Review Article

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Quick Response Code:

Website: www.ajts.org

DOI: 10.4103/ajts.AJTS_124_18

Umbilical cord blood quality and quantity: Collection up to transplantation

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Abstract:

Umbilical cord blood (UCB) is an attractive source of hematopoietic stem cells for transplantation in some blood disorders. One of the major factors that influence on transplantation fate is cord blood (CB) cell count, in addition to human leukocyte antigen similarity and CD34+ cell number. Here, we review the factors that could effect on quality and quantity of CBUs. Relevant English-language literatures were searched and retrieved from PubMed using the terms: CB, quality, collection, and transplantation. The numbers of total nucleated cells (TNCs) and CD34+ cells are good indicators of CB quality because they have been associated with engraftment; thereby, whatever the TNCs in a CB unit (CBU) are higher, more likely they led to successful engraftment. Many factors influence the quantity and quality of UCB units that collect after delivery. Some parameters are not in our hands, such as maternal and infant factors, and hence, we cannot change these. However, some other factors are in our authority, such as mode of collection, type and amount of anticoagulant, and time and temperature during collection to postthaw CBUs and freeze-and-thaw procedures. By optimizing the CB collection, we can improve the quantity and quality of UCB for storage and increase the likelihood of its use for transplantation.

Keywords:

Cord blood bank, cord blood stem cell transplantation, cryopreservation, hematopoietic stem cell, umbilical cord blood

Introduction

Hematopoietic stem cell transplantation (HSCT) is one of the main strategies to treat some disorders such as malignant and nonmalignant blood diseases and also immunodeficiency disorders.[1] Bone marrow and peripheral blood have been two main sources for transplantation; however, due to the absence of fully human leukocyte antigen (HLA)-matched donors and the high risk of graft-versus-host disease (GVHD), only a few patients can benefit from these sources.[2-4] Hence, the first time in 1988, cord blood (CB) was used as an alternative source of HSCT in a boy with Fanconi anemia, which had several benefits such as ability to tolerate HLA-mismatched transplants, lower risk of acute and chronic GVHD, and lower risk of blood-transmitted infectious diseases.[5] Despite all the benefits, the uses of this source are limited because of the low numbers of cells in one unit.[6,7] As engraftment is closely correlated with the number of infused cells, attempts should be made to process and cryopreserve only those units that have optimum total nucleated cell (TNC) and CD34+ cell numbers, as processing and storage of umbilical CB (UCB) are a time-consuming and costly affair.[8,9] Strategic procedures for collection until transplantation of CB [Figure 1] not only avoid wasting costs and save valuable storage spaces

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Submission: 08-10-2018
Accepted: 16-06-2019
Published: 03-12-2019

How to cite this article: Mousavi SH, Zarrabi M, Abroun S, Ahmadipanah M, Abbaspanah B. Umbilical cord blood quality and quantity: Collection up to transplantation. Asian J Transfus Sci 2019;13:79-89.

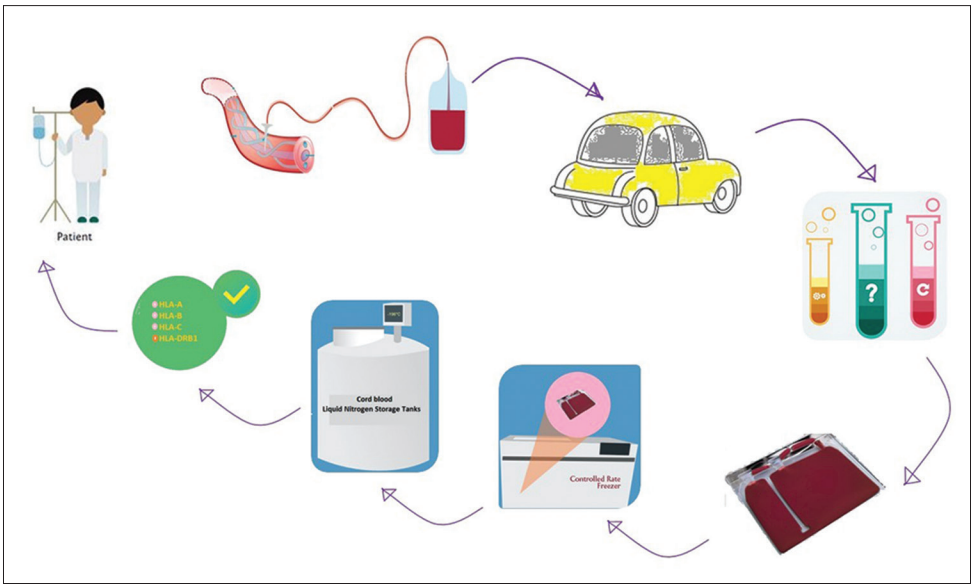

Figure 1: Procedure for collection of umbilical cord blood until transplantation

for good-quality units but also enable better potential for efficacious cognitive behavioral therapy (CBT) in recipients.[10]

While various parameters such as mode of collection, storage time and temperature, transport, processing, and freeze-and-thaw techniques have been reported to affect the quality and quantity of CBUs, we aimed to investigate these factors in this study.

Phlebotomy Training

The quality of CB is primarily determined during collection, and the recent consensus of the American College of Obstetricians and Gynecologists to recommend delayed cord clamping for term and preterm infants could reduce the proportion of units with an acceptable TNC.[11,12] Time of clamping must be performed using extreme caution because the alteration in placental hemodynamics could cause hypervolemia in newborn, and also, this time is at the discretion of the obstetric team.[11] Furthermore, the interval between placenta delivery and CB collection has significantly influenced on CBU's volume.[13,14] Askari *et al*. showed that, collection within not greater than 5 minutes of placental delivery produced higher volume and TNC count. Increased TNC count was also seen in Caucasian women, prim gravidae, female newborns, and collection duration of more than 5 minutes. A time between delivery of newborn and placenta of not greater than 10 minutes predicted better volume and CD34+ count.[15] Ballin *et al*. showed that CBUs collected during 10 min after cord clamping had lower thrombin activation within time.[16]

In addition, CBUs collected using an open system were associated with a significant risk of bacterial and maternal cell contamination as compared to closed collection system.[17,18] Clark *et al*. in 2012 indicated that CB collected by CB bank staff resulted in a significantly lower contamination rate than when collected by an obstetric staff.[19] Elchalal *et al*. showed that the CB collection by syringe causes higher number of TNC and also volume rather than collecting by bag.[19] However, there was no statistically difference in the percentage of CD34+ cells. Even though the open collection system is technically easier, it led to a greater risk of microbial contamination.[20,21]

Using active syringe/flush/syringe method, for collecting UCB before placental delivery, results in greater volumes and lower discard rate.[22] According to our experience, using multiple puncturing causes higher volumes of UCBs, but these techniques also increase the risk of contamination. Thus, the collection of UCB in within 10 min after cord clamping in the closed system and without manipulation has higher volume, higher TNC, lower coagulation activity, and less contamination [Figure 2].

Mode of Collection

There are two main methods for CB collecting: *in utero and ex utero*. *In utero* CB collection is generally performed by cord blood bank (CBB) expert or obstetrician or midwife after the newborn is delivered and assessed, the cord is clamped and cut, and the collection is started immediately. This procedure does not disturb the natural course of birth or the postpartum period and has lower macroscopic clots than *ex utero* CBUs collected,[23-26,27] but this can interfere to normal delivery process. With *ex utero* collections, the placenta can be removed from the

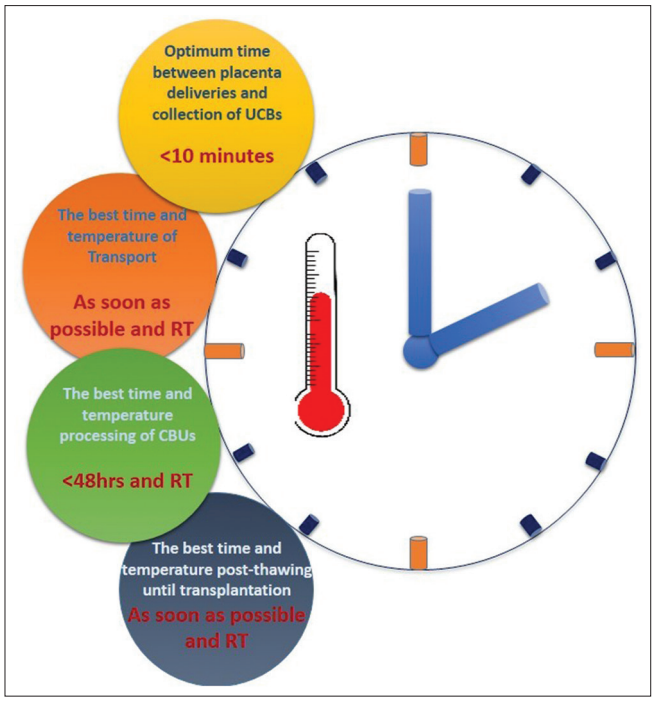

Figure 2: The best time and temperature in different steps of umbilical cord blood collection until transplantation

delivery suite and transported to a nearby clean room for the collection. The cord should be clamped within 3–5 s of the infant's delivery, and the placenta was taken immediately by bank staff to a suitable site where it may be suspended in a device to allow collection of blood by gravity.[28] Although the *ex utero* collection is easier, it may increase bacterial contamination.[26] Fact-Netcord standard determines that CB collections shall only occur in uncomplicated deliveries and CB units (CBUs) collected *in utero* shall only be obtained from infant donors after a minimum of 34 weeks' gestation.[27]

Several studies showed that *in utero* CB collection had a greater volume,[26,29-36] TNC,[25,26,29-34,36] CD34+cell count,[26,34,37] colony-forming units (CFUs),[25,26,34-36] and viability of nucleoid cells[32] and higher monocytes and granulocytes[25] than *ex utero* CB collection.[28] Wong *et al.* hypothesized that a higher incidence of microscopic clots with *ex utero* collections consequently led to lower nucleated cell counts and CFUs.[25] In addition, in some studies, *in utero* CBUs collected in the vaginal delivery compared to *ex utero* CBUs collected in cesarean section had significantly higher TNC,[24,26] higher CD34+ %, better TNC recovery, and higher volume.[26] Some researchers demonstrated that the mode of delivery has no impact on volume, TNC, CD34+,[25,33] and CFU-granulocyte, macrophage (GM)[34,38] and there was no significant difference between mode of collection and delivery in CB collection.[26,31,39,40] The presence of hemorrhage in the delivered placenta and clots forming in the fetal placental vessels could explain the lower levels of hematopoietic progenitors and also TNC in UCB collected in *ex utero* technique. Furthermore, the optimal time of cord clamping is related to the higher volume, TNC, and CD34+ cells in *in utero* mode. This was probably a result of the squeezing action exerted from the uterine contractions, which favored CB collections.[17]

Thus, *in utero* collection mode, with a single puncture of the umbilical vein, performed by the closed system is the greatest method for collecting good-quality CBUs.

Type and Amount of Anticoagulant

Virtually, all UCB is collected in sterile bags with usually 16-gauge needles and with anticoagulant-based citrate, such as citrate phosphate dextrose (CPD) or CPD-adenine 1 (CPD-A1). However, some CBBs suggest the use of heparin instead of CPD or CPD-A1 anticoagulants. Amount and type of anticoagulant are variable.[13,26,27,31,32,38,40-60] A study showed that anticoagulant concentration had no impact on cell viability although cell viability reduces gradually after storage for 25–48 h and higher.[61] Pope *et al*. suggested that the ratio of anticoagulant could cause decrease cell viability in CBUs with the volume lower than 60 ml. Further, it was suggested that lower WBC viability in low volume CBUs could be due to a higher ratio of anticoagulant.[57]

Kraus *et al*. compared the effect of CDP and dry heparin on CBU's parameters. They found significantly higher preprocessed TNC count, postprocessing TNC count, % CD34+cell, and number of CD34+cells in the CPD than heparinized units. Interestingly, viability was significantly higher in the postprocessed heparin units than CPD CBUs. The viability of the CD34+ cells decreased in CBUs that collected in heparin than CDP anticoagulant.[62] They suggested that CPD has a dual role as an anticoagulant and a preservative as it contains dextrose which provides a substrate for glycolysis and preserves the metabolism in the cells. Heparin, which does not specifications of CPD and because it is broken down over long periods of time, only could be useful for blood that is to be transfused within 12 h of the collection. In addition, the use of dry heparin may adversely affect the osmolality of the CBU.[62] Harris *et al*. showed that CBUs collected in CPD had a significant decline in TNC yield at 24 and 48 h. There was a significant but lower decline in TNC yield in the CBUs collected in heparin between 0 and 24 h and no significant difference thereafter. The results of this study indicated that heparin was more biocompatible than CPD as measured by cell viability endpoints and CPD caused significant acidosis of the blood and prolonged exposure to an acidic environment could be affected on CBUs. Thereby, it is important to ensure that CBUs

quality will be maintained during transport regardless of the size of the collection volume.[63]

CB-collecting bags are single- or double-closed sterile with usually 16-gauge needles. Some studies suggested that using a syringe is good for CB collection.[64] According to the AABB Technical Manual[28] and our experience, using 250 ml bag, 35 ml of CPD-A1 adequately prevents clotting. The use of heparin requires more studies.

Time and Temperature of Collection and Transport

Temperature of transport and storage before processing had minor but sometimes significant effects on cell viability. Different studies suggested various storing temperature ranges such as 4°C,[26,39,47] 4°C–7°C,[58] 4°C–10°C,[52,54] 4°C–24°C,[33] and room temperature (RT).[32,44,65] Furthermore, there are controversies about the shipping temperature of CBUs and some believe that CBUs must be transported at 15°C–25°C,[60,66] and some other recommend 20°C ± 2°C to processing facility within 48 h after collection[58,67] and RT.[32,44,59,68]

For newly collected UCB, the transit temperature requirements are not well defined, leaving each facility to determine transport temperature criteria and acceptable limits. Thus, fresh UCB may be transported at RT, on ice, or with insulated precooled stabilizing packs.[28] Wada *et al*. served a 1% decrease in viability for every 4-h increase in transport time for newly collected UCB units that were shipped at ambient temperature.[69] Storage at either RT or 1°C–4°C does not seem to make a large different, but lower temperature may minimize growth of any contaminating bacteria. Hence, according to this review and our experience, the best temperature for collection and transport is RT and UCB should routinely transport to processing laboratories within 24 h [Figure 2].

Time and Temperature before Processing

Several studies informed that CBUs could be stored in RT[44,59,68,70] or 4°C[17,26,27,31,33,39,46,53,71-73] or 15°C–25°C[57] before processing. Numerous studies showed that processing should be performed within 48 h[26,45,47,60,66,74] [Figure 2].

A study reported that TNC at RT was significantly higher than 4°C regardless of the time interval.[75] In addition, TNCs recovery after 48 h[76,77] and mononuclear cell counts (MNCs) recovery after 24 h at 25°C were significantly higher than at 4°C.[76] Some studies demonstrated that storage at RT for up to 24 h led to significant losses of nucleated cells;[78] the recovery rate of viable MNCs, hematopoietic progenitor cells (HPCs), and CFU-forming potency[79] and the recovery of MNCs at 4°C were significantly higher than RT in 24 and 72 h.[73] Pamphilon *et al*. showed that the samples that stored at 4°C had higher average TNC, CD45 + viability, and MNC count rather than RT.[80] Louis *et al*. indicated that storage of cord blood units at room temperature before processing and cryopreservation altered in vivo hematopoietic reconstitution in mice, although in vitro hematopoietic colony-forming unit potential was changed.[56]

Researchers of another study showed that CD34+cells were significantly higher at RT than 4°C regardless of the time interval.[75,77] A reason for the increase of CD34+cells at RT could be producing antiapoptotic factors by these cells in combination with cytokine-promoting cell maintenance.[77]

Pereira-Cunha *et al*. performed an analysis on manipulated and unmanipulated UCB in 24, 48, and 96 h before freezing, at RT (20°C–22°C). In this study, all cell subsets remained viable until 96 h after collection. CD34+cells and T-lymphocytes increased, probably due to the loss of other subsets. CFU growth during the period analyzed and confirmed stem cell functionality, despite the decrease at 96 h. Results demonstrated that UCB units could probably be processed up to 96 h after collection.[81]

Multiple studies have investigated some parameters that have influence on PT viability, such as storage temperature between collection and processing, temperature transient of cryopreserved cells, thawing techniques, and time of thawing to infusion.[48,60,80,82-84] The US Food and Drug Administration recommended that prefreezing-nucleated viability is a quality control parameter and must be higher than 85%.[85]

Isoyama *et al*. revealed that TNCs viability maintained significantly at RT for 24 h or longer.[86]

Recovery rates after 72 h at RT were declined, and all variants after 24 h at 4°C had >80% recovery.[79]

Fry *et al*. described that samples maintained at refrigerated temperatures resulted in higher recoveries than those at room temperature in all variables assessed. Specifically, when assessing for CFU yields after thawing, the impact of time on BM resulted in a significant loss as soon as 24 hours. This decrease was also observed for PBSCs and CB but at 48 hours of fresh storage.[48]

Louis *et al*. demonstrated that PT results had similar *in vitro* characteristics between immediate processing and 4°C storage for cell recovery and viability and both significantly higher than RT storage. They showed that storage of CBUs at RT before processing and

cryopreservation profoundly altered *in vivo* hematopoietic reconstitution in mice, although *in vitro* hematopoietic CFU potential was unaltered.[56] In 2014, Guttridge *et al*. indicated that refrigerated storage at 4°C can prolong TNCs viability presumably by slowing metabolism and preserve nutrient supply, particularly in mature blood cells.[87]

Many factors impact on time from collection to processing, including the distance between collection sites to the processing laboratory, techniques in processing laboratory, and availability of processing staff.[88] The viability of CBUs was significantly affected by the time between collection and processing. Several studies showed that viability decreased significantly by the time.[32,48,50,59,61,79,89] Furthermore, TNC count, CD45+ viability, and MNC count change over time and these changes differed for each storage temperature.[80]

Page *et al*. stated that mean overall viability, CFUs, CD34+, and the post-TNC count processed within 24 h were significantly higher than CBUs processed after 24 h.[88] Another study showed that delay over 36 h to cryopreservation was in association with a significantly lower viability of the units, whereas there was no significant effect on CD34+cell count.[89]

The results of the COBLT study showed that viability, CD34+ cell count, and post-TNC remain stable for more than 48 h at RT, and also, there was no negatively effect on recovery of TNC and CD34+cell concentration after processing.[90]

Time has a continuous impact on CFUs in fresh and postthawed samples that stored at 4°C. A significant reduction was observed after 48 h both in fresh and postthawed samples.[48]

There was no correlation between the time from collection to processing and CD34+ cell count and TNC.[59] The recovery rate of TNC was declined at 48 h at RT and at 24 h at 4°C.[75]

However, evidence showed that viability of CD34+ cell count and CFU-GM decrease over time, regardless of the temperature.[80]

Pope *et al*. demonstrated that time from collection to freezing in more than 24 h had a significant influence on the viability of the final products, especially in CBUs with volume lower than 100 ml and in more than 36 h, viability decline in CBUs with volumes higher than 100 ml.[57] Some studies mentioned the effect of volume, TNC, and CD34+ cell count on CBUs viability. They showed that volume under 80 ml, TNC $<50 \times 10^7$, and CD count $<0.5 \times 10^6$ had lower cell viability than other units.[59]

A study showed that stored CBUs at 4°C significantly increase the percentage of cell apoptosis and have a lower expression of CXCR-4 in CD34+ cells compared to samples stored at RT for 24 and 48 h.[77]

Before cryopreservation maybe, decrease in viability is not apparent, probably due to sublethal damage from prolonged preprocessing storage that is only apparent after cryopreservation. This is maybe related to the nutrient depletion and metabolic variations during storage dropping the potential of cells to tolerate cryopreservation. This unfavorable impact may be made worse in lower-volume donations with higher anticoagulant concentrations, perhaps through depletion of calcium and magnesium ions affecting metabolic processes.[87]

Probably, this contradiction is because of difference in mode of collection, the experience of staff, the ratio of anticoagulant to blood, transportation, processing technique, freeze in bag or tube, lack of attention to volume, different analysis methods, and some other factors.

According to our experience, storing CBUs before processing at RT has higher TNC recovery, higher viability, higher CD34 count, and greater CFUs rather than 4°C. Moreover, the quality of CBUs is better in samples that proceed within 24 h.

Processing Technique

Today, UCB processing laboratories use a variety of techniques for volume reduction, removal of red cells, or both. Most methods involve centrifugation, sedimentation, and/or filtration for reducing the red cell content, plasma volume, or both. The most common means of reducing red cell content has been the use of sedimenting agents such as hydroxyethyl starch (HES), gelatin, poligeline, and dextran.[28] The process that is done manually or automatically should lead to produce samples with high cell count and quality. It is determined that PT viability has a correlation with the percentage of neutrophil, total red blood cell (RBC), hemoglobin, hematocrit (Hct), red cell distribution width, percentage of viability after processing, volume of plasma in freeze mix, total freeze volume, freeze rate, time of collection to freezing, and PT CD34% recovery.[60] Final products with a great count of RBCs have shown significantly lower progenitor cell recovery after cryopreservation.[91] Furthermore, the impact of RBCs on clonogenic assay and recrystallization of extracellular ice masses in density-packed RBCs during thawing process of CBUs could reduce recovery.[32] Thereby, recovery of TNC and Hct of final products is the most important indicator of CBB processing quality.[32]

According to our experience, Pablo Rubinstein method is a good procedure to reduce red cell count using HES and depletes the units of plasma and anticoagulant to minimize the final product and cryoprotectant volumes. In addition, automation technology incorporated into the Sepax CB processing system offers a closed and sterile processing system that efficiently harvests stem cells from UCB and adaptable to a large-scale processing environment.

Freezing Technique

Freezing and storage methods must be robust enough to ensure that the quality of UCB unit is maintained for many years.[28] Dimethyl sulfoxide (DMSO) is used by most CBBs, and although confirmed that it does not have a direct toxic effect on hematopoietic cells, a damaging impact due to osmotic shock has been postulated.[67] According to the protocol published by Rubinstein *et al*., DMSO should be added slowly (up to a final concentration of 10%; samples are kept at 4°C at all times) to prevent an osmotic shock to the cells.[21] Radke *et al*. investigated the effect of addition DMSO on CD34+ cell apoptosis over time. After performing volume reduction, the amount of the final concentration of DMSO and dextran in the bag was 10% and 15%, respectively. The CBUs were thawed using a 37°C water bath and diluted 1:3 with the NYCBB washing solution containing 50% dextran 40, 12.5% human serum albumin 20%, and 37.5% phosphate buffered saline. They were not observed any significant difference in nonviable cells between bag, aliquot, and segment. However, the percentage of early apoptotic and necrotic/late apoptotic cells differed (the segment was highest other than). They showed that between temperatures, the percentage of viable CD34+ cells did not reveal a notable difference. They demonstrated that the addition of DMSO could lead to an induction of apoptosis. The immediately or progressive addition of DMSO had no impact on the percentage of annexin V-positive cells.[67] Several studies showed that the optimal DMSO concentration for UCB freezing is 10%.[92-94] Skoric *et al*. demonstrated that concentration of 5% DMSO without further additives is sufficient for cryopreservation of CBU cells.[22]

The viability of cells is known to be critically affected by the cooling rate.[73,95] Djuwantono *et al*. compared the effect of rapid and slow cooling on MNCs viability, apoptosis level, and CD34+ enumeration. They showed that the viability was significantly higher in rapid cooling than slow cooling while had reverse effect on CD34+ enumeration, and he difference was not significant in apoptosis level.[96] The optimal freezing rate for CBUs has been found 1°C–2.5°C/min[73] with following procedure: I = –5°C/min, to 0°C; II = 0°C/min, for 5 min (equilibration); III = –2°C/min, for 5 min; IV = –1°C/min, for 30 min and V = –5°C/min, for 5 min[22] [Figure 3]. In a study, they investigated the impact of different freezing methods and DMSO concentrations on CBUs cell recovery. Controlled-rate comparable uncontrolled-rate freezing and DMSO at 5 or 10% final concentration were used. CD34+ recovery, CFU-GM, and BFU-E recovery in the CBUs were the highest when controlled-rate freezing procedure and 5% DMSO were applied.[22] Donaldson *et al*.[95] suggested that good recovery of CBU hematopoietic stem cells can be achieved with 5%–10% DMSO at a controlled-rate freezing of 1°C/min.[22] Hunt *et al*. described a significant difference in recovery between cooling at 1°C and 5°C/min in favor of 1°C/min.[92] It has been well established that slow cooling in a programmable controlled-rate freezing device at rate of 1 C/min will result in adequate recovery of HPCs.[66] Shlebak *et al*. showed that there were no differences in MNC and CFU-GM recovery after controlling versus uncontrolled-rate freezing.[23]

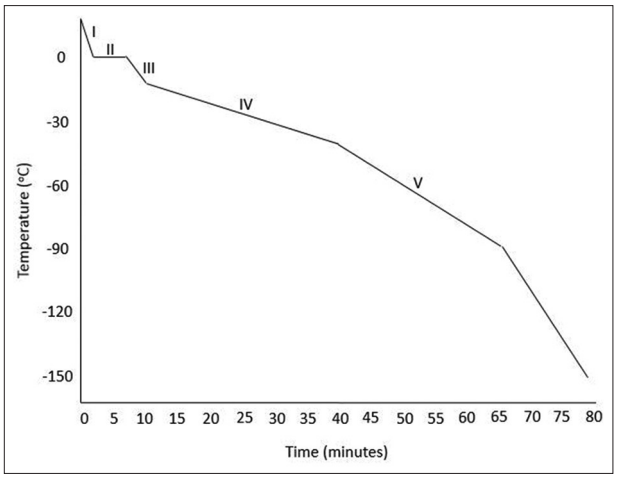

Figure 3: The optimal freezing rate for umbilical cord blood unites

When the transition period from liquid to the solid phase was prolonged, a higher degree of cell destruction has been observed.[22] A study confirms that more immature progenitors are unchanged after successive cryopreservation procedures.[97]

A study recommended that CBUs can be transferred to an –80°C freezer anytime during controlled - rate cooling but should only be transferred to liquid nitrogen vapor phase when the samples have been cooled to –40°C or lower.[98]

Both AABB and FACT-Netcord have defined storage temperature limits for cryopreserved UCB to be <–150°C. Once frozen, UCB units are typically transferred to long-term storage into a monitored liquid nitrogen container, either immersed in liquid (at –196°C) or in vapor phase to minimize the potential for cross-contamination.[28]

Thawing Technique

The UCB product is carefully removed from the storage tank, and a thorough inspection is performed to evaluate the integrity of the container. The unit is sealed within a clean or sterile transparent bag and submerged in a 37°C water bath containing clean or sterile water or saline. Gentle kneading of UCB as it is thawing helps to accelerate the process, preventing recrystallization and consequential cell damage or death.[28] Current practices for preparing UCB products for infusion includes the traditional thaw-and-wash method, the thaw-and-dilution technique, or bedside thawing.[99,100] Miura *et al*. showed that thawing process reduces the viability of TNC, whereas the amount of CD34+ cells remained less affected.[94] Some studies demonstrated that PT cell viability declined over time and these differences were statistically significant.[26,82,83,101] In addition, a study showed that count of early apoptotic CD34+ and CD45+ PI(−) over time was similar and the difference was not significant.[83] Recovery of CFUs after thawing significantly decreased and the number of CFUs at the time point PT 30 min declined compared to immediately after thaw.[83,102,103] A study suggested that macroscopic clumping and gel formation after thawing process were because of granulocytes lyse and release nucleoprotein and lysosomal enzyme. To solve these problems and reduce the loss of progenitor cells, extracellular cryoprotectants, such as HES, have been used in combination with DMSO during freezing.[75] Laroche *et al*. evaluated the effect of PT and postwash (PW) on CBUs. Briefly, UCB units were taken out of the liquid nitrogen storage container, put in a plastic bag, and immersed in a 37°C water bath. They explained that CD cell count declined after thawing and increased after washing, but this difference was not statically significant. CFUs declined after PT but increased after PW, and this difference was statically significant. In addition, they showed that viability declined after thawing and washing had no effect on viability. They demonstrated that TNC count and recovery declined over time and became significantly lower at 2 h and 5 h of PW. There was no significant difference in CD34 counts and recovery after PW. The significant increase in the number of CFU after PW compared to PT was maintained through 5 h.[72] Barker *et al*. showed that thawing CB with albumin-dextran dilution (without washing) compared to washing technique diminishes unit manipulation, decreases cell loss, speeds time to infusion, and is associated with endurable infusion reaction and a higher rate of sustained engraftment in CBT recipients >20 kg.[104]

Furthermore, homing of stem cells to the bone marrow microenvironment is critical for the successful transplant. In a study showed that L-selectin, VLA-4, VLA-5, H-CAM, and CXCR4 expression on CD34+/CD38 – cells did not change after two freeze-thaw cycles, while LFA-1 expression actually increased.[97]

More recently, because the majority of products are red cell reduced and because of concern for cell loss at the wash step, a dilution or simple reconstitution approach has gained support.[105]

Time and Temperature Postthawing

In a study, three methods were compared for thawing of frozen CBUs. These methods were (1) traditional washed with albumin reconstitution (dilution method), (2) thaw-only (without dilution or wash), and (3) wash. There was a significant difference in recovery between the thaw-only and wash methods at 0 and 48 h compared with the wash being lower [Figure 2]. The viability of CD cell declined at 24 h in the second group. However, dilution methods had more CD34+ recoveries at all points in times. This group demonstrated that the number of CFUs was better preserved in dilution-and-wash methods over time. In addition, they confirmed that viability was highest for the dilution method and lowest for thaw only technique. They assessed the intuitional toxicity and reported that there was no significant difference in severity and frequency of adverse effects at the time of CB infusion and time to neutrophil recovery among three methods. Median PT TNC count/kg was significantly higher in dilution group compared to the thaw-only and wash groups. This group suggested that dilution-and-wash methods provide more stability of viable CD34+ cells and functional HPC than thaw-only and decreased significantly after just 2 h.[82]

Conclusion

As with any emerging technology over time, UCB banking has evolved to become a more established, standardized practice. However, significant quality issues remain. Up till now, the infused cellular dose and the favorable phase of disease were the fundamental factors for transplant success. In this review, we emphasize the need to amplify quality control according to the international standards such as FACT-Netcord and AABB. Hence, having well-trained personals for CB collection and also CB processing is the important point that should be considered in all CBBs. Using the collection bags with an optimal amount of anticoagulant, mode of delivery, time from clamping to the collection, controlling time and temperature of transportation and storage the CBUs, processing the CBUs according the standards, careful use of DMSO, having an optimum freezing rate and freezing technique, thaw procedures are other critical

parameters. To maximize the likelihood that a CBU is suitable for use, a quality product must be collected, produced, and stored regardless of its intended recipient.

Acknowledgments

We are grateful to all our colleagues in Cord Blood Bank of Royan Stem Cell Technology Institute.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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