

## Factors Associated with Umbilical Cord Blood Derived Mononuclear Cells Banking in Morocco: A Preliminary Study

Research Article

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### Abstract

Umbilical cord blood (UCB) is commonly used as the main hematopoietic stem cell (HSC) source for allogeneic transplantation. Immediately available when compared to bone marrow (BM), UCB is now widely processed and stored as rigorously qualified units. These units were firstly estimated before processing using specific criteria for the donor's choice, and by parameters such as Total Nucleated Cells (TNC), Mononuclear Cells (MNC), CD34+ cells and Colony Forming Units (CFU) for final processing. Appropriate assessment of factors related to maternal, obstetrical and neonatal variables showed these variables as the mainly contributors to a better UCB quality management. This preliminary study aimed to identify the effects of parameters such as maternal age, weight, gravid status, delivery mode, neonatal weight and gender on the MNC count in more 18 years old aged consent, and 33 full-term gravid donors. MNC count did not depend on gravid status and maternal age and weight as well as delivery mode and gender. However, neonatal weight and MNC counts appeared slightly associated independently to maternal age. This work might be helpful in developing and controlling procedures prior to UCB manipulation and would be improved for more UCB eligibility after bank creation.

**Keywords:** Umbilical Cord Blood Banking; Maternal and Neonatal Factors; MNC, Morocco.

**Abbreviations:** BM: Bone Marrow; CFU: Colony Forming Unit; HLA: Human Leukocyte Antigen; HSC: Hematopoietic Stem Cells; MNC: Mononuclear Cells; UCB: Umbilical Cord Blood; TNC: Total Nucleated Cells.

### Introduction

Umbilical Cord Blood (UCB) is increasingly used as an alternative source of stem cells since the first successful transplantation into a patient with a Fanconi's Anemia [1]. These cells are able to reconstitute stem cell disorders *in vivo*, and are the firstly considered for allogeneic transplantation and banking [2]. Several thousand of clinical applications using UCB have been conducted despite of the low blood volume collected and the non eligibility of collected units. Indeed, developing practical and efficient methods have showed that only 29% of collected UCB units remained suitable for banking [3]. To ensure their quality, different variables such as donor choice, collection process, collected volume, Total Nucleated Cells (TNC), Mononuclear Cells (MNC), total CD34+ cell counts were assessed [2]. However, there is still controversy regarding evaluation of UCB unit's safety and potency. Using

standardized protocols and conditioning regimens, outcome and engraftment in UCB transplantation were primarily dependent on HLA disparity [4]. But, infused TNC or MNC dose have been repeatedly associated with engraftment success [5, 6]. At this fact, collected volume is strongly considered as the first indicators for stem cell abundance [4, 6, 7], allowing thus decision to proceed and bank. Interestingly, other variables such as maternal age, weight, parity, type of delivery, gestational age, newborn weight, height, sex [5, 8-10] and also pre-birth characteristics [11] might influence differently CD34+ and TNC or MNC counts. These aspects were analysed in many UCB banks to develop standard common procedures and quality control tools for banking.

In Morocco, published data on UCB banking are still missing. Some population characteristics are widely shared in North Africa such as fertility, caesareans deliveries [12] and obesity [13] and

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might be used as UCB banking indicators having to be assessed. This preliminary study aims to evaluate the donors profile as the mainly selection parameter for the UCB quality assessment. A specific questionnaire about the mostly associated maternal and neonatal characteristics with birth was developed.

## Materials And Methods

### Criteria Collection of Donors

Data regarding Moroccan family and medical history were reported in a questionnaire for each donor. Exclusion criteria consisted of smoking, positive infections with HIV, Hepatitis B and C viruses, Cytomegalovirus, blood and organ transplantation history, inherited diseases, and pathological pregnancies. Inclusion criteria were Moroccan nationality, age higher than eighteen ( $\geq 18$ ) years old, and a gestational age of at least 33 weeks ( $\geq 33$ ).

### Collection of UCB Units

The institutional requirements of the Institut Pasteur Ethical Committee have been respected and its authorization granted and signed consent forms from informed pregnant women are received. Samples were then collected either after normal vaginal or caesarean deliveries from the candidate for donation. Units were collected in a conservation medium composed with RPMI (Gibco, Invitrogen) at 10% EDTA (Gibco, Life Technologies), 5% Bovine Foetal Sera (BFS, Gibco Invitrogen), 2% Peni-streptomycin (Gibco, Life Technologies) and 1% fungisone (Gibo, Life Technologies). Only units with volume exceeding 70 ml were included in the study.

### Separation of MNC

UCB was delayed on Ficoll-Paque (1,077 g/ml, Biotech GmbH) and the buffy coat was harvested and cells washed twice in RPMI supplemented with 5% BFS. Collected MNC were counted and tested for viability using the Trypan blue (Gibco, Invitrogen) dye exclusion method. Samples containing less than 95% viable cells were excluded.

### Statistics

Associations between parameters were analysed by Mann-Whitney U and Anova test. Multivariate linear regression analysis was also realized to test the correlation between MNC and the quantitative variables. A value of  $P < 0.05$  was considered to be significant.

## Results

### Maternal and Neonatal Characteristics in UCB Donation

More than 250 UCB units have been collected, but only 158 UCB units were included in the study as summarized in Table 1. The mean maternal age was 30 years old with a range of 18-47 years. Forty three percent of them were less than 30 years old (group  $< 30$ ) with a mean of 24.1; while 57 % exceeded 30 years old (group  $\geq 30$ ) with a mean of 36.2 years old. Maternal weight ranged from 58-102 Kg (mean 77.09 kg) while neonatal weight counted for 2.7-4.1 kg meaning 3.25 kg. The donors present 1 or 2 gravid in 61.2 % of the cases and  $\geq 3$  gravid in 38.8 %. The babies are 59% female and 41% male and are mostly born after normal delivery (51.61%). The mean MNC count was  $310.9 \times 10^6$  with values ranging from  $8 \times 10^6$  to  $3200 \times 10^6$  ( $3.2 \times 10^9$ ) cells.

### The Effects of Neonatal and Maternal Weight on MNC Count

MNC count increased regarding maternal and neonatal weight in the donors group  $< 30$  years (Table 2). Inversely, babies with less than 3.2 kg were associated with higher MNC counts in the donors group  $\geq 30$  years. Nevertheless, significant associations between maternal or neonatal weight with MNC count were not observed for both women groups.

### Relationships Between Maternal Age and Gravid Status on the MNC Count

Table 3 showed higher MNC count in donors group  $< 30$  years

**Table 1. Maternal and Neonatal Characteristics Relative to Collected MNC.**

	Number	Mean or (%)	S.D.	Minimum	Maximum	Median
Consent donor	158					
Maternal age (years)	158	30	7.3	18	47	31
< 30	68	24.1 (43)	3.2	18	29	24
$\geq 30$	90	36.2 (57)	4.6	30	47	36
Gravid status	98					
[1-2]	60	61.2				
$\geq 3$	38	38.8				
Delivery type	93					
Normal	48	51.61				
Caesarean	45	48.39				
Maternal weight	98	77.09	10.9	58	102	74.5
Neonatal weight	98	3.25	0.3	2.7	4.1	3.2
Total MNC Recovery $\times 10^6$	98	310.9	457.1	8	3200	186.5

Table 2. The effects of Neonatal and Maternal Weight on Total MNC Count.

Maternal age	Neonatal and Maternal weights	Number	UCB MNC x10 <sup>6</sup> (Median [IQR])	Mann-Whitney test P-value
< 30 years	Neonatal weight	40		
	< 3.2	23	154 [84-273]	
	≥ 3.2	17	246 [87-621]	0.1629 (< 3.2 vs ≥ 3.2)
	Maternal weight	40		
≥ 30 years	< 74.5	29	158 [87-273]	
	≥ 74.5	11	310 [62-621]	0.4134 (< 74.5 vs ≥ 74.5)
	Neonatal weight	58		
	< 3.2	26	223 [94-438]	
≥ 30 years	≥ 3.2	32	170 [101.5-405]	0.9938 (< 3.2 vs ≥ 3.2)
	Maternal weight	58		
	< 74.5	20	126 [88-398]	
	≥ 74.5	38	209 [100-410]	0.3596 (< 74.5 vs ≥ 74.5)

IQR: Interquartile Range.

Table 3. Mutual Effects of Maternal Age Interval and Gravid Status on MNC Count.

Number of gravid	Maternal age	Number	UCB MNC x10 <sup>6</sup> (Median [IQR])	P-value* (< 30 years vs ≥ 30 years)	P value * n gravid vs 1 gravid	P value * n gravid vs 2 gravid
1	< 30 years	14	224 [87-366]	0.4713		0.5247
	≥ 30 years	15	186 [120-280]			
2	< 30 years	11	140 [62-284]	0.9835	0.5247	
	≥ 30 years	20	129 [80-470]			
3	< 30 years	10	156 [96-621]	0.5581	0.5085	0.2122
	≥ 30 years	14	234.5 [104-438]			
4	< 30 years	5	120 [62-310]	0.4636	0.7032	0.5036
	≥ 30 years	8	315 [113-66]			

\*Anova P-value

IQR: Interquartile range.

presenting 1 or 2 gravid when compared to the older ones. Indeed, these later having 3 or 4 gravid produced more MNC in their UCB. However, none of the maternal age, nor the gravid status affected significantly the MNC count ( $P > 0.05$ ). Also, MNC count was not related to the number of gravid in both maternal groups (gravid1 vs 2, 3, 4  $P > 0.05$ ; gravid 2 vs 3, 4  $P > 0.05$ ).

#### Multivariate Analysis of Maternal and Neonatal Factors and MNC Count

The multivariate linear regression analysis showed that maternal age and weight, and gravid status were not influencing MNC in both maternal group (Table 4). However, newborn weight appeared to be the factor affecting significantly MNC count in the total women population ( $P=0.035$ ).

#### Discussion

Advances in UCB therapeutics resulted in identifying protocols for the screening of collection units and their storage. In this con-

text, two axis might be proposed, the feasibility of the process by testing the donors pre-selection, and the evaluation of safety and efficiency of collected units. Thus, most of UCB bank or processing laboratories use, in their registry inventory a multi-parameters combination such as volume and TNC as the main selection factors for suitable units. In this study, pre-selection process have been assessed.

In our series, donors aged  $\geq 30$  years old had likely more chance to have a girl than a boy and this trend appeared to be common in all age intervals in Morocco, but baby's gender could not be considered as parameter influencing MNC count (unpublished data). However, higher yield of TNC or CD34+ cells with female newborn than male counterparts have been reported [14, 15]. Elsewhere, delivery mode via caesarean sections allowed significant increase in collected units volumes as compared to vaginal deliveries [16] which was not supported by others [7, 15]. Our analysis did not focus on the collection volume of units and suggested that successful banked units might be collected either in utero or in ex utero as reported [17, 18].

Table 4. Multivariate Linear Regression Analysis of Factors Associated with MNC.

Group	Parameter	Coefficient	P-value
Total Population	Maternal Age	-3.54	0.611
	Maternal Weight	-1.29	0.814
	Gravid Status	77.01	0.101
	New Born Weight	317.58	0.035
Maternal Age < 30	Maternal Age	-5.18	0.730
	Maternal Weight	-1.20	0.879
	Gravid Status	49.26	0.421
	New Born Weight	356.14	0.084
Maternal Age ≥ 30	Maternal Age	-14.89	0.310
	Maternal Weight	-0.11	0.989
	Gravid Status	90.30	0.204
	New Born Weight	261.85	0.217

Previous reports have indicated that in primigravid aged  $\geq 25$  years old, low density (LD) cell counts (seemed as MNC), appeared to be significantly higher than those of  $\leq 24$  years old [9], suggesting that primigravid fact is in favour to higher LD cell especially in women aged  $\geq 25$  years old [19]. We reported that MNC count was relevant in donors group < 30 years with 1 or 2 gravid and that maternal age and weight might not be useful as parameters highlighting UCB quality. Also, our primigravid women did not consent to UCB donation in spite of the given cord blood information and have afraid for their first babies.

The relationships between maternal weight/obesity and UCB banking were not reported anywhere, but we showed that this parameter might be correlated indirectly to higher MNC count. At this fact, bigger babies from normal exceeding-weight donors were associated with higher yields of MNC according to other reports [20], and this association was relevant when the total donor population was considered.

This potential UCB donation profile might be considered in Morocco because it did not depend on maternal age. Especially in rural locations, many people do not have yet an official birth state thus introducing a doubt on their age. In addition, neonatal weight might be a parameter defining the first donor's profile identification helpful for UCB higher quality. The low cost characterization reported in this study is an important tool in the banking start up for a successful pre-selection of UCB units with high MNC counts. Additional testing of hematopoietic potential will be assessed by conventionally used outcomes such as CFU and CD34+ cell counts.

## Conclusion

UCB banks are created worldwide according to specific standards protocols regarding stem cell manipulation and cryopreservation while donor choice remained less standardized. The growing interest in therapeutic applications of UCB have emphasize the importance of donors search in establishing UCB banking process. To do this in Morocco, many efforts have to be displayed to involve medical authorities, professional associations, and governmental and non-governmental organizations for supporting stem

cell research and banking [21].

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