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Toxoplasmosis as an early complication of allogeneic hematopoietic cell transplantation

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Abstract

Among 419 consecutive allogeneic hematopoietic cell transplant recipients, we observed 17 (4.0%) cases of toxoplasmosis at a median time of day 45 (6-322) after transplant. Seven of these 17 cases occurred before day 30 after transplant. Because of the lack of PCR screening and trimethoprim-sulfamethoxazole prophylaxis before engraftment, the diagnosis of toxoplasmosis was late and five of these seven patients died. Analyzing these cases, early *Toxoplasma* blood PCR screening, starting from transplant, is crucial.
Introduction

Toxoplasmosis is a rare but well-known complication that can occur after allogeneic hematopoietic cell transplantation (HCT). It almost exclusively occurs through the reactivation of previously seropositive recipients (R+), especially when the donor is seronegative or the patient has been transplanted with cord blood.\textsuperscript{1,2} The incidence varies widely according to several parameters: (a) the seroprevalence of toxoplasmosis in the country—low in North America, over 50% in France\textsuperscript{3}—(b) the administration and compliance to trimethoprim-sulfamethoxazole (TMP-SMX),\textsuperscript{1} and (c) whether a regular screening with blood real-time PCR (qPCR) is performed or not. In centers that do not screen their R+ patients using a PCR analysis after transplant, the incidence rate is reported to be around 0.2–2%,\textsuperscript{4,5} and the diagnosis is often done at autopsy.\textsuperscript{2} In centers screening their R+ patients, the incidence is much higher, between 6% and 16%\textsuperscript{1,4} but with more cases of infection than cases of disease. Indeed, a weekly blood qPCR screening of R+ patients allows for the detection of the circulating DNA of the parasite before the appearance of symptoms. Despite the lack of a randomized study, qPCR \textit{Toxoplasma} screening is recommended by international HCT guidelines for the early diagnosis of \textit{Toxoplasma} reactivation—especially when TMP-SMX is not given—to anticipate \textit{Toxoplasma}.\textsuperscript{6}

Most cases of \textit{Toxoplasma} are observed between 2 to 6 months after transplant, and even in the larger studies, early cases occurring before day 30 are very rare.\textsuperscript{1,5,7} Because of recent, severe, and early cases of toxoplasmosis in our department, we reviewed all our \textit{Toxoplasma} cases over the last 11 years, focusing on cases observed before day 30 after HCT. The aim of the study was to identify the causes leading to this unexpected finding of early toxoplasmosis and reconsider the pertinence of our procedures accordingly.
Methods

From the laboratory data, we retrospectively collected all cases of *Toxoplasma* infection or disease in allogeneic HCT recipients in our department between January 2007 and June 2018, identifying all patients with a positive qPCR on any body fluid and/or a microscopic examination showing tachyzoites. All patients and donors were assessed for *Toxoplasma* serology before transplant. *Toxoplasma* infection and disease were defined according to the European Society for Blood and Marrow Transplantation’s guidelines. Briefly, *Toxoplasma* infection was defined as a positive qPCR with or without fever but with no organ involvement. *Toxoplasma* disease was defined as *Toxoplasma* infection plus clinical, radiological, or pathological evidence of organ involvement by *T. gondii*. Proven *Toxoplasma* disease was defined as the histologic or cytologic demonstration of tachyzoites in tissue samples obtained either by biopsy or bronchoalveolar lavage (BAL) or at autopsy. Patients who had clinical and radiologic evidence of *Toxoplasma* disease plus ≥1 positive PCR test from blood, CSF, or BAL but who had no histologic confirmation were classified as having probable disease. Disseminated toxoplasmosis was defined as clinical, radiological, or histologic evidence of disease in > 1 organ. The cases were considered early before day 30 after transplant.

All R+ patients were screened, irrespectively of prophylaxis, by qPCR in blood, targeting a 529 bp-repeat-DNA fragment (AF146527) per previously reported methods and with the same qPCR technic all over the study time. According to our local procedures from 2000, this screening was performed weekly from engraftment to day 100, then every 1-2 weeks from 3 to 6 months, then at each consultation, according to the outpatient’s visits, until the patient was withdrawn from any immunosuppressant.

A positive blood qPCR triggered a diagnostic workup, including fungoscopic eye examination, brain CT scan, or magnetic resonance imaging, lung CT scan, and additional investigations according to the clinical presentation. Asymptomatic but ≥ 1 positive qPCR patients were preemptively treated until
there were two consecutive negative qPCR tests; the treatments were by daily doses of trimethoprim-sulfamethoxazole (TMP-SMX) if they did not receive TMP-SMX at diagnosis or by pyrimethamine (P) with sulfadiazine (S), atovaquone, or dapsone if the diagnosis was done under TMP-SMX. Patients with Toxoplasma disease were treated with PS, P-clindamycin, or high-dose TMP-SMX for 3 weeks and then put on secondary prophylaxis. According to French Health Public Law (CSP Art L1121-1.1), the current type of investigation does not require specific informed consent or ethics committee approval.

Results

Four hundred and nineteen patients received an allogeneic HCT during the study period. Data were censored starting on October 30, 2018 so that all patients had a minimal follow-up of 4 months after transplant. Among these 419 patients, 17 (4.0%) developed toxoplasmosis, seven (1.6%) before day 30 (median (range) time: day 22 (6–28)) and 10 (2.4%) after day 30 (median (range) time: day 172 (43–322)) (Table 1). Briefly, all 17 patients were seropositive (R+) for Toxoplasma, and all donors (D) except one were seronegative.

Among the seven patients with early toxoplasmosis, none had received TMP-SMX prophylaxis just before, or since transplant, and only two had been screened for qPCR before diagnosis. In these two patients, the second qPCR of the screening was positive at day 25 and day 28, respectively. These patients were preemptively treated, and none of them developed Toxoplasma disease. In the five other early cases, the diagnosis was suspected based on clinical manifestations—mostly fever or pneumonia —when qPCR blood screening was not routine. Three patients were neutropenic, and only one had graft-versus-host disease (GvHD). All the seven patients have developed febrile neutropenia between transplant and the diagnosis of toxoplasmosis, including six cases of fever of unknown origin. Two patients have another documented infection before the diagnosis of toxoplasmosis which could have delayed the diagnosis: one probable pulmonary aspergillosis and
one pseudomonas bacteremia. Only one patient had a co-infection at time of toxoplasma infection (pulmonary aspergillosis with pleural effusion which was positive for galactomanne and negative for toxoplasma PCR in the pleural liquid). The delay between first symptoms and toxoplasmosis diagnosis was 0 to 6 days. All five (71%) patients with Toxoplasma disease (proven: 1, probable: 4) had pulmonary toxoplasmosis diagnosed at a median of day 22 (range: 6–28). All five patients died one to 33 days after the diagnosis from respiratory (n=2) or multivisceral failure because of disseminated toxoplasmosis (n=3).

Among the 10 cases observed after day 30, there was only one Toxoplasma disease (10%) in a patient who was being treated for TMP-SMX prophylaxis but suffering from gut GvHD. At day 319, this patient who refused to come to the hospital during one month, developed seizures, revealing brain toxoplasmosis, which was proven by histology and qPCR on a brain biopsy, while a previous blood qPCR tested negative 4 weeks before. This patient was successfully treated and survived. The other nine patients were preemptively treated based on positive qPCR results. Two of them died within 3 months after the diagnosis of Toxoplasma infection, one from leukemia relapse (n=1) and the other from unexplained multivisceral failure with pericarditis (n=1), but both of these patients had a negative blood qPCR at the time of death. A third patient developed Toxoplasma infection at day 45 after HCT, concomitantly with uncontrolled acute grade III GvHD and multivisceral failure and remained positive for qPCR in blood until death despite preemptive treatment and without evidence of Toxoplasma disease. The median (range) number of toxoplasma PCR performed before the diagnosis - first positive PCR - of toxoplasma infection or disease was 0 (0-1) in the 7 early cases and 25 (5-29) in the 10 cases occurring after day 30.

Discussion

We report that 41% of our toxoplasmosis cases occurred within 30 days after allogeneic transplant when the patients usually did not receive any post-transplant TMP-SMX prophylaxis and were not
screened by qPCR, both being started after engraftment according to our procedures. Although the small size of our patient cohort precludes any statistical comparison, the concomitant lack of prophylaxis and of screening probably explains the high proportion of *Toxoplasma* disease and of the early deaths with or from toxoplasmosis in these early cases when compared with the later cases. Noteworthy, six of these seven patients developed toxoplasmosis when they had no GvHD and had not received any steroid therapy. On the other hand, the 10 patients with toxoplasmosis after day 30 benefited from a regular screening so that nine of them could have been treated preemptively before the onset of any symptom. The only patient who developed CNS toxoplasmosis 10 months after transplant while on treatment of active GvHD had refused consultations during four weeks while he should have benefited from a weekly PCR screening.

In previous series, most toxoplasmosis cases occurred during the second or third months after allogeneic HCT, with a risk persisting across the first year of transplant and sometimes even later.\textsuperscript{10,11} Cases occurring during the first 4 weeks of transplant have been rarely reported but seem to have a high mortality rate.\textsuperscript{1,5,7} Our series clearly identifies toxoplasmosis as an early complication of allogeneic HCT.

However, these cases should have been detected earlier, before any toxoplasma disease, using a weekly blood qPCR screening from transplant. Therefore, in R+ patients, we recommend starting this screening immediately after transplant, not from the point of engraftment. Whether the administration of TMP-SMX given before transplant for Pcp prophylaxis in some centers may prevent early toxoplasmosis in HCT recipients has not been addressed in the literature and cannot be determined from our series but seems unlikely as Bactrim cannot be efficient on dormant cysts.

The main limit of the current study is that because of the small number of cases, it was not possible to search for the risk factors for developing early—rather than late—toxoplasmosis among our whole cohort of 419 patients. However, our findings are consistent with previous studies identifying cord blood transplant, donor’s seronegativity, and lack of TMP-SMX prophylaxis as risk factors for *Toxoplasma* reactivation.\textsuperscript{1,4}
For a long time, because of the length and deepness of neutropenia after myeloablative conditioning, our efforts during the first 3–4 weeks of transplant have been focused on the optimal management of bacterial and fungal infections, which were the main fears of the transplanters. More recently, because of the predominant use of reduced-intensity conditioning, other infections, initially considered to be complications of the “second” postengraftment phase of transplant, have emerged as major, early problems. Indeed, we recently reported the early onset of pneumocystosis in three out of 18 cases because of the lack of TMP-SMX before engraftment. Similarly, *Toxoplasma* infection may develop early after transplant and should be detected using a weekly qPCR screening from transplant to avoid the development of early *Toxoplasma* disease. From 3 months after transplant, we recommend to go on PCR screening, every 1-2 weeks, as long as the patient receives immunosuppressants.

**Conclusion**

Because there is no reliable *Toxoplasma* prophylaxis except for TMP-SMX, which should not be given before engraftment because of its hematologic toxicity, we strongly recommend starting a weekly qPCR screening with the transplant and eventually from conditioning in R+ patients to catch early reactivation. The timing between the sample and result is also crucial to optimally start a preemptive treatment and avoid the development of *Toxoplasma* disease.
Conflicts of interest: The authors declare that they have no conflicts of interest.

Author contributions:
CR and CC conceived and designed the study. RR, ML, FB, CP, AT and SM generated and provided the clinical data. CA, FF, and FB provided the laboratory data. CR, LC and CC assembled the data, and ran the analysis. CR, SM, FB and CC analyzed and interpreted the data and drafted the manuscript. All authors approved the final version.

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Allogeneic stem cell transplantation in therapy-related myelodysplasia after autologous transplantation for lymphoma: a retrospective study of the SFGM-TC

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autologous stem cell transplantation, lymphoma.

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ABSTRACT

Therapy-related myelodysplastic syndrome (t-MDS) after autologous stem-cell transplantation (ASCT) is a rare complication with no curative option. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) may be considered for eligible patients and has been understudied in t-MDS. We report 47 consecutive patients with t-MDS after an ASCT who underwent allo-HSCT with a median age of 58 years (range: 30-71) at transplantation and a median follow-up of 22 months (range: 0.7-107). The median overall survival (OS) was 6.9 months (95% confidence interval, 0-19). OS rates were 45% (29-60%) and 30% (15-45%) at 1 and 3 years after transplantation, respectively. On univariate analysis prior therapy for t-MDS before allo-HSCT (p=0.02) and mismatched donors (p=0.004) were associated with poor OS. Three-year non-relapse mortality (NRM) and relapse rates were 44% (25-63%) and 41% (22-61%), respectively. Mismatched donors (p<0.001) were associated with higher NRM and a high-risk MDS (p=0.008) with a higher relapse risk. On multivariate analysis HLA mismatch was associated with higher NRM (HR 6.21; 95% CI 1.63-23.62; p=0.007).

In conclusion, our results suggest that one third of the patients who develop t-MDS after an ASCT for lymphoma are cured after an allo-HSCT. The use of mismatched donors with standard GVHD prophylaxis should be avoided in such indication for allo-HSCT. It will be worth to see if the implementation of CY post-transplantation will improve the outcome with mismatched donors.
INTRODUCTION

Treatment options for lymphoid neoplasms include autologous stem-cell transplantation (ASCT). ASCT may be used for the treatment of relapsed or refractory follicular, diffuse large B-cell, Hodgkin’s, or T-cell lymphoma and is associated with improved remission rates and prolonged survival.\textsuperscript{1-3} Adequate patient selection and advances in supportive care have improved outcomes of intensive chemotherapy in recent years. However, such prolongation of survival is also associated with late complications, such as the development of myeloid neoplasm consecutive to the treatment received, including the conditioning chemotherapy of ASCT.\textsuperscript{4-6}

The risk of developing therapy-related myelodysplastic syndrome (t-MDS) or acute myeloid leukemia (t-AML) secondary to the use of both alkylating agents and topoisomerase inhibitors ranges from 5 to 7\% in most series, although there are studies with very variable and extreme incidences between 1\% at 30 months to 11.7\% at six years and may continue to increase until 12-15 years after ASCT.\textsuperscript{7,8}

Therapy-related MDS is associated with a high incidence of cytogenetic abnormalities, with frequent deletions or monosomies of chromosomes 5 and 7.\textsuperscript{8,9} These abnormalities have been described after the use of alkylating agents, confirming their role in the development of myelodysplasia.\textsuperscript{7,8} Most cytogenetic alterations and acquired mutations are associated with a poor prognosis linked to a low response to chemotherapy and short duration of remission.\textsuperscript{10,11,12}

Although allo-SCT has been widely studied in the context of t-AML studies focusing on t-MDS are rare. Furthermore, the best time to perform allogeneic allo-HSCT in this group of patients is unknown as well as predictors that might help patient selection are lacking.\textsuperscript{13,14}
We thus launched a retrospective multicentric study to evaluate the results of all consecutive allogeneic transplantation in population MDS secondary to autologous SCT for lymphoma.

**PATIENTS AND METHODS**

**Data collection**

The registry coordinated by the Francophone Society of Bone Marrow Transplantation and Cellular Therapy (SFGM-TC) of the European Project Manager Internet Server database (ProMISE) was used as the data source. An electronic letter of authorization for the collection and use of the data for this retrospective study was sent to each center. All patients had given written consent before transplant for data collection in ProMISE for future research, in accordance with the Declaration of Helsinki. The scientific council of the SFGM-TC approved this study on February 2, 2017.

**Patient selection**

For this retrospective study, we considered all consecutive adult patients who received an allogeneic transplant for the treatment of t-MDS to ASCT for lymphoid neoplasms registered from 2006 to 2016 in the ProMISE SFGM-TC database.

Patients who received an ASCT and developed secondary t-AML or those who had t-MDS which progressed to t-AML before allo-HSCT were not included. Patients who received ASCT due to neoplasms other than lymphoid neoplasms were not included.

**Definitions**
Lymphoid neoplasms were categorized according to the WHO 2008 classification. The types of MDS followed the WHO 2008 criteria for MDS and were adapted to the WHO 2016 classification. The cytogenetic classification was assessed according to the IPSS score. Lower-risk MDS comprised MDS with low-risk and intermediate-1 risk IPSS scores, and higher risk MDS those with intermediate-2 and high-risk IPSS scores. MDS-EB-1 and MDS-EB-2 were defined according to WHO and were analyzed together. The other categories of MDS with <5% blasts in bone marrow (MDS SLD, MDS MLD, MDS-RS-SLD, MDS-RS-MLD, MDS del(5q), MDS-U) were analyzed together. The response criteria in MDS in patients who received some type of treatment after or before allo-HSCT was defined according to the International Working Group response criteria. Acute GvHD was described according to the criteria of the International Bone Marrow Transplantation Registry. For the analysis of CMV serostatus, the most hazardous combination, defined as a CMV-seronegative recipient and CMV-seropositive donor, was compared with the other possible combinations. Intensity of the allo-HSCT conditioning regimen was analyzed according to previously established working definitions. Only two categories were considered in the analysis: myeloablative and reduced-intensity conditioning, due to the multiple schemes and doses used. HLA mismatch was defined as the presence of at least one difference in the HLA-A, -B, -Cw, DR, or DQ loci. For the analysis, no mismatch HLA comprised identical-sibling donor and matched unrelated donor 10/10 (MUD), and HLA mismatched those with mismatched unrelated donor 9/10 (MMUD) and cord blood units 4/6 and 5/6.
Overall Survival (OS) was defined as the period from the day of allo-HSCT until the
day of death from any cause or date of the last recorded follow-up.

Non-relapse mortality (NRM) was defined as death from any cause other than relapse
of MDS, including progression to AML, from the day of allo-HSCT.

Relapse was defined as a relapse of MDS or progression to AML according to the
WHO criteria.

Statistical analysis
The characteristics of the patients and the factors related to lymphoma, ASCT, t-
MDS, and allo-HSCT are summarized with descriptive statistics. The primary
endpoint of the study was OS, which was calculated using the Kaplan-Meier method.
The frequency of NRM and relapse was calculated by cumulative incidences.

The prognostic effects of the factors with respect to OS, NRM, and relapse were
analyzed with a log-rank test (Mantel-Cox) by univariate analysis. The multivariate
analysis was performed using the potential predicting factors that were significant by
univariate analysis with Cox proportional hazards regression models. The calculations
were performed with SPSS software version 23.0.

RESULTS
To date, the French ProMISe database includes 74,779 autologous transplant and
38,860 allogeneic transplant observations from 98 centers in France, Belgium, and
Switzerland. We searched the French ProMISe database for all registered patients
from January 2006 to December 2016. A total of 47 patients who met the inclusion
criteria were included.
Lymphoma and ASCT

The initial neoplasm for eight patients (17.0%) was a Hodgkin Lymphoma, whereas it was a Non-Hodgkin Lymphoma for 37 (78.7%) (Table 1). Fourteen patients (29.8%) received more than two lines of chemotherapy before autologous transplantation (range 0-8). Conditioning consisted in Carmustine – Etoposide – Cytarabine – Melphalan (BEAM) for 80.8% of patients and other regimens for only 19.2%. Thirty-seven patients (78.7%) achieved complete remission. There were no patients with relapsed lymphoma at the time of allo-HSCT, but it is unknown whether there were patients who relapsed or progressed from lymphoma between ASCT and allo-HSCT. There were no patients who relapsed from lymphoma after allo-HSCT.

MDS

The median time from ASCT to the diagnosis of t-MDS was 74.4 months (range 2.2-259). Eleven patients (23.5%) had MDS-EB-1, 12 (25.6%) MDS-EB-2, and 22 (46.7%) other types of MDS. No diagnostic information was obtained for two patients (4.2%).

Cytogenetic data were obtained for 41 patients: 82.9% had at least one cytogenetic abnormality. The most frequent cytogenetic anomalies were on chromosomes 7 (11 patients, 23.4%) and 5 (six patients, 12.8%) or both of those (fifteen patients, 31.9%). Among the patients, 23.4% were considered to be at low risk and 59.6% at high-risk. Sixty-eight percent of patients received at least one treatment line before allo-HSCT: 16 (34.1%) received hypomethylating agents and 11 (23.4%) AML-like induction treatment. Ten patients (21.3%) achieved complete remission before transplantation and 36 (76.6%) were not in complete remission prior to allo-HCST.
The characteristics of the patients at allo-HSCT are shown in Table 2.

The median age at allo-HSCT was 58 years (range: 30 - 71 years) and most of the patients were male (78.7%). The median time interval from diagnosis of t-MDS to allogeneic transplantation was 7.9 months (range: 2.5 - 16.8).

Peripheral blood stem-cells (PBSC) were the source used for 87.4% of patients. Nineteen patients (40.5%) were transplanted from an HLA-identical sibling donor and 17 (36.2%) from a matched unrelated donor. 10 patients (21.2%) had an mismatched unrelated donor including three patients (6.3%) received umbilical cord blood transplantation (one with a double cord). None of them received a haploidentical donor. Myeloablative conditioning (MAC) was used in nine patients (19.1%), four received a combination of busulfan/cyclophosphamide regimen (two BuCy and two CyBu), with conventional doses (12.8 mg/kg busulfan IV, 120 mg/kg cyclophosphamide), three a combination of fludarabine/busulfan with ATG (150 mg/m^2 fludarabine, 12.8 mg/kg busulfan and various doses of ATG between 2.5 mg–5 mg/kg), one fludarabine/busulfan without ATG (160 mg/m^2 fludarabine, 9.6 mg/kg busulfan), and one fludarabine and TBI (120 mg/m^2 fludarabine, 8 Gy TBI).

Thirty-eight patients (80.9%) received reduced intensity conditioning (RIC). 22 patients received a combination of fludarabine-busulfan-based RIC with ATG or ALG (100–150 mg/m^2 fludarabine, 3.2–6.4 mg/kg busulfan, 2.5-5 mg/kg ATG or 5–20 mg/kg ALG). 4 patients received fludarabine-busulfan-based RIC without ATG. Eight patients received fludarabine-TBI-based RIC (2Gy–8 Gy) and four patients received sequential FLAMSA-RIC.
Eighteen patients (38.3%) received prophylaxis with cyclosporine (CsA) and mycophenolate mofetil (MMF) and 11 (23.4%) cyclosporine and methotrexate (MTX). Twelve patients (25.5%) received only cyclosporine and six (12.8%) received other regimens. The most frequent serological status for cytomegalovirus (CMV) was 29.7% R+/D+. Thirteen transplants (27.1%) were performed with the combination R+/D+.

Performance status was assessed using the Karnofsky Performance Score (KPS): 29 patients (61.8%) had a score of 90-100 before transplantation.

Response to allo-HSCT and complications.

The median duration of post-transplant follow-up was 22 months (range 0.7-107) and median survival 6.9 months (95% CI 0-19).

Thirty-four patients (72.3%) were in complete response after allo-HSCT and nine patients (19.1%) had a relapse/progression post-transplantation. The response was not evaluated for four patients (8.6%) because of the early death of three and lost to follow-up one of them.

Acute GvHD occurred in 20 patients (42.5%), seven (14.8%) had grade I, and 13 (27.7%) had a grade requiring treatment (Grade II-IV). Ten patients (21.3%) developed chronic GvHD, of whom four (8.6%) had extensive and six (12.7%) limited involvement. At the time of the analysis in August 2017, there were 15 relapses or progressions of which 13 died: 12 related to relapse and one could not determine the cause. 11 patients in complete remission died due to transplant complications, mainly infectious. 3 patients had early death due to
transplant complications without knowing the response obtained to the transplant. No deaths due to veno-occlusive disease or graft rejection were reported. The causes of death are listed in Table 3.

**Univariate and Multivariate analysis of patients**

OS for all patients was 45% (95% confidence interval 29-60) at first year, 39% (95% CI 24-55) at 2 years and 30% (95% CI 15-45) at 3 years (Table 4 and Figure 1). Univariate analysis found that prior therapy of t-MDS with hypomethylating agents before allo-HSCT (p=0.02) and the presence of an HLA mismatch (p=0.004) were associated with poorer OS (Table 5). Multivariate analysis showed only a non-statistically significant association with poorer OS for patients receiving a treatment based on hypomethylating agents (HR, 3.55; 95% CI, 0.97 - 12.97; p=0.06), and no other clinically significant factors (Table 6).

NRM was 35% (95% CI 18–51) at first year, 39% (95% CI 21–56) at 2 years, and 44% (95% CI 25–63) at 3 years (Table 4 and Figure 1). Univariate analysis identified gender (p=0.02), graft type (p=0.02) mismatched unrelated donor type (p<0.001) and the presence of an HLA mismatch (p=0.001) to be significant risk factors (Figure 2). Multivariate analysis showed an association between the use of a mismatch unrelated donor (MMUD) and shorter survival after transplantation, relative to identical siblings or matched unrelated donors (MUD) (HR, 6.21; 95% CI, 1.63– 23.62; p=0.007)

The risk of relapse was 35% (95% CI 18–53) at first year, 41% (95% CI 22–61) at 2 years and 41% (95% CI 22–61) at 3 years (Table 4 and Figure 1). The type of MDS and presence of marrow blasts (p=0.008) were the most significant predictive factor of relapse in univariate analysis, however multivariate analysis did not identify the presence of marrow blasts as a significant factor of relapse.
DISCUSSION

This retrospective study of the SFGM-TC, which examined the experience of allo-HSCT in patients, with t-MDS after an ASCT for lymphoid neoplasm, over 10 years, showed donor mismatch to adversely affect OS. This is the first series published in this specific population. In other studies, such patients account for 7 - 32% of the sample.13,14,19-26 Most of the survival data of these studies show results for a mix of patients, including those with t-AML and t-MDS exposed to multiple treatments (chemotherapy and/or radiotherapy, and not necessarily previous ASCT) and various primary diseases (solid organ neoplasms, lymphoid neoplasms, myeloid neoplasms, and congenital anomalies).

Although allo-HSCT has been used as a curative therapeutic modality for eligible patients, data published by several groups have shown poor long-term survival.13,14,19-21 In our series, the median overall survival was 6.9 months with OS at first and third year of 45 and 30%, respectively. The high NRM and relapse rates at three years (44% and 41%, respectively) were similar to those previously reported by other studies, consistent with the poor prognosis of these patients.

Relapse or progression of t-MDS was the main cause of mortality (44.4%) in our study. Patients with MDS-EB-1 and MDS-EB-2 had a higher risk of relapse by univariate analysis. The association of these aggressive variants of myelodysplasia with higher relapse rates has been described in other studies on patients with various primary diseases. It is not known whether treating t-MDS before transplantation can decrease the risk of relapse or whether maintenance treatment should be started after transplant to prevent relapse. In our study, the only type of pre-transplant treatment associated with improved survival was AML-like therapy even if they had not
achieved CR before transplantation, in univariate analysis (p= 0.02). The multivariate analysis shows a non-statistically significant association of hypomethylating agents before transplantation with a poorer OS (HR 3.55, 95% CI 0.97-12.97, p=0.06) regardless of the response they obtained with this treatment before transplantation, in contrast to patients with de novo MDS and poor risk cytogenetics, who normally benefit from this approach.28 Our results are not valued for the sample size and the retrospective analysis. Prospective studies are necessary to determine the benefit of a type of treatment before transplantation27. A retrospective analysis that included more patients with t-AML than t-MDS, reports relapse rates of 42% and 44% at 5 and 10 years with OS of 38% and 24% respectively, when AML-like chemotherapy is used before transplantation.25

No patient in our study received post-transplant maintenance therapy. The use of maintenance therapy, with low doses of azacytidine after allo-HSCT28 and azacytidine29 or decitabine with infusion of donor lymphocytes30, has been published recently and may be worth considering. Targeted therapies, directed against mutated oncogenes, such as IDH-1, IDH-2, or FLT-3 genomic alterations, may improve the outcome of specific subsets of patients in the future.

Infections and GVHD were the main cause of non relapse mortality. In our series there were 27 deaths, 12 were due to relapse, 8 due to infections and 3 due to GVHD. The few deaths due to GVHD show the intensity of the immunosuppressive prophylaxis used, limiting the graft-versus-tumor effect, increasing the possibility of relapse and the appearance of infections. Multiple schemes and doses used in conditioning and immunosuppressive prophylaxis, testing the usual drugs, have not allowed to identify whether one scheme is superior to
A significant factor to improve survival was the absence of a mismatch by using either HLA identical-sibling or matched unrelated donors, as previously reported.\textsuperscript{14} A prospective study of the SFGM-TC reported better OS (37\% vs 15\%, p=0.02) of patients with high-risk MDS who had identical HLA donors \textit{versus} those who did not.\textsuperscript{31} In our series, there was a lower frequency of NRM in patients with 10/10 donors than 9/10 donors (HR, 6.21, 95\% CI, 1.63-23.62, p=0.007), although was not significative in OS by multivariate analysis. NRM associated with mismatched HLA donors could be improved with new prophylactic strategies to counter GvHD, such as post-transplant cyclophosphamide (PT-Cy). Reports over the last years on a small number of patients with \textit{de novo} MDS/AML and t-MN who underwent allo-HSCT show similar results for HLA identical-sibling, MUD, or haploidentical donors.\textsuperscript{32} A recent retrospective series of EBMT in patients with MDS who underwent haploidentical transplants reported better OS for patients treated with PT-Cy than those who were not (OS at three years of 38\% vs 28\%), but with high NRM (41\% vs 55\%).\textsuperscript{33} This modality may be an acceptable option, although the risk of relapse remains high and variations in the dose of PT-Cy have even been tested in high-risk patients with refractory MDS/AML.\textsuperscript{34} Indeed, older patients were recently reported to have OS of 42\%, and a relapse rate of 24\% at two years.\textsuperscript{35} Strategies to improve progression-free survival and decrease NRM using PT-Cy have been implemented. The ALTERGREF trial (NCT03250546), currently in the inclusion phase, will evaluate the effect of PT-Cy for prevention of GvHD in haploidentical and HLA-9/10 mismatched unrelated donors transplant.

The proportion of patients with high risk cytogenetic has been previously reported to be 17 - 49\%.\textsuperscript{13,14,23,24} In our study, 68\% (32 patients) had adverse cytogenetic
alterations, especially abnormalities in chromosomes 5 and 7. However, the results were probably not significant for the few patients in the other risk categories.

The best time to perform the transplant is unknown. It is possible that rapid transplantation could reduce the risk of t-MDS/t-AML-related deterioration. Although a higher frequency of NRM is possible if the transplant is performed beyond six months (50% vs 12.5%, p=0.03) caused by toxicity and infections due to multiple chemotherapy cycles, we found no difference in OS or NRM. Although the median age of 58 years is higher than for other large related series and 60% of patients had a KPS ≥ 90, these factors did not play a significant role in survival, as in other series.

Our data also do not support that factors related to the primary disease and its treatment can influence survival. Interestingly, no veno-occlusive disease was observed in this population having received a previous autologous transplantation.

A retrospective report of EBMT found better results in the period between 1998 and 2006 than for transplants performed before 1998 (40% vs 29%, p=0.02). We found no differences between the periods of 2006-2010 and 2011-2016, probably because support care has not changed as much in the last 10 years as during the transition from the 80’s to 90’s.

Treatment options are still limited for patients who are not candidates for allo-HSCT. Other therapeutic strategies have been tested using azacitidine or clofarabine associated with chemotherapy with encouraging results.

The limitations of our study include the collection of retrospective data and the absence of a historical group for comparisons. Patient selection may have varied
between centers. The small number of patients and the low incidence of secondary myelodysplasia makes it difficult to perform a prospective study.

In summary, although the number of patients in this study was small, the results suggest that patients receiving an ASCT for a lymphoid neoplasm who develop t-MDS have short OS after allo-HSCT, with few long-time survivors. The use of MMUD donors with standard GvHD prophylaxis should be avoided in such indications for allo-HSCT. Studies that attempt to determine whether the implementation of Cy post transplantation would improve these outcomes with mismatched donors are still ongoing. It remains necessary to explore more alternatives and transplant strategies in this critical population.

AUTHOR CONTRIBUTIONS
EG and DJA designed the study, analyzed the data, and wrote the paper. All authors provided patient data, approved the study design, critically reviewed the manuscript and approved the final draft.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.
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