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Impact of Delayed Infusion Time in Umbilical Cord Blood Transplantation

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In umbilical cord blood (UCB) transplantation, UCB units are typically thawed, washed, and infused into the patient as rapidly as possible. In some instances there is a delay in the time from the unit thaw and wash procedure to infusion into the patient. Therefore, we examined the effect of thaw duration time on engraftment outcomes in 567 patients undergoing UCB transplantation. With a range of 32 to 523 minutes, a prolonged thaw duration had no obvious effect on the incidence of neutrophil engraftment or time to recovery. This was true for recipients of single UCB transplantation (incidence: 97% versus 93%, $P = .13$; time to neutrophil recovery: 21 days versus 21 days, $P = .32$; and platelet recovery: 79% versus 78%, $P = .48$), and similar results were observed in double UCB transplantation (time to neutrophil engraftment: 20 days versus 19 days, $P = .71$). However, there was a trend toward better platelet recovery in recipients of double UCB transplants with prolonged thaw duration (HR, 1.28; $P = .06$). In conclusion, this study demonstrates prolonged thaw duration has no detrimental effect on engraftment after single or double UCB transplantation.

INTRODUCTION

By convention, umbilical cord blood (UCB) units are typically infused as soon as possible after thawing or thawing and washing because of concern for a loss of viability. However, possible reasons for delaying the infusion after thawing and washing include instances in which the segment used for HLA typing is not attached to the UCB unit. In such cases, to confirm the identity of the UCB unit, we have performed "confirmatory HLA typing" on the thawed UCB mononuclear cells using monoclonal antibodies directed against MHC I [1]. Although this practice provides reassurance of unit identity, it also results in a prolonged thaw duration, because rapid serologic HLA class I typing takes approximately 3 hours. Other situations that may delay cord infusion once thawed include issues with transport from the clinical laboratory to the patient's bedside, patient instability, or human error.

We hypothesized that these situations all have the potential to negatively impact cell viability after thaw and, hence, transplantation outcomes. Considering the association between nucleated cell (or $CD34^+$ cell) dose and the probability and/or timing of neutrophil and platelet engraftment [2-4], these concerns are relevant. Here we set out to understand whether the time from UCB thawing and washing to infusion into the patient, henceforth referred to as "thaw duration," impacts clinical outcomes.

METHODS

We conducted a retrospective review of 869 patients who underwent either single or double UCB (dUCB) transplantation at the University of Minnesota between 1992 and 2013. The goal of the analysis was to determine the effect of thaw duration on unit engraftment and other clinical outcomes. In recipients of dUCB transplantation, the focus was unit dominance and its rate of recovery. Therefore, we excluded those with persistent mixed donor chimerism (n = 29), absence of documentation of unit predominance (ie, due to mortality or lack of testing [n = 113]), or primary graft failure occurred (n = 69). Another 91 patients were excluded because either the time the UCB unit was removed from cryopreservation or the time the UCB unit was infused into the patient could not be confirmed. Patients were treated on protocols approved by the University of Minnesota institutional review board, and written consent was obtained from all patients, their parents, or guardians in accordance with the Declaration of Helsinki.

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UCB units were processed as per our previous report [5], including undergoing a thaw and wash as per the method of Rubinstein et al. [6]. In cases where the UCB bank did not perform confirmatory HLA typing on an integrally attached segment, post-thaw testing of HLA class I was performed on a sample from the UCB unit itself on the day of thaw.

This analysis included determination of thaw duration, defined as the time the UCB unit was removed from local frozen storage (ie, the start of unit thawing) to the time infusion was initiated. For both single and dUCB transplants, a prolonged thaw duration was defined as being greater than the median time from thaw to infusion for each data set (180 minutes for single UCB transplants and 200 minutes for dUCB transplants). As above, in dUCB transplants the thaw duration was analyzed for the engrafted UCB unit. A comparison of thaw duration was also performed for both units, with the engrafted UCB unit compared with the nonengrafted UCB unit.

Baseline patient and transplant characteristics and transplant outcomes were prospectively collected and recorded in the University of Minnesota Blood and Marrow Transplant database. Primary endpoints for this study were achieving neutrophil engraftment by day +42 and platelet recovery by 1 year post-transplant. Neutrophil engraftment was defined as having an absolute neutrophil count of 500×10^6/L at day +42 posttransplant. Platelet recovery was defined as a platelet count $> 50 \times 10^9$/L by day +180 unsupported by transfusion for at least 7 days. Secondary endpoints were 1 year transplant-related mortality (TRM) and overall survival (OS). Other transplant outcomes were defined as previously described [7,8].

Statistical comparison of categorical variable was performed using the chi-square test, and the Kruskal-Wallis (Wilcoxon) rank-sum test was used for comparison of continuous variables. The Kaplan-Meier method [9] was used to estimate the probabilities of OS, and the log-rank test was used for univariate comparisons. A cumulative incidence estimator was used to calculate the probabilities of neutrophil engraftment and platelet engraftment reflecting the nonevent deaths as a competing risk [10]. The cumulative incidence of TRM was also calculated reflecting relapse as a competing risk. Fine and Gray regression analyses were used to compare the differences between cumulative incidence curves for the endpoints of neutrophil engraftment, platelet engraftment, and TRM [11].

Patient- and transplant-related variables (UCB unit age, year of UCB unit collection, conditioning intensity, gender, age at transplant, total nucleated cell (TNC) dose at infusion, $CD34^+$ cell dose at infusion, cell viability post-thaw, UCB colony-forming units, blood group status, HLA match, recipient cytomegalovirus status, year of transplant, bank UCB unit was sourced from, and presence of hypertension on UCB infusion) were all tested in the univariate analysis. Those with a $P < .2$ or with clinical interest were included in the multivariate analysis. Prognostic factor models for all endpoints were created using a backward selection method considering a $P < .2$. The cut-off significance level for all P values was .05. Statistical analysis was performed using SAS version 9.3 (SAS Institute, Cary, NC).

RESULTS

Single UCB Transplants

In 208 single UCB transplants, the median thaw duration was 179 minutes (range, 101 to 506; Table 1). There was no significant difference in thaw duration between patients who experienced graft failure and those who did not (188 versus 177 minutes, $P = .34$). Having a prolonged thaw duration had no impact on the incidence of neutrophil engraftment at day +42 on univariate analysis (97% versus 93% $P = .13$). Also, a prolonged thaw duration had no impact on the time to neutrophil engraftment (21 days versus 21 days, $P = .32$, Figure 1A). Looking at patients receiving UCB units with a TNC dose in the lowest 25% of the cohort, prolonged thaw duration had no impact on the incidence of neutrophil engraftment (100% versus 100%, $P = .9$) or the time to neutrophil engraftment (24 days versus 24 days, $P = 1.0$). Additionally, a prolonged thaw duration also had no significant impact on the incidence of platelet recovery at 1 year (79% versus 78%, $P = .48$; Figure 2A). This remained not significant on multivariate analysis ($P = .53$). Prolonged thaw duration also had no significant impact on TRM after single UCB transplant (17% versus 21%, $P = .55$; Figure 3A) or acute graft-versus-host disease (GVHD), disease-free survival, relapse, or OS in single UCB transplantation (data not shown).

Table 1
Thaw Duration for Single and dUCB Units

	n
Single UCB patients	208
Unit thaw duration (min)	
101-160	47
160-179	56
179-204	51
204-506	53
Median thaw duration	179 min
dUCB transplants	359
Winning unit thaw duration (min)	
32-178	88
178-200	93
200-240	89
240-523	91
Median thaw duration	200 min

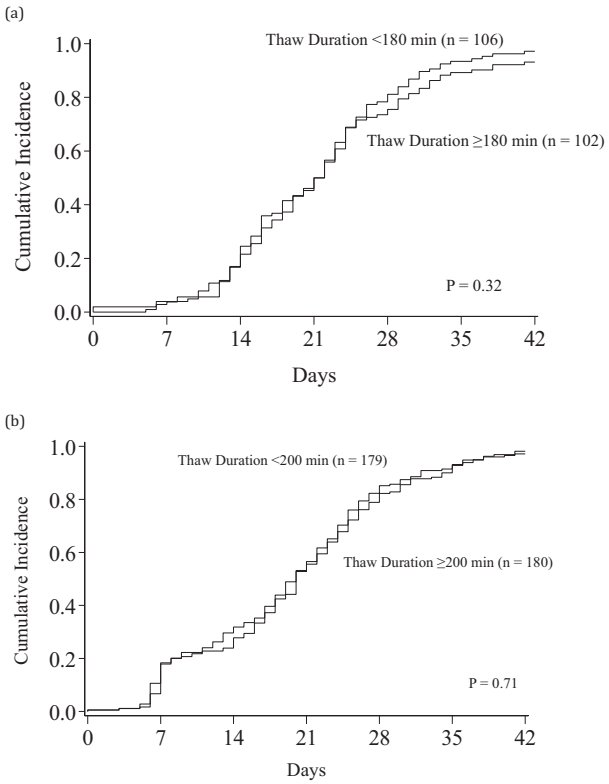

Figure 1. Time to neutrophil engraftment for (A) single and (B) dUCB transplants. Note that patients with graft failure were excluded from the dUCB analysis.

dUCB Transplants

In 359 dUCB transplants, the median thaw duration for the engrafted UCB unit was 200 minutes (range, 32 to 523; Table 1). Thaw duration had no effect on unit predominance ($P = .54$). Looking at median thaw duration for the 2 units, there was no significant difference in patients who experienced graft failure and those who did not (206 versus 205 minutes, $P = .98$).

There was no significant impact of prolonged thaw duration on time to neutrophil engraftment (20 days versus 19 days, $P = .71$; Figure 1B). Segregating patients who had a winning UCB unit in the lowest TNC quartile of the cohort showed that prolonged thaw duration also had no impact on the incidence of neutrophil engraftment (93% versus 98%, $P = .46$) or the time to neutrophil engraftment (22 days versus