrow (BM) in 34 cases (10%), and umbilical cord blood (UCB) in 49 cases (14%).

Graft manipulation was performed in 206 transplants (58%). Since 2005, ex vivo graft engineering has been used in our center in most peripheral blood transplants. All immunomagnetic procedures were performed according to the manufacturer's standard protocol using the CliniMACS device (Miltenyi Biotec, Germany).

Three different methods of ex vivo T-cell depletion were used in our study: positive selection of CD34+ cells was used in 82 cases (40%), CD3/CD19 depletion was used in 102 cases (49%), and TCR$\alpha\beta$/CD19 depletion was performed in 22 cases (11%) (Table 2 provides transplant characteristics).

Study Design and Definitions

Myeloid recovery was defined as the first of 3 consecutive days on which the absolute neutrophil count was $>0.5 \times 10^9$/L. Platelet recovery time was considered the first of 3 consecutive days on which platelet counts $>20 \times 10^9$/L were achieved, with no transfusion requirements.

For the analysis of risk factors, the primary endpoint was cumulative incidence of relapse. Relapse was defined as morphologic evidence of recurrence in the peripheral blood or in BM (>5% blasts in BM), or clinical evidence at extramedullary sites. Late relapse was defined as that developed >1 year after transplant. Secondary endpoints were disease-free survival (DFS), overall survival (OS), and nonrelapse mortality (NRM) that was defined as any cause of death other than disease.

In an attempt to discriminate any impact of disease status at the time of transplantation on relapse, we separately analyzed and compared the cumulative incidence of relapse for patients transplanted in the first CR, second CR, or beyond. We found a relapse cumulative incidence in the first CR of 19 ± 4%, in the second CR of 36 ± 5%, and in those beyond the second CR or refractory disease of 39 ±5%, with no statistical differences between the second or more advanced disease phase groups ($P = .5$). Indeed, when grouping together patients in the first CR versus more advanced disease and comparing outcomes, we found statistical differences in terms of RI (a first CR of 19 ± 4% versus beyond the second CR of 38 ± 3%; $P = .0002$). For the analysis of relapse, we decided to establish 2 groups of patients: early phase for patients in the first CR and an advanced phase for the remaining patients.

Chimerism was evaluated by the short tandem repeat PCR method at the time of engraftment and periodically following transplantation. Donor chimerism was determined from whole blood and BM cell subsets [5]. Immune reconstitution after transplantation was performed by flow cytometry. Acute GVHD was graded according to standard criteria [6]. Chronic GVHD was defined with the criteria established in the 2005 National Institutes of Health (NIH) consensus conference. This consensus conference recognized 2 main categories of GVHD, each with 2 subcategories [7]. The broad category of acute GVHD included classic acute GVHD and persistent, recurrent, or late-onset acute GVHD occurring >100 days after transplantation. The category of chronic GVHD included classic chronic GVHD and an overlap syndrome. The severity of chronic GVHD was graded as mild, moderate, and severe according to these consensus criteria.

Table 1
Patients and Disease Characteristics

Variable	Training Cohort N (%)	Validation Cohort N (%)	*P* Value
Patients	294	84	
No. of allo-HSCTs	353	90	
Age (y)			
Median (range)	9 (1-20)	9 [1-18]	ns
Gender			
Male/female	212 (60%)/141 (40%)	49 (55%/41 (45%)	ns
Disease			
ALL	213 (60%)	44 (49%)	ns
AML	108 (31%)	43 (47%)	
CML/JMML	32 (9%)	3 (4%)	
Disease status			
First CR	120 (34%)	32 (36%)	ns
Second CR	126 (36%)	28 (31%)	
Beyond the third CR	44 (12%)	6 (6%)	
No. in remission	63 (18%)	24 (27%)	
Disease phase			
Early stage	120 (34%)	34 (38%)	ns
Advanced stage	233 (66%)	56 (62%)	
Lansky-Karnofsky score			
Median (range)	90% (60-100)	90% (50-100)	ns
No. of transplants			
First	266 (75%)	76 (84%)	ns
Second/third	78/9 (25%)	12/2 (16%)	
Type of previous transplant			
Autologous	24 (27%)	1 (11%)	ns
Allogeneic	63 (72%)	8 (89%)	
Follow-up (mo)			
Median (range)	54(6-294)	15(3-29)	

JMML indicates juvenile myelomonocytic leukemia; ns = not significant.

Table 2
Transplant Characteristics

Conditioning Regimen	N (%)
Myeloablative conditioning	188 (53%)
Reduced-intensity conditioning	165 (47%)
Use of serotherapy	
Yes	88 (25%)
No	265 (75%)
Fludarabine based	
Yes	240 (68%)
No	113 (32%)
Total body irradiation based	
Yes	38 (11%)
No	315 (89%)
Graft manipulation	
No	147 (42%)
Yes	206 (58%)
CD34 selection	82
CD3/CD19 depletion	102
TCR$\alpha\beta$/CD19 depletion	22
Stem cell source	
Peripheral blood	270 (76%)
BM	34(10%)
Umbilical cord blood	49(14%)
Type of donor	
Matched related donor	121 (34%)
Haploidentical related donor	105 (30%)
Unrelated donor	127 (36%)

Statistical Analysis

Patient, disease, and transplantation-related variables were expressed as medians and ranges, or as percentages, as appropriate. Occurrence of acute GVHD and chronic GVHD, as well as NRM and RI, were expressed as CI curves to adjust the analysis for competing risks. Death from any cause and graft rejection were competing risks to estimate the CI of acute GVHD and chronic GVHD. Death in remission was treated as a competing event to calculate the cumulative RI. Relapse was considered the competing event for calculating the CI of NRM. The Gray test was used to assess differences between RI and NRM.

In the univariate analysis of risk factors for relapse, the following variables were evaluated: age and gender, Lansky-Karnofsky score at transplant, type of disease and disease phase, number of transplants, conditioning regimen, graft manipulation, type of manipulation, stem cell source, type of donor, and presence of acute GVHD or chronic GVHD. Late relapse was defined as an event occurring >12 months after transplant. For the multivariate analysis of relapse probability, the Cox hazard regression model was used, including in the models all variables with $P < .1$ in univariate analysis. Hazard ratios (HRs) were calculated with a 95% confidence interval (CI). A P value <.05 was considered statistically significant. Patients were censored at the time of relapse, death, or last follow-up.

A personalized prognostic relapse risk score was assigned based on the weight of HRs for each variable in the final Cox model. We validated the results in an independent cohort of patients transplanted from 2016 to the present. The probability of DFS was estimated by the Kaplan-Meier product-limit method. The significance of differences between the DFS curves was estimated by the log-rank test (Mantel-Cox). The database was locked on May 31, 2016. Statistical analysis of the data was performed using the statistical package SPSS for Macintosh version 20.0 (IBM, Armonk, NY) and the R software (R Foundation, Austria) package for Macintosh (version R 3.3.3).

RESULTS

Descriptive Analysis

The median time to neutrophil and platelet engraftment was 14 and 12 days, respectively. There were 20 cases (6%) of primary graft failure and 23 cases of secondary graft failure (6%).

A total of 186 patients (53%) developed acute GVHD (grade 1, n = 26; grade 2, n = 62; grade 3, n = 40; and grade 4, n = 39). The cumulative incidence of acute GVHD was 51 ± 2% at a median time of 28 days (7 to 180 days). A total of 98 patients (28%) developed chronic GVHD (mild in 24 cases, moderate in 47 cases, and severe in 27 cases). The cumulative incidence of chronic GVHD was 36 ± 3% at a median time of 4 months (2 to 20 months). In addition, 180 patients (51%) developed some type of GVHD (classic acute GVHD in 71 patients; persistent, recurrent, or late-onset acute GVHD in 11 patients; classic chronic GVHD in 64 patients; and an overlap syndrome in 34 patients).

Relapse Characteristics

A total of 104 patients relapsed after allogeneic transplantation with a cumulative incidence of relapse of 31 ± 2%. The median time to relapse was 4 months (0 to 54 months).

The cumulative incidence of relapse according to disease phase was 19 ± 4% in the first CR, 36 ± 5% in the second CR, and 39 ± 5% beyond the second CR or refractory disease, with no statistical differences between the second or more advanced disease phase groups (P = .5). Indeed, when we grouped together patients in the first CR versus more advanced disease and compared outcomes, we found statistical differences in terms of RI (first CR of 19 ± 4% versus the second CR and beyond of 38 ± 3%; P = .0002). Because of this, for relapse analysis, we decided to establish 2 groups of patients: an early phase for patients in the first CR and an advanced phase for the remaining patients.

According to the pattern of relapse, there were 79 medullary relapses, 12 extramedullary relapses, and 13 combined relapses. There were no statistically significant differences when comparing relapse kinetics according to the pattern of relapse (the median time to relapse in medullary relapse was 4 months, 7 months in extramedullary relapse, and 5 months in combined relapse). If we consider the disease status at the time of transplantation, there were no statistically significant differences in the relapse kinetics between the patients transplanted in the first CR, the second CR, or beyond the second CR (5, 6, and 3 months, respectively). Finally, there were no statistically significant differences in the kinetics of relapse according to the type of donor (data not shown). Of the 104 relapses, there were 19 late relapses. The cumulative incidence of late relapse was 14 ± 3%.

Univariate Analysis of Relapse

The following variables influenced the cumulative incidence of relapse in the univariate analysis (Figure 1): Lansky-Karnofsky score (a score ≥80% [26 ± 3%] versus a score <80% [38 ± 4%]; P = .02), disease phase at the time of transplantation (early phase 19 ± 3% versus advanced phase 38 ± 3%; P = .0002), in the case of second and third transplants having received a previous autologous transplant or an allogeneic transplant (13 ± 7% versus 45 ± 6%, respectively; P = .009), presence or absence of acute GVHD (20 ± 3% versus 43 ± 3%, respectively; P = .001), and presence or absence of chronic GVHD (12 ± 3% versus 40 ± 3%, respectively; P = .0001). We found a correlation between the early phase of disease and better performance status (Lansky-Karnofsky score ≥80%; OR, 3; P = .001). According to the classification of GVHD of the NIH consensus conference, the variables that influenced the cumulative incidence of relapse in univariate analysis were the presence of classic chronic GVHD (12 ± 4%) and the presence of overlap syndrome (12 ±5%) compared with patients without any type of GVHD (49 ± 4%) (P = .0001).

Cox Multivariate Analysis of Relapse

In the multivariate analysis, only the disease phase at the time of transplantation and GVHD were found to be associated with the relapse cumulative incidence (Table 3).

Prognostic Risk Score for Relapse

A personalized prognostic risk score that could be applied to an individual patient was developed to link the patient's significant risk factors with relapse (Table 4).

Applying the personalized risk score in the training cohort, the cumulative incidence of relapse in a patient with a score of 3 was 70 ± 5%, 40 ± 6 with a score of 2, and 24 ± 6% with a score of 1; in those patients with a score of 0, the cumulative incidence of relapse was 6 ± 4%. The model was tested using the validation cohort. Risk score groups established in the training set were used to define the same 4 risk groups in the validation set with scores of 0, 1, 2, and 3 in 13, 16, 41, and 20 patients, respectively. There was a slightly lower incidence of relapse in the validation cohort, perhaps owing to a shorter follow-up, but the difference was not significant (Figure 2A,B).

The score is reflective of outcomes across all diseases included. We evaluated the score separately in ALL and AML cohorts to strengthen the conclusions. For ALL, the RI with risk scores of 0,1, 2, and 3 points was 5%, 28%, 49%, and 63%, respectively; for AML, the RI with risk scores of 0, 1, 2, and 3, points was 7%, 29%, 36%, and 68%, respectively.

DFS and OS

With a median follow-up of 54 months, the DFS and OS rate were 37 ± 3% and 45 ± 4%, respectively. In the univariate analysis, the DFS rate for patients in the early phase of disease was 60 ± 5%;

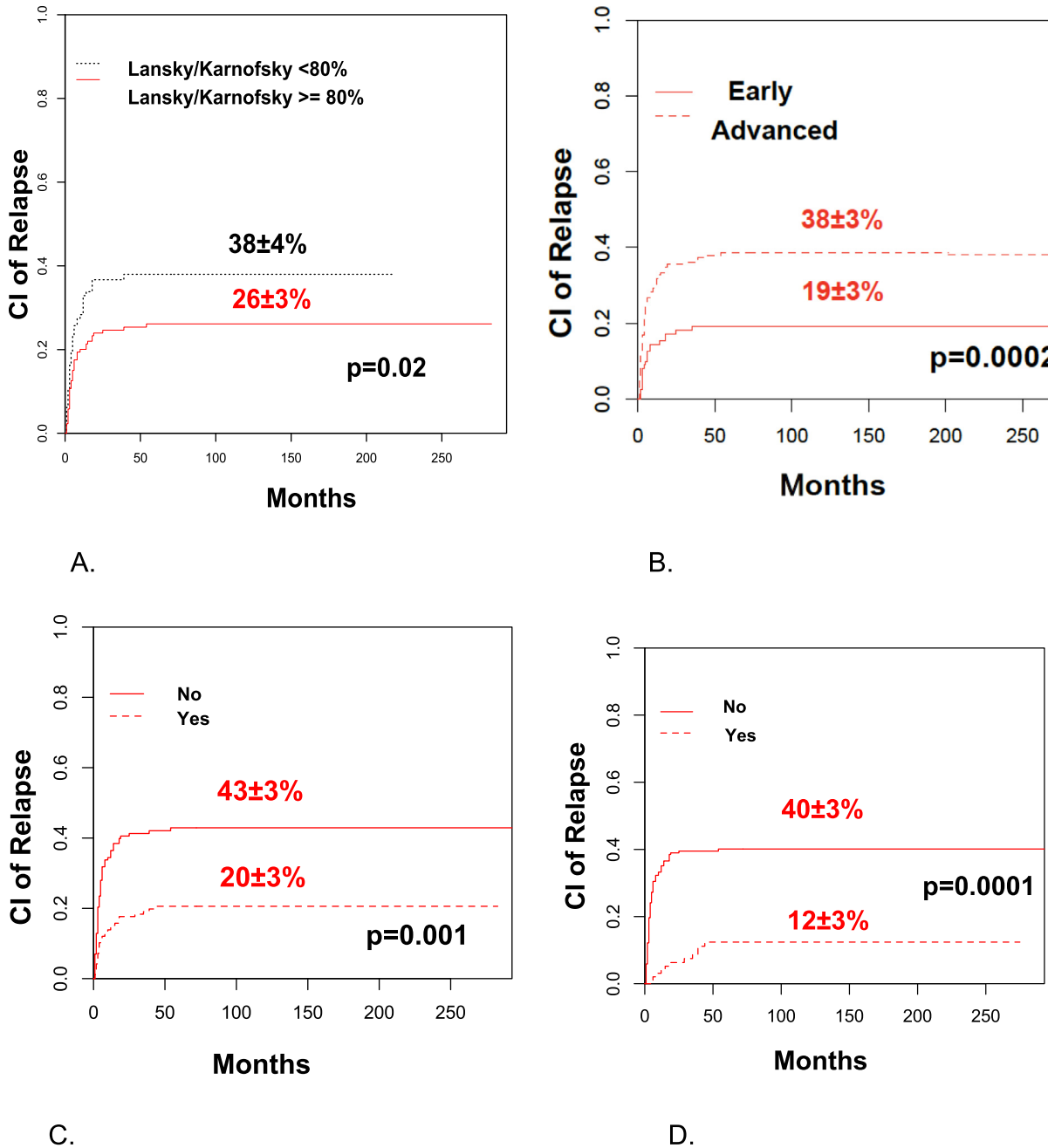

Figure 1. Univariate analysis of relapse according to performance status (A), according to disease phase (B), according to acute GVHD (C), and according to chronic GVHD (D).

Table 3
Multivariate Analysis of Prognostic Factors for Relapse

Variable	HR	CI (95%)	*P* Value
Disease phase			
Early phase	1		
Advanced phase	2.84	1.76-4.57	.001
GVHD classification			
Chronic GVHD	1		
Acute GVHD	4.27	1.99-9.15	.0001
No GVHD	6.86	3.63-12.97	.0001

Table 4
Risk Score for Relapse

	Points
Disease Phase	
Early	0
Advanced	1
GVHD	
Chronic GVHD	0
Acute GVHD	1
No GVHD	2

in the advance phase of disease, the DFS rate was 26 ± 3% (P = .0001). With regard to GVHD, in patients with classic chronic GVHD the DFS rate was 82 ± 5%, for those patients without GVHD the rate was 35 ± 5%, for overlap syndrome patients the rate was 28 ± 8%, for acute GVHD patients the rate was 13 ± 4%, and for persistent, recurrent, or late-onset acute GVHD the rate was 0% (P = .0001). The main reason for the worst DFS was an increase of NRM in patients with late-onset acute GVHD.

When we analyzed DFS according to the severity of chronic GVHD according to the NIH classification, DFS for patients with mild GVHD was 84 ± 9%, for moderate GVHD it was 77 ± 6%, and with severe GVHD it was 21 ± 8% (Figure 3). There were statistically significant differences in DFS between patients with severe GVHD and those patients with mild and moderate GVHD (P = .0001).

The following variables influenced the DFS rate in multivariate analysis: Lansky-Karnofsky score (≥80% versus <80%, HR, 1.45; 95% CI, 1.06 to 1.96; P = .019), disease status (advanced phase versus early, HR, 0.49; 95% CI, 0.34 to 0.71; P = .0001), type of donor (unrelated versus related, HR, 0.64; 95% CI, 0.47 to 0.87; P = .004), acute GVHD (yes versus no, HR, 0.53; 95% CI, 0.38 to 0.73; P = .0001), and presence of chronic GVHD (yes versus no, HR, 4.15; 95% CI, 2.72-6.34; P = .0001). The cumulative incidence of NRM was 29 ± 2%.

DISCUSSION

Allo-HSCT can cure a substantial proportion of pediatric patients with hematological malignancies. Despite decades of research and significant reductions in NRM, the risk of relapse has not decreased significantly and is the leading cause of treatment failure for most hematological malignancies treated with allo-HSCT in children and adolescents. Given the poor outcomes of postransplantation relapse, it is necessary to identify those patients at high risk of relapse, in whom relapse prevention strategies may be indicated. Several studies have associated different risk factors with postransplant relapse, but most of the analyses have been conducted in adult patients, not including children and adolescents [8]. This retrospective study was designed to identify risk factors predictive of postransplant relapse in pediatric patients. In our study, the only risk factors associated with relapse in the multivariate analysis were disease status and the presence of GVHD.

First, as expected, those patients transplanted in an advanced stage (in the second or beyond the second, or not in morphologic remission) had an almost 3 times greater risk of relapse than those transplanted in the first CR, as has been published in the pediatric population [8-10]. In recent years, measurements of MRD status before transplantation have acquired great relevance as being predictive of post-transplantation relapse, both in ALL and in AML [11,12]. One of the limitations of our study was that, being a retrospective study with patients transplanted before 2000, MRD could not be analyzed as a risk factor for relapse.

GVHD is strongly associated with the allogeneic antineoplastic effect of HSCT, graft-versus-leukemia (GVL) effect, or graft versus tumor (GVT) effect [13]. Although separable in murine studies, in humans the distinction between GVHD and

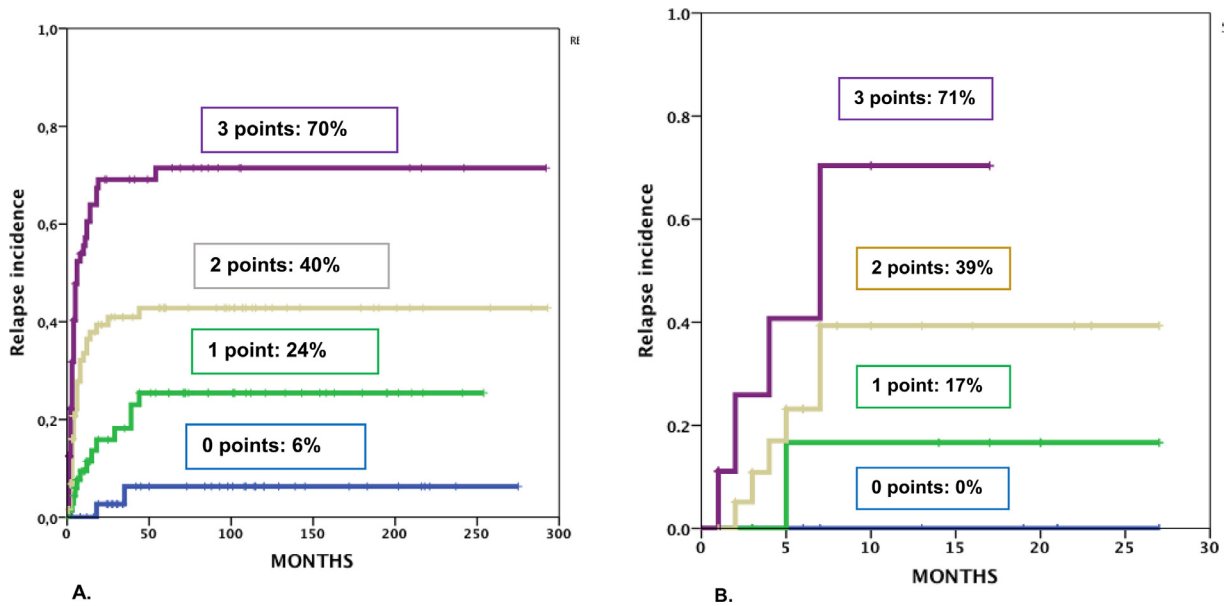

Figure 2. (A) RI according to the risk score based on the Cox multivariate analysis results in the training cohort. (B) RI according to the risk score based on the Cox multivariate analysis results in the validation cohort.

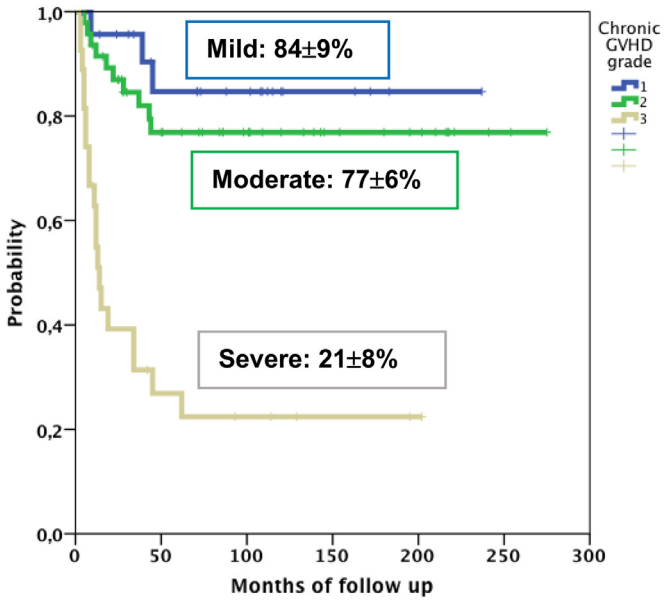

Figure 3. DFS according to the grade of chronic GVHD in the NIH classification.

GVT is less apparent. In our retrospective study, GVHD, mainly chronic GVHD, was the most influential risk factor in the development of relapse. Those patients without GVHD had 6 times higher risk of relapse than those with some type of chronic GVHD. Numerous reports have suggested a reduced risk of relapse in patients with mild to moderate GVHD, but mortality from severe GVHD precludes a survival benefit despite its accompanying GVT [14-16]. However, most of them were published without considering the NIH classification of GVHD [7]. Traditionally, the diagnosis of GVHD has been based on the time of onset, either $<$100 or $>$100 days after HSCT [6]. However, advances in HSCT practice including reduced-intensity conditioning regimens and donor lymphocyte infusion, have altered the natural history and presentation of acute GVHD and chronic GVHD. The NIH consensus criteria for the diagnosis of chronic GVHD emphasize the manifestations of GVHD instead of the time of onset after HSCT. We retrospectively reclassified historically defined GVHD of those patients who received an allogeneic transplantation before 2010, and those patients transplanted after this date were classified prospectively with the NIH consensus criteria. We analyzed the impact of the NIH classification of acute GVHD and chronic GVHD on relapse. Several retrospective studies have investigated the role of the consensus criteria GVHD subtypes on major outcomes [17-20], but most of them only included adult patients. In our study, in the univariate analysis, the presence of acute or chronic GVHD was associated with a reduction of relapse. Using the NIH consensus criteria, classic acute GVHD, classic chronic GVHD, and overlap syndrome were associated with reduction of relapse. These findings have not been reported previously in the pediatric population, although we must consider the limitations present in this retrospective study where patients were reclassified according to the NIH consensus conference retrospectively.

Another finding in our study was that the presence of acute features in patients with chronic GVHD is a marker of adverse prognosis owing to the high NRM as reported previously [17, 20, 21]. The DFS in those patients with classic chronic graft versus host disease was 82 $\pm$ 2%, and in those patients with overlap syndrome it was 28 $\pm$ 8%, suggesting that patients with chronic GVHD but with acute features had an elevated NRM. In addition, we found in this study that the severity of GVHD was not associated with increased GVL and less relapse, as other authors have already published [22,23]. Patients with mild and moderate chronic GVHD had a DFS of 84 $\pm$ 9% and 77 $\pm$ 6%, respectively, versus 21 $\pm$ 8% in those patients with severe chronic GVHD with an NRM of 0%, 7%, and 77%, respectively.

Another finding was that performance status represented with the Lanky-Karnofsky score was found to influence the relapse rate in the univariate analysis, because patients with low performance status have more advanced disease. The Spanish Transplant Group, and more recently the Center for International Blood and Marrow Transplant Research, previously published the influence of Lansky score in post-transplant relapse [24,25].

With promising immunological and cellular therapeutic approaches coming into general use, it is important to define timing and target populations for intervention to prevent relapse after HSCT. In this retrospective study, we found that the median time to relapse was 4 months; therefore, it would be in this period where we could intervene therapeutically to avoid relapse in those patients considered high risk. Pulsipher et al [4] reported, in a multicenter prospective study, risk factors and timing of relapse after allogeneic transplantation in a pediatric population diagnosed with ALL. They identified strong interactions between leukemia risk groups, the occurrence of acute GVHD and MRD pre- and post-HSCT in determining relapse risk, and survival in children with ALL after HSCT. They concluded that there was a window between day +55 and day +100 to 200 when most high-risk patients have not relapsed yet and that this population could benefit from measures to avoid relapse at that time.

Finally, we developed a risk score to allow for a personalized estimation of relapse after allogeneic transplantation for children. We found only 1 study that developed a personalized risk score for long-term survival in children undergoing an allogeneic transplantation owing to ALL or AML but not for relapse [26]. These data offer updated prognostic estimates that could be shared with families during clinic information at diagnosis and during the evolution. Scores were assigned

based on HRs in the final Cox model in the multivariate analysis. We assigned a numeric score to each significant risk factor, giving greater value to the presence of GVHD with respect to the disease phase, because this variable was seen to have greater influence in post-transplantation relapse. Those patients with the highest score, not having GVHD and transplanted at an advanced stage, would have an accumulated incidence of relapse of 70 ± 5% versus those patients with the lowest score, having chronic GVHD and transplanted at an early stage, whose cumulative incidence of relapse was very low (6 ± 4%). The validity of the score was verified using an independent validation cohort of 90 allo-HSCTs performed in our unit from 2016 to the present.

Disease status at transplantation and GvHD have been found to be the only variables associated with post-transplant relapse in our study. Disease status at transplantation is an unmodifiable factor. MRD status in patients in CR may affect the risk of relapse after allogeneic transplantation, but even in MRD-positive patients, allogeneic transplantation provides high survival rates in patients with very high risk leukemia, mainly AML [11]. Chronic GVHD is an important cause of morbidity and mortality, but it has been associated with a low risk of relapse, especially in the first-year post-transplant [27]. However, for severe chronic GVHD, there was no survival benefit of a GVHD-associated GVL effect owing to increased NRM. As there are no established clinical strategies for chronic GVHD management for each and every transplanted patient, it would be useful to know more precisely which GVHD setting is most beneficial in terms of leukemia control and which is associated with an increased risk of NRM.

The main limitations of our study are its retrospective nature. Despite this, several conclusions can be drawn. First, post-transplant relapse is clearly related to the disease phase and the presence of chronic GVHD. Second, the best timing to apply measures to prevent the appearance of relapse in high-risk patients would be the first 3 months after transplantation, given that this is the period where most patients have not yet relapsed. Third, the association of GVHD with GVL is clearly established in our study, but the only form of GVHD that decreases relapse and also has an impact on a better DFS is the classical form of chronic GVHD according to the NIH classification.

ACKNOWLEDGMENTS

The authors thank Dr. Lucas Moreno for his help with English grammar.

Conflict of interest statement: There are no conflicts of interest to report.

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