



### THE RELATIONSHIP BETWEEN THE UMBILICAL CORD LENGTH AND IT'S DIAMETER WITH THE TOTAL CD34+ AND TOTAL NUCLEATED CELL (TNC) AS A PARAMETER OF CORD BLOOD SELECTION

M Indriani<sup>1\*</sup>, Y Rahadiyanto<sup>2</sup>, Y Effendi<sup>3</sup>, BW Putra<sup>4</sup>, Z Hafy<sup>1,5</sup>

<sup>1</sup>Magister Program of Biomedical Science, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia

<sup>2</sup>Department of Clinical Pathology, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia

<sup>3</sup>Department of Obstetrics and Gynecology- Mohammad Hoesin Hospital, Palembang, Indonesia

<sup>4</sup>Prodia Stem Cell Indonesia (ProStem), Jakarta, Indonesia

<sup>5</sup>Department of Histology, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia

\*Corresponding author : [meiindriyani1505@gmail.com](mailto:meiindriyani1505@gmail.com)

#### ABSTRACT

**Background:** The stem cell transplantation successful influenced by the quality of the umbilical cord blood which includes the number of CD34 + and Total Nucleated Cells (TNC).

**Objectives:** The study aims to determine the correlation between umbilical cord length and it's diameter, with the number of CD34 + and TNC cells as indicators of the quality of cord blood storage feasibility. Several other factors as maternal age, gestation period, and infant birth weight also examined.

**Methods:** Thirty four of umbilical cords from the delivered woman in Dr. Mohammad Hoesin Hospital (RSMH) Palembang were collected from May to June 2018. The length and diameter of the cord were immediately measured after delivery. The evaluated cells were counted with a flow cytometer at Klaster Stem Cell and Tissue Engineering Research Centre (SCTE) IMERI Faculty of Medicine University of Indonesia (FKUI).

**Results:** Spearman correlation test show that there was no correlation between the length and diameter of the umbilical cord, maternal age, gestational period and infant weight, with the number of CD34 + and TNC cells in the cord ( $p > 0.05$ ). However, the profile analysis indicated that the longer and larger the diameter of the umbilical cord, the higher concentration of the CD34+ and TNC cells.

**Conclusion:** This study suggested that the younger maternal age, older gestational age, and higher infant birth weight, also normal hemoglobin level, tend to increase the number of CD34+ and TNC cells in the cord blood.

**Keywords :** CD34+, Total nucleated cells, Umbilical cord blood

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## **INTRODUCTION**

Stem cells are naive cells that can divide and develop into various types of cells in the body and potentially replace various damaged[1] body tissues with two characteristics of self renew and differentiated. There are 3 types of stem cells that can be obtained from the human body: bone marrow, peripheral blood, and cord blood.[2]

Umbilical cord blood (UCB) is the blood that was left over in the placenta shortly after the baby was born and the umbilical cord has been cut. This cord blood is one of the potential sources of hematopoietic stem cells that has a greater number of Progenitor cells compared to the peripheral blood or bone marrow. UCB is also rich in Haematopoietic Stem Cells (HSCs) which will be responsible for the formation of blood and the immune system.[3]

To ensure the quality of cord blood well maintained for a long time, Umbilical Cord Blood Banking (UCBB) was established.[4] Studies indicated that the success of stem cell transplantation is influenced by several parameters such as the number of TNC and CD34+ cells.[5,6] TNC and CD34+ cell numbers reflect the hematopoietic potential of cord blood. Stem Cell transplants success rate are higher when CD34+ cell counts and TNC numbers are high.[7] Accurate CD34+ cell counts are needed to calculate the dosage needed for Stem Cell Transplantation. So, the success of Stem Cell transplantation is very dependent on CD34+ cell count calculations. TNC doses that will provide during transplantation is also a very important factor for the use of hematopoietic stem cells in cord blood transplantation. Higher cost of TNC can increase the success of cord blood transplantation.[8] A number of factors

such as the length and diameter of the umbilical cord, maternal age, gestation, hemoglobin of the preterm mother and infant weight at birth may affect the number of TNCs and CD34+ cells.[9]

This study is designed to determine the correlation of umbilical cord length and diameter, and other factors as maternal age, gestational age, pre-maternal hemoglobin and infant birth weight to the number of CD34+ and TNC cells as indicators of the quality of the feasibility of cord blood storage.

## **MATERIAL AND METHODS**

Thirty four of umbilical cords from the delivered woman in Dr. Mohammad Hoesin Hospital (RSMH) Palembang were collected from May to June 2018. Subjects were required to sign an informed consent form before samples were taken. This study was ethically approved by Health Research Review Committee of Mohammad Hoesin Central Hospital and Faculty of Medicine, Sriwijaya University (No.49/kepkrsmhfkunsri/2018)

### **Cord blood collection and umbilical cord measurement**

Hemoglobin levels was measure from venous blood before the subject give birth. Shortly after the baby was born, the umbilical cord that connects the baby and mother is clamped and cut and the umbilical cord that still attached to the mother is disinfected using Povidone Iodine. Next, UCB was collected by obstetricians or midwives that aided the process of delivery within-utero technique. The placenta was removed and the umbilical cord was measured starting from the base of the placenta and then moving towards the cutting site using a gauge. The diameter of the umbilical cord was measured by placing the surface of the

umbilical cord into a caliper and read the diameter on it.

### Sample handling

The cord blood that has been taken then immediately sent to the laboratory for sample handling. The sample is centrifuged to obtain a buffy coat and stored in a -80°C freezer until the number of samples is fulfilled.

### TNC and CD34+ cells counting

Samples that have been prepared are stained using Trypan Blue and examined under a microscope to see the number of nucleated cells. The potential of existing stem cells then evaluated using the flow cytometry method for TNC and CD34+ cell markers. Each cell will be read and shot by a laser beam one by one through their respective channels. The shot will be read by two detectors to describe the size and content of the cell.

### Statistical analysis

The statistical analysis for this study is performed using SPSS 16.0. Since most the continuous data were not normally distributed (based on the Shapiro-Wilk normality test), we used the Spearman correlation analysis test to measure the relationship between the umbilical cord length and diameter with the number TNC and CD34+ cell and the other study variables.

## RESULTS

Descriptive statistics were carried out on CD34+ cell counts, TNC, cord length and diameter, maternal age, gestation period, hemoglobin of preterm mothers and infant body weight (Table 1).

Based on the data distribution analysis, there were some outliers in the

dataset of each variable on the study as showed by the boxplot diagram below (Figure 1). There is one outlier in the lower side and one outlier in the upper side of the umbilical cord length dataset, 65cm and 28cm, respectively. In the TNC group, there were 2 outliers in the upper side of the dataset group, and one of them with very high TNC count number (3179520 cells/uL) showed by a longer upper whisker on the boxplot. In the CD34+ cell group, there were also 2 outliers on the upper side of the data group, one of them with the CD34+ cell concentration more than 2.5 million cells per uL. The infant birth weight dataset group showed one upper outlier and one lower outlier value, 4100 grams, and 1350 grams, respectively (Figure 1).

The longer and the larger the diameter of the umbilical cord, the higher the concentration of TNC and CD34+ cells, and vice versa. For example, one case with short umbilical cord (28cm) only contained very low number of TNCs and CD34+ cells which were 17760 cells/uL and 100640 cells/uL, respectively. The cases with short umbilical cords may be related to inadequate nutritional intake during pregnancy. A condition where the mother lacks nutritional intake during pregnancy is considered as a factor that plays a role in the process of TNC and CD34+ cell transport from the maternal blood circulation to the umbilical cord which in turn causes low TNC and CD34+ cell concentrations. Further studies might be needed to support this finding.

The study showed that there was no correlation between the length and diameter of the umbilical cord, maternal age, gestation period, hemoglobin of preterm mothers and infant birth weight with TNC and CD34+ cells (Table2).

Table 1. Descriptive characteristics of subjects

Characteristics	N	Min	Max	Mean value $\pm$ SD
Umbilical cord length (cm)	34	28	65	51.4 $\pm$ 6.8
Umbilical cord diameter (cm)	34	0.6	2.5	1.4 $\pm$ 0.5
Maternal age (years)	34	21	35	28.4 $\pm$ 3.6
Gestation period (weeks)	34	29	42	36.7 $\pm$ 3.1
HB level (g/dL)	34	8.5	13.2	10.9 $\pm$ 1.3
Neonates Birth Weight (gram)	34	1300	4100	2677.5 $\pm$ 593.7

Tabel 2. Spearman Correlation Test

Outcome Variables	Predictor Variables	Number of samples (n)	<i>p</i> value	Coeffisien Correlation ( <i>r</i> )
<b>CD34+</b>	Umbilical cord length	34	0.148	0.253
	Umbilical cord diameter	34	0.543	0.108
	Maternal age	34	0.683	-0.073
	Gestation period	34	0.067	0.318
	Hb level	34	0.685	0.072
	Neonates birth weight	34	0.107	0.282
<b>TNC</b>	Umbilical cord length	34	0.165	0.244
	Umbilical cord diameter	34	0.831	0.038
	Maternal age	34	0.175	-0.238
	Gestation period	34	0.156	0.249
	Hb level	34	0.538	0.109
	Neonates birth weight	34	0.072	0.313

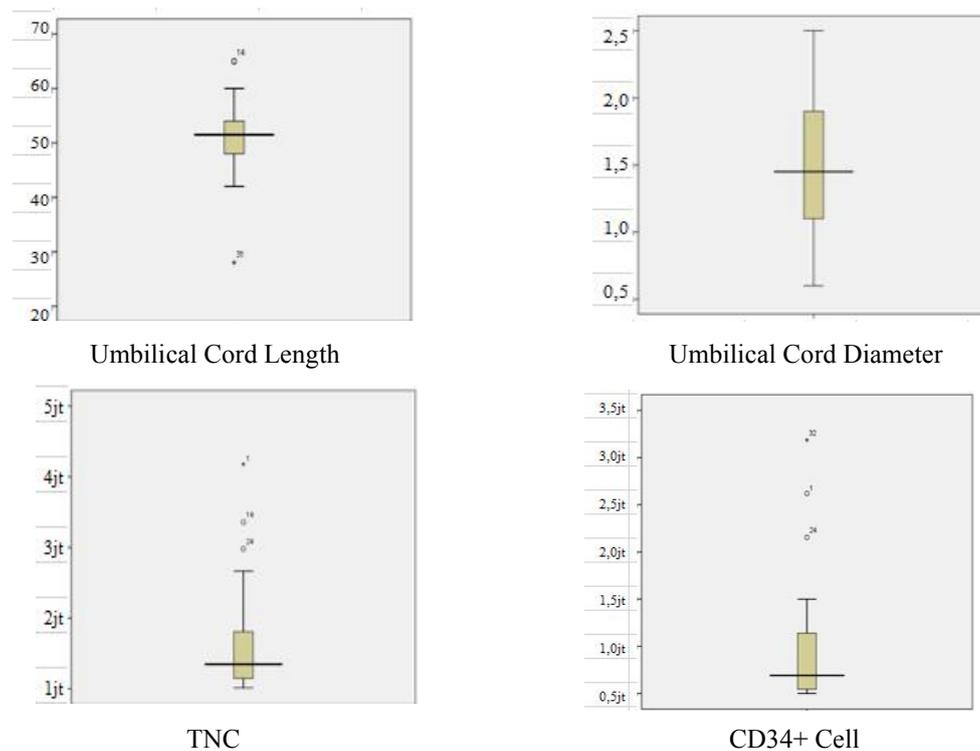


Figure1. Data distribution of each research variable

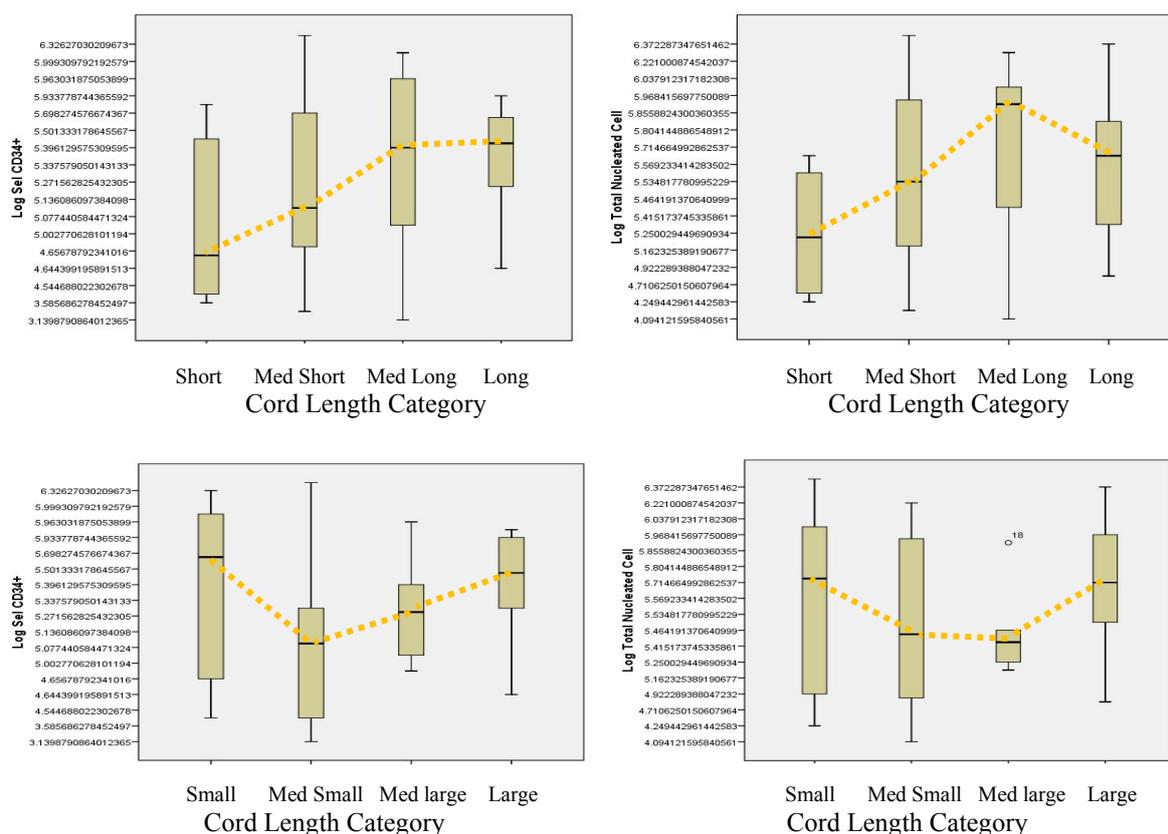


Figure2. CD34+ and TNC cell profiles in each variable

**DISCUSSION**

The number of TNC and CD34+ cells when collecting cord blood that will be used for storage and transplantation have to be assured exactly because high amounts of TNC and CD34+ cells will provide good transplant results.[8]

The finding of the highest value above was followed by several other supporting data such as 30 weeks gestation, Hemoglobin for preterm mothers 10.3 g/dL, infant weight of only 1600 grams with leukocytes reaching  $42.15 \times 10^3/uL$ , and accompanied by fetal history, premature rupture of membranes. Increasing leukocytes in the mother's blood and the increase of TNC and CD34+ cells in cord blood shows that Stem Cell plays a role in repairing small

tissues/organs due to injury or inflammation in the mother's body.[9] In this study, it's look like the UCB with a very high number of CD34+ cells and TNC do not automatically have a higher quality of the stem cell if accompanied by a history of abnormalities in both the mother and fetus. This discovery needs to be further investigated to prove it.

From the boxplot analysis on TNC and CD34+ cell profiles (Figure 2), it shows that the longer the cord, the higher concentration of CD34+ cells and TNC. But when the length of the umbilical cord has exceeded, the TNC concentration will decline. It observed that CD34+ cells and TNC profiles on the umbilical cord diameter are quite fluctuating. In the beginning, CD34+ cell and TNC concentrations were high and then

decrease, but then increases along with the change of cord diameter to be bigger. This result were in line with basic theory about the development of Stem Cells on the umbilical cord. For the maternal age category, TNC and CD34+ cell concentrations will decrease with age, related to the productive age of the reproductive organs and physiological functions. Non-productive age mothers (> 35 years) have a higher risk for pregnancy complications. For the gestational period, both time past (premature) or past time (post term) shows lower CD34+ cell concentration. This is inversely proportional with TNC where TNC concentrations are increases along with mother's pregnancy. This is in line with the research conducted by Nakagawa *et al.* and Wen SH *et al.*[10] In the Hemoglobin category of preterm mothers, TNC and CD34+ cell concentrations seen to be higher as maternal Hemoglobin levels are closer to the normal category. In the infant weight category, the profile of TNC and CD34+ cell concentrations increases along with increasing infant weight at birth, but these will decreases when babies are born in excess weight, which is in line with the research of Mancinelli *et al.* and Phillip J *et al.*[11,12]

There was no correlation between the length of the umbilical cord and the diameter of the umbilical cord to the number of TNC and CD34+ cells, in line with research conducted in Indonesia and Jordania.[13,14] There were no correlation between maternal age with TNC and CD34+ cells. This result was different from the research conducted by Philip J *et al.*,1 related to the difference of samples number. For gestational variables, no correlation was found between gestation and TNC and CD34 cells, in line with research conducted by Urciuoli P *et al.*[15] There was no correlation between the pre-

maternal Hemoglobin of the number of TNC and CD34+ cells, different from the study of Djuwantono *et al.*,4 related to the difference in isolation techniques from the samples examined. There was no correlation between infant body weight to TNC and CD34+ cell numbers, in line with research by Cannabaro *et al.*[16]

## CONCLUSION

In summary, the study found that no correlation appear between the length and diameter of the umbilical cord with the number of TNC and CD34+ cells. However, the concentration of TNC and CD34+ cells is directly proportional to the length and diameter of the umbilical cord, gestational age, hemoglobin levels of the preterm mother, and the infant's weight at birth. Contrast with maternal age, that the older the maternal age, the lower the concentration of TNC and CD34+ cells.

We suggest using the longer and larger UCB for more optimal CD34+ concentration. For more optimal TNC concentration, we recommend using larger diameter of umbilical cord. TNC concentration will decline in excessive cord length, ages and birth weight.

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