Human Immunology 80 (2019) 976–982

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

The impact of transfused blood products on deceased donor HLA typing

Reuben P. Jacob[a], Christina L. Dean[b], Scott M. Krummey[b], Zuleikha Shah[b], Nathaniel Sutherland[b], Casey Orear[b], Howard M. Gebel[b], Robert A. Bray[b], Harold C. Sullivan[b,*]

[a] Department of Pathology, Stanford University, Stanford, CA, United States
[b] Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA, United States

ARTICLE INFO

Keywords:
Deceased donor
HLA typing
Leukoreduced
Deceased donor organs
Transplantation
Transfusion

ABSTRACT

Accurate deceased donor HLA typing assumes that the blood sample tested contains only DNA from the organ donor. Prior to procurement, many organ donors are transfused at least one unit of red blood cells (RBC). Non-organ donor DNA acquired from transfusions may result in incorrect and/or ambiguous HLA typing. To address this question, we investigated the impact of RBC transfusion on organ donor HLA typing by using different in vitro transfusion models: leukoreduced (LR) and non-LR RBCs. Various quantities of LR and non-LR RBCs were added to normal peripheral blood and HLA typing was performed by real time PCR. Our results show that HLA typing of deceased donors can be impacted dependent upon the type and quantity of transfused RBCs. Importantly, if LR RBCs are given, HLA typing is unlikely to be affected, precluding the need to delay typing and obtain an alternative source of donor DNA.

1. Introduction

Deceased donor (DD) organs comprised approximately 82% (28,587) of organ transplants performed in the U.S in 2017 [1]. The HLA system, discovered by Dausset in 1952 [2], began to play an important role in successful transplantation in 1969 after Patel and Terasaki demonstrated that positive crossmatches were associated with hyperacute rejection of kidney allografts [3]. Due to the importance of the HLA system in DD organ allocation and transplant outcomes, accurate HLA typing of DDs is vital. Laboratories that perform DD HLA typing are required to perform molecular typing and report results at the level of serological splits to the organ procurement organization (OPO) [4]. The shift in HLA typing from serologic based assays to molecular based assays followed studies that identified the HLA gene structure and protein sequence and the development of rapid typing methods utilizing polymerase chain reaction (PCR) technology. PCR allowed for the amplification of specific HLA DNA fragments, which led to better accuracy and improved resolution.

DD HLA typing is predicated on the assumption that the whole blood (WB) sample obtained for testing exclusively contains the DNA of that organ donor. However, prior to procurement, organ donors frequently receive blood products as part of life saving treatment or to maintain organ viability for donation. Studies on deceased organ donor characteristics reveal that many deceased organ donors receive at least one RBC transfusion before organ procurement [5]. Anecdotally, it is not uncommon to have a DD receiving more than 20 units of blood products prior to organ procurement. As of 2015, 90% of red blood cells (RBCs) in the U.S. are leukoreduced (LR), and therefore, individual units contain little blood-donor DNA [6]. However, if an organ donor were to receive non-LR RBCs prior to a blood sample being acquired for HLA typing, the presence of blood-donor DNA could result in incorrect and/or ambiguous HLA typing, potentially leading to disastrous consequences for the organ recipient. This is especially true given increased organ sharing and the emergence of the virtual crossmatch in pre-transplantation evaluation.

The clinical impact of transfusion on DD grafts is quite complex. One study reported an inverse relationship between organ recovery and the number of transfusions the DD received, with transfusions serving as a surrogate for hemodynamic instability in the DD [5]. Another study found a 23% decrease in the odds of recipients developing delayed graft dysfunction if the DD was transfused; the effect was more pronounced with increasing numbers of blood transfusions [7]. In contrast, another

Abbreviations: HLA, human leukocyte antigen; DNA, deoxyribonucleic acid; OPTN, organ procurement and transplantation network; UNOS, united network for organ sharing; DD, deceased donor; LR, leukoreduced/leukoreduction; RBC, red blood cells; PRBC, packed red blood cells; WB, whole blood; PCR, polymerase chain reaction; OPO, organ procurement organization; TBV, total blood volume; EDTA, ethylenediaminetetraacetic acid; SSP, sequence specific primer; SSO, sequence specific oligonucleotide probe

* Corresponding author at: Department of Pathology, Emory University Hospital, Atlanta, GA 30322, United States.
E-mail address: hcsulli@emory.edu (H.C. Sullivan).

https://doi.org/10.1016/j.humimm.2019.10.001
Received 16 March 2019; Received in revised form 3 October 2019; Accepted 7 October 2019
Available online 15 October 2019

study showed no difference in transplant vasculopathy or incidence of post-transplant rejection in heart transplant recipients whose DD received blood transfusions versus those that did not [8]. Just as the clinical effects of transfusion in DD transplantation are unclear, its impact on laboratory testing, e.g., HLA typing, has not been well studied.

According to the OPTN/UNOS Histocompatibility Committee, "Blood transfusions administered to the donor in the immediate pre-collection period may have an impact on the accuracy of HLA typing." (https://unos.org/wp-content/uploads/unos/Histo_Brochure.pdf). Indeed, the effects of microchimerism – the presence of small numbers of cells originating from a genetically different individual – have previously been described after transfusion [9–11] and pregnancy [12] and could theoretically be detected by sensitive HLA typing methods; yet there is little evidence that HLA typing submitted to UNOS post transfusion might affect the accuracy of HLA typing of DDs. Herein, we investigated the potential impact of RBC transfusion on the integrity of HLA typing by utilizing different transfusion scenarios.

2. Material & methods

RBC transfusion was simulated in vitro by mixing studies using LR blood obtained from the Emory University Hospital blood bank's expired inventory and non-LR blood from healthy volunteers. This study was reviewed and approved by the Emory University Institutional Review Board. Seven HLA typed healthy volunteers were assigned as recipients or donors in the mock transfusion scenarios. HLA typing results of each transfusion scenario were then compared to the original typing results of each volunteer. To replicate the total blood volume (TBV) of an organ donor (average of 5L), an in vitro model of 5 ml was used. Varying mixtures of LR and non-LR donor RBCs (e.g., 0.25 ml in vitro = 1 RBC unit in vivo) were then made to simulate different transfusion scenarios.

In this study, six transfusion scenarios were performed. Three scenarios were designed to simulate non-LR WB transfusions, representing the worst-case situations (i.e., largest presence of white cells). However, most red cell products transfused in the US are packed red blood cells (PRBC). Therefore, two additional scenarios simulated non-LR PRBC transfusions. For these simulations, donor blood was centrifuged at 3500 rpm for 10 min and the buffy coat was removed. These PRBCs were not filtered, and not considered LR. In one of the PRBC scenarios, the recipient received blood from three different donors, simulating an organ donor receiving multiple transfusions, each likely to come from a different donor. The final scenario was the LR RBC transfusion scenario in which the recipient received varying amounts of LR RBC from expired units from the blood bank.

Blood samples were collected in EDTA tubes and treated and tested as if they were deceased organ donor samples. DNA extraction was performed using the EZ1 Advanced XL automated nucleic acid purification system (Qiagen, Valencia, CA). HLA typing was performed by real time PCR using Linkage Biosciences (South San Francisco, CA) HLA typing kit (11 loci+: HLA – ABCDRDQDP SABR™ 384 Kits) and software (SureTyper™). White counts were measured using the ABX Micros 60 (HORIBA Medical, Irvine, CA) and the XE-2100 - Sysmex (Sysmex America Inc, Lincolnshire, IL).

3. Results

The various mixtures representing different transfusion scenarios between recipient blood with non-LR donor blood and recipient blood with LR donor blood are detailed in Table 1. The HLA types of the recipients and the donors are provided in Tables 2–6. Each table also displays the number of antigens/alleles present at each HLA locus between the recipient and donor. Subsequent columns represent the different transfusion volumes (i.e., 10 units, 2 units, 1 unit) and show the number of antigens/alleles detected at each locus and whether those antigens/alleles were recipient or donor derived as evidenced by positive donor- and recipient-specific wells on the real time PCR tray.

In transfusion scenario 1 (Table 2), recipient and donor were homozygous at multiple loci; this situation limited the number of antigens per locus to 2, one from the recipient and one from the donor. In this setup, contaminating donor DNA could be detected even when 1 donor unit was simulated. Transfusion scenario 2 depicts a recipient:donor pair that is HLA disparate with only a few shared antigens/alleles (Table 3); whereas transfusion scenario 3 presents a recipient:donor pair that is more similar in the HLA typing with many shared antigens/alleles (Table 4). The same recipient:donor pair from transfusion scenario 3 was used in transfusion scenario 4 except the donor red cells were spun down to simulate a PBRC transfusion (Table 5).

In regard to analysis, loci at which > two antigens/alleles were present were unable to be assigned an HLA type by the SureTyper™ software. For the non-LR WB and PRBC transfusion scenarios, evidence of donor DNA was present no matter the number of simulated transfused red cell units. Indeed, the real time PCR method could detect donor DNA even when donor units were PRBC (Tables 5 and 6), which had WBC counts of 0.01–0.46 $\times 10^3/\mu l$ after removal of the buffy coat. Our PCR method also detected donor DNA from each of the 3 donors in transfusion scenario 5 (Table 6), which mimics the clinical reality of a recipient receiving RBC units from multiple donors. Interestingly, the number of positive donor-specific wells decreased as the number of mock units transfused decreased. At times, especially with the 1 RBC unit transfusion simulations, some donor antigen/alleles were no longer discernable as all donor-specific wells for the donor antigens/alleles were negative.

In contrast to non-LR RBCs, LR RBCs do not appear to impact HLA typing. In simulations of transfusing 16 or 19 LR RBC units (i.e., almost total replacement of recipient blood volume), non-ambiguous recipient typing was obtained. No evidence of non-recipient DNA was detected by the real time PCR software (data not shown). This was not unexpected since the WBC counts of LR units were repeatedly 0.00 $\times 10^3/\mu l$. To further investigate the potential influence of donor DNA from LR RBC, a LR RBC was HLA typed in isolation to determine if HLA typing could be obtained. DNA extraction yielded a low concentration of 1.3 ng/dL of DNA. At this low concentration, 14 extractions were required to yield sufficient volume to run the real time PCR assay, and even then, no HLA typing was rendered (Fig. 1A). In fact, the majority of wells resulted as errors and even the well-specific control gene failed to amplify (Fig. 1B); and the wells that were considered positive had abnormal melting curve tracings that were not representative of true positive reactions (Fig. 1C).

4. Discussion

DNA typing by molecular methods such as PCR has revolutionized the fields of transfusion and transplantation medicine. Genotyping has eliminated a number of ambiguities that occur when determining the phenotype of recently transfused recipients by immunologic and biochemical typing [13]. Several studies have examined the effect of RBC transfusions on genotyping [13,14]. Wenk et al. used Southern blot analysis of variable number of tandem repeats (VNTR) as well as PCR dot-blot visual examination for alleles of the DQA1, LDLR, GYPA, HBGG, G7S8 and GC loci [13] while Reid et al. used PCR amplification to analyze blood group related genes [14]. Both studies demonstrated that DNA typing of recipient blood can reliably be determined after transfusion of RBCs even without the widespread use of LR. Explanations from these studies include dilution of the donor's white blood cells (WBCs) in the recipient's circulation and a selective advantage in the recovery of the recipient's own cells. These two studies from 1997 and 2000, respectively, focused mainly on transfusions and its effects on VNTRs and RBC genotyping. The role of transfusions and its effects exclusively on HLA typing has not been previously addressed, which is

Table 1
Varying Mixtures of LR and non-LR Donor RBCs to Simulate Different Transfusion Scenarios.

Transfusion Scenarios 1–3 (Non-LR WB donor)	Volume of recipient blood	Volume of donor blood
10 units RBCs	2.5 ml	2.5 ml
2 units RBCs	4.5 ml	0.5 ml
1 unit RBC	4.75 ml	0.25 ml
Transfusion Scenario 4 (Non-LR PRBC donor)	Volume of recipient blood	Volume of donor blood
10 units RBCs	2.5 ml	2.5 ml
2 units RBCs	4.5 ml	0.5 ml
1 unit RBC	4.75 ml	0.25 ml
Transfusion Scenario 5 (Multiple non-LR PRBC donors)	Volume of recipient blood	Volume of each donor blood
3.33 units PRBCs per donor (10 units total)	2.5 ml	0.83 ml
Transfusion Scenario 6 (LR donor)	Volume of recipient blood	Volume of donor blood
16 units RBCs	1.0 ml	4.0 ml
19 units RBCs	0.25 ml	4.75 ml

Table 2
Transfusion Scenario 1: Non-LR, WB transfusion between recipient and donor who are homozygous at multiple loci.

HLA Locus	Recipient A HLA Type WBC: 10.0 × 10^3/μl		Donor A HLA Type WBC: 4.3 × 10^3/μl		No. of distinct antigens/alleles detected at locus	Recipient and donor antigens/alleles detected in 10 unit transfusion scenario		Recipient and donor antigens/alleles detected in 2 unit transfusion scenario		Recipient and donor antigens/alleles detected in 1 unit transfusion scenario	
						R*	D**	R	D	R	D
A	*01	*03	*23	*30	4	*01, *03	*23, *30	*01, *03	*23, *30	*01, *03	*23
B	*08	XX	*07	*44	3	*08	*07, *44	*08	*07, *44	*08	*07
C	*07	XX	*04	*15	3	*07	*04, *15	*07	*15	*07	*15
DRB1	*03:01(17)	XX	*11	XX	2	*03:01	*11	*03:01	*11	*03:01	*11
DRB3/4/5	DRB3*01	XX	DRB3 *02	XX	2	DRB3*01	DRB3*02	DRB3*01	DRB3*02	DRB3*01	None
DQA1	*05:01	XX	*01	XX	2	*05:01	*01	*05:01	*01	*05:01	*01
DQB1	*02	XX	*06:02	*06:03	3	*02	*06:02, *06:03	*02	*06:02	*02	*06:02
DPA1	*01	*02:01	*01	*03	3	*02:01, *01 (I***)	*03, *01 (I)	*02:01, *01 (I)	*03, *01 (I)	*02:01, *01 (I)	*03, *01 (I)
DPB1	*04:02	*09:01	*03:01	*40:01	4	*04:02, *09:01	*03:01, *40:01	*04:02, *09:01	*03:01, *40:01	*04:02, *09:01	*40:01

* R = recipient allele detected based on positive recipient-specific wells.
** D = donor allele detected based on positive donor-specific wells.
*** I = indeterminant; DNA source could not be determined due to recipient and donor sharing antigen/allele at that locus.

a concern since technological advances in PCR technology, especially with regard to amplifying small quantities of DNA, has significantly advanced. To our knowledge, this is the first study to specifically address the effect of transfusion on HLA typing and whether LR impacts the results.

The clinical relevance of the current study cannot be understated. Assuming a majority of the approximately 28,000 organ donors per year receive at least one transfusion, 10% of which are non-LR RBCs, the HLA typing of approximately 2,800 donors could theoretically be impacted/compromised by DNA derived from the transfusion donor(s) as opposed to the organ donor. The results obtained in this study suggest that HLA typing for DDs is differentially impacted based on the LR status of the RBC administered. Whereas, non-LR RBC transfusion could result in ambiguous or incorrect donor typing, transfusion with LR RBC does not appear to impact donor HLA typing. Ambiguity in recipient HLA typing was found after transfusion simulation of only 1 non-LR RBC unit, pointing to the level of detection in current PCR technologies. As evidenced by results obtained in the 19 unit LR transfusion scenario where the DNA concentration was 3 ng/μl, real time PCR can detect low levels of DNA. While the Council of Europe requires LR RBC units to contain $< 1.0 \times 10^6$ per unit, the FDA has a less stringent criteria of $< 5.0 \times 10^6$ residual leukocytes per unit [15]. As such, LR units in the U.S. contain more white blood cells; nonetheless, from our study it appears that this residual amount of DNA is inconsequential to achieving accurate typing.

Limitations of this study include the use of an in vitro model, which may not be reflective of in vivo events. This study was a closed system, whereas the human body is much larger with both intra- and extravascular spaces contributing to the distribution of blood. In our system, the distribution of transfused RBCs likely overestimates the concentrations of blood donor leukocytes in the peripheral system. Furthermore, in bleeding DDs, transfused blood may be eliminated. Thus, donor DNA may not be as detectable as in our closed system. Also, we focused on non-LR WB transfusion scenarios, whereas > 90% of the transfusions in the US are LR PRBCs. The goal in focusing on non-LR, WB transfusions was to present worse-case scenarios in which the transfusion of unfiltered donor white cells would provide the highest likelihood of impacting HLA typing. Of note, in recent years WB transfusions have made a resurgence in usage, especially in the trauma setting [16]; and starting in the 31st edition of the AABB Standards for Blood Banks and Transfusion Services, low-titer group O WB is considered the standard for transfusing out-of-group WB, or when the recipient's blood type is unknown [17], meaning transfusion of WB may become more clinically relevant.

In addition, we only thoroughly examined one molecular method. However, we did perform sequence specific primer (SSP) and sequence specific oligonucleotide probe (SSO) assays in one of the 10 RBC unit transfusion simulations (recipient B/donor B) for proof of principle. Similar to real time PCR, both SSO and SSP detected antigens/alleles from both the recipient and donor and final HLA typing could not be properly assigned. Other methods such as next-generation sequencing (NGS) may be more sensitive at lower concentrations of donor DNA; however, NGS is not regularly employed for DD typing due to time constraints required by UNOS. Finally we had the advantage of having donor typing to help us determine ambiguity. As reflected in our LR RBC transfusion scenarios, donor typing will not always be readily

Table 3
Transfusion Scenario 2: Non-LR, WB transfusion between recipient and donor who share few antigens/alleles.

HLA Locus	Recipient B HLA TypeWBC: 4.9 × 10^3/μl		Donor B HLA Type WBC: 6.1 × 10^3/μl		No. of distinct antigens/alleles detected at locus	Recipient and donor antigens/alleles detected in 10 unit transfusion scenario		Recipient and donor antigens/alleles detected in 2 unit transfusion scenario		Recipient and donor antigens/alleles detected in 1 unit transfusion scenario	
						R*	D**	R	D	R	D
A	*02:01	*23	*02:02	*68	4	*23	*02:02, *68	*23	*02:02, *68	*23	*02:02, *68
B	*15:01 (B62)	*57	*07	*15:03 (B72)	4	*15:01, *57	*07, *15:03	*15:01, *57	*07, *15:03	*15:01, *57	*07, *15:03
C	*03:03 (C9)	*06	*07	*12	4	*03:03, *06	*07, *12	*03:03, *06	*07, *12	*03:03, *06	None
DRB1	*07	*14	*03:01 (DR17)	*15	4	*07, *14	*03:01, *15	*07, *14	*03:01, *15	*07, *14	*03:01, *15
DRB3/4/5	DRB4 *01:03 N	DRB3 *02	DRB3 *02	DRB5 *01	3	DRB4 *01:03 N, DRB3 *02 (I***)	DRB5*01 DRB3 *02 (I)	DRB4 *01:03 N, DRB3 *02 (I)	DRB5*01 DRB3 *02 (I)	DRB4 *01:03 N, DRB3 *02 (I)	DRB5*01 DRB3 *02 (I)
DQA1	*01:04	*02:01	*01:02	*05:01	4	*01:04, *02:01	*01:02, *05:01	*01:04, *02:01	*01:02, *05:01	*01:04, *02:01	*01:02, *05:01
DQB1	*03:03 (DQ9)	*05	*02	*06	4	*03:03, *05	*02, *06	*03:03, *05	*02, *06	*03:03, *05	*02, *06
DPA1	*01:03	*01	*01:03	*02	2	*01:03 (I)	*01:03 (I)	*01:03 (I)	*01:03 (I)	*01:03 (I)	*01:03 (I)
DPB1	*04:01	XX	*01:01	*4:01	2	*04:01 (I)	*04:01 (I), *01:01	*04:01 (I)	*04:01 (I), *01:01	*04:01 (I)	*04:01 (I), *01:01

* R = recipient allele detected based on positive recipient-specific wells.
** D = donor allele detected based on positive donor-specific wells.
*** I = indeterminant; DNA source could not be determined due to recipient and donor sharing antigen/allele at that locus.

Table 4
Transfusion Scenario 3: Non-LR, WB transfusion between recipient and donor who share several antigens/alleles.

HLA Locus	Recipient C HLA Type WBC: 6.13 × 10^3/μl		Donor C HLA Type WBC:5.3 × 10^3/μl		No. of distinct antigens/alleles detected at locus	Recipient and donor antigens/alleles detected in 10 unit transfusion scenario		Recipient and donor antigens/alleles detected in 2 unit transfusion scenario		Recipient and donor antigens/alleles detected in 1 unit transfusion scenario	
						R*	D**	R	D	R	D
A	*01	*68:01	*01	*68:02	3	*01 (I***)	*01 (I), *68:02	*01 (I),	*01 (I), *68:02	*01 (I), *68:01	*01 (I)
B	*08	*57	*08	*15:10 (B71)	3	*57, *08 (I)	*15:10, *08 (I)	*57, *08 (I)	*15:10, *08 (I)	*57, *08 (I)	*15:10, *08 (I)
C	*06	*07	*03:04 (C10)	*07	3	*06, *07 (I)	*03:04, *07 (I)	*06, *07 (I)	*03:04, *07 (I)	*06, *07 (I)	*07 (I)
DRB1	*03:01 (DR17)	*07	*03:01 (DR17)	XX	2	*07, *03:01 (I)	*03:01 (I)	*07, *03:01 (I)	*03:01 (I)	*07, *03:01 (I)	*03:01 (I)
DRB3/4/5	DRB3 *01	DRB4 *01:03 N	DRB3 *01	DRB3 *02	3	DRB4 *01:03 N, DRB3 *01 (I)	DRB3*02, DRB3 *01 (I)	DRB4 *01:03 N, DRB3 *01 (I)	DRB3*02, DRB3 *01 (I)	DRB4 *01:03 N, DRB3 *01 (I)	DRB3 *01 (I)
DQA1	*02:01	*05	*05:01	*05	2	*02:01, *05 (I)	*05/05:01	*02:01, *05 (I)	*05/05:01	*02:01, *05 (I)	*05/05:01
*DQB1	*02	*03:03 (DQ9)	*02	XX	2	*02 (I), *03:03	*02 (I)	*02 (I), *03:03	*02 (I)	*02 (I), *03:03	*02 (I)
DPA1	*01:03	*01	*01:03	*02	2	*01:03/*01 (I)	*02, *01:03 (I)	*01:03/ *01 (I)	*01:03 (I)	*01:03/*01 (I)	*01:03 (I)
DPB1	*04:01	XX	*01:01	*04:02	3	*04:01	*01:01, *04:02	*04:01	*01:01, *04:02	*04:01	*01:01, *04:02

* R = recipient allele detected based on positive recipient-specific wells.
** D = donor allele detected based on positive donor-specific wells.
*** I = indeterminant; DNA source could not be determined due to recipient and donor sharing antigen/allele at that locus.

Table 5
Transfusion Scenario 4: Non-LR, PRBC transfusion between recipient and donor who share several antigens/alleles.

HLA Locus	Recipient C HLA Type WBC: 6.1 × 10^3/μl		Donor C HLA Type WBC: 0.5 × 10^3/μl		No. of distinct antigens/alleles at locus	Recipient and donor antigens/alleles detected in 10 unit transfusion scenario		Recipient and donor antigens/alleles detected in 2 unit transfusion scenario		Recipient and donor antigens/alleles detected in 1 unit transfusion scenario	
						R*	D**	R	D	R	D
A	*01	*68:01	*01	*68:02	3	*01 (I***)	*01(I), *68:02	*01 (I), *68:01	*01(I)	*01 (I), *68:01	*01 (I),
B	*08	*57	*08	*15:10 (B71)	3	*57, *08 (I)	*15:10, *08 (I)	*57, *08 (I)	*15:10, *08 (I)	*57, *08 (I)	*15:10, *08 (I)
C	*06	*07	*03:04 (C10)	*07	3	*06, *07 (I)	*03:04, *07 (I)	*06, *07 (I)	*07 (I)	*06, *07 (I)	*07 (I)
DRB1	*03:01 (DR17)	*07	*03:01 (DR17)	XX	2	*07, *03:01 (I)	*03:01 (I)	*07, *03:01 (I)	*03:01 (I)	*07, *03:01 (I)	*03:01 (I)
DRB3/4/5	DRB3 *01	DRB4 *01:03 N	DRB3 *01	DRB3 *02	3	DRB4 *01:03 N, DRB3 *01 (I)	DRB3*02, DRB3 *01 (I)	DRB4 *01:03 N, DRB 3*01 (I)	DRB3*01 (I)	DRB4 *01:03 N, DRB3 *01 (I)	DRB3*01 (I)
DQA1	*02:01	*05	*05:01	*05	2	*02:01, *05 (I)	*05/05:01	*02:01, *05 (I)	*05/05:01	*02:01, *05 (I)	*05/05:01
DQB1	*02	*03:03 (DQ9)	*02	XX	2	*02 (I), *03:03	*02 (I)	*02 (I), *03:03	*02 (I)	*02 (I), *03:03	*02 (I)
DPA1	*01:03	*01	*01:03	*02	2	*01:03/*01 (I)	*01:03 (I)	*01:03 /*01 (I)	*01:03 (I)	*01:03/ *01 (I)	*01:03 (I)
DPB1	*04:01	XX	*01:01	*04:02	3	*04:01	*01:01, *04:02	*04:01	*01:01, *04:02	*04:01	*01:01, *04:02

* R = recipient allele detected based on positive recipient-specific wells.
** D = donor allele detected based on positive donor-specific wells.
*** I = indeterminant; DNA source could not be determined due to recipient and donor sharing antigen/allele at that locus.

Table 6
Transfusion Scenario 5: Non-LR, PRBC transfusion between recipient and multiple donors.

HLA Locus	Recipient D Type WBC: 3.35 × 10^3/μl		Donor D1* Type WBC: 0.01 × 10^3/μl		Donor D2** Type WBC: 0.03 × 10^3/μl		Donor D3*** Type WBC: 0.01 × 10^3/μl		No. of distinct antigens/alleles at locus	Recipient and donor antigens/alleles detected in transfusion scenario			
										R****	D1	D2	D3
A	*24	*31	*01	*68:01	*02:01	*23	*02:01	*02:05	7	*24, *31	*01, *68:01	*02:01 (I***), *23	*2:01 (I), *2:05
B	*35	*39	*08	*57	*15:01 (B62)	*57	*50	*52	7	*35, *39	*08, *57 (I)	*15:01, *57 (I)	*52
C	*04	*07:02	*06:02	*07:01	*03:03 (C9)	*06:02	*06:02	*12	6	*04, *07:02	*06:02 (I), *07:01	*06:02 (I), *03:03	*06:02 (I)
DRB1	*04:04	*11	*03:01 (DR17)	*07:01	*07:01	*14	*07:01	*15	6	*04:04, *11	*03:01, *07:01 (I)	*07:01 (I), *14	*07:01 (I), *15
DRB3/4/5	DRB4 *01	DRB3 *02	DRB3 *01	DRB4 *01:03 N	DRB4 *01:03 N	DRB3*02	DRB4*01	DRB5 *01	5	DRB4*01 (I), DRB3*02 (I)	DRB3*01, DRB4 *01:03 N (I)	DRB4 *01:03 N (I), DRB3*02 (I)	DRB4 *01 (I), DRB5*01
DQA1	*03:01	*05:03	*02:01	*05:01	*01:01	*02:01	*01:03	*02:01	6	*03:01, *05:03	*05:01, *02:01 (I)	*02:01 (I)	*02:01 (I)
DQB1	*03:01 (DQ7)	*03:02 (DQ8)	*02:01	*03:03 (DQ9)	*03:03 (DQ9)	*05	*02:02	*06:01	7	*03:01, *03:02	*03:03 (I)	*03:03 (I), *05	*02:02, *06:01
DPA1	*01:03	XX	*01:03	*01	*01:03	*01	*01:03	*01	1	*01:03 (I)	*01:03/*01 (I)	*01:03/*01 (I)	*01:03/*01 (I)
DPB1	*04:01	*04:02	*04:01	XX	*04:01	XX	*03:01	*04:01	3	*04:01 (I), *04:02	*04:01 (I)	*04:01 (I)	*03:01, *04:01 (I)

*****I = indeterminant; DNA source could not be determined due to recipient and donor (s) sharing antigen/allele at that locus.
* D1 = donor 1.
** D2 = donor 2.
*** D3 = donor 3.
**** R = recipient.

Fig. 1. A. Test Results Table: SureTyper™ software unable to assign HLA typing for all loci. B. Tray Viewer: Majority of wells (323/384; 84%) failed to amplify including the positive control well. The remaining wells were either called positive, negative or questionable. C. Representative melting curve of positive well: Though there were 18 wells called positive, the majority demonstrated an abnormal melting curve not corresponding with the expected positive curve.

available especially when using RBCs from an outside blood supplier/distributor.

As the present study demonstrates, determining the LR status of RBCs given to the organ donor prior to procurement can be critical information to help confirm whether the HLA typing obtained has the potential to be confounded by the presence of transfused leukocytes from the blood donor(s). This information may be obtained from the blood bank at each institution; however, the logistics of obtaining such information may be challenging. Nonetheless, if this information can be obtained and the donor HLA typing results are unambiguous, it may be appropriate to modify current UNOS OPO requirements to include the LR status of transfused units received by DDs. Thus, if non-LR RBCs were transfused, HLA typing from alternative tissue sources may be warranted. These sources include those that require invasive sampling, such as lymph node or splenic tissue, or less invasive sampling, such as buccal swab testing, which has been shown to demonstrate identical typing to blood-derived DNA [18]. Conversely, if LR RBCs were transfused, even in large quantities, a peripheral blood sample is sufficient for reliable HLA typing. Finally, the findings in this study may have implications in determining reliability of HLA typing in other populations receiving transfusions, particularly in transfusion-dependent hematologic patients being evaluated for hematopoietic progenitor cell transplantation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge Vanessa M. Williams (MT, AMT) and Willie S. Williams (CHT, ABHI) for their work and contribution to this project.

References

[1] Organ Procurement and Transplantation Network (Internet), (cited 2018 Dec 27). Available from: https://optn.transplant.hrsa.gov/.
[2] J. Dausset, Immuno-hematologie des leucocytes, Bibl Paediatr. 14 (1958) 29–56.
[3] R. Patel, P.I. Terasaki, Significance of the positive crossmatch test in kidney transplantation, Available from: N. Engl. J. Med. 280 (14) (1969) 735–739.
[4] Organ procurement and transplantation network: policies, Rockville (MD), Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, Richmond (VA), United Network for Organ (Internet), (cited 2018 Dec 27). Available from: https://optn.transplant.hrsa.gov/media/1200/optn_policies.pdf.
[5] F.W. Selck, P. Deb, E.B. Grossman, Deceased organ donor characteristics and clinical interventions associated with organ yield, Available from: Am. J. Transplant 8 (5) (2008) 965–974.
[6] S. Rajbhandary, B.I. Whitaker, PG, The 2014-15 AABB Blood Collection and Utilization Survey Report, 2018.
[7] J.S. de la Cruz, M.B. Sally, J.R. Zatarain, M. Crutchfield, K. Ramsey, J. Nielsen, et al., The impact of blood transfusions in deceased organ donors on the outcomes of 1,884 renal grafts from United Network for Organ Sharing Region 5, Available from: J. Trauma Acute Care Surg. 79 (4 Suppl 2) (2015 Oct) S164–S170.
[8] C.M. Rosner, R. Singh, G. Panjrath, P. Shah, S.S. Desai, A.J. Rongione, Effect of donor blood transfusions on post heart transplant outcomes, J. Card. Fail. 21 (8) (2015) S41, https://doi.org/10.1016/j.cardfail.2015.06.146.
[9] P.T. Adams, R.D. Davenport, D.A. Reardon, M.S. Roth, Detection of circulating donor white blood cells in patients receiving multiple transfusions, Available from: Blood 80 (2) (1992) 551–555.
[10] T.H. Lee, T. Paglieroni, H. Ohro, et al., Longterm multi-lineage chimerism of donor leukocytes in transfused trauma patients (abstract), Blood 88 (Suppl 1) (1996).
[11] T.H. Lee, E. Donegan, S. Slichter, M.P. Busch, Transient increase in circulating donor leukocytes after allogeneic transfusions in immunocompetent recipients compatible with donor cell proliferation, Available from: Blood 85 (5) (1995) 1207–1214.
[12] D.W. Bianchi, G.K. Zickwolf, G.J. Weil, S. Sylvester, M.A. DeMaria, Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum, Available from: Proc. Natl. Acad. Sci. U.S.A. 93 (2) (1996) 705–708.
[13] R.E. Wenk, P.A. Chiafari, DNA typing of recipient blood after massive transfusion, Available from: Transfusion 37 (11–12) (1997) 1108–1110.
[14] M.E. Reid, M. Rios, V.I. Powell, D. Charles-Pierre, V. Malavade, DNA from blood samples can be used to genotype patients who have recently received a transfusion, Available from: Transfusion 40 (1) (2000) 48–53.
[15] M.K. Fung, A.F. Eder, S.L. Spitalnik, et al., Technical Manual, 19th ed., AABB, Bethesda (MD), 2017.
[16] E.G. Pivalizza, C.T. Stephens, S. Sridhar, S.D. Gumbert, S. Rossmann, M.F. Bertholf, et al., Whole blood for resuscitation in adult civilian trauma in 2017: a narrative review, Available from: Anesth. Analg. 127 (1) (2018) 157–162.
[17] Standards for Blood Banks and Transfusion, 31st ed., AABB, 2018.
[18] M.P.M. Adriaanse, A.C.E. Vreugdenhil, V. Vastmans, L. Groeneveld, S. Molenbroeck, D.A. Schott, et al., Human leukocyte antigen typing using buccal swabs as accurate and non-invasive substitute for venipuncture in children at risk for celiac disease, Available from: J. Gastroenterol. Hepatol. 31 (10) (2016) 1711–1716.