

Flow cytometric analysis of stem cells derived from umbilical cord blood – CD34+/CD45+ enumeration and viability

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Dissertation in fulfillment of the requirements for the degree
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Introduction

Early methods of cord blood separation resulted in a significant loss of progenitor cells

Objectives in haematopoietic cell separation:

- ensure maximum UCB cell count storage
- ensure successful engraftment
- optimize storage space

•Stem cell dose

Cord Blood Transplantation (COBLT) banks (2004):

TNC dose : $8.7 \times 10^7/\text{kg}$

CD34 cell dose $2.4 \times 10^5/\text{kg}$

Cellular Therapy Society (2006)

TNC dose : $3.5 \times 10^7/\text{kg}$

CD34 cell dose $1.5 \times 10^5/\text{kg}$

Relevance and motivation for this study

This study is the first in a series aimed at establishing criteria for umbilical stem cell processing and storage in South Africa which are benchmarked against international standards of the cord blood industry

Aim

To determine processing standards for collection volume, cell count, cell viability to compare these with UCB banks internationally

- H_0 = Maternal age has an influence on parameters
- H_0 = Infant gender influences parameters

METHODOLOGY

Ethics approval

Faculty of Health Sciences Committee of the
University of Pretoria

- research protocol
- informed consent

Samples

913 Netcells clients (January 2007- July 2008)

Cord Blood Collection

- Collected from the umbilical vein into a 250ml Baxter bag (containing 35ml CPDA-1)
- Couriered in a temperate coolant kit and processed within 48 hours



Cord Blood Processing

Sepax Cell Processing System (Biosafe) in combination with a single use CS-530 separation kit



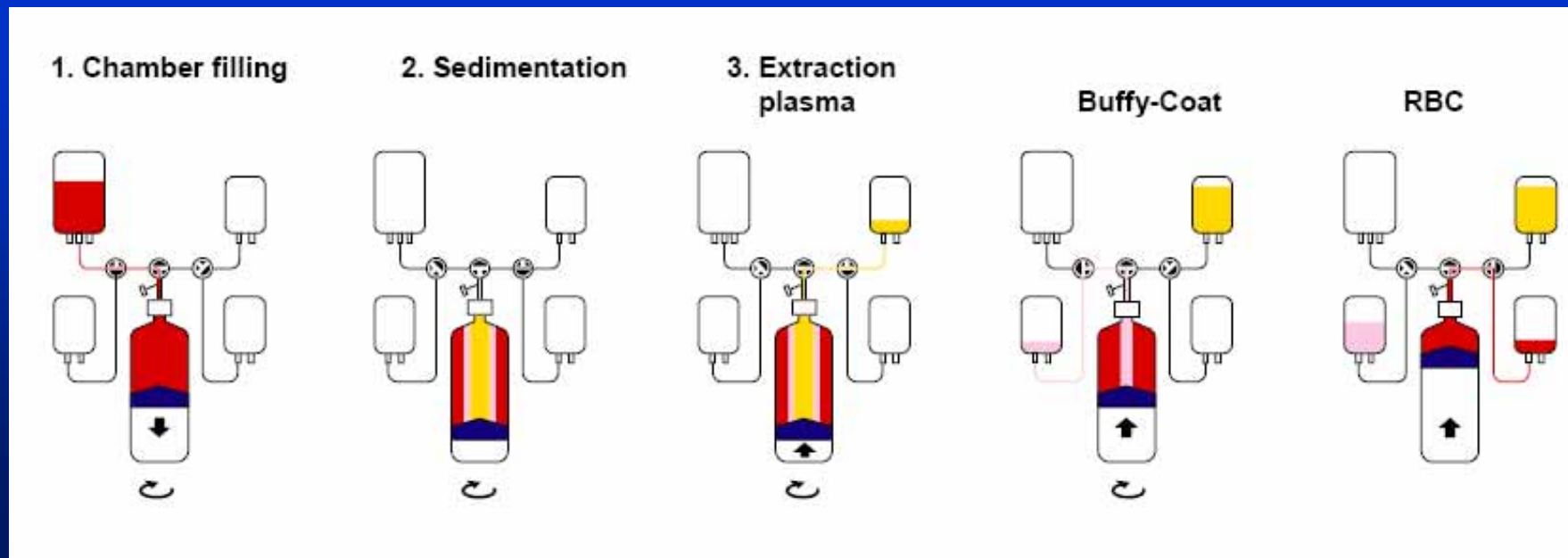
Cord Blood Processing

- Sepax is used by 70% of Netcord cord blood banks
- Sepax is used by over 50% of HRSA cord blood banks (banks funded by US government to increase cord blood inventory)
- Sepax has been used in over 300,000 procedures in 40 countries



Cord Blood Processing

- Automated process and closed system
- Aseptic environment - laminar airflow
- 6% Hydroxethyl starch - red cell removal
- Blood separation through centrifugation - Sepax Processor rotating syringe
- Component transfer effected through displacement of the piston position of the syringe
- Cryogenic bag : buffy coat (enriched CD34+ stem cells and CD45+ nucleated cells)



Quality Assurance

- Processed UCB aliquot – identification, enumeration and viability of CD34+ and CD45+ cells
- Sterility testing of the processed UCB - Bact/Alert® SN Culture bottles
- Viability testing after cryopreservation prior to stem cell transplantation
- DNA and plasma – HLA typing

Cryopreservation

- 10% Dimethyl Sulfoxide (DMSO)
- Kryo 560-16 Controlled Rate Freezer -180°C
- Stored at -196°C in a vapor phase Nitrogen storage vessel MVE



Flow Cytometry

Stem-Kit™ Reagents consists of:

- Two color fluorescent (CD45-FITC, CD34-PE) murine monoclonal antibody reagent
- CD45-FITC, PE isoclonic control
- Nucleic acid 7-AAD viability dye
- Lysing solution (NH₄Cl)

Beckman Coulter Cytomics FC500 Flow Cytometer with CXP System Software

Quality Assurance

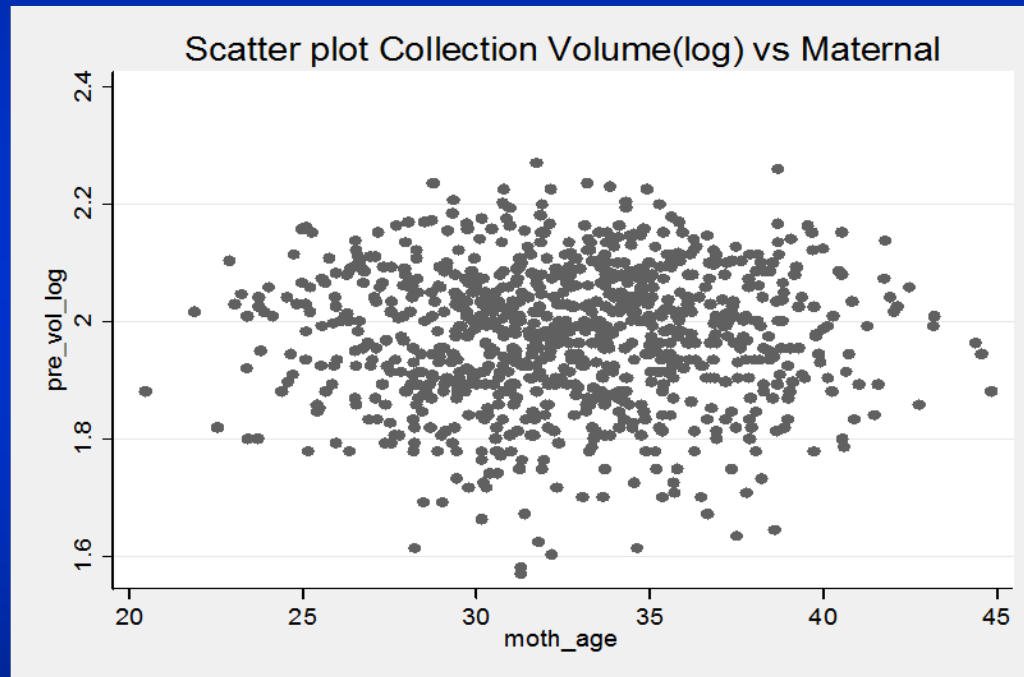
- Flow Check - light scatter and laser alignment
- Colour Compensation - standardize fluorescence intensity
- Stem Trol QC
- External Quality Assurance Program of the RCPA (Royal College of Pathologist of Australia)
- The ISHAGE (International Society of Hematherapy and Graft Engineering) guideline for CD34+ cell determination

Results

Gender	♂♀	♂♀ 95% Conf. Interval	♂	♀	T-test Conclusion
Sample Size	913		492 (53.9%)	421 (46.1%)	♂ > ♀
Mean Collection Volume (ml)	97.8	96.2 - 99.5	97.6	98.1	♂ < ♀
Mean Leukocyte/ml	2.5×10^7	2.4 - 2.6 $\times 10^7$	2.5×10^7	2.5×10^7	♂ = ♀
Mean Leukocyte Total	6.7×10^8	6.3 - 7.1 \times 10^8	5.5×10^8	5.7×10^8	♂ < ♀
Mean CD34+/ml	94,557	88,449 - 100,663	97,407	91,224	♂ > ♀
Mean CD34+ Total	2.5×10^6	2.4 - 2.7 \times 10^6	2.6×10^6	2.5×10^6	♂ > ♀
Mean Leukocyte Viability %	86.6	85.9 - 87.2	86.8	86.2	♂ > ♀
Mean %CD34+ Viability	95.9	95.7 - 96.3	96.1	95.7	♂ > ♀
Mean Maternal age (y)	32.7	32.4 - 32.9	32.5	32.9	♂ < ♀

Results

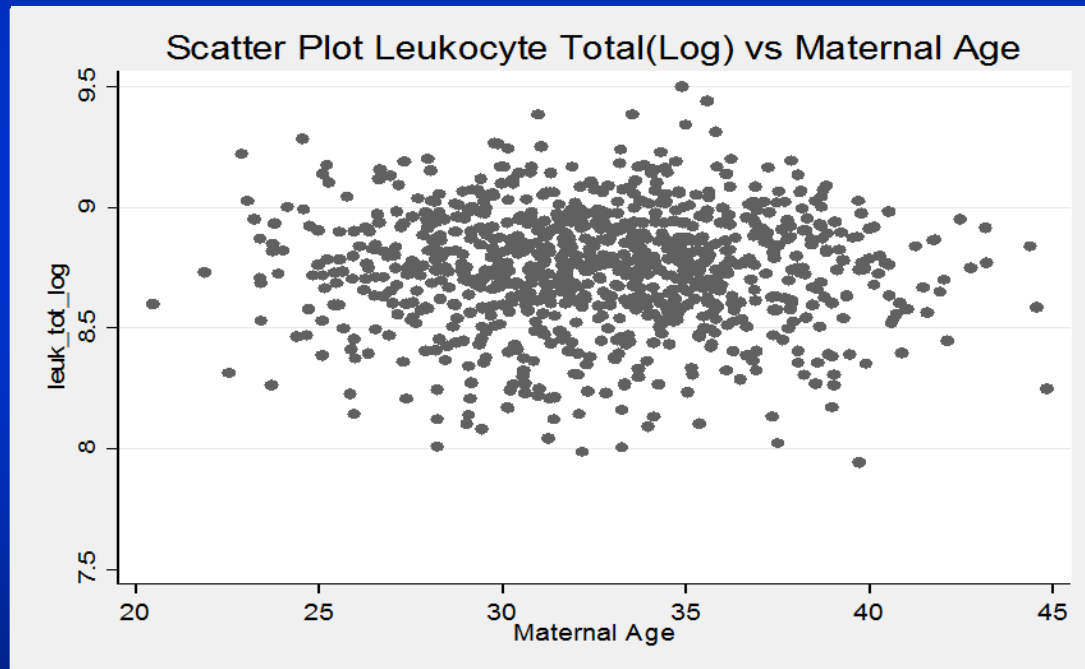
Collection volume and maternal age



No correlation between maternal age and collection volume

Results

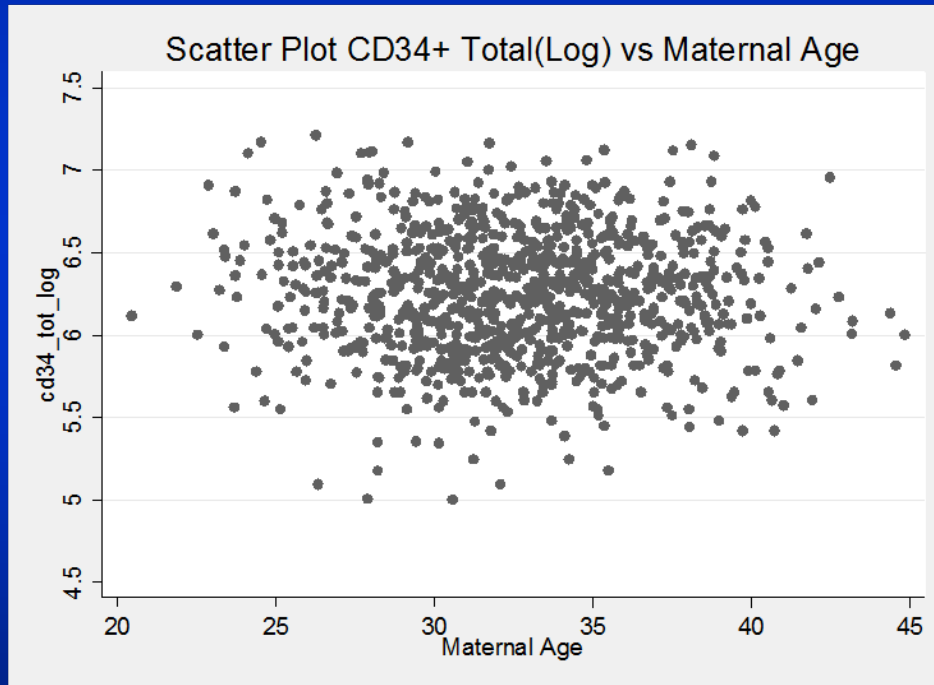
Total leukocytes and maternal age



No correlation between maternal age and total leukocytes

Results

Total CD34+ and maternal age



No correlation between maternal age and total CD34+

Conclusions

- Maternal age has no influence on collection volume, CD34+/CD45+ cell count or viability
- Gender does have an influence on collection volume, CD34+/CD45+ cell count and viability
- Netcells Cryogenics standards and values for collection volume, CD34+/CD45+ cell count and viability compare favourably with UCB banks internationally