20. Dallas MH, Triplett B, Shook DR, et al. Long-term outcome and evaluation of organ function in pediatric patients undergoing haploidentical and matched related hematopoietic cell transplantation for sickle cell disease. *Biol Blood Marrow Transplant*. 2013;19:820-830.
21. Bolaños-Meade J, Fuchs EJ, Luznik L, et al. HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease. *Blood*. 2012;120:4285-4291.
22. Oshrine BR, Olson TS, Bunin N. Mixed chimerism and graft loss in pediatric recipients of an alemtuzumab-based reduced-intensity conditioning regimen for non-malignant disease. *Pediatr Blood Cancer*. 2014;61:1852-1859.
23. Marsh RA, Vaughn G, Kim MO, et al. Reduced-intensity conditioning significantly improves survival of patients with hemophagocytic lymphohistiocytosis undergoing allogeneic hematopoietic cell transplantation. *Blood*. 2010;116:5824-5831.
24. Shenoy S, Eapen M, Wu J, et al. A multicenter phase II trial of unrelated donor reduced intensity bone marrow transplantation for children with severe sickle cell disease (SCURT): results of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN 0601) study. 2015;126:619.
25. Locatelli F, Kabbara N, Ruggeri A, et al. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. *Blood*. 2013;122:1072-1078.
26. Eapen M, Klein JP, Sanz GF, et al. Effect of donor-recipient HLA matching at HLA-A, -B, -C, and -DRB1 on outcomes after umbilical-cord blood transplantation for leukaemia and myelodysplastic syndrome: a retrospective analysis. *Lancet Oncol*. 2011;12:1214-1221.
27. Koura DT, Horan JT, Langston AA, et al. In vivo T cell costimulation blockade with abatacept for acute graft-versus-host disease prevention: a first-in-disease trial. *Biol Blood Marrow Transplant*. 2013;19:1638-1649.
28. Saliba RM, Rezvani K, Leen A, et al. General and virus-specific immune cell reconstitution after double cord blood transplantation. *Biol Blood Marrow Transplant*. 2015;21:1284-1290.
29. Shereck EB, Cooney E, van de Ven C, Della-Lotta P, Cairo MS. A pilot phase II study of alternate day ganciclovir and foscarnet in preventing cytomegalovirus (CMV) infections in at-risk pediatric and adolescent allogeneic stem cell transplant recipients. *Pediatr Blood Cancer*. 2007;49:306-312.
30. Bhatia M, Jin Z, Baker C, et al. Reduced toxicity, myeloablative conditioning with BU, fludarabine, alemtuzumab and SCT from sibling donors in children with sickle cell disease. *Bone Marrow Transplant*. 2014;49:913-920.
31. Leen AM, Bollard CM, Mendizabal AM, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood*. 2013;121:5113-5123.
32. Melenhorst JJ, Leen AM, Bollard CM, et al. Allogeneic virus-specific T cells with HLA alloreactivity do not produce GVHD in human subjects. *Blood*. 2010;116:4700-4702.
33. Parikh SH, Brochstein J, Martin PL, et al. Successful engraftment of donor umbilical cord blood (UCB) cells after co-transplantation of Nicord® (ex vivo expanded UCB progenitor cells with nicotinamide) and a second unmanipulated cord blood unit after myeloablative chemotherapy in pediatric patients with severe sickle cell disease. *Blood*. 2016;128:3651.
34. Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. *N Engl J Med*. 2014;371:339-348.
35. Horwitz ME, Chao NJ, Rizzieri DA, et al. Umbilical cord blood expansion with nicotinamide provides long-term multilineage engraftment. *J Clin Invest*. 2014;124:3121-3128.

The Case for High Resolution Extended 6-Loci HLA Typing for Identifying Related Donors in the Indian Subcontinent

Rajat Kumar Agarwal [1,2,*], Ankita Kumari [1], Amit Sedai [2], Lalith Parmar [1], Rakesh Dhanya [1], Lawrence Faulkner [3]

[1] *Sankalp India Foundation, Bangalore, India*
[2] *Jagriti Innovations, Bangalore, India*
[3] *Cure2Children Foundation, Florence, Italy*

Article history:
Received 1 March 2017
Accepted 30 May 2017

Key Words:
Bone marrow transplantation
Histocompatibility testing
HLA matching
Related donor
Consanguinity

A B S T R A C T

Three-loci low-resolution (LR) or intermediate-resolution HLA typing is generally considered adequate in the related blood and marrow transplantation (BMT) context. However, a single high-resolution (HR) mismatch may have a similar adverse impact on BMT outcome as an LR one. We sought to determine the frequency of mismatches that may go undetected when standard typing (LR or 3-loci HR) is used compared with 6-loci HR typing for related donor compatibility testing, and to assess its impact on relevant BMT outcomes. We analyzed data from a total of 2554 6-loci (HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1) HR sequence-based typing (full typing [FT]) from 754 patients, 1011 siblings, and 789 parents done at DKMS Germany (www.DKMS.de) between January 1, 2014, and June 21, 2016. We also studied 38 cases in which the family had undergone 3-loci HLA typing (standard typing [ST]). Patients were from India (70%), Pakistan (22%), and Sri Lanka (8%). The IMGT/HLA database (www.ebi.ac.uk/ipd/imgt/hla) was used to tease out nonpermissive DPB1 mismatches. HLA disparity–related outcomes, such as rejection and graft-versus-host disease (GVHD) were assessed in a retrospective matched-pair cohort of 50 patients (25 with ST and 25 with FT) who underwent BMT for severe thalassemia from compatible related donors. We found fully matched (either 12/12 HR matches or with a single permissive DPB1 mismatch) related donors for 285 patients (38%). Of these donors, 89% were siblings and 11% were parents. The likelihood of matching on an individual locus on LR but not on HR was found to be 5%. A total of 9 donors (3%; 7 siblings and 2 parents) who would have been considered a full match by HR typing on A, B, and DRB1 alone were not a match by extended 6-loci HR typing. Five of these 9 donors had a mismatch on C or DQB1, and 4 had a nonpermissive DPB1 mismatch. In this group, 5 donors (56%) belonged to a consanguineous family, in 2 donors (22%) there was no reported consanguinity, and in 2 donors (22%) consanguinity was unknown. We identified 18 donors (6%; 13 siblings and 5 parents) who would have

Financial disclosure: See Acknowledgments on page 1595.
Category:
Histocompatibility
Pediatric (Matched Donors)
Contributors: The manuscript has been read and approved by all the authors.

* Correspondence and reprint requests: Rajat Kumar Agarwal, BE, Sankalp India Foundation, #304-D, Chamundi Emerald, 1st Main, 7th Cross, N. S. Palya, B.T.M. 2nd Stage, Bangalore 560076, India.
E-mail address: rajat@jagriti.co.in (R.K. Agarwal).

been considered a 12/12 match by LR HLA typing alone but were found not to match on extended HR typing. In this group, 11 donors (61%) were from consanguineous families, 3 donors (17%) had no reported consanguinity, and in 4 donors (22%) consanguinity was unknown. Outcome analysis showed that the actuarial proportion of patients with GVHD was 4% in the FT group compared with 16% in the ST group, with log-rank $P = .1952$. The ST group included 2 patients with grade III-IV acute GVHD and 1 patient each with moderate and severe chronic GVHD, whereas the FT group only 1 patient with grade III acute GVHD. We conclude that even in the context of related donors, the use of LR and/or 3-loci (A, B, and DRB1) HR HLA typing might result in a sizable risk of missing a clinically relevant mismatch, which may have an adverse impact on transplantation outcomes. In the Indian subcontinent, this observation is not limited to putatively compatible parents or consanguineous families; we recommend full 6-loci HR HLA typing even for matched related BMTs.

INTRODUCTION

Matched-related transplantation remains by far the most common form of transplantation in the developing world, where the chance of finding a HLA-compatible relative can be as high as 74% depending on family size and consanguinity rate [1-3]. In a related context, DNA-based 3-loci low-resolution (LR) or intermediate-resolution typing for HLA-A and -B, and high-resolution (HR) typing for -DRB1 is considered an acceptable standard by most centers, on the basis of the presumptive extended HR match identity of inherited whole haplotypes [4]. Most centers continue to use LR HLA typing to determine matches within a family. It has been shown that a single HR HLA mismatch may have a similar adverse impact as a single LR HLA mismatch on the outcome of related hematopoietic stem cell transplantation [5-9].

We sought to quantify the potential for missing relevant HLA disparities between matched related donors by comparing standard typing with 6-loci (A, B, C, DRB1, DQB1, and DPB1) HR typing in the Indian subcontinent, where consanguinity or close ethnicity may result in a high frequency of partially overlapping haplotypes. We also attempted to assess the clinical impact of typing level on HLA disparity-associated outcomes such as graft-versus-host disease (GVHD) and graft rejection in a relatively homogeneous group of patients who underwent transplantation for severe thalassemia.

METHODS

HLA Typing and Patient Characteristics

A total of 2554 6-loci (HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1) HR sequence-based full typing (FT) analyses for 754 patients, 1011 siblings, and 789 parents were performed at DKMS Germany (www.dkms.de) between January 1, 2014, and June 21, 2016. In addition, we studied 38 cases in which the family had also undergone less than 6-loci typing, including 4 with 3-loci HR typing, 22 with 2-loci HR and 4-loci LR typing, and 12 with LR typing restricted to A, B, and DRB1 only. Of these 38 cases, 25 underwent transplantation and could fit into a matching cohort for comparison with transplantations done in patients who underwent FT. Families were from India (70%), Pakistan (22%) and Sri Lanka (8%).

To assess the clinical impact of standard typing (ST) (DNA-based 3-loci LR, intermediate-resolution, or HR typing for A, B, and DRB1) versus FT (HR typing for A, B, C, DRB1, DQB1, and DPB1), we retrospectively compared 2 matching cohorts of 25 patients each with severe thalassemia syndromes who underwent related compatible BMT on a homogeneous protocol from within a total of 86 evaluable patients who had undergone transplantation at Sankalp India Foundation and Cure2Children Foundation collaborating institutions (People Tree Hospitals, Bangalore, India; South East Asia Institute for Thalassemia, Jaipur, India; Kokilaben Dhirubhani Ambani Hospital, Mumbai, India; Pakistan Institute of Medical Sciences, Islamabad, Pakistan; Central Asiri Hospital, Colombo, Sri Lanka; and Nawaloka Hospital, Colombo, Sri Lanka). Patient cohorts where grouped based on age at transplantation (≤3 years, 3 to 10 years, and >10 years) and donor–recipient sex mismatch. All the patients in both the groups were low risk (defined as liver <2 cm from costal margin and no splenomegaly).

Study Design

For the 754 patients who received FT, the HLA reports were analyzed for matches by comparing all 6 alleles. An attempt to find matches was also done comparing only HLA-A, -B, and -DRB1 as is done using HR ST and comparing the reports on LR alone, that is, comparing only the first 2 digits of the HLA typing reports. Discrepancies in outcomes between the various strategies were noted.

The IMGT/HLA database (www.ebi.ac.uk/ipd/imgt/hla) was used to tease out permissive mismatches by DPB1 T cell epitope (TCE groups) algorithms. We excluded 12 families in which certain alleles were not resolved or were unnamed (new alleles).

All patients underwent transplantation between August 2013 and June 2016 after conditioning with a combination of rabbit anti-thymocyte globulin, busulfan, and cyclophosphamide followed by the infusion of freshly harvested granulocyte colony- stimulating factor–primed donor marrow on day 0. Rejection/GVHD prophylaxis consisted of cyclosporin A, methotrexate, and low-dose prednisone [10].

An event was defined as the onset of acute GVHD grade III or IV, moderate or severe chronic GVHD according to standard criteria [11,12], or rejection (defined as inability to detect ≥5% donor DNA in recipient's peripheral blood 30 days from BMT or thereafter). The median duration of follow-up was 13 months (range, 0.7 to 22.7 months) in the FT cohort and 16.7 months (range, 1 to 30.7 months) in the ST cohort.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 5 (www.graphpad.com/prism/Prism.htm). Kaplan–Meier survival curves were compared using the log-rank (Mantel–Cox) test. BMTPlus (www.bmtplus.net) was used prospectively for data collection [13], and Microsoft Excel (Microsoft, Redmond, Washington) was used to compare codes and establish potential matches/mismatches.

RESULTS

Among the 754 patients who received FT and were analyzed for matches, we found fully matched (either 12/12 HR matches or a single permissive DPB1 mismatch) related donors for 285 patients (38%). Of these, 89% were siblings and 11% were parents. The chance of matching on an individual locus on LR but not on HR as an average of different loci was 5%, which locus-wise was 2.6% for HLA-A, 3.3% for -B, 3.5% for -C, 4.5% for -DRB1, 9.0% for -DQB1, and 6.7% for -DPB1. A total of 9 donors (7 siblings and 2 parents) who would have been considered to have a full match by HR typing on A, B, and DRB1 alone were not a match by extended 6-loci HR typing. Of these 9 donors, 5 had a mismatch on C or DQB1, and 4 had a nonpermissive DPB1 mismatch. Within this comparison, all the mismatches were limited to 1 allele. Three mismatches were in the graft-versus-host (GVH) direction, and the other 6 were bidirectional mismatches. This group included 5 donors (56%) from consanguineous families, 2 donor (22%) with no reported consanguinity, and 2 donors (22%) with unknown consanguinity.

We identified 18 donors (13 siblings and 5 parents) who would have been considered a full 12/12 HLA match using LR HLA typing alone but were found not to be a full match