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Original Article

Safety and feasibility of umbilical cord blood collection from preterm neonates after delayed cord clamping for the use of improving preterm complications

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Abstract: Background: Umbilical cord blood (UCB) is a new and convenient source of stem cells reported to be safe and effective in preventing and treating preterm complications. The initial processing step for this therapy involves cord blood collection and isolation of the mononuclear cell (MNC) layer. However, there is limited information regarding the feasibility and safety of cord blood collection in preterm infants, and whether cord blood cell quality and quantity are adequate for treating complications in preterm infants. UCB units from preterm infants are currently discarded due to safety concerns regarding collection and owing to the harvesting of inadequate volumes for banking. This study aimed to investigate the feasibility and safety of UCB collection following delayed cord clamping (DCC) for preventing and treating complications in preterm infants. Methods and Materials: Singleton preterm infants below 35 weeks gestation were assigned to two cohorts: cord blood collection and non-cord blood collection groups. Mortality and preterm complications in the two groups were compared to evaluate the safety of cord blood collection in preterm infants. The characteristics of the cord blood cells in preterm infants were investigated by comparing the cord blood parameters before and after processing with those of term infants born during the same period. Results: There were 90 preterm infants and 120 term neonates enrolled in this study. Compared to those of the term group, the preterm neonates had significantly less cord blood volume and fewer cell numbers. Nevertheless, the MNC number in the preterm group was $1.92\pm1.35\times10^8$ per kg, which fulfilled the previously reported targeted cell dose (5×10^7 cells/kg) suitable for application to improve preterm complications. There was no significant difference regarding complications in the preterm neonates with or without cord blood collection. Conclusions: The collection of UCB after DCC in preterm infants is feasible and safe. The cell numbers and quality fulfill the criteria for use in improving preterm complications. Cord blood MNCs from preterm neonates should be reconsidered as an ideal source for use in stem cell therapy for preterm complications.

Keywords: Cord blood collection, delayed cord clamping, preterm infants, safety, feasibility

Introduction

Preterm complications including mental and physical retardation as well as chronic lung diseases often become lifetime issues affecting survivors [1-4]. The current treatments are usually either targeted at single organs or symptoms. Previous studies, including our previous investigation revealed that cord blood mononuclear cell (MNC) administration is as effective as systemic, multi-organ targeted treatments for preterm complications [5-8]. The foundation of this treatment involves cord blood collection and isolation. Delayed cord clamping (DCC) is recommended for neonates, especially preterm infants [9-11]. However, cord blood collection after DCC may not be feasible in preterm infants, and an attempt to increase CB unit volume may impact delivery practices [12]. There is concern whether the cord blood collection procedure is safe for preterm infants, and it is necessary to investigate the feasibility of

using preterm umbilical cord blood (UCB) cells for prevention and treatment of preterm complications.

Previous analyses of preterm cord blood cells focused on fulfilling the requirement of UCB transplantations in hematological diseases [12, 13]. However, in recent years, cord blood cells are frequently used in clinical trials to treat non-hematological diseases [14], particularly for preterm-associated complications [15-18]. The safety of cord blood collection in preterm infants during DCC, and the quantity of MNCs (rich in stem cells), CD34+ cells, and colony forming unit-granulocytes and macrophages (CFU-GM) should be reassessed. Our previous study revealed the safety of autologous cord blood MNC infusion to prevent preterm complications [5]. Therefore, we conducted this study to investigate the feasibility and safety of cord blood collection after DCC in preterm infants for preventing and treating preterm-associated complications. The safety of cord blood collection in preterm infants, mortality, and complications were compared in preterm infants with cord blood collection (PCBC group) and without cord blood collection (N-PCBC group). The feasibility of cord blood collection, cord blood volume, UCB cell characteristics, and the weight of the MNCs were compared between the PCBC and the term infants (TCBC group).

Methods

Patients

For this prospective study, infants born between January 1 and June 1, 2019 at Guangdong Women and Children's Hospital were eligible if they fulfilled the following criteria: 1) singleton birth, 2) without congenital abnormalities (detected using prenatal ultrasound), 3) without maternal chorioamnionitis, 4) without asphyxia, 5) mother tested negative for hepatitis B (HBsAg and/or HBeAg) and C virus (anti-HCV), syphilis, HIV (anti-HIV-1 and -2) and IgM against cytomegalovirus, rubella, toxoplasma and herpes simplex virus, 6) mother did not experience gestational diabetes or preeclampsia, and 7) DCC was conducted (at least 30 s after birth).

Informed consent was obtained before delivery. Cord blood was collected from preterm infants below 35 weeks gestation who fulfilled the above criteria, and they were assigned to the PCBC group if consent for cord blood collection was obtained from their parents or guardians. If consent was not obtained, the cord blood was not collected, and the parents could choose to deal with the cord blood as they preferred. However, if they agreed to participate in the study, their basic clinical characteristics were collected and analyzed. Cord blood was collected from term infants fulfilling the above criteria, for whom consent was obtained, and the infants were assigned to the TCBC group.

Clinical outcomes including mortality and preterm complications were compared between the PCBC and N-PCBC groups. Cord blood characteristics (cord blood volume, number and concentration of MNCs, CFU-GM, CD34+ cells, and cell viability) were compared between the PCBC group and the TCBC group; this was conducted at the Guangdong Cord Blood and Stem Cell Bank. The study protocol was approved by the ethics committee of Guangdong Women and Children's Hospital. All patients in the study were administered intensive care therapy in accordance with departmental guidelines. All clinical diagnoses were defined according to a standard reference [19].

Cord blood collection process

The cord blood collection procedure was performed as previously described [5, 20]. The Guangdong Cord Blood and Stem Cell Bank is a public provincial blood bank affiliated to the Guangdong Women and Children's Hospital, and cord blood was collected at the hospital with the consent of participants. After umbilical cord clamping was delayed for at least 30 s, cord blood was collected using a blood-collection bag (WEGO, China) containing 28 mL of citrate-phosphate-dextrose anticoagulant before the placenta was delivered. The umbilical vein was sterilized and punctured with a 17-gauge needle. UCB collection was performed by trained obstetricians or by cord blood bank collection staff. When collection was complete, the blood bag tubing was closed and sealed. Before processing, the cord blood volume was calculated. Established cord blood bank procedures using the SEPAX S-100 automated processing system (Biosafe, Geneva, Switzerland) were followed. The samples were incubated with 6% Hespan (Bethlehem, USA) for 30 min to

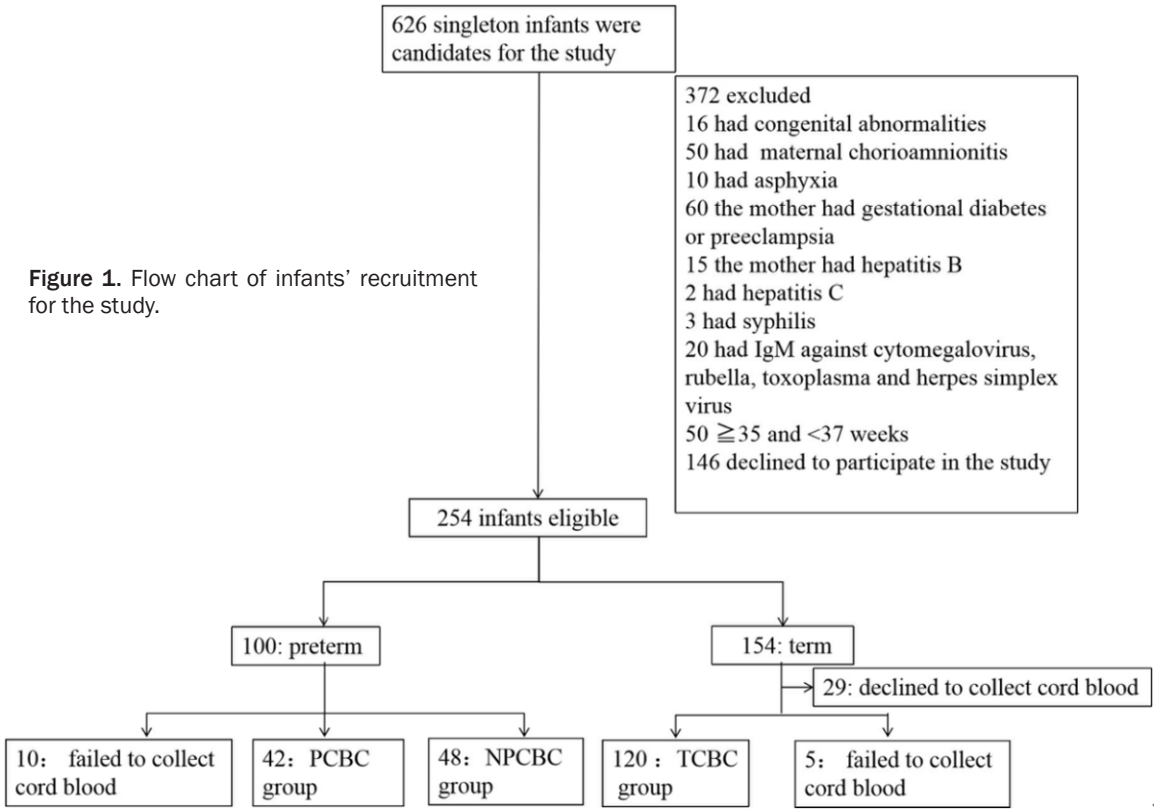

Figure 1. Flow chart of infants' recruitment for the study.

reduce the volume and precipitate the red blood cells. Cord blood volume, MNCs, CD34+ cells, and CFU-GM number were evaluated. Cell viability was determined using the 7-aminoactinomycin D (7-AAD) detection kit through flow cytometry analysis (BD Bioscience, USA). Processing was performed within 48 h of sample collection. Routine blood tests and blood gas analysis of peripheral blood were performed 24 h after birth.

Statistical analyses

Statistical analyses between the two groups were performed using an unpaired two-tailed Student's t-test or chi-squared test, as appropriate. A Kolmogorov-Smirnov test was employed to investigate the normality distribution of the variables. A value of $P<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS 21.0 (IBM).

Results

Patient recruitment and baseline characteristics

There were totally 626 singleton deliveries at Guangdong Women and Children's Hospital from January 1 to June 1, 2019. Two hundred and ten infants were enrolled in the study. There were 42 preterm infants in the PCBC group, 48 preterm infants in the N-PCBC group, and 120 term neonates in the TCBC group. The numbers of infants deemed eligible for the study and the number assigned to each group are shown in **Figure 1**. Cord blood collection failed in 10 preterm and 5 term infants, owing to the need for urgent delivery (e.g., placental abruption) and umbilical cord abnormality. There was no significant difference in the baseline characteristics and status of the infants in the two preterm groups (**Table 1**).

Safety of cord blood collection after delayed cord clamping in preterm infants

There were no significant differences in mortality and preterm complications such as bronchopulmonary dysplasia, intraventricular hemorrhage (IVH), necrotizing enterocolitis, retinopathy of prematurity, ventilation-associated pneumonia, hypoxic ischemic encephalopathy (HIE), late onset sepsis and, and anemia in the PCBC and N-PCBC groups. Details are shown in **Table 2**. Additionally, no significant differences in laboratory investigations 24 h after birth

Table 1. Baseline characteristics and status of the preterm infants

Characteristics	PCBC Group (N=42)	N-PCBC Group (N=48)	*P* value
Male (n, %)	26 (61.9%)	30 (62.5%)	0.954
GA (weeks)	31±2	31±1	0.829
BW (grams)	1642±400	1596±200	0.704
Cesarean Section (n, %)	10 (23.8%)	11 (22.9%)	0.920
Apgar Score			
1 minute	8	9	0.186
5 minutes	9	9	0.332

PCBC group = preterm cord blood collection group, N-PCBC group = preterm without cord blood collection group. GA = gestational age, BW = birth weight.

Table 2. Outcomes and complication comparison between PCBC Group and N-PCBC Group

complication	PCBC Group (N=42)	PCBC Group (N=48)	P
IVH (n, %)	2 (4.8)	3 (6.3)	0.758
BPD (n, %)	1 (2.4)	1 (2.1)	0.924
NEC (n, %)	2 (4.8)	3 (6.3)	0.758
ROP (n, %)	1 (2.4)	1 (2.1)	0.924
LOS (n, %)	1 (2.4)	1 (2.1)	0.924
VAP (n, %)	4 (9.5)	5 (10.4)	0.888
HIE (n, %)	1 (2.4)	0 (0)	0.282
Anemia (n, %)	15 (35.7)	13 (27.1)	0.378
Mortality (n, %)	1 (2.4)	1 (2.1)	0.924

PCBC: preterm infants with cord blood collection. N-PCBC: preterm infants without cord blood collection. IVH: intraventricular hemorrhage. BPD: bronchopulmonary dysplasia. NEC: necrotizing enterocolitis. ROP: retinopathy of prematurity. LOS: late onset sepsis. VAP: ventilation-associated pneumonia. HIE: hypoxic ischemic encephalopathy.

were observed between the PCBC and N-PCBC groups (**Table 3**).

Feasibility of cord blood cell collection after delayed cord clamping in preterm infants

The volume of cord blood collected, cell numbers, and cell concentrations, before and post processing were lower in the PCBC group compared to those in the TCBC group ($P<0.01$); The total number and number per kg of MNC, CFU-GM, and CD34+ cells were also lower in PCBC group compared to those in the TCBC group. Cell viability in the PCBC group was higher, ranging from 99.5 to 100%, mean (99.7± 0.17%). The MNCs per kg were $1.92\pm1.35\times10^8$ and $4.82\pm1.23\times10^8$ in the PCBC and TCBC groups, respectively. The CD34+ cells per kg were $0.90\pm0.24\times10^6$ and $1.5\pm1.0\times10^6$ in the PCBC and TCBC groups, respectively. Details are shown in **Table 4**.

Discussion

In our study, cord blood collection was performed in 42 preterm and 120 term infants after DCC. Although DCC is believed to be beneficial for preterm infants [21, 22], it results in decreased cord blood volume and total nucleated cell counts in cord blood donations [23, 24]. Considering the limited cord blood volumes of preterm infants, concerns exist regarding whether cord blood collection after DCC is feasible in preterm neonates [10-12]. Furthermore, attempts to increase CB unit volume may impact delivery practices [12, 24-27]. The previous safety study from the preterm group was not during the DCC era [24-26]. In our study, we observed no significant differences in circulatory parameters such as pulse, blood pressure, and blood gas immediately following cord blood collection. No adverse effects of cord blood collection including anemia and other common preterm complications were observed in the PCBC compared to the NCBC group. These results demonstrate the feasibility and safety of cord blood collection for the prevention and treatment of preterm complications in preterm neonates after DCC.

For the cord blood cell characteristics, all parameters except for cell viability before and after processing were significantly lower in the preterm infants compared to those of term infants which is consistent with a previous report [12]. The number of CD34+ cells per kg in the PCBC group was $0.90\pm0.24\times10^6$ compared with $1.5\pm1.0\times10^6$ in the TCBC group. The number of MNCs per kg in the PCBC group was 1.92×10^8 compared with 4.82×10^8 in the TCBC group, which fulfilled the previously reported targeted cell dose of (5×10^7 cells/kg) for application in improving preterm complications. None of the preterm UCB units collected satisfied the criteria for processing and cryopreservation for clinical application in hematologic diseases even when the limit for accepting UCB units for banking of TNC was as low as $6* 10^8$.

Currently, the total nucleated cell content per kg is the parameter used for UCB selection for unrelated transplantation [12, 13]. UCB from

Table 3. Laboratory investigations 24 h after birth between PCBC and N-PCBC Group (Data were presented as mean ± SEM)

LAB Investigation 24 h after birth	PCBC Group (N=42)	N-PCBC Group (N=48)	P
Hemoglobin (g/dl)	165.2±25.4	167.8±18.1	0.73
White cell count (*10^9/L)	13.32±6.4	9.91±5.1	0.11
Hematocrit	47.75±7.7	50.27±5.7	0.3
Platelet Count (*10^6/L)	305.33±51.2	257.02±95.8	0.924
Albumin (g/L)	41.2±6.1	49.00±19.4	0.16
AST (u/l)	54.0±28.9	61.56±29.9	0.48
ALT (u/l)	7.8±3.1	6.25±2.7	0.14
Blood urea nitrogen (mmol/L)	3.2±1.63	4.41±1.9	0.07
Creatinine (umol/L)	58.4±14.6	58.36±18.3	0.99
pH	7.32±0.1	7.31±0.1	0.756
PaO_2 (kpa)	8.39±2.3	7.89±3.1	0.253
$PaCO_2$ (kpa)	5.98±1.3	6.57±1.0	0.362

PCBC group = preterm cord blood collection group, N-PCBC group = preterm without cord blood collection group. SEM = standard error of the mean, LAB = laboratory, AST = glutamic-pyruvic transaminase, ALT = glutamic-oxalacetic transaminase.

Table 4. Cord blood collection related parameters comparison between PCBC Group and TCBC Group

	PCBC Group (N=42)	TCBC Group (n=120)	P
Volume Collected before processing (BP) (ml)	47.06±19.10	111.6±19.28	<0.01
Cord blood volume after processing (AP) (ml)	23.27±0.63	22.06±0.11	<0.01
MNC BP (*10^8)	3.11±2.16	16.69±4.27	<0.01
MNC AP (*10^8)	2.87±1.86	14.47±3.68	<0.01
MNC Concentration BP (*10^6 cell/ml)	5.90±0.45	15.06±0.29	<0.01
MNC Concentration AP (*10^6 cell/ml)	11.97±6.96	65.34±15.35	<0.01
MNC/kg of birth weight (*10^8)	1.92±1.35	4.82±1.23	<0.01
CFU-GM*10^5	3.71±3.25	118.17±54.11	<0.01
CFU-GM/kg of birth weight (*10^5)	2.28±2.0	35.18±15.80	<0.01
CD34+ (*10^6)	1.42±1.5	5.21±3.62	<0.01
CD34+/kg of birth weight (*10^6)	0.90±0.24	1.5±1.0	<0.01
Viability	99.80±0.14	97.24±2.11	<0.01

PCBC group = preterm cord blood collection group, TCBC group = term cord blood collection group, MNC = mononuclear cell, BP = before processing, AP = after processing, CFU-GM = colony forming unit-granulocyte and macrophage. Kg = kilogram.

preterm infants particularly those below 34 weeks gestation contain very limited MNC and is, therefore, not feasible for routine unrelated cord blood banking [23, 27]. However, since the first UCB transplantation was successfully conducted 32 years ago [13], UCB MNCs have been used as a new and convenient source of stem cells, for prevention and treatment of preterm complications [15-18]. An increasing number of studies have revealed that stem cells interact with injured tissue through the release of soluble bioactive factors but not via engraftment and replacement of the targeted organs or cells [28-30]. This evidence laid the foundation for the use of MNCs as a new systemic, multi-organ targeted therapy for preterm complication without the necessity of engraftment, which occurs in hematologic diseases.

Animal studies have demonstrated the beneficial effect of cord blood stem cell application in preterm related complications such as BPD, sepsis and HIE [31-35]. Evidence from several clinical trials demonstrated the efficacy and safety of cord blood cell infusion in neonates and children [5, 17, 18]. MNCs from UCB are rich in stem cells, which are believed to be the most potent cells for improving preterm compli-

cations by secreting various bioactive factors [28-30]. UCB derived stem cells demonstrated multiple advantages including easier extraction, lower immunogenicity, and higher proliferation capacity without harming the donor [14, 34]. Kurtzberg and colleagues demonstrated that a single intravenous autologous infusion of 1-5*10^7 total nucleated cells per kg could improve the cognitive outcomes of children with cerebral palsy [17]. Another clinical trial performed by the same group also showed autologous umbilical cord blood cells (1-5*10^7 cells/dose, up to 4 doses) infusion to neonates with hypoxic-ischemic encephalopathy had beneficial long term effects on neurodevelopmental outcomes [18]. Machaliński demonstrated that whole autologous cord blood infusion (15 mL/kg) within 48 h after birth reduced incidences of IVH and anemia in preterm newborns [9]. Mesenchymal stromal/stem cells (MSCs) were successfully isolated and expanded in vitro from small-volume umbilical cord blood units, which did not qualify for banking, of preterm infants [27]. UCB derived MSCs have been used for severe intraventricular hemorrhage and BPD and in extremely low birth weight preterm infants [6, 7, 18]. Our previous phase II trials also demonstrated that a single intravenous autologous infusion of 5*10^7 MNC per kg could reduce the duration of respiratory support and, therefore, had the potential to prevent BPD in very preterm neonates [5]. The cell doses used in previously reported studies ranged from 0.1*10^7/kg to 5*10^7/kg and can be safely used up to 5*10^7/kg [5-8, 36], which is the effective dose applied in allogeneic transplant after myeloablative chemotherapy in a study of autologous UCB cells in neonates with HIE [8]. In this study, the average volume of UCB was 47.06 mL, and the MNC after processing was 1.92±1.35×10^8/kg. All the cord blood units collected fulfilled the current requirements reported in the published literature (>5*10^7 MNC per kg) for application in autologous usage. The latest NETCORD/FACT International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release also reconsidered CB collection in very preterm infants, which could be performed after a comprehensive evaluation of infant donor safety [23]. Based on our current study, cord blood collection is feasible and safe in preterm neonates. Considering the adverse outcomes of preterm complications and the benefit of cord blood MNC, preterm delivery should not be a reason to not collect UCB.

There are several limitations in this study. Usually, neonates who require stem cell therapy to improve preterm complications are extremely premature infants (GA<32 weeks). In this study, the lowest gestational age in enrolled preterm patients was 28 weeks and 2 days. Many of the enrolled infants were more mature than previously expected. More extremely preterm infants should be enrolled in future studies. Approximately 400 singleton births were not eligible for study. The relatively low percentage (about 1/3) of final enrollment weakened the generalizability of the population. The low incidence of complications in the two preterm groups may also lower the confidence. This is partly due to the parents' concern about potential impact of cord blood collection on the safety of their infants. As informed consent was obtained immediately before delivery, the timing of obtaining informed consent limited more comprehensive communication with the parents.

In conclusion, we demonstrated that cord blood collection in preterm neonates is safe and feasible following DCC. The MNCs in cord blood units are sufficient for application in treatment of preterm complications. Currently, discarded potentially life-saving umbilical cord blood units in very preterm infants should be used for treating preterm complications. The safety and feasibility of cord blood collection provided an important first step toward the safe clinical translation of cord blood derived cell therapy for this special population of preterm neonates.

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Disclosure of conflict of interest

None.

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