Research article/Raziskovalni prispevek

ANALYSIS OF CORD BLOOD UNITS DONATED TO THE SLOVENIAN CORD BLOOD BANK

ANALIZA POPKOVNIČNE KRVI, DAROVANE V SLOVENSKO BANKO POPKOVNIČNE KRVI

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Abstract

Background *Umbilical cord blood (CB) as well as bone marrow has been used to treat a variety of haematopoietic disorders. The most important factors that influence the outcome of haematopoietic stem cell transplantations are the HLA match and the number of transplanted nucleated cells. The aim of our research was to analyse CB units donated to the Slovenian cord blood bank.*

Methods *The concentrations of nucleated cells (NC) and haematopoietic stem cells (CD34+ cells) in CB were measured with a haematology analyser and flow cytometry. Growth of colony-forming cells (CFC) was determined in cell culture. The results were statistically evaluated using the Pearsons correlation analysis.*

Results *The median NC concentration in CB was 10.6 × 10^9/L (range: 4.07–26.2 × 10^9/L), and the median number of NC was 721 × 10^6 (221–2157 × 10^6). The median collected volume of CB including 21 mL of anticoagulant was 73 mL (32–150 mL). The median concentrations of CD34+ cells and CFC were 35.5/μL (6.6–142.8/μL) and 17.7/μL (2.2–55.9/μL), respectively. The ratio of CD34+/CFC was 1.9 (range, 1.0–13.6), with a statistically significant positive correlation coefficient of 0.87. While there was a good correlation between the concentrations of NC and CD34+ cells, no correlation was observed between those concentrations and the collected volume of CB.*

Conclusions *Of donated CB units, 46 % contained more than 750 × 10^6 nucleated cells and fulfilled the criteria for CB storage in our bank.*

Key words: *cord blood; haematopoietic stem cells; nucleated cells; CD34+ cells; public cord blood bank*

Izvleček

Izhodišča *Popkovnična kri kot tudi kostni mozeg se uporabljata kot vir krvotvornih matičnih celic (KMC) za zdravljenje nekaterih krvnih bolezni, levkemij, limfomov, plazmacitoma, aplastične anemije, imunskih pomanjkljivosti in presnovnih bolezni. Najpomembnejša dejavnika, ki vplivata na uspešnost presaditve sta število presajenih KMC in ujemanje v antigenih HLA med darovalcem in prejemnikom celic. Javne banke popkovnične krvi obdelujejo in shranjujejo darovano nesorodno popkovnično kri ter zagotavljajo celične pripravke za zdravljenje bolezni, ki potrebujejo presaditev KMC. Začetki nastajanja javnih bank (New*

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York, Milano, Düsseldorf) segajo v leto 1992 ali 3 leta po objavi prve uspešne presaditve KMC iz popkovnične krvi otroku z aplastično anemijo. V Sloveniji smo jo odprli v aprilu 2008 na Zavodu RS za transfuzijsko medicino v Ljubljani, kjer deluje v okviru Enote za shranjevanje popkovnične krvi (ESPOK). V tej raziskavi smo ovrednotili popkovnično kri, ki smo jo v preteklem letu sprejeli v našo banko.

Metode *Koncentracijo jedrnih in krvotvornih matičnih celic v popkovnični krvi smo določili s hematološkim analizatorjem in pretočnim citometrom. Krvotvorne celice, ki in vitro tvorijo kolonije (CFC), smo določili v celičnih kulturah v gojišču Methocult. Rezultate smo statistično ovrednotili in izračunali Pearsonovo korelacijo in frekvenčno porazdelitev števila jedrnih celic v popkovnični krvi.*

Rezultati *Mediana koncentracije jedrnih celic v popkovnični krvi je bila 10,6 × 10^9/L (4,07–26,2 10^9/L) in skupnega števila jedrnih celic 721 × 10^6 (221–2157 × 10^6). Mediana volumna zbrane popkovnične krvi z 21 mL antikoagulanta je bila 73 mL (32–150 mL), koncentracije celic CD34+ 35,5/µL (6,6–142,8/µL) in CFC 17,7/µL (2,2–55,9/µL). Popkovnično kri so zbrali, preden se je izločila posteljica. Mediana razmerja CD34+/CFC je bila 1,9 (1,0–13,6). Med seboj sta bili pozitivno povezani koncentraciji jedrnih celic in celic CD34+, ki pa nista bili povezani z volumnom odvzete krvi. Mediana razmerja CD34+/NC je bila 0,28 % in ni bila povezana s koncentracijo jedrnih celic. Absolutno število celic pa je bilo močno odvisno od volumna krvi (n = 100, r = 0,77, P < 0,0001).*

Zaključki *V banko smo v povprečju shranili 46 % enot darovane popkovnične krvi, tj. le tiste, ki so vsebovale več kot 750 × 10^6 jedrnih celic, kar zadostuje za zdravljenje prejemnika s KMC, ki ima največ 25 kg, oziroma več, če enota vsebuje še več celic. V povprečju smo zavrgli 54 % enot. Povprečen volumen popkovnične krvi je v Sloveniji manjši od količine, ki jo odvzamejo v Španiji ali Italiji.*

Ključne besede *popkovnična kri; krvotovorne matične celice; jedrne celice; celice 34+; javna banka popkovnične krvi*

Introduction

Cord blood (placental blood) has been used since 1988 as a source of stem cells for haematopoietic cell transplantation in paediatric, and later in adult, patients to treat acute and chronic leukaemia, lymphoma, myeloma and several non-malignant diseases such as aplastic anaemia and severe combined immunodeficiency and metabolic diseases.[1,2] Public CB banks process and store donated unrelated CB. The first public CB banks were established in 1992 in New York, Milan and Düsseldorf. Storage of related CB started at the Blood Transfusion Centre of Slovenia in 1999[3,4] and the Slovenian public cord blood bank was started at the same institution in April 2008. More than 347,000 units of CB have been stored in public CB banks worldwide, and more than 3,938 children, plus 4,133 adults have received grafts from these banks during the years 1988 through 2009, as reported by Bone Marrow Donors Worldwide and NetCord.[5,6] The most important factors that determine the fate of engraftment are the total nucleated cell dose and the HLA match between donor and recipient. A high cell dose significantly increases post-transplant survival. A cell dose of 2 × 10^7 NC/kg of recipient body weight at infusion (3 × 10^7 NC/kg at collection of CB) is estimated to be the minimum for successful engraftment.[2] Haematopoietic stem cells can be cultured *ex vivo* in a medium containing haematopoietic growth factors, where they proliferate in colonies and could be enumerated and identified as colony-forming cells (CFC).

In this study we determined the volume, NC, CD34+ cell count and CFC in CB, and investigated the relationships between these parameters. The frequency distribution of NC concentration and total number of NC of cord blood was examined.

Methods

CB collection

CB was collected from consenting mothers using the standard technique with the placenta *in utero* during vaginal or Caesarean delivery. Blood was collected by gravity into a cord blood collection bag (MacoPharma, France) containing 21 mL of anticoagulant CPD (citrate/phosphate/dextrose) and transferred to the Slovenian public CB bank.

Cell count and CB volume

All cell counts were performed within 48 hours of CB collection. The total volume of CB including 21 mL anticoagulant was estimated by subtracting the tare weight of the bag and then dividing the resulting value by blood density (1.050). The concentration of NC was measured by using an automated haematology analyser (Cell-DYN 3200, Abbott) without correction for nucleated red blood cells. CD34+ cells were enumerated by flow cytometry using the ISHAGE gating protocol[7] and a stem cell CD34+ enumeration kit (Immunotech Beckman Coulter) The performance of the

analyses was evaluated two times a year in United Kingdom National External Quality Assessment Schemes for Leucocyte Immunophenotyping (UK NEQAS) and INSTAND (Germany) with very good results.

Colony-forming assay

The colony-forming assay was performed with MethoCult medium (StemCell Technologies, Canada) as instructed by StemCell Technologies. The assay was set up in duplicates in 35 mm culture dishes. CB was diluted (1:100) in a 0.9 % NaCl *solution,* and 0.3 mL of cell suspension was transferred into 3 mL of MethoCult medium and plated into two dishes. After 14 to 16 days of incubation at 37 °C with 5 % CO_2, the colonies on the culture dishes were enumerated.

Statistical analysis

Data analysis was performed with GraphPad Prism 5.00 for Windows, GraphPad Software (San Diego, CA, U.S.A.). Correlations were evaluated with Pearson's correlation. A two-sided P value < 0.05 was considered significant.

Results

Nucleated and CD34+ cells

Results of the analysed CB parameters are presented in Table 1. The median volume of collected CB containing 21 mL of CPD was 73 mL (n = 100; range, 32–150 mL) and the median NC concentration was 10.6×10^9/L (n = 100; range, 4.07–26.2 × 10^9/L) (Figure 1). No correlation was observed between the NC concentration and either the CB volume (r = 0.07; P = 0.49) (Figure 2) or the percent of CD34+/NC (r = –0.03; P = 0.82) (Figure 3). A value of 26.2 × 10^9NC/L in a 38 mL sample of CB was obtained from a premature twin infant (m = 1400 g) born at 33 weeks' gestation. The median absolute number of nucleated cells per collected CB unit was 721 × 10^6 (n = 100; range, 221–2,157 × 10^6). The frequency distribution (Figure 4) showed that 46 % of the collected units contained more than 750 × 10^6 NC, which is the minimal cell dose for transplantation of a 25 kg patient (3 × 10^7 NC/kg at collection).[2] Only 15 % of the units fulfilled the criteria for a recipient weighing 40 kg.

The median concentration of CD34+ cells was 35.5/µL (n = 76; range, 6.6–142.8/µL). It was positively correlated with the NC concentration (r = 0.30; P = 0.01), but not with the CB volume (r = –0.09; P = 0.42) (Table 2). The median ratio of CD34+/NC was 0.28 % (0.07–1.19). As expected, total cell counts were strongly positively correlated with the collected volume, as N = volume × concentration.

Table 1. *Summary of CB parameters.*

Tab. 1. *Povzetek parametrov popkovnične krvi.*

	n Št.	Mean ± SD Srednja vrednost	Median Mediana	Range Razpon
Volume (with 21 mL CPD) Volumen (z 21 mL CPD)	100	78 ± 24 mL	73 mL	32–150 mL
NC conc. Konc. JC	100	11.1 ± 3.5 × 10^9/L	10.6 × 10^9/L	4.07–26.2 × 10^9/L
NC total number Absolutno št. JC	100	847 ± 396 × 10^6	721 × 10^6	221–2157 × 10^6
CD34+ conc. Konc. 34+	76	41.2 ± 2.8/µL	35.5/µL	6.6–142.8/µL
CD34+/NC CD34+/JC	76	0.32 ± 0.21 %	0.28 %	0.07–1.19%
CFC conc. Konc. CFC	58	20.0 ± 12.2/µL	17.6/µL	2.2–55.9

Legend: CPD (citrate/phospate/dextrose); NC conc. (concentration of nucleated cells); CD34+ (haematopietic stem cells); CFC (colony forming cells).

Legenda: CPD (citrat/fosfat/dekstroza); Konc. JC. (koncentracija jedrnih celic); CD34+ (krvotvorne matične celice); CFC (celice, ki tvorijo kolonije).

Table 2. *Summary of correlations.*

Tab. 1. *Povzetek korelacij.*

	n prevod	Pearson r prevod	P prevod	Correlation prevod
CD34+ conc. vs. volume Konc. CD34+ proti volumnu	76	–0.09	0.42	No Ne
NC conc. vs. volume Konc. JC proti volumnu	100	0.07	0.49	No Ne
NC conc. vs. % CD34+/NC Konc. JC proti % CD34+/JC	76	–0.03	0.82	No Ne
NC conc. vs. CD34+ conc. Konc. JC proti konc. CD34+	76	0.30	0.01	Yes Da
CD34+ vs. CFC CD34+ proti CFC	58	0.87	< 0.0001	Yes Da
NC vs. volume JC proti volumnu	100	0.77	< 0.0001	Yes Da

Legend: NC conc. (concentration of nucleated cells); CD34+ (haematopietic stem cells); CFC (colony forming cells).

Legenda: Konc. JC (koncentracija jedrnih celic); CD34+ (krvotvorne matične celice); CFC (celice, ki tvorijo kolonije).

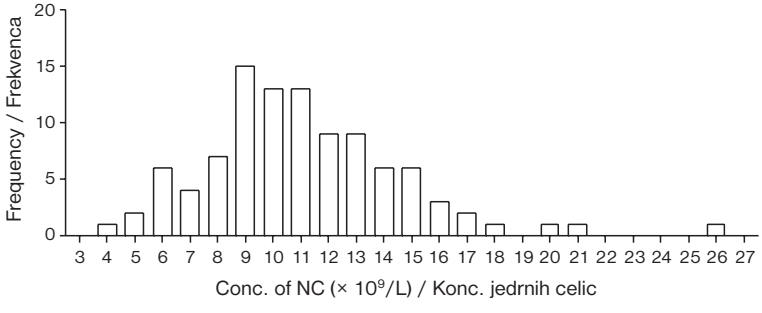

Figure 1. *Frequency distribution of cord blood NC concentration (n = 100).*

Sl. 1. *Frekvenčna porazdelitev koncentracije jedrnih celic v popkovnični krvi (n = 100).*

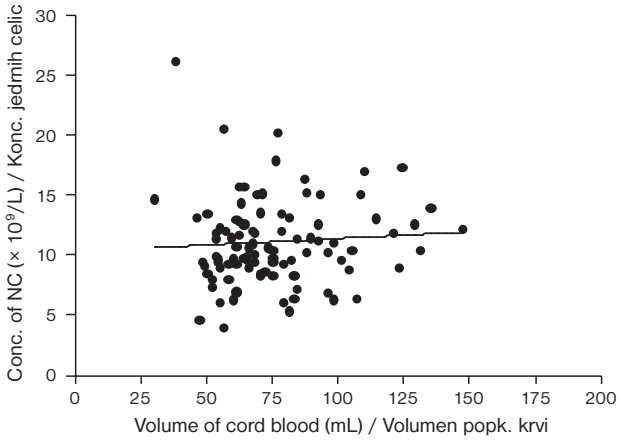

Figure 2. *Correlation between NC concentration and volume of CB (including 21 mL CPD) (n = 100, r = 0.07, P = 0.49). Linear regression curve.*

Sl. 2. *Povezava med koncentracijo jedrnih celic in volumnom popkovnične krvi, skupaj z 21 mL CPD (n = 100, r = 0,07, p = 0,49). Linearna regresijska premica.*

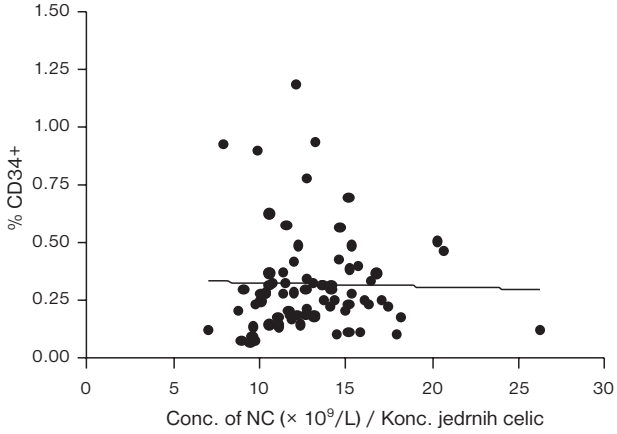

Figure 3. *Correlation between percent of CD34+ and concentration of NC (n = 76, r = –0.03, P = 0.82). Linear regression curve.*

Sl. 3. *Povezava med % CD34+ in koncentracijo jedrnih celic (n = 76, r = –0,03, p = 0,82). Linearna regresijska premica.*

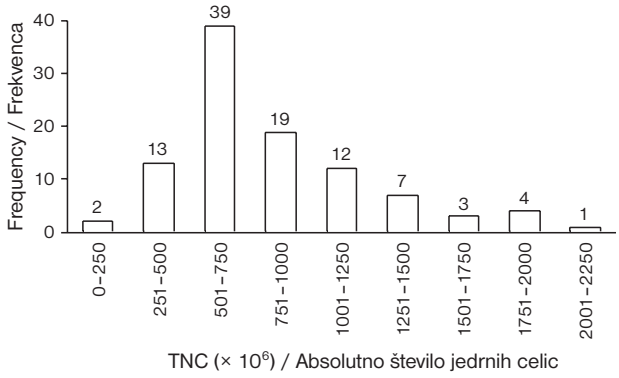

Figure 4. *Frequency distribution of total number of nucleated cells in cord blood units (n = 100).*

Sl. 4. *Frekvenčna porazdelitev absolutnega števila jedrnih celic v popkovnični krvi (n = 100).*

Colony-forming cells

Colony-forming cells were estimated as the sum of granulocyte-macrophage, erythroid and mixed colonies. The median concentration of CFC was 17.6/μL (n = 58; range, 2.2–55.9/μL). The median ratio of CD34+cells/CFC was 1.9 (range, 1.0–13.6), with a strongly positive correlation coefficient of 0.87; P < 0.0001 between the number of CD34+ cells and CFC. On average, every second CD34+ cell formed a colony. A ratio value of 13.6 was obtained from the sample of the premature twin infant.

Discussion

The Slovenian Act on quality and safety of human tissues and cells for the purposes of medical treatment[8] allows public and private companies to process and store CB. At the moment, four commercial private banks operate in Slovenia. Three of them store blood outside the country. The Slovenian public bank also offers storage of directed CB when a sibling has a disease treatable by haematopoietic stem cell transplantation and upon the request of a physician.

From April 2008 to April 2009 our bank collected 190 unrelated units of CB. Eighty-seven units, which contained more than 750 × 10^6 nucleated cells, were processed, cryopreserved and stored. The data of cell concentrations and correlations presented in this study were comparable with previously published data.[9–12] The only exception was the correlation between the NC concentration and the ratio of CD34+/NC, which we found not to be correlated, contrary to the results published by Solves.[13] The median CB volume collected in Slovenia was 52 mL, less than the volume reported from Milan (87 mL, personal communication with Dr. P. Rebulla), from the Madrid bank (81.7 mL),[14] and from Jerusalem (76 mL).[15] The collection technique was the same, with the placenta *in utero.* Assuming that the average weight of a newborn is similar throughout Europe (including Slovenia) and that the volume of CB is positively correlated with the newborn's weight,[9, 11, 16] we believe that the smaller volume of collected CB may be the consequence of slight differences in the collection technique.

In this paper, we showed the frequency distribution of the total number of nucleated cells in collected CB units donated to our public bank. We estimate that the same distribution could be found in private banks, where CB units with low and high numbers of nucleated cells are stored. Parents are often misled by private banks, which do not inform them that the applicability of the units depends on the number of nucleated cells. It is important to provide complete information to parents who are making the decision whether to donate CB to a public bank or pay around EUR 1,800 to store blood in a private bank for 20 years. Only 8 % of units are suitable for recipients weighing 50 kg (e.g. a 20-year-old person), and only 37 % of units reach the target dose for a 30 kg child. Several professional associations have expressed their concerns about private cord banking for autologous use.[17–19]

Conclusions

Public CB banking plays a crucial role in unrelated haematopoietic cell transplantations. In order to provide more units that are suitable for older or heavier patients, the CB collection technique needs to be improved. Additional training of obstetric personnel and determination of obstetric inclusion criteria for CB donation is necessary to pave the way for collection of units with larger volumes. Increased volumes will translate into more cells and more life.

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