Predicting Overall Viability of Cord Blood Harvests

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Introduction

Since the 1980's it has been recognised that stem cells collected from umbilical cord blood can be used as an alternative to stem cells collected from bone marrow (BM) or peripheral blood (PB)[1]. A child with Fanconi anaemia was successfully transplanted in 1988 with cord blood stem cells donated by his human leukocyte antigen (HLA) matched sister[2] . Since then cord blood stem cells have been used to treat a wide range of malignant and non-malignant disorders[3]. These include leukaemia, Fanconi anaemia, severe aplastic anaemia, myelodysplastic syndrome, haemoglobinopathies, BM failure syndromes, primary immunodeficiency disease (PID) and inherited metabolic disorders (IMD)[3,4]. There is evidence that pluripotent cord blood stem cells could potentially contribute to organ and tissue regeneration[5]. There is potential for cord blood stem cells to treat diseases including diabetes, arthritis, burns, neurological disorders and myocardial infarction[6].

Umbilical cord blood stem cells have been found to possess higher ex-vivo proliferative capacity and increased replication capability compared with bone marrow[1,2]. Cord blood stem cells are more primitive and possess longer telomeres than those found in adult cells[2]. Their immunological naivety and high expansion potential enables a higher tolerance to 1-2 HLA mismatches. This tolerance allows for a better chance of finding donor stem cells for transplantation in cases of ethnic minorities or rare haplotypes[5,7]. A lower severity of graft versus host disease (GVHD) and a lower risk of latent virus transmitted infection make cord blood stem cells a transplantation source to be considered[8,9]. However, only a limited volume of cord blood can be collected and the associated lower number of stem cells can delay the time to engraftment and immune reconstitution compared with other sources of stem cells[10]

An understanding of the factors that influence the quality of the stem cell harvest will help in predicting which collections will have maximal yields and may provide opportunities to optimise the collection, post collection storage conditions and processing techniques of the cord blood in order to minimise cell loss and viability.

Aim

The aim of this study is to investigates factors that affect WBC viability after collection and before freezing and storage of the harvested CB derived stem cells.

Methods

Background Sydney Adventist Hospital (SAH), Pathology Department established the Stem Cell Unit (SCU) to provide a service to process, freeze, test and store Haematopoietic Progenitor Cells (HPC) collected from umbilical cord blood (CB) for autologous use only. SAH provided these services under contractual agreements to a number of private cord blood companies in Australia between June 2003 and December 2010. The Stem Cell Unit was licensed by the Therapeutic Goods Administration (TGA).

Participants 9918 CB collections were studied, collected between 2003 and 2010.

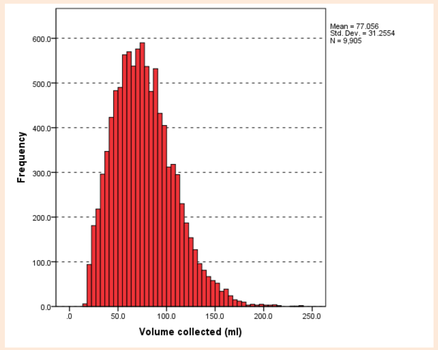
Sample Collection CB was collected in-utero or ex-utero from caesarean or vaginal birth in dispersed clinical locations by local staff trained to standard methods. The cord blood was collected into Maco Pharma triple cord blood bag containing 29ml of CPD. CB was transported to the central lab under controlled conditions for analysis, processing and freezing.

Processing The CB was centrifuged and volume reduced using an Optipress II Automated Blood Component Separator

CD34+ cell enumeration and Viability studies CD34+ enumeration kit (Beckman Coulter) was used to test the CB. The samples were run on either an Epics XL or Cytomics FC500 (Beckman Coulter) using STEMONE Software (Beckman Coulter) to enumerate CD34+ cells and viability.

Results-1

Volume of CB collected (ml) between 2003 and 2010



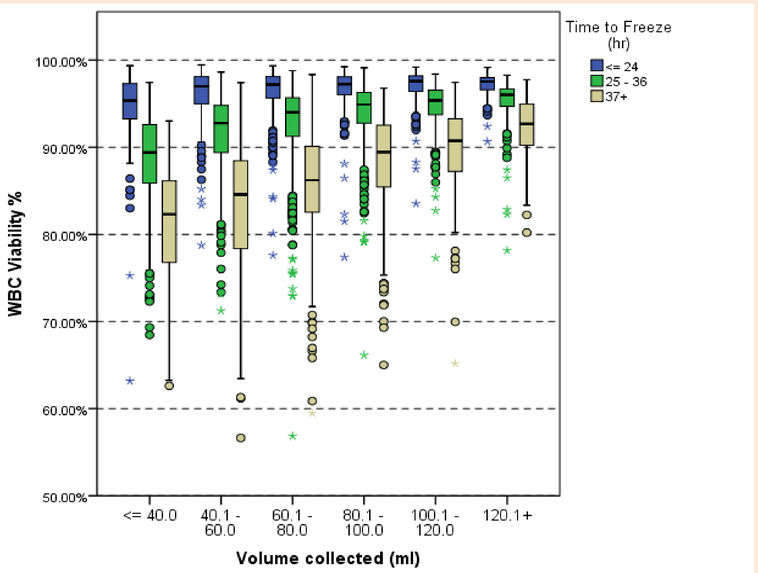
Results-2

Summary statistics based on volume of CB collected (ml)

Volume collected (ml)	Number of CB Collected	% of CB collections	Overall WBC Viability %	Total WBC x10[8]	Total CD34 count x10[6]	%CD34
<=40	1077	10.9	88.4 (7.4)	4.0 (1.8)	1.4 (1.2)	0.36 (0.25)
40.1-60.0	2142	21.7	90.8 (6.6)	7.0 (2.5)	2.3 (1.7)	0.34 (0.21)
60.1-80.0	2471	25.0	92.2 (6.0)	9.6 (3.3)	3.4 (2.4)	0.35 (0.21)
80.1-100.0	2049	20.8	93.2 (5.1)	12.3 (4.1)	4.6 (3.5)	0.38 (0.23)
100.1-120.0	1226	12.4	94.1 (4.4)	14.9 (4.9)	5.8 (4.0)	0.39 (0.21)
120.1-140.0	548	5.6	94.6 (3.7)	17.0 (5.0)	7.2 (4.6)	0.42 (0.23)
140.1-160.0	229	2.3	95.2 (3.0)	20.6 (6.2)	9.3 (6.1)	0.44 (0.23)
160.1+	127	1.3	95.5 (2.8)	22.3 (7.1)	11.4 (8.7)	0.50 (0.31)

Results-3

Relationship between volume collected, WBC viability and Time to Freeze (TTF)



- This graph shows that the lower the volume, the more impact TTF had on overall WBC viability.
- TTF of greater than 24h had a significant impact on the viability of the final product especially at lower volumes of less than 100 ml.
- These interaction is are statistically significant (p<0.005)

Results-4

The following tables shows the proportion of CB units able to achieve viabilities of >95%, >90% and >80% respectively.

>95% Viability

TTF (hr)	Cord Blood Volume collected (ml)					
	<40	40-60	60-80	80-100	100-120	>120
<24	54.0	74.8	82.3	86.7	88.2	92.1
24-36	6.0	23.4	36.1	49.0	57.8	68.9
37+	0.0	1.5	4.6	7.8	12.8	22.3

If CB is frozen within 24hrs and volume is >60ml, there is a >80% of maintaining >95% WBC viability.

At 37+ hrs, 0% of CB units of <40ml had >95% viability.

If the collected CB is frozen within 24 h, there is a >90% likelihood of maintaining >90% viability regardless of volume.

However with greater TTF it is less likely that WBC viability will be maintained especially at lower volumes

>90% Viability

TTF (hr)	Cord Blood Volume collected (ml)					
	<40	40-60	60-80	80-100	100-120	>120
<24	90.2	97	96.8	98.3	98.5	100
24-36	44.6	71.2	81.5	88.6	93.6	97.4
37+	4.2	15	25.3	45.5	57.4	76.8

>80% Viability

TTF (hr)	Cord Blood Volume collected (ml)					
	<40	40-60	60-80	80-100	100-120	>120
<24	98.8	99.7	99.8	99.7	100	100
24-36	90.9	98.4	98.3	99.1	99.7	99.7
37+	67.3	70.3	84.7	88.7	95.3	100

80% WBC viability can be maintained in >80% of CB volumes regardless of TTF except for volumes of <60ml, frozen after 36hrs

Conclusions

This paper summarises the experience from our laboratory and uses the data obtained from 9918 collected cord bloods to develop predictive tables for obtaining a quality cord blood product. These studies identify the relationship between volume, time from collection, viability, WBC and CD34+ cell count of cord blood units. This information could be used by both private and public cord blood banks alike to help prioritise workload and enable more informed choices on the long term storage of individual collected cord blood units.

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