Donor-specific anti-HLA antibodies (DSAs) in patients undergoing allogeneic hematopoietic stem cell transplantation from mismatched donors on behalf of GITMO and AIBT

Ursula La Rocca[1,2*], Roberto Ricci[1], Alfonso Piciocchi[3], Walter Barberi[1], Elena Oldani[4], Alida Dominietto[5], Raffaella Cerretti[6], Alessandra Picardi[7,8], Francesca Bonifazi[9], Riccardo Saccardi[10], Maura Faraci[11], Giovanni Grillo[12], Lucia Farina[13], Benedetto Bruno[14], Anna Grassi[4], Anna Proia[15], Elena Tagliaferri[16], Giuseppina De Simone[17], Michele Malagola[18], Michela Cerno[19], Simone Cesaro[20], Paolo Bernasconi[21], Lucia Prezioso[22], Paola Carluccio[23], Nicola Mordini[24], Matteo Pelosini[25], Attilio Olivieri[26], Patrizia Chiusolo[27], Stella Santarone[28], Michele Cimminiello[29], Roberto Crocchiolo[12], Franco Papola[30], Gianni Rombolà[31], Nicoletta Sacchi[32], Valeria Miotti[33], Lia Mele[34], Benedetta Mazzi[35], Fabio Ciceri[36], Massimo Martino[37], Anna Paola Iori[1*]

[1]*Hematology, Department of Translational and Precision Medicine, Sapienza, Rome, Italy;* [2]*National Blood Center, Italian National Institute of Health, Rome, Italy;* [3]*GIMEMA Data Center, Rome, Italy;* [4]*Bone Marrow Transplant Unit, ASST Papa Giovanni XXIII, Bergamo, Italy;* [5]*IRCCS Ospedale Policlinico S.Martino, Genoa, Italy;* [6]*Stem Cell Transplant Unit, Policlinico Tor Vergata, Rome, Italy;* [7]*Stem Cell Transplant Program AORN Cardarelli, Naples, Italy;* [8]*Biomedicine and Prevencion Department of Tor Vergata University, Rome, Italy;* [9]*IRCSS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy;* [10]*Cell Therapy and Transfusion Medicine Department, Careggi University Hospital, Florence, Italy;* [11]*Hematopoietic Stem Cell Transplantation Unit, IRCSS Istituto Giannina Gaslini, Genoa, Italy;* [12]*Hematology Department, ASST Great Metropolitan Hospital Niguarda, Milan, Italy;* [13]*Foundation IRCCS-Italian National Cancer Institute, Milan, Italy;* [14]*Division of Hematology and Stem Cell Therapy Unit, University of Turin, Turin, Italy;* [15]*Unity of Hematology and Stem Cell Transplant, Azienda Ospedaliera S. Camillo Forlanini, Rome, Italy;* [16]*Hematology and BMT Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy;* [17]*Unità Operativa Complessa di Trapianto di Cellule Staminali Emopoietiche e Terapie Cellulari, Azienda Ospedaliera di Rilievo Nazionale Santobono-Pausilipon, Naples, Italy;* [18]*Adult Bone Marrow Transplant Unit; Program of Cellular Therapy and Research in Hematology; ASST Spedali Civili di Brescia, University of Brescia, Brescia, Italy;* [19]*Division of Hematology and Stem Cell Transplantation, Azienda Sanitaria Universitaria Friuli Centrale, Udine, Italy;* [20]*Pediatric Hematology Oncology, Department of Mother and Child, Azienda Ospedaliera Universitaria Integrata, Verona, Italy;* [21]*Division of Hematology, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy;* [22]*Hematology and Stem Cell Transplant Unit, Ospedale Maggiore, Parma, Italy;* [23]*Unit of Hematology and Stem Cell Transplantation, AOUC Policlinico, Bari, Italy;* [24]*Hematology Division, Azienda Ospedaliera S. Croce e Carle, Cuneo, Italy;* [25]*Clinical and Experimental Medicine, Section of Hematology, University of Pisa, Pisa, Italy;* [26]*Hematology Department, University of Ancona, Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona, Ancona, Italy;* [27]*Department of Radiological and Hematological Sciences, A. Gemelli University Polyclinic Foundation IRCCS, Catholic University of the Sacred Heart, Rome, Italy;* [28]*Department of Hematology, Transfusion Medicine and Biotechnologies, Ospedale Civile, Pescara, Italy;* [29]*Hematology and Bone Marrow Transplantation Unit, S. Carlo Hospital, Potenza, Italy;* [30]*Regional Centre of Immunohematology and Tissue Typing, S.Salvatore Hospital, L'Aquila, Italy;* [31]*Azienda Ospedaliero-Universitaria Careggi, University of Florence, Florence, Italy;* [32]*Italian Bone Marrow Donor Registry, E. O. Ospedali Galliera, Genoa, Italy;* [33]*Laboratory of Immunogenetics, Santa Maria della Misericordia University Hospital, Udine, Italy;* [34]*HLA Laboratory and stem cell processing, A.O. SS Antonio e Biagio e C. Arrigo, Alessandria, Italy;* [35]*Immunogenetics Laboratory, Immuno-Hematology and Transfusion Medicine Unit, San Raffaele Scientific Institute, Milan, Italy;* [36]*Department of Onco-Hematology, Hematology and Bone Marrow Transplantation, IRCCS San Raffaele Scientific Institute and Vita-Salute San Raffaele University, Milan, Italy;* [37]*Hematology and Stem Cell Transplantation and Cellular Therapies Unit (CTMO), Stem Cell Transplant Program CIC 587, Department of Hemato-Oncology and Radiotherapy, Grande Ospedale Metropolitano "Bianchi-Melacrino-Morelli", Presidio Morelli, Reggio Calabria, Italy*

***Ursula La Rocca and Anna Paola Iori are both Corresponding Author**

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Correspondence: Ursula La Rocca
e-mail: ursula.larocca@uniroma1.it

Background - Antibodies directed against donor-specific HLA allele(s)/antigen(s) (DSAs) represent a known risk factor for hematopoietic stem cell transplantation (HSCT) engraftment. Still, the overall management needs to be standardized.

Material and methods - GITMO and AIBT ran a survey on DSAs in Italian Transplant Programs including mismatched HSCT performed between January 2014 and June 2017.

Results - One-thousand-thirty-three patients were proposed for the study, 804 were evaluable. Overall, 355 (44%) were screened: 91/355 (25.6%) showed anti-HLA antibodies, 23 DSAs (6.5%). Female gender and at least 4 previous pregnancies showed an impact on alloimmunization. Eleven patients with DSAs underwent desensitization. In seven cases no desensitization was employed. An alternative donor was selected for five patients. Neutrophil and platelet engraftment were obtained in 93.6% and 86.6% of the whole population, respectively, and were statistically associated with the absence of anti-HLA antibodies, ABO match, a higher number of infused nucleated cells and lack of a-GvHD. In addition, significant factors for platelet engraftment were the use of leuco-depleted transfusions, HLA match, younger age of the patient. Graft failure (GF) was associated with bone marrow stem cell source, and a lower number of infused CD34+. The detection of antibodies directed against both HLA classes, donor and patient age, the hematologic and molecular remission at HSCT, HLA match, ANC and PLTS engraftment, full donor engraftment within 28 days after HSCT, early and late GF, grade>II a-GVHD showed an impact on OS.

Discussion - Anti-HLA antibodies and DSAs were confirmed as risk factors affecting OS. DSAs were managed with various approaches resulting in stable engraftment in 81.9% of patients. Our study supports the clinical relevance of DSAs detection and management in mmHSCT. A standardized approach of DS is warranted.

Keywords: *anti-HLA antibodies, donor selection, engraftment, desensitization strategy.*

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a definitive treatment for many hematologic diseases. The selection of partially HLA-matched donors has increased the availability of suitable donors[1]. The most important limitations of mismatched (mm) HSCT are the consequences of intense bidirectional alloreactivity reactions, with

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increased risk of host-*vs*-graft (HVG) and graft failure (GF), and graft-*vs*-host disease (GvHD)[2]. GF is a major complication following HSCT, with an incidence of 4-20%[2] according to the different HSCT settings and a high risk of poor outcomes.

GF pathogenesis is based on either cellular rejection through chemo-resistant recipient T lymphocytes or NK cells directed against mismatched donor cells or humoral rejection involving antibody-dependent cell-mediated cytotoxicity or complement-mediated cytotoxicity[2-5]. Other factors involved as predisposing or causative factors are the use of myelosuppressive drugs, the onset of viral or bacterial infections, and major or bidirectional ABO mismatches. In addition, myeloablative conditioning regimens, peripheral blood stem cell sources (PBSCs), or non-T-cell-depleted grafts may facilitate engraftment[6-14].

The presence of anti-HLA antibodies directed against donor-specific HLA allele(s) or antigen(s) (DSA) has been recognized as a barrier for stem cell engraftment, leading to GF or delayed engraftment. The European Society for Blood and Marrow Transplantation (EBMT) consensus guidelines for the detection and treatment of DSAs in haploidentical HSCT suggest DSA testing via the Luminex technology (Luminex, Austin, TX, USA) and/or cell-based assays in all patients who are candidates for haploidentical or mismatched HSCT; in the case of DSAs with >1,000 MFI (mean fluorescence intensity), C1q (complement) testing or cell-based assays should be performed; DSA levels >1,000 of MFI require desensitization, especially if MFI is >5,000 and C1q positive, if an alternative donor is not available. Desensitization strategies should be chosen according to local experiences[2,6].

However, many aspects should be further investigated, including clinically significant MFI cutoff values considered significant for engraftment, the role of DSAs against each of the HLA loci on post-transplant outcomes, and the unavailability of shared desensitization protocols and donor selection strategies.

Hence, the Italian Group for Blood and Marrow Transplantation (GITMO) and the Italian Society for Immunogenetics and Transplantation Biology (AIBT) conducted this study among Italian Transplant Programs (ITPs), with the aim of analyzing the policy for anti-HLA antibody and DSA detection and approaches for positive results in terms of donor selection criteria and desensitization strategies in hematologic patients who were candidates for HSCT. A clear scenario of DSA management policies adopted by the ITPs will allow for the definition of shared consensus strategies.

MATERIALS AND METHODS

The GITMO/AIBT "Donor-specific anti-HLA antibodies (DSAs) in patients undergoing allogeneic hematopoietic stem cell transplantation from mismatched donors" (ClinicalTrials.gov identifier NCT04469985) is a retrospective, observational, multicentric, non-interventional and non-pharmacological study that included all patients (adults and children) who received mismatched HSCT from January 2014 to June 2017. Among the 52 GITMO Italian allogeneic transplant centers, 35 agreed to participate in the study, and 26 transplant programs (74%) submitted their data regarding anti-HLA antibodies and DSA detection, monitoring, and management.

Primary outcomes were the evaluation of anti-HLA antibodies and DSA detection and monitoring activities in hematologic patients undergoing mmHSCT, including methods employed for antibody screening, the cut-off of MFI values considered detrimental for engraftment, donor selection criteria according to DSA status, and the desensitization protocol employed.

Secondary outcomes included the impact of DSAs on neutrophil and platelet engraftment, the onset of GF up to the last follow-up (FU), and the overall survival (OS) rate in patients who underwent HSCT with or without DSAs.

Neutrophil and platelet engraftment were defined as the first of three consecutive days with an absolute neutrophil count (ANC) $\geq 0.5\times10^9$/L and the first of seven consecutive days with an absolute PLT $\geq 20\times10^9$/L without platelet transfusion in the last five days, respectively[7].

Primary GF was defined as the lack of initial donor cell engraftment, characterized by a peripheral blood ANC $<0.5\times10^9$/L by day +28 after HSCT without evidence of relapse[7] and by day +42 after CB HSCT. Secondary GF is characterized by the loss of donor cells after initial engraftment, with recurrent ANC $<0.5\times10^9$/L[7]. 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 full donor chimerism and in the absence of active severe GvHD or relapse[7].

The study was conducted according to the ethical principles derived from the Declaration of Helsinki's current national and European legislation on clinical trials and the principles of good clinical practice. Following the approval of the competent Ethics Committee, according to the current legislation on observational, non interventional studies, all patients enrolled in the study signed informed consent forms. All patients included in the registry provided informed consent for data registration in the EBMT ProMISe database. The data were collected through a dedicated case report form using the web application Redcap (Vanderbilt University, Nashville, TN, USA) and included data on pre-HSCT blood transfusion, pregnancies, anti-HLA and DSA testing, anti-HLA and DSA testing methods, a cutoff of MFI considered detrimental for engraftment, DSA and MFI values, and desensitization strategies. Moreover, data from all HSCTs registered in the European ProMISe database were extracted, including date of birth, age at HSCT and sex, diagnosis, disease stage at HSCT, patient and donor HLA mismatch, patient and donor ABO mismatch, donor date of birth (age at HSCT) and sex, HSCT characteristics, such as conditioning regimen, stem cell source and number of CD34+ and total nucleated cell (TNC) infusions, the onset of acute GvHD, date of neutrophil and platelet engraftment, disease relapse, follow-up, and the date and cause of death. Additional queries were submitted to each Centre to minimize missing data.

Statistical analysis

Subject characteristics for the whole population and stratified by anti-HLA antibodies were summarized by means of the variables (with %) for categorical variables or by means of quantiles and means with standard deviations (SDs) for continuous variables. All percentages were evaluated on available data. In univariate analysis, non parametric tests were performed for comparisons between groups (chi-square and Fisher's exact tests for categorical variables or response rates and Mann-Whitney and Kruskal-Wallis tests for 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 (ORs) and 95% confidence intervals (CIs) were reported as parameter results of the logistic regression models. Survival distributions (e.g., TRM and 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 and OS were evaluated by means of the log-rank test or Cox regression model in univariate and multivariate analyses, respectively, after assessment of the proportionality of hazards. Hazard ratios (ORs) and 95% confidence intervals (CIs) were reported as parameter results of the Cox regression models. All covariates were evaluated in univariate models, and all factors with univariate associations with p values <0.1 were considered in the multivariate models. Backward and stepwise methods were applied to identify the multivariate models with a step-by-step iterative construction that involved the selection of independent variables to be considered in the final model. All tests were 2-sided, with $p<0.05$ indicating statistical significance, and confidence intervals were calculated at the 95% level. All analyses were performed using R software (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). Data collection: GITMO provided, through EBMT ProMISe, the clinical and outcome data of the patients enrolled in the study and the characteristics of the donors.

RESULTS

Patients and transplant characteristics

Twenty-six out of the 35 Italian TPs who were initially involved submitted their data regarding anti-HLA antibody and DSA detection, monitoring, and management. One thousand thirty-three patients were included in the study, 955 were actively enrolled, and 804 were evaluable, according to the inclusion criteria and on the completeness of the submitted data. The median age was 48 years (range: 0-72 years). Most patients undergo transplantation due to hematologic malignancies or non-malignant diseases. Patient and transplant characteristics are shown in **Table I**.

Methods employed for anti-HLA antibodies

Anti-HLA antibodies

Three hundred fifty-five of the 804 patients (44%) underwent anti-HLA antibody detection before HSCT.

Table I - *Characteristics*

Characteristics	No. = 804
Age, median (range)	48 (0-72)
Gender, No. (%)	
F/M	287 (36%) / 517 (64%)
Pregnancies No. (%)	127 (55%)
Abortions No. (%)	12 (4.9%)
Diagnosis, No. (%)	
Acute leukemia	313 (41.1%)
Bone marrow failure	24 (3.2%)
Chronic lymphocytic leukemia	9 (1.2%)
Chronic myeloid leukemia	12 (1.6%)
Hemoglobinopathy	5 (0.7%)
Lymphoma	103 (13.5%)
Inherited disorder, metabolism	3 (0.4%)
Myelodysplastic syndrome/ Myeloproliferative neoplasm	10 (1.3%)
Multiple myeloma	23 (3.0%)
Myelodysplastic syndrome	66 (8.7%)
Myeloproliferative neoplasia	28 (3.7%)
Plasma cell leukemia	1 (0.1%)
Prolymphocytic leukemia	1 (0.1%)
Precursor lymphoid neoplasms	149 (20%)
Primary immune deficiency	14 (1.8%)
Not specified	43
Pre-HSCT blood transfusions, No. (%)	630 (88%)
Leucodepleted blood transfusions, No. (%)	545 (86%)
Regimen intended to be myeloablative (full intensity), No. (%)	584 (77%)
TBI, No.(%)	96 (12%)
Stem cell source, No. (%)	
BM	347 (45.4%)
Cord	42 (5.5 %)
PBSC	375 (49.1%)
Not specified	40
HLA match, No. (%)	
Mismatched relatives	352 (44%)
Unrelated	452 (56%)
Degree of mismatch in related donors, No. (%)	
≥2 HLA loci mismatch	341 (98%)
1 HLA locus mismatch	7 (2%)
Not specified	4
ABO match, No. (%)	
Matched	147 (25%)
Major incompatibility	207 (35%)
Minor incompatibility	204 (35%)
Bidirectional incompatibility	27 (4.6%)
Not specified	219
CMV serological status	
Negative Negative	110 (15%)
Positive Negative	75 (10%)
Negative Positive	215 (30%)
Positive Positive	319 (44%)
Not specified	85
Age of the donor, median (range)	33 (10-66)
Donor sex, No. (%)	
F/M	280 (35%)/516 (65%)

Characteristics	No. = 804
Gender mismatch, No. (%)	
Female Female	107 (13%)
Male Female	173 (22%)
Female Male	174 (22%)
Male Male	342 (43%)
Not specified	8
Anti-HLA Ab tested before HSCT, No. (%)	355 (44%)
Anti-HLA Ab result, No. (%)	
Negative	264 (74.3%)
Positive - class II	26 (7.3%)
Positive - both classes	23 (6.4%)
Postive - class I	42 (12%)
DSA, No. (%)	23 (6.5%)
Strategy employed, No. (%)	
Desensitization strategy	11 (47.8%)
Alternative donor selection	5 (21.7%)
No strategies employed	7 (30.5 %)
ANC engraftment, No. (%)	736 (93.6%)
PLT engraftment ≥20×10^9/L, No. (%)	586 (86.6%)
PLT engraftment ≥50×10^9/L, No. (%)	447 (87.1%)
Full donor engraftment within 28 days, No. (%)	442 (60%)
Early graft loss, No. (%)	17 (2.7%)
Late graft loss, No. (%)	15 (2.2%)
aGVHD, No. (%)	
Grade I	105 (14%)
Grade II	118 (15%)
Grade III	52 (6.8%)
Grade IV	29 (3.8%)
Present, grade unknown	1 (0.1%)

*Percentages were calculated based on available data.
Ab: antibody; aGvHD: acute graft-*vs*-host disease; ANC: absolute neutrophil count; BM: bone marrow; CMV: cytomegalovirus; DSA: donor specific anti-HLA antibodies; HLA: human leukocyte antigen; HSCT: hematopoietic stem cell transplantation; PBSC: peripheral blood stem cell; PLT: platelet; TBI: total body irradiation.

One hundred thirty-six (38%) were females, and two hundred nineteen were males (62%). Three hundred twenty (91%) patients had received blood transfusions. Ninety-one out of 355 patients had anti-HLA antibodies (25.6%): 42 had class I antibodies, 26 had class II antibodies, and 23 had both class I and class II antibodies. Requests for anti-HLA antibody detection were more frequent in patients who were candidates for HSCT with previous pregnancies (p=0.028), had previous blood transfusions (p=0.007), had leucodepleted (p<0.001) or irradiated (p<0.001) blood components and were receiving a MAC regimen (p=0.040). Female sex (p=0.003) and at least four previous pregnancies (p=0.024) were significantly correlated with anti-HLA antibody positivity (**Tables I and II**).

Table II - *Statistical associations (anti-HLA antibodies screening policy and results)*

Characteristics	Anti-HLA antibodies screening p-value[1]	Anti-HLA antibodies result p-value[1]
Gender (F)	0.17	0.003
Pregnancies	0.028	0.89
Number of pregnancies (≥4)	0.25	0.024
Abortions	0.95	0.077
Number of abortions	0.27	0.22
Pre-HSCT blood transfusions	0.007	0.064
Leucodepleted blood transfusions	<0.001	0.78
Irradiated blood transfusions	<0.001	0.58
Regimen intended to be myeloablative (full intensity)	0.040	>0.99

[1]Pearson's Chi-squared test. HLA: human leukocyte antigen; HSCT: hematopoietic stem cell transplantation.

Testing and monitoring

The Luminex platform was used to test and monitor anti-HLA antibodies in most patients (93.5%). Other methods employed included flow cytometry, ELISA, and CDC assays. A complement (C1q/C3d) assay was performed in only 26 patients (7.6%). The MFI considered the cutoff varied between 1,000 and 5,000, according to the assay employed.

DSA and desensitization strategies

Twenty-three out of 355 patients had DSAs (6.5% of the evaluable patients; 25.3% of the anti-HLA-positive patients), with a median MFI of 8,300 (range: 257-23,000). DSA-specific antibodies against A2 were detected in 3patients;anti-A*29:02,anti-B15,anti-DQ3,anti-DRB*10:01, anti-DQA1*01:03, anti-DQB1*03:01, anti-DRB1*09:01, anti-DRB1*04:01, anti-DRB1*04:05 and anti-DP14 were detected in 1 patient each. Eleven patients received desensitization, with various schedules; 7 patients did not receive treatments; and an alternative donor was selected for the remaining 5 patients.

Among the different TCs, the desensitization strategies employed were as follows:

- Rituximab (375 mg/m^2) was administered on day -15, followed by plasmapheresis (PP) on days -9 and -8 and intravenous immunoglobulin (IVIG) on day -7. Selected platelet transfusions were employed in the case of antibodies directed against class I HLA.
- PP procedures on days -10, -8, and -1.
- PP procedures using EC30 W filters (days not specified).
- Weekly PP procedures and rituximab 375 mg/m^2 (repeated for four weeks) were administered.
- Rituximab 375 mg/m^2/week for 4 weeks and IVIG 1 g/kg/day for 2 consecutive days were administered, followed by the infusion of donor-irradiated PLT transfusions.
- Rituximab 375 mg/m^2 on day -7.
- IVIG followed by selected platelet transfusion in patients with antibodies directed against class I HLA.
- PP procedures on two consecutive days followed by IVIG.

Complete antibody clearance was obtained in 7 out of the 11 desensitized patients, with a median time of 12 days after HSCT (range: 0-30). All patients achieved ANC engraftment, and PLT engraftment was observed in all but one patient. Two patients showed a reduction in antibody levels, both of whom achieved engraftment. In the first case, characterized by an MFI reduction from 10,725 to 498, early graft loss was observed 26 days after HSCT; further DSA evaluations were not reported. Two patients showed antibody persistence: one who was engrafted, and the other who died due to GF and sepsis. Notably, the latter patient showed a positive complement binding assay, with an MFI of 12,200. All 5 patients for whom an alternative donor was selected experienced full donor engraftment. Among the patients who did not receive desensitization treatments, 2 did not achieve platelet engraftment. One patient, after achieving PLT engraftment on day +21, developed secondary thrombocytopenia starting on day +60 (**Figure 1**).

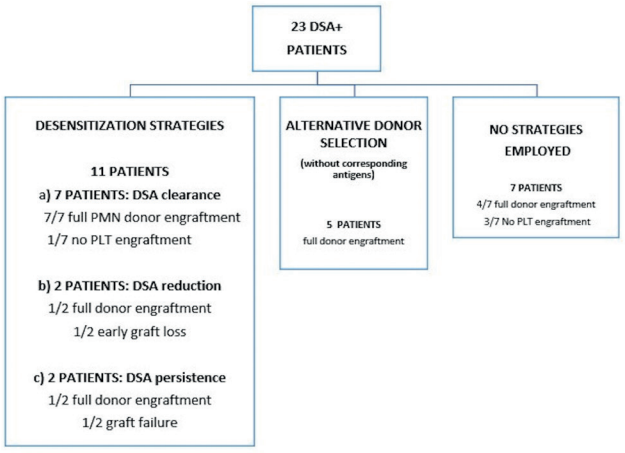

Figure 1 - DSA and desensitization strategies
DSA: donor specific anti-HLA antibodies; PLT: platelet.

Engraftment

Neutrophil engraftment was obtained in 736 patients (93.6%). Statistical analysis revealed a significant impact on ANC engraftment for the following variables: anti-HLA antibody detection against both HLA classes I and II (p=0.002), ABO incompatibility (p<0.001), the total number of infused nucleated cells (p=0.006), and the onset of a-GvHD (p=0.001). Platelet engraftment (PLT ≥20×10^9/L) was achieved in 586 patients (86.6%). Factors affecting PLT engraftment were the use of leucodepleted blood components (p=0.005), anti-HLA antibody detection (p<0.001), HLA mismatch between the donor and recipient (p<0.001), older age of the patient (p=0.021), ABO compatibility (p=0.002), the number of infused nucleated cells (p=0.029), and the onset of a-GvHD (p=0.009). Regarding the achievement of a PLT count ≥50×10^9/L, we detected anti-HLA antibodies (p=0.030), HLA matching between donors and recipients (p=0.010), the dose of infused nucleated cells (p=0.003), hematologic remission before HSCT (p=0.006), the CMV serological status of donors and recipients (p=0.038) and the onset of aGVHD (p=0.030). Overall, 442 patients achieved full donor engraftment for ANC and PLTs within 28 days after HSCT. Variables associated with full donor engraftment were leucodepletion of blood components (p=0.006), HLA match between the donor and recipient (p<0.001), degree of HLA mismatch in related donors (p=0.014), anti-HLA antibody detection (p<0.022), number of total infused nucleated cells (p=0.004) and CD34+ (p=0.046), stem cell source (p=0.017), intensity of the conditioning regimen (p=0.009), and ABO incompatibility (p=0.004) (**Table III**).

Table III - *Engraftment: statistical associations between variables*

Characteristic	ANC engraftment p-value[1]	PLT engraftment ≥20×10^9/L p-value[1]	PLT engraftment ≥50×10^9/L p-value[1]	Full donor engraftment within 28 days p-value[1]
Pregnancies	0.21	>0.99	>0.99	>0.99
Abortions	0.19	0.68	0.69	0.97
Leucodepletion of blood components	0.14	0.005	0.050	0.006
HLA match	0.13	<0.001	0.010	<0.001
Degree of mismatch in related donors	0.92	0.42	0.75	0.014
Age of the donor	0.73	0.086	0.27	0.90
Pre-HSCT hematological remission	>0.99	0.56	0.006	0.88
Age at HSCT	0.085	0.021	0.24	0.17
Stem cell source	0.44	0.88	0.31	0.017
Conditioning regimen intended to be myeloablative (full intensity)	>0.99	0.69	0.93	0.009
ABO match	<0.001	0.002	0.18	0.004
CMV patient and donor status	0.94	0.24	0.038	0.28
Gender mismatch	>0.99	0.18	0.93	0.16
Anti-HLA Ab results	0.002	<0.001	0.030	0.022
Total infused nucleated cells	0.006	0.029	0.003	0.004
Number of infused CD34 positive cells	0.34	0.24	0.80	0.046
aGVHD	0.001	0.009	0.030	0.31
aGvHD maximum grade	0.002	<0.001	0.021	0.10

[1]Pearson's Chi-squared test; Wilcoxon rank sum test; Wilcoxon rank sum exact test.
All variables analyzed: gender; number of pregnancies; pre-HSCT blood transfusions; anti-HLA tested before HSCT; DSA; donor gender; TBI; number of infused CD3 positive cells (T-cells)×10^6/kg; cytogenetic remission; molecular remission; diagnosis; TBI total dose.
Ab: antibody; aGvHD: acute graft-*vs*-host disease; ANC: absolute neutrophil count; CMV: cytomegalovirus; DSA: donor specific anti-HLA antibodies; HLA: human leukocyte antigen; HSCT: hematopoietic stem cell transplantation; PLT: platelet; TBI: total body irradiation.

Early and late graft loss

Seventeen patients (2.7%) experienced early graft loss (35% females, 65% males). Late graft loss was observed in 15 patients, accounting for 2.2% of our population (40% females, 60% males). Early and late graft loss were not associated with the detection of anti-HLA antibodies (p=0.63 and p=0.80, respectively) or DSAs (p=0.99 and p=0.60, respectively). We found a significant association between late graft loss and the use of bone marrow as a stem cell source (p=0.032) and a lower number of infused CD34+ cells (p=0.033).

Overall survival

The median follow-up was 51.32 months (range 0.0-93.9). The 48- and 60-month OS rates were 57.4 and 56.5%, respectively (**Figure 2**). The following variables had an impact on OS according to univariate analysis: the presence of antibodies directed against both HLA classes I and II (p<0.001); donor (p<0.001) and patient (p<0.001) age; hematological (p<0.001) and molecular (p<0.001) remission before HSCT; the HLA match between donor and recipient (p=0.007); neutrophil (p<0.001) and platelet engraftment (p<0.001); full donor engraftment within 28 days after HSCT (p<0.001); the occurrence of both early (p<0.001) and late graft loss (p<0.001); and the onset of a-GVHD >II (p=0.017). Multivariate analysis revealed that the presence of antibodies directed against both HLA classes II and I (p=0.006), patient age at HSCT (p=0.020), achievement of complete donor engraftment (p=0.010), and onset of early graft loss (p=0.004) affected OS (**Table IV**).

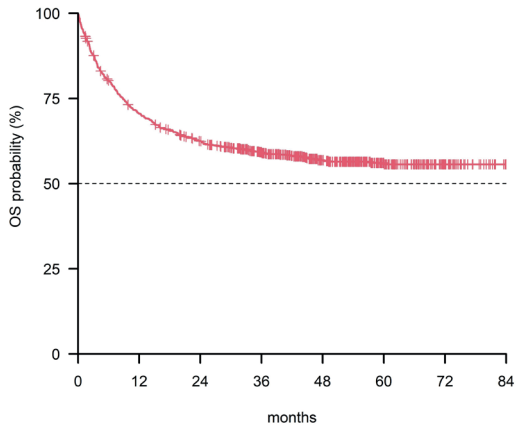

Figure 2 - Overall survival

Table IV - *Overall survival univariate*

Characteristics	HR[1]	95% CI[1]	p-value
Overall survival			
Anti-HLA Ab result Negative Positive- class II Positive - both classes Postive- class I	 0.61 2.80 1.13	 0.28, 1.31 1.65, 4.73 0.70, 1.85	 0.20 <0.001 0.61
Age at HSCT	1.02	1.01, 1.03	<0.001
Age of the donor	1.00	1.00, 1.00	<0.001
Hematological remission before HSCT	0.24	0.13, 0.44	<0.001
Molecular remission before HSCT	0.54	0.34, 0.88	0.013
HLA match	0.74	0.60, 0.92	0.007
PLT engraftment	0.95	0.93, 0.96	<0.001
ANC engraftment	0.93	0.91, 0.95	<0.001
Full donor engraftment within 28 days after HSCT	0.31	0.24, 0.41	<0.001
Early graft loss	3.74	2.04, 6.88	<0.001
Late graft loss	4.18	2.33, 7.50	<0.001
Grade > II a-GVHD	1.94	1.12, 3.33	0.017
Cox multivariate			
Negative **Positive- class II** **Positive – both classes** **Postive- class I**	 0.74 2.37 1.41	 0.32, 1.70 1.28, 4.41 0.84, 2.34	 0.48 0.006 0.19
Age at HSCT	1.01	1.00, 1.03	0.020
Full donor engraftment within 28 days after HSCT	0.62	0.43, 0.89	0.010
Early graft loss	2.99	1.42, 6.26	0.004

[1]HR: hazard ratio, CI: confidence interval. All variables analyzed: gender; pregnancies; No. of pregnancies; abortions; No. of abortions; pre-HSCT blood transfusions, leucodepleted blood transfusions; anti-HLA tested before HSCT; anti-HLA result; DSA detection; cytogenetic remission before HSCT; ABO mismatch; donor and recipient CMV status; patient gender; donor gender; gender mismatch; TBI; stem cell source; No. of infused CD34 positive cells; number of infused CD3 positive cells (T-cells); full donor engraftment within 28 day; early graft loss; late graft loss; aGVHD.

DISCUSSION

For many years, the role of anti-HLA antibodies and DSAs and the mechanisms of alloantibody generation have been extensively studied in solid organ transplantation[15-18]. Over time, their importance has also been well recognized in the setting of allogeneic HSCT. DSAs represent a risk factor for GF, PGF, and GR in patients with hematologic diseases undergoing mismatched HSCT[6-14].

The increasing selection of haploidentical and partially HLA-matched donors highlighted new interest in this topic, representing a new hurdle[8].

However, for years, there has been a lack of recommendations for DSA management, with consequent heterogeneous policies among transplant centers, mainly based on approaches described by groups with more experience[18].

In 2018, the EBMT Group published consensus guidelines[6] for the management of DSAs, recommending testing for DSAs using the Luminex platform/cell-based assays in all patients before HSCT. They considered MFI >1,000 as positive; to better assess the risk of GF the Authors have suggested to perform C1q test and/or cell assays. The expert panel stated that DSA analysis should be included in the donor selection process. In the case of DSAs with MFI >1,000, if other suitable donors are not available, desensitization of patients is recommended, especially for those with MFI >5,000 and/or C1q positivity. The choice of desensitization approach may be based on the experience of each transplant center. Recently, the Spanish Group of Hematopoietic Transplant and Cell Therapy published a survey of patients who underwent haplo-HSCT between 2012 and 2021 regarding assay use, monitoring strategies, criteria and strategies for desensitization and transplant outcomes[19]. The study included 1,454 transplants; 69 patients (4.7%) were DSA positive, and 46 (67%) had an MFI >5,000. Multivariate analysis identified a baseline MFI >20,000 as an independent risk factor for survival and an increase in titer after infusion as an independent risk factor for GF. However, to date, several aspects, including the established detrimental MFI cut-off value, the importance of DSAs directed against different HLA loci on post-transplant outcomes, and the best desensitization strategy to be applied in all mismatched transplant settings (unrelated donor, mismatched family donor, cord blood), require further investigation.

The first step of this project, promoted by GITMO and AIBT, was the proposal of a survey to investigate the management of anti-HLA antibodies and DSAs in ITPs, followed by a retrospective analysis to compare the different practices regarding this issue among the centers. We decided to observe a 4-year period (from 2014 to 2017) to limit, as much as possible, patient heterogeneity in terms of transplant policy in each ITPs.

To the best of our knowledge, this study reports the largest retrospective study including DSA analysis in patients who were candidates for different types of mmHSCT. The results highlight a very large variety in anti-HLA antibody screening and DSA management in Italy: only 44% of patients underwent anti-HLA antibody and DSA detection before HSCT, probably because of the high risk of immunization. In fact, we observed a significant correlation between the number of anti-HLA antibody tests requested and a history of previous pregnancies (p=0.028) or previous blood transfusions (p=0.007).

Different methods were employed to study anti-HLA antibodies: most of them (93.5%) are Luminex bead assays, with different MFI cut-offs considered, varying between 1,000 and 5,000, according to the different kits employed. Other tests included flow cytometry, ELISA, and CDC assays. In 7.6% of the patients, a complement binding assay (C1q/C3d) was performed. Among the 355 patients screened, 91 (25.6%) showed anti-HLA positivity. DSAs were found in 6.5% of the evaluable patients, according to the reported incidence in Spain. These data reflect the prevalence of anti-HLA antibodies among hematologic patients undergoing HSCT and underline the importance of testing patients before transplantation.

The main causes of alloimmunization are pregnancy, blood transfusion or previous allogeneic transplantation[2]. In our country, the factors predictive of alloimmunization were female sex (p=0.003) and at least four previous pregnancies (p=0.024). This data confirms what was reported by other studies. Triulzi *et al.*[22] conducted a study on 7,920 volunteer donors and detected anti-HLA antibodies in 17.3% of the female donors and in 24.4% of those with a history of pregnancy; this percentage increased progressively with the number of pregnancies, reaching 32.2% in the case of more than 4 pregnancies. No correlation was found between previous transfusions and HLA antibody development due to the policy to use universal pre-storage leukoreduction. This observation is in accordance with data reported by Seftel *et al.*[23], who reported alloimmunization in 19% and 7% of patients receiving post-storage or pre- and post-storage leucodepleted blood products, respectively. Considering that more than half of the patients in the ITPs group were not tested for anti-HLA antibodies before transplantation, the observed impact of pretransplant not leucodepleted

blood transfusions on not obtaining full donor engraftment within 28 days after HSCT (p=0.006) could be the consequence of undiagnosed alloimmunization.

Regarding the MFI cut-off value considered as detrimental for engraftment, the likelihood of developing rejection increases as the MFI levels increase[6]. Previous studies reported an MFI >20,000 as a risk factor for survival[20,21]. We cannot confirm these data, considering the low number of positive patients included, the low MFI median value observed (8,300; range 257-23,000), and the efficacy of the desensitization strategies used, allowing us to obtain stable engraftment in 9 out of 11 treated patients. Due to the absence of guidelines or recommendations, significant heterogeneity of approaches was employed, even within the same center. Therefore, it is not possible to evaluate the effectiveness of each approach. However, the absence of a correlation between DSAs and engraftment or GF underlines the important role of desensitization for patients with high levels of DSAs in mitigating the detrimental effects of alloimmunization on engraftment reported in the literature[6,8-14].

Multivariate analysis revealed that concomitant positivity of both classes of anti-HLA Abs was a risk factor affecting OS (p=0.006). We cannot explain this finding, but we can speculate that this patient population represents a cohort with a complex immunological status, beyond DSAs, requiring a dedicated strategy of transfusion support and desensitization. However, further studies are needed to analyze these aspects.

CONCLUSIONS

The results obtained by our analysis underline the heterogeneity in anti-HLA antibodies and DSA testing, monitoring, and desensitizing on the national scale, confirming the absence of common strategies. Therefore, a harmonized policy and approach is needed. We suggest testing for anti-HLA antibodies if mismatched donors are selected and repeating the test before starting the conditioning regimen to have time to plan a desensitization strategy. Antibody detection and monitoring should be performed in dedicated laboratories to avoid limits due to the unavailability of "in-house" testing and to standardize results in terms of complete antibody characterization and interpretation. For DSA detection, an alternative donor should be selected. In the case of the unavailability of alternatives, a desensitization strategy should be employed, according to the MFI level and complement assay results. Moreover, to better define the shared desensitization strategy, in case of DSA positivity, it is very important to have a multidisciplinary approach between transplant physicians, immunogenetics and transfusion specialists.

AUTHORSHIP CONTRIBUTIONS

API, RR, VM and ULR conceived and designed the study. All Authors collected the data. API, ULR, RR and AP analyzed the data and wrote the article. All the Authors revised the article and gave their final approval for submission.

The Authors declare no conflicts of interest.

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