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Hematopoietic profile of the umbilical cord blood of Congolese neonates

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Abstract

Context: Several emerging diseases, including diabetes, cancer and others, are still incurable. It is shown today that there are new therapeutic potential of stem cells contained in the wall of the umbilical cord. The use of these cells has contributed to therapeutic advances in regenerative medicine. Objectives: The purpose of this study was to determine the hematopoietic profile of the umbilical cord blood of newborns. Material and methods: Blood samples were collected at the maternity ward of the CMDC medical center by clamping. The evaluation of the hematological and morphological parameters were analyzed respectively by the hematological automaton and the electron microscope. Results: The study involved samples of 16 newborns. The erythrocyte constants show: MCV (90.12 \pm 9.12 fl), MCHC (33.35 \pm 5.36 g / dl) and MCHC (29.83 \pm 5.45pg). The average value of blood platelets is 349.62 \pm 267.03.109 / L. The anemia of the erythrocyte line is the most represented. The most frequent leucocyte lineage differences are monocytosis, lymphocytosis and neutropenia. As for the platelet lineage, there was thrombocytosis and thrombocytopenia. Conclusion: Umbilical cord blood contains stem cells, capable of producing all other blood cells. Hence its use in regenerative medicine..

Keywords: Hematopoiesis, stem cells, umbilical cord, DRC.

INTRODUCTION

Umbilical cord blood is very rich in stem cells, whose properties are used in the treatment of blood diseases and certain cancers. Used in the treatment of nearly 80 blood diseases, hematopoietic stem cells contained in the cord are now providing great hopes for treatment [1, 2].

Scientists are convinced that these famous cells would allow the reconstitution of all kinds of tissues, with the support, the prospect of overcoming diseases still incurable [3, 4].



Figure 1: Image of a fetus and its umbilical cord [5]

Umbilical cord blood is an easy source for hematopoietic stem cells, which are increasingly used as an alternative to bone marrow or peripheral stem cells for transplantation to treat diseases malignant and benign in children and adults [6,7].

The umbilical cord blood is contained in the placenta. It is collected at the birth in the umbilical veins. After being qualified, the cells are stored and frozen in a bank where they can be kept indefinitely. The

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cells thus harvested have certain advantages over those derived from the embryo, bone marrow or peripheral blood [4, 8].

In addition to hematopoietic stem cells, cord blood contains the elements normally present in the blood (red blood cells, white blood cells, platelets and plasma) [9, 10].

The particularities of the African blood count are well established at present. Leukopenia with neutropenia, as well as relative lymphocytosis, have been observed in African adults and children [11, 12]

This leuko-neutropenia was not found in the African newborn at birth. On the other hand, the influence of the environment on the hematological parameters is well established [13, 14].

Studies on the biological standards of Africans are rare if not almost non-existent. Already, other studies have shown that there are differences between the average values of certain biological parameters of the African and the European. These differences are due, among other things, to nutritional and environmental variations. If we add the notion of intra- and inter-individual biological variations, we understand that we can not transpose reference values from one country to another [14].

Thus, in the course of an international cooperative study on the transferability of reference values, it was concluded that reference values adapted to geographical origin and taking into account the ethnic factor into account should be established. Africa. Therefore, the establishment of baseline values is of paramount importance for a given population at the scientific, diagnostic, and therapeutic levels [15, 6].

Our work aimed to evaluate the profile of the blood cord blood count of the newborn in Lubumbashi (DRC), while determining the erythrocyte, leukocyte and platelet values of the cord blood; evaluating the variation of the parameters of the hemogram and identifying the frequency of abnormalities of the hemogram in relation to pre-existing reference values.

ENVIRONMENT, MATERIALS AND METHODS

i) Field of study

This work was carried out in the maternity ward and the hematology laboratory at the CMDC clinic (medical center of the city center) in Lubumbashi commune. It is in this entity that the sampling as well as the determination of the hematological parameters were carried out.

ii) Study material

We used in this study the umbilical cord blood of the newborn. Several hematological parameters were evaluated. These are erythrocytes, leucocytes, platelets, erythrocyte constants and the leucocyte formula.



Figure 2: The cord blood in the EDTA tube

iii) Methods of the study

We conducted a cross-sectional prospective study based on the

analysis of various parameters of the cord blood count in 16 neonates received from May 18 to July 20, 2016.

Was included in our study, any mature newborn with a weight greater than 2500g and whose mother did not have any particular complaints, nor developed infections in the third trimester of pregnancy; and who gave his free and informed consent to participate in the study [2,5].

Excluded from our study were any newborn malformed or whose mother presented with third trimester bleeding (placenta previa, premature detachment of membranes) [12].

The hematological analyzer automat using the Coulter principle was used; impedance analysis made it possible to count red blood cells, leucocytes and platelets. Electron microscopy made it possible to evaluate the morphology and the leucocyte formula of different samples $^{[16,17]}$.

RESULTS AND DISCUSSION

The following results reveal an abundant presence of blood cells in umbilical cord blood; this also suggests the presence of stem cells in this blood [17]. Hematopoietic stem cells (HSCs) are capable of producing all types of blood cells: red blood cells, white blood cells and platelets. They are responsible for maintaining the production of blood cells during life. They have been used for years in bone marrow transplants for the treatment of blood diseases [8, 18].

Here are the highlights of our study:

Table 1: Blood cell counts

	Erythrocytes		Leuk	cocytes	Platelets		
	N	%	N	%	N	%	
Normal	8	50	14	87.5	10	62.5	
drop	7	43.75	1	6.25	3	18.75	
Increase	1	6.25	1	6.25	3	18.75	
Total	16	100	16	100	16	100	

Table 1 reveals the presence of all major groups of blood cells in umbilical cord blood. This observation deserves a lot of attention and rationalizes the use of cord blood in the management of certain diseases.

Passweg *et al* (2010) and Refsum *et al* (2017) have shown in their studies that cord blood is mainly used for cases of leukemia, certain immune deficiency and certain forms of anemia. These corroborate the observation of our analyzes, which shows the abundant and sometimes exaggerated presence of leucocytes and erythrocytes ^[6,7].

It should be noted that in the field of tissue engineering, we are now able to manufacture certain blood cells in vitro; It is with this in mind that a group of researchers recently presented a method of producing large quantities of red blood cells from umbilical cord blood that could be used in clinical care [11, 15].

We also think, as some researchers believe, that the presence of all these large groups of cells stem from stem cells. Thus stem cells present in the cord blood are known to be "naïve" cells, which means that they have the capacity to transform into a specific type of blood cells such as white blood cells, red blood cells or platelets, for the purpose of cell therapy [10, 19].

Table 2: Characteristics of erythrocyte constants

		Hb		Ht		MCV		МСНС		МСНС	
	N	%	N	%	N	%	N	%	N	%	
Normal	13	81.25	12	75	14	87.5	13	81.75	15	93.75	
Drop	3	18.75	3	18.75	2	12.5	3	18.75	1	6.25	
Increase	-	-	1	6.25	-	-	-	-	-	-	
Total	16	100	16	100	16	100	16	100	16	100	

Table 2 reveals the behavior of erythrocyte constants. In general, the erythrocyte constants are normal, compared to the norm. However, for some parameters, the values of GR, Hb and Hte vary from one study to another ^[8].

Abo-Elkheir *et al* (2017) and Jaing (2014) have shown in their research that in the neonatal period, the interpretation of hemoglobin, hematocrit and red blood cell counts must take into account different parameters that influence the results. These values are particularly dependent on the time required to ligate the umbilical cord $^{[8,\ 12]}$, latency between birth and peripheral blood sampling, oxytocin administration to the mother at delivery, birth weight or the intensity of fetal distress caused by hypoxia. At the time of delivery, blood flow from the placenta can increase the total blood volume of the newborn by 50% to 60% $^{[3,\ 10]}$.

The researchers have developed a method that promotes the proliferation of umbilical cord blood cells and their differentiation into red blood cells. It consists of isolating cells from umbilical cord blood and placing them in a specific culture medium, adding elements that will improve their growth and then their differentiation into red blood cells. To do this, the cells are stored in a special rotary incubator for 21 days. After optimizing the production process; researchers found that the characteristics of the cells obtained, for example hemoglobin levels and oxygen transport capacity, were similar to those of red blood cells produced in vivo [7, 16].

The researchers then tested the safety and functionality of red blood cells produced *in vitro* in animals. The safety of these cells has been demonstrated, no adverse effects have been reported. In a model of haemorrhagic anemia in non-human primates, red blood cells produced in vitro have demonstrated their ability to carry oxygen and accelerate haematopoietic recovery ^[9, 13].

Table 3: Leukocyte count

	Moi	Monocyte		neutrophil		lymphocyte	
	N	%	N	%	N	%	
Normal	6	37.5	13	81.25	10	62.5	
Drop	-	-	3	18.75	-	-	
Increase	10	62.5	-	-	6	37.5	
Total	16	100	16	100	16	100	

Table 3 gives the results of the leucocyte formula. It turns out that the rate of basophils is zero. This result is consistent with the confirmation of Ratajczak *et al.* (2013), who state that basophil levels are virtually zero at birth.

Our study revealed the presence of immune cells in this case lymphocytes; however this would not interfere with the use of cord

blood in therapy; because for a large part, these lymphocytes are immature. Even in non-identical transplants in the HLA system, very few reactions of the transplant against the host have been observed, which has encouraged the creation of cord blood banks for use in children, but perhaps also in adults [16, 17].

Table 4: Abnormalities of the erythrocyte lineage

Anomaly of the erythrocyte lineage	N	%
Normocytic and normochromic anemia	2/16	12.5
Hypochromia without anemia	1/16	6.25
Polycythemia	1/16	6.25
Microcytic anemia	2/16	12.5
Microcytosis without anemia	1/16	6.25

Table 4 shows some abnormalities of the erythrocyte lineage including anemia, polycythemia and microcytosis. However, these anomalies are considered as such, when comparing the results obtained with the standard. Jaing (2014) and Angulskie *et al* (2017) have shown that the abundance of immature cells, also reflecting the abundance of stem cells, would give the impression of a blood abnormality; which is not sometimes the case.

Note also that our analyzes showed the presence of various elements of the erythrocyte lineage such as erythroblasts, reticulocytes and erythrocytes (Figure 3, 4, and 5), thus encouraging us to quantify stem cells.

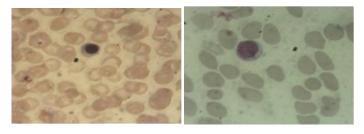


Figure 3: Erythroblasts

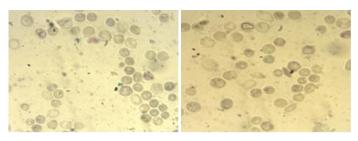


Figure 4: Reticulocytes

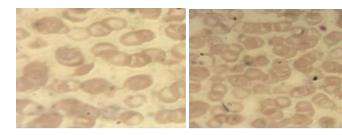
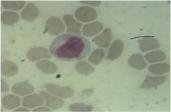


Figure 5: Erythrocytes

Table 5: Abnormalities of the leukocyte lineage

Anomaly of the leukocyte lineage	N	%
Leukopenia	1/16	6.25
Leukocytosis	1/16	6.25
Lymphocytosis	6/16	37.5
Neutropenia	3/16	18.75
Monocytosis	10/16	62.5

Table 5 reveals some abnormalities of the leukocyte lineage including leukopenia, leukocytosis, lymphocytosis, neutropenia and monocytosis. As in the erythrocyte lineage, it is difficult to conclude absolutely whether it is really abnormalities, where just a normal process of leukopoiesis. Nevertheless, our biological analyzes showed the presence of different leucocytes (Lymphocytes, monocytes and neutrophils) except basophils (Figure 6, 7 and 8).



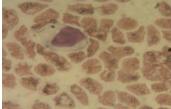


Figure 6: Lymphocytes

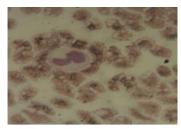




Figure 7: Monocytes

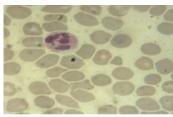




Figure 8: Neutrophiles

Table 6: Abnormalities of the platelet lineage

Anomaly of the platelet lineage	N	%
Thrombocytosis	3/16	18.75
Thrombocytopenia	3/16	18.75

Table 6 reveals some small abnormalities of the platelet lineage in six out of sixteen samples.

The difference in the values of the hemogram parameters observed between our study and those of the other studies could be explained by the size of the sample, the length of clamping of the umbilical cord likely to impact the results of the hemogram, the also measuring devices that are different [18, 19].

CONCLUSION

Umbilical cord blood contains stem cells that can be used in the regeneration of all blood cells.

The hematological analyzes of the present work show the richness of the blood cells in the umbilical cord blood; and these results support the abundance of hematopoietic stem cells.

The use of umbilical cord blood in regenerative medicine would be justified by the capacity of the stem cells that this blood contains; this ability leads to regeneration into several cell types.

Limitation of the study

The lack of a clear profile of umbilical cord blood stem cells is the limitation of this study; it would have been better to also quantify hematopoietic stem cells from cord blood to arrive at definitive conclusions before any further experimentation.

Contribution of the authors

All authors have contributed effectively to this research. They read and approved the final version.

Conflict of interest

The authors declare that there is no conflict of interest.

Thanks

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