

# A Review of Procedures Involved in Human Umbilical Cord Blood Banking and Transplantation

Nikougoftar Zarif M<sup>1\*</sup>

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.

\*Corresponding Author: Nikougoftar Zarif M, Email: nikougoftar@ibto.ir

Submitted: 16-05-2014, Accepted: 29-08-2014

## Abstract

Cord blood hematopoietic stem cells are widely used as an alternative source for hematopoietic stem cells transplant. Increasing rate of patients who need hematopoietic stem cells transplant and many advantages of cord blood in comparison to bone marrow hematopoietic stem cells, have promoted banking of cord blood units. Cord blood banking requires accurate steps in donor selection, cord blood collection, processing, cryopreservation and finally screening and testing of the units. The main limitation of umbilical cord blood transplantation is cell dose and resolving this limitation is vital in avoiding frequent problems in hematopoietic stem cells transplant. Transplantation of more than one unit and ex vivo expansion of umbilical cord blood units, decrease these limitation. Furthermore, the international standard guidelines have been published by the Foundation for the Accreditation of Cellular Therapy (FACT)-NetCord to improve the quality of units and simulation of cord blood banks activities. This review presents a general overview on cord blood banking and related activities and its improvement during last two decades.

**Keywords:** Blood banking, cord blood, hematopoietic stem cells.

## Introduction

Today, hematopoietic stem cells transplant (HSCT) is used as a treatment method in a variety of malignancies as well as benign, inherited and dysplastic disorders<sup>1,2</sup>. The main source of hematopoietic stem cells (HSCs) is bone marrow, but these cells are also present in peripheral blood and Umbilical Cord Blood (UCB) in a lower count<sup>3,4</sup>. Since the first successful UCB transplant in 1988 to a patient with Fanconi's anemia, the UCB has been known as an alternative source for HSCT<sup>5</sup>. The special properties of UCB-HSCs including immediate availability of CB units, low risk of donation for mothers and infants, less HLA restriction for donors, potentially reduced risk of Graft Versus Host Diseases (GVHD), low risk of viral infection and more expression of proliferation and expansion transcript factors, make it as a preferable source for HSCT<sup>6-10</sup>. These properties caused much attention to harvest and storage of CB units and CB banks were established all over the world<sup>11,12</sup>. Some of the CB banks are private and store the CB units for the same family, public CB banks collect

and store the donated CBs for allogeneic HSCT, and hybrid CB banks do both activities. Because of increasing rate of diseases that need HSCT and limited HLA identical donors, there are many patients who do not find HLA matched units. Therefore, the necessity of expanding CB banks is clear. The main objective in CB banking is to store high quality units for successful HSCT, so, they work under strict quality control rules. Donor selection is an important step to select safe units. For volume reduction some manual or automatic procedures are used and high cell recovery is desired<sup>13</sup>. After the CB unit volume reduction, Total Nucleated Cell (TNC) count, CD34 absolute count, viability tests and Colony Forming Unit (CFU) assay are performed on separated buffy coats<sup>14-16</sup>. CB banks usually define cut offs for some criteria, for instance; on TNC and CD34+ cell counts and viability tests<sup>17,18</sup>. If the processed unit is qualified, all the infectious screening tests and HLA typing are done and the HLA results will be added to the database<sup>19</sup>. The aim of the present study is to provide an overview

of CB banking procedures involved in human UCB banking and transplantation as well as current issues in resolving some limitations.

### Donor selection

Donor selection is an important step in CB bank's activity. The quality of units requires extensive and accurate donor information. Healthy donor mothers are informed that UCB donation is voluntary and consent is obtained. The family medical history is reviewed and CB from single birth, low-risk mothers, with normal obstetric practices is selected. Some excluding criteria are as follows: presence of congenital abnormalities, hematologic or immune deficiency disorders, inherited coagulopathies, metabolic disorders, history of high risk sexual behavior, drug abuse and blood transfusion within 12 months before delivery<sup>20</sup>. Mother blood samples also should be obtained for infectious disease screening, including; microbial culture, cytomegalovirus, Syphilis, Hepatitis virus B and C, Human Immunodeficiency Virus 1 and 2 and some other tests according to the local policies. There are some documents showing that following these strict criteria leads to collection of only about 30% of units<sup>20,21</sup>. Furthermore, other studies have indicated that only 10-15% of banked cord blood units contain sufficient TNC and CD34+ cells for adult HSC transplant<sup>22,23</sup>.

### Cord blood collection

The CB units are collected by trained staff. The cord blood is harvested from venous cord in utero or ex utero, after careful disinfection of the umbilical cord, in order to reduce risk of contamination. Venous cord blood flows by gravity into a CB bag containing adequate volume of anticoagulant reagent. In some centers open CB collection is used, but the rate of contamination is more than closed method (14% VERSUS 4%)<sup>24</sup>. The labeled cord blood and mother units as well as detailed documents are transported to the CB bank in appropriate temperature for the processing<sup>25</sup>.

### Cord blood selection for volume reduction and processing

Some collected CBs do not contain adequate cells; therefore, the cord blood volume, TNC count, and CD34+ cell count are performed on aliquots separated from CB units before volume reduction.

The cut off for each criterion is characterized by the bank and usually are different within the banks.

### Volume reduction

In order to save the space and decrease the CB bank costs, plasma and red blood cells are removed from CB units. In volume reduction processing, a method that leads to more TNC recovery and also cost effectiveness is preferred<sup>26</sup>. There are two main methods, manual and automatic. In both methods usually Hydroxyethyl Starch (HES) is used for better RBC sedimentation, as Robinstein et al., have showed<sup>27</sup>. HES can recover 98% of CFUs. Other experiments using density gradient centrifugation, gelatin and HES sedimentation have resulted in low loss of HPCS<sup>27</sup>. In the manual method, after adding HES 6% to CB in a ratio of 1:4, buffy coat is separated by two step centrifugation<sup>28</sup>. The mixture of plasma and HES, also sediment RBCs are transferred to the satellite bags and buffy coat is collected to a freezing bag, usually adjusted to 20 ml. The automated instruments have also been designed for buffy coat separation in a close system. The mixture of CB and HES connect to a single use kit, containing a chamber or a bag that is housed in a device or instrument and buffy coat layer is formed under centrifugation. An optical sensor controls buffy coat fraction separation of the unit. In automated methods usually a two step centrifugation is performed and finally buffy coat is shifted to the freezing bag in a closed system. In some studies the TNC recovery by automated methods has been reported to be as high as 77% to 78%<sup>13,18</sup>, whereas, in manual method it rises to more than 80%<sup>29,30</sup>. Furthermore another investigation has pointed to CFU recovery of 98% in manual method<sup>27</sup>. All together, the rate of recovery in manual method is slightly higher than automated method, but processing in a closed method with lower contamination is an important advantage of automated techniques.

### Cryopreservation

For cryopreservation of UCBs, the units are placed in a mixing and cooling device to add cryopreserving solution containing Dimethyl Sulfoxide (DMSO) and dextran 40 (1:1). The separated buffy coat is then mixed with DMSO in a mixture of 4:1 and 2 reference samples are drawn from the unit. For the better cell freezing,

a control rate freezer is used. This instrument decreases the unit temperature from 4°C to -100°C in a programmed rate<sup>31</sup>. Until the screening test results are gained, units are saved at gas phase of liquid nitrogen, and if there are no any positive test results, they will be transferred to a liquid phase for long term storage. The cooling rate of units, also protectant reagent are the main factors governing the survival of HSCs, so, many studies have been performed to optimize cryopreservation protocols<sup>32,33</sup>.

### **Cord blood testing**

All the infectious diseases screening tests are performed on the plasma fraction and ABO and hemoglobinopathies are detected on sediment RBCs. TNC count of the main product is evaluated using automated cell counter and nucleated RBCs are excluded. The CD34+ absolute count is calculated using flow cytometry according to ISHAGE protocol. Cell viability testing by trypan blue dye exclusion or DNA fluorescent dyes have been studied<sup>25,34</sup>. CFU assay in a semisolid culture media is then used to characterize the quality of CB units. The HLA typing for the allele A and B and DRB1 is performed on DNA extracted from buffy coats using a PCR based method and in some cases sequence based typing is carried out<sup>35,36</sup>.

### **Cord blood transplantation**

At first UCBT was only performed in sibling donors, even with the HLA mismatch at one of the three loci. After a short time UCBTs with unrelated donors were reported with high rate of engraftment and low rate of GVHD<sup>37</sup>. Several studies have focused on correlation of engraftment with CB volume, TNC, CD34+ cell dose, and CFU assay to define some criteria for the successful transplantation<sup>17,38-40</sup>.

### **New insights in Cord blood banking**

The most important limitation of CB units is cell dose, that implies low potential for widespread use of CB units since a threshold of 2×10<sup>7</sup> TNC/kg of recipient body is required for successful transplantation<sup>41</sup>, many methods have been used for ex vivo expansion of UCB HSC<sup>42,43</sup>. The culturing of UCB for expansion has showed that committed progenitors and immature cells, also LTC-ICs were substantially amplified without any effect on proliferative potential<sup>44</sup>. The previous

studies focused on CD34+/CD38- HSCs expansion, because they highly proliferate in the presence of cytokine cocktails and generate more and contain long telomeres<sup>45</sup>. Separation of this population needs ex vivo manipulation of UCB units that increase the risk of contamination. However, recent studies have demonstrated that the whole buffy coat expansion causes short recovery of BM after UCBT. Today using two CB units compensate the low cell dosage<sup>46</sup>. In order to minimize the GVHD, transfusion of donor T cells to the recipients, and decreasing the risk of GVHD, haploidentical mesenchymal stem cells are added to the UCB units to increase the HSCs homing and lower the immunological responses<sup>47,48</sup>. In addition to CB expansion, ex vivo megakaryocyte differentiation of HSCs to overcome late platelet recovery after CBT has been performed<sup>49,50</sup>. Genetic manipulation of UCB-HSCs, because of their self renewability and differentiation into progenitor cells, is an interesting therapeutic source in patients suffering from diseases such as: Fanconi's anemia, cycle cell anemia, thalassemia and some other hematologic disorders. Several investigations have described successful introduction of specific genes into UCB-HSCs<sup>51</sup>. Finally, unrestricted somatic stem cells derived from UCB, reside in an early differentiation state, and after an appropriate stimulation treatment, can display a wide differentiation capacity in vitro and in vivo<sup>52</sup>.

### **Conclusion**

UCB transplantation has been known as a viable source of HSCs for patients with malignant and non malignant hematopoietic disorders. To collect and storage these valuable HSC sources, many UCB banks have been established worldwide. Banking of UCB units gives a chance to patients who do not have a HLA matched sibling or unrelated stem cell donor to find a ready HSC source in a short time. Storage of all valuable units needs large spaces and has lead to introduction of some procedures to reduce the unit's volume and consequently presentation of automated cell separation methods that are helpful in CB bank activities. As the CB banking cost is high, it is important to bank the qualified units; therefore, there have been some efforts to improve the size and quality of CB banks to store more and safer units.

## References:

1. Armitage JO. Bone marrow transplantation. *N Engl J Med*. 1995;330(12):827-38.
2. Boncimino A, Bertaina A, Locatelli F. Cord blood transplantation in patients with hemoglobinopathies. *Transfus Apher Sci*. 2010;42(3):277-81.
3. Bertolini F, Battaglia M, Lanza A, Palermo B, Cuomo A, Preti P, et al. Hematopoietic stem cells from different sources: biological and technical aspects. *Bone Marrow Transplant*. 1998;21 Suppl 2:S5-7.
4. Rocha V, Kabbara N, Ionescu I, Ruggeri A, Purtill D, Gluckman E. Pediatric related and unrelated cord blood transplantation for malignant diseases. *Bone Marrow Transplant*. 2009;44(10):653-9.
5. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with Fanconi anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med*. 1989;321(17):1174-8.
6. Wagner JE, Kernan NA, Steinbuch M, Broxmeyer HE, Gluckman E. Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease. *Lancet*. 1995;346(8969):214-19.
7. Kurtzberg J, Laughlin M, Graham ML, Smith C, Olson JF, Halperin EC, et al. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. *N Engl J Med*. 1996;335(3):157-66.
8. Rubinstein P. Cord blood banking for clinical transplantation. *Bone Marrow Transplant*. 2009;44(10):635-42.
9. Gluckman E, Rocha V. Cord blood transplantation: state of the art. *Haematologica*. 2009;94(4):451-4.
10. Francese R, Fiorina P. Immunological and regenerative properties of cord blood stem cells. *Clinical Immunology*. 2010;136(3):309-22.
11. Brand A, Rebullia P, Engelfriet CP, Reesink HW, Beguin Y, Baudoux E, et al. Cord blood banking. *Vox Sang*. 2008;95(4):335-48.
12. Querol S, Gomez SG, Pagliuca A, Torrabadella M, Madrigal JA. Quality rather than quantity: the cord blood bank dilemma. *Bone Marrow Transplant*. 2010;45(6):970-8.
13. Solves P, Planelles D, Mirabet V, Blanquer A, Carbonell-Uberos F. Qualitative and quantitative cell recovery in umbilical cord blood processed by two automated devices in routine cord blood banking: a comparative study. *Blood Transfus*. 2013;11(3):405-11.
14. Kalodimou VE, Bikos L, Kourenti C, Fifa A, Vlachousis A. Direct measurement of CD34+ cord blood stem cell absolute counts, viability and WBC using flow cytometry. *Health Science Journal*. 2011;5(4):306-19.
15. Sarang Sh, Joshi M, Nazareth V, Sapre R, Mehnawat R, Ramdasi S, et al. Functional assessment of cord blood units using two different assays. *International Journal of Hematology Oncology and Stem Cell Research*. 2012;6(3):20-26.
16. Querol S, Gomez SG, Pagliuca A, Torrabadella M, Madrigal M. Quality rather than quantity: the cord blood bank dilemma. *Bone Marrow Transplant*. 2010;45(6):970-78.
17. Jaime-Perez J, Monreal-Robles R, Colunga-Pedraza J, Mancías-Guerra C, Rodríguez-Romo L, Gómez-Almaguer D. Cord blood banking activities at a university hospital in northeast Mexico: an 8-year experience. *Transfusion*. 2012;52(12):2606-12.
18. Jamali M, Atarodi K, Nakhlestani M, Abolghasemi H, Sadegh H, Faranoosh M, et al. Cord blood banking activity in Iran National Cord Blood Bank: A two years experience. *Transfus Apher Sci*. 2014;50(1):129-35.
19. Parco S, Vascotto F, Visconti P. Public banking of umbilical cord blood or storage in a private bank: testing social and ethical policy in northeastern Italy. *J Blood Med*. 2013;4:23-29.
20. McCullough J, Herr G, Lennon S, Stroncek D, Clay M. Factors influencing the availability of umbilical cord blood for banking and transplantation. *Transfusion*. 1998;38(5):508-10.
21. Tamburini A, Malerba C, Picardi A, Amadori S, Calugi A. Placental/umbilical cord blood: experience of St. Eugenio Hospital collection center. *Transplant Proc*. 2005; 37(6):2670-2.
22. Donaldson C, Armitage WJ, Laundry V et al. Impact of obstetric factors on cord blood donation for transplantation. *Br J Haematol*. 1999;106(1):128-32.
23. Migliaccio A, Adamson JW, Rubinstein P, Stevens C. Correlations between progenitor cell dose, likelihood to engraft and time to myeloid engraftment in 79 unrelated placental/cord blood transplants.. *Exp Hematol*. 1997;25:356a. [abstract]
24. Bertollini F, Lazzari L, Lauri E, Corsini C, Castelli C, Gorini F, et al. A comparative study of different procedures for the collection and banking of umbilical cord blood. *J Hematother*. 1995;4(1):29-36.
25. Fasouliotis SJ, Schenker JG. Human umbilical cord blood banking and transplantation: a state of the art. *Eur J Obstet Gynecol Reprod Biol*. 2000;90(1):13-25.
26. Harris DT, Schumacher MJ, Rychlic S, Booth A, Acevedo A, Rubinstein P, et al. Collection, separation

- and cryopreservation of umbilical cord blood for use in transplantation. *Transplant*. 1994;13(2):135-43.
27. Rubinstein P, Dobrila L, Rosenfield RE, Adamson JW, Migliaccio G, Migliaccio AR et al. Processing and cryopreservation of placental /umbilical cord blood for unrelated bone marrow reconstitution. *Proc Natl Acad Sci U S A*. 1995;92(22):10119-22.
  28. Rubinstein P, Taylor PE, Scaradavou A, Adamson JW, Migliaccio G, Emanuel D, et al. Unrelated placental blood for bone marrow reconstitution: organization of the placental blood program. *Blood Cells*. 1994;20(2-3):587-96.
  29. Solves P, Mirabet V, Larrea L, Carbonell-Uberos F, Torres A, Guillén M. Cord blood volume reduction with AXP system. *Biology and Clinical Applications of Cord Blood Cells*. Mandelieu, France; October 2008. [abstract]
  30. Lapierre V, Pellegrini N, Bardey I, Malugani C, Saas P, Garnache F, et al. Cord blood volume reduction using an automated system (Sepax) vs. a semi-automated system (Optipress II) and a manual method (hydroxyethyl starch sedimentation) for routine cord blood banking: a comparative study. *Cytotherapy*. 2007;9(2):165-9.
  31. Donaldson C, Armitage W, Denning-Kendall P, Nicol A, Bradley B, Hows J, Optimal cryopreservation of human umbilical cord blood. *Bone Marrow Transplant*. 1996;18: (4)725-31.
  32. Li Y, Ma T. Bioprocessing of cryopreservation for large-scale banking of human pluripotent stem cells. *Biores Open Access*. 2012;1(5):205-14.
  33. Sputtek A, Korber C. Cryopreservation of red blood cells, platelets, lymphocytes and stem cells. In: Fuller BJ, Grout BWW, editors, *Clinical application of cryobiology*, Boca Raton: CRC Press; 1991. pp. 95–147.
  34. Picardi A, Arcese W. Quality assessment of cord blood units selected for unrelated transplantation: A Transplant Center perspective. *Transfus Apher Sci*. 2010;42(3):289-97.
  35. Laurenti L, Perrone MP, Bafti MS, Ferrari F, Screnci M, Pasqua I, et al. HLA typing strategies in a cord blood bank. *Haematologica*. 2002;87(8):851-4.
  36. Kotelevskaya EA, Smirnova SA, Smolyaninov AB. Low and high-resolution HLA typing of cord blood samples of register of donors in St. Petersburg. *Cellular Therapy and Transplantation*. 2009;2(5)5: Abstract 72.
  37. Wagner JE, Rosenthal J, Sweetman R, Shu XO, Davies SM, Ramsay NK, et al. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease. *Blood*. 1996;88(3):795-802.
  38. Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson JW, Migliaccio AR, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med*. 1998;339(22):1565-77.
  39. Gluckman E, Rocha V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R, et al. Outcome of cord-blood transplantation from related and unrelated donors. *N Engl J Med*. 1997;337(6):373-81.
  40. Al-Sweedan S, Musalam L, Obeidat B. Factors predicting the hematopoietic stem cell content of the umbilical cord blood. *Transfus Apher Sci*. 2013;48(2):247-52.
  41. Rocha V, Gluckman E; Eurocord-Netcord registry and European Blood and Marrow Transplant group. Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft-andtransplantation-related factors. *Br J Haematol*. 2009;147(2):262-74.
  42. Shpall E, Quinones R, Giller R, Zeng C, Baron AE, Jones RB, et al. Transplantation of ex vivo expanded cord blood. *Biol Blood Marrow Transplant*. 2002;8(7):368-76.
  43. Guzmán P, Sánchez V, Mayani H. Concise Review: Ex Vivo Expansion of Cord Blood-Derived Hematopoietic Stem and Progenitor Cells: Basic Principles, Experimental Approaches, and Impact in Regenerative Medicine. *Stem Cells Transl Med*. 2013;2(11):830-8.
  44. Ballen K, Gluckman E, Broxmeyer H. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood*. 2013;122(4):491-8.
  45. Soleimani M, Mozdarani H, Pourfathollah AA, Mortazavi Y, Alimoghaddam K, Nikougoftar M, et al. Expansion of Non-Enriched Cord Blood Stem/Progenitor Cells CD34+ CD38- Using Liver Cells. *Iranian Biomedical Journal*. 2005;9(3):111-16.
  46. Georges G, Lesnikov V, Baran S, Aragon A, Lesnikova M, Jordan R, et al. A Preclinical Model of Double- versus Single-Unit Unrelated Cord Blood Transplantation. *Biol Blood Marrow Transplant*. 2010;16(8):1090-8.
  47. Okas M, Gertow J, Uzunel M, Karlsson H, Westgren M, Kärre K, et al. Clinical expansion of cord blood-derived T cells for use as donor lymphocyte infusion after cord blood transplantation. *J Immunother*.

**Nikougoftar**

- 2010;33(1):96-105.
48. Yong-Tao L, Zhang Y, Liu M, Qiuwaxi J, Ashwood P, Charles S, et al. Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. *Journal of Translational Medicine J Transl Med.* 2013;11:196.
  49. Nikougoftar Zarif M, Soleimani M, Abolghasemi H, Amirizade N, Abroun S, Kaviani S. The High Yield Expansion and Megakaryocytic Differentiation of Human Umbilical Cord Blood CD133+ Cells. *Cell Journal(Yakhteh).* Cell J. 2011;13(3):173-8.
  50. Nikougoftar Zarif M, Soleimani M, Abolghasemi H, Amirizade N, Arefian E, Rahimian A. Megakaryocytic differentiation of CD133+ hematopoietic stem cells by down-regulation of microRNA-10a. *Hematology.* 2013;18(2):93-
  51. Schuhmann NK, Pozzoli O, Sallach J, Huber A, Avitabile D, Perabo Let al. Gene transfer into human cord blood\_derived CD34 cells by adeno-associated viral vectors *Experimental Hematology.* 2010;38(9):707-17.
  52. Greschat S, Schira J, Küry P, Rosenbaum C, de Souza Silva MA, Kögler G, et al. Unrestricted somatic stem cells from human umbilical cord blood can be differentiated into neurons with a dopaminergic phenotype. *Stem cell development. Stem Cells Dev.* 2008;17(2):221-32.