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CORD BLOOD

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Autologous cord blood cell infusion in preterm neonates safely reduces respiratory support duration and potentially preterm complications

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Abstract

Preterm birth and its complications are the leading cause of neonatal death. The main underlying pathological mechanisms for preterm complications are disruption of the normal maturation processes within the target tissues, interrupted by premature birth. Cord blood, as a new and convenient source of stem cells, may provide new, promising options for preventing preterm complications. This prospective, nonrandomized placebo controlled study aimed at investigating the effect of autologous cord blood mononuclear cells (ACBMNC) for preventing preterm associated complications. Preterm infants less than 35 weeks gestational age were assigned to receive ACBMNC (5×10^7 cells/kg) intravenous or normal saline within 8 hours after birth. Preterm complication rates were compared between two groups to demonstrate the effect of ACBMNC infusion in reducing preterm complications. Fifteen preterm infants received ACBMNC infusion, and 16 infants were assigned to the control group. There were no significant differences when comparing mortality and preterm complication rates before discharge. However, ACBMNC infusion demonstrated significant decreases in duration of mechanical ventilation (3.2 days vs 6.41 days, $P = .028$) and oxygen therapy (5.33 days vs 11.31 days, $P = .047$). ACBMNC infusion was effective in reducing respiratory support duration in very preterm infants. Due to the limited number of patients enrolled, powered randomized controlled trials are needed to better define its efficacy.

KEYWORDS

autologous cord blood cells, effect, preterm complication, prevention

Abbreviations: ACBMNC, autologous cord blood mononuclear cells; BPD, bronchopulmonary dysplasia; CP, cerebral palsy; GA, gestational age; HIE, hypoxic ischemic encephalopathy; IV, intravenous; IVH, intraventricular hemorrhage; LOS, late onset sepsis; MSC, mesenchymal stem cells; NEC, necrotizing enterocolitis; PS, pulmonary surfactant; RBC, red blood cells; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity; SPC, stem and progenitor cells; UCB, umbilical cord blood; VAP, ventilation-associated pneumonia.

Ren Zhuxiao and Xu Fang contributed equally to this study.

1 | INTRODUCTION

Preterm birth, a rising significant health problem around the world, affects 5% to 18% of babies born.[1] Preterm birth complications are the first major direct cause of neonatal death.[2] The main underlying

pathological mechanisms for preterm complications are disruptions of normal organ maturation, which is partly caused by target tissue stem cell depletion and damage from oxygen toxicity, infection/inflammation, and so on.[3–5] The brain and lungs are two organs commonly affected, the potential pathophysiology of which is neuron demyelination and alveolar dysmorphia, respectively.[4,5] With the improvement of intensive care intervention, mortality rates of preterm babies decreased. However, many survivors still face a lifetime of disability, including long-term respiratory complications, learning disabilities, and visual and hearing problems, even in developed countries.[2] Current treatment strategies are single-organ targeted, and could not address the underlying multi-organ structural simplification.

Cord blood mononuclear cells layer is rich in valuable stem and progenitor cells (SPC).[4–6] On the one hand, they are capable of self-renewal; on the other hand, they have the potential to differentiate into various cellular phenotypes.[5] In addition, their paracrine effect contributes to tissue repair and immune modulation.[7,8] Animal studies have demonstrated the effect of cord blood stem cells infusion in prevention and treatment of preterm-related complications such as bronchopulmonary dysplasia (BPD), sepsis, and hypoxic ischemic encephalopathy (HIE).[9,10] Evidence from several clinical trials proved the safety and feasibility of autologous cord blood infusion in neonates.[4,11–15] However, there are very limited data about preterm neonates. This led us to hypothesize that intravenous (IV) infusion of autologous cord blood mononuclear cells (ACBMNC) would lower the risk of developing preterm complications. The safety and feasibility of ACBMNC infusion in preterm infants has been demonstrated in our previous study.[4] In this prospective study, we analyzed whether ACBMNC could reduce preterm complications.

Significance statement

The results from this prospective nonrandomized study found that autologous cord blood cells infusion substantially reduced the duration of mechanical ventilation and oxygen supplement. Fewer preterm complications were observed in the cord blood cell infusion group, although there was no significant difference. Preterm birth complications are the first major direct cause of neonatal death, and no curative therapies are available to alleviate the symptoms of preterm complications. Stem cells as a treatment provides regrowth to their underdeveloped organs and therefore could improve the outcomes of preterm infants by adding more stem/progenitor cells. This may be helpful to prevent preterm complications.

2 | MATERIALS AND METHODS

2.1 | Study design

This study included 31 preterm neonates less than 35 weeks gestational age (GA). Between 1 December 2009 and 1 July 2010, we conducted a single-center, phase II, prospective, nonrandomized, placebo-controlled trial of a single IV ACBMNC infusion in preterm infants less than 35 weeks GA in Guangdong Women and Children Hospital. Fifteen patients underwent ACBMNC infusion. Sixteen patients underwent the same volume of normal saline infusion (control group). The study was approved by the ethics committee of Guangdong Women and Children Hospital.

Trial registration: ClinicalTrials.gov, NCT03053076, registered 02/10/2017, retrospectively registered, https://register.clinicaltrials.gov/prs/app/action/SelectProtocol?sid=S0006WN4&selectaction=Edit&uid=U0002PLA&ts=2&cx=9y23d4

2.2 | Participants and methods

Inborn infants admitted to the Neonatal Intensive Care Unit of Guangdong Women and Children Hospital were eligible if they were: (a) born in the study hospital, (b) singleton birth, (c) less than 35 weeks GA, (d) without congenital abnormalities (through prenatal screening), (e) without clinical chorioamnionitis, and (f) the mother was 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. If the neonates fulfilled the above criteria, we would talk with their parents before the delivery. If the consent for IV ACBMNC infusion was obtained from their parents or guardians, the cord blood would be collected. And the infants will be assigned to the ACBMNC infusion group if their umbilical cord blood (UCB) cells after processing were available. If the consent for IV ACBMNC infusion was not obtained, the cord blood would not be collected for this trial, and the parents could choose to deal with the cord blood as they preferred. These infants were assigned to the control group if their parents or guardians consented. Those assigned to the ACBMNC group received an infusion of ACBMNC with in 8 hours after birth. Those in the control group received an infusion of a placebo solution, which is normal saline of the same volume. Cell dose for all patients was targeted at 5×10^7 cells per kilogram.

2.3 | Procedures

Right before the preterm infant was delivered, written consent was signed by the parents. The procedure of cord blood collection was performed in accordance with cord blood bank guidance which was reported previously.[4] Guangdong Cord Blood and Stem Cell Bank is a public provincial blood bank affiliated to the Guangdong Women and Children's Hospital, which collects cord blood according to the consents in this hospital. Therefore, the cord blood of the neonates with consents had been routinely collected during the delivery. The procedure of cord blood collection and transfusion was performed in accordance with cord blood bank guidance.[16] The umbilical cord was clamped for the collection using a blood-collection bag (WEGO, China) containing 28 mL of citrate-phosphate-dextrose anticoagulant right after the baby was born and before the placenta was delivered. The umbilical vein was sterilized and punctured with a 17-gauge needle. UCB collection was made by

trained obstetricians or cord blood bank collection staff who were present at hospital during weekdays for 8-12 hours per day. When collection was completed, the blood bag tubing was closed and sealed. Cord blood labeled with full name of donor, group type, and volume of the blood product was stored in 4°C and sent to the Cord Blood and Stem Cell Bank for processing immediately. Before processing, 2 mL of samples were taken from all collected CB units to test for the presence of virus (human immunodeficiency virus, hepatitis B virus, hepatitis C virus, cytomegalovirus) and bacterial infections (*Treponema pallidum* included). A sample of peripheral blood was collected from the mother and tested for the presence of maternal transmissible diseases. And the results were obtained soon before transfusion started. After the sample was taken, it was volume and red blood cell (RBC) was reduced after 30 minutes incubation with 6% Hespan (Bethlehem) following established CBB procedures using the SEPAX S-100 automated processing system (Biosafe, Geneva, Switzerland) if the unit contained >30 mL of UCB or manually if the unit was <30 mL. The mononuclear layer was isolated by density gradient centrifugation (1000*g*, 30 minutes, RT, Beckman, American), then was transferred to cryobags. Excessive nucleated cell-poor plasma was expelled. Meanwhile, MNC count, CD34 cell, CFU-GM, and sterility detection (Sheldon Manufacturing Inc., Cornelius, Oregon) were performed. Cell viability was measured via 7-aminoactinomycin D (7-AAD) detection kit through flow cytometry analysis (BD Bioscience). After processing, the ACBMNC were sent back to Guangdong Women and Children Hospital. All infusions were administered in Guangdong Women and Children Hospital. The cells or normal saline were administered through a peripheral or central IV catheter over 15 minutes, followed by a 2 mL saline flush to clear the intravascular line. Infusate and subject identities were double-checked by research and clinical nursing staff. Infusions were also monitored by research and clinical staff. All patients in the study were given intensive care therapy in accordance with departmental guidelines which including positive pressure mechanical ventilation, noninvasive respiratory support, oxygen therapy, and exogenous pulmonary surfactant (PS) (Curosurf, Chiesi, Parma, Italy) replacement. Chest radiographs were performed at admission and 8 hours after ACBMNC infusion on the first day of life in all surviving patients. Blood gas was monitored at least every 24 hours until weaning from ventilation. All clinical diagnoses were defined according to a standard reference.[17] Assessment of safety were conducted at 12 and 24 hours after infusion, as well as during hospitalization and return visits. Laboratory investigations in peripheral blood were monitored and kept stable during the whole treatment period. Infusion reactions and signs of circulatory overload were checked. A hospital ethics committee reviewed the study data during the trial. None of them were involved in the study. The staff who collected and analyzed the patients data were not aware of the treatment assignment.

2.4 | Outcomes

The primary outcome of this study is the mortality rate before discharge. The secondary outcomes are preterm complications rate before discharge. Other outcomes included duration of mechanical ventilation and oxygen therapy, reintubation, and time of return to BW. Preterm complications included BPD, intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), respiratory distress syndrome (RDS), ventilation-associated pneumonia (VAP), HIE, late onset sepsis (LOS), and anemia. All clinical diagnoses were defined according to a standard reference.[17]

2.5 | Statistical analysis

Statistical analyses between the two groups were performed using an unpaired two-tailed Student's *t* test or Chi-squared test as appropriate. A value of $P < .05$ was considered statistically significant. All statistical analyses were done using SPSS 21.0 (IBM).

3 | RESULTS

3.1 | Study infants and intervention

A total of 31 infants were enrolled between 1 December 2009 and 1 July 2010 in our single center. All patients were followed up to their first discharge home. There were no significant differences in the baseline characteristics and status of the infants in the two groups (Table 1).

3.2 | Study intervention

The characteristics of the ACBMNC and the use of PS or oxygen in the two groups are shown in Tables 2 and 3. Study infants received the ACBMNC at a median hours after birth of 6.77 ± 1.52 hours, and

TABLE 1 Baseline characteristics and status of the infants in two groups

Characteristics	Control group (N = 16)	Autologous cord blood group (N = 15)	*P* value
Sex no. (%)			.411
Male	12 (75%)	13 (86.7%)	
Female	4 (25%)	2 (13.3%)	
GA (weeks)	31.05	31.17	.829
BW (g)	1641.56	1596.00	.704
Delivery mode no. (%)			.519
Vaginal delivery	6 (37.5%)	4 (26.7%)	
Cesarean section	10 (62.5%)	11 (73.3%)	
Apgar score			
1 minute	7.81	8.6	.186
5 minutes	8.94	9.47	.332

TABLE 2 Characteristics of the ACBMNC

No.	Volume collected	Volume postprocessing	Cells collected (108)	Cells postprocessing (108)	Cell concentration postprocessing*106/mL	CFU-GM/105	CD34+/106	Viability	Infused NC*107/kg	Infused age (H)	Infused volume	Pathogen detection
1	34	22	1.82	1.46	6.65	1.84	1.1	100	4.48	5	11	Negative
2	35	20	1.6	1.32	5.82	1.98	0.7	99.5	5	6	13	Negative
3	61	24	2.44	1.67	6.95	2.3	1.14	99.8	5	7	12	Negative
4	33	16	0.97	0.86	5.4	0.72	0.31	99.8	5	4	14	Negative
5	36	22	1.43	1.21	5.5	1.57	0.59	99.8	5	9	13	Negative
6	28	16	1.43	1.23	7.7	1.53	0.9	99.5	5	4.5	11	Negative
7	55	28	5.81	5.43	26.3	11.27	5.69	99.8	5	6	3	Negative
8	65	20	2.99	2.37	11.85	1.66	1.66	99.9	5	7	8	Negative
9	81	20	6.14	4.33	21.65	9.96	3.9	99.9	5	8	5	Negative
10	27	28	2.21	1.97	7.05	3.35	0.55	99.8	5	8	28	Negative
11	46	24	2.96	2.65	13.6	3.32	1.01	99.5	5	7	6	Negative
12	37	24	1.51	1.4	5.85	1.04	0.08	99.9	5	9	12	Negative
13	24	22	1.72	1.58	8.53	2.82	1.1	99.8	5	6	9	Negative
14	69	24	8.11	7.83	40.8	6.08	16.22	99.8	5	7	2	Negative
15	76	30	5.3	4.7	22.8	6.39	1	99.5	5	8	7	Negative
Mean	47.13	22.67	3.10	2.67	13.10	3.72	2.40	99.7	4.97	6.77	10.27	
SD	19.10	4.05	2.17	2.00	10.35	3.25	4.10	0.17	0.13	1.52	6.18	

the volume transfused were 10.27 ± 6.18 mL. The control group received normal saline at 6 hours after birth and the volume transfused were 10 mL. The transfusion lasts 15 to 30 minutes. Twelve patients received PS treatment in each group and one infant needed two doses of PS in control group. All infants in both groups received mechanical ventilation and oxygen therapy because of clinical symptoms and signs of RDS. The use of mechanical ventilation and oxygen therapy for infants in the ACBMNC group were discontinued approximately 3 and 6 days earlier than in the control group, respectively (P = .028 and .047 for each comparison).

3.3 | Outcomes

3.3.1 | Primary outcome (mortality)

All infants survived to discharge in both groups (Table 3).

3.3.2 | Secondary outcome

The secondary outcomes and other outcomes in the two groups before discharge are shown in Table 3. No BPD occurred in the ACBMNC infusion group. There was one patient suffered from mild BPD in control group (P = .962). We found that of the 15 infants who were assigned to ACBMNC infusion, the average duration of mechanical ventilation is 3.20 days as compared with 6.41 days in control group (P = .028). And the average duration of oxygen supplement is 5.33 days compared with 11.31 days in control group (P = .047). ACBMNC infusion significantly reduced the duration of mechanical ventilation and oxygen therapy. No NEC, ROP, or HIE was observed in ACBMNC infusion group. Two patients had stage 2a NEC, one patient had stage 1 ROP, and one patient had mild HIE in control group. Only one patient (6.7%) needed reintubation in ACBMNC infusion group compared with three patients (18.8%) in control group. It took similar days for infants in both groups to return to birth weight (19.81 days in ACBMNC infusion group vs 19.47 days in control group). One infant had grade 1 IVH and one patient had LOS in ACBMNC infusion group. The rates of anemia, VAP, and RDS grade more than 2 did not differ significantly between the two groups. Duration of Hospitalization was similar in both groups.

During the first 24 hours, infants in the ACBMNC infusion group had more white blood cells (WBC) than infants in the placebo group (Table 4). No significant differences in hemoglobin (HB), platelet (PLT), and blood gas after 24 hours were observed.

4 | DISCUSSION

In this prospective nonrandomized study, we analyzed whether ACBMNC infusion soon after birth could reduce preterm complications in preterm neonates. This article focuses on short-term outcomes before discharge. We found no infants were dead before

TABLE 3 Outcomes before discharge

Outcome	Control group (N = 16)	Autologous cord blood group (N = 15)	*P*
IVH, n (%)	1 (6.3%)	1 (6.7%)	.962
BPD, n (%)	1 (6.3%)	0 (0%)	.325
NEC, n (%)	2 (12.5%)	0 (0%)	.875
ROP, n (%)	1 (6.3%)	0 (0%)	.325
LOS, n (%)	0 (0%)	1 (6.7%)	.294
VAP, n (%)	4 (25%)	5 (33.3%)	.609
HIE, n (%)	1 (6.3%)	0 (0%)	.938
Anemia, n (%)	11 (68.8%)	10 (66.7%)	.901
RDS grade > 2, n (%)	9 (56.3%)	8 (53.3%)	.87
PS treatment, n (%)	12 (75%)	12 (80%)	.739
Duration of mechanical ventilation (day)	6.41	3.20	**.028**
Duration of oxygen therapy (day)	11.31	5.33	**.047**
Reintubation, n (%)	3 (18.8%)	1 (6.7%)	.316
Time return to BW (day)	19.81	19.47	.898
Duration of hospitalization (day)	28.44	30.00	.851

Note: Bold entries denote ACBMNC infusion demonstrated significant decreases in duration of mechanical ventilation (3.2 days vs 6.41 days, *P* = .028) and oxygen therapy (5.33 days vs 11.31 days, *P* = .047).

TABLE 4 Laboratory investigations before and after infusion

Laboratory tests	Control group (N = 16)	Autologous cord blood group (N = 15)	*P*
HB baseline (g/L)	166.4	157.3	.22
HB 24 h (g/L)	136.7	139.8	.69
WBC baseline ($\times 10^9$/L)	9.5	13.0	.096
WBC 24 h ($\times 10^9$/L)	10.2	12.3	**.042**
PLT baseline ($\times 10^9$/L)	214	296	**.002**
PLT 24 h ($\times 10^9$/L)	288.3	293.0	.924
PH baseline	7.31	7.32	.728
PH 24 h	7.41	7.40	.756
PO_2 baseline (kPa)	7.89	8.39	.618
PO_2 24 h (kPa)	6.77	7.39	.253
PCO_2 baseline (kPa)	6.57	5.98	.275
PCO_2 24 h (kPa)	4.52	4.18	.362
FiO_2 baseline	0.36	0.37	.787
FiO_2 24 h	0.22	0.22	.829

Note: Bold entries denote, during the first 24 hours, infants in the ACBMNC infusion group had more white blood cells (WBC) than infants in the placebo group (*P* = .042). Infants in the ACBMNC infusion group had more platelets before infusion (*P* = .002).

discharge in both groups. Less preterm complications were observed in ACBMNC infusion group, although there is no significant difference. ACBMNC infusion substantially reduced the duration of mechanical ventilation and oxygen supplement.

Preterm complications continue to be a significant cause of morbidity and mortality for premature infants.[2] The underlying pathological mechanisms for preterm complications are disruptions of normal organ maturation.[3] The brain and lung are two organs commonly affected, clinical presented as HIE and BPD, respectively.[3,5,9,18–24] Until now, the treatments for these complications are single-organ or symptom targeted.[22,23] We are looking for a therapy that has the potential to cope with these multiple factors. Previous study reported the sudden lack of circulating cord blood at the time of preterm birth results in a lack of progenitor cells for normal tissue maturation.[9,21] However, recent study showed the disruption of the normal maturation processes within the target tissues, interrupted by premature birth, which is partially responsible for abnormal end organ development.[3] Stem cells have the potential to give rise to multiple differentiated cellular phenotypes. However, later research showed stem cells engraftment seems to be a rare event,[3,22,23] stem cells as a treatment probably does not provide regrowth of underdeveloped organs directly.[7,12] Growing number of studies have shown that stem cells interact with injured tissue through the release of soluble bioactive factors, which have immunomodulatory and anti-inflammatory effects.[3,4,7,9,25] This evidence laid the foundation for MNCs as a new systemic, multi-organ targeted therapy for preterm complication prevention.

BPD is considered as one of the major complications of preterm birth.[3,4] The new definition of BPD addressed that it is a chronic lung disease associated with an arrest in lung development including alveolar and vascular structural development. Despite the rapid advance of neonatal care, BPD remains a significant burden for the preterm population, which is lack of effective intervention.[3,4] Ahn and the colleagues had demonstrated that intratracheal transplantation of allogeneic hUCB-derived mesenchymal stem cells (MSCs) is safe, feasible, and effective in preterm infants at high risk for BPD.[11,13] Matthay and team showed that IV allogeneic BM-MSCs was safe in adults with ARDS.[26] Compared with other stem cell types in cord blood, MSCs are more attractive to researchers, as it is non-immunogenic and can be expanded rapidly ex vivo.[7,27] With these properties, allogeneic transplantation of MSCs are used for treatment of diseases in adult and neonates.[11,26,28] ACBMNC, as a convenient source of MSC, has never been studied in prevention or treatment of BPD in preterm. Valuable SPC mainly exits in MNC layer.[12,27] It is considered as the most effective component in cord blood. However, as MSCs are present at a low frequency in UCB,[27] ACBMNC may not only act through the effect of MSCs presented. The beneficial effect of ACBMNC could be the integration of all the SPCs and their soluble bioactive factors in UCB. Compared with intratracheal transplantation of allogeneic hUCB-derived MSCs, IV ACBMNC infusion can not only supply with stem cells but also secret growth factor through paracrine which contribute to improved lung structure and maintenance of pulmonary development.[3,7,8,29–31] More studies on the optimal cell

source including cord blood MNC vs MSCs are warranted. Oxygen- and mechanical ventilation-mediated lung injury have been implicated in the pathogenesis of BPD.[5,32] In the management of BPD, mechanical ventilation is the main method to maintain lung volume in preterm, however, all methods of ventilation can damage the epithelium and increase inflammation response. In addition, even oxygen supplementation at low levels can cause oxidative stress and damage the development of the immature lung.[32,33] Our study is the first research observing the effect of ACBMNC as a potential treatment option for BPD. We found no one had BPD in 15 infants receiving ACBMNC infusion; furthermore, ACBMNC infusion reduced mechanical ventilation and oxygen supplement duration obviously, and therefore could shorten exposure to oxygen and attenuate lung injury associated with oxidative stress. However, more research regarding the underlying mechanisms are needed.

Preterm infants are vulnerable to brain injury, including HIE and IVH. HIE and IVH can lead to cerebral palsy (CP) causing functional impairments and lifelong disabilities.[12,19,33–37] SPC in cord blood obtained several advantages over the use of other SPC source, including immunomodulatory capacity, anti-fibrosis activity, anti-apoptotic activity, endogenous repair, and growth promoting activity.[9,27] Recently, both autologous and allogeneic cord blood infusion are applied in the treatment of HIE, IVH, and CP.[12,15,35] Cotten and colleagues in Duke University tested feasibility and safety of providing autologous UCB cells to neonates ≥35 weeks with HIE and found that UCB cells infusion could improve neurodevelopmental outcomes after 1-year follow-up.[12] Kotowski transfused whole cord blood in preterm neonates to treat anemia and compared IVH rate in infants infused RBCs, and found lower IVH rate in cord blood infusion group.[15] Kurtzberg demonstrated that appropriately dosed ACB infusion improves motor function in young children with CP,[36] and they suggested improvements in motor function resulted from new brain connectivity induced by paracrine signaling of ACB cells. In a recent research, Wasielewski and the colleagues found inflammatory activation decreased after UCB cells treatment in an HIE rat model.[38] They and other researchers also showed that UCB cells could improve secretion of vascular endothelial growth factor, reduce apoptosis, and accelerate angiogenesis post brain injury.[38–40] These previous studies suggested that cord blood stem cell therapy has beneficial effect on premature brain injury. Compared with near-term neonates and older children, very preterm infants, with immature blood brain barrier and simpler cerebral structure, are more prone to brain damage related to fluctuations in cerebral oxygenation.[34,37] Preterm infants often require mechanical or assisted ventilation after birth. This can cause brain injury through oxidative stress.[37] Preoligodendrocytes are highly vulnerable to oxidative stress.[9] We infused ACBMNC in very preterm neonates, and found beneficial effect on reducing duration of mechanical ventilation and oxygen usage, which may protect brain tissue via attenuating oxidative stress. However, the underlying cellular mechanisms on how UCB cells improve neurodevelopment are complex. More research on our mechanistic understanding of paracrine and non paracrine pathways for UCB cells therapy should be conducted.[41,42]

Rare studies evaluated the stem cell therapy effect on other common complications such as LOS, RDS, NEC, and ROP, which resulted from immaturity of immune system, pulmonary, intestine, and retina, respectively. Although the immunomodulatory and therapeutic effects of stem cells for the lung injury and sepsis were reported in adults,[7] no exact data were reported about the special population of neonates. Our study first reported the concrete number of patients diagnosed with various complications in ACBMNC infusion and control group. No significant difference was found which may be due to the limited number of participants. Regarding the incidence of ROP, the study group was not with high risk of this complication development since there were no babies below 29 GA, and since the complication may develop later than 30 days after the birth, the longer observation is required to definitely exclude ROP incidence.

For the laboratory tests, during the first 24 hours, infants in the ACBMNC infusion group had more WBC than infants in the placebo group, which may be partly attributed to the infusion of MNCs from cord blood compared with no cells in normal saline infused in control group. Other mechanisms including immunoregulation may also play a role; however, more research is needed to understand the underlying reasons.

To our knowledge, this is the first study to assess ACBMNC transfusion very soon after birth in very preterm neonates in relation to the rate of prematurity-related complications. We reported here for the first time that ACBMNC infusion soon after birth was safe and feasible, and demonstrated significantly great decreases in duration of mechanical ventilation and oxygen therapy. No BPD, NEC, ROP, and HIE were observed in ACBMNC infusion group, indicating that ACBMNC may have the potential to reduce the rate of prematurity complications.

This study certainly has some limitations. First, the sample size is small and the study group does not involve substantial number of immature babies (eg, ~1000 g of birth weight). These characters could make the conclusion less accurate and may lead to loss of truth in significant difference regarding complication rate in two groups. Second, this study is nonrandomized and single-blind, and this may cause bias. Considering these limitations, further multicenter, randomized, double-blinded, placebo controlled trials in more immature neonates are needed to prove the effectiveness.[43]

CONFLICT OF INTEREST

The authors indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Z.R.: contributed in writing the manuscript, read and approved the final manuscript; F.X., C.Z., J.M., M.K., W.W., T.M., Q.W.: performed the trials, read and approved the final manuscript; X.Z., X.X.: read and approved the final manuscript; Q.Z., L.L., J.W., G.L.: analyzed data and collected the references, read and approved the final manuscript; K.L.: performed the trials; designed the study and edited the manuscript, read and approved the final manuscript; J.Y.: designed the study and edited the manuscript, read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Lancet Neonatal Survival Steering Team, Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? *Lancet*. 2005;365(9462):891-900.
2. WHO WEBSITE, http://www.who.int/topics/preterm_birth/en/
3. Niver D. Bronchopulmonary dysplasia: structural challenges and stem cell treatment potential. *Adv Neonatal Care*. 2014;14(1):E1-E11.
4. Yang J, Ren Z, Zhang C, et al. Safety of autologous cord blood cells for preterms: a descriptive study. *Stem Cells Int*. 2018;2018: 5268057.
5. Möbius MA, Freund D, Vadivel A, et al. Oxygen disrupts human fetal lung mesenchymal cells: implications for bronchopulmonary dysplasia. *Am J Respir Cell Mol Biol*. 2019;60:592-600.
6. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood*. 2013;122(4): 491-498.
7. Walter J, Ware LB, Matthay MA. Mesenchymal stem cells: mechanisms of potential therapeutic benefit in ARDS and sepsis. *Lancet Respir Med*. 2014;2(12):1016-1026.
8. Pierro M, Thebaud B, Soll R. Mesenchymal stem cells for the prevention and treatment of bronchopulmonary dysplasia in preterm infants. *Cochrane Database Syst Rev*. 2017;11:CD011932.
9. Alessandro Borghesi, Claudia Cova, Diego Gazzolo1, Mauro Stronati, Stem cell therapy for neonatal diseases associated with preterm birth, *J Clin Neonatol* 2013 2 1 1-7
10. Mezey E, Nemeth K. Mesenchymal stem cells and infectious diseases: smarter than drugs. *Immunol Lett*. 2015;168(2):208-214.
11. Chang YS, Ahn SY, Yoo HS, et al. Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial. *J Pediatr*. 2014;164(5):966-972.e6.
12. Cotten CM, Murtha AP, Goldberg RN, et al. Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. *J Pediatr*. 2014;164(5):973-979.e1.
13. Ahn SY, Chang YS, Kim JH, Sung SI, Park WS. Two-year follow-up outcomes of premature infants enrolled in the phase I trial of Mesenchymal stem cells transplantation for bronchopulmonary dysplasia. *J Pediatr*. 2017;185:49-54.e2.
14. Kotowski M, Litwinska Z, Klos P, et al. Autologus cord blood transfusion in preterm infants—could its humoral effect be the key to control prematurity-related complications ? A preliminary study. *J Physiol Pharmacol*. 2017;68(6):921-927.
15. Rudnicki J, Kawa MP, Kotowski M, et al. Clinical evaluation of the safety and feasibility of whole autologous cord blood transplant as a source of stem and progenitor cells for extremely premature neonates: preliminary report. *Exp Clin Transplant*. 2015;13(6):563-572.
16. National Health and Family Planning Commission of the People's Republic of China. Umbilical cord blood and stem cell bank management regulation. Website: http://www.Moh.Gov.cn/zhuzhan/wsbmgz/201308/9da745d55fe749a2b82b95ee65294b55.shtml (Chinese).
17. Gomella T, Cummingham M, Fabien E. *Neonatology*. 6th ed. New York: Lange; 2009.
18. Baker CD, Balasubramaniam V, Mourani PM, et al. Cord blood angiogenic progenitor cells are decreased in bronchopulmonary dysplasia. *Eur Respir J*. 2012;40(6):1516-1522.
19. Efstathiou N, Soubasi V, Koliakos G, et al. Mobilization of circulating progenitor cells following brain injury in premature neonates could be indicative of an endogenous repair process. A pilot study. *Hippokratia*. 2015;19(2):141-147.
20. Machalinska A, Modrzejewska M, Kawa M, et al. Potential contribution of mobilized circulating endothelial progenitor cells to development of retinal neovascularization in preterm infants with ROP. *Klin Oczna*. 2013;115(3):194-198.
21. Kourembanas S. Stem cell-based therapy for newborn lung and brain injury: feasible, safe, and the next therapeutic breakthrough? *J Pediatr*. 2014;164(5):954-956.
22. Solves P, López M, Mirabet V, Blanquer A, Roig R, Perales A. Characteristics of umbilical cord blood units collected from preterm deliveries. *Gynecol Obstet Invest*. 2009;68(3):181-185.
23. O'Reilly M, Thébaud B. Stem cells for the prevention of neonatal lung disease. *Neonatology*. 2015;107:360-364.
24. Larijani B, Esfahani EN, Amini P, et al. Stem cell therapy in treatment of different diseases. *Acta Med Iran*. 2012;50(2):79-96.
25. Ho MS, Mei SH, Stewart DJ. The immunomodulatory and therapeutic effects of mesenchymal stromal cells for acute lung injury and sepsis. *J Cell Physiol*. 2015;230(11):2606-2617.
26. Matthay MA, Calfee CS, Zhuo H, et al. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial. *Lancet Respir Med*. 2019;7(2):154-162.
27. Lee MW, Jang IK, Yoo KH, Sung KW, Koo HH. Stem and progenitor cells in human umbilical cord blood. *Int J Hematol*. 2010;92(1):45-51.
28. Blau HM, Daley GQ. Stem cells in the treatment of disease. *N Engl J Med*. 2019;380(18):1748-1760.
29. Ortiz LA, Dutreil M, Fattman C, et al. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci USA*. 2007; 104:11002-11007.
30. Gupta N, Krasnodembskaya A, Kapetanaki M, et al. Mesenchymal stem cells enhance survival and bacterial clearance in murine *Escherichia coli* pneumonia. *Thorax*. 2012;67:533-539.
31. Raff aghello L, Bianchi G, Bertolotto M, et al. Human mesenchymal stem cells inhibit neutrophil apoptosis: a model for neutrophil preservation in the bone marrow niche. *Stem Cells*. 2008;26:151-162.
32. Popova AP. Mesenchymal cells and bronchopulmonary dysplasia: new insights about the dark side of oxygen. *Am J Respir Cell Mol Biol*. 2019;60(5):501-502.
33. wang J, dong W. Oxidative stress and bronchopulmonary dysplasia. *Gene*. 2018;678:177-183.
34. Womble TA, Green S, Shahaduzzaman M, et al. Monocytes are essential for the neuroprotective effect of human cord blood cells following middle cerebral artery occlusion in rat. *Mol Cell Neurosci*. 2014;59:76-84.
35. Baud O, Trousson C, Biran V, et al. Association between early low-dose hydrocortisone therapy in extremely preterm neonates and neurodevelopmental outcomes at 2 years of age. *JAMA*. 2017;317(13): 1329-1337.
36. Sun JM, Song AW, Case LE, et al. Effect of autologous cord blood infusion on motor function and brain connectivity in young children with cerebral palsy: a randomized, placebo-controlled trial. *Stem Cells Translational Medicine*. 2017;6(12):2071-2078.
37. Paton M, McDonald CA, Allison BJ, Fahey MC, Jenkin G, Miller SL. Perinatal brain injury as a consequence of preterm birth and intrauterine inflammation: designing targeted stem cell therapies. *Front Neurosci*. 2017;11:200.
38. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the nichd neonatal research network. *Pediatrics*. 2010;126:443-456.
39. Wasielewski B, Jensen A, Roth-Härer A, Dermietzel R, Meier C. Neuroglial activation and Cx43 expression are reduced upon transplantation of human umbilical cord blood cells after perinatal hypoxic-ischemic injury. *Brain Res*. 2012;1487:39-53.
40. Rosenkranz K, Kumbruch S, Tenbusch M, et al. Transplantation of human umbilical cord blood cells mediated beneficial effects on

apoptosis, angiogenesis and neuronal survival after hypoxic-ischemic brain injury in rats. *Cell Tissue Res*. 2012;348:429-438.
41. Yasuhara T, Hara K, Maki M, et al. Mannitol facilitates neurotrophic factor up-regulation and behavioural recovery in neonatal hypoxic-ischaemic rats with human umbilical cord blood grafts. *J Cell Mol Med*. 2010;14:914-921.
42. Nitkin CR, Rajasingh J, Pisano C, Besner GE, Thébaud B, Sampath V. Stem cell therapy for preventing neonatal diseases in the 21st century: current understanding and challenges. *Pediatr Res*. 2019. https://doi.org/10.1038/s41390-019-0425-5
43. Mueller M, Kramer BW. Stem cells and bronchopulmonary dysplasia - the five questions: which cells, when, in which dose, to which patients via which route? *Paediatr Respir Rev*. 2017;24:54-59.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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