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Third Allogeneic Stem Cell Transplantation in Children: A Multicenter Analysis From the Spanish GETH-TC Pediatric Group

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ABSTRACT

Third allogeneic hematopoietic stem cell transplantation (HSCT3) is rarely performed in pediatric patients and is associated with high toxicity and mortality. Data on outcomes in this setting remain scarce. We conducted a retrospective multicenter study within the Spanish GETH-TC Pediatric group including 29 children and adolescents who underwent HSCT3 between 2011 and 2024 for malignant ($n = 21$) or nonmalignant ($n = 8$) diseases. Patient-, disease-, and transplant-related variables were analyzed, and survival outcomes were estimated using Kaplan–Meier methods. Engraftment was achieved in 22 patients (75.8%). Grade III–IV acute GvHD occurred in 7% and chronic GvHD in 11%. Severe viral infections were frequent (48%), as were bacterial (34%) and fungal infections (21%). By day +100, TRM was 31%, and overall mortality at last follow-up was 66%, predominantly transplant-related. Estimated 12-month overall survival (OS) and event-free survival (EFS) were 39.5% and 32.7%, respectively. Outcomes differed by indication: patients with malignant diseases had limited survival, particularly when transplanted with measurable residual disease, whereas outcomes were more favorable in nonmalignant disorders, with a 12-month EFS of 50%. Survival was also associated with preserved performance status. HSCT3 in children remains a high-risk procedure. Meaningful survival is restricted to carefully selected patients and appears more achievable in nonmalignant indications.

1 | Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an established curative therapy for numerous malignant and nonmalignant diseases. However, performing a third allo-HSCT (HSCT3) in the same patient is exceptionally challenging due to the cumulative toxicity of prior treatments, the heightened risk of graft-versus-host disease

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(GVHD), and severe infectious complications from prolonged immunosuppression. In relapsed malignancies after a first transplant, only around 15% of patients achieve long-term relapse-free survival at 5 years post HSCT2 [1]. A third allo-HSCT may be considered in scenarios such as relapse of the original disease or graft failure after a second transplant [2]. The decision to proceed with a third transplant depends on the individual patient's clinical status (e.g., remission state, organ function, performance status) and the availability of a suitable donor. Published outcomes for patients undergoing a HSCT3 are notably poor, with most series reporting long-term survival of only 10%–20% and frequent early deaths from relapse or transplant-related toxicity [2–4].

In pediatric patients, multiple transplants pose additional challenges related to immune recovery and the cumulative toxicities of previous conditioning regimens with the consequent risk for significant late effects on growth and organ development [5].

Nearly all published data on third allogeneic transplants come from relapsed malignant diseases (mainly acute leukemias) with very little literature addressing HSCT3 in nonmalignant conditions. In nonmalignant disorders (such as severe aplastic anemia, immunodeficiencies, or other bone marrow failure syndromes), multiple transplants may occasionally be needed, usually due to graft rejection or failure of engraftment rather than disease relapse, but such cases are uncommon and reported mostly as single-case experiences. Overall, there is currently no clear consensus or guideline on the indications for HSCT3 in either malignant or nonmalignant settings, and current knowledge is drawn largely from individual center experiences and retrospective studies.

In light of the formidable challenges and knowledge gaps described above, we performed a multicenter study, in the context of GETH-TC (Grupo Español de Trasplante Hematopoyético y Terapia Celular) Pediatric working party, to collect and analyze the experience with HSCT3 in the pediatric population, encompassing both malignant and non-malignant diseases. We aimed to better characterize the clinical outcomes and the spectrum of complications associated with HSCT3 in children and adolescents. Through this study, our goal is to glean insights that could inform improved therapeutic strategies and patient selection criteria for this high-risk procedure.

2 | Methods

2.1 | Study Design and Setting

We conducted a retrospective, observational, multicenter study across eight Spanish pediatric transplant centers participating in the GETH-TC Pediatric group. The study included 29 consecutive patients who underwent HSCT3 between the years 2011 and 2024.

2.2 | Eligibility

Children and adolescents who received a third allogeneic HSCT for malignant or nonmalignant indications. The study complied with the Declaration of Helsinki; local IRBs approved the protocol with waivers of consent due to the retrospective design.

2.3 | Definitions

Neutrophil engraftment was defined as the first of three consecutive days of achieving a sustained peripheral blood neutrophil count of $>0.5\times10^9$/L. Platelet engraftment was defined as independence from platelet transfusion for at least 7 days with a platelet count of $>20\times10^9$/L. Full donor chimerism was defined as ≥95% donor-derived hematopoiesis in peripheral blood and/or bone marrow, according to institutional chimerism assays. In patients with nonmalignant diseases, including hemoglobinopathies and primary immunodeficiencies, chimerism status was collected as reported by each participating center, without specification of lineage-specific assessment. Graft failure (or rejection) was defined as either primary graft failure, characterized by the absence of donor-derived myeloid cells by day +30 or autologous hematopoietic recovery, or secondary graft failure, defined as the loss of donor-derived hematopoiesis after initial engraftment, as evidenced by declining or absent donor chimerism, with or without subsequent autologous reconstitution. For patients with malignant diseases, complete remission (CR) was defined according to standard criteria as the absence of morphological evidence of disease with hematological recovery, and no evidence of measurable residual disease (MRD) by flow cytometry and/or molecular techniques when available. In patients transplanted in the context of graft failure, where hematopoietic recovery is absent by definition, disease status was assessed based on the absence of morphological and/or molecular evidence of active disease, irrespective of bone marrow aplasia. Acute and chronic GvHD diagnosis was made clinically and confirmed by biopsy whenever possible, with grading based on standard criteria (modified Glucksberg and NIH criteria) [6, 7]. SOS (sinusoidal obstruction syndrome) was diagnosed following the pediatric EBMT criteria [8]. Transplant-associated microangiopathy (TA-TMA) was diagnosed according to the Jodele criteria [9]. Relapse was defined as morphological and/or phenotypical and/or molecular evidence of recurrence in the peripheral blood, bone marrow, or extramedullary sites. Overall survival (OS) was defined as survival from the time of transplantation until death by any cause. Disease-free survival (DFS) was defined as survival from the time of transplantation without evidence of disease relapse in case of malignant disease. Event-free survival (EFS) was defined as time to relapse/progression, engraftment failure, or death. GvHD-free/relapse-free survival (GRFS) was defined as survival from the time of transplantation without evidence of any chronic GvHD, relapse, or death from any cause. Transplant-related mortality (TRM) was defined as any cause of death related to transplantation other than disease. Severe infections were defined as infectious episodes requiring hospitalization and intravenous antimicrobial therapy. Severe viral infections included those requiring systemic antiviral treatment and/or associated with end-organ disease. Severe bacterial infections were defined as infections requiring intravenous antibiotics and associated with sepsis or hemodynamic instability. Severe fungal infections included

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proven or probable invasive fungal infections according to EORTC/MSG criteria.

2.4 | Statistical Analysis

Statistical analyzes were performed using 4.2.2 R software [10]. Qualitative data were summarized as absolute frequencies and percentages, while quantitative data were expressed as means with standard deviations or medians with interquartile ranges, depending on the data distribution. Group comparisons were made using chi squared or Fisher's exact test as appropriate for categorical variables, and either the ANOVA or Kruskal–Wallis test for continuous variables, based on the distribution of the data. The probabilities of overall survival (OS), disease-free-survival (DFS), and GvHD-free/relapse-free survival (GRFS) was estimated using the Kaplan–Meier method, and univariable comparisons among the groups were performed using the log-rank test. Endpoints were calculated at the time of last contact, loss to follow-up or death. We conducted a univariate analysis to assess the factors influencing OS, DFS, and GRFS using univariate and multivariable Cox regression models. Characteristics selected for inclusion in the multivariate model were those with $p < 0.20$ in univariate analysis. A p-value of < 0.05 was considered statistically significant. Patients who were alive and event-free at last follow-up were censored at the time of last contact.

3 | Results

3.1 | Overall Cohort

A total of 29 children and adolescents who underwent an HSCT3 were included in the study. The median age at the time of the HSCT3 was 6.2 years (interquartile range [IQR] 3.9–10.6), with the majority of patients being male (79%).

Haploidentical family donors were the most frequent donor type for HSCT3 ($n = 22$; 75.9%), followed by mismatched unrelated donors (MMUD) ($n = 5$; 17.2%) and HLA-identical siblings (SD) ($n = 2$; 6.9%). Overall, five patients (17.2%) received all three grafts from the same donor, while 24 (82.7%) had at least one donor change across transplants.

Conditioning intensity, defined as myeloablative conditioning (MAC) or reduced-intensity conditioning (RIC), varied considerably across the three procedures. Only 10/29 (34.5%) patients received the same intensity across all three transplants (MAC → MAC → MAC in 6; RIC → RIC → RIC in 4), whereas 19/29 (65.5%) experienced at least one shift, most commonly toward RIC by HSCT3. Specifically, between HSCT2 and HSCT3, 21/29 (72.4%) maintained the same intensity (MAC → MAC in 9; RIC → RIC in 12), while 8/29 (27.6%) switched (MAC → RIC in 4; RIC → MAC in 4).

Baseline performance status (PS) at the time of HSCT3, available for 23 patients, was generally preserved across both malignant and nonmalignant groups, with the majority of patients (82.6%) presenting a Karnofsky/Lansky score ≥ 80. CMV serostatus was evaluable in 28 pairs, with double seropositivity (recipient and donor) being the most common (64.3%).

The median follow-up for the overall cohort, calculated from the HSCT3 to last contact or death, was 5 months (IQR 1–28). In the analysis of the entire cohort, the estimated probability of OS at 6 and 12 months was 50% (95% CI 35–73) and 39.5% (95% CI 25–63), respectively.

Given the heterogeneity of underlying diseases, patient characteristics, transplant features, and outcomes are presented separately for malignant and nonmalignant indications below.

Patient- and transplant-related characteristics are summarized in Tables 1 and 2, while detailed individual data are provided in Table S1.

3.2 | Malignant Diseases

3.2.1 | Patients' and Transplant Characteristics

Twenty-one patients (72%) underwent HSCT3 for malignant diseases, predominantly acute leukemias (acute lymphoblastic leukemia [ALL], $n = 8$; acute myeloid leukemia [AML], $n = 7$). Other diagnoses included myelodysplastic syndrome (MDS) ($n = 2$), juvenile myelomonocytic leukemia (JMML) ($n = 2$), anaplastic large cell lymphoma ($n = 1$) and metastatic osteosarcoma ($n = 1$). Notably, one patient initially diagnosed with MDS had progressed to AML by the time of HSCT3. The main indication for HSCT3 was graft rejection ($n = 12$), with disease relapse accounting for the remaining cases ($n = 9$). Among patients with acute leukemia, 12 were in complete remission at the time of HSCT3, while 4 had measurable residual disease (MRD-positive).

Conditioning regimens were heterogeneous, with a balanced distribution between MAC ($n = 10$; 47.6%) and RIC ($n = 11$; 52.4%). Total body irradiation (TBI) was used in 7 patients (33.3%).

The median interval between HSCT1 and HSCT3 was 4 months (IQR 3–29), while the median interval between HSCT2 and HSCT3 was 2 months (IQR 1–9).

Regarding donor strategy, three patients (14.2%) received all three transplants from the same donor, whereas 18 (85.7%) had at least one donor change.

3.2.2 | Clinical Outcomes

Engraftment after HSCT3 was achieved in 18 of 21 patients (85.7%). Among evaluable cases, graft rejection occurred in three patients (14.3%), all of whom had been transplanted due to prior graft failure.

The median time to neutrophil recovery was 13 days (IQR 9–17), and the median time to platelet recovery was 14 days (IQR 8–44). All patients who achieved engraftment attained full donor chimerism.

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TABLE 1 | Patient- and transplant-related characteristics.

Variable	Overall ($N=29$)	Malignant ($N=21$)	Nonmalignant ($N=8$)
Sex			
Male	23 (79.3%)	16 (76.2%)	7 (87.5%)
Female	6 (20.7%)	5 (23.8%)	1 (12.5%)
Age at HSCT3, years			
Median (IQR)	6.2 (3.8–10.6)	6.0 (3.7–10.6)	6.2 (4.5–6.8)
Year of HSCT3			
≥ 2018	15 (51.7%)	11 (52.4%)	4 (50.0%)
≤ 2017	14 (48.3%)	10 (47.6%)	4 (50.0%)
Donor type (HSCT3)			
Haploidentical	22 (75.9%)	19 (90.5%)	3 (37.5%)
Identical sibling	2 (6.9%)	1 (4.8%)	1 (12.5%)
MMUD	5 (17.2%)	1 (4.8%)	4 (50.0%)
Stem cell source			
PBSC	23 (79.3%)	19 (90.5%)	4 (50.0%)
BM	4 (13.8%)	1 (4.8%)	3 (37.5%)
UCB	2 (6.9%)	1 (4.8%)	1 (12.5%)
Conditioning (HSCT3)			
MAC	13 (44.8%)	10 (47.6%)	3 (37.5%)
RIC	16 (55.2%)	11 (52.4%)	5 (62.5%)
TBI (HSCT3)			
Yes	7 (24.1%)	7 (33.3%)	0 (0%)
No	22 (75.9%)	14 (66.7%)	8 (100%)
Interval HSCT1 → HSCT3, months			
Median (IQR)	4 (3–24)	4 (3–29)	15 (11–20)
Interval HSCT2 → HSCT3, months			
Median (IQR)	2 (1–13)	2 (1–9)	13 (10–13)
Karnofsky/Lansky ≥ 80%			
Available data about PS in $n=23$	19 (82.6%)	16 (94.1%)	3 (50%)
Disease status at HSCT3 (for acute leukemia; $n=16$)			
CR	12 (75%)	12 (75%)	NA
MRD+	4 (25%)	4 (25%)	NA
Donor change across transplants			
Same donor ×3	5 (17.2%)	3 (14.2%)	2 (25%)
≥ 2 donors	24 (82.7%)	18 (85.7%)	6 (75%)

Note: Percentages are calculated within each column (overall, malignant, and nonmalignant groups) unless otherwise specified. For variables with missing data or restricted applicability, percentages are calculated based on available cases. Karnofsky/Lansky performance status was available in 23 patients overall (17 malignant and 6 nonmalignant). Disease status at HSCT3 was assessed only in patients with acute leukemia ($n=16$).
Abbreviations: BM: bone marrow; CR: complete remission; MAC: myeloablative conditioning; MMUD: mismatched unrelated donor; MRD: minimal residual disease; NA: not applicable; PBSC: peripheral blood stem cells; RIC: reduced-intensity conditioning; TBI: total body irradiation; UCB: umbilical cord blood.

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TABLE 2 | Clinical outcomes after HSCT3.

Outcome	Overall ($N=29$)	Malignant ($N=21$)	Nonmalignant ($N=8$)
Engraftment achieved	22 (75.8%)	18 (85.7%)	4 (50.0%)
Time to neutrophils > 500/μL, days			
Median (IQR)	13.5 (10–17)	13 (9–17)	21.5 (10–25)
Time to platelets > 20×10^9/L, days			
Median (IQR)	15 (12–26)	14 (8–44)	25 (10–53)
aGVHD III–IV	2 (6.8%)	2 (9.5%)	0 (0%)
Moderate–severe cGVHD	2 (6.8%)	2 (9.5%)	0 (0%)
Severe viral infection	14 (48.3%)	12 (57.1%)	2 (25.0%)
Severe bacterial infection	10 (34.5%)	7 (33.3%)	3 (37.5%)
Severe fungal infection	6 (20.7%)	5 (23.8%)	1 (12.5%)
SOS	5 (17.2%)	4 (19.0%)	1 (12.5%)
TA-TMA	3 (10.3%)	3 (14.2%)	0 (0%)
TRM at day + 100	9 (31.0%)	6 (28.6%)	3 (37.5%)
Relapse	7 (33.3%)	7 (33.3%)	NA
Alive at last follow-up	10 (34.5%)	6 (28.6%)	4 (50.0%)
Cause of death			
TRM	13 (68.4%)	9 (60.0%)	4 (100%)
Relapse	6 (31.6%)	6 (40.0%)	NA

Note: Percentages are calculated within each column (overall, malignant, and nonmalignant groups) unless otherwise specified.
Abbreviations: aGVHD: acute graft versus host disease; cGVHD: chronic graft versus host disease; NA: not applicable; SOS: sinusoidal obstruction syndrome; TA-TMA: transplant-associated thrombotic microangiopathy; TRM: transplant-related mortality.

Acute GvHD Grade III–IV occurred in two patients (9.5%), and moderate–severe chronic GvHD was also observed in two cases (9.5%).

Severe viral infections were frequent ($n=12$; 57.1%), while severe bacterial and fungal infections occurred in 33.3% ($n=7$) and 23.8% ($n=5$) of patients, respectively. Endothelial complications included SOS in 19.0% ($n=4$) and TA-TMA in 14.2% ($n=3$).

3.2.3 | Survival

Survival outcomes remained limited. At last follow-up, six patients (28.6%) were alive. Among deaths, 60% ($n=9$) were attributed to transplant-related causes and 40% ($n=6$) to disease relapse. By day +100, TRM was 28.6%. Estimated OS was 47.6% (95% CI 30.4–74.6) at 6 months and 38.1% (95% CI 22.1–65.7) at 12 months (Figure 1A). Corresponding EFS was 38.1% (95% CI 22.1–65.7) and 28.6% (95% CI 14.5–58.2), respectively, while GRFS was 33.3% (95% CI 18.2–61.0) and 23.8% (95% CI 11.0–51.1) at 12 and 24 months.

In patients receiving grafts from ≥ 2 different donors, estimated OS was 47% (95% CI 30–75) and 41.2% (95% CI 23–73) at 12 and 24 months, respectively, while DFS was 33.3% and 29.4% at the same time points (Figure 1B). Notably, no patient who underwent three consecutive transplants from the same donor survived beyond the first year. Among patients with acute leukemia, none of the four transplanted with MRD-positive disease survived after HSCT3.

3.3 | Nonmalignant Diseases

3.3.1 | Patients' and Transplant Characteristics

Eight patients (28%) underwent HSCT3 for nonmalignant indications, including β-thalassemia major ($n=1$), acquired aplastic anemia ($n=2$), chronic granulomatous disease (CGD) ($n=2$), and hemophagocytic lymphohistiocytosis (HLH) ($n=3$). The indication for HSCT3 in this group was graft failure after the second transplant.

Conditioning regimens were predominantly RIC ($n=5$; 62.5%), while MAC was used in three patients (37.5%).

The median interval between HSCT1 and HSCT3 was longer than in malignant diseases (15 months, IQR 11–20), as was the interval between HSCT2 and HSCT3 (13 months, IQR 10–13).

Two patients (25%) received all three transplants from the same donor, whereas the remaining 6 (75%) underwent transplantation from at least two different donors.

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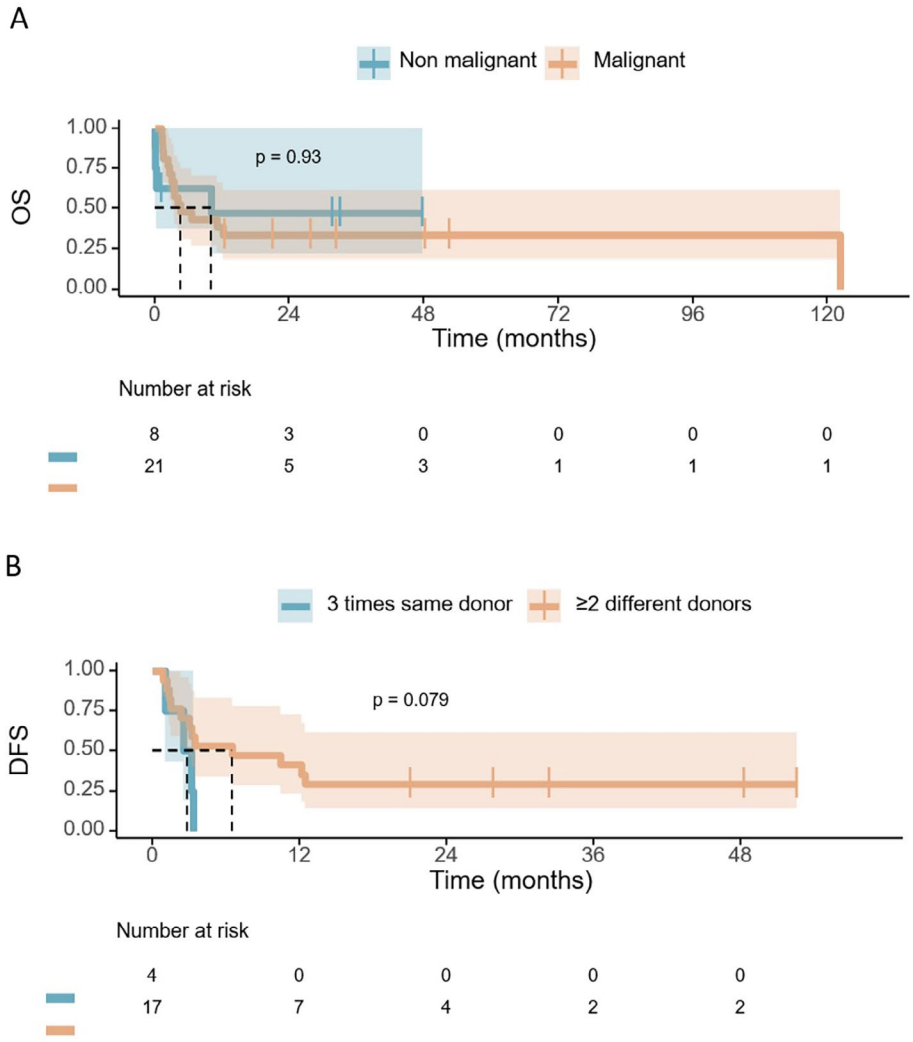


FIGURE 1 | (A) Overall survival according to disease type; (B) disease-free survival (DFS) in malignant diseases group according to donor change.

3.3.2 | Clinical Outcomes

Engraftment after HSCT3 was achieved in four patients (50.0%) and all of them attained full donor chimerism. The median time to neutrophil recovery was longer compared to malignant cases (21.5 days, IQR 10–25), as was platelet recovery (25 days, IQR 10–53).

No cases of Grade III–IV acute GvHD or moderate–severe chronic GvHD were observed.

Severe viral infections occurred in 25.0% ($n = 2$) of patients, while severe bacterial and fungal infections were reported in 37.5% ($n = 3$) and 12.5% ($n = 1$), respectively. SOS occurred in 12.5% ($n = 1$) of patients, and no cases of TA-TMA were observed.

3.3.3 | Survival

At last follow-up, four patients (50.0%) were alive, all of whom had achieved engraftment. All deaths in this group were attributed to transplant-related causes. By day +100, TRM was 37.5%. Estimated OS was 62.5% (95% CI 36.5–100) at 6 months and 46.9% (95% CI 21.5–100) at 12 months (Figure 1A) while EFS remained stable at 50% at both timepoints (95% CI 25–99.9).

3.4 | Impact of Donor Change (Overall Cohort)

We next explored whether donor change across transplants influenced survival outcomes. At 12 months, estimated OS was 20% (95% CI 3–100) in patients receiving all three grafts from the same donor, compared with 43.9% (95% CI 28–70) in those with at least one donor change ($p = 0.39$). This difference persisted over time, with OS plateauing at approximately 39% in the donor-change group, whereas no survivors remained in the same-donor group beyond the first year.

3.5 | Univariate and Multivariate Analyzes

Univariate and multivariate Cox regression analyzes for OS were performed in the overall cohort (Table 3). Year of transplant and performance status were associated with OS in univariate

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TABLE 3 | Univariate and multivariate analysis of factors associated with overall survival (OS).

Variable	Comparison	Univariate HR (95% CI)	*p*	Multivariate HR (95% CI)	*p*
Disease type	Nonmalignant versus malignant	1.05 (0.34–3.21)	> 0.9	—	—
Performance status	≥ 80 versus < 80	0.20 (0.06–0.68)	**0.01**	0.65 (0.14–3.10)	0.6
Donor change	≥ 2 donors versus same donor	0.61 (0.20–1.89)	0.4	—	—
Conditioning	RIC versus MAC	1.41 (0.55–3.58)	0.5	—	—
Year of transplant	< 2018 versus ≥ 2018	7.41 (2.5–22)	**< 0.001**	15.1 (0.5–457)	0.12

Note: Analyzes were performed in the overall cohort. Hazard ratios (HR) with 95% confidence intervals (CI) are reported. Variables not included in the multivariate model are indicated by (–). Bold values indicate statistically significant associations ($p < 0.05$).

analysis, but no independent predictors were identified in the multivariate model. Due to the limited sample size, subgroup-specific analyzes were not performed.

Patients transplanted from 2018 onwards had significantly superior survival compared to those transplanted earlier. When analyzed as a continuous variable, a later year of transplantation was associated with improved OS (HR 0.85 per year increase, 95% CI 0.76–0.95; $p = 0.005$). Consistently, when analyzed as a categorical variable, patients transplanted before 2018 had a significantly higher risk of death compared to those transplanted from 2018 onwards (HR 7.41, 95% CI 2.50–22.0; $p < 0.001$).

Baseline PS also played a key role. Children with a Karnofsky/Lansky score ≥ 80 had a significantly reduced risk of death compared to those with poorer performance (< 80). In univariate analysis, the HR for OS was 0.20 (95% CI 0.06–0.68; $p = 0.01$), and for DFS 0.24 (95% CI 0.07–0.79; $p = 0.02$).

Consistent with OS, similar findings were observed for DFS. When analyzed as a continuous variable, a later year of transplantation was associated with improved DFS (HR 0.75 per year increase, 95% CI 0.65–0.87; $p < 0.001$). In addition, transplantation performed before 2018 was associated with significantly worse DFS (HR 15.3, 95% CI 4.15–56.7; $p < 0.001$), whereas a performance status ≥ 80 was associated with improved DFS (HR 0.24, 95% CI 0.07–0.79; $p = 0.019$). Other variables, including diagnosis (malignant vs. nonmalignant), donor type, stem cell source, conditioning intensity (MAC vs. RIC), use of TBI, time interval from prior transplant, and CMV serostatus, were not significantly associated with survival outcomes. Similarly, donor change across transplants did not reach significance in this analysis.

4 | Discussion

In this multicenter retrospective study, we analyzed the outcomes of a third allogeneic HSCT in a pediatric cohort including both malignant and non-malignant diseases. Our results confirm that HSCT3 remained a high-risk procedure. However, meaningful survival can be achieved in carefully selected patients, and importantly, outcomes differed according to the underlying disease.

Transplant-related mortality was the predominant cause of failure after HSCT3, with many deaths occurring early due to severe infections and regimen-related complications including endothelial syndromes. This underscores that reducing toxicity and strengthening supportive care are central challenges in HSCT3. In this context, RIC approaches may be required in many HSCT3 candidates to mitigate cumulative regimen-related toxicity. However, de-intensification must be balanced against disease control, especially in malignant indications. Consistent with this trade-off, we observed a higher relapse incidence in patients who switched from MAC at HSCT2 to RIC at HSCT3, highlighting the need for careful selection of candidates for de-intensified conditioning and for maximizing disease control before HSCT3. Reported outcomes after HSCT3 remain poor across series, with long-term survival often in the 10%–20% range in acute leukemia cohorts. Our 12-month OS (~40%) in the overall cohort may reflect pediatric selection and inclusion of nonmalignant indications, but early toxicity remains substantial [2, 4].

Beyond transplant-related factors, baseline performance status emerged as one of the strongest clinical determinants of outcome in our study. Virtually all long-term survivors had a Karnofsky/Lansky score ≥ 80% at HSCT3, whereas patients with poorer functional status rarely survived the procedure. This observation is consistent with previous reports [11, 12]. Rank et al. [3] identified Karnofsky scores > 80 as a significant predictor of OS after HSCT3, and the Japanese registry analysis [4] likewise showed improved outcomes in patients with preserved functional status. A high PS likely reflects both better organ reserve to tolerate cumulative transplant toxicity and less aggressive underlying disease. From a practical standpoint, our findings support the use of PS as a gatekeeping criterion when considering HSCT3. Proceeding to a HSCT3 in children with Karnofsky/Lansky scores < 80% was rarely associated with benefit and should prompt careful reconsideration of alternative strategies.

In patients transplanted for malignant disorders, outcomes remained limited and were influenced by disease status at HSCT3. None of the patients with acute leukemia and transplanted with detectable MRD survived beyond 6 months, whereas a substantial proportion of those transplanted in CR achieved meaningful survival. Although limited by small numbers, this finding strongly argues against proceeding to HSCT3 in the presence of active disease and supports considering MRD negativity as a key prerequisite.

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The interval from the previous transplant may further refine prognosis, as a longer remission after HSCT2 likely reflects less aggressive disease biology. Larger registry studies have shown improved outcomes in patients relapsing later after a second transplant, although our cohort was underpowered to confirm this association [4, 13–14]. It is notable that in the adult series by Rank et al. [3] only patients with AML (and not ALL) achieved survival beyond 1 year, hinting that disease subtype also matters. In our pediatric series, numbers were too small to draw disease-specific conclusions, but ALL patients historically have very poor outcomes if needing a third transplant, which should be considered in risk assessments.

In contrast to malignant indications, our study included a small but informative subgroup of patients transplanted for nonmalignant disorders. Survival outcomes in this cohort appeared more favorable, with a 12-month EFS of 50%. These findings indicate that HSCT3 for nonmalignant disorders should be considered a distinct clinical scenario, rather than extrapolated from malignant indications. Most of the available literature on repeated transplants in nonmalignant conditions focuses on second procedures, particularly in severe aplastic anemia, primary immunodeficiencies, and thalassemia, where long-term survival rates of 60%–80% have been reported after graft failure [15–18]. Evidence on HSCT3 in this setting remains very limited and largely anecdotal [19]. In this context, the comparatively favorable outcomes observed in our cohort suggest that HSCT3 may be more readily justified in selected nonmalignant cases with graft failure, provided that organ function and performance status are preserved.

An important question in serial transplants is whether changing the donor between transplants can improve outcomes by invoking a new alloreactive response [20]. In our cohort, most patients underwent HSCT3 from a different donor, and survival was numerically higher in this group, although differences did not reach statistical significance. Patients who had ≥2 different donors across their three transplants had higher 1-year OS (~44%) compared to those who used the same donor all three times (1-year OS ~20%). Similar trends have been reported in previous studies [3], suggesting that donor change may help overcome immune escape or tolerance to the original graft. However, given the limited sample size and the potential trade-off with increased toxicity, particularly when alternative donors are haploidentical, donor strategy should be individualized rather than considered a primary determinant of outcome after HSCT3.

Our multicenter data suggest that survival after HSCT3 has improved in recent years. We noted a striking difference when comparing transplants performed in 2018 or later to those in earlier years. Patients transplanted in more recent years showed markedly better early outcomes, with significantly improved short-term OS. Similar observations have been reported in other series, where more recent year of transplant emerged as a favorable prognostic factor [3]. This temporal trend likely reflects advances in supportive care, improved recognition and management of transplant-related complications, and more stringent patient selection. It is also worth noting that the therapeutic landscape for relapse has evolved: from 2017 onward, novel agents (e.g., blinatumomab, inotuzumab, CAR-T cell therapy for ALL, FLT3 and other targeted inhibitors for AML) have become available [21–27]. These modalities can be used to bridge patients to transplant in a better remission, or even obviate the need for an immediate third transplant in some cases.

This study has several limitations inherent to its retrospective design and small sample size, which restricted the statistical power of subgroup analyzes and limited multivariable modeling. Due to the very low incidence of both acute and chronic GvHD in our cohort, we were unable to assess any potential association between GvHD and disease control, including a possible graft-versus-leukemia effect. In addition, the multicenter nature of the study introduced heterogeneity in transplant strategies, conditioning regimens, donor selection, and supportive care. These factors warrant cautious interpretation of our findings.

In conclusion, a third allogeneic HSCT in children remains a high-risk procedure, with TRM representing the major barrier to success. Meaningful survival appears restricted to highly selected patients, particularly those with preserved performance status and, in malignant disease, deep remission at the time of HSCT3. Outcomes were comparatively more favorable in non-malignant disorders, supporting the concept that HSCT3 represents a distinct clinical scenario in this setting. Conditioning intensity and donor strategy should be individualized, balancing cumulative toxicity against disease control. Despite its risks, HSCT3 may offer a curative opportunity in carefully selected pediatric patients.

Author Contributions

Luisa Sisinni designed the study and wrote the paper. Odelaisy León-Trianaanalyzed the data., Pascual Balsalobre López contributed to coordination of the study within the GETH-TC group, Luisa Sisinni, Marta Villalon Herrera, Sara Vinagre Enríquez, Mercedes Plaza Fornieles, Iván López Torija, Andrea Urtasun Erburu, Laura Alonso García, Alexandra Regueiro García, Maria Trabazo del Castillo, Eduardo J. Bardón-Cancho, Carla Miñarro Chacón collected the data. José Luís Fuster Soler, Antonio Pérez-Martínez, Marta Gonzalez-Vicent, Cristina Díaz de Heredia revised critically the paper.

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The authors have nothing to report.

Ethics Statement

The study was approved by the institutional ethics committee of the coordinating center, with acceptance by the participating centers, and was conducted in accordance with the Declaration of Helsinki.

Consent

Due to the retrospective nature of the study and the use of anonymized data, the requirement for informed consent was waived by the ethics committee.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Individual patient characteristics, treatments, and transplant course.

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