Impact of pretransplant donor-specific anti-HLA antibodies in HLA-mismatched peripheral blood stem cell transplantation

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Article

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Abstract

The cut-off levels of donor-specific anti-HLA antibodies (DSAs) that are considered to predict a high risk of graft failure remain unclear. Using peripheral blood stem cell transplantation (PBSCT) data from the Japanese Society for Transplantation and Cellular Therapy/Japanese Data Center for Hematopoietic Cell Transplantation (JSTCT/JDCHCT), we examined the role of DSAs, and performed a retrospective analysis of patients whose recipients underwent related PBSCT between 2010 and 2014 with pre-transplant anti-HLA antibodies. Patients were divided into 3 groups using a mean fluorescence intensity (MFI) of 5,000 as a cut-off value: DSA positive (n = 8), anti-HLA antibody-positive (n = 137) and anti-HLA antibody-negative (n = 3657). There was a significant difference in the number of CD34-positive cells (median: 4.31, 3.97, and 5.33×10^6/kg, respectively; $p < 0.05$). Regarding the eight DSA-positive patients, only two underwent therapeutic intervention, and neutrophils were engrafted in all but one patient (median, 10 days). Although there was a statistically significant difference in neutrophil and platelet engraftment among the 3 groups (both $p < 0.05$), neutrophil engraftment was faster in the DSA group, with no significant difference in the overall survival ($p = 0.46$). Our results, based on JSTCT/JDCHCT data, suggest that DSAs may not affect the risk in related PBSCT.

Introduction

Anti-human leukocyte antigen (HLA) antibodies, particularly donor-specific anti-HLA antibodies (DSAs), cause graft failure in HLA-mismatched hematopoietic stem cell transplantation[1–3]. The European Society for Blood and Marrow Transplantation Consensus Guidelines recommend screening for DSAs before transplantation and that donors without DSAs be selected[4]. Since an elevated mean fluorescence intensity (MFI) is reported to generally increase the possibility of graft failure[5, 6], the MFI values of DSA only decrease prior to transplantation when unavoidable (e.g., when no optimal donor is available or a donor with HLA antibody corresponding to the mismatched recipient antigen can be selected).

Administering neutralizing antigens has been suggested to reduce anti-HLA antibodies for DSA-positive transplantation. Platelet concentrate with HLA antigens corresponding to DSAs (DSA-PC) are considered to be typical neutralizing antigens[7]. However, since platelets have only HLA-class I antigens[8], they cannot be neutralized by antibodies against HLA-class II antigens. Thus, a method of neutralizing antibodies by administering irradiated donor lymphocytes with both class I and class II antigens has been reported[9].

There is no international consensus on the MFI threshold for DSA positivity; however, the cut-off value for DSA positivity in CBT is > 500 or > 1,000[1, 6, 11, 10], and 3 studies on haploidentical stem cell transplantation have used cut-off values of 1,500–5,000[7, 8, 11]. Thus, the cut-off values for graft failure may vary with different transplant sources[7, 12]. It is therefore important to clarify the cut-off values of DSAs according to the transplant source and to develop guidelines for patients with DSAs. Ciurea et al.[3] showed no significant difference in the engraftment rate between bone marrow (BM) and peripheral blood (PB) as sources for DSA-positive transplantation. Conversely, Cluzeau et al.[13] reported that PB-

derived CD34 + cells had significantly lower HLA-DP antigen levels compared to BM-derived CD34 + cells, suggesting that PBSCT may be advantageous in transplants with DSA with HLA-DP antibodies. However, whether PB or BM should be used in such situations remains controversial.

Peripheral blood stem cells were preferentially selected as a stem cell source in HLA-mismatched related transplantation with HLA antibodies in the Japanese Society for Transplantation and Cellular Therapy/Japanese Data Center for Hematopoietic Cell Transplantation (JSTCT/JDCHCT) database (PBSCT, n = 225; BMT, n = 19). We assessed the impact of DSAs on primary graft failure after related PBSCT to provide comprehensive recommendations for clinical practice, including a strategy for desensitizing DSAs to improve the likelihood of successful transplantation.

Patients And Methods

Data collection

A total of 6,472 patients received HLA-mismatched BM transplantation (BMT), PBSCT, or both from a related donor enrolled in the JSTCT/JDCHCT Transplant Registry Unified Management Program (TRUMP) in 2010–2014. After excluding cases with BMT (n = 2491) and mixed BM and PBSC transplantation (n = 99) 3,882 patients were included in this study (Fig. 1). Among these, pre-transplant HLA antibodies (including DSA) were detected in 225 patients. After excluding 69 patients who did not give their informed consent, and 11 patients without detailed data on MFI values, 145 patients were classified as HLA antibody (including DSA)-positive and were analyzed as the HLA antibody-positive group; 3657 patients who underwent PBSCT only without anti-HLA antibodies prior to transplantation and for whom death/survival information was available at the final confirmation date were analyzed as the anti-HLA antibody-negative group (Table 1). Patients with anti-HLA antibodies against HLA-DP, HLA-DQ, and HLA-DRB3/4/5 were not included in the DSA or anti-HLA antibody-positive group because donor data for these HLA loci were unavailable.

This study was approved by the institutional review boards of the JSTCT and Tama-Hokubu Medical Center, Tokyo Metropolitan Health and Medical Treatment Corporation.

Definitions

At each institution, the presence of anti-HLA antibodies and the MFI for each HLA allele were determined using a Luminex assay. In this study, “DSA-positive” was defined by HLA antibodies with MFI ≥ 5,000 that corresponded to mismatched donor antigen, based on a previous report from Japan[7]. Patients with HLA antibodies that did not correspond to mismatched donor antigens, irrespective of their MFI, or with HLA antibodies corresponding to mismatched donor antigens but with MFI ≥ 5,000, were considered anti-HLA antibody-positive. Conversely, the anti-HLA antibody-negative group comprised patients who underwent PBSCT without HLA antibodies. This donor stratification has been previously reported[7, 12].

Neutrophil and platelet engraftment after transplantation were defined as an absolute neutrophil count of $> 0.5\times10^9$/L on the first of 3 consecutive days, and a platelet count of $> 20\times10^9$/L without transfusion.

Endpoints

The primary endpoint was to evaluate the impact of anti-HLA or DSAs on neutrophil or platelet engraftment in HLA-mismatched related PBSCT. The secondary endpoint was to investigate the current status of therapeutic interventions against anti-HLA antibodies in Japan.

Statistical analyses

Differences between groups were tested using an analysis of variance (ANOVA) and Fisher's exact test. The following factors were compared: recipient sex, age at transplantation, performance status, disease, disease status (remission or not), hematopoietic cell transplantation-comorbidity index (HCT-CI), history of previous transplantation, conditioning regimen (myeloablative conditioning [MAC] or reduced-intensity conditioning [RIC]) and total body irradiation [TBI] contained or not), sex combination (female to male, male to female or match), ABO mismatch (match, major mismatch, minor mismatch, or major + minor mismatch), GVHD prophylaxis (tacrolimus-based or cyclosporine-based), use of *in vivo* T-cell depletion, HLA disparity as assessed by serological typing of HLA-A, -B, -C, and -DR in the graft versus-host (GVH) and host-versus-graft (HVG) directions and number of infused CD34 + cells. The cumulative incidence was used to estimate the incidence of neutrophil and platelet engraftment by treating non-event deaths as a competing risk. The cumulative incidence of each group was evaluated using the Gray test. For significant differences the Bonferroni test was used to analyze differences between groups. A Kaplan–Meier survival analysis was conducted, with differences assessed using a log-rank test. P values of < 0.05 were considered statistically significant. According to the Bonferroni method, since we performed three statistical tests at once in this study and adjusted the alpha (α) = 0.05 for each test, the α level (0.017) was used to determine the probability value for the significance test of each comparison pair.

All statistical analyses were performed using EZR (Saitama Medical Centre, Jichi Medical University; http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html), a graphical user interface for R (version 3.0.2; The R Foundation for Statistical Computing, Vienna, Austria)[14].

Results

Characteristics of patients with anti-HLA antibody or DSA

We investigated 145 patients with anti-HLA antibody or DSA (male, n = 59; female, n = 86; median age, 49 years [range, 3–68 years]). The median dose of CD34-positive cells was 3.54×10^5/kg (range, 0.74×10^5/kg-15.10×10^5/kg). The disease status before transplantation was complete remission (CR) in 86 patients and non-CR in 59, excluding aplastic anemia (AA), myeloproliferative disorders (MPD), and Epstein-Barr virus-associated lymphoproliferative disorder (EBV-LPD). MAC (n = 35) and RIC (n = 110) were administered as conditioning regimens. Underlying diseases included acute lymphoblastic leukemia

(ALL, n = 20), acute myeloid leukemia (AML, n = 84), other leukemia (n = 5), myelodysplastic syndrome (n = 21), chronic myeloid leukemia (n = 2), MPD (n = 1), AA (n = 2), malignant lymphoma (n = 9), and EBV-LPD (n = 1). A tacrolimus-based (n = 130) or cyclosporine-based (n = 15) combination was administered for GVHD prophylaxis. The median follow-up period of survivors was 203 days (range, 1–1,933 days).

The MFI of anti HLA antibodies was measured by the HLA Foundation Laboratory (n = 77), the Japanese Red Cross Society (n = 12), Repro CELL Incorporated (n = 5), cord blood bank (n = 1), and by the treating institution (n = 48) (Figure. 2).

When an MFI of 5,000 was used as the cut-off value, 8 patients and 137 patients were classified into the DSA-positive and anti-HLA antibody-positive groups, respectively. The patient characteristics are summarized in Table 1.

Engraftment

The details of all 8 cases in the DSA group are shown in Table 2. Although most of cases (7/8) were non-CR before transplantation and only two cases underwent therapeutic intervention for DSA, neutrophil engraftment was observed in 7 cases. In the one case with failed engraftment, the patient underwent transplantation due to failure of induction chemotherapy for AML; however, she died on day 30 without engraftment due to sepsis.

The cumulative incidence of neutrophil and platelet engraftment according to the HLA antibody status is shown in Fig. 3. The point estimates for neutrophil engraftment at day 42 were 0.850 (95%CI: 0.839–0.862), 0.869 (95%CI: 0.798–0.916) and 0.875 for the anti-HLA antibody-negative, antibody-positive and DSA-positive groups, respectively. Although there was a significant difference in neutrophil engraftment among the three groups ($p < 0.05$), Bonferroni's test showed no significance; however, neutrophil engraftment occurred earlier in the DSA group. The point estimates for platelet engraftment at day 100 were 0.654 (95%CI: 0.638–0.669), 0.612 (95%CI: 0.524–0.689) and 0.375 (95%CI: 0.070–0.697), respectively. Although a significant difference existed in platelet engraftment among the three groups ($p < 0.05$), Bonferroni's test showed no significance. Figure 3C shows the overall survival (OS). There was no significant difference among the three groups ($p = 0.46$).

We also examined cut-off values of 3,000 and 1,000 (Supplementary Figs. 1 and 2). No significant difference in neutrophil engraftment or OS existed with these cut-off values; however, there were statistically significant differences in platelet engraftment at both cut-off values (both $p < 0.05$). When both cut-off values for the MFI were used, a significant difference in platelet engraftment was observed among the three groups; however, Bonferroni's test showed no significant difference. Furthermore, with cut-off values of 3,000 and 1,000, there were no significant differences in OS among the three groups ($p = 0.42$, 0.17, respectively).

When the cut-off value for the MFI was set at 5,000 and only cases tested in the HLA Foundation Laboratory (where MFI was most frequently measured) were evaluated (Table 1), all significant

differences in neutrophil and platelet engraftment and OS disappeared (p = 0.06, 0.45, 0.35, respectively) (Supplementary Fig. 3).

DSA positivity at MFI ≥ 10,000

Among our 3 cases with an MFI of ≥10,000 (MFI, 28,302, 15,742, 14,441), no patients received *in vitro* T-cell-depletion, and all 3 achieved neutrophil engraftment (day 10, n = 2; day 14, n = 1) (Table 2). One patient (#1) died of early recurrence within 28 days. The remaining two patients (#2 and #3) showed recurrence at 90 and 132 days, respectively. Platelet engraftment was not achieved in two cases (#1 and #2), while it was on day 21 in case #3.

Intervention for DSA

Among 145 cases, 10 received treatment to reduce DSA levels (rituximab, n = 5 [2 received rituximab + bortezomib with one also receiving plasma exchange]; rituximab with plasma exchange and intravenous immunoglobulin [IVIG], n = 1; rituximab alone, n = 2) (Table 3). Three patients received platelet concentrate from healthy-related donors with DSAs corresponding to HLA antigens (DSA-PC) to absorb DSAs; one also received IVIG. Two received high-dose platelet concentrate from healthy donors without DSAs corresponding to HLA antigens; one also received plasma exchange.

Discussion

We analyzed HLA-mismatched related PBSCT cases registered in the TRUMP data of the JSTCT/JDCHCT and found that DSAs did not affect the risk of neutrophil engraftment failure or OS. Although previous studies reported that the presence of DSAs influenced engraftment and survival, the cut-off values for the MFI of DSAs varied. The cut-off value for DSA positivity in CBT is defined as > 500 or > 1,000[1, 6, 10], yet three studies on haploidentical stem cell transplantation used cut-off values of 1,500–5,000[7, 8, 11]. Ciurea et al.[11] analyzed all cases in which T-cell-depletion was performed. We adopted a cut-off value of MFI ≥ 5,000, as reported by Yoshihara et al.[7] Since cases without T-cell depletion show a much lower risk of graft failure than those with T-cell depletion, our study population only included 30 cases with T-cell depletion, and no T-cell depletion in our DSA-positive group. Chang et al. showed that DSA positivity at MFI ≥ 10,000 strongly correlated with primary graft rejection[8]. A Japanese single-center report showed that although 2 of 3 PBSCT patients with pre-transplant DSA positivity at MFI ≥ 10,000 developed graft failure (with no therapeutic intervention to lower their DSA), but no patients with an MFI of 5,000–10,000 developed graft failure[7]. Conversely, three of our cases (MFI: 28,302, 15,742, 14,441), showed neutrophil engraftment without any delay (Table 2). Platelet engraftment was not reached in two cases (#1 and #2) due to early recurrence, while it was observed on day 21 in one case. Additionally, all three patients showed successful engraftment without therapeutic intervention.

To improve engraftment, many studies have shown the beneficial effects of various types of intervention to reduce the anti-HLA antibody load, mainly through combined approaches[15]. Our study included 10 patients who received therapeutic intervention, mainly using a combined approach (Table 3). It is unclear

whether these interventions were effective, as they were not necessarily limited to patients with DSAs with high MFI values. Yoshihara et al.[7] reported on pre-transplantation desensitization strategies, including a combination of plasmapheresis, rituximab, antibody adsorption with platelet concentrate from healthy donors selected for DSA-PC, and bortezomib administration (a proteasome inhibitor). Among these strategies, DSA-PC most effectively reduced DSAs. Similarly, Yamashita et al.[16] used a single-dose rituximab, IVIG and DSA-PC transfusion, and reported that DSA-PC transfusion was the most effective approach. Accordingly, they recommended using an antibody-producing cell depleting agent (e.g., rituximab) to stop HLA antibody production, in combination with antibody neutralizing treatment. Since platelets have only class I HLA antigens, they can only absorb the DSAs of class I HLA antigens[8]. Additionally, since HLA class I molecules are expressed at very low levels on erythrocytes[17], when large numbers of donor erythrocytes are transfused into the recipient, antigen-antibody reactions of DSAs are expected to occur, although the desensitization effect is weaker than the effect of platelets. Maruta et al. showed that the infusion of irradiated donor lymphocytes to adsorb both class I HLA antibodies and class II HLA antibodies improved engraftment[9].

The donor sources in four reports investigating BMT or PBSCT associated with DSA positivity before transplantation showed low DSA sequestration rates. The donor sources in those reports of DSA-positive transplants were as follows: among the 11 cases reported by Yoshihara et al.[7] 6 were PB and 5 were BM; all cases reported by Ciure et al.[11] were BM; all cases reported by Chang et al.[8] were mixed BM and PB; among the 134 cases reported by Bramanti et al.[18], 65 were PB and 69 were BM. In this study, we investigated PBSCT as the source of transplantation in all patients, which has not been reported. We hypothesized that because PBSC contains more platelets and lymphocytes than BM or cord blood transplantation, these cells may act as neutralizing antigens and improve survival. Consequently, when the transplantation source was restricted to PBSCT, the results contradicted the findings of previous studies reporting that DSA had a negative effect on neutrophil engraftment. Since there are inter-institutional differences when measuring the MFI for HLA antibodies, depending on the measurement system, we considered it to be potentially important to evaluate the results using the same measurement system. However, we also found that when the cut-off value of MFI $\geq$ 5000, which was limited to specimens measured at one-inspection laboratory, there were no significant differences (Supplementary Fig. 3).

Besides graft failure, studies have shown that patients with DSAs had a significantly lower event-free survival and OS than those without DSAs[1, 5, 6]. However, in our study, the OS of the DSA-positive group did not differ from that of the groups without DSAs or anti-HLA antibodies. This indicates that the existence of DSAs themselves is not an adverse prognostic factor, though stable neutrophil engraftment, with or without DSAs, improves the prognosis. In other words, even if platelet engraftment was unstable, it might not affect the prognosis, as it could be managed by platelet transfusion.

The present study was associated with several limitations. First, the size of the DSA group was small. Furthermore, DSAs directed against HLA-DP and DQ loci were not evaluated. Piyanuch et al. reported that

the expression levels of anti-HLA antibodies against these loci were low, but still had an impact on graft rejection[19]. For additional information on HLA-DP antigen, the CD34 + cells from PB show lower antigen expression levels than those from BM[13], suggesting that PBSCT may provide some advantages in HLA antibody-positive transplants. Since HLA typing of these loci is not commonly performed before HSCT in Japan, we could not detect these DSAs in the present study.

Anti-HLA antibodies were reported to disappear with the discontinuation of transfusion[20, 21] or the use of cryopreserved autologous platelets[22] or HLA-matched platelets[20, 24]. Surprisingly, anti-HLA antibodies often disappear in the course of treatment, despite continued random donor platelet transfusion[20, 22–28]. Often, in patients in whom a previously detected HLA antibody had disappeared, the antibody was not reproduced when subsequently transfused[23]. Also, as described earlier, the MFI of DSA is easily changed if the administered PC unexpectedly contains DSA antigens and antibody adsorption occurs. Taken together, DSA detection is expected to be quite dependent on the timing of sampling. Thus, in future studies to detect DSAs, the timing when the MFI of DSAs is determined should be strictly defined.

Allogeneic hematopoietic stem cell transplantation using haploidentical related donors represents an alternative treatment for patients who lack a related or unrelated HLA-matched donor. Based on historical data, graft failure due to the presence of DSAs is a major complication that makes physicians hesitant to pursue transplantation using these donors. We herein found that DSA was not an adverse factor for neutrophil engraftment or OS in PBSCT. Even with the Japanese registry data, the number of DSA cases was too small to conclude that PBSCT could overcome the adverse effects of DSA. However, we believe that the present study nevertheless adds valuable new evidence to the research on DSA-positive transplantation, which is still far from complete. Further studies are needed to determine precisely how to appropriately intervene in cases in which the MFI of DSA is extremely high.

Declarations

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Author contributions

TH, KI and JK designed the research, analyzed data, and wrote the paper. HT and YK designed the research. KK, TF, YK, MS, ND, HN, KM, YM, HS, TE, TK, YH, TI, YA provided data. All authors have read the manuscript and agreed to submission.

Compliance with ethical standards

Conflict of interest

Hirohisa Nakamae received research funding from Astellas Pharma Inc., Novartis Pharma K.K., and honoraria from Astellas Pharma Inc., Kyowa Kirin Co., Ltd., NIPPON SHINYAKU Co., Ltd., and Novartis Pharma K.K. The remaining authors declare no competing financial interests.

References

1. Takanashi M, Atsuta Y, Fujiwara K, Kodo H, Kai S, Saito H, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. Blood 2010;116:2839–2846. DOI: 10.1182/blood-2009-10-249219
2. Spellman S, Bray R, Rosen-Bronson S, Haagenson M, Klein J, Flesch S, Vierra-Green C, Anasetti C. The detection of donor-directed, HLA-specific alloantibodies in receipients of unrelated hematopoietic cell transplantation is predictive of graft failure. Blood. 2010;115(13):2704–8. doi: 10.1182/blood-2009-09-244525
3. Ciurea SO, Thall PF, Wang X, Wang SA, Hu Y, Cano P, et al. Donor-specific anti-HLA Abs and graft failure in matched unrelated donor hematopoietic stem cell transplantation. Blood. 2011;118(22):5957–64. doi: 10.1182/blood-2011-06-362111.
4. Ciurea SO, Cao K, Fernandez-Vina M, Kongtim P, Malki MA, FuchsE, Luznik L, Huang XJ, Ciceri F, Locatelli F, et al. Consensus Guidelines for the Detection and Treatment of Donor-specific Anti-HLA Antibodies (DSA) in Haploidentical Hematopoietic Cell Transplantation. Bone Marrow Transplant. 2018;53(5):521–534. doi: 10.1038/s41409-017-0062-8
5. Ruggeri A, Rocha V, Masson E, Labopin M, Cunha R, Absi L, et al. Impact of donor-specific anti-HLA antibodies on graft failure and survival after reduced intensity conditioning-unrelated cord blood transplantation: a Eurocord, Societe Francophone d'Histocompatibilite et d'Immunogenetique (SFHI) and Societe Francaise de Greffe de Moelle et de Therapie Cellulaire (SFGM-TC) analysis. Haematologica. 2013;98:1154–60. doi: 10.3324/haematol.2012.077685.
6. Cutler C, Kim HT, Sun L, Sese D, Glotzbecker B, Armand P, Koreth J, Ho V, et al. Donor-specific anti-HLA antibodies predict outcome in double umbilical cord blood transplantation. Blood. 2011;118(25):6691–7. doi: 10.1182/blood-2011-05-355263.
7. Yoshihara S, Maruya E, Taniguchi K, Kaida K, Kato R, Inoue T, et al. Risk and prevention of graft failure in patients with preexisting donor-specific HLA antibodies undergoing unmanipulated haploidentical SCT. Bone Marrow Transplant. 2012;47(4):508–15. doi: 10.1038/bmt.2011.131.
8. Chang YJ, Zhao XY, Xu LP, Zhang XH, Wang Y, Han W, Chen H, Wang FR, Mo XD, Zhang YY, Huo MR, Zhao XS, Y K, Liu KY, Huang XJ. Donor-specific anti-human leukocyte antigen antibodies were associated with primary graft failure after unmanipulated haploidentical blood and marrow transplantation: a prospective study with randomly assigned training and validation sets. J Hematol Oncol. 2015;8:84. doi: 10.1186/s13045-015-0182-9
9. Maruta A, Fukawa H, Kanamori H, Harano H, Noguchi T, Kodama F, Kase N, Matsuzaki M, Miyashita H, Motomura S, et al. Donor-HLA-incompatible marrow transplantation with an anti-donor cytotoxic

antibody in the serum of the patient. Bone Marrow Transplant. 1991;*7*(*5*):397–400.

10. Brunstein CG, Noreen H, DeFor TE, Maurer D, Miller JS, Wagner JE. Anti-HLA antibodies in double umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2011;17(11):1704–1708. doi: 10.1016/j.bbmt.2011.04.013.
11. Ciurea SO, de Lima M, Cano P, Korbling M, Giralt S, et al. High risk of graft failure in patients with anti-HLA antibodies undergoing haploidentical stem-cell transplantation. Transplantation. 2009;*88*(*8*):1019–24. *doi*: 10.1097/TP.0b013e3181b9d710.
12. Ciurea SO, Thall PF, Milton DR, Barnes TH, Kongtim P, Carmazzi Y, et al. Complement-binding donor-specific anti-HLA antibodies and risk of primary graft failure in hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2015;21:1392–8. DOI: 10.1016/j.bbmt.2015.05.001
13. Cluzeau T, Lambert J, Raus N, Dessaux K, Absi L, Delbos F, et al. Risk factors and outcome of graft failure after HLA matched and mismatched unrelated donor hematopoietic stem cell transplantation: a study on behalf of SFGM-TC and SFHI. *Bone Marrow Transplant.* 2016;*51(5):687 – 91. doi: 10.1038/bmt.2015.351.*
14. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48(3):452–8. doi: 10.1038/bmt.2012.244
15. Marfo K, Lu A, Ling M, Akalin E. Desensitization protocols and their outcome. Clin J Am Soc Nephrol. 2011;6(4):922–36. doi: 10.2215/CJN.08140910.
16. Yamashita T, Ikegame K, Kojima H, Tanaka H, Kaida K, Inoue T and Ogawa H. Effective desensitization of donor-specific HLA antibodies using platelet transfusion bearing targeted HLA in a case of HLA-mismatched allogeneic stem cell transplantation. Bone Marrow Transplant. 2017;52(5):794–796. doi: 10.1038/bmt.2017.10.
17. Rees L, Kim JJ. HLA sensitisation: can it be prevented?. Pediatr Nephrol. 2015;30(4):577–87. doi: 10.1007/s00467-014-2868-6.
18. Bramanti S, Calafiore V, Longhi E, Mariotti J, Crespiatico L, Sarina B, De Philippis C, Nocco A, Santoro A, Castagna L. Donor-Specific Anti-HLA Antibodies in Haploidentical Stem Cell Transplantation with Post-Transplantation Cyclophosphamide: Risk of Graft Failure, Poor Graft Function, and Impact on Outcomes. Biol Blood Marrow Transplant. 2019;25(7):1395–1406. doi: 10.1016/j.bbmt.2019.02.020.
19. Kongtim P, Cao K, Ciurea SO. Donor Specific Anti-HLA Antibody and Risk of Graft Failure in Haploidentical Stem Cell Transplantation. Adv Hematol. 2016;2016:4025073. doi: 10.1155/2016/4025073.
20. Murphy MF, Metcalfe P, Ord J, Lister TA, Waters AH. Disappearance of HLA and platelet-specific antibodies in acute leukaemia patients alloimmunized by multiple transfusions. *Br J Haematol.* 1987;*67(3):255 – 60. doi*: 10.1111/j.1365-2141.1987.tb02344.*x.*
21. Howard JE and Perkins HA. The natural history of alloimmunisation to platelets. Transfusion. 1978;18(4):496–503. doi: 10.1046/j.1537-2995.1978.18478251250.x.
22. Dutcher, JP, Schiffer CA, Aisner J and Wiernik, PH. Longterm follow-up of patients with leukaemia receiving platelet transfusions: identification of a large group of patients who do not become

alloimmunised. Blood. 1981;*58*(*5*):1007–11.

23. McGrath K, Wolf M, Bishop J, Veale M, Ayberk H, Szer J, Cooper I and Whiteside M. Transient platelet and HLA antibody formation in multitransfused patients with malignancy. Br J Haematol. 1988;68(3):345–50. doi: 10.1111/j.1365-2141.1988.tb04212.x.
24. Brand A, Claas FH, Voogt PJ, Wasser MN and Eernisse JG. Alloimmunisation after leukocyte-depleted multiple random donor platelet transfusions. Vox Sang. 1988;54(3):160–6. doi: 10.1111/j.1423-0410.1988.tb03892.x.
25. Djerassi I and Farber S. Conference on obstacles to the control of acute leukemia. Control and prevention of hemorrhage: platelet transfusion. Cancer Res. 1965;25(9):1499 – 503.
26. Tejada F, Bias WB, Santos GW, Zieve PD. Immunologic response of patients with acute leukaemia to platelet transfusions. Blood. 1973;42(3):405–12.
27. Green D, Tiro A, Basiliere J and Mittal K. Cytotonic antibody complicating platelet support in acute leukaemia. JAMA. 1976 Aug 30;236(9):1044–6.
28. Holohan TV, Terasaki P, Deisseroth AB Suppression of transfusion-related alloimmunization in intensively treated cancer patients. Blood. 1981;58(1):122–8.

Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

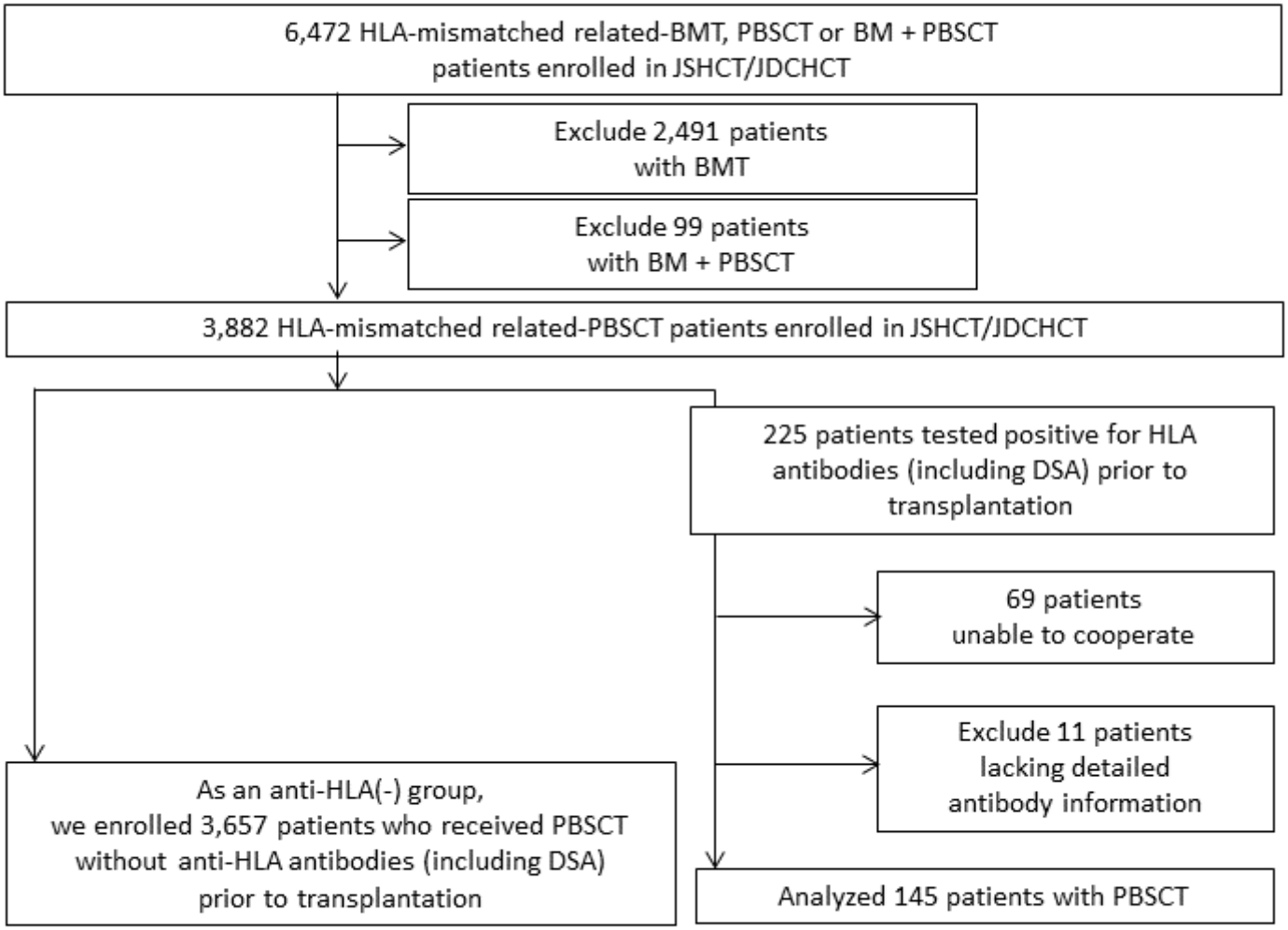

Figure 1

Patient flow-chart.

A total of 6,472 patients who received HLA-mismatched BMT, PBSCT, or both from blood donors were enrolled in the JSTCT/JDCHCT Transplant Registry Unified Management Program between 2010 and 2014. Among these, 2,491 patients with BMT and 99 patients with mixed BM and PBSC transplantation were excluded. A total of 3,882 patients with HLA-mismatched BM PBSCT from a related donor were analyzed in this study. Although 225 patients with anti-HLA antibodies detected before transplantation were selected, 69 patients did not provide their consent for participation in the study, while the records of 11 patients lacked detailed antibody data from their institutions; these cases were excluded. Thus, a total of 145 DSA or anti-HLA antibody-positive patients were eligible for inclusion in the study. Furthermore, of those 3,882 patients, after excluding the above patients, a total of 3,657 patients without anti-HLA antibodies detected before transplantation were used as a control group for this study.

Abbreviations: HLA, human leucocyte antigen; BM, bone marrow; PB, Peripheral blood; JSTCT/JDCHCT, the Japan Society for Hematopoietic Cell Transplantation/Japanese Data Center for Hematopoietic Cell Transplantation; DSA, donor specific HLA antibody

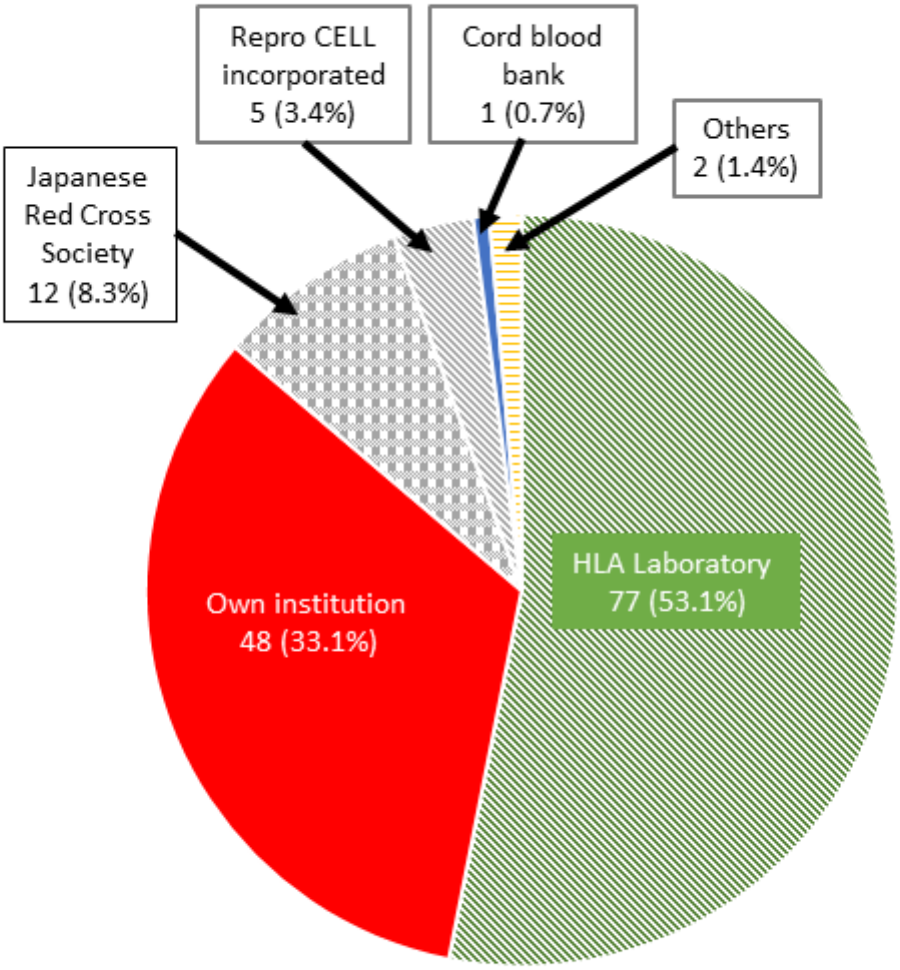

Figure 2

Percentage of inspection laboratories of DSAs.

The MFI of anti HLA antibodies was measured by the HLA Foundation Laboratory (n=77), the Japanese Red Cross (n=12), Repro CELL (n=5), a cord blood bank (n=1) cases, and by the treating institution (n=48).

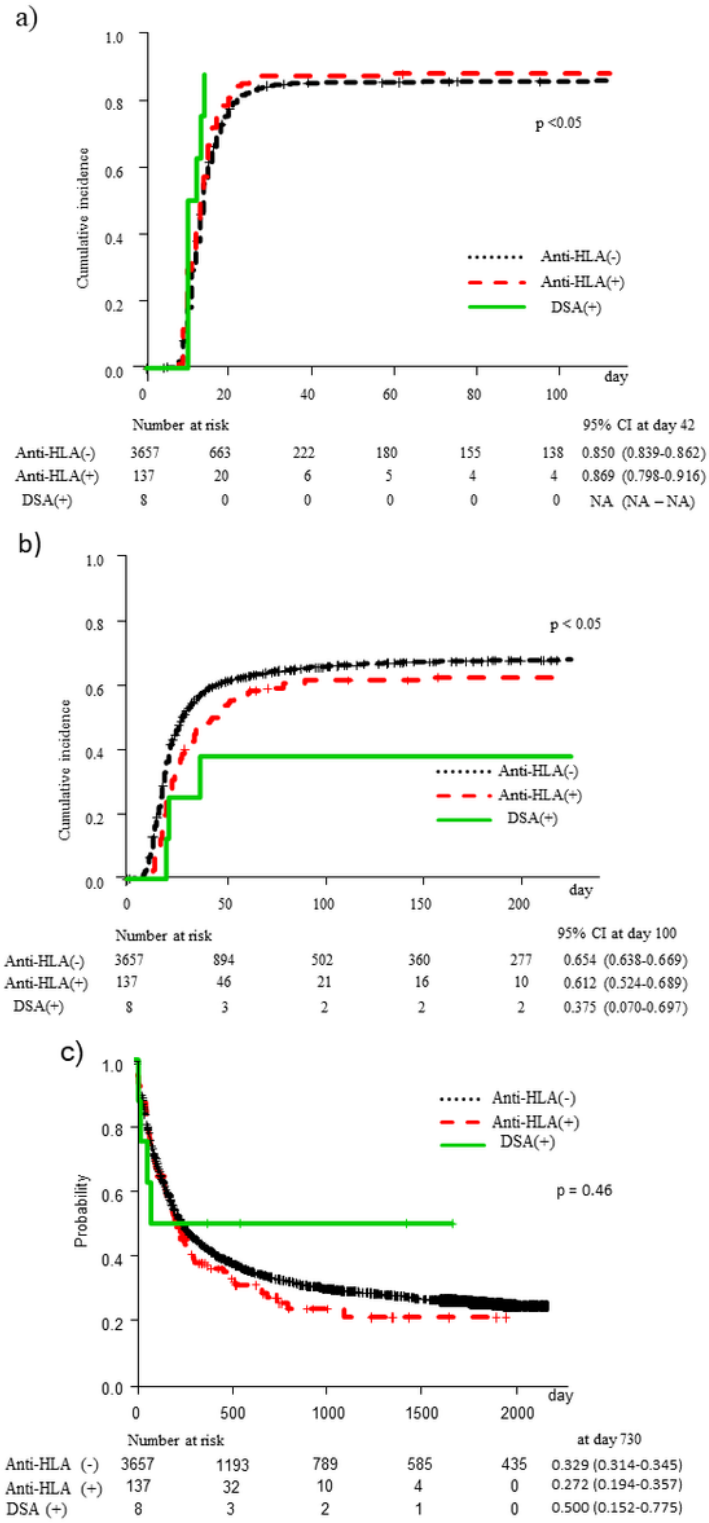

Figure 3

Cumulative incidence of a) neutrophil engraftment and b) platelet engraftmentand c) overall survival with/without pretransplant DSAs or anti-HLA antibody.

All patients were classified into three groups: patients without anti-HLA antibodies (Group 1, n=3,657, dotted line), patients positive for anti-HLA antibodies (Group 2, n=137, dashed line) and patients positive

for DSAs (Group 3, n =8, solid line). There were significant differences in the neutrophil and platelet engraftment among the groups (both $p<0.05$), but the difference in the Kaplan-Meier survival curves was not statistically significant ($p=0.46$). However, neutrophil engraftment was faster in the DSA group than in the other groups.

Abbreviations: HLA, human leucocyte antigen; DSA, donor specific HLA antibody.

Supplementary Files

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

- DSATable1.xlsx
- DSATable2.xlsx
- DSATable3.xlsx
- DSASupInfo.pdf