The impact of pre-transplant anti-HLA antibodies in transplants from HLA-identical sibling donors: a multicenter study

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Article

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Abstract

Few studies have performed comparative analysis of the outcome of hematopoietic stem cell transplantation from HLA-identical sibling donors (ISD-HSCT) in patients with or without anti-HLA Abs. In this study we retrospectively collected data from a multicenter study to analyze the distribution and impact of the pre-existing anti-HLA Abs in ISD-HSCT. Among 402 recipients, 111 were tested with anti-HLA Abs. Gender, time from diagnosis to transplantation and distribution of primary disease might be risk factors for the occurrence of anti-HLA Abs. We found that patients with anti-HLA Abs had delayed neutrophil engraftment and were more vulnerable to experience Cytomegalovirus (CMV) reactivation. The presence of anti-HLA Abs was proved to be an independent risk factor for neutrophil engraftment (HR 1.42 95%CI 1.13-1.80, P=0.003) and CMV reactivation (HR 2.03 95%CI 1.19-3.46, P=0.009). We found that anti-HLA Abs have a negative impact on the prognosis in the early period after transplantation from sibling donors and anti-HLA Abs was also an independent risk factor for the overall survival (OS) at 180 days (HR 2.32, 95%CI 1.03-5.27, P=0.042) among female recipients. In conclusion, anti-HLA Abs have a negative impact on the prognosis early after ISD-HSCT.

Introduction

Anti-HLA Abs are antibodies directed against class I and II HLA (Human Leukocyte Antigen) antigens, and are formed after exposure to foreign antigens occurring during pregnancy, transfusions, or solid organ transplantations. Proinflammatory states, such as infections, are associated with an increase of these antibodies(1). Allo-sensitization is a major problem in transplantation(2, 3). Multiparous women are frequently allo-immunized to HLA and their HLA Abs may either persist or become gradually undetectable. In addition, primary HLA allo-immunization by blood transfusion is caused by leukocytes contained in the cellular blood products(4, 5). Another risk factor for humoral sensitization is allotransplantation(6).

Anti-HLA Abs have been shown to be associated with graft rejection in solid-organ transplantation(7-11). More recently, association between anti-HLA Abs and graft failure has been observed in hematopoietic stem cell transplantation (HSCT)(12). Several studies have investigated donor specific antibodies (DSAs) in HLA-mismatch transplantation(12-14). Yet elimination of anti-HLA Abs was seldom adopted in ISD-HSCT (HSCT from HLA-Identical Sibling Donor) owing to the consensus and traditional rules. The role of anti-HLA Abs pre-existing in recipients before sibling transplantation is vague.

So far, few studies have comparatively analyzed the outcome of ISD-HSCT in patients with or without anti-HLA Abs. Early studies mainly focused on DSAs in transplantation. However, we hypothesized that the pre-existing anti-HLA Abs, especially non-DSASs, may play a role in transplantation. In this study, we retrospectively collected data from a multicenter study and analyzed the distribution of the pre-existing anti-HLA Abs in ISD-HSCT. We then compared clinical outcomes between patients with and without anti-HLA Abs.
Patients And Methods

Patients

We retrospectively collected data on patients undergoing ISD-HSCT between January 2018 and December 2020 from six centers. The latest follow up data are from January 2022. Before transplantation, histocompatibility was determined by serology for HLA-A, HLA-B and HLA-C loci and by DNA typing for HLA-DRB1 locus in all donor-recipient pairs. Data about patients, donors, the presence of anti-HLA Abs or not, characteristics of the disease, and transplantation outcome were collected. Informed consent was obtained from all patients or their legal guardian in accordance with the Declaration of Helsinki. The study was approved by the institutional review board/ethical committee of each participating Center.

Measurement of anti-HLA Abs

Pre-existing anti-HLA Abs are detected before transplantation. Collected serum samples were first examined by mixed screening of anti-HLA Abs (MIX). When the mixed screening result was positive, specific anti-HLA-I or II Abs tests were performed to detect anti-HLA Ab type (LABScreen@Single Antigen HLA Class I (LS1 A04) and HLA Class II (LS2A01), One Lambda) and MFI intensity. LABScreen Single Antigen Kit can detect 124 different specific antibody sites, including 21 anti-HLA-A sites, 43 anti-HLA-B sites, 15 anti-HLA-C sites, 18 anti-HLA-DR sites, 7 anti-HLA-DQ sites, and 20 anti-HLA-DP sites. Anti-HLA antibody reactivity was detected using the Luminex platform (Luminex, Canoga Park, CA).

A value of mean fluorescence intensity (MFI) of anti-HLA-Ab below 1000 was considered negative. Positive results were divided into different intensity levels based on MFI values: 1000<MFI<2000 weakly positive (WP), 2000<MFI<5000 positive (P), 5000<MFI<8000 intermediately positive (IP), and 8000<MFI<20000 strongly positive (SP)(15). For MFI values above 20000, the serum sample was diluted and tested again. Based on the laboratory results, patients were divided into positive and negative anti-HLA Abs groups.

Transplantation Procedure

Recipients were treated with conditioning regimens for transplantation in each center. All patients received standard antibiotics, antifungal agents, and blood products according to the protocols of each institution.

Assessment of engraftment, GVHD, and survival

The date of neutrophil recovery was defined as the first of three consecutive days in which the absolute neutrophil count (ANC) exceeded 0.5 \( 10^9/\text{L} \). The date of platelet recovery was defined as the first of seven consecutive days during which the platelet count was at least 20 \( 10^9/\text{L} \) without platelets transfusion. Acute and chronic GVHD (Graft-Versus-Host-Disease) (aGVHD and cGVHD) were diagnosed and graded according to standard clinical criteria. TA-TMA was diagnosed in HSCT recipients with either
histologic evidence of TMA on tissue sample or diagnostic markers appearing at the same time: (1) lactate dehydrogenase above normal for age; (2) schistocytes on blood smear; (3) de novo thrombocytopenia or increased transfusion requirements; (4) de novo anemia or increased transfusion requirements; (5) hypertension >99% for age (<18 years) or >140/90 (18 years of age); (6) proteinuria ≥30 mg/dL or random urine protein/ creatinine ratio≥ 2 mg/mg; and (7) elevated soluble terminal complement complex activity (plasma sC5b-9 above normal of ,244 ng/mL)(16). Relapse was defined as a recurrence of the underlying hematologic malignant disease. Overall survival (OS) was calculated for all the patients and measured from the date of transplantation to the date of death (any cause). Non-relapse mortality (NRM) was defined as death without preceding relapse(17).

Statistical analysis

Descriptive statistical analysis was performed to assess variables related to patients, primary diseases and transplant characteristics. The χ² test was used for categorical variables, and the nonparametric test was used for continuous variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of EBV and CMV reactivation, aGVHD, cGVHD, relapse and NRM. For EBV and CMV reactivation, death before the occurrence of EBV and CMV was the competing event; for GVHD, relapse and death without GVHD were the competing events; for relapse, death without relapse was the competing event; and for NRM, relapse was the competing event. The probabilities of OS were calculated using the Kaplan-Meier method and the log-rank test was used for group comparisons of OS. All P values were two-sided and the results were considered statistically significant when P <0.05. Analyses were carried out using the R statistical package (version 4.2.1).

Results

1. Characteristics and distribution of anti-HLA Abs

Among 402 patients, 111 (27.61%) recipients had anti-HLA Abs. The details of the distribution of anti-HLA Abs are shown in Table 1. The proportion of females in the positive anti-HLA Abs group is significantly higher than in the negative anti-HLA Abs group (P<0.0001). Time from diagnosis to transplantation in the positive anti-HLA Abs group was longer than in the negative anti-HLA Abs group (P=0.017). In addition, the distribution of primary diseases was significantly different between the two groups (P=0.023). There was no significant difference in age between the positive and negative anti-HLA Abs groups (P=0.353). Gender, time from diagnosis to transplantation and distribution of primary disease are potential risk factors for the occurrence of anti-HLA Abs.

We found that 48 patients (46.95%) had a single anti-HLA-I Abs, 11 (10.81%) a single anti-HLA-II Abs and 44 (39.64%) had anti-HLA-I+II Abs. We further explored the distribution of specific anti-HLA Abs in different primary diseases. Most of the patients with single anti-HLA-I Abs were diagnosed with AML (Acute myeloid leukemia), while most of the patients with single anti-HLA-II Abs were diagnosed with AA (aplastic anemia). In addition, most anti-HLA-I+II Abs patients were also found in AML, as shown in Table S1. There were 73 (65.76%) patients with MFI>5000, while 57 (51.35%) had MFI>8000(Table S2).
We compared the baseline transplant-related characteristics of patients between the positive and negative anti-HLA Abs groups, as shown in Table 2. There were significant differences in conditioning regimens between the two groups (P=0.001). In addition, there was a statistical difference in donor–recipient relationship (female to male) (P=0.0008). Patients in the negative anti-HLA Abs group (N=85, 21.57%) were more likely to receive the graft from female to male than positive anti-HLA Abs group (N=13, 3.3%). The two groups were comparable in terms of status of disease, stem cell source, transfusion number of MNC, CD34+ and CD3+ cells, and ABO match.

2. Relationship between anti-HLA Abs and clinical events

We observed that the presence of anti-HLA Abs delayed the engraftment of neutrophils (P=0.0014, Figure 1A). In addition, the presence of anti-HLA Abs was proved to be an independent risk factor for the neutrophil engraftment in univariate and multivariate analysis (HR 1.42 95%CI 1.13-1.80, P=0.003, Table S3). The proportion of patients achieving platelet engraftment was similar in the positive and negative anti-HLA Abs groups (P=0.21, Figure 1B). Patients with anti-HLA Abs were more vulnerable than patients without anti-HLA Abs to experiencing CMV reactivation (P=0.039, Figure 2A), but not EBV reactivation (P=0.15, Figure 2B). Anti-HLA Abs was identified as an independent risk factor for CMV reactivation in both univariate and multivariate analysis. (HR 2.03 95%CI 1.19-3.46, P=0.009, Table S4). Yet there were no significant differences in aGVHD (P=0.73, Figure 2C) and cGVHD (P=0.31, Figure 2D) between positive and negative anti-HLA Abs groups.

3. Relationship between anti-HLA Abs and Prognosis

We found that the probability of overall survival at 180 days in the positive anti-HLA Abs group was lower than in the negative anti-HLA Abs group (P=0.012, Figure 3A). As we had observed that gender was a significant factor in the discrimination between negative and positive anti-HLA Abs groups, we analyzed the effect of anti-HLA antibodies in subgroups divided by gender. Univariate and multivariate analyses indicated that the presence of anti-HLA Abs is an independent risk factor for the OS at 180 days (HR 2.32, 95% CI 1.03-5.27, P=0.042, Table 3) among female recipients, while among male recipients there is no significant difference (HR 0.46, 95%CI 0.06-3.52, P=0.457, Table 3).

In addition, there was a statistical difference in the cumulative incidence of NRM at 180 days between positive and negative anti-HLA Abs groups (P=0.018, Figure 3B). The presence of anti-HLA Abs did not significantly affect the results of univariate and multivariate analyses for NRM 180 days between different genders (Female recipients: HR 2.25, 95%CI 0.79-6.41, P=0.127; Male recipients: P=0.998, univariate analysis, Table S5).

There were no significant differences in the overall survival (P=0.98, Figure 4A), relapse (P=0.77, Figure 4B) and NRM (P=0.21, Figure 4C) between the positive and negative anti-HLA Abs groups.

We further analyzed the causes of death, the details of the causes of death at 180 days and until the end of the study period in the positive and negative anti-HLA Abs groups, the results are presented in Table 4.
The main cause of death was disease recurrence (28.57% of patients with anti-HLA Abs and 71.43% of patients without anti-HLA Abs). Deaths caused by relapse ranked first both in positive and negative anti-HLA Abs groups. TA-TMA, infection and GVHD were the other main causes of death.

Discussion

Since the 1966 findings of the presence of anti-HLA Abs in kidney allografts(18), there has not been a lot of focus on anti-HLA Abs in bone marrow transplants(19). Previous studies have mainly focused on DSAs (donor specific antibodies), which may play a pivotal role in the rejection of allografts in allogenic hematopoietic stem cell transplantation (allo-HSCT)(20, 21). Only a few studies researched the role of non-DSA in allo-HSCT. We conducted this retrospective, multicenter study to better understand the role of anti-HLA Abs in ISD-HSCT.

Firstly, we summarized the distribution of anti-HLA Abs in sibling recipients. We found that gender, period of the diseases and other underlying diseases are related to the occurrence of the anti-HLA Abs before transplantation in recipients. Previous studies had noticed how the risk factor of the production of the anti-HLA Abs is more likely to occur in females, after multiple pregnancies and multiple blood transfusions (1, 4, 22, 23). In our study, we hypothesize that the reasons why the stage of the disease and underlying diseases are related to the production of the antibodies might be previous multiple blood transfusions in the treatment of the disease. A previous study found that the diagnosis is an independent risk factor for the presence of antibodies, which was confirmed in our study(6). Anti-HLA Abs were detected in 27.61% of ISD transplantation recipients, making it a common occurrence in stem cell transplantation.

We demonstrated that the presence of anti-HLA Abs in recipients delayed the recovery of neutrophils in ISD-HSCT, while it didn't impact the engraftment of platelets. The mechanisms behind anti-HLA Abs and graft failure are not clear(24). Previous studies have found that the presence of donor-specific anti-DPB1 DSAs in matched unrelated hematopoietic stem cell transplantation(25) and anti-HLA Abs against the mismatched HLA Antigens (HLA-Ags) in haploidentical transplantation patients are correlated with graft failure(13), which demonstrates that allo-sensitization did not significantly increase the risk of graft failure unless Abs were directed against the donor HLA-Ags. This suggests that DSAs are the key to the development of graft failure(26). Yet the role of non-DSAs has been neglected in research. Although Ciurea et al found that DSA was the only risk factor for graft failure(25), the direct role of anti-HLA Abs (non-DSA) should be better studied in HID-HSCT.

Recent studies have found that the nonspecific AHAs (anti-HLA antibodies) have no influence in neutrophil or platelet engraftment in single-unit umbilical cord blood transplantation (UCBT)(27). In our study there were no direct donor HLA-Ags to attack because of the same phenotype of the donor and the recipient. Interestingly, the presence of anti-HLA Abs in ISD-HSCT delayed the cumulative incidence of neutrophil engraftment. HLA class I is expressed on the surface of nucleated cells, while HLA class II is expressed on B lymphocytes, antigen-presenting cells and activated T lymphocytes(19, 28, 29). Little is
known about the expression of HLA proteins on the cells that mediate engraftment. In the early phase of engraftment, the anti-HLA Abs might act on the nucleated cells(2, 24, 29), which may cause the delayed engraftment of neutrophils. Recent studies have hypothesized that immune complexes or antibodies against neutrophil antigens may be responsible for neutropenia in Felty’s syndrome and in systemic lupus erythematosus (SLE)(30, 31). In addition, clinical observations have found that anti-SSA antibodies are strictly associated with leukopenia/ lymphopenia in both SLE and Sjogren syndrome (SS)(32, 33). The presence of these autoantibodies may represent an interesting link between low white blood cell numbers and atherosclerosis(33). In unrelated donor HCT, DSAs cause graft failure and the presence of antibodies against HLA-DP was quite prominent, being observed in 60% of antibody-positive failures(25). In a study on data from the database of Japan Society for Hematopoietic Cell Transplantation (JSHCT), researchers have demonstrated that DSAs were associated with an increased risk of graft failure after CBT(34). In our study, we found that the presence of anti-HLA Abs does not have an impact on platelet engraftment, although the mechanisms behind this need further studies(35).

In solid organ transplants, especially in renal and heart transplants, patients suffer antibody-mediated rejection (AMR) in the presence of anti-HLA Abs(8-10, 36, 37). Acute graft-versus-host disease (aGVHD) is a rare but frequently lethal complication after solid organ transplantation(11). Yet there are only a few studies on the relationship between GVHD and DSAs in haploidentical transplantation(38, 39). Theoretically, recipient-specific anti-HLA antibodies (RSAs) might affect the development of GVHD(13). In a study among voluntary unrelated donors (VUDs), recipients of a graft from an anti-class II immunized donor had a higher cumulative incidence of a first episode of either acute or chronic GVHD(22). In results from UCBT studies, anti-HLA Abs did not have a significant effect on grade II-IV acute GVHD(40). In our study, the presence of anti-HLA Abs in sibling recipients didn't have an impact on the development of GVHD.

The presence of anti-HLA Abs increased the incidence of CMV reactivation, while it did not have an impact on EBV reactivation. Considering the signaling pathways associated with reactivation of CMV, Liu et al. have hypothesized that the transcription factor NF-κB may be involved in CMV reactivation. Smith et al. have found that anti-HLA Abs could induce NF-KB activation and thus promote endothelial cell proliferation(41). A recent study has reported that HLA-A2 Abs could activate NF-κB by stimulating the phosphorylation of the non-receptor tyrosine kinase Src(42). However, CMV activation is more dependent on failure of the host's antiviral immune responses in recipients with latent infection. Whether anti-HLA Abs participate in the immune response of transplant recipients is still an unresolved question. In addition, a previous study has shown by multifactorial analysis a significant correlation between neutropenia (more than seven days) and CMV infection. Given that neutrophils act as antimicrobial actors in the immune response, the delayed engraftment of neutrophil increases the incidence of infection-related incidents(43).

In conclusion, anti-HLA Abs have a negative impact on the prognosis in the early period after transplantation from sibling donors. We have clearly shown that the presence of anti-HLA Abs is the only risk factor for overall survival at 180 days among female recipients in both univariate and multivariate
analyses. Patients without neutrophil engraftment have been found to have worse outcomes (1-year OS, 19.5%), which confirms our observation that engraftment after CBT is crucial (15). Lower engraftment or delayed neutrophil recovery observed in anti-HLA Abs group could have affected the higher NRM in HLA-Mismatched Single CBT, although we cannot exclude the presence of other factors that have an impact on engraftment or early mortality (27). We analyzed the composition of the causes of deaths in positive and negative anti-HLA Abs groups at 180 days and at the final date of this study. In the early period after transplantation, we found that the incidence of infection-related diseases and TA-TMA causing death in the positive anti-HLA Abs group were higher than in the negative anti-HLA Abs group, while GVHD and relapse were a little higher in the negative anti-HLA Abs group. There was no difference in the prognosis later than 180 days after transplantation.

This multicenter, retrospective study demonstrates that the presence of anti-HLA Abs, mainly non-donor specific antibodies, in transplantation from a sibling donor delays the engraftment of neutrophil, increases the incidence of CMV reactivation and is the only risk factor for OS in the early phase after transplantation. There is now sufficient evidence supporting the negative impact of DSA in mismatched HSCT. Our study is limited by its retrospective design and multicenter sources, therefore we recommend a more detailed prospective study is carried out.

Declarations

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Author contributions: Depei Wu and Xiaojin Wu designed the study. Yingjun Chang, Xiaoyu Zhu, Xiaoxia Hu, Rong Guo, Yanming Zhang, Xiao Ma, Yue Han, Ying Wang, Huiying Qiu performed the research and collected the data. Xiya Wei wrote the paper and analyzed data.

Competing Interests: The authors have no conflict of interest.

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Supporting information statement

Additional supporting information may be found online in the Supporting Information section.
References


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**Tables**

Tables 1 to 4 are available in the Supplementary Files section.

**Figures**

**Figure 1.** The engraftment of neutrophils and platelets. A The presence of anti-HLA Abs delayed the engraftment of neutrophils\( (p=0.0014) \). B There was no difference in the cumulative incidence of platelet recovery between the two groups\( (p=0.21) \).

**Figure 1**

See image above for figure legend.
Figure 2. The cumulative incidence of CMV, EBV, aGVHD and cGVHD. A Patients with anti-HLA antibodies were more vulnerable to experience CMV infection than negative anti-HLA antibodies patients (p = 0.039). B There was no difference in the cumulative incidence of EBV infection between the two groups (p = 0.15). C There was no significant difference in the cumulative incidence of aGVHD between the two groups (p = 0.73). D There was not significant in the cumulative in cGVHD between the two groups (p = 0.31).

Figure 2

See image above for figure legend

A

B

Figure 3. The OS and NRM at day +180. A The survival rate at day +180 in patients with anti-HLA antibodies was lower than patients without anti-HLA antibodies (p = 0.012). B The non-relapse mortality at day +180 in positive anti-HLA antibodies group was higher than in negative anti-HLA antibodies group (p = 0.018).
Figure 3
See image above for figure legend

Figure 4. The cumulative incidence of relapse, NRM and overall survival. **A** There was no difference in the cumulative incidence of relapse between the two groups (P=0.77). **B** There was not different in the cumulative incidence of non-relapse mortality between the two groups (P=0.21). **C** There was no difference in overall survival probability between the two groups (P=0.82).

Figure 4
See image above for figure legend

**Supplementary Files**
This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- TableS2.xlsx
- TableS3.xlsx
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- TableS5.xlsx
- Table1.xlsx
- Table2.xlsx
- Table3.xlsx
- Table4.xlsx