Vietnam Journal of Biotechnology **22**(1): 35-44, 2024. DOI: 10.15625/vjbt-20713

CHARACTERIZATION OF CORD BLOOD IMMUNE AND STEM CELLS FROM VIETNAMESE INFANTS

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Received: 11.12.2023
Accepted: 12.03.2024

ABSTRACT

Cord blood has emerged as a valuable and versatile resource, holding within its grasp a myriad of immune and stem cell types with immense therapeutic potential. Moreover, immune profiling in cord blood, a burgeoning field of research, offers profound insight into the early immune development of newborns, paving the way for understanding immune health, disease susceptibility, and therapeutic interventions. Therefore, in this research, we aimed to characterize the diverse immune and stem cell types present in the cord blood of Vietnamese infants. In this study, a total of 59 cord blood samples were examined for immune cell populations. Cell populations were identified using flow cytometry based on the expression of specific markers on the cell surface. The results showed that the $CD3^+/CD4^+$ population accounted for the greatest proportion of lymphocytes (46.71 ± 8.99%), followed by the $CD3^+/CD8^+$ population (22.58 ± 6.73%). The mature B-cell population ($CD3^-/CD19^+$) accounted for 9.52 ± 3.28%, while $CD3^+/CD19^+$ cells accounted for an average of 8.59 ± 5.77%. Moreover, the NK population ($CD3^-/CD56^+$ cells) accounted for 6.44 ± 4.9%, T lymphocytes carrying both CD4 and CD8 markers ($CD3^+/CD4^+/CD8^+$) accounted for 0.63 ± 0.41%, and NKT cells ($CD3^+/CD56^+$) accounted for only 0.30 ± 0.53%. Moreover, the number of hematopoietic stem cells (HSCs) tends to increase in umbilical cord blood units with a high volume and number of TNCs and MNCs. In contrast, the number of HSCs was negatively correlated with the ratio of B lymphocytes. The TCD4 ratio

was positively correlated with the number of HSCs but negatively correlated with the NK ratio ($p = 0.01$). The study initially characterized the diversity of some stem and immune cell types present in the umbilical cord blood of Vietnamese infants. These findings laid the groundwork for developing novel immune profiles and expanding the potential applications of umbilical cord blood in cellular therapy.

Keywords: Immune cells, Immune profiling, Cord blood, Hematopoietic stem cells, Natural killer cells, Natural killer T cells, T lymphocyte, B lymphocyte.

INTRODUCTION

In the fields of regenerative medicine and immunotherapy, cord blood has emerged as a valuable and versatile resource containing a myriad of immune cell types and hematopoietic stem cells (HSCs) with great therapeutic potential. As we delve deeper into the stem and immune cell types in cord blood, we discover a world of possibilities. The intricate interplay between immune cells and stem cells holds promise for new therapies for countless diseases and conditions. From harnessing the power of immune cells for immunotherapy to using stem cells for tissue regeneration, cord blood offers an unparalleled opportunity to advance the frontiers of medicine (Kim *et al.*, 2011; Roura *et al.*, 2015).

Moreover, immune profiling in cord blood, a burgeoning field of research, provides insight into the early immune development of newborns, paving the way for understanding immune health, disease susceptibility, and therapeutic interventions. One of the most intriguing aspects of immune profiling in cord blood is its ability to shed light on the early developmental milestones of the immune system. During fetal life, the immune system undergoes a series of intricate processes, from the generation of diverse immune cell populations to the establishment of immune tolerance toward self-antigens. Cord blood provides a snapshot of this developmental journey, offering insights into the maturation of T cells, B cells, NK cells, and other immune cells, and the establishment of immune memory (Olin *et al.*, 2018; Anderson *et al.*, 2021; Eisner *et al.*, 2022). Furthermore, immune profiling in cord blood has great potential for predicting the future immune health of newborns. Variations in immune cell populations and functional characteristics observed in cord blood may correlate with the risk of developing immune-mediated diseases later in life (Olin *et al.*, 2018).

In this research, we characterized the diverse immune and stem cell types present in the cord blood of Vietnamese newborns. By understanding the unique signatures of these immune and stem cell populations, we aim to pave the way for innovative therapies, personalized medicine, and better predictions of future immune health in infants.

MATERIALS AND METHODS

Research subjects

Forty-eight donated cord blood samples were collected, and 11 partially donated cord blood samples from healthy infants were included in this study. The mothers were pregnant from week 37 onward; had undergone screening tests; and did not have

hepatitis B, hepatitis C, HIV, CMV or syphilis viruses. Pregnant women signed a consent form to voluntarily donate umbilical cord blood samples.

Cord blood collection

Samples were collected immediately after the birth. All samples were collected from the umbilical cord vein and stored in specialized collection bags containing 35 ml of CPDA-1 (citrate phosphate dextrose adenine) anticoagulant.

TNC and MNC count

To determine the density of white blood cells in umbilical cord blood, we performed a complete blood count (CBC) using an automated hematology analyzer (Nihon Kohden 3700, Japan). The total nucleated cells (TNCs) were calculated by multiplying the TNC by the total volume of the collection bag. The total mononuclear cell (MNC) counts were calculated by adding the absolute lymphocyte and monocyte counts reported in the CBC and then multiplying this value by the total volume of the cord blood collection bag.

Immune cell and hematopoietic stem cell phenotyping

Following cord blood collection, a fresh sample of whole blood was lysed from red blood cells (RBCs) by lysing solution and then stained with antibodies to examine the proportion of immune cells and the density of HSCs, such as CD45, CD34, CD3, CD4, CD8, CD19, and CD56 (Beckman Coulter). The samples were analyzed on a Navios Beckman Coulter 10-color flow cytometer (Figure 1). To determine the number of HSCs, the number of white blood cells was defined as $CD45^+$, and the number of HSCs was determined based on CD34+ and low site scatter (SS) counts.

To determine immune cells, the lymphocyte population was determined by $CD45^+$ and site scatter (SS) counts. Based on the gating lymphocytes, the proportions of $CD4^+$ T cells were determined by $CD3^+$ and $CD4^+$; $CD8^+$ T cells were determined by $CD3^+$ and $CD8^+$; $CD4^+$ $CD8^+$ T cells were determined by $CD3^+$, $CD4^+$, and $CD8^+$; B cells were determined by $CD19^+$ and $CD3^-$; and NK cells were determined by $CD3^-$ and $CD56^+$ (Figure 1).

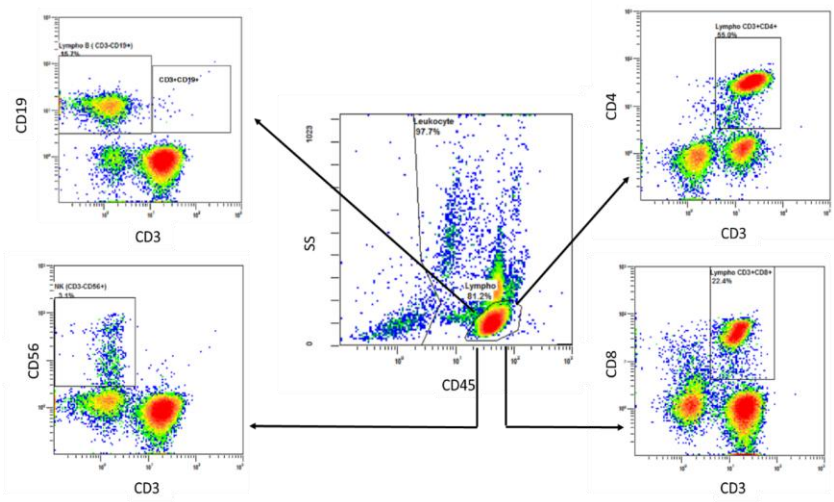

Figure 1. Strategy for analyzing immune cell populations.

Data analysis

Descriptive statistics were used to present data on sex, gestational age, and test indicators. Student's t test, the Mann–Whitney test and linear regression were used to test the differences and correlations between indicators, respectively.

RESULTS AND DISCUSSION

Characteristics of the samples

Overall, 59 infants were recruited for this study, including 48 total cord blood donors and 11 partial cord blood donors. The average gestational age was 38.8 ± 0.9 weeks, ranging from 37 to 40 weeks. The average weight of the infants was 3.2 ± 0.3 (range: 2.6–4.2) kg, 28.8% of whom weighed ≤ 3 kg, and 71.2% of whom weighed ≥ 3 kg. In a total of 48 cord blood samples collected, the average cord blood sample volume was 94.49 ± 30.35 ml, excluding anticoagulants (Table 1).

TNC and MNC counts

Total Nuclear Cells (TNCs): The average total nucleated white blood cell (TNC) count of the cord blood units collected was 11.48 ± 3.93 × 10^8 cells (n = 48), ranging from 5.10 × 10^8 cells to 55.7 × 10^8 cells. The average count of total mononuclear cells (MNC) in the collected cord blood samples was 4.34 ± 1.66 × 10^8 cells, ranging from 1.64 × 10^8 cells to 8.42 × 10^8 cells (Table 2).

Table 1. Characteristics of sex, weight, gestational age, and cord blood volume.

Characteristic		**Frequency (n = 59)**	**Ratio (%)**
Gender	*Boy*	31	52.5
	Girl	28	47.6
Gestational age	*≤ 38 weeks*	18	30.5
	> 38 weeks	41	69.5
Weight	*≤ 3 kg*	17	28.8
	> 3 kg	42	71.2
Characteristic	**Mean ± SD**	**Min**	**Max**
Gestational age (weeks)	38.8 ± 0.9	37	40
Weight (kg)	3.2 ± 0.3	2.6	4.2
Volume (ml)	94.49 ±30.35	52	176
CB volume of boy (ml)	95.32 ± 31.11	52	176
CB volume of girl (ml)	89.94 ± 26.46	55,9	140

Table 2. Average number of TNCs and MNCs by gender.

Characteristic		Frequency	Mean ± SD	Min	Max	P value
TNC (× 10^8)	Total	48	11.49 ± 3.97	5.10	19.82	p > 0.05
	Boy	28	11.20 ± 3.85	5.10	19.82	
	Girl	20	11.89 ± 4.10	5.18	19.82	
MNC (× 10^8)	Total	48	4.34 ± 1.66	1.64	8.42	p > 0.05
	Boy	28	4.48 ± 1.71	1.65	8.42	
	Girl	20	4.15 ± 1.56	1.64	7.21	

HSC count

Survey results on 48 umbilical cord blood samples showed that the average number of HSCs was 3.64 ± 2 × 10^6 cells, with a range from 0.81 × 10^6 to 9.79 × 10^6 cells. In particular, the number of HSCs in the umbilical cord blood of boys was 4.12 ± 2.23 × 10^6 cells (n = 28), which was greater than that in the umbilical cord blood of girls (2,97 ± 1,51 × 10^6 cells; n = 20) (p < 0.05). Furthermore, the number of HSCs in cord blood with a volume greater than 100 ml (excluding anticoagulants) was 5.26 ± 1.74, which was greater than that in cord blood units less than 100 ml (2.83 ± 1.65; p < 0.05) (Table 3).

Table 3. The average number of HSCs in cord blood.

Characteristic	HSC *(x10^6 cells)*			
	Mean ± SD	Min	Max	P value
Total	3.6 ± 2.0	0.81	9.79	
Girl	2.97 ± 1.51	0.87	9.79	< 0.05
Boy	4.12 ± 2.23	0.81	6.90	
< 100 ml	2.83 ± 1.65	0.81	7.31	1.12×10^{-5}
> 100 ml	5.26 ± 1.74	3.36	9.79	

Immune cell proportions

Analysis of 59 umbilical cord blood samples revealed that the $CD4^+$ T-cell population ($CD3^+/CD4^+$) accounted for the greatest proportion (46.71 ± 8.99%) of lymphocytes, followed by the $CD8^+$ T-cell population (CD3+/CD8+) (22.58 ± 6.73%). The mature B-cell population ($CD3^-/CD19^+$) accounted for 9.52 ± 3.28%, and $CD3^+/CD19^+$ cells accounted for an average of 8.59 ± 5.77%. Moreover, the NK population ($CD3^-/CD56^+$) accounted for 6.44 ± 4.9%, T lymphocytes carrying both CD4 and CD8 markers ($CD3^+/CD4^+/CD8^+$) accounted for 0.63 ± 0.41%, and the NKT population ($CD3^+/CD56^+$) accounted for only 0.3 ± 0.53% (Figure 2).

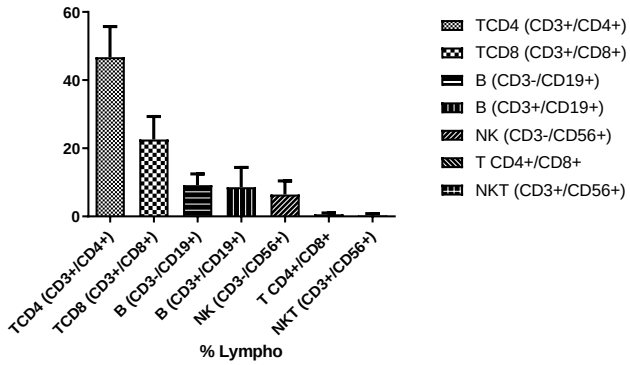

Figure 2. Proportion of the immune subpopulation in the lymphocyte cord blood. TCD4 (CD3+/CD4+) accounted for 46.71 ± 8.99%, TCD8 (CD3+/CD8+) accounted for 22.58 ± 6.73%, mature B cells (CD3-/CD19+) accounted for 9.52 ± 3.28%, CD3+/CD19+ accounted for 8.59 ± 5.77%, NK (CD3-/CD56+) accounted for 6.44 ± 4.9%, T CD4/CD8 (CD3+/CD4+/CD8+) accounted for 0.63 ± 0.41%, and NKT (CD3+/CD56+) accounted for 0.3 ± 0.53%.

Relationships between immune populations and other factors

Analysis of correlation coefficients between indicators using linear regression showed that factors such as gestational age, birth weight, proportion of $CD3^+CD19^+$ B cells, $CD8^+$ T cells, and NK cells did not correlate linearly with the number of HSCs. However, the sample volume, number of TNCs, and number of MNCs strongly correlated with the number of HSCs, with r values of 0.67, 0.60, and 0.57, respectively ($p < 0.01$). The proportion of $CD4^+$ T cells was moderately positively correlated with the number of HSCs ($r = 0.35$; $p = 0.01$), and the number of B cells ($CD3^-/CD19^+$) was negatively correlated with the number of HSCs ($r = -0.3$; $p = 0.03$) (Table 4).

Table 4. Correlations between factors and the number of HSCs.

Characteristic	Number of HSCs (n = 59)	
	Correlation coefficients (r)	P value
Gestational age (weeks)	-0.08	0.59
Birth weight (kg)	0.14	0.34
Sample volume (ml)	0.67	0.00
TNCs (10^8)	0.60	0.00
MNCs (10^8)	0.57	0.00
$CD19^+/CD3^+$ B cells	-0.16	0.28
B-cell ($CD19^-/CD3^+$)	-0.30	0.03
TCD4 cells ($CD3^+/CD4^+$)	0.35	0.01
TCD8 cells ($CD3^+/CD8^+$)	-0.11	0.44
NK cells	-0.23	0.11

In addition, the $CD4^+$ T-cell percentage was moderately negatively correlated with the fraction of NK cells (r = -0.42; p = 0.01), and the percentage of $CD8^+$ T cells had a moderate negative correlation with the proportion of NK cells (r = -0.27; p = 0.03). Moreover, factors such as gestational age, birth weight, sample volume, number of TNCs, MNCs, B cells ($CD3^+/CD19^+$) and B cells ($CD3^-/CD19^+$) had no linear correlation with the number of NK cells (Table 5).

Table 5. Correlations between factors and the NK proportion.

Characteristic	NK proportion (n = 59)	
	Correlation coefficients (r)	P value
Gestational age (weeks)	0.10	0.46
Birth weight (kg)	-0.03	0.83
Sample volume (ml)	- 0.23	0.12
TNCs (10^8)	- 0.01	0.99
MNCs (10^8)	- 0.11	0.46
$CD19^+/CD3^+$ B cells	- 0.26	0.05
B-cell ($CD19^-/CD3^+$)	0.09	0.50
TCD4 cells ($CD3^+/CD4^+$)	- 0.42	0.01
TCD8 cells ($CD3^+/CD8^+$)	- 0.27	0.03

The volume of cord blood collected and the average number of nucleated cells (TNCs), mononuclear cells (MNCs) and $CD34^-$ expressing HSCs in cord blood in our study were consistent with the results reported by Nguyen *et al.*, 2020 (n = 403) (Nguyen *et al.*, 2020) because we both studied the Vietnamese population. However, Lee *et al.* suggested that the average number of HSCs (CD34+) was equivalent between boys and girls, which contradicts our findings that HSCs are greater in boys than in girls ($p < 0.05$) (Lee *et al.*, 2012).

Umbilical cord blood has long been known not only as a source of HSCs but also as a rich source of immune cells. These nascent immune cells are directly related to a baby's health during early life. B lymphocytes (B cells) are essential for the adaptive immune system's ability to recognize, target, and eliminate pathogens through the production of antibodies. They also contribute to the establishment of immunological memory, which provides long-lasting protection against recurring infections. Although the immune system of infants, especially newborns, is not yet fully developed, infants are able to respond more quickly to humoral stimuli than are adult B lymphocytes (Halista *et al.*, 1998). $CD4^+$ T cells, also known as helper T cells or T helper cells, play important roles in supporting B and $CD8^+$ T lymphocytes in performing immune functions. TCD4 is capable of recognizing peptides from foreign elements such as bacteria and viruses and responds by producing cytokines and carrying out other reactions to help activate other cells in the immune system. $CD8^+$ T lymphocytes are called cytotoxic T cells. They detect and

recognize foreign antigens by binding to human leukocyte antigen (HLA) molecules on the surface of presenting cells, and they use perforin and granzyme to kill abnormal cells directly. The function of natural killer (NK) cells is crucial for immune surveillance and defense. NK cells detect and eliminate virus-infected cells and cancerous cells by recognizing stress-induced ligands. They release cytotoxic granules containing perforin and granzymes, inducing apoptosis in target cells. Additionally, NK cells produce cytokines such as INF-γ, regulating immune responses. NKT cells play an essential role in immune regulation and help activate T cells and NK cells in the immune system. Furthermore, NKT cells are specialized cells that regulate heterogeneous immune responses, including allergic and inflammatory responses. In this study, we found that the $CD4^+$ T-cell population accounted for the most significant proportion of lymphocytes (46.71% ± 8.99%), followed by the $CD8^+$ T-cell population ($CD3+/CD8^+$), which accounted for 22.58 ± 6.73%. Mature B cells (CD3-/CD19+) accounted for 9.52 ± 3.28%, and NK cells ($CD3^-/CD56^+$) accounted for 6.44 ± 4.9%. Moreover, T CD4/CD8 cells ($CD3^+/CD4^+/CD8^+$) accounted for 0.63 ± 0.41%, and NKT cells ($CD3^+/CD56^+$) were relatively modest, accounting for only 0.3% ± 0.53% of lymphocytes. The B and T lymphocyte hybrid cell population that coexpresses both CD3 and CD19 has thus far been little mentioned in the medical literature, and its role remains unclear. Only a few publications, such as Schultz *et al.*, have described a patient who previously received a haploidentical HSC transplantation and subsequently received a CAR-T-cell product with $CD3^+CD19^+$ B lymphocytes (Schultz *et al.*, 2020). Another publication was by Li *et al.*, 2021 on patients infected with HIV and Mycobacterium (Li *et al.*, 2021). All publications focused on the detection of $CD3^+/CD19^+$ populations in adult peripheral blood, and there were no publications on cord blood lymphocyte populations. In this study, this birth cell population accounted for 8.59% ± 5.77% of lymphocytes, which was slightly lower than the percentage of lymphocytes in the B lymphocyte $CD3^-/CD19^+$ population.

In addition, when analyzing the relationships between cell types, we observed that the number of HSCs tended to increase in umbilical cord blood units with high volume and a high number of TNCs and MNCs. In contrast, the number of HSCs was negatively correlated with the ratio of B lymphocytes. Moreover, the $CD4^+$ T-cell ratio was positively correlated with the number of HSCs but negatively correlated with the NK-cell ratio ($p = 0.01$). The results of the correlation between CD4 T cells and HSCs were also similar to those of Putrawan *et al.* (2022), who reported a correlation coefficient ($r = 0.64$ ($p < 0.001$). However, their study was limited to Wistar rats (Putrawan *et al.*, 2022). The relationship between HSCs and $CD4^+$ T cells has long been studied, and the interaction between $CD4^+$ T cells and HSCs is a complex process that includes the maintenance of HSCs and the regulation of the immune system. This relationship is significant, contributes to the success of hematopoietic stem cell transplants and is necessary for the normal functioning of the immune system (Mahmud *et al.*, 2011, Mueller *et al.*, 2014). These correlation patterns of cell proportions are still not well studied; however, they can be a premise for studying and building immune profiles from the newborn period and modeling to predict future diseases.

CONCLUSION

In this study, we initially characterized the diversity of stem and immune cell types present in the umbilical cord blood of Vietnamese newborns and demonstrated correlations between them. These findings serve as the foundation for developing new immune profiles and expanding the applications of umbilical cord blood in cell therapy.

ACKNOWLEDGMENTS

This study was funded by project DT-01 from Vinmec Healthcare System, Vingroup Corporation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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