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## Cellularity of the umbilical cord blood of hypertensive pregnant women: a case control study

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### ABSTRACT

**Aim:** To analyze the relationship between the cellularity of placental and umbilical cord blood of hypertensive and non-hypertensive pregnant women. **Method:** This is a case-control study that used a quantitative approach. The sample consists of 73 pregnant women and it was conducted from March to September 2011. The data are part of the study approved by the Research Ethics Committee under protocol number 126/10. **Results:** It was found that 80% of the Umbilical Cord and placental blood bags collected from pregnant women with hypertension presented cellularity  $\geq 5 \times 10^8$ . This is the appropriate amount in relation to the totality of nucleated cells, given the criteria established by Resolution 56. **Conclusion:** The results contributed to the identification of factors that make it possible to obtain an adequate number of hematopoietic stem cells, resulting in the increased number of hematopoietic stem cell transplantation in Brazil.

**Descriptors:** Hypertension, Pregnancy-Induced; Fetal blood; Hematopoietic Stem Cells; Placenta.

## INTRODUCTION

Bone marrow transplantation (known as hematopoietic stem cell transplantation) is a special therapy used to treat patients with hematological diseases and certain genetic changes - for which different therapeutic alternatives were considered and excluded<sup>(1)</sup>. The main limitation of allogeneic hematopoietic stem cell transplantation is the lack of a compatible donor. In the absence of an identical family member, non-familial volunteers supported by the HLA (Human Leukocyte Antigen) can be donors<sup>(2)</sup>. The HLA antigens are encoded in the short arm of chromosome 6, and its primary function is the connection and presentation of self peptides and tumor antigens or infectious microorganisms for T lymphocytes, triggering the adaptive immune response and allowing the immune system to differentiate the specific from the non-specific<sup>(2)</sup>. In addition, HLA molecules represent the main alloantigens related to organ transplant rejection and grafting versus host disease in hematopoietic stem cell transplantation<sup>(2)</sup>.

In order to offer a growing and continuous number of compatible donors, many Umbilical Cord and Placenta Blood Banks (UCPBB) around the world were created. As an example of these initiatives, the umbilical cord and placenta blood bank network was implemented in Brazil, through the Ministerial Decree No. 2381/GM of 28 June 2004<sup>(3)</sup>. This project aims to set up a bank network of distribution in several Brazilian states, in order to capture the national sampling of this blood type. The first UCPBB was inaugurated in Brazil in February 2001, at the National Cancer Institute (INCA), which is currently regulated by RBD (Resolution of the Board of Directors), No. 56 of 16 December 2010<sup>(4)</sup>.

Through the collection of this blood, hematopoietic stem cells are obtained. In INCA,

this procedure is carried out by the nurse, who subsequently sends the bag to the Processing and Cryopreservation Laboratory. There the initial count of the total number of nucleated cells (white blood cell and packed cell volume count) of each bag is performed and, in possession of these results, the processing of the blood from the umbilical cord and placenta is initiated. Soon after, this material is frozen<sup>(5)</sup>.

According to the criteria established by the National Health Surveillance Agency through the RBD No. 56, the blood can only be accepted for processing if the total number of nucleated cells in the unit is equal to or greater than  $5 \times 10^8$ , and its use is limited to the fixed cellularity. The RBD No. 56 also states that the UCPBB may decide to increase the minimum amount accepted for processing an umbilical cord blood unit in its facilities, as defined by its quality policy. The UCPBB of INCA established the total number of nucleated cells as being equal to or greater than  $7.5 \times 10^8$  for processing<sup>(4)</sup>.

As to the inclusion and exclusion of pregnant women in terms of the donation of umbilical cord and placental blood, the RBD No. 56 establishes several criteria, including the exclusion of pregnant women who have diseases that interfere with placental vitality. Considering that hypertension is one of the diseases that interfere with placental vitality and decrease uterine blood flow, the Brazilian public banks of umbilical cord and placental blood exclude pregnant women with donation hypertension.

The placentas of hypertensive women have obstructive lesions of the deciduous arteries. Such lesions, regardless of the type of hypertension, are associated with the lowest blood flow depending upon the arteriolar spasm, due to the hypoxic state. It is suggested that the oxygen deficit shows that the

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placental circulation of hypertensive patients is decreased<sup>(6)</sup>.

Due to decreased blood flow in cases of hypertension and the suspicion that this fact will also reduce the cellularity in the blood of the umbilical cord and placenta, pregnant women with hypertension have been excluded from donation.

In the Maternity Hospital Oswaldo de Nazareth some specific tests were performed, such as ultrasound with Dopplerfluxometry in women with hypertension. After this examination, in some cases of pregnant women with hypertension, placental flow was unchanged. This fact leads to the questioning of the relationship between high blood pressure in its different classifications and the actual decrease in placental flow in each case, and this may or may not lead to the reduction of blood cellularity.

Considering the need for clarification on the subject, this research had as its study object the initial cellularity (total nucleated cells) from the umbilical cord and placental blood of pregnant women with hypertension. The hypothesis is that these women do not have an initial decrease in the cellularity of blood. The objective was to analyze the relationship between the cellularity of umbilical cord and placental blood of hypertensive and non-hypertensive pregnant women.

## METHOD

This is a case-control study that used a quantitative approach. Data collection, which was based upon 98 beds, was held in an institution located in the city of Rio de Janeiro. On average, there are three hundred eighty (380) births monthly in this institution, which means an average of 13 births per day.

The sample size was based on a sample size calculation, with an estimated 80 pregnant women. To calculate the sample size, we considered: the significance level of 5% (bilateral); the statistical test of 80% and the expected difference between the groups being relatively "moderate" ( $\geq 25\%$ ), known as *effect size*, obtained by prior knowledge of a pilot study of 19 cases. Of the total sample, there was a loss of seven pregnant women, due to fetal complications (severe fetal distress) after the baby's birth, which, according to the RBD 56, is an exclusion criterion for the collection of umbilical cord and placental blood. The sample comprised 73 pregnant women, which included 25 pregnant women with high blood pressure in the treatment group and 48 women without hypertension in the control group. The inclusion and exclusion criteria of the survey were based on the RBD 56.

- Inclusion criteria: maternal age over eighteen (18) years; pregnant women who have undergone at least two documented antenatal visits; gestational age equal or higher than 35 weeks; bag route for less than 18 hours; labor presenting no abnormalities.
- Exclusion criteria: severe fetal distress; fetus with congenital abnormalities; infection and/or maternal temperature higher than 38°C during labor; pregnant women with increased risk for communicable infections through blood; presence of disease(s) that may interfere with placental vitality, with the exception of hypertension; pregnant women using hormones or drugs that are deposited in the tissues; pregnant women with a personal history of systemic autoimmune disease or neoplasia and pregnant women and their families, biological fathers and their families, or biological siblings of the newborn, who feature a history of inherited diseases of the hematopoietic

system, such as the chronic granulomatous disease, immunodeficiency, dementia and degenerative, metabolic or genetic neurological diseases.

The correlation between the use of drugs and cellularity of the umbilical cord and placental blood in both groups did not become the object of study for this research.

Data collection was prospective, conducted by researchers from March to September 2011, according to the existing standard procedure protocol at UCPBB/INCA, divided into the following steps:

- 1<sup>st</sup> – Analysis of medical records to identify potential donors;
- 2<sup>nd</sup> – Explanation in terms of blood donation and the request for signing the Free and Informed Consent;
- 3<sup>rd</sup> – Verification of the size and weight of the placenta;
- 4<sup>th</sup> – Blood collection of the umbilical cord and placental vessels and its packaging in a specific bag;
- 5<sup>th</sup> – Forwarding of the biological material for the Processing and Cryopreservation Laboratory of INCA;
- 6<sup>th</sup> – Laboratory testing;
- 7<sup>th</sup> – Recording of the laboratory results of the cellularity of the umbilical cord and placental blood of each bag. Two instruments, a checklist for the selection of pregnant women and a semi-structured form composed of seven parties, were used.

The following methods were included in the statistical analysis:

- The comparison of maternal variables, of newborns and placenta or umbilical cord characteristics between groups of hypertensive and non-hypertensive pregnant women was analyzed by means of the

Student's t test for independent samples or the Mann-Whitney test. For numerical comparison of the initial cellularity between two subgroups (or categories) the Mann-Whitney test was used;

- Between three subgroups, the Kruskal-Wallis ANOVA was applied;
- For the association of initial cellularity according to the ratings of the RBD 56 and UCPBB/INCA with the hypertensive and non-hypertensive pregnant women, we used the  $\chi^2$  test or Fisher's exact test.

We used non-parametric methods, because the initial cellularity did not present normal distribution (Gaussian distribution), due to the dispersion and rejection of the hypothesis of normality according to the Kolmogorov-Smirnov test. The significance adopted was at the level of 5%. Statistical analysis was performed using the SAS® System software version 6.11 (SAS Institute, Inc., Cary, North Carolina).

This study was approved by the Research Ethics Committee of INCA, protocol 126/10. All participants were informed about the project and signed the Free and Informed Consent. The procedure was performed through the collection of blood from the umbilical cord and placenta, which was withdrawn after birth, thus not offering any risk to the mother and the baby.

## RESULTS

The data of seventy three (73) pregnant women were collected. Of this total, 34.2% (48) were non-hypertensive pregnant women and 65.8% (25) were hypertensive. Of these hypertensive women, 8% had chronic hypertension, 64% had gestational hypertension, 20% had pre-eclampsia and 8% had

**Table 1** - Comparison of the numerical variables of hypertensive and non-hypertensive pregnant women: data related to mothers and newborns and the characteristics of the placenta and umbilical cord. Rio de Janeiro 2011.

Variable	Hypertensive				Non hypertensive				p valor a
	mean	±	DP	median	mean	±	DP	median	
MATERNAL									
Maternal age (years)	26,0	±	7,1	25	25,6	±	6,4	25	0,99
No. of pregnancies	3,00	±	2,25	3	2,42	±	1,32	2	0,54
Number of births	1,64	±	1,93	1	1,06	±	1,06	1	0,51
Maternal BMI Before pregnancy (kg / m²)	28,7	±	7,5	28	24,1	±	4,6	23,9	0,004
Current maternal BMI (kg / m²) *	34,3	±	9,3	34,7	28,6	±	4,9	27,9	0,002
NEWBORN									
Capurro (days)	275,8	±	7,7	278	276,4	±	9,6	278,5	0,57
Fetal Weight (g)	3300,0	±	455,2	3390	3340,4	±	427,2	3260	0,71
FEATURES PLACENTA / CORD									
Placental weight (g)	743,7	±	185,5	745	719,9	±	153,6	720	0,56
Placental diameter (cm)	18,5	±	2,6	18	18,6	±	1,5	19	0,64
Cord length (cm)	40,9	±	13,9	42	36,0	±	11,3	35	0,17

SD: Standard Deviation

a Student's *t* test for independent or Mann-Whitney's samples.

**Table 2** - Initial cellularity - hypertensive and non-hypertensive pregnant women. Rio de Janeiro, 2011.

Variable	Hypertensive		Non hypertensive		p valor a
Initial cellularity (number)	11,2 ± 8,3 (8,8)		10,4 ± 5,5 (9,7)		0,86
Initial cellularity (DRC 56)					
< 5 x 10 <sup>8</sup>	5	20%	5	10,4%	0,21
≥ 5 x 10 <sup>8</sup>	20	80%	43	89,6%	
Initial cellularity (BSCUP-INCA)					
< 7,5 x 10 <sup>8</sup>	9	36%	17	35,4%	0,96
≥ 7,5 x 10 <sup>8</sup>	16	64%	31	64,6%	

a Mann-Whitney's test for numerical cellularity and Fisher's  $\chi^2$  or Fisher's exact test for categorical cellularity.

The numerical cellularity was expressed as mean ± SD (median) and the categorical cellularity was expressed by the rate (n), and percentage (%).

superimposed pre-eclampsia when compared to chronic hypertension. The comparison of the numerical variables of hypertensive and non-hypertensive pregnant women regarding maternal and newborns' characteristics are described in Table 1.

According to the results obtained in Table 1, the maternal variables (age, number of pregnancies, number of births), of newborns (fetal weight, Capurro) and the characteristics of the placenta and umbilical cord (described as placental weight and length of the cord) showed

no significant difference at 5% between the two groups. It was observed that the group of hypertensive women had a body mass index (BMI) ( $p = 0.004$ ) prior to pregnancy and the current ( $p = 0.002$ ) is significantly higher than the group of non-hypertensive women.

The initial cellularity of the umbilical cord and placental blood obtained from hypertensive and non-hypertensive pregnant women is described in Table 2, according to criteria established by the RBD 56 and the UCPBB/INCA.

According to the results described in Table 2, there was no significant difference in the numerical cellularity ( $p = 0.86$ ), in the initial cellularity recommended by the RBD 56 ( $p = 0.21$ ) and in the initial cellularity used in UCPBB/INCA ( $p = 0.96$ ) among hypertensive and non-hypertensive pregnant women.

## DISCUSSION

Hypertension is a disease considered to be a public health problem, due to its high socio-medical costs<sup>(7)</sup>. In the present study, there are a great number of pregnant women with gestational hypertension (64%), which is considered one of the major complications of pregnancy and puerperium, with an incidence of 6% to 30% of pregnant women and it results in a high risk of maternal and perinatal morbidity and mortality<sup>(8)</sup>.

Hypertensive disorders that occur during pregnancy are classified as chronic hypertension, pre-eclampsia/eclampsia and pre-eclampsia superimposed on chronic hypertension and gestational hypertension. The latter is the most common disorder and it is associated with few maternal-fetal clinical complications. Pre-eclampsia can occur in 5% of pregnancies and its severe forms occur in 1% of cases, resulting in convulsions (eclampsia)

in 0.05% of cases. These syndromes occur in 6% to 8% of pregnancies, contributing to prematurity and perinatal morbidity and mortality due to intrauterine hypoxia<sup>(9)</sup>.

Through this study, it was found that the initial cellularity of the umbilical cord blood of hypertensive pregnant women showed no significant difference, compared with the cellularity of non-hypertensive pregnant women. From 25 umbilical cord and placental blood bags collected from hypertensive pregnant women, 80% had cellularity  $\geq 5 \times 10^8$  (according to the standards established by RBD 56) and 64% had cellularity  $\geq 7,5 \times 10^8$  (UCPBB/INCA default).

In a study carried out with hypertensive pregnant women, the umbilical cord blood obtained from women with pre-eclampsia showed a decrease in volume, in the total of nucleated cells and CD34+ in the cell count, compared with those who did not have hypertension<sup>(10)</sup>. Another study shows that the volume and cellularity of the umbilical cord and placental blood can be influenced by obstetric and neonatal factors<sup>(11,12)</sup>. Despite the decreased cellularity of this blood in pregnant women with pre-eclampsia, there are few studies noting the actual decrease in the total nucleated cells in terms of the blood of pregnant women with different types of hypertension.

Assuming that the decrease in blood flow to the placenta can reduce this cellularity, hypertensive pregnant women were excluded from donation (RBD 56); however, this study found that the umbilical cord and placental blood of women with hypertension met the criteria established by RBD 56<sup>(4)</sup>. From the results of this research, we suggest a reassessment of the selection criteria with regard to pregnant women with hypertension.

In this study, participants had a mean age

of 26 and 25.6 years. This shows that women are starting their procreative phase whilst still young and extending this stage to maturity, whereas the reproductive age ranges from 15 to 44 years<sup>(13)</sup>.

The behavior of fertility by age shows a changed process due to the sharp decrease in the rates of younger women in several metropolitan areas. The acceleration of the fertility decline in the five years between 2000 and 2005, the decrease in the number of births and consequently the reduction in the size of new cohorts became part of the age pyramid of the current Brazilian population. The biggest drop occurred in the age group between 20 and 24 years, but there were also significant falls in the group between 15 and 19 years. There were no changes in the level of rates for women at older ages<sup>(14)</sup>.

As for the number of pregnancies and deliveries, in this study the participants had a median of three pregnancies (hypertensive) and two pregnancies (non-hypertensive). Studies have shown that the average number of children has diminished over the years in Brazil. According to the Secretary of Health Surveillance, in 1970 Brazilian women had on average 5.8 children. Thirty years later, that average is 2.3 children. In the world, in the late twentieth century, the fertility rate was 2.9 children per woman; in the most developed countries the rate was 1.5, and in the less developed countries it was around 3.2<sup>(15)</sup>.

It was noted also that the higher the level of family income, the lower the probability of a woman having many children. Once again, it is worth noting that the existing relationship between income and education has already been proven in several studies of the economic area. In this case, people with higher incomes are generally better educated and more concerned with the education and standard

of living that they can offer to their children throughout their existence. Thus, it is expected that they have fewer descendants<sup>(15)</sup>.

The practice of cesarean section has a higher percentage compared to vaginal delivery. According to this study, 46.6% of the women had vaginal delivery and 53.4% had cesarean section. These results contradicted the recommendation of the World Health Organization that cesarean deliveries should not exceed 15% of total births<sup>(14)</sup>.

With respect to BMI, we observed in this study that the BMI before pregnancy and the current BMI were significantly higher in the group of hypertensive pregnant women, compared to the group of non-hypertensive pregnant women. This weight gain not only contributes to obesity, but is also associated with complications.

Another study also adds that being overweight or even obese are major public health problems in society, due to the growth of such conditions in all age groups and its association with various chronic diseases, especially hypertension<sup>(16)</sup>.

It is estimated in Brazil that 38.8 million people aged 20 years or older are overweight. This number corresponds to 40.6% of the population in this age group, of which 10.5 million are obese. Research indicates that many diseases of the modern era are associated with excess body fat, including cardiovascular, renal, liver and digestive diseases, as well as diabetes and orthopedic problems. The incidence of these diseases is twice as high among obese men and four times higher among obese women, when compared to the non-obese population<sup>(13)</sup>.

Women who are overweight or obese during pregnancy and childbirth have a significant risk during the prenatal, childbirth, postpartum and neonatal stages. Complica-



tions in the prenatal stage include recurrent miscarriages, birth defects, gestational hypertension, pre-eclampsia, gestational diabetes and venous thrombo-embolism<sup>(17)</sup>.

Regarding newborns, the average weight and Capurro corresponded to the normal standard levels. To estimate more accurately the risk of the child in terms of having certain diseases or dying, it became necessary to examine two major variables together: gestational age and weight. At birth, the weight of healthy newborns must be greater than 2,500 g, and the Capurro above 37 weeks<sup>(18)</sup>.

According to this research, some children had less weight than that recommended by the Ministry of Health, which regards every newborn baby born weighing less than 2,500g as underweight. The incidence varies depending on the country or region, according to socioeconomic conditions. In Brazil, the average is 10.2%, with a variation between 8.6% and 12.2% according to the region. It is also highly variable, the ratio between newborns with low weight, premature and newborn infants with gestational ages equal or higher than 37 weeks with intrauterine growth retardation<sup>(18)</sup>. This ratio is related to the socioeconomic status. Prematurity and low birth weight are key factors in neonatal mortality, the development of infections, increased hospitalization rates, postnatal neuropsychological deficit and poor school performance, in addition to the repercussions on the health conditions during adult life<sup>(19)</sup>.

This study showed that the greater the weight of the newborn, the greater the cellularity found in the umbilical cord blood of hypertensive pregnant women. Based on these results, more attention should be given to the weight of the baby in order to get a higher quantity of cells after collection, and also to intensify research related to neonatal factors.

With regard to the characteristics of the placenta and umbilical cord, the weight and diameter of the placenta and the umbilical cord length presented normal standards. The term placenta presents a diameter between 15 and 20 cm and a mean weight of 450g and the umbilical cord length ranges from 50 to 60 cm<sup>(20)</sup>.

## CONCLUSION

This study aimed to analyze the initial amount of nucleated cells in the umbilical cord and placenta blood of hypertensive and non-hypertensive pregnant women, based on criteria established by the RBD.

We concluded that there is no statistically significant difference between the number of blood stem cells of hypertensive pregnant women and non-hypertensive women; 80% of the collected bags of pregnant women with hypertension presented cellularity  $\geq 5 \times 10^8$ , that is, an adequate amount of the total nucleated cells, meeting the criteria established by the RBD 56. The cellularity also met the standards used in UCPBB-INCA, that is, 64% of the collected bags showed cellularity  $\geq 7,5 \times 10^8$ . Thus, the blood of pregnant women with hypertension had adequate cellularity for both standards.

From the results obtained in this study, the PUCB cellularity of hypertensive pregnant women must be investigated in future research, because much has been studied about the influence of hypertension in the flow and changes in the placental bed, but until the end of this study, little was known in terms of the changes in the blood of these women. Based on these results and with the intensification of research in this field, we suggest the reevaluation in terms of the criteria for umbilical cord

and placental blood donation of pregnant women with hypertension.

The results shown in this study indicate the need for further research to elucidate questions concerning the influence of the different hypertensive disorders in the cellularity of umbilical cord and placental blood.

The observation of factors that can influence the cellularity of this blood type is included in the many skills of nurses working in UCPBB. Detailed knowledge of the operating procedure for UCPBB, relational competence with the patient so that he/she accepts the idea of donating blood, the creativity and subtlety to deal with pregnant women who often find themselves in labor at the time they are being addressed and the identification of factors that may influence the cellularity, are indicators of the quality of the material obtained.

The research results have contributed to the identification of factors that allow the attainment of an adequate quantity of hematopoietic stem cells, resulting in the increased number of transplantation of these cells in Brazil. In the process, the commitment of nurses along with the staff of the public banks of umbilical cord and placental blood is important for achieving better results with a larger number of donors and increased satisfaction of the patients and their families.

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