# Immunophenotypic evaluation, and physiological and laboratory correlations of hematopoietic stem cells from umbilical cord blood

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**ABSTRACT:** The use of umbilical cord blood stem cells is an efficient alternative for the transplantation of hematopoietic progenitor cells. A number of factors can influence the volume and amount of CD34$^+$ cells, which are considered as immature and capable of proliferation. Quantification of CD34$^+$ cells, evaluation of CD38 and c-kit molecules on these cells, as well as correlations of such factors as maternal age, gestational age, newborn sex and weight, umbilical cord length, placental weight with increased volume and concentration of immature cells, among others, were performed in 70 blood samples from term newborns. The mean volume of umbilical cord blood collected was 53.8±33.6 mL, where 30.96±18.9 CD34$^+$/μL UCB cells were found, of which 16.66±8.32% were CD34$^+$CD38$^-$ cells, and 47.23±24.0% were CD34$^+$CD117$^-$ cells. Newborn weight and placental weight were positively correlated with increased volume of collected UCB. The volume of collected blood was found to affect the absolute count of CD34$^+$ cells and the relative value of these among total nucleated cells, as well as the percentage of CD34$^+$CD117$^+$ and CD34$^+$CD117$^-$ cells. CD34$^+$ cells were positively correlated with leukocytes, and gestational age was negatively correlated with the number of CD34$^+$ cells. Our results confirm the importance of the accurate quantification of CD34$^+$ cells and their subsets, and that many factors may be related to the higher number of hematopoietic stem cells, which are crucial for successful transplantation.

## Introduction

Umbilical cord blood (UCB) represents an alternative source of hematopoietic stem cells (HSC) for use in allogeneic transplantation of patients affected by hematological disorders, inherited immunodeficiencies,

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and metabolic diseases. This source of stem cells has been successfully used to replace bone marrow and apheresis in transplants since the first transplantation using UCB, performed by Gluckman and colleagues *et al.*, in 1989. Since then, the studies have progressed as to the procedures of collection, processing, characterization, quantification, cryopreservation, thawing, and transportation of UCB around the world.

Parameters commonly used to evaluate a UCB unit and predict transplant outcomes have been total nucleated cells (TNCs) and CD34$^+$ cells counts. CD34 anti-

gen is a membrane glycoprotein that characterizes the hematopoietic progenitor cell, along with other markers of the cell surface, such as the presence of the c-kit (CD117) and the absence of molecule CD38. Thus, in several reference centers, marker CD34 has been used to identify hematopoietic stem cells (Mayani and Landsdorf, 1998).

In addition to the presence of CD34, it has been demonstrated that phenotype CD34⁺CD38⁻ characterizes a cell as a candidate of being a "true hematopoietic stem cell". Among the three available sources – bone marrow, peripheral blood and umbilical cord blood – the frequency of CD34⁺CD38⁻ cells is greater in the UCB (De Bruyn *et al.*, 1998). This fact explains the success of its use in transplants, since these cells are responsible for long term graft survival (Larghero *et al.*, 2006).

The aims of the present study were to quantify UCB CD34⁺ cells, with an analysis of expression of CD38 molecules and c-kit. Also, correlations between physiological, hematological and immunophenotypical parameters were determined in order to check for potential influences in the volume of collected blood or in the quantification of hematopoietic stem cells.

## Material and Methods

### Studied samples

UCB samples were obtained by puncture of the umbilical vein of umbilical cords of term neonates (after 37 weeks) born in normal or cesarean delivery and collected into blood bags containing CPDA-1 as anticoagulant. A total of 70 UCB samples were collected at the obstetrics center of the Hospital das Clínicas of Porto Alegre, after signing of informed and free consent form. The blood samples were processed within 24 hours post-collection.

### Hematological Parameters

A UCB aliquot was collected in EDTA for a complete blood count through hematological counter Pentra 120 (ABX Diagnostics), at the Hematology Laboratory.

### CD34⁺ staining

The samples were processed according to Brocklebank and Sparrow (Brocklebank and Sparrow, 2001). CD34⁺ cells values and UCB volume were corrected, using the sample's anticoagulant dilution factor.

**Absolute and relative CD34⁺ cells count:** 100 μL of blood were incubated with 10 μL of conjugated monoclonal anti-CD45 antibody with FITC (clone HI30, Becton Dickinson (BD) Biosciences, San Jose, CA) and conjugated monoclonal anti-CD34 antibody with PE (clone 581, BD Biosciences, San Jose, CA) in Trucount tubes (BD Biosciences) for 20 min at room temperature (23ºC) and in darkness. Subsequently, 2 mL of lysis solution (Quicklysis – Cytognos) and 2 μL of 7AAD (7-Amino Actinomycin D) (Molecular Probes, Eugene) (at a concentration of 1 μg/mL) in DMSO (dimethyl sulfoxide) were added and incubated for 15 min at room temperature (23ºC) and in darkness. The samples were read in flow cytometry within 15 min, at the most, from the termination of incubation, with acquisition of 200,000 CD45⁺ events. All samples were processed in duplicate. **Relative count of CD38 and CD117 cells among CD34⁺ cells:** The control was done using one tube with 100 mL of UCB cells only, and a second tube with cells and isotype control for FITC (anti-mouse IgG1 FITC) and PE (anti-mouse IgG2A PE) (clone X40, X39). A third tube with 100 μL of UCB was incubated with anti-CD34 FITC (clone 8G12, BD, San Jose, CA), anti-CD38 PE (clone HIT2, Pharmingen, BD) and anti-CD45 PerCP (clone 2D1, BD) and, in a fourth tube, 100 μL of UCB was incubated with anti-CD34 FITC, anti-CD117 PE (clone 104D2, BD) and anti-CD45 PerCP. All tubes were incubated in darkness for 25 min at room temperature. Afterwards, 2 mL of lysis solution (Facs Lysing Solution – BD) was added and incubated for another 15 min at room temperature and in darkness. Thereafter the samples were washed twice and re-suspended in 1 mL of PBS with 10% paraformaldehyde. The samples were then read, with acquisition of 50,000 events within the selected gate.

### Acquisition and analysis through flow cytometry

Data acquisition was performed using flow cytometer FACSCalibur (BD) with 488-nm argon laser and the analysis was carried out with CELLQuest 3.1 software (BD). For absolute and percentage CD34⁺ determination the Trucount-ISHAGE gating strategy was used according to Brocklebank and Sparrow. For percentage analysis of CD34⁺, CD38⁻ and CD117⁺ cells, initially the control tube and the tube containing only cells were analyzed in order to define negativity and positivity. Afterwards, a strategy was used that was similar to that of the ISHAGE guidelines, in which the CD45⁺ population was selected and then, among these, we selected those that possessed CD34, and of these

we ascertained those that marked for CD38 or CD117. By analyzing the percentage in the given quadrants we obtained the CD34$^+$CD38$^-$, CD34$^+$CD117$^+$ and CD34$^+$CD117$^-$ cells.

*Correlation between physiological, hematological and immunophenotypical parameters*

A total of 64 correlations was performed by analyzing physiological, hematological and immunophenotypical parameters of UCB. The parameters: volume of UCB, gestational age, newborn (NB) weight, TNC, CD34$^+$, CD34$^+$CD38$^-$, CD34$^+$CD117$^+$ and CD34$^+$CD117$^-$ cells, and number of erythroblasts were all correlated between one another and between the variables: type of delivery, NB sex, cord length, and maternal age. In addition, the number of erythroblasts was related to hemoglobin, MCV, RDW (red blood cell distribution width), while gestational age was correlated with MCV, erythrocytes, neutrophils and lymphocytes, and, finally, NB weight with number of erythrocytes. Of these, we have specified only those presenting significant correlations.

*Statistical analysis*

Groups were compared using the Student's t-test and the evaluation of quantitative variables using Pearson's correlation, using SSPS for statistical analisys with p< 0.05 or 001.

**Results**

Of the 70 UCB samples analyzed, 37 (53%) were obtained in cesarean section and 33 (47%) in vaginal delivery. Concerning newborn sex distribution, 43 infants (61%) were males and 27 (39%) were females. The characteristics of mothers, newborns, UCB samples, and the values obtained for the UCB cell surface markers investigated are presented in Table 1. In 49 of these 70 samples, molecules CD38 and CD117 on CD34$^+$ cells were investigated. There was no significant difference between the samples processed in duplicate concerning CD34 values for a confidence interval of 95% by the Student's t-test for paired samples.

**TABLE 1.**

**Gestational and maternal ages, newborn weight, cord length, placental weight, and collected UCB volume. Quantification of CD34$^+$, CD34$^+$CD38$^-$, CD34$^+$CD117$^-$, and CD34$^+$CD117$^+$ cells**

|  | Number | Mean ± standard deviation | Median | Range |
|---|---|---|---|---|
| Maternal age (years) | 70 | 25.7 ± 6.4 | 25.0 | 16 - 40 |
| Gestational age (weeks) | 70 | 39.1 ± 1.3 | 38.6 | 37 - 42 |
| Newborn weight (g) | 70 | 3,369.1 ± 498.0 | 3,350.0 | 1,930 - 4,530 |
| Umbilical cord length (cm) | 70 | 59.6 ± 15.1 | 55.5 | 35 - 105 |
| Placental weight (g) | 66 | 629.7 ± 169.3 | 600.5 | 300 - 1210 |
| Volume of UCB (mL) | 70 | 53.8 ± 33.6 | 48.0 | 20 - 206 |
| CD34$^+$ (cells/mL) | 70 | 30.96 ± 18.9 | 28.17 | 7.9 - 93.9 |
| CD34$^+$ (%)* | 70 | 0.24 ± 0.14 | 0.22 | 0.06 - 0.65 |
| CD34$^+$CD38$^-$ (%)** | 49 | 16.66 ± 8.32 | 16.58 | 1.44 - 38.39 |
| CD34$^+$CD117$^-$ (%)** | 49 | 47.23 ± 24.0 | 47.12 | 7.67 - 92.11 |
| CD34$^+$CD117$^+$ (%)** | 49 | 52.77 ± 24.0 | 52.88 | 7.89 - 92.33 |

\* Among CD45$^+$ cells     \*\* Among CD34$^+$ cells

The hematological profile of 62 full-term cord blood samples showed (mean±standard deviation): Erythrocytes: 4.37±0.45 x$10^{12}$/L; Hemoglobin: 14.89±1.37 g/dL; Hematocrit 44.83±4.24%; MCV: 102.67±5.16 fL; MCH: 34.13±1.80 pg; MCHC: 33.24±0.84 g/dL; RDW: 14.33±1.29%; Leukocytes: 13.63±3.84 x $10^9$/L; Band Neutrophils: 2.19±3.03%; Segmented Neutrophils: 50.62±10.61%; Lymphocytes 35.77±10.03%; Erythroblasts (N=57): 7.52±10.35 in 100 leukocytes; Platelets (N=33): 268.30±72.93 x$10^9$/L.

Among the 64 correlations investigated, 14 were statistically significant. As the volume, cord length and the variables CD34$^+$, CD34$^+$CD38$^-$, CD34$^+$CD117$^-$, and CD34$^+$CD117$^+$ cells were compared with type of delivery and NB sex, no statistically significant difference was observed between the groups by the Student's t-test for independent samples. As to the quantity of CD34$^+$ and CD34$^+$CD38$^-$ samples, there was no correlation according to Pearson's test.

The use of Pearson's test revealed a correlation between the collected volume of UCB and: the newborn weight, the placental weight, the absolute number of CD34$^+$ cells per microliter of UCB and the relative number of CD34$^+$ cells among CD45$^+$ cells. CD34$^+$ cells per microliter of UCB was negatively correlated with the gestational age, but positively correlated with the absolute number of total leukocytes. A significant positive correlation was found between the relative number of CD34$^+$CD117$^-$ cells and: the volume of UCB, the CD34$^+$ cells, the leukocytes, and the CD34$^+$CD38$^-$ cells. These correlations are shown in figure 1. A significant negative correlation was found between the relative number of CD34$^+$CD117$^+$ cells and: the volume of UCB (r= -0.41, p<0.01), the relative number of CD34$^+$ cells (r = -0.41, p<0.01), the number of leukocytes (r = -0.45, p<0.01), and the relative number of CD34$^+$CD38$^-$ cells (r = - 0.29, p<0.01), (data not shown). All of the other variables investigated were not significantly correlated (data not shown).

## Discussion

UCB has been used as an alternative source of hematopoietic stem cells to that of bone marrow for almost two decades. The characterization of UCB units facilitates the understanding of factors affecting the quality and improvement of transplant outcomes (Aroviita *et al.*, 2005).

The values of hematopoietic stem cells, considered as CD34$^+$ cells, found in the present study were smaller than those reported by Brocklebank and Sparrow (Brocklebank and Aparrow, 2001), who found from 22 to 600 7AAD negative CD34$^+$ cells per microliter of UCB. On the other hand, the results obtained in this study were similar to those reported by Chin-Yee (1997), Barnett (1998), and Pranke (2006), and colleagues, who found 15 to 148, 14 to 63, and 13 to 85 CD34$^+$/μL cells in UCB, respectively. The present study detected 0.24 ± 0.14 CD34$^+$ cells among CD45$^+$ cells, similarly to other studies which found 0.23% (Espínoza *et al.*, 2006), 0.27% (Larghero *et al.*, 2006) 0.28 ± 0.15% (Pranke *et al.*, 2006) of these cells. Differences in the quantification of CD34$^+$ cells may be a result of the heterogeneity proper of cord blood cells as well as of differences between the techniques used by the various groups (Yap *et al.*, 2000).

Furthermore, a number of factors have been described that may influence the quantification of UCB CD34 cells and that may account for the variations in the reported results. Some reports have shown that cesarean delivery provides collection of a higher volume of blood increasing the absolute value of CD34$^+$ cells (Yamada *et al.*, 2000). It has also been shown that factors like newborn weight and sex as well as maternal age can affect the concentration of CD34$^+$ cells (Aroviita *et al.*, 2005; Nakagawa *et al.*, 2004). In the present study, no connection was detected between type of delivery and volume, neither between NB sex and weight or maternal age, and quantity of CD34$^+$ cells, although there was a positive correlation of volume of collected UCB with NB weight, which can thus result in an increase in the absolute number of CD34$^+$ cells. On the other hand, the quantification of CD34$^+$ cells performed without using 7AAD, may be one of the factors responsible for the higher number of CD34$^+$ cells in the UCB reported in other studies.

The percentage of CD34$^+$CD38$^-$ cells found in this study (around 17% of total CD34$^+$ cells) is in agreement with data from a few other authors, such as D'Arena *et al.* (1996), Hao *et al.* (1995) and Timeus *et al.* (1998a), despite the overall great variability of the results reported for these cells, ranging from 2.6% or 3.9% (De Bruyn *et al.*, 1998; Pranke *et al.*, 2005) to around 35% (Almici *et al.*, 1997), among CD34$^+$ cells.

However, the use of 7AAD shows controversial results too when protocols using or not using it are compared. It is known that 7AAD identifies dead or apoptotic cells (Philpott *et al.*, 1996). Cabezudo *et al.* (1999), however, comparing different methods for counting CD34 cells, did not find differences between those
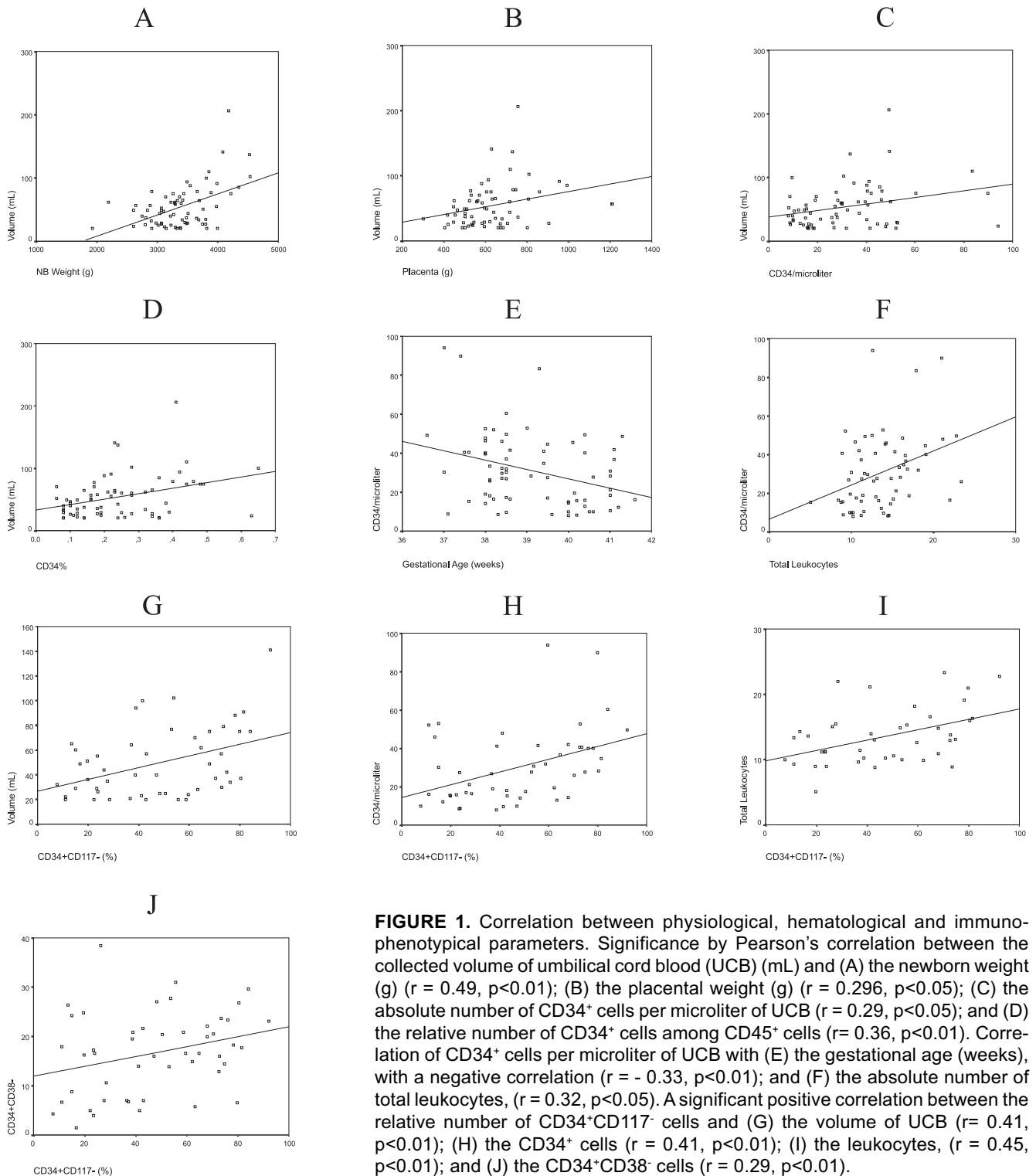
**FIGURE 1.** Correlation between physiological, hematological and immuno-phenotypical parameters. Significance by Pearson's correlation between the collected volume of umbilical cord blood (UCB) (mL) and (A) the newborn weight (g) (r = 0.49, p<0.01); (B) the placental weight (g) (r = 0.296, p<0.05); (C) the absolute number of CD34$^+$ cells per microliter of UCB (r = 0.29, p<0.05); and (D) the relative number of CD34$^+$ cells among CD45$^+$ cells (r= 0.36, p<0.01). Correlation of CD34$^+$ cells per microliter of UCB with (E) the gestational age (weeks), with a negative correlation (r = - 0.33, p<0.01); and (F) the absolute number of total leukocytes, (r = 0.32, p<0.05). A significant positive correlation between the relative number of CD34$^+$CD117$^-$ cells and (G) the volume of UCB (r= 0.41, p<0.01); (H) the CD34$^+$ cells (r = 0.41, p<0.01); (I) the leukocytes, (r = 0.45, p<0.01); and (J) the CD34$^+$CD38$^-$ cells (r = 0.29, p<0.01).

using and not using 7AAD as regards the absolute number of CD34^{+} cells. Nevertheless, notwithstanding these conflicting results and the detection of unviable cells remaining problematic, there are situations in which the use of 7AAD may be justified. In cases where CD34 cells are clinically used, such as in umbilical cord blood banks, the measurement of cell viability through 7AAD is important, since the number of these cells is used to choose an umbilical cord blood sample. Also, in cases where the samples are cryopreserved, as in UCB banks, or after cell cultivation, whose aim is to transplant frozen or expanded cells, cell viability may be much affected (Philpott *et al.*, 1996).

The success of UCB cells transplantation is largely related to the number of TNC and CD34^{+} cells. On the one hand UCB CD34^{+}CD38^{-} cells possess high potential of proliferation and expansion of CD34^{+} cells, suggesting possible advantages concerning the homing and engrafment of more undifferentiated cells (Timeus *et al.*, 1998b).

In the present study the quantity of TNCs was positively correlated with the percentage value of CD34^{+} cells per microliter of blood, as shown in other studies (Nakagawa *et al.*, 2004). A more rapid engraftment and better graft survival are obtained when the cord blood provides at least $2.0 \times 10^7$ TNCs and $2.0 \times 10^5$ CD34^{+} cells per kilogram of the recipient's body weight (Gluckman *et al.*, 2005).

Our results show the existence of a positive correlation between collected volume and number of CD34^{+} cells both per microliter of UCB and in the percentage found among CD45^{+} cells. In a study, where 1,200 donors were evaluated, it was observed that high volume samples were correlated with high doses of TNCs, CD34^{+} cells and colony forming units of granulocytes and macrophages (CFU-GM) (Bradley and Cairo, 2005). In another study, in which 9,205 cases were analysed, factors like newborn and placental weight, umbilical cord length, cesarean section and advanced gestational age were found to significantly influence the volume of collected blood and the number of TNCs (Jones *et al.*, 2003). Our results also indicate a positive correlation of newborn and placental weight with higher collected volume, as reported by some authors (Jones *et al.*, 2003; Nakagawa *et al.*, 2004), but not in the other parameters. Cairo *et al.* (2005) did not observe any association of cesarean delivery with number of TNC, CD34^{+} cells and CD34^{+}CD38^{-} cells, as in the present study. Cairo and colleagues have also shown a positive correlation between the number of erythroblasts with CD34^{+} cells and CD34^{+}CD38^{-} cells, which were not found in the present study.

In our study, we found that the quantity of CD34^{+} cells per microliter of UCB was inversely proportional to gestational age, as reported in several studies by other authors (Gasparoni *et al.*, 2000; Yap *et al.*, 2000).

We also found negative correlations concerning the relative presence of CD34^{+}CD117^{+} cells among the CD34^{+} cells population with the following parameters: volume, total leukocytes, number of CD34^{+} cells per microliter of UCB, and percentage of CD34^{+}CD38^{-} cells. On the other hand, the same parameters presented positive correlations with CD34^{+}CD117^{-}. The CD117 molecule is the receptor of the stem cells growth factor and is involved in the interactions of CD34^{+} cells with stromal and other cells in the bone marrow. Its expression among CD34^{+} cells may range from 58% (Sakabe *et al.*, 1998) to 81% (Gluckman *et al.*, 2005), and among CD34^{+}CD38^{+} and CD34^{+}CD38^{-} cells, the c-kit is expressed in 80±10% and 56±24% of these cells, respectively (Pranke *et al.*, 2005), in the UCB. The findings in the literature, however, are still conflicting as to whether these more primitive, hematopoietic stem cells express this molecule in higher (Williams, 2000) or lower levels (Xiao and Dooley, 2003), or do not express it at all (Sakabe *et al.*, 1998). We can thus suggest that cells presenting CD34^{+}CD117^{-} phenotype, or low expression on the surface, appear to vary with the volume obtained, as well as with the presence of TNC and CD34^{+} and CD34^{+}CD38^{-} cells.

We can thus suggest that, as the volume of collected UCB directly affects the quantity of CD34^{+} cells per microliter of blood and possibly the quantity of total nucleated cells.Research has shown that the UCB may in the future be a source of stem cells to treat several diseases, since investigations into the plasticity of stem cells have demonstrated the importance of these cells in restoring various other organs. Another advantage of using UCB is that, according with some studies, the risk of developing acute and chronic GVHD in UCB recipients with up to two HLA incompatible alleles is similar to that of a recipient of bone marrow from an identical HLA donor (Gluckman *et al.*, 2004; Grewal *et al.*, 2003). Nonetheless, further investigation into the biological properties of UCB cells will make it possible to take full advantage of their potential in transplantation procedures and increase the number of patients who can be successfully treated.

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# References

Almici C, Carlo-Stella C, Wagner JE, Mangoni L, Garau D, Rizzoli V (1997). *Biologic and phenotypic analysis of early hematopoietic progenitor cells in umbilical cord blood*. Leukemia. 11: 2143-2149.

Aroviita P, Terano K, Hiilesmaa V, Kekomäki R (2005). *Cord blood progenitor cell concentration and infant Sex.* Transfusion, 45: 613-621.

Barnett D, Granger V, Storie I, Peel J, Pollitt R, Smart T, *et al* (1998). *Quality assessment of CD34+ stem cell enumeration: experience of the United Kingdom National External Quality Assessment Scheme (UK NEQAS) using a unique stable whole blood preparation*. Br J Haematol. 102: 553-565.

Bradley MB, Cairo MS (2005). *Cord Blood Immunology and Stem Cell Transplantation.* Hum Immunol. 66: 431-446.

Brocklebank AM, Sparrow RL (2001). *Enumeration of CD34+ cells in cord blood: a variation on a single-platform flow cytometric method based on the ISHAGE gating strategy*. Cytometry. 46: 254-251.

Cabezudo E, Querol S, Concelas JA, Garcia J (1999). *Comparison of volumetric capillary cytometry with standard flow cytometry for routine enumeration of CD34+ cells.* Transfusion. 39: 864-872.

Cairo MS, Wagner EL, Fraser J, Cohen G, Van de Vem C, Carter SL, *et al* (2005). *Characterization of banked umbilical cord blood hematopoietic progenitor cells and lymphocyte subsets and correlation with ethnicity, birth weight, sex, and type of delivery: A Cord Blood Transplantation (COBLT) Study report*. Transfusion. 45: 856-866.

Chin-Yee I, Anderson L, Keeney M, Sutherland DR (1997). *Quality assurance of stem cell enumeration by flow cytometry*. Cytometry. 30: 296-303.

D'Arena G, Musto P, Cascavilla N, Di Giorgio, Zendoli F, Carotenuto M (1996). *Human umbilical cord blood: immunophenotypic heterogeneity of CD34+ hematopoietic progenitor cells*. Haematologica. 81: 404-409.

De Bruyn C, Delforge A, Bron D, Brernier M, Massy M, Ley P, *et al.* (1998). *Comparison of the coexpression of CD38, CD33 and HLA-DR antigens on CD34+ purified cells from human cord blood and bone marrow*. Stem Cells. 13: 281-288.

Espínoza LR, Romano BP, Flores AG, Garcia MOG, Armora FC, Argüelles AR (2006). *Instrument and protocol dependent variation in the enumeration of CD34+ cells by flow cytometry.* Transfusion. 46: 530-536.

Gasparoni A, Ciadella L, Avanzini MA, Bonfichi M, Di Mario M, Piazzi G, *et al.* (2000). *Immunophenotypic changes of fetal cord blood hematopoietic progenitor cells during gestation*. Pediatr Res. 47: 825-829.

Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, *et al.* (1989). *Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling*. N Engl J Med. 321: 1174-1178.

Gluckman E, Koegler G, Rocha V (2005). *Human Leukocyte Antigen Matching in Cord Blood Transplantation. Semin Hematol.* 42: 85-89.

Gluckman E, Rocha V, Arcese W, Michael G, Sanz G, Chan KW, *et al.* (2004). *Eurocord Group. Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice.* Exp Hematol. 32: 397-407.

Grewal SS, Barker JN, Davies SM, Wagner JE (2003). *Unrelated donor hematopoietic cell transplantation: marrow or umbilical cord blood?* Blood. 101: 4233-4244.

Hao QL, Shah AJ, Thiemann FT, Smogorzewska EM, Crooks GM (1995). *A functional comparison of CD34+CD38- cells in cord blood and bone marrow*. Blood. 86: 3745-3753.

Jones J, Stevens CE, Rubinstein P, Robertazzi RR, Kerr A, Cabbad MF (2003). *Obstetric predictors of placental/umbilical cord blood volume for transplantation.* Am J Obstet Gynecol. 188: 503-509.

Larghero J, Rea D, Brossard Y, Van Nifterik J, Delasse V, Robert I, Biscay N, Chantre E, Raffoux E, Socie G, Gluckman E, Benbunan M, Marolleau JP (2006). *Prospective flow cytometric evaluation of nucleated red blood cells in cord blood units and relationship with nucleated and CD34+ cell quantification*. Transfusion. 46:403-406.

Mayani H, Landsdorf PM (1998). *Biology of umbilical cord blood-derived hematopoietic stem/progenitor cells*. Stem Cells. 16: 153-165.

Nakagawa R, Watanabe T, Kawano Y, Kanai S, Suzuka H, Kaneko M, *et al.* (2004). *Analysis of maternal and neonatal factors that influence the nucleated and CD34+ cell yield for cord blood banking*. Transfusion. 44: 262-267.

Philpott NJ, Turner AJC, Scopes J, Westby M, Marsh JCW, Gordon-Smith EC, *et al.* (1996). *The use of 7-amino actinomycin D in identifying apoptosis: simplicity of use and broad spectrum of application compared with other techniques.* Blood. 87: 2244-2251.

Pranke P, Hendrikx J, Debnath G, Alespeiti G, Rubinstein P, Nardi N, *et al.* (2005). *Immunophenotype of hematopoietic stem cells from placental/umbilical cord blood after culture*. Braz J Med Biol Res. 38: 1775-1789.

Pranke P, Hendrikx J, Alespeiti G, Nardi N, Rubinstein P, Visser J (2006). *Comparative quantification of umbilical cord blood CD34+ and CD34+ bright cells using the ProCOUNT^TM - BD and ISHAGE protocols.* Braz J Med Biol Res. 39: 901-906.

Sakabe H, Yahata N, Kimura T, Zeng ZZ, Minamiguchi H, Kaneko H, *et al.* (1998). *Human cord blood-derived primitive progenitors are enriched in CD34+c-kit- cells: correlation between long-term culture-initiating cells and telomerase expression*. Leukemia. 12: 728-734.

Timeus F, Crescenzio N, Basso G, Ramenghi U, Saracco P, Gabutti V (1998a). *Cell adhesion molecule expression in cord blood CD34+ cells*. Stem Cells. 16: 120-126.

Timeus F, Crescenzio N, Marranca D, Dall'Aglio M, Fazio L, Di Marco M, *et al.* (1998b). *Cell adhesion molecules in cord blood hematopoietic progenitors*. Bone Marrow Transplant. 22: 61-62.

Williams DA (2000). *Stem Cell Model of Hematopoiesis* in: Hematology - Basic Principles and Practice, Churchill Livingstone. Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, McGlave P (Eds.), pp 126-138.

Xiao M, Dooley DC (2003). *Assessment of cell viability and apoptosis in human umbilical cord blood following storage.* J Hematother Stem Cell Res. 12: 115-122.

Yamada T, Okamoto Y, Kasamatsu H, Horie Y, Yamashita N, Matsumoto K (2000). *Factors affecting the volume of umbilical cord blood collections.* Acta Obstet Gynecol Scand. 79: 830-833.

Yap C, Loh MT, Heng KK, Tan P, Yu SL, Chan SH, *et al.* (2000). *Variability in CD34+ cell counts in umbilical cord blood: implications for cord blood transplants*. Gynecol Obstet Invest. 50: 258-259.