

Predictors of Cord Blood Unit Cell Content in a Volume Unrestricted Large Series Collections: a Chance for a Fast and Cheap Multiparameter Selection Model

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Abstract

Background

Cord blood plays a very important role in stem cell transplantation and therapy, with an emerging implication also in regenerative medicine. The number of cells available in a single cord blood unit (CBU), in particular the CD34 + and total nucleated cell (TNC) content, influences the transplantation clinical outcome. We analysed a very large series of CBUs, collected for private banking without any specific volume restriction, to deeply investigate the best predictors of cord blood stem cells content.

Methods

Maternal and neonatal clinic laboratory data of a total 2583 UCBs were obtained from the InScientiaFides cord blood bank based in Republic of San Marino. Univariate and multivariate analysis were conducted to better interpret the data and to build a predictive model to select, the CBU with high CD34 + content.

Results

Our univariate analysis show that seasonality and the geographical area affect the quality of umbilical cord blood. Gestational age, baby's gender and birth weight have a positive correlation with CB TNC content. The baby's birth weight affect positively also CD34 + content and CBU volume while the cesarean delivery affect the CB volume only. Our predictive model, based on multivariate analysis, shows that male baby's, gestational age lower to 39 weeks, cesarean delivery and CBUs with a content of TNC higher than $3,44 \times 10^8$ (group A) have a significant higher CD34 + content than group B (female baby's, gestational age higher than 39 weeks and vaginal delivery). The group A have a 37,5% of CBUs with a concentration of CD34 + $> 2 \times 10^6$, while no CBUs with high concentration of CD34 + were detect in group B.

Conclusion

This study, conducted on a very large series of CBUs without any specific volume constraint, highlighted the prenatal and maternal factors that significantly influence the quality of the CBU collected. Specifically, it highlights that volume is not the best predictor of CD34 + CBU content; for this reason it cannot be taken into consideration alone for the analysis of the collected samples. Our final aim is to identify relevant factors, immediately available, that help to choice UCB with high CD34 + cell content, especially in simultaneous deliveries.

Background

The first cord blood (CB) transplant for hematopoietic reconstitution, occurred 33 years ago in France for a child with Fanconi anemia [1] and represented the groundwork for CB positioning as alternative source of stem cells. Nowadays, CB plays a very important role in stem cell transplantation and cell therapy, with an emerging implication also in regenerative medicine. Recent studies showed how umbilical stem cells exerted therapeutic effects also for the treatment of autism [2], type 1 diabetes [3], acquired sensorineural hearing loss in children [4] and cerebral palsy [5]. Beside the well-known advantages related to the use of CB, this source of hematopoietic stem cells (HSC) contains a higher proportion of primitive HSC, resulting in longer lasting potency and long-term reconstitution [6]. Clinical outcomes after cord blood stem cell transplantation are influenced by the number of cells available in a single cord blood unit (CBU), in particular of CD34+, CD45+ and total nucleated cell (TNC). Some national networks or public banks have adopted a fixed threshold for CB volume to determine the best CBU: most institutions for example discard units with a volume lower than 60 to 80 mL. Actually, is a matter of debate whether the volume of a cord blood is the best predictor for HSC content. In fact, in reports where CB were collected and analysed irrespective of their initial volume, as performed in autologous and family-related context, some smaller collection showed more stem cells than a slightly larger one. For this reason, it is important to widen the analysis of predictive factors to have access to CBU endowed with adequate reconstituting/cellular potential. With respect to TNC content, international institutes adopt a range of CBU eligibility ranging from 5×10^8 to 10×10^8 [7–8]. In recent years, several private structures have started cord HSC programs of conservation for autologous use (or extended for family use) for future treatments. In this case, the retention policies are less stringent. In unrelated allogeneic setting, recent reports indicate that a dose ranging for 1.0×10^5 to 1.2×10^5 / kg CD34+ cells or higher confers to CBU the capacity to reconstitute stable haematopoiesis after transplantation [9–10]. For autologous CB the safe minimum dose of CD34+ cells is unknown but, due to the lack of histocompatibility diversities, is supposed to be less than that for allogeneic transplantations. Previously, we observed that volume is a less important predictor than TNC for CD34+ cell content and may not be used as a selection criterion [11]. In order to establish a CBU quality, the number of CD34+ and TNC cells in each CBU represent well defined predictors but also maternal and neonatal factors should be evaluated for a more accurate quality prediction of individual CBU, also considering future perspectives where CBU might be used in autologous or family related settings for new therapeutic indications. In this report, we analysed a very large series of CBUs, collected for private banking (autologous and family related), without any specific volume restriction, to deeply investigate the best predictors of stem cells taking also into account some maternal and neonatal factors such as: maternal age, gestational period, mode of delivery (vaginal vs. cesarean delivery), baby's birth weight, infant's gender, birth season, geographical area of birth and child blood group.

Methods

CBU selection and collection

For this study, a retrospective analysis of 2583 processed samples was performed from July 2016 to June 2021 (study sample); 180 CBUs collected from July 2021 to December 2021 were analyzed to test a

predictive model (test sample). The samples were collected in various Italian (birth units) collection points, in both private and public hospitals and subsequently shipped with a dedicated courier for the transport of organs and tissues at a controlled temperature and then processed at the InScientiaFides cord blood bank (CBB) based in Republic of San Marino (accredited FACT-NETCORD on 5 October 2012). All pre and post-natal data, together with those related to the CBU have been made available by the Foundation InScientiaFides (non-profit organization which operates in the Republic of San Marino) that promotes research in the field of stem cell procurement and conservation. All participants provided a written informed consent. Parents were given a kit, complete with all the material necessary for the collection of the CB. The collection kits are produced and packaged in compliance with the Italian Ministry of Health rules for the safety and transport of infected materials and diagnostic samples. The kit components are certified according to directive 93/42/EEC implemented in Italy with legislative decree 46/97. CB collections were performed by trained collection staff using either in-utero or ex-utero collection procedures. At the time of collection, the umbilical cord was stabilized and sterilized with Neoxinal 0.05%; (Nuova Farmec, Settimo di Pescantina (VR), Italy). The umbilical vein was punctured with a 12-gauge needle attached to the collection bag containing 21 ml of CPD anticoagulant (Maco Pharma, Mouvaux, France). The CB was drained by gravity into the collection bag and gently rocked on a rotating scale to ensure adequate mixing. The sample was placed in the refrigerated kit (Dryce, Milan, Italy), validated for maintenance the temperature between 2 and 8°C for at least 60 hours and then sent to the CBB.

The shipping time, on average, was 35 hours 60 minutes, while the entire average processing time was 43 hours 56 minutes, never exceeding 72 hours, as required by the Italian law "State-Regions Agreement April 2011 " and the FACT-NETCORD provisions.

CBU processing

The processing of the samples took place in a clean room (Grade B GMP classification) while the collection bag was opened under a sterile hood in laminar flow conditions. All CBUs were processed by manual method which provides the red blood cell depletion and plasma reduction as previously reported by Kristin M. Page, et al. 2014 [12]. Briefly, the CBU treatment begins with the isolation of the buffy coat (BC) fraction by transferring the bag content in a suitable number of 50 ml tubes (Euroclone, Pero, Italy) using a 50 mL syringe with a G18 needle and subsequently centrifuged at 3500 rpm for 12 minutes with acceleration value = 2 and braking value = 0 in a Eppendorf centrifuge 5804 (Eppendorf SE, Hamburg, Germany). After the separation of the components, the plasma was removed with a 10 ml syringe (RAYS S.P.A., Osimo, Italy) and the BC collected using a pasteur pipette; then, BC was inserted into another 50mL tube until a volume of 20 ml was reached. TNC and CD34 + were enumerated in the BC to determine final cell count recovery. DMSO/Dextran (10% dimethyl sulfoxide in 5% dextran 40, WAK-Chemie Medical GmbH, Steinbach/Ts, Germany) was added as a cryoprotectant and the product was cryopreserved by controlled rate freezing in a 25-mL double-compartment cryopreservation bag (Pall Corporation, Covina, CA). Cryopreserved CBUs were stored in air phase of liquid nitrogen.

CBU TNC count

TNC analyses were performed through an automated haematology system (ADVIA-2120, Siemens Healthcare Diagnostics, Munich, Germany), which is based on peroxidase staining to provide white blood cell (WBCs) counts. Leukocyte count, red blood cells (RBC), hemoglobin (Hb), hematocrit, mean red blood cell volume, average Hb content in red blood cells, red blood cell distribution width, platelets (PLT) count, mean volume of PLT, distribution width of PLT, and nucleated red blood cells were analyzed. Differential white blood cell count included lymphocytes, monocytes, neutrophils, eosinophils, basophils and large unstained cells. CBU's TNC counts were obtained by multiplying the absolute WBC counts by CBU volumes.

CBU CD34 + cell count and viability

Quantification of CD34 + cells was performed with a BD FACSCanto™ II flow cytometer using reagents of BD Stem Cell Enumeration Kit (BD Biosciences, San Jose, CA). This kit uses a combination of CD45 FITC antibodies (clone 2D1), and CD34 PE (clone 8G12). The assay simultaneously enumerates the total viable dual-positive (CD45+/CD34+) hematopoietic stem cells giving an absolute count of CD34 + cells/ μ l and the percentage of viable CD45+/CD34 + cells. Dead cells were excluded from the count by co-staining with 7-aminoactinomycin-D (BD Biosciences, San Jose, CA).

ABO group Rh type confirmation

DiaClon ABD-Confirmation was used for the ABO/RhD blood group control of CB units (DiaMed GmbH, Cressier, France). Briefly, 50 μ l of whole blood has been added to 0.5ml of diluent (DiaMed GmbH, Cressier, France) in a 1.5 mL tube. Subsequently, 12.5 μ l of suspension has been withdrawn and pipetted into the microtubes of the ABD cards containing the monoclonal antibody. After centrifugation, (10min) ABD cards were analysed and blood group determined according to the manufacturer's instructions.

Statistical analysis

For the statistical analysis of predictors (independent variables) for distinct CD34 + and TNC targets (dependent variables), the age of the mother, the type of birth, the age of gestation, the birth weight of the newborn, the volume of CB, the blood group of the newborn, the geographical area of birth, the birth season and the sex were processed. In a first set of analysis, all continuous and dichotomic independent variables were evaluated. Linear regression and one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference post hoc test ($p < 0.05$) were used. Two multivariate analysis models were then conducted using the number of CD34 + and TNC as independent variables, against all other dependent variables using multiple linear regression analyses. Moreover, based on multivariate results for CD34 + content, we built a predictive model stratifying the test samples (see "CBU selection and collection") in two groups: group A (male babies, gestational age lower to 39 weeks, cesarean delivery and CBUs with a content of TNC higher than 3.44×10^8 ; this TNC threshold was chosen considering the median value of the study sample) and group B (female babies, gestational age higher than 39 weeks, vaginal delivery and a count of CBUs TNC lower to 3.44×10^8). Data were analyzed using the Student's t-test to check for a significant difference between groups ($p < 0.05$). All descriptive data were presented as mean, median, range and standard deviation. Univariate and multivariate regression analysis as well as

comparisons by one-way ANOVA and Student's t-test were carried out by Minitab® 16.2 (RMIT University, Melbourne, Australia).

Results

CBU characteristics

The analyses of CBUs collected and processed at InScientiaFides from July 2016 to June 2021 (study sample) demonstrated that the 51.06% of the CBU collected derived from males, while the remaining 48.93% from females. Furthermore, the majority of samples resulted from vaginal (62.95%) compared to 36.43% of caesarean deliveries, respectively, with a median birth weight of 3.17 kg (range, 1.02–5.04 kg). The majority of pregnant women give birth at 39 (median value with a range 30–45) weeks of gestation with a median age of 38 years (range 20–58 years). The demographic Italian analysis showed that 40.81% of the samples come from the north of Italy, while 27.84%, 21.45% and 9.81% from the center, south and islands, respectively. In addition, the birth distribution of our series in the various seasons was 23.23% in winter, 27.91% in autumn, 24.47% in spring and 24.51% in summer (Table 1).

Table 1
Clinical characteristics of maternal and infant CBU.

	N (%)	Median (range)	Mean (\pm SD)
Number of CBU collected	2583 (100%)		
Babies' gender			
Male	1319 (51.06%)		
Female	1264 (48.93%)		
Infant gestational age (weeks)		39 (30–45)	39.09 (\pm 1.34)
Maternal age (years)		38 (20–58)	37.56 (\pm 5.42)
Birth weight (kilograms)		3.17 (1.02–5.04)	3.29 (\pm 1.23)
Type of delivery			
Caesarean	941 (36.43%)		
Vaginal	1555 (62.95%)		
Geographical birth area (Italy)			
South	554 (21.45%)		
Islands	256 (9.91%)		
Center	719 (27.84%)		
North	1054 (40.81%)		
Season of birth			
Spring	632 (24.47%)		
Summer	633 (24.51%)		
Autumn	721 (27.91%)		
Winter	600 (23.23%)		

The clinical characteristics of the samples were the following: the CBUs collected had a median volume of 72 ml (range, 15–205 ml) with a median time to processing from birth to freezing of 43 hours (range, 6–127 hours). After processing, the CBUs median TNC and CD34 + content were 3.44×10^8 (range, $2.40 \times 10^6 - 18.83 \times 10^9$) and 7.69×10^5 (range, $2.40 \times 10^3 - 1.35 \times 10^7$), respectively. Post-process CD34 + viability was, on average, 95.1% while the analysis of CB blood group showed that 44.25% of babies had 0 blood group respect to the 36.93%, 10.84% and 3.25% A, B and AB group, respectively (Table 2).

Table 2
 Characteristics of the processed cord blood units including post-processing cellular content.

	N (%)	Mean (\pm SD)	Median (range)
Number of CBU collected	2583 (100%)		
Collection volume (ml)		74.46 (\pm 26.54)	72 (15–205)
Time to processing from birth to freezing (hours)		43.67 (\pm 14.66)	43 (6-127)
CBU TNC count ($\times 10^8$)		3.86 (\pm 2.14)	
			3.44
			(0.002–88.3)
CBU CD34 + count ($\times 10^6$)		1.14 (\pm 1.18)	
			0.769
			(0.0002- 13.5)
CD34 ⁺ Viability (%)		95.10	
Blood group of babies:			
A	954 (36.93%)		
B	280 (10.84%)		
O	1143 (44.25%)		
AB	84 (3.25%)		

Post process CBU analyses

A first set of single-regression analysis strongly confirms in our large-series CBU (study sample) the correlation previously reported by us [11] between CBU TNC and CD34+ ($r = 0.5368$, $p < 0.001$), TNC and CBU volume ($r = 0.5149$, $p < 0.001$) and between volume and CD34+ ($r = 0.3175$, $p < 0.001$). A regression analysis between CD45 + cells and TNC count was performed to confirm the accuracy of our TNC count, showing high correlation with TNC ($r = 0.8022$, $p < 0.001$) (Fig. 1).

Maternal and prenatal data analyses

Taking into account the gestational age, we found a positive correlation with CBU TNC content ($r = 0.0213$, $p = 0.0001$) while no correlations were found with CD34 + and volume ($r = 0.00008$, $p = 0.65$; and $r = 0.0008$, $p = 0.15$, respectively). No correlations were obtained for TNCs and CD34 + content with mother age ($r = 0.0006$, $p = 0.198$; $r = 0.002$, $p = 0.442$, respectively) while significant was the correlation with

volume ($r = 0.0024$, $p = 0.013$,) and mother age. Then, we confirmed the correlation between birth weight and CD34+ ($r = 0.0772$, $p < 0.005$), also showing significant as well as correlation of birth weight with TNC and volume ($r = 0.1152$, $p < 0.005$ and $r = 0.1024$, $p < 0.005$, respectively) (Fig. 2).

One-way ANOVA analyses showed that child gender affects CBU TNC content while birth modality affects only the CBU volume. Particularly, females ($3.97 \times 10^8 \pm 2.2 \times 10^8$) showed a higher TNC content than males ($3.75 \times 10^8 \pm 2.2 \times 10^8$). Concerning the type of delivery, it influences CBU volume only: cesarean deliveries showed higher volumes ($79.76 \text{ mL} \pm 28 \text{ mL}$) compared to vaginal ($71.06 \text{ mL} \pm 24.47 \text{ mL}$) (Fig. 3).

The blood group of the child did not show any correlation with sample characteristics considering TNC, CD34 + and CBU volume (Data not shown).

Seasonal and geographical data analyses

The birth seasonal analyses demonstrated that the CBU volume collected in winter and autumn ($76.71 \text{ mL} \pm 28.23 \text{ mL}$ for winter and $75.63 \text{ mL} \pm 25.84 \text{ mL}$ for autumn, on average) are more abundant than those collected in the hot months ($71.74 \text{ mL} \pm 24.12 \text{ mL}$ for spring, and $73.82 \text{ mL} \pm 26.11 \text{ mL}$ for summer, on average)(Fig. 4). No significant differences were observed in TNC and CD34 + amount during the seasonal analysis. Finally, the evaluation of Italian geographical area showed (Fig. 4) that samples collected in the middle-south have higher volumes and higher TNC/CD34 + content than those of north area. For CBU collected in islands, we did not observe any significant difference for TNC and CD34 + counts as compared to the rest of Italy, while lower volumes were observed as compared to CBU collected in the middle-south.

Multivariate analyses

In order to determine the best predictor of CBU quality, a multivariate analysis was performed taking into account the influence of maternal, pre and postnatal data, as well as geographical and seasonal data. As highlighted in Table 3, the independent variables explain 71% of the variability of the content of TNC ($p = 0.000$).

Table 3
Clinical characteristics predictive of TNC content in multiple regression analyses.

Donor-related variables	Standardized Coefficients β	t	p -value
SEX ^c	-0.06	-6.08	0.000
GESTATIONAL AGE	0.15	14.01	0.000
DELIVERY METHOD ^d	0.10	8.30	0.000
VOLUME	0.45	35.16	0.000
GEOGRAFICAL AREA ^e	0.00	0.13	0.899
CD34+	0.49	37.74	0.000
SEASON ^f	0.00	-0.27	0.790
MOTHER AGE	0.01	0.71	0.478
BIRTH WEIGHT	-0.01	- .48	0.633
Significant correlation are shown in bold.			

c Female as the reference group

d Cesarean delivery as the references group

e South as the references group

f Winter as the references group

The content of CD34+ ($p = 0.000$) and volume ($p = 0.000$) are the main factors that positively influenced the CB TNC content, as well as delivery method ($p = 0.000$), gestational age ($p = 0.000$) and baby's gender ($p = 0.000$), (vaginal higher than cesarean, higher gestational age higher than lower, females higher than males). The independent variables considered in multiple regression explain 56% of the variability of the CD34 + content ($p = 0.000$). TNC count represents the most important variable that influenced the CD34 + cells. The gestational period had negative correlation with the CD34 + cell content ($p = 0.000$). Concerning type of delivery ($p = 0.004$) and baby's gender ($p = 0.000$), we found that cesarean delivery and male infants increase the CD34 + cells content (Table 4).

Table 4
Clinical characteristics predictive of CD34 + content in multiple regression analyses.

Donor-related variables	Standardized Coefficients β	<i>t</i>	<i>p</i> -value
SEX ^c	0.07	5.14	0.000
GESTATIONAL AGE	-0.09	-6.23	0.000
DELIVERY METHOD ^d	-0.04	-2.91	0.004
VOLUME	0.02	0.91	0.362
GEOGRAFICAL AREA ^e	-0.01	- .91	0.364
TNC	0.74	37.01	0.000
SEASON ^f	0.00	-0.21	0.831
MOTHER AGE	-0.02	-1.32	0.189
BIRTH WEIGHT	0.02	1.48	0.140
Significant correlation are shown in bold.			

c Female as the reference group

d Cesarean delivery as the references group

e South as the references group

f Winter as the references group

In order to validate our results, a predictive model based on results of our multivariate analyses was built using the CBUs data collected from July to December 2021 (test samples; see Materials and Method). *T*-*student* test results highlighted that CD34 + content of group A (male babies, gestational age lower to 39 weeks, cesarean delivery and CBUs with a content of TNC higher than 3.44×10^8) was significant higher than group B (female babies, gestational age higher than 39 weeks, vaginal delivery and a count of CBUs TNC lower to 3.44×10^8) (t-value = 7.18; $p < .00001$); in addition 37.5% of CBUs had a CD34 + content $> 2 \times 10^6$ in group A, while no CBUs with a content of CD34 + $> 2 \times 10^6$ was detected in group B (Table 5).

Table 5
Application of the predictive model on the test CBU sample.

	GROUP A (± SD)	GROUP B (± SD)	<i>p-value</i>
N°	36	144	-
CD34 + mean (x 10⁶)	1.63 (± 0.78)	0.44 (± 0.27)	< .00001
Frequency of CB with CD34 + > 2x10⁶	37.5%	0%	-
Comparison of CBU of group A (male babies, gestational age lower to 39 weeks, cesarean delivery and CBUs with a content of TNC higher than 3.44x 10 ⁸) versus group B (female babies, gestational age higher than 39 weeks, vaginal delivery and a count of CBUs TNC lower to 3.44x 10 ⁸).			

Discussion

To date, the success of a transplant strongly depends on the injected cell dose. CB stem cells have a several strengths: immediate availability, low risk of graft versus host disease and an adequate level of hematopoietic progenitor cells. For these reasons, cord blood represents an alternative source to bone marrow and peripheral stem cells, albeit with a smaller quantity of cells. Investigators suggest some ways to solve this problem: (i) screening and selection cell-rich CB for public banks, (ii) choosing the optimal methods to collect, (iii) increasing the recovery rate of CB in processing, and (iv) promoting an ex vivo expansion of CB stem cells. Numerous studies have been carried out with the aim to identify the factors that influence the cord blood sampling and therefore the content of stem cells. In this retrospective study, we examined a very large series of CBUs, collected in Italy for private banking without any specific volume restriction, to deeply investigate the best predictors of stem cell content, taking into account some maternal and neonatal factors such as: maternal age, gestational period, type of delivery, baby's birth weight, infant's gender, birth season, geographical birth area and child blood group. Worldwide, CB banks adopt multiple criteria of selection, including TNC count and volume of collected CBUs. The threshold for volume ranges between 60 and 80 mL: in this way, only CBUs with a higher volume were analysed and enrolled based on the number of TNCs. Actually, higher CB volumes appear as a good predictor of CD34 + cell content in collection series with a volume higher than 80 mL, also in multivariate analysis [12]. However, for a better understanding of independent factors influencing CB TNC/CD34 + cell content in large series, CB volume restrictions or stratifications during analysis could affect or weaken the impact of all analysed variables. In a first set of analyses, we performed univariate linear regression that shows as several maternal, obstetric, and neonatal factors were related to the TNC and CD34 + content of CBU. This data show a significantly higher TNC count in heavier babies. In particular, the increase of one kg of baby weight seems to increase of around 7.66x 10⁷ CD34 + content, of 19.5 mL volume and of 2.00x 10⁸ TNC [13–14]. Like Sparrow et al. [15], we report a significantly higher CB volume in caesarean compared to vaginal deliveries. On the contrary, delivery modality does not influence the content of TNC and CD34+, at variance with univariate results reported by Mohyeddin et al.

in a very small series where vaginal deliveries lead to an increase in TNC content [14]. In accordance with our findings, Ballen et al. [16] showed that newborns with longer gestational age had higher TNC counts but lower CD34 + cells at univariate regression analyses. Our univariate data also show that the female babies had more TNC content than male babies. A very slight correlation between mother age and the CBU volume was observed, even though age does not affect the CBU TNC/CD34 + content. In our univariate analyses, the UCB collected in the months of winter and autumn have higher volumes than those collected in spring and summer. We also demonstrated that the samples collected in central-southern Italy have higher CBU volumes and higher TNC/CD34 + content than those of northern Italy, while for CBUs collected in Italian islands no significant differences were observed for TNC and CD34 + content. The relations between obstetric and neonatal factors and CBU cell composition were further investigated on a multiple regression analyses. By the multivariate approach, gestational age, CB volume, babies' gender and type of delivery show a significant positive influence on the TNC content accordingly to Wen et.al [13]. Recent reports indicate that a dose of 1.0×10^5 or 1.2×10^5 / kg CD34 + cells or higher confers to CBU the capacity to reconstitute stable haematopoiesis after transplantation [9–10]. Hence, considering the relevance of CD34 + for engraftment, we focused our attention on relations between them and obstetric and neonatal factors. In reference to this, male babies, short gestational age and cesarean delivery significantly increase CD34 + UCB content in our multivariate approach. Thus, a predictive model based on these criteria was applied on a second CBU sample (test sample) to assess its ability to select CBU with high CD34 + cell content. As a result, we found that group A of CBU including male babies, gestational age lower than 39 weeks, cesarean delivery had a very higher CD34 + content as compared to group B (female babies, gestational age higher than 39 weeks and vaginal delivery).

Conclusion

This study, conducted on a very large series of CBUs without any specific volume constraint, highlighted the prenatal and maternal factors that significantly influence the quality of the collected UCBs. Specifically, it demonstrated that volume does not represent the best predictor of CBU quality and, for this reason, it cannot be taken into consideration alone for the choice of the CB sample to be collected. Our final aim is to identify relevant factors, immediately available, that help to choice UCB with high CD34 + cell content, especially in simultaneous deliveries. Thus, the best scenario for UCB collection is male baby, a gestational age < 39 weeks, a caesarean delivery and the cheap and fast evaluation of a TNC content high than 3.44×10^8 .

Abbreviations

CBU

Cord blood unit

TNC

total nucleated cell

CB

cord blood
HSC
hematopoietic stem cells
CBB
cord blood bank
BC
buffy coat
WBC
white blood cell
RBC
red blood cells
PLT
platelets

Declarations

Ethics approval and consent to participate

All participants provided written informed consent prior to the data analyses. Consent for children data analyses were provided by parents/guardians.

Consent for publication

All authors contributed to the manuscript and approve its submission.

Availability of data and materials

The datasets used and/or analysed during the current study are available on request, by contacting the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SF, LP collected the clinical data. SF, AL, GL, PL, PL, contributed to data analysis. SF, LP analyzed results, planned the study and wrote the manuscript. All authors critically revised the manuscript and approved the final version.

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Figures

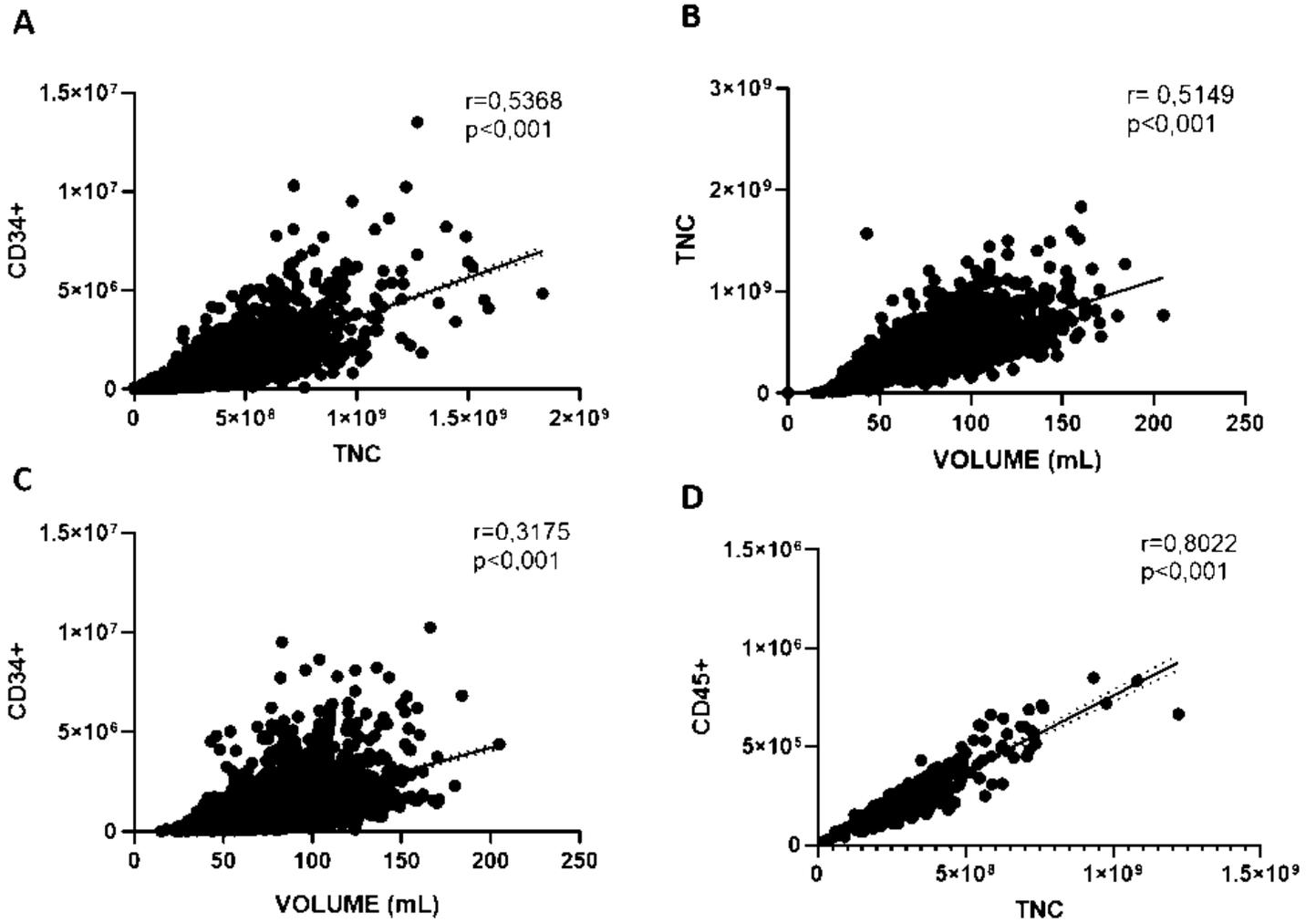


Figure 1

Correlation among the UCB characteristics (N=2583). Significant positive correlations were observed between (A) CD34+ and TNC ($r=0.5368$; $p<0.001$); (B) volume and TNC ($r=0.5149$; $p<0.001$); volume and CD34+ ($r=0.3175$; $p<0.001$). (D) Positive correlation observed among the TNC content and CD45+ ($r=0.8022$; $p<0.001$).

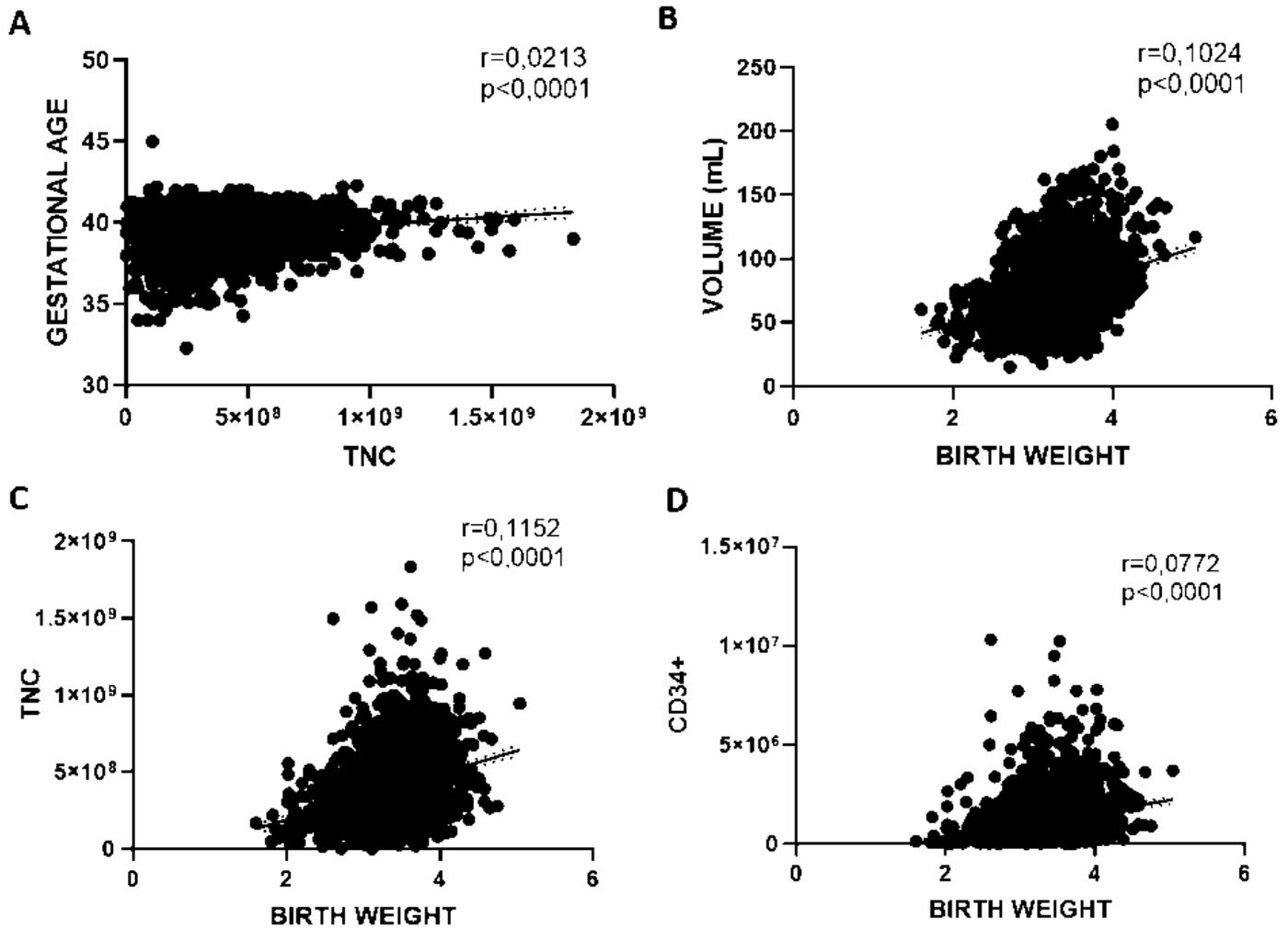


Figure 2

Correlation among the UCB characteristics (N=2583) and obstetrics and neonatal factors. Significant positive correlation were observed between (A) TNC and gestational age ($r= 0.0213$; $p<0.0001$); birth weight and volume (B) ($r=0.1024$; $p<0.0001$); TNC (C) ($r= 0.1152$; $p<0.0001$) and CD34+ (D) ($r= 0.0772$; $p<0.0001$).

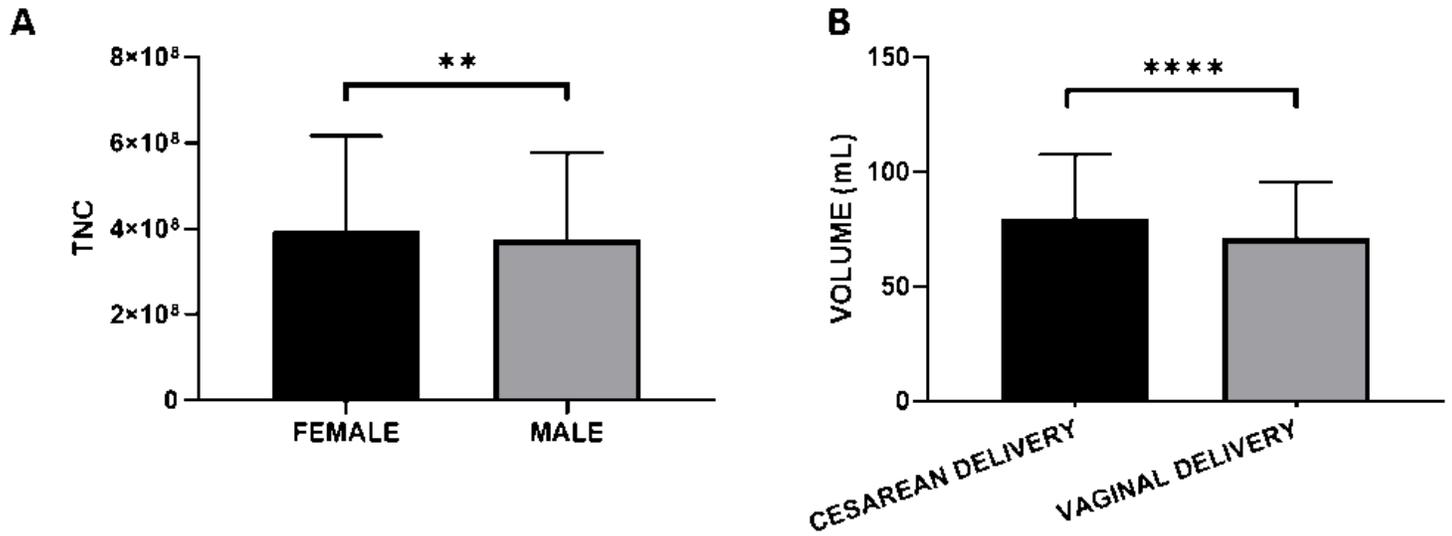


Figure 3

Relation between babies gender and type of delivery with UCB volume and TNC. One-way ANOVA analysis (using Tukey Method post hoc test) showed that child gender affects TNC content (A) while birth modality affects the volume (B). Particularly females showed a higher TNC content than males (A) and the cesarean delivery (B) showed more abundant volumes compared to vaginal. All reported values are expressed as mean ± standard deviation (**p<0.005; **** p<0.0001).

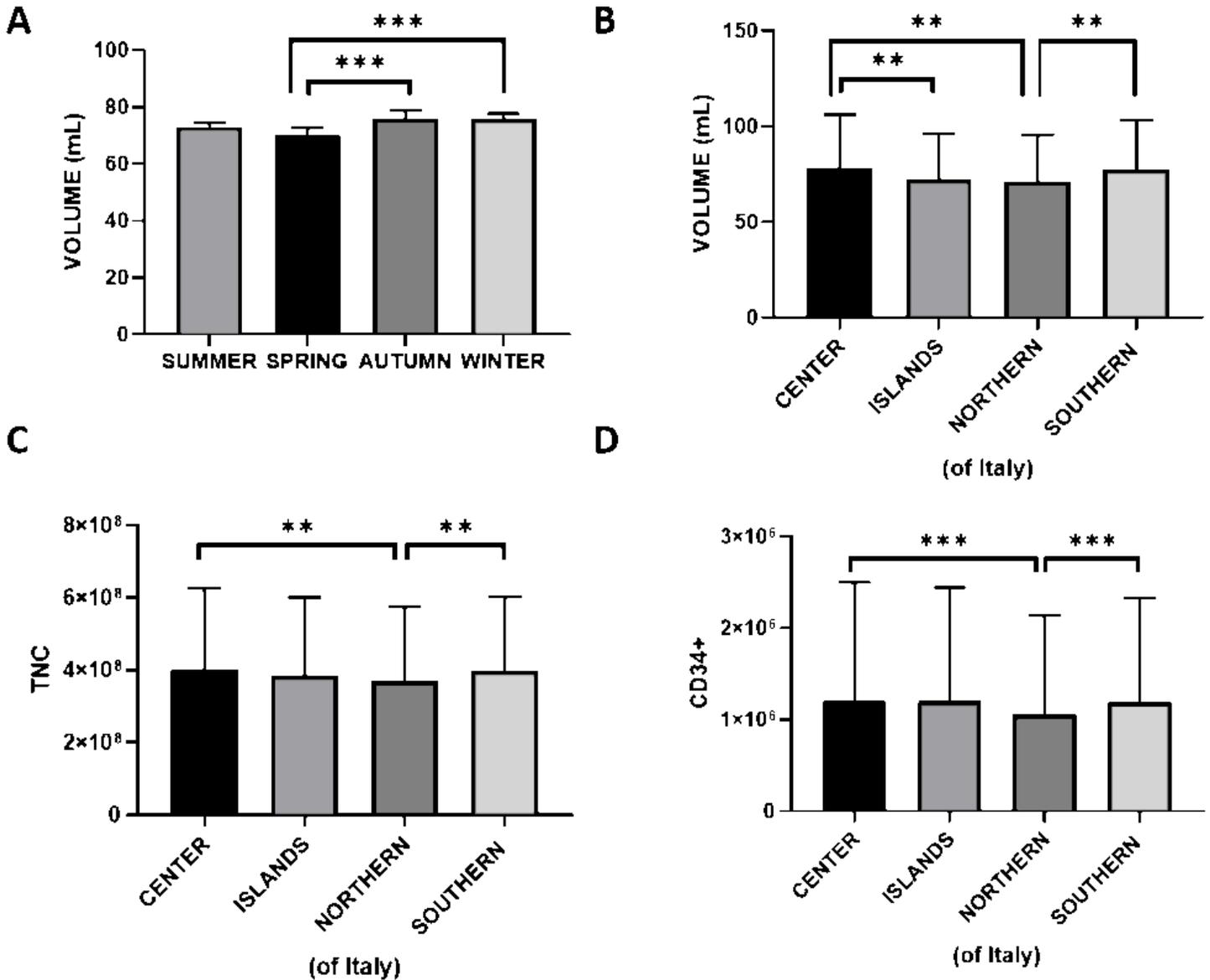


Figure 4

Correlation among the UCB characteristics and seasonal and geographical data. One-way ANOVA analysis (using Tukey Method post hoc test) showed that CBU volume collected in winter and autumn are more abundant than those collected in spring. No significant differences were observed between UCB collected in summer and those collected in other months (A). CBU collected in central-southern Italy showed higher volumes and higher TNC/CD34+ content than those of northern Italy. For CBU collected in Italian islands we did not observe any significant difference as compared to the rest of the country in terms of volume and TNC/CD34+. All reported values are expressed as mean ± standard deviation (**p<0.005; *** p<0.001).