Indian Journal of Biotechnology
Vol 7, October 2008, pp. 547-549

Short Communications

The stem cell in the umbilical cord blood is not related to volume and nucleated cell count

A Mahantappa[1], Avijit Banik[2], Jyothsna Shivshankar[2] and S G A Rao[1]*

[1]Cryo Stem Cell Karnataka Pvt Ltd and [2]Sri Raghavendra Biotechnologies Pvt Ltd, 72, SBM Colony, Anandnagar, Bangalore 560 024, India

Received 6 March 2007; revised 14 February 2008; accepted 20 April 2008

The percent CD 34^+ cells in umbilical cord tremendously varied from sample to sample. Study conducted on 100 cryopreserved samples for their volume, nucleated cell count and CD 34^+ cell count showed no correlation between the three. Correlation study between volume and nucleated cell count showed poor correlation, where as correlation between volume and CD 34^+, nucleated cell count and CD 34^+ cell count showed no/negative correlation. Graphical representation of volume vs. nucleated cell count, volume vs. CD 34^+ cell count, and nucleated cell count vs. CD 34^+ cell count show the same results. These results have important bearing in umbilical cord blood banking, since sample acceptance/rejection at present is based only on volume of cord blood collected, which instead should be based on the number of CD 34^+ stem cells.

Keywords: Autologous banking, CD 34^+ cells, haematopoietic stem cells, umbilical cord blood

Umbilical cord blood derived stem cells (UCBSC) have been shown as a viable alternative to bone marrow derived haematopoietic stem cells for transplantation, a successful treatment of several haematopoietic diseases, such as, leukemia and genetic anemias. However, the umbilical cord blood stem cells are available only at the time of child birth. Therefore, they need to be cryopreserved in stem cell banks. Since the umbilical cord blood stem cells were used only for transplantation, the number of stem cells present in the volume of cord blood collected was critical. It was believed that volume less than 40 mL was not suitable for banking, since they do contain optimum number of stem cells for successful transplantation. The optimum number was shown to be 1.5×10^7 nucleated cells/kg body wt and 1.7×10^5 CD 34^+ cells/kg body wt of the recipients[1-10]. In this paper, authors show that volume of umbilical cord blood less than 40 mL may contain the optimum number of CD 34^+ stem cells for transplanting a recipient weighing 10 kgs. Further, there is a poor co-relation between volume, nucleated cell count and CD 34^+ count. Since it is CD 34^+ count, which is crucial, the acceptability of the umbilical cord blood units cannot be decided only on the volume collected or nucleated cell count, and CD 34^+ count is mandatory. Furthermore, with the discovery that stem cells present in bone marrow and umbilical cord blood can differentiate into tissues other than haematopoietic makes these stem cells valuable in regenerative medicines. The minimum number of stem cells needed for these applications is not yet clear; therefore, no minimum acceptable number for banking can be fixed as of now.

Cord blood was collected in a validated closed system developed by the authors called "stem cord" (Fig. 1), which was packed in a collection kit (Fig. 2) containing heparin 10,000 IU, vaccutainer for mother's child for testing of infectious diseases (ID), 2 mL syringe, gloves and spirit. Collection of umbilical cord blood was done by trained medical person under validated standard operating procedures. Before collecting the umbilical cord blood, collection team examined the collection kit to check the collection device (stem cord) for its integrity. The collection device should be sterile, closed, sealed and leak proof. Before collecting the blood, 2000 IU of heparin was added into collection device through 'Y' connector.

Once the baby was out of mother's uterus, the umbilical cord was clamped 5-6 cm away from the baby's side. Collection began after the birth of the baby. As the baby was separated from the umbilical cord, the cord should remain clamped at the cut end. After surface sterilizing the cord, it was punctured with the needle provided with stem cord collecting bottle. Blood started flowing into the device, which was held lower level than the placenta so that by gravitational force the blood could flow (SOP No.C01, Replacement: C01, Revision:3rd, Effective From: 1st Sep, 2007). After collecting the umbilical cord blood, around 2 cc blood was collected from the expelled placenta in a vaccutainer without heparin to screen the blood for infectious diseases. After collection, umbilical cord blood sample was transported to the cGMP-facilitated clean room within 24 h for its further processing.

Before processing umbilical cord blood, the samples were screened for infectious diseases like

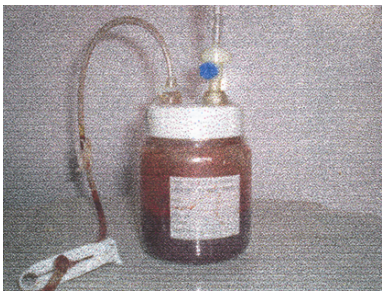

Fig. 1—Stem cord bottle showing collected UCB for processing

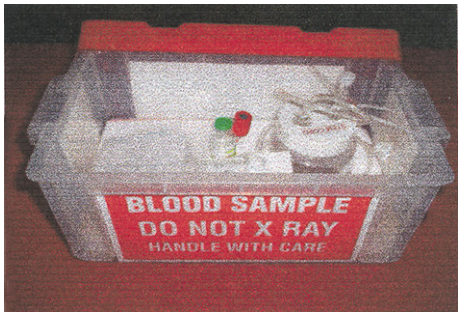

Fig. 2—UCB collection kit used for sample collection

HIV1, HCV, HbsAg and Syphilis. Screening the umbilical cord blood was a part of QC work, so ID test was carried out in QC lab, which was also maintained under cGMP norms (Class 10000). Rapid test kits were used for ID tests, HIV1 & 2, HbsAg, HCV (Biomed Industries), Syphilis (ACON Laboratory Inc., San Diego, CA, USA). After the screening, umbilical cord blood samples were sent for further processing. If umbilical cord blood sample was found to be positive for any of the above-mentioned infectious diseases, the sample was rejected and replaced with another sample. In total 100 clean samples were included in the present study.

Screened blood was carried inside the cGMP regulated clean room at class10000, provided with a HEPA fitted blowers. Clean room was designed and maintained as per scheduled 'M' standard for injectable drugs under the Drugs & Cosmetics Act 2005 [As amended by Drugs & Cosmetics 7th Amendment Rules, 2005]. Clean room was provided with pass box for entry and exit of finished products. Umbilical cord blood was processed under the LAF inside the clean room. The blood was mixed with hydroxy ethyl starch (6%, w/v; Claris or Fresenious kabi) in the ratio of 1:4 in a sterile processing kit developed by Cryo Stem Cell Karnataka Pvt Ltd and mixed well for 30 min. RBC was then allowed to sediment up to 30 min. Most of the RBC's sediment at the bottom, whereas nucleated cells remained in supernatant. After 30 min sedimentation, supernatant was collected and centrifuged for 25 min, at 500 rpm. The pellets were pooled and suspended in autologous plasma.

Chilled cryopreservent, dimethyl sulphoxide (DMSO) in hydroxy ethyl starch (1:1) was added slowly to the pellet to get a 10% (v/v) solution. Before adding DMSO, about 100 µL cell suspension was kept for nucleated cell count, viability and CD 34$^+$ analysis. After the cell pellet was mixed with cryoprotectant, it was subjected to slow cooling, where temperature decreases up to –196°C at a rate of 1-2°C per min using slow cooling device developed by Sri Raghavendra Biotechnologies Pvt Ltd.

10 µL of sample was mixed with WBC diluting fluid (90 µL), and then sample was diluted 10 times. WBC diluting fluid contains acetic acid, which lyses all the RBCs. After 4-5 min, 10 µL from the diluted sample was loaded on to haemocytometer, and total number of nucleated cells was counted. Viability count gives the real count of living and dead cells. Viability of blood cells was checked by using Trypan blue as it stains only dead cells, whereas all living cells appear colourless. Percent viability can be calculated using the following formula:

$$\% \text{ Viability} = \frac{\text{Total no. of cells - No. of dead cells}}{\text{Total no. of cells}} \times 100$$

For enumeration of CD 34$^+$ cells, BD Procount Progenitor Cell Enumeration Kit (BD Bio-Sciences-Sanjose, USA) was used. Kit contains Fluorochrome-conjugated monoclonal antibodies directed against the CD 34 molecules used to identify CD 34$^+$ cells by flowcytometry. Flowcytometry application for CD 34$^+$ cells identification and enumeration provided a rapid quantitative and reproducible method to evaluate the progenitor cell population. Kit contains the vials, test and control tubes. Test Vial contains: Nucleic acid dye and phycoerythrin (PE)-labeled murine monoclonal CD 34$^+$ Abs and peridinin chlorophyll protein (perCP)-labeled murine monoclonal CD45 Abs. BD Trucount tubes, each containing a lyophilized pellet of 4.2 µm fluorescent-dye beads. Analysis of stained sample was done within 12 h on a BD FACSCAN using the ISHAGE protocol.

In the present study, 100 umbilical cord blood samples were studied. The volume of cord blood collected varied from as low as 10 mL (sample no. 46) to a high of 125 mL (sample no. 65; detailed data is not shown). Total nucleated cell count also showed a variation between 26×10^6 (sample no. 41) to 1797×10^6

(sample no. 65). The variation in CD 34^+ cell count was between 20×10^4 (sample no. 11) to 904.2×10^4 (sample no. 51). Correlation of coefficient calculated between volumes of umbilical cord blood collected vs. total nucleated cells harvested from them showed a positive but poor correlation (r=0.616), whereas no correlation was observed between volumes of umbilical cord blood collected vs. CD 34^+ stem cells. When total nucleated cell count was compared with CD 34^+ stem cells, there was a negative correlation (r= –1.426). The lack of correlation between the three parameters—volume of umbilical cord blood, total nucleated cells and CD 34^+ stem cells—are more clear when we look at the graphic representation (Figs 3-5). In Fig. 3, which compares volume with total nucleated cells, sample no. 41 with 15 mL volume has a low nucleated cell count of 26×10^6, whereas sample no. 46 with 10 mL volume has 220×10^6 nucleated cells. This lack of correlation is further substantiated when volume of umbilical cord blood collected is compared to CD 34^+ stem cells harvested (Fig. 4). Sample no. 46 with 10 mL volume has 55×10^4 CD 34^+ stem cells, but sample no. 78 with 50 ml volume has only 15×10^4 CD 34^+ stem cells. The same is true when total nucleated cells harvested is compared with CD 34^+ stem cells (Fig. 5); Sample no. 6 with 366×10^6 nucleated cells has 1.26×10^6 CD 34^+ cells (0.35%), while Sample no. 6 with 309×10^6 nucleated cells has $4.57\times10^+$ CD 34^+ cells (1.48%). These results fairly state that the volume of umbilical cord blood collected and total nucleated cells harvested do not have any correlation with CD 34^+ stem cells present. Thus, on the basis of volume of umbilical cord blood and number of total nucleated cells, acceptability of collected sample cannot be decide for transplantation, since the deciding factor is number of CD 34^+ stem cells. It has been shown that 1.7×10^5 CD 34^+ stem cells/kg body weight is needed for transplantation. Therefore, the suitability of the sample for transplantation should be based on the number of CD 34^+ stem cells instead of the volume of cord blood collected.

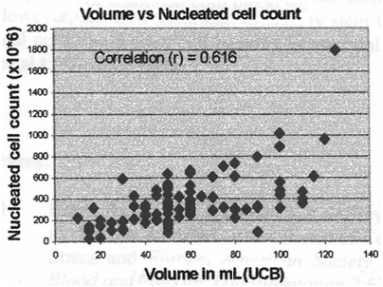

Fig. 3—Poor correlation between volume (mL) of UCB and nucleated cell count($\times10^6$)

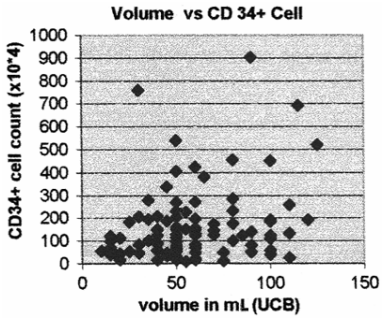

Fig. 4—Graph showing lack of correlation between volume (mL) of UCB and no. of CD 34^+ cell

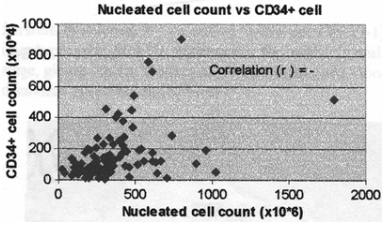

Fig. 5—Negative correlation between nucleated cell count ($\times10^6$) and CD 34^+ cell count ($\times10^4$)

References

1 Ballen K, Broxymeyer H E, McCullough J, Piaciabello W, Rebulla P *et al*, Current status of cord blood banking and transplantation in the United States and Europe, *Biol Blood Marrow Transplant*, 7 (2001) 635-645.

2 Wagner J E, Barker J N, DeFor T E, Baker K S, Blazar B R *et al*, Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: Influence of CD 34^+ cell dose and HLA disparity on treatment-related mortality and survival, *Blood*, 100 (2002) 1611-1618.

3 Nishio Y, Koda M, Kamada T & Someya Y, Use of human cord blood stem cells to promote restoration of spinal cord tissues in rats, *J Neurosurg Spine*, 5 (2006) 424-433.

4 Peterson D A, Umbilical cord blood and brain stroke injury, *J Clin Invest,* 1114 (2004) 312-314 (doi: 10.1172/JC1200422540).

5 Borlongan C V & Hadan M, Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke, *Stroke*, 35 (2004) 2385.

6 Sanberg P R, Willing A E, Garbuzova-Davis S, Saporta S, Liu G *et al*, Umbilical cord-derived stem cells and brain repair*, Stem Cell Biol: Dev Plasticity*, 1049 (2005) 67-83 (doi:10.1196/annals.1334.008).

7 Rao S G A, Desai S S, Goud A P, Deora A, Amin M K *et al*, Umbilical cord blood as a source of CD^+ haemopoietic stem cells, *Indian J Med Res*, 101 (1995) 28-30.

8 Dravid G & Rao S G A, *Ex vivo* expansion of stem cells from umbilical cord blood, *Stem Cells*, 20 (2002) 183-189.

9 Tanavde V M, Desai S S & Rao S G A, Erythrocyte depletion of human umbilical cord blood using dextran sedimentation*, Ind J Med Res*, 106 (1997) 16-19.

10 Dravid G G, *Analysis of stem cell populations from human umbilical cord blood and their expression in vitro*. Ph D Thesis, Cancer Research Institute, Parel, Mumbai, April 2001.