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Anti-HLA Donor-Specific Antibodies are Associated to Infection and Not to the Engraftment Rate in Outpatient Haploidentical Hematopoietic Cell Transplantation

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ABSTRACT

Background: Recipients of a related haploidentical stem cell transplant (haplo-SCT) can have preformed antibodies to HLA donor's antigens. **Objective:** The aim of the study was to evaluate the engraftment rate and major clinical associations of anti-HLA donor-specific antibodies (DSA) at two mean fluorescence intensity (MFI) thresholds in recipients of an outpatient haplo-SCT. **Methods:** Seventy haplo-HCT recipients were analyzed. A virtual crossmatch was performed using the donor HLA typing and the recipient's anti-HLA DSA test results. Data for anti-HLA-A, -B, -C, and -DR were analyzed. Recipients with DSA ≥ 500 MFI were considered positive, and those with < 500 were considered negative; the same was adopted for MFI ≥ 1000. **Results:** Post-transplant infection was higher in recipients with DSA ≥ 500 MFI (84.6%, p = 0.041). First-year mortality was higher in DSA-positive patients ≥ 500 MFI, p = 0.004, and DSA ≥ 1000 MFI, p = 0.022, than in DSA-negative recipients. Graft failure in the first 100 days was not associated with DSA ≥ 500 or ≥ 1000 MFI. There was no difference in acute (a-GVHD) or chronic (c-GVHD) graft versus host disease between DSA-positive and negative patients. **Conclusions:** There was no association of anti-HLA DSA at MFI ≥ 500 and ≥ 1000 with graft failure, however, increased infection and 1st-year mortality were documented in related haplo-HCT at the MFI cutoffs studied. (REV INVEST CLIN. 2023;75(5):249-58)

Keywords: Anti-HLA donor-specific antibodies. Graft failure. Haploidentical stem cell transplant. Mean fluorescence intensity. Single-antigen assay. Virtual crossmatch.

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INTRODUCTION

Hematopoietic cell transplantation (HCT) represents a potential cure for various malignant and non-malignant hematological diseases[1]. When there is no HLA-identical donor, a haploidentical one is a valid option, usually a first-degree relative[2]. According to the recommendations of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), donation from parents, siblings, or other relatives who share a single HLA-A, -B, -C, and -DRB1 haplotype typed by intermediate or high-resolution with the recipient is defined as haploidentical related[3]. This type of donation carries the risk of primary graft failure[4-6], rejection, and acute graft-versus-host disease (GVHD) in patients allosensitized against HLA antigens. Furthermore, guidelines from the NMDP/CIBMTR state that DSA leads to detrimental effects after HCT[7]. Therefore, it is necessary to test for specific antibodies in the recipient against donor HLA antigens (donor-specific antibodies, DSA) before haplo-HCT[3,8].

In non-myeloablative conditioning regimens, the receptor's T and NK cells, responsible for the cellular immune response, can cause bidirectional alloreactions, as in the case of GVHD[2,5]. With the selective depletion of T cells, the risk of GVHD and graft failure in haploidentical transplants decreases[2] at the cost of increased relapse; however, the trend is to use allografts without manipulation[5]. In developing countries, there is also the prohibitive cost of T cell depletion.

Haploidentical transplantation has been related to a higher frequency of graft rejection and GVHD[9]. Anti-HLA donor-specific antibodies (anti-HLA DSA) can generate graft failure in 2-15% of cases. DSA specificity is routinely investigated in solid-phase assays, such as LIFECODES™ single antigen assay (LSA). The results are expressed in mean fluorescence intensity (MFI) values. These assays provide a practical, specific, and sensitive approach since they contain a higher density of the specific antigen per bead for evaluating the risk of graft rejection mediated by antibodies through the virtual crossmatch[10,11]. The clinical decisions derived from the DSA testing results are based on certain MFI thresholds. Despite their importance, no international consensus exists on DSA cut-off levels[6]. Moreover, fluorescence is not calibrated against a standard, making interlaboratory standardization difficult. A DSA threshold ≥ 1000 MFI is recommended in the European Bone Marrow Transplant (EBMT) guidelines[12].

MATERIAL AND METHODS

The files and electronic records of patients who attended a university hospital hematology reference center, self-identified as Hispanic mestizos with malignant or non-malignant hematological pathologies, undergoing their first haploidentical transplant of hematopoietic progenitors obtained from the peripheral blood after mobilization with subcutaneous granulocyte colony-stimulating factor, 10 µg/Kg/day for 4 days, collected on day 5 by a single large volume leukapheresis, from June 2017 to December 2020 were analyzed. All individuals had a single antigen assay to detect anti-HLA DSA carried out within 1 month before transplantation. The Ethics and Research Committee at the institution approved the study, and informed consent was waived due to the study's retrospective nature.

The conditioning regimen was administered in the outpatient clinic as described[13]. It consisted of fludarabine 25 mg/m^2 and cyclophosphamide (Cy) 350 mg/m^2 from days −5 to −3, and melphalan, 70-100 mg/m^2 on days −2 and −1, with or without 2 Gy of total body irradiation. In aplastic anemia cases, melphalan was not used; instead, Cy 50 mg/kg/day on days −2 and −1 was administered.

Posttransplant GVHD prophylaxis included Cy, 50 mg/kg/day on day +3 and +4, mycophenolic acid 1 g/day from day +5 to day +35, and oral CsA through day +18; CsA levels were measured weekly, adjusting for a target level of 150-250 ng/mL, and later tapered over 30-60 days.

Collected data included high-resolution HLA typing of recipients and donors by sequencing-based typing and anti-HLA antibodies (LSA, LIFECODES™ single antigen, Immucor, Waukesha, WI, USA) present in recipients' serum before transplant, hematological diagnosis, disease status at the time of transplant, conditioning scheme received, the dose of CD34+ cells/kg infused, and ABO compatibility. Days to the recovery of neutrophils and platelets, the development of cytokine release syndrome (CRS), GVHD, and death in the first 100-day post-transplant, among

other relevant clinical variables, were also entered in the database.

Determination of anti-HLA donor-specific antibodies

The LSA test was conducted on a LABScan fluoroanalyzer from the Luminex Labscan 100 platform (LuminexTM, Austin, TX, USA). In short, 10 µL of serum was incubated with 40 µL beads mix for 30 min. After washing, the diluted anti-human IgG PE conjugate was added to the beads. After 30 min of incubation, wash buffer was added to the wells, the plate was placed in the Luminex instrument for reading, and MFI results were recorded. Data were analyzed in Match it! Antibody Software (Immucor GTI Diagnostics, Inc. Waukesha, WI, USA). A virtual crossmatch (VXM) was performed using the donor's HLA typing and DSA values against each allele HLA-A, -B, -C, and -DRB1. Two groups of recipients were integrated for the analysis, those with ≥ 500 and ≥ 1000 MFI for each HLA locus analyzed individually. The association of both DSA levels with relevant clinical variables was investigated. The control group included DSA-negative patients and those in whom donor's HLA antigens were included in the kit and had values < 500 and < 1000 MFI. Foundation for the Accreditation of Cellular Therapy (FACT) related donor haploidentical transplant standards for HLA typing was followed[14].

Graft evaluation

The percentage of chimerism at +30- and +100-day post-transplant was determined by capillary electrophoresis for short tandem repeat (STR) analysis in same-sex donor-recipient pairs, X and Y-chromosomes by FISH for different-sex donor-recipients[8]. Myeloid recovery was defined as the days required to achieve an absolute neutrophil count ≥ 500/µl for 2 consecutive days and a platelet count ≥ 20,000/µl for 2 consecutive days, at least 7 days after the last platelet transfusion.

Statistical analysis

A descriptive analysis of patients' characteristics was performed. Continuous variables were described as medians and interquartile ranges after evaluating the normality of the data distribution with the Kolmogorov–Smirnov test. For categorical variables, frequencies and percentages were analyzed, in addition to the association between groups with Fisher's exact test or Pearson's Chi-square test. The Mann–Whitney test was used to calculate the differences between variables and compare data from both groups. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics software for Windows v. 25.0 (IBM Corp., Armonk, NY).

RESULTS

Information from 70 HLA haploidentical patient/donor pairs who received mobilized HCT in an outpatient setting from June 2017 to December 2020 was analyzed. Patients' characteristics are described in table 1. Thirty-eight patients (54.3%) were male, and the median age was 32 years (15-38).

Regarding donors, 58.6% were males, and the median age was 32 years (24-43); the kinship of the donor with the recipient was brother/sister in 30 cases (42.9%), son/daughter in 13 (18.6%) and father/mother in 27 (38.6%). The donor and recipient match in 16 cases was female /female (22.9%), female/male in 13 (18.6%), in 16 cases, male /female (22.9%), and in 25 cases, the recipient and donor were males (35.7%).

Two MFI thresholds, DSA ≥ 500 and DSA ≥ 1000, were evaluated for HLA loci A, B, C, and DR. In the study group, 13 (18.6%) DSA-positive patients were found for the threshold DSA ≥ 500 MFI, while for the DSA ≥ 1000 MFI cut-off, only 4 (5.7%) cases were encountered. Of the 13 cases with ≥ 500 MFI, 6 (46.2%) were men, and 7 (53.8%) were women (Table 1).

The median MFI observed for DSA anti-HLA-A was 148 (102.3-231.7), HLA-B 186 (114.7-284.2), HLA-C, 229 (159.7-332.5), and HLA-DR, 113 (62.5-215.7). The characteristics of DSA-positive cases are detailed in table 2.

Transplant characteristics

All patients received the reduced-intensity conditioning regimen on an outpatient basis. A median CD34+ cell dose of 9.5×10^6/kg (range 9-12.7) was infused. In all cases, the graft was obtained from the donors' peripheral blood by apheresis and was not depleted of T cells.

Table 1. Transplant patient characteristics by donor-specific antibody (DSA) thresholds

Characteristic	n = 70	DSA > 1000 MFI			DSA ≥ 500 MFI		
		Yes (n = 4)	No (n =66)	p	Yes (n = 13)	No (n =57)	p
Sex		–		0.625			0.364
Male	38 (54.3%)	2 (50%)	36 (54.5%)		6 (46.2%)	32 (56.1%)	
Female	32 (45.7%)	2 (50%)	30 (45.5%)		7 (53.8%)	25 (43.9%)	
Age	23 (15–38)	10 (3–35)	25 (16–38)	0.143	35 (22–41)	22 (13–35)	0.174
Diagnosis				0.237			0.039
B-ALL	27 (38.6%)	0 (0%)	27 (40.9%)		1 (7.7%)	26 (45.6%)	
T-ALL	2 (2.9%)	0 (0%)	2 (3%)		0 (0%)	2 (3.5%)	
NHL	5 (7.1%)	0 (0%)	5 (7.6%)		1 (7.7%)	4 (7%)	
HL	5 (7.1%)	0 (0%)	5 (7.6%)		0 (0%)	5 (8.8%)	
CML	2 (2.9%)	0 (0%)	2 (3%)		1 (7.7%)	1 (1.8%)	
AML	16 (22.9%)	2 (50%)	14 (21.2%)		7 (53.8%)	9 (15.8%)	
MDS	4 (5.7%)	0 (0%)	4 (6.1%)		0 (0%)	4 (7%)	
SAA	6 (8.6%)	2 (50%)	4 (6.1%)		3 (23.1%)	3 (5.3%)	
CLL	1 (1.4%)	0 (0%)	1 (1.5%)		0 (0%)	1 (1.8%)	
MYF	1 (1.4%)	0 (0%)	1 (1.5%)		0 (0%)	1 (1.8%)	
H IgM S	1 (1.4%)	0 (0%)	1 (1.5%)		0 (0%)	1 (1.8%)	
Disease status				0.325			0.231
Active	31 (44.3%)	3 (75%)	28 (42.4%)		8 (61.5%)	23 (40.4%)	
First remission	18 (25.7%)	0 (0%)	18 (27.3%)		3 (23.1%)	15 (26.3%)	
Second remission	13 (18.6%)	0 (0%)	13 (19.7%)		0 (0%)	13 (22.8%)	
Refractory	8 (11.4%)	1 (25%)	7 (10.6%)		2 (15.4%)	6 (10.5%)	
Transfusion of blood products							
Red blood cell concentrates	39 (55.7%)	4 (100%)	35 (53%)	0.09	10 (76.9%)	29 (50.9%)	0.079
Platelet concentrates	24 (34.3%)	4 (100%)	20 (30.3%)	0.012	8 (61.5%)	16 (28.1%)	0.026
Platelet apheresis	15 (21.4%)	1 (25%)	14 (21.2%)	0.628	5 (38.5%)	10 (17.5%)	0.103
Pregnancy before HCT	12 (17.1%)	1 (25%)	11 (16.7%)	0.537	3 (23.1%)	9 (15.8%)	0.391
IgG anti-CMV-positive status (n = 24)	18 (75%)	2 (100%)	16 (72.7%)	0.554	4 (80%)	14 (73.7%)	0.634

MFI: mean fluorescence intensity; B-ALL: B-cell acute lymphoblastic leukemia; T-ALL: T-cell acute lymphoblastic leukemia; NHL: non-Hodgkin lymphoma; HL: Hodgkin lymphoma; CML: chronic myeloid leukemia; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; SAA: severe aplastic anemia; CLL: chronic lymphocytic leukemia; MYF: myelofibrosis; H IgM S: hyper IgM syndrome; HCT: hematopoietic cell transplant; CMV: cytomegalovirus.

Table 2. DSA-positive patients according to HLA specificities

Patient	Sex	Age	Diagnosis	DSA anti–HLA–A	DSA anti–HLA–B	DSA anti–HLA–C	DSAanti–HLA–DRB1
1	F	42	AML	–	–	–	4853
27	F	32	NHL	–	848	–	–
28	F	43	AML	634	874	903	–
31	M	70	AML	–	–	600	–
34	M	39	AML	–	–	538	616
35	F	40	AML	–	626	–	–
36	M	29	B–ALL	–	–	721	–
37	M	2	AML	1125	1033	–	–
40	M	35	SAA	712	714	990	–
48	F	5	SAA	–	–	–	8714
52	F	40	AML	–	–	592	–
56	M	16	SAA	–	–	–	1219
64	F	30	CML	–	924	–	556

AML: acute myeloid leukemia; NHL: non-Hodgkin lymphoma; B-ALL: B-cell acute lymphoblastic leukemia; SAA: severe aplastic anemia; CML: chronic myeloid leukemia; DSA: donor-specific antibody.

The median days to achieve the myeloid graft were 16 (14-18). The same was true for platelet engraftment, 16 (14-20). In 58 (82.9%) patients, chimerism was reported at 30 days; 45 (77.6%) achieved complete chimerism, 6 (10.3%) mixed chimerism, and in 7 (12.1%), there was no chimerism. In 42 (60%) patients, chimerism was determined at 100 days; 33 (78.6%) had complete chimerism, and 9 (21.4%) mixed. Null chimerism was not found at this time (Table 3).

Association of donor-specific antibodies with the study variables

Regarding baseline characteristics for the group with DSA ≥ 500 MFI, statistical significance was found only for diagnosis (p = 0.039). Of the DSA-positive patients, 7 (53.8%) had AML and 3 (23.1%) severe aplastic anemia (SAA), in contrast to the control group (15.8 and 5.3%, respectively). The most frequent diagnosis in the negative DSA group, including 26 cases (45.6%), was B-ALL (Table 1).

Regarding pre-HCT transfusion requirements for the two MFI thresholds, the demand for platelet concentrates was greater in DSA-positive patients (MFI ≥ 500, p = 0.026; MFI ≥ 1000, p = 0.012); all DSA-positive patients ≥ 1000 MFI received platelet transfusion support, as did 61.5% of those with DSA ≥ 500 MFI (Table 1).

There was no significant association between donor characteristics and anti-HLA DSA MFI values or HCT features (Table 3). Regarding complications after HCT (Table 4), the incidence of post-transplant infections was higher in the DSA ≥ 500 MFI group with 11 patients (84.6%, p = 0.041). In addition, mortality in the 1st year was higher in patients with DSA positive for both thresholds, DSA ≥ 500 MFI, p = 0.004; DSA ≥ 1000 MFI, p = 0.022 (Table 4).

Regarding the evaluation of graft functioning, no significant difference was observed in the days of myeloid and platelet engraftment in the DSA-positive groups compared to the DSA-negative patients for both MFI thresholds. Furthermore, no higher graft failure, GVHD, fever, neutropenia, or other adverse clinical events were documented in DSA-positive patients after HCT.

Table 3. Principal graft and hematopoietic stem cell transplant characteristics in 70 patients according to donor-specific antibodies cutoff

Characteristic	n = 70	DSA > 1000 MFI			DSA ≥ 500 MFI		
		Yes (n = 4)	No (n =66)	p-value	Yes (n = 13)	No (n =57)	p-value
CD34+ $\times 10^6$	10 (9–12.7)	9.6 (8.1–13.7)	10 (9–12.7)	0.687	10 (8.5–12.6)	10 (9–12.8)	0.825
ABO mismatch	–	–		0.664			0.959
Major	6 (8.6%)	0 (0%)	6 (9.1%)		1 (7.7%)	5 (8.8%)	
Minor	12 (17.1%)	0 (0%)	12 (18.2%)		2 (15.4%)	10 (17.5%)	
Compatible	51 (72.9%)	4 (100%)	47 (71.2%)		10 (76.9%)	41 (71.9%)	
Major and minor	1 (1.4%)	0 (0%)	1 (1.5%)		0 (0%)	1 (1.8%)	
Myeloid engraftment	57 (81.4%)	3 (75%)	54 (81.8%)	0.569	10 (76.9%)	47 (82.5%)	0.452
Days to myeloid engraftment	16 (14–18)	14 (14–14)	16 (14–18)	0.506	14 (14–16)	17 (14–18)	0.137
Platelet engraftment	57 (81.4%)	3 (75%)	55 (83.3%)	0.537	10 (76.9%)	48 (84.2%)	0.391
Days to platelet engraftment	16 (14–20)	18 (17–18)	16 (14–20)	0.23	16 (14–20)	16 (14–20)	0.916
Chimerism at 30 days (n = 58)	–	–		0.633			0.465
Null	7 (12.1%)	0 (0%)	7 (12.7%)		1 (10%)	6 (12.5%)	
Complete	45 (77.6%)	3 (100%)	42 (76.4%)		9 (90%)	36 (75%)	
Mixed	6 (10.3%)	0 (0%)	6 (10.9%)		0 (0%)	6 (12.5%)	
Chimerism at 100 days (n = 42)	–	–		0.613			0.472
Null	0 (0.0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
Complete	33 (78.6%)	2 (100%)	31 (77.5%)		5 (71.4%)	28 (80%)	
Mixed	9 (21.4%)	0 (0%)	9 (22.5%)		2 (28.6%)	7 (20%)	

Adverse clinical outcomes and their relationship with the presence of donor-specific antibodies

Complications in the entire group (Table 4) included CRS in 45 (64.2%) patients; Grade 1 was the most common (66.7%). Acute GVHD occurred in 27 patients (38.6%), Grade 1 in 12 cases (44.4%), and 21 cases had a cutaneous presentation. Chronic GVHD developed in 20 cases (28.6%); in 13, the presentation was cutaneous. Post-HCT viral, fungal, or bacterial infection was reported in 42 patients (60%).

Of the 70 recipients, 21 (30%) had a CMV infection confirmed by polymerase chain reaction; fever and neutropenia were reported in 53 cases (75.7%). The post-HCT transfusion requirement included packed red blood cells in 19 (27.1%) cases, apheresis platelets transfused in 20 (28.6%) patients, and platelet concentrates in 19 (27.1%). The need for platelet transfusion support was statistically higher for patients with anti-HLA DSA levels above both thresholds studied, 0.012 and 0.026 for cutoffs > 1000 and 500 MFI, respectively.

In 3 patients (4.3%), a second HCT was necessary due to graft failure in one and disease relapse in two. Regarding the mortality observed in the cohort, 15 patients (21.4%) died at 100 days and 28 (40%) in the 1st year. Death was transplant-related in 13 (18.6%) patients (Table 4).

DISCUSSION

This study analyzed the characteristics of haploidentical HCT and its association with engraftment and adverse clinical events in the context of anti-HLA MFI thresholds ≥ 500 and ≥ 1000. A graft malfunction can be related to the presence of anti-HLA DSA with greater relevance in haploidentical transplants, and anti-HLA DSA at high MFI values can have a role in antibody-mediated rejection, engraftment delay, and poor graft survival[6,8,15].

The incidence of anti-HLA DSA in our cohort was 18.6%. This rate compares to a study that found 18% (22 of 122)[4]. The previous reports have found rates from 13.9% (11 of 79 patients) (11), 14.2% (18 of 134 patients)[8], to 21% (5 of 28 patients)[11]. A history of pregnancy in DSA-positive women at the two thresholds explored was comparable, 23.1% (DSA ≥ 500 MFI) and 25% (DSA ≥ 1000 MFI). Other groups have documented that DSA is significantly higher in patients with a history of pregnancy[16,17]; a higher percentage of women (86%) than in our cohort (45.7%) has been reported[4].

A test is usually considered positive with DSA values ≥ 1000 MFI, but this cutoff point varies between transplant centers[2,8,18]). The poor functioning of the graft and the rejection mediated by antibodies are accentuated when the MFI values are ≥ 5000[5,8]. Due to this variability, it is recommended that each HLA typing laboratory establish its cutoff values[18]. The European Society for Blood and Marrow Transplantation (EBMT) guide for the detection and treatment of DSA[2] strongly recommends local laboratory assay validation and standardization for the detection of DSA, including MFI cutoffs. The same guideline recommends that the anti-HLA DSA test be performed within 1 month before the transplant, allowing time to select the best-related donor in the presence of high DSA levels.

The association between the hematological diagnosis and the presence of anti-HLA DSA has been observed in other studies, being more frequent with AML and MDS. Ciurea et al. found 76% versus 55%[4], and Bramanti et al. 58% versus 33% in these diagnoses[8]. This relationship is probably related to sensitization by transfusion of blood products, as intensive support treatment for these diseases is required[19,20]. In our patients, the administration of platelet concentrates was significantly higher in the DSA-positive groups and more notable in those with DSA ≥ 1000 MFI. After AML, severe aplastic anemia presented a higher incidence of sensitization against HLA antigens, and ATG was added to the conditioning regimen to minimize graft rejection[21].

In Mexico, 78% of HCTs are carried out in public institutions[22]; however, the test is not performed routinely due to budget and infrastructure limitations in the health system[23]. Our findings are important as they can help determine in which cases the search for DSA is clinically relevant and to establish a meaningful MFI cutoff.

Graft failure has been reported in patients with DSA at 2000 MFI and higher[24]; however, this study is the first reporting clinically relevant adverse outcomes for thresholds of 1000 MFI and lower, with the incidence of infection being significantly higher in the DSA-positive group with a threshold ≥ 500 MFI; it is possible that post-transplant infection was associated to the use of Cy; however, the finding that it was statistically associated to MFI values ≥ 500 suggests that there was a relationship between anti-HLA DSA and infection.

Infection in the 1st year after haploidentical HCT is associated with high mortality, increasing the probability of graft failure or delayed engraftment[25,26].

Table 4. Complications observed in 70 patients after outpatient haplo-identical hematopoietic stem cell transplantation according to two DSA thresholds

Characteristic	n = 70	DSA > 1000 MFI			DSA ≥ 500 MFI		
		Yes (n = 4)	No (n =66)	p–value	Yes (n = 13)	No (n =57)	p–value
Cytokine release syndrome	45 (64.2%)	2 (50%)	43 (65.2%)	0.451	9 (69.2%)	36 (63.2%)	0.472
Grade	–						
1	30 (66.7%)						
2	13 (28.9%)						
3	2 (4.4%)						
Acute GVHD	27 (38.6%)	2 (50%)	25 (43.9%)	0.602	6 (66.7%)	21 (40.4%)	0.135
Grade	–						
1	12 (44.4%)						
2	8 (11.4%)						
3	6 (22.2%)						
4	1 (3.7%)						
Acute GVHD location	–						
Oral cavity	2						
Cutaneous	21						
Gastrointestinal	10						
Liver	5						
Eyes	1						
Chronic GVHD	20 (28.6%)	2 (50%)	17 (28.8%)	0.350	5 (41.7%)	14 (27.5%)	0.264
Chronic GVHD location	–						
Oral cavity	8						
Cutaneous	13						
Gastrointestinal	3						
Liver	6						
Eyes	3						
Lungs	1						
Joints	1						
Infections	42 (60%)	3 (75%)	39 (59.1%)	0.473	11 (84.6%)	31 (54.4%)	0.041
CMV (+) PCR	21 (30%)	1 (25%)	20 (30.3%)	0.653	5 (38.5%)	16 (28.1%)	0.335
Fever and neutropenia	53 (75.7%)	4 (100%)	49 (74.2%)	0.319	12 (92.3%)	41 (71.9%)	0.113

(Continues)

Table 4. Complications observed in 70 patients after outpatient haplo-identical hematopoietic stem cell transplantation according to two DSA thresholds *(continued)*

Characteristic	n = 70	DSA > 1000 MFI			DSA ≥ 500 MFI		
		Yes (n = 4)	No (n =66)	p–value	Yes (n = 13)	No (n =57)	p–value
Transfusion requirements post-TCH							
Red blood cell concentrates	19 (27.1%)	2 (50%)	17 (25.8%)	0.296	3 (23.1%)	16 (28.1%)	0.506
Platelet apheresis	20 (28.6%)	2 (50%)	18 (27.3%)	0.321	3 (23.1%)	17 (29.8%)	0.455
Platelet concentrate	19 (27.1%)	2 (50%)	17 (25.8%)	0.296	2 (15.4%)	17 (29.8%)	0.245
Graft failure	16 (22.8%)	1 (25%)	15 (22.7%)	0.655	4 (30.8%)	12 (21.1%)	0.337
Relapse	14 (20.0%)	1 (25%)	13 (19.7%)	0.599	2 (15.4%)	12 (21.1%)	0.490
Progression	4 (5.7%)	0 (0%)	4 (6.1%)	0.786	0 (0%)	4 (7%)	0.431
Second transplant	3 (4.3%)	0 (0%)	3 (4.5%)	0.836	0 (0%)	3 (5.3%)	0.535
Mortality							
100-day post-HCT	15 (21.4%)	0 (0%)	15 (22.7%)	0.372	3 (23.1%)	12 (21.1%)	0.566
First-year post-HCT	28 (40%)	4 (100%)	24 (36.4%)	0.022	10 (76.9%)	18 (31.6%)	0.004
Transplant-related mortality	13 (18.6%)	0 (0%)	13 (19.7%)	0.431	2 (15.4%)	11 (19.3%)	0.548

Infection prevalence was recently reported in our patients receiving an outpatient haplo-HCT; it was the cause of death in 49.4%, with severe infections in the pre-engraftment period developing in 22.4% of the recipients; 14.9% were viral and 12.1% fungal. The 100-day and 2-year cumulative incidence of infection-related mortality (IRM) was 15%; fungal agents contributed to infection-related mortality in 33.3%[26].

The major adverse clinical event in both thresholds in our cohort was death during the 1st year after HCT, seen in 40%, comparable to other reports. This finding was not observed during the 1st month and was part of late-stage events. Chimerism and the success of myeloid and platelet grafting were analyzed at 1 month and after 1 year. Graft failure and mortality in the first 100 days were not related to a DSA ≥ 500 or ≥ 1000 MFI; 1st-year post-HCT mortality, however, was associated to both cutoffs.

As expected, patients with graft failure did not present acute or chronic GVHD. Their mortality in the 1st 100 days and 1 year after HCT was 62.5% and 75%, respectively, underscoring the severity of this event.

In conclusion, Class I and Class II anti-HLA DSA present in a cohort of Hispanic haploidentical transplant recipients at two MFI cutoff levels were not associated with delayed engraftment or graft failure; however, the presence of DSA at these levels was associated with infection and 1st-year mortality, supporting the need to establish clinically meaningful thresholds for local reporting of assay results at histocompatibility laboratories.

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