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blood advances

Variable CD34$^+$ recovery of cryopreserved allogeneic HPC products: transplant implications during the COVID-19 pandemic

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Key Points

- CD34$^+$ cell recovery and viability are variable after thawing cryopreserved hematopoietic progenitor cell products.
- Reduced CD34$^+$ cell recovery and viability are linked to longer storage time and higher white cell concentration before cryopreservation.

Donor registries and transplantation societies recommend cryopreservation of unrelated donor hemopoietic progenitor cell (HPC) products before the recipient commences conditioning therapy to mitigate the donor and travel risks associated with the COVID-19 pandemic. However, little is known regarding the postthaw quality of such allogeneic products or the effect of precryopreservation storage and processing on these characteristics. We investigated the postthaw CD34$^+$ cell recovery and viability of 305 allogeneic HPC products cryopreserved at 9 laboratories across Australia. Median postthaw CD34$^+$ cell recovery was 76% and ranged from 6% to 122%. Longer transit time before cryopreservation, white cell count (WCC) during storage, and complex product manipulation before cryopreservation were independently associated with inferior postthaw CD34$^+$ cell recovery. Longer precryopreservation transit time and WCC were also associated with inferior postthaw CD34$^+$ cell viability. We conclude that although postthaw CD34$^+$ cell recovery and viability of cryopreserved allogeneic HPC is generally acceptable, there is a significant risk of poor postthaw product quality, associated with prolonged storage time, higher WCC, and complex product manipulation precryopreservation. Awareness of expected postthaw recovery and practices that influence it will assist collection, processing, and transplant centers in optimizing outcomes for transplant recipients.

Introduction

Cryopreservation of hematopoietic progenitor cell (HPC) products is currently recommended by many professional bodies before the allogeneic stem cell transplant recipient commences conditioning, to reduce risks that have developed during the COVID-19 pandemic.[1-3] These risks include the possibility of donor or recipient infection and disrupted transport routes. Cryopreservation and infusion of thawed HPC products are well established for autologous HPCs and cord blood. In the setting of limited cell dose with umbilical cord blood transplantation, the postthaw CD34$^+$ cell dose has been demonstrated to influence recipient engraftment.[4] Furthermore, postthaw graft quality of cord blood units is influenced by factors such as volume of storage, processing technique, and processing facility accreditation.[5] In the adult donor setting, less is known regarding the effect of precryopreservation characteristics on postthaw graft quality of frozen allogeneic HPC products. In particular, the possibility of prolonged liquid storage time

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related to travel from collection center to cryopreservation laboratory is unique to this setting and does not usually occur before cryopreservation of autologous HPC or cord blood. Although prolonged liquid storage of HPC has not been shown to adversely affect engraftment time for products infused while "fresh," it is not known whether the same is true of cryopreserved products.[6] Therefore, we investigated factors influencing the postthaw quality of cryopreserved allogeneic HPCs, to inform practice for transplantation, collection, and processing centers during the current COVID-19 pandemic.

Methods

We collected data on allogeneic bone marrow (HPC-M) or G-CSF–mobilized peripheral blood (HPC-A) products cryopreserved at participating Australian cell processing laboratories between 2015 and 2019 inclusive. Where the product had been split, data were collected for the cryopreserved portion only. Transit time was defined as the time from completion of collection to commencement of cryopreservation. All products were cryopreserved in controlled rate freezers, and $CD34^+$ cell quantitation was performed with single-platform assays. However, composition of cryopreservation mixture and postthaw processing of samples before viable $CD34^+$ cell quantitation were not standardized between laboratories (supplemental Methods). Regression analysis was used as described. Medians were compared by using the Mann-Whitney *U* test, and the effect of categorical variables on binary outcomes was assessed by the χ^2 test. Analysis was performed with SPSS, version 24.0 (IBM, Chicago, IL).

Results

Nine centers contributed data on 305 products (n = 17 HPC-Ms and n = 288 HPC-As). Precryopreservation product characteristics are summarized in Table 1. Transit time was >36 hours for 110 products (36%) and >48 hours for 28 (9%). All HPC-M products underwent volume reduction by buffy coat enrichment before cryopreservation. The majority of HPC-As (n = 274) underwent no manipulation or only plasma reduction before cryopreservation, whereas the remainder were CD34 selected (n = 10) or washed (n = 4).

Postthaw $CD34^+$ recovery

Postthaw product quality as assessed on pilot samples is summarized in Table 1. The median $CD34^+$ cell recovery (precryopreservation/postthaw viable $CD34^+$ cell count) ranged widely from 6% to 122%. Forty-seven products (15%) had <50% and 4 products (1%) had <20% $CD34^+$ recovery after thawing, but here was no apparent enumeration, transportation, or processing mishap to explain such poor postthaw recovery. On univariate linear regression analysis, a weak correlation was observed between $CD34^+$ cell recovery and transit time, initial white cell count (WCC), product type, and precryopreservation manipulation, whereas no correlation was observed with initial product volume (Table 2). On multivariate analysis, however, only transit time, initial WCC, and manipulation status were associated with $CD34^+$ cell recovery with statistical significance (Table 2).

Products that had been collected >36 hours (estimated threshold for overseas products) before cryopreservation had a lower median $CD34^+$ cell recovery (72% vs 77%; $P = .061$) and a higher likelihood of poor (<50%) $CD34^+$ cell recovery (21% vs 12%; $P = .049$). Poor (<50%) recovery was even more common for products collected >48 hours before cryopreservation (43% vs 13%; $P < .001$). Furthermore, of 11 products with travel time >36 hours that then underwent manipulation, 7 (60%) had $CD34^+$ cell recovery <50%.

Similarly, products with an initial WCC >300 × 10^9/L, the recommended maximum for transportation of HPC-As,[7] were more likely to have <50% $CD34^+$ cell recovery (25% vs 13%; $P = .021$). Conversely, of 75 products with WCC in the lowest quartile (<180 × 10^9/L), 56% had excellent (>80%) $CD34^+$ cell recovery, compared with only 34% of samples with WCC ≥180 × 10^9/L ($P < .001$).

Table 1. Pre- and postcryopreservation characteristics of products

	n	Median	Range
Precryopreservation			
Transit time, h	305	26	0.25-85
Product volume, mL			
HPC-A	288	263	42-751
HPC-M	17	892	92-2040
Product WCC, ×10^9/L	305	222	13-901
$CD34^+$ cell viability, %	96	99	73-100
Postcryopreservation			
$CD34^+$ cell recovery, %			
Total	305	76	6-122
HPC-A	288	75	6-122
HPC-M	17	87	36-110
Infused as HPCs*	80	82	34-119
Infused as DLI	17	68	6-96
Stored	187	72	7-122
Disposed	7	85	31-99
$CD34^+$ cell viability, %			
Total	139	87	6-99
HPC-A	135	91	6-99
HPC-M	4	84	17-91
Infused as HPCs*	61	95	47-99
Infused as DLI	6	84	6-94
Stored	59	89	13-98
Disposed	7	91	42-97
$CD34^+$ cells, ×10^6/kg per recipient			
Total	297	3.1	0.1-20
HPC-A	280	3.1	0.1-18.7
HPC-M	17	2.6	0.2-20
Infused as HPCs*	80	5	0.94-15.7
Infused as donor leukocytes	16	5.3	1.9-16.8
Stored	184	3.4	0.6-19.5
Disposed	7	3.4	1.2-7.2

*n = 14 products combined at infusion and excluded from this summary.

Postthaw $CD34^+$ viability

Postthaw $CD34^+$ cell viability (percentage of viable events within the $CD34^+$ cell gate) was available for 139 samples from 6 centers.

Table 2. Univariate and multivariate linear regression analysis of CD34$^+$ cell recovery

		Univariate analysis			Multivariate analysis*		
	Reference	r^2	Regression coefficient (95% CI)	P	r^2	Regression coefficient (95% CI)	P
Transit time	NA	0.038	−0.25† (−0.39 to −0.11)	.001	0.121	−0.26 (−0.41 to −0.12)	>.001
Initial WCC	NA	0.032	−0.03 (−0.05 to −0.01)	.002	0.121	−0.04 (−0.06 to −0.02)	>.001
Initial volume	NA	0	0 (−0.01 to 0.012)	.664	—	N/A	—
Product type	HPC-A	0.020	13.36 (2.85 to 23.89)	.013	—	N/A	—
Manipulation	Plasma reduction/none	0.050	−23.07 (−34.42 to −11.72)	<.001	0.121	−18.5 (−29.80 to −7.20)	.001

CI, confidence interval.
*Variables included in original model: transit time, initial WCC, initial volume, product type, manipulation, processing center.
†The predicted CD34$^+$ recovery decreased by 0.25% for each additional hour of transit time.

Both transit time and WCC were associated with postthaw CD34$^+$ cell viability (Figure 1).

Infused products

As of 20 March 2020, 111 products (36%) had been thawed and infused (Table 1). Of 80 products infused alone as HPCs for transplantation, neutrophil recovery occurred in 75 (94%) at a median of 19 days after infusion (range, 12-33 days). All 5 infused products with <50% CD34$^+$ cell recovery achieved neutrophil recovery (median, 16 days; range, 12-33 days). At the time of this writing, only 1 of 9 products with <20% recovery had been infused, as donor lymphocytes.

Discussion

In this analysis of 305 samples from cryopreserved allogeneic HPC products, we observed a median CD34$^+$ cell recovery of 74%, with poorer recovery associated with longer duration of liquid storage, higher WCC during storage, and complex cell processing (eg, CD34$^+$ cell selection) before cryopreservation. Although the magnitude of each of these effects was small in our analysis, they were incremental, such that the likelihood of poor CD34$^+$ cell recovery in a manipulated product with long liquid storage time was relatively high.

Our findings differ from those reported in 2 previous studies, in which allogeneic products cryopreserved at single centers had mean CD34$^+$ cell recoveries of >90%.[8,9] The smaller sample sizes in the earlier studies and different HPC processing and CD34$^+$ cell quantitation practices may account for this discrepancy.

CD34$^+$ cell viability showed a greater correlation with transit time than CD34$^+$ cell recovery and also was associated with WCC of the product during transit. Further study of the relationship of storage conditions and duration of transport with postthaw CD34$^+$ cell viability is warranted.

Although most products infused as HPCs achieved neutrophil recovery, the number of infused products was too small to allow for meaningful investigation of the effect of pre- or postthaw parameters on clinical efficacy. Registry studies suggest that cryopreservation

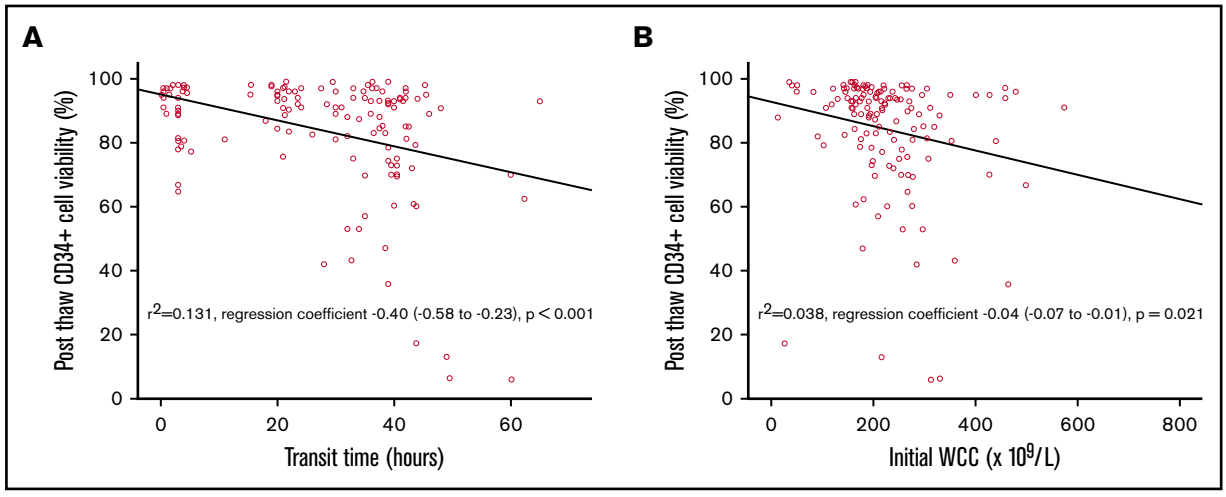

Figure 1. Postthaw CD34$^+$ cell viability. Viability data is plotted against time from collection to cryopreservation (A) and initial WCC (B).

itself may not alter clinical outcomes, with the exception of transplantation for severe aplastic anemia.[10-12] However, the effect of precryopreservation conditions was not examined in these studies.

Our observations support the conclusion that transplant centers should request a higher $CD34^+$ cell dose from donors whose products are to be cryopreserved and suggest that optimization of precryopreservation factors, such as transit time and WCC, during storage may improve postthaw yield. Arrangements for cryopreservation of HPC products at collection centers or regional hubs before long-distance shipment should result in fewer products with long (eg, >36 hour) liquid storage times before cryopreservation. However, some registries continue to recommend cryopreservation of unrelated donor products at transplant centers, a process that may increase risks of poor postthaw graft quality caused by the increased transit time.[2] Our data also support diluting products to a maximum WCC of 300×10^9/L before transport and cryopreservation. Ultimately, however, these factors accounted for only a small proportion of the overall variability in $CD34^+$ cell recovery ($r^2 = 0.121$; Table 2). Thus, as recently recommended by the World Marrow Donor Association,[13] transplant centers must be cognizant of the risk of unexpectedly poor postthaw $CD34^+$ cell recovery and take steps to mitigate it, including examination of thawed pilot vials before the commencement of conditioning therapy.

Authorship

Contribution: D.P. designed the research, analyzed the data, and wrote the manuscript; N.H. designed the research and wrote the manuscript; and V.A., P.C., D.T., E.O., A.B., K.K., S.L., S.T., C.H., D.J.C., G.A.K., A.-M.W., L.B., M.G., and D.J.G. wrote the manuscript.

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References

1. European Society for Blood and Marrow Transplantation. Coronavirus disease COVID-19: EBMT recommendations update April 21, 2020 Available at: https://www.ebmt.org/sites/default/files/2020-04/EBMT_COVID-19-guidelines_v.7.1%282020-04-21%29.pdf. Accessed 29 April 2020.
2. National Marrow Donor Program: BeTheMatch. Donor Product Cryopreservation: updated March 23, 2020. Available at: https://www.ebmt.org/sites/default/files/2020-04/EBMT_COVID-19-guidelines_v.7.1%282020-04-21%29.pdf. Accessed 29 April 2020.
3. Hamad N. Bone Marrow Transplant Society of Australia and New Zealand COVID19 Consensus Position Statement 15th April 2020. Available at: https://www.bmtsanz.org.au/bone-marrow-transplant-society-of-australia-and-new-zealand-covid19-consensus-position-statement-15th-april-2020/. Accessed 29 April 2020.
4. Avery S, Shi W, Lubin M, et al. Influence of infused cell dose and HLA match on engraftment after double-unit cord blood allografts. *Blood*. 2011;117(12):3277-3285.
5. Purtill D, Smith K, Devlin S, et al. Dominant unit $CD34^+$ cell dose predicts engraftment after double-unit cord blood transplantation and is influenced by bank practice. *Blood*. 2014;124(19):2905-2912.
6. Patton WN, Nivison-Smith I, Bardy P, et al. Graft Transit Time Has No Effect on Outcome of Unrelated Donor Hematopoietic Cell Transplants Performed in Australia and New Zealand: A Study from the Australasian Bone Marrow Transplant Recipient Registry. *Biol Blood Marrow Transplant*. 2017;23(1):147-152.
7. Australian Bone Marrow Donor Registry. ABMDR-STD-NCU-10 Transporting Fresh Haemopoietic Progenitor Cells. 2019. https://www.abmdr.org.au. Accessed 26 May 2020.
8. Berens C, Heine A, Müller J, et al. Variable resistance to freezing and thawing of CD34-positive stem cells and lymphocyte subpopulations in leukapheresis products. *Cytotherapy*. 2016;18(10):1325-1331.
9. Fisher V, Khuu H, David-Ocampo V, et al. Analysis of the recovery of cryopreserved and thawed CD34+ and CD3+ cells collected for hematopoietic transplantation. *Transfusion*. 2014;54(4):1088-1092.
10. Eapen M, Zhang MJ, Tang XY, et al. Hematopoietic Cell Transplantation with Cryopreserved Grafts for Severe Aplastic Anemia. *Biol Blood Marrow Transplant*. 2020;26(7):e161-e166.
11. Medd P, Nagra S, Hollyman D, Craddock C, Malladi R. Cryopreservation of allogeneic PBSC from related and unrelated donors is associated with delayed platelet engraftment but has no impact on survival. *Bone Marrow Transplant*. 2013;48(2):243-248.
12. Hamadani M, Zhang MJ, Tang XY, et al. Graft cryopreservation does not impact overall survival after allogeneic hematopoietic cell transplantation using post-transplantation cyclophosphamide for graft-versus-host disease prophylaxis. *Biol Blood Marrow Transplant*. 2020;26(7):1312-1317.
13. World Marrow Donor Association. SPEAR Rapid Alert: May 2020. Available at: https://share.wmda.info/display/LP/Welcome?preview=%2F20748021%2F344873012%2F20200501-SEAR-Rapid+Alert.pdf#/. Accessed 29 June 2020.