Original article

Donor specific anti-HLA antibodies in hematopoietic stem cell transplantation. Single Center prospective evaluation and desensitization strategies employed

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Background - In the setting of mismatched-hematopoietic stem cells transplantation, the detection of antibodies directed against donor-specific HLA allele(s) or antigen(s) (DSA) represents a barrier for engraftment. It is thus necessary to plan an immunosuppressive strategy, or to select an alternative donor. This prospective study aimed at evaluating the efficacy of our strategy for testing DSAs and the efficacy of the desensitization strategy (DS) employed between November 2017 and November 2020.

Materials and methods - The anti-HLA antibody search was performed using the Luminex bead assays (Lifecode ID and LSA I/II-Immucor) and expressed as mean fluorescence intensity (MFI >1,000 positive). If the patient had DSAs and no alternative donors, a DS was employed with rituximab (day −15), 2 single volume plasmaphereses (PP; days −9 and −8), intravenous immunoglobulins (day −7) and infusion of HLA selected platelets, if persistent DSAs were directed against class I HLA. DS was scheduled with or without PP, according to the DSA MFI (>1,000 or <5,000) and FCXM (flow cytometry crossmatch).

Results - Twenty-two out of 126 patients (17.46%) showed anti-HLA antibodies, 5 of them DSAs (3.97% of total); 3 patients underwent DS obtaining engraftment. Female gender (p=0.033) and a history of previous pregnancies or miscarriages (p=0.009) showed a statistically significant impact on alloimmunization. Factors associated with a delayed neutrophil engraftment were patient's female gender (p=0.039), stem cell source (p=0.025), and a high HSCT-specific comorbidity index (p=0.028). None of the analyzed variables, including the DSA detection, influenced engraftment.

Conclusions - Our study confirms the importance to test DSAs in mismatched-hematopoietic stem cells transplantation The DS used proved successful in removing DSAs. Prospective multicenter studies are needed to better define and validate consensus strategies on DSA management in HSCT.

Keywords: *hematopoietic stem cell transplantation, anti-HLA antibodies, donor selection, engraftment, desensitization strategy.*

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) from a HLA-mismatched (MM) donor represents a treatment option for patients with hematologic malignancies who lack a human leukocyte antigen (HLA) matched donor. The alloreactivity related to the HLA mismatch between donor and recipient leads to the risk of both host-versus-graft (HVG), causing graft failure (GF), and graft-versus-host disease (GvHD)[1]. GF ranges between 4% and 20%[2], with a high risk of poor outcomes. It may be due to hematopoietic stem cell rejection caused by chemo-resistant recipient T lymphocytes, NK cells against mismatched donor cells (cellular rejection) or antibody-mediated rejection (humoral rejection), involving either antibody-dependent cell-mediated cytotoxicity or complement-mediated cytotoxicity[2-5]. The role of anti-HLA antibodies directed against donor-specific HLA allele(s) or antigen(s) (DSA) has been well analyzed in acute and chronic graft rejection (GR) of solid organ transplantations and in transfusion medicine[6-9]. Recently, their role has been better recognized in the setting of HSCT[6]. The presence of DSAs represents a barrier for stem cell engraftment, leading to primary GF or delayed engraftment. Over the years, several methods have been developed to obtain a precise detection and characterization of anti-HLA antibodies in HSCT recipients[2]. In January 2018, the European Society for Blood and Marrow Transplantation (EBMT) published the Consensus Guidelines for the detection and treatment of DSAs in haploidentical hematopoietic cell transplantation[10]. The panel of experts suggest desensitizing patients with DSAs before HSCT, if another suitable donor is not available. However, a defined desensitizing schedule is not proposed. In fact, even if different strategies have been reported, most of them are anecdotal or referred to small series of patients, with different approaches employed[10]. To date, there is no standard desensitization strategy recommended. We hereby report a prospective evaluation of anti-HLA antibodies testing, monitoring and desensitization strategies employed in patients who were candidates to an allogeneic mismatched HSCT at the Hematology Center and Transfusion Medicine Unit of the "Sapienza" University between November 2017 and November 2020.

MATERIALS AND METHODS

From November 2017 to November 2020, all consecutive patients candidates at our Center in Rome for an allogeneic HSCT from mismatched related donors, mismatched unrelated donors, umbilical cord blood (UCB) and lacking an HLA identical donor, or patients for whom an HLA identical donor was still not identified, were included. All patients or their parents or legal guardians provided consent for anti-HLA antibodies testing, desensitization protocol, if necessary, and data collection. Anti-HLA antibodies analysis was performed at the time of the confirmatory test, in case of haploidentical donors, or when starting the search of an alternative donor to guide the selection. The tests were repeated at least 30 days prior to the HSCT date, as reported in our previous experience[6]. According to our Institutional Policy, the donor selection algorithm was first based on the HLA match, followed by donor age, patient and donor body weight ratio, gender, CMV serological status and blood group. Starting from 2020, in case of haploidentical donor selection, the EBMT consensus recommendations for donor selection in haploidentical HSCT[11] were followed. In case of DSA identification, if the patient had more than one available donor, a donor without the corresponding HLA antigen was selected; if there was only one available donor, a desensitization protocol was employed.

Anti-HLA antibodies and DSAs testing

The search for anti-HLA antibodies was carried out using the multianalyte bead assay, with the Luminex platform (Luminex, Austin, TX, USA), including Lifecode ID and LSA I/II (Immucor Inc., Norcross, GA, USA). The results were expressed as mean fluorescence intensity (MFI); as in the preliminary experience, a MFI >1,000 was considered as positive[6]. Antibody search was repeated every 7 days after HSCT for the first month, and then 60, 180 and 365 days after HSCT for all patients. In case of a mismatched donor and DSAs detection, a flow cytometric crossmatch (FCXM) test was performed.

Conditioning regimen

The conditioning regimen consisted of thiotepa, fludarabine and busulfan. The latter was infused over 3 days (9.6 mg/kg) in the myeloablative conditioning regimens (MAC) and over 2 days (6.4 mg/kg) in reduced intensity conditioning (RIC) regimens. In the case of young patients (≤30 years old), with acute lymphoblastic

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leukemia, total body irradiation (TBI, total dose 12 Gy) and fludarabine were employed[12-14]. In patients with aplastic anemia the conditioning regimen was cyclophosphamide and fludarabine with the addition or not of TBI 2 Gy on day –1[15].

GvHD prophylaxis

All patients received the combination of cyclosporine (CyA), short course methotrexate and anti-thymocyte globulin (ATG, Thymoglobulin, 6 or 7.5 mg/kg) in cases of an unrelated donor transplant. In patients receiving an haploidentical HSCT, mycophenolate mofetil (MMF), CyA and post-transplant cyclophosphamide (PT-Cy, 50 mg/kg on days +3 and +4) were employed. Acute (a) and chronic (c) GvHD were defined according to consensus criteria[16,17].

Graft manipulation

Graft manipulation (plasma removal, erythrocyte sedimentation or both) was performed according to the ABO match between donor and recipient. Female and previously transfused male donor grafts were plasma depleted in order to avoid transfusion-related acute lung injury (TRALI)[18,19]. As of March 2020, according to EBMT, Italian group for blood and marrow transplantation (GITMO), and Italian Bone marrow Donor registry (IBMDR) recommendations, in case of a high risk of donor infection by community-acquired COVID-19, cryopreserved peripheral blood stem cell (PBSC) were employed[20].

Definitions

Immunological events have been defined as the occurrence of previous pregnancies, miscarriages, or blood transfusions. Neutrophil recovery was defined as the achievement of an absolute neutrophil count $\geq 0.5\times10^9$/L for 3 consecutive days; platelet recovery was the achievement of $\geq 20\times10^9$/L, with no platelet transfusion requirement in the previous 7 days[21]. Graft failure (GF) was defined as "primary" in case of an absent initial donor cell engraftment, a peripheral neutrophil absolute count $<0.5\times10^9$/L by day +28 after PBSC or bone marrow (BM) HSCT without relapse, and by day +42 after a CBU transplant. GF was defined as "secondary" if the loss of donor cells, after an initial engraftment, and a recurrent absolute neutrophil count $<0.5\times10^9$/L were observed[21,22]. Poor graft function (PGF) was defined as severe cytopenia involving at least two cell lines and/or a transfusion requirement, in the presence of hypoplastic or aplastic bone marrow, with a full donor chimerism, in the absence of active severe GvHD or relapse[21]. Overall survival (OS) was defined as the time from treatment to death. Transplant related mortality (TRM) was defined as death due to any transplantation-related cause other than disease relapse.

Desensitization strategy

The full desensitization strategy (**Figure 1A**) was scheduled with a combination of:

- anti-CD20 monoclonal antibody rituximab (375 mg/m^2) on day –15 to inhibit antibody production by CD20+ B lymphocytes[6];
- two single volume plasmapheresis (PP) procedures, to remove preformed anti-HLA antibodies[6], on days –9 and –8 if ATG was employed; when ATG was not required, PP were performed on days –1 and 0 (**Figure 1B**);
- intravenous immunoglobulins (IVIG) on day –7 (800 mg/kg), to limit antibody rebound[18], followed by the infusion of HLA selected platelets for DSA absorption, if persistence of antibodies directed against class I HLA antigens.

In the case of persistent DSA levels before the administration of ATG (day –4), additional single volume PP procedures were employed with immune-adsorption techniques (plasma-treatment [PT]), using columns to selectively remove IgG antibodies, avoiding the removal of chemotherapy (**Figure 1A**).

According to the EBMT Consensus Guidelines for the detection and treatment of DSAs[10], considering the strong association between high DSA levels (MFI >5,000) and complement activation, and our preliminary experience[6] we decided to differentiate the desensitization strategy employed in DSA positive patients with MFI >5,000 and/or a positive crossmatch, and those with an MFI between 1,000 and 5,000, and a negative crossmatch.

In the latter cases, we employed the following schedule (**Figure 1C**):

- rituximab (375 mg/m^2) on day –15[6];
- IVIG on day –7 (800 mg/kg)[6];
- infusion of HLA selected platelets for DSA absorption in the case of persistence of antibodies directed against class I HLA antigens.

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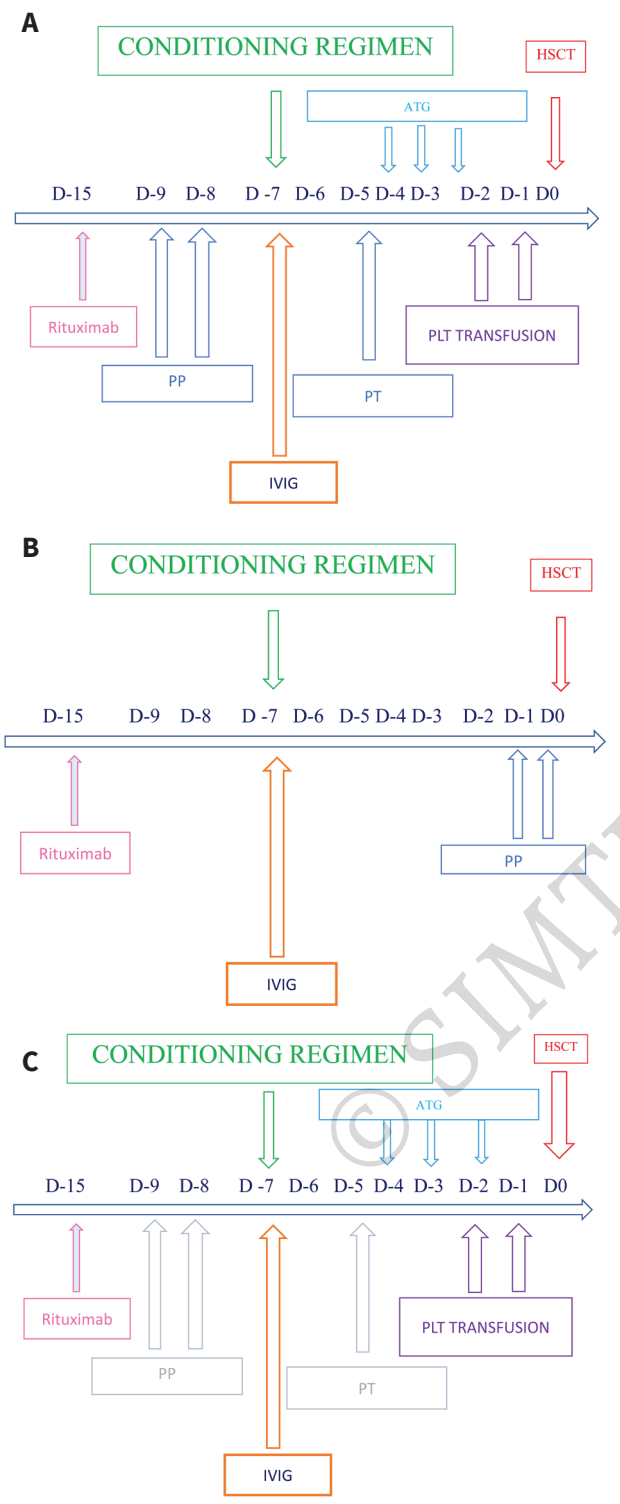

Figure 1 - Desensitization strategy
ATG: anti-thymocyte globulin; HSCT: hematopoietic stem cell transplantation; IVIG: intravenous immunoglobulin; PLT: platelet PP: plasmapheresis; PT: plasma-treatment.

Statistical analysis

Subjects' characteristics for the whole population and stratified by anti-HLA antibodies were summarized by medians of the variables (with%) for categorical variables or by means of quantiles and mean with standard deviation (SD) for continuous variables. In univariate analysis, non-parametric tests were performed for comparisons between groups (Chi-Squared and Fisher Exact test in case of categorical variables or response rate, Mann-Whitney and Kruskal-Wallis test in case of continuous variables). Logistic regression models were used in univariate and multivariate analyses to assess whether the clinical and biological parameters were associated with response outcomes. Odds ratios (OR) and 95% confidence intervals (CI) were reported as parameter results of the logistic regression models. Survival distributions (e.g. TRM, OS) were estimated using the Kaplan-Meier Product Limit estimator. Subgroup comparisons with clinical and biological parameters were performed for descriptive purposes. Differences in terms of time to response, TRM and OS were evaluated by means of the Log- Rank test or Cox regression model, in univariate and multivariate analyses, after assessment of proportionality of hazards. Hazard ratios (HR) and 95% CI were reported as parameter results of the Cox regression models. All tests were 2-sided, accepting $p<0.05$ as statistically significant, and confidence intervals were calculated at 95% level. All analyses were performed using the R software.

RESULTS

From November 2017 to November 2020, 126 hematologic patients (58 females/68 males), median age 48 years (range 5-69), were prospectively enrolled in the study. Twenty-two showed anti-HLA antibodies (17.46%): 9 for class I HLA antigens, 5 for class II, and 8 for both class I and II. Seventy-nine patients (76%) had received blood transfusions, with a median of 16 units (range: 0-200) of RBCs, 13 (range: 0-61) platelet concentrates and 2 (range: 0-54) plasma units. Among female patients, 70% had had previous pregnancies or miscarriages. Female gender ($p=0.033$) and a history of previous pregnancies or miscarriages ($p=0.009$) showed a statistically significant impact on alloimmunization (**Table I**). In our experience, most patients had a complex immunization, directed against a median of 13 antibodies each (range 1-40). Looking at the epitope's correspondence, we observed a

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Table I - *General characteristichs (patients undergoing antibodies' screening)*

Characteristics	Overall No.=126	Anti-HLA antibodies		p-value[1]
		No No.=104	Yes No.=22	
Age, median (range)	48 (5, 69)	48 (8, 69)	55 (5, 61)	0.56
Gender, No. (%) Female Male	58 (46%) 68 (54%)	43 (41%) 61 (59%)	15 (68%) 7 (32%)	0.033
Pregnancies, No. (%)	33 (70%)*	21 (60%)	12 (100%)	0.009
Pre-HSCT blood transfusions, No. (%)	79 (76%)**	62 (74%)	17 (85%)	0.39
RBC transfusions, median (range)	16 (0, 200)	16 (0, 200)	15 (0-71)	0.69
PLT transfusions, median (range)	13 (0, 61)	14 (0, 61)	10 (1-45)	0.87
Plasma transfusions, median (range)	2 (0, 54)	2 (0, 54)	5 (0-31)	0.72

[1]Wilcoxon rank sum test; Fisher's exact test. All percentages are evaluated on available data. *Data available on 47 patients; **Data available on 104 patients.

median of 4 epitopes involved for each patient (range 1-11). Five patients presented with DSAs (3.97% of the total), directed against class I and II HLA antigens in 3 and 1 case, respectively. One patient had antibodies directed against both class I and class II HLA antigens. Four of the 5 patients with DSAs underwent a HSCT. One patient died before receiving the transplant, due to disease progression.

HSCT characteristics

Eighty-five out of the 126 patients underwent a HSCT (*Online Supplementary Content*, **Figure S1**). The donor was unrelated in 55 patients (65%) (10/10 HLA matched in 34, 9/10 in 21), and a haploidentical donor in 26 (31%). Four patients (4.7%), who underwent a transplant from an identical HLA related donor, were also included in the observation, since the anti-HLA antibody research was performed together with the family HLA typing. Thirty-two donors (38%) were female, fifty-three were males (62%), with a median age of 33 years (range 19-67) (**Table II**).

Anti-HLA antibodies, DSA in patients who underwent a HSCT and desensitization strategy

Nine of the 85 patients who underwent a HSCT showed anti-HLA antibodies (10.59%): 5 for class I HLA antigens, 1 for class II, and 3 for both class I and II.

Table II - *General characteristics of patients undergoing HSCT*

Characteristics	Overall, No.= 85
Age, median (range)	48 (5, 69)
Gender, No. (%) Female Male	36 (42%) 49 (58%)
Diagnosis, No. (%) Lymphoproliferative disorder Myeloproliferative disorder Aplastic anemia	25 (29%) 59 (69%) 1 (1.2%)
Donor, No. (%) Haplo MUD- MMUD Sibling	26 (31%) 55 (65%) 4 (4.7%)
HLA match, No. (%) MUD 10/10 MMUD 9/10 Haplo Sibling	34 (40%) 21 (24.7%) 26 (30.6%) 4 (4.7%)
ABO, No. (%) Matched, No. (%) Major MM, No. (%) Minor MM, No. (%) Bidirectional MM, No. (%)	42 (49%) 15 (18%) 23 (27%) 5 (5.9%)
HSC source, No. (%) BM PBSC PBSC cryopreserved	21 (25%) 59 (69%) 5 (5.9%)
Sorror, No. (%) 0-2 3-4 5-6	67.2% 26.4% 6%
Conditioning regimen, No. (%) MAC RIC	56 (66%) 29 (34%)
Graft manipulation, No. (%) No manipulation Plasma-depletion Plasma-depletion + HES Plasma-depletion + cryopreservation Cryopreservation	38 (45%) 40 (47%) 2 (2.4%) 2 (2.4%) 3 (3.5%)
Donor gender, No. (%) Female Male	32 (38%) 53 (62%)
Donor age, median (range)	33 (19-67)
GvHD prophylaxis, No. (%) ATG+CyA+MTX CyA+MMF+PTCy CyA+MTX	57 (67%) 26 (31%) 2 (2.4%)

Before starting the conditioning regimen 4 of the 9 patients with anti-HLA antibodies showed DSA (4.70% of the total) directed against class I HLA (Cw6-MFI 1,667; A2-MFI 1,109; A2-MFI 2,263) and class II HLA antigens (DR7-MFI 1,200) in 3 and 1 patient, respectively (*Online Supplementary*

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Content, **Table SI**). In 1 of the 4 patients with DSAs (A2-MFI 1,109), both the antibody detection and the FCXM, repeated before starting the conditioning regimen, were negative and a desensitization strategy was thus not necessary. Donor neutrophil and platelet engraftments were reached on days +18 and +27, respectively. Seven months after the HSCT, the patient is in good clinical conditions, with a full donor chimerism. The three remaining patients underwent the desensitization strategy. Since all of them showed an MFI >1,000 and <5,000 (cut-off values, according to our internal protocol), and a negative FCXM, we employed the second desensitization strategy option. DSA levels, monitored during and after the desensitization treatment (every 7 days after HSCT for the first month, and then 60, 180 and 365 days) in all treated patients, showed a decreasing profile before stem cell infusion, without antibody rebound. All patients obtained a donor neutrophil engraftment within 20 days after the transplant and a platelet engraftment was reached at a median of 27 days (range 25-64) (*Online Supplementary Content*, **Table SI** and **SII**).

Donor engraftment

Neutrophil engraftment occurred at a median of 20 days (range 10-37) after HSCT. Factors associated with a delayed neutrophil engraftment were patient's female gender (p=0.039), bone marrow as stem cell source (p=0.025), and a high HSCT specific comorbidity index (p=0.028)[23]. No variable was statistically correlated with platelet engraftment, observed at a median of 24 days (range 11-178) after HSCT. A full donor chimerism was observed in 69 patients (81.18%) within 20 days after HSCT; the employment of a MAC conditioning regimen showed a statistically significant association with the probability of obtaining a full chimerism at 20 days after HSCT (p=0.046%). Three patients died within 20 days after HSCT, without showing trilinear engraftment. Only 2 patients (2.35%), who did not present anti-HLA antibodies, experienced a GF. Eleven patients (12.94%) had a PGF. None of the variables analyzed, including the detection of anti-HLA antibodies or DSAs, influenced the risk of developing a GF or a PGF (**Table III**).

HSCT and overall outcomes

The median follow-up was 22.76 months (range 0.92-35.0). The 2-year and 3-year OS were 68.7% and 58.9%, respectively (**Figure 2**). Patients' age (p=0.027), a PBSC stem cell source

Table III - *Statistical correlations*

Characteristics	p-value[1]
Neutrophil engraftment	
Gender	0.039
HSC source	0.025
Sorror	0.028
PLT engraftment	No statistical correlation among the evaluated variables
Day +20 chimerism	
MAC conditioning regimen	0.046
Poor graft function	No statistical correlation among the evaluated variables

[1]Wilcoxon rank sum test; Fisher's exact test.
All variables analyzed: age, median (range); aGvHD, No. (%); anti-HLA Ab, No. (%); bacterial infections, No. (%); CD3+, median (range); CD34+, median (range); conditioning regimen, No. (%); diagnosis, No. (%), donor, No. (%); donor age, median (range); donor gender, No. (%); DSA, No. (%); fungal infections, No. (%); graft manipulation, No. (%); GvHD prophylaxis, No. (%); HLA match, No. (%); ABO match, No. (%); HSC source, No. (%); pregnancies, No. (%); pre-HSCT blood transfusions, No. (%); Sorror, No. (%); viral infections, No. (%).

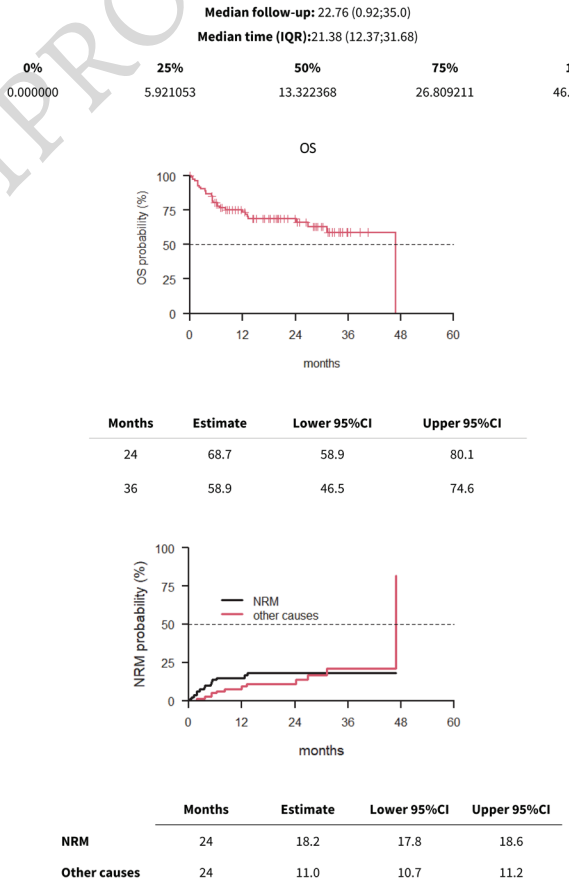

Figure 2 - Median follow-up, OS and TRM
OS: overall survival; TRM: transplant related mortality.

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Table IV - *Overall survival*

OS univariate			
Characteristics	**HR[1]**	**95% CI[1]**	**p-value**
Age	1.02	1.00, 1.04	0.027
PBSC	6.80	1.60, 28.9	0.009
Sorror as continuous	1.44	1.13, 1.83	0.003
CyA+MTX	6.01	1.36, 26.5	0.018
OS multivariate			
Characteristics	**HR[1]**	**95% CI[1]**	**p-value**
Sorror_n	1.33	1.04, 1.70	0.022
HSC source (PBSC)	5.17	1.20, 22.3	0.027

[1]HR: hazard ratio; CI: confidence interval.
All variables analyzed: age, median (range); aGvHD, No. (%); anti-HLA Ab, No. (%); bacterial infections, No. (%); CD3+, median (range); CD34+, median (range); conditioning regimen, No. (%); diagnosis, No. (%), donor, No. (%); donor age, median (range); donor gender, No. (%); DSA, No. (%); fungal infections, No. (%); graft manipulation, No. (%); GvHD prophylaxis, No. (%); HLA match, No. (%); ABO match, No. (%); HSC source, No. (%); pregnancies, No. (%); pre-HSCT blood transfusions, No. (%); Sorror, No. (%); viral infections, No. (%).

(p=0.009), the Sorror HCT-specific comorbidity index[23] (p=0.003), and the GvHD prophylaxis platform (p=0.018) showed a statistical correlation with OS. Multivariate analysis confirmed that the HSCT-specific comorbidity index (p=0.022) and the stem cell source (p=0.027) had the most significant impact on OS (**Table IV**). The 2-year NRM was 18.2% (**Figure 2**). The TRM rate at 100 days was 8.2%. In our experience, the overall OS and TRM did not differ between patients with or without anti-HLA antibodies and DSAs.

DISCUSSION

Considering the increasing use of partially HLA-matched donors, DSAs represent a new hurdle in HSCT, being a risk factor for GF, PGF, and GR[10,24-30]. Many aspects should be investigated to define risk factors for developing anti-HLA antibodies. The main causes are pregnancies, blood transfusions or previous allogeneic transplantation[2], but the impact of each one is not well clarified. The effect of pregnancies and transfusion on alloimmunization have been analyzed in studies on the prevalence of anti-HLA antibodies in blood donors[31-35]. A study on 7920 volunteer donors showed a similar prevalence of anti-HLA antibodies in non-transfused (1%) and transfused male donors (1.7%)[36]. This low prevalence of HLA immunization in transfused males is probably related to the long interval (median 22 years) between a previous transfusion and blood donation[36-38]. Anti-HLA antibodies were detected in 17.3% of all female donors and in 24.4% of those with a history of pregnancies, increasing progressively with the number of pregnancies, up to 32.2% if more than 4 pregnancies were reported in the personal history. Our study confirms that the female gender (p=0.033) and a history of previous pregnancies or miscarriages (p=0.009) had a statistically significant impact on alloimmunization. In a large retrospective analysis on 617 patients with hematologic diseases, Seftel *et al.*[37] reported an alloimmunization in 19% and 7% of patients receiving post-storage or pre-and post-storage leukoreduced blood products, respectively. Thanks to the introduction of online universal leukoreduction in 2008, we observed no statistically significant differences in alloimmunization between transfused and non-transfused patients (p=0.39). However, it is possible to detect "natural antibodies" also in non-transfused males, due to cross reactivity with bacterial antigens, foods, and inhaled allergens[31-33]. Moreover, HLA antibodies appear to be dynamic, with reactivation of DSA without re-exposure[39]. In our analysis, the number of patients without immunizing events is too limited to analyze this aspect. Further studies are required to understand better the factors capable of influencing HLA immunogenicity and antigenicity. Besides, it is still unknown whether antibodies directed against class I or class II HLA antigens are more implicated in the pathogenesis of engraftment failure. Many aspects are involved, including their expression and density on the different cellular surfaces[24,39]. Another unmet need is to establish the prohibitive levels of DSA. MFI levels generally considered positive are those >500-1,000[2], even if some groups have recently reported that the presence of low DSA levels in the absence of desensitization did not correlate with the risk of developing a GF or a PGF[40]. Regarding necessary monitoring activities, considering the persistent negative results without antibody rebound obtained after desensitization in all treated cases in both of our experiences, we consider as sufficient to repeat DSA detection within the first two months without retesting at 180 and 365 days after HSCT. Antibody detection should be considered in case of disease relapse. The expansion of anti-HLA antibody testing methods has allowed to obtain a higher sensitivity. Nowadays, solid-phase assays are the most frequently employed

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methods, providing semi-quantitative results. The main limitations are related to the lot-to-lot variability in the amounts of coated proteins and the inter-assay variabilities. These aspects limit the possibility of establishing stringent cut-off levels, thus highlighting the role of local laboratories' experience and standardization[39]. Overall, solid phase assays require an advanced understanding of molecular polymorphism and serologic patterns of cross-reactivity[39]. The epitope's specificity identification could represent an important tool to be evaluated in patients with a complex immunization. Moreover, many studies have demonstrated the critical role of complement-binding DSA in the development of GR in haploidentical HSCT recipients and solid organ transplantation[10,29,30]. In our experience, using solid-phase assays in conjunction with FCXM allowed to better evaluate DSA's sensitivity, specificity, and strength. Moreover, the DSA testing and monitoring strategy during donor selection allowed to identify patients at a high risk of developing GF. Hence, we had time to select alternative donors, as first option, or to start our desensitization protocol, avoiding any interference with the pharmacokinetics of the conditioning regimen and ATG administration. This strategy was tuned thanks to our previous experience on 65 consecutive patients screened for anti-HLA antibodies, candidates for a mismatched HSCT: the desensitization strategy was effective and safe in 2 treated patients[6]. Our strategy permits us to overcome DSA obstacles: GF was observed only in 2 patients (2.35%) who did not present anti-HLA antibodies nor DSAs. PGF, observed in 12% of patients, did not correlate with DSAs. Factors associated with a delayed neutrophil engraftment were the patient's female gender (p=0.039), the use of bone marrow as stem cell source (p=0.025), and a high HSCT–specific comorbidity index (p=0.028). We did not observe any statistical correlation among the evaluated variables on platelet engraftment. OS and TRM did not differ between patients with or without anti-HLA antibodies and DSAs.

CONCLUSIONS

Our analysis shows that anti-HLA antibodies are frequent in hematologic patients (17.46%), with a high probability of identifying DSAs. We confirm the importance of routine testing and monitoring for anti-HLA antibodies and DSAs in mismatched HSCT procedures and the need to plan desensitization strategies if other donors are unavailable. To date, there is no recommended standard desensitization strategy. The desensitization schedule employed at our Center appears safe and effective in obtaining a stable engraftment. Future prospective studies are needed to standardize anti-HLA antibodies and DSA management and to test our tailored strategy on larger series of patients, taking into account HLA match, conditioning regimen, and MFI. To make this possible, a strong collaboration between transplant physicians, transfusion and immunogenetics specialists is required.

AUTHORS' CONTRIBUTIONS

API and ULR conceived the idea of the study and the protocol and wrote the manuscript; ULR collected the data; AP performed the statistical analysis. MPP, ULR, PG, LL, PC, GiG and CC performed and analyzed tests and results. API, WB and RR managed HSCT and follow up. DC and NC performed the hematopoietic stem cell manipulation. MG and MSB performed the desensitization strategies. RR, GG, RF, MM, SC, and API revised the data, tables, figures and manuscript. All the Authors approved the manuscript.

The Authors declare no conflicts of interest.

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