63

CD34+ ENUMERATION FROM VENA PUCTURE VS FINGER PRICK COLLECTION FOR STEMCELLS THERAPY

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Background: CD34+ had been used to determine the right time for harvesting stem cell in therapy. Venipunctured usually used to collect samples for CD34+ enumeration using flow cytometry. However, puncturing the veins has some disabilities such as collapse veins and could effect the angiogenesis in stem cell therapy. Finger pricked was suggested as an alternative for sampling blood of CD34+.
Objective: This study is used to compare the CD34+ enumeration from samples collected from finger prick and venipuncture.
Method: Four healthy subjects were used to determine the enumeration of CD34+ in the body using CD34+ using flow cytometry. Blood was collected for finger prick and veins on each subjects using EDTA tube at minimal volume of 1 mL. On the same day, CD34+ enumeration was perfomed using Stem Cell Enumeration Kit from BD Biosciences. Samples were run on FACS Canto II and FACS Canto Clinical Software.
Result: As the comparison in healthy patient (n = 4) the mean of CD34+ enumeration from venipuncture sample is lower than finger prick. The mean of Venipuncture CD34+ is 0.75 SD 0.28 and Finger Prick is 1.11 SD 1.49. Statistic analysis using compare T-test shows $t_{count} = 1.052$ and $t_{table} = 2.571$. The t_{count} is lower than t_{table} show ($1.052 < 2.571$).
Conclusion: There is no difference between the technique sampling from venipuncture and finger prick for CD34+ enumeration.
Keywords: CD34+ enumeration, venipuncture, Finger Prick collection, stemcells therapy.

64

EXPRESSION OF HSC AND VSEL POPULATIONS IN MANUAL VS AUTOMATED PROCESSED UMBILICAL CORD BLOOD

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Background and Objectives: Umbilical cord blood (UCB) is one of the richest sources of primitive stem cells. A recent study showed that not only Hematopoietic Stem Cells (HSC) but also very small embryonic-like stem cells (VSEL) found in UCB. These rare cells have high potential interest for regenerative medicine and play an important role in tissue or organ injury repair. For therapy purposes, it is important to know the best method to produce high populations of stem cells. In this study, we compare UCB stem cells processed by manual

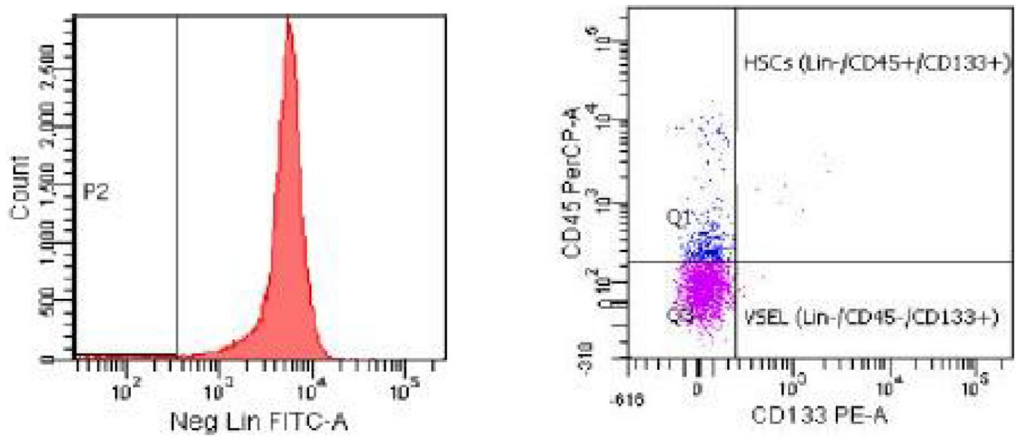

Figure 1. Expression of HSC and VSEL in manual processed UCB.

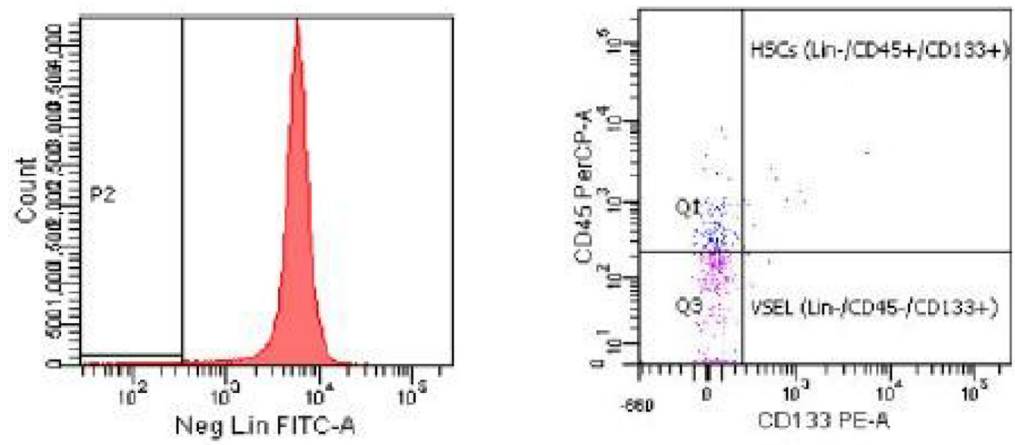

Figure 2. Expression of HSC and VSEL in automated processed UCB.

Table I. HSC and VSEL expression in manual vs automated processed UCB.

	Manual	Automated
HSC	0.9%	3.3%
VSEL	0.5%	1.0%

and automated processing method to observe the expression of HSC and VSEL populations.

Methods and Results: We compared manual and automated UCB processing method to detect population of HSC and VSEL in UCB samples. HSC were characterized as $Lin^-/CD45^+/CD133^+$, while VSEL were characterized as $Lin^-/CD45^-/CD133^+$. Lineage negative-FITC, CD45-PerCP, and CD133-PE antibody were used in flowcytometri assay. In comparison of processing UCB with manual and automated procedure, the population of HSC and VSEL are higher in automated procedure. In manual processed UCB sample, expression of HSC is about 0.9% from the population whereas in the automated processed UCB sample, the expression is about 3.3% from the population. For the expression of VSEL is 0.5% in manual processed UCB sample and 1.0% in automated processed UCB sample.

Conclusions: We conclude by using automated processing method, the stem cells had higher populations of HSC and VSEL compared to manual processing method (Figures 1 and 2, Table I).

65

IMMEDIATE CRYOPRESERVATION OF HUMAN PRIMARY HEMATOPOIETIC CELLS PROVIDES THE BEST SOLUTION FOR GLOBAL RESEARCH AND DISTRIBUTION

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Human hematopoietic cells are critical raw materials for research and manufacturing of cell therapy products. Fresh products maintain acceptable viability and functionality for the first 24 hours of transport. However, beyond 24 hours, the number of viable cells decreases dramatically. When considering global transportation and the cell therapy supply chain, the need for a practical shipping option is essential.

Hence, we set out to evaluate fresh leukopaks (LP), upon collection and at 24 hours to simulate shipment. We also evaluated cryopreserved leukopaks frozen on day 0, and frozen on day 1, allowing for flexible shipping options.

For this comparison we evenly split leukopaks from multiple donors into halves. One half was immediately cryopreserved using a control rate freezing process; one half was held at room temperature for 24 hours and then cryopreserved. We isolated CD3 and CD14 cells and put them into functional assays.

Total lymphocyte (CD45) counts were measured on each condition. When LPs are shipped fresh, there is approximately a 30% decrease in total lymphocyte count. However, when LPs are frozen using HemaCare's control rate freezing process immediately post collection, there is approximately a 17% decrease in total lymphocyte count. When cryopreservation is performed after shipment, there is approximately a 70% loss in total lymphocyte count. Viability of the cells, measured by flow cytometry, was greater than 99% in all groups.

We found the highest recovery, viability, and functionality of target cells resulted from the immediate cryopreservation of leukopaks collected per the HemaCare collection and processing model. HemaCare shipping containers accurately maintain the integrity of cryopreservation for up to 10 days. This presents a scalable option for emerging autologous and allogeneic cell therapies that require apheresis shipments from HemaCare to cell therapy processing facilities around the world.

66

HERC2 MUTATIONS IDENTIFIED BY NEXT GENERATION SEQUENCING IN AN ATAXIA TELANGIECTASIA PATIENT WITH NK/T CELL LYMPHOMA

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Introduction: Ataxia telangiectasia is due to mutation in ATM serine/threonine kinase (*ATM*) gene. A novel mutation of HECT and RLD domain containing E3 ubiquitin protein ligase 2 (*HERC2*) gene was found by next generation sequencing to also result in ataxia telangiectasia.

Methods: A 17 year old patient with intention tremors, Henoch-Schonlein purpura, recurrent sinopulmonary infections and conjunctival injections was diagnosed with extranodal NK/T cell lymphoma. Since *ATM* gene sequencing done under the suspicion of ataxia telangiectasia was negative, whole exome sequencing (WES) and Sanger directed sequencing was performed on the patient and his parents. Genomic DNA was captured on the NimbleGen 2.1M human exome array and was subjected to 74 base paired-end reads on the Illumina HiSeq instrument. Sequence reads were mapped to the reference genome (hg19) using the ELAND program. SAMtools was used to call the variants. Among these, common variants that are listed in the public databases (dbSNP build 137, 1000 Genomes Project release 10.31.2012 and NHLBI Exome Sequencing Project) were excluded and only the rare variants were considered as potential causative variants. The variants were validated by PCR and direct Sanger sequencing.

Results: WES revealed compound heterozygous mutations of c.4625G>A (p.Arg1542His, rs112385654) and c.10474T>C (p.Ser3492Pro, rs185865505) in *HERC2*. The former was maternally inherited and was not listed in the Exome Aggregation Consortium (ExAC, release 0.3, http://exac.broadinstitute.org) while the latter was paternally inherited and was found in the ExAC with frequency of 0.00077 among which none were homozygous, making the likelihood of both variants being carried highly unlikely to happen by chance ($<1.2 \times 10^{-8}$). The altered amino acid residues were highly conserved across the vertebrates and the amino acid changes were predicted to be damaging by PolyPhen-2 and SIFT (Sorting Intolerant From Tolerant) programs. Given the known function of *HERC2* in coordinating ubiquitin-dependent assembly of DNA repair factors on damaged chromosomes, its pathogenicity is plausible. The patient underwent chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT) and is currently 1.5 year from HSCT with no evidence of disease.

Conclusions: Rare *HERC2* mutations were identified in an ataxia telangiectasia patient with NK/T cell lymphoma by WES.

67

PUBLIC AWARENESS OF CORD BLOOD BANKING IN SAUDI ARABIA

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Introduction: Cord blood (CB) has proven to be a valuable source of hematopoietic stem cells for transplantation to treat many hematological disorders. Thus, many CB banks have been established worldwide. However, CB is not routinely preserved and only parents aware of CB banking have the option to bank their child's CB. Our aim was to estimate the level of public awareness of CB banking in Saudi Arabia.

Methodology: an anonymous, self-administered, questionnaire was distributed. The questionnaire consisted of demographic data; awareness measure; attitude toward banking preference and donating for research.

Results: A total of 1146 participants have completed the questionnaire. The majority of the participants were young female (26%), college graduates (57%). The subjective assessment of the overall knowledge about CB banking was inadequate (66%). For the objective assessment, 12 questions were asked about CB source, collection, storage and usage. Only half of the subjects (52%) knew that CB is a source of stem cells. More than half of the subjects did not know the main use of CB. About the half did not know the method of collection nor the condition of storing. The main source of knowledge was from social media (58%). Most people (81%) are willing to store their CB and would choose a public bank to benefit any patient in need, and would donate their child's CB to research if it is not suitable for transplantation.

Conclusion: This study shows a high lack of knowledge about CB banking. More than have of the subject were unaware of CB banking and its uses. However, most subjects are accepting CB storage, which anticipates great impact and efficacy of educational programs. Moreover, the data demonstrated that health professionals were not the source of knowledge. Therefore, it is recommend