Original article

Cord blood hematological reference values in term and late preterm infants from the Mediterranean island of Sardinia: a preliminary study

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Abstract

Background: Even though umbilical cord blood (UCB) has become an important source of hematopoietic stem cells for transplant, little is known about normal hematological values at birth. The aim of our preliminary retrospective study was to determine reference values for the main hematological parameters of UCB, stratified by gender, gestational age and delivery route in the Sardinian neonatal population.

Method: In our retrospective study we reviewed hematological blood counts from UCB samples collected consecutively at the Neonatal and Obstetric Division of San Gavino Hospital, Sardinia, Italy, between January 1, 2013, and June 30, 2014. Unsuitable samples (insufficient blood amount, clotted or hemolyzed samples) were excluded from the analysis.

Results: A total of 439 UCB samples from newborn infants with gestational age ranging from 34 to 42 weeks were included in the study. Newborn infants from vaginal delivery and preterm infants showed higher erythrocyte and leukocytes values, while no differences in hematological values were found between males and females. Interestingly, reticulocyte hemoglobin content was specifically evaluated and its values were found not to be influenced by gender, gestational age, and delivery route.

Conclusions: These preliminary findings may contribute to define reference values for UCB hematological parameters in term and late preterm infants from the Mediterranean island of Sardinia, thus favoring a wider use of these parameters in the clinical management of the newborn.

Keywords

Complete blood count, umbilical cord blood, reference values, hematological parameters, newborn infants.

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How to cite

Introduction

Complete blood count (CBC) is one of the most commonly used laboratory tests to evaluate potentially pathological newborn infants. In Neonatal Care Units, it is normally performed by using capillary, venous and, more rarely, arterial blood. In newborns, venous blood can be drawn by peripheral vein puncture, but it is sometimes difficult to obtain a sufficient blood sample. On the other hand, the heel prick sampling is commonly used in Neonatal Units due to its relative easiness; however, it can be associated with pain for the newborn [1, 2] and anxiety for parents [3], as well as with complications such as calcaneal osteomyelitis and secondary calcaneal deformity [4], bruising and hematomas [5]. Additionally, blood sample hemolysis is common, with potential influences on the reliability of laboratory results. Therefore, if a skilled phlebotomist is available, venipuncture is the preferred technique for obtaining blood in full-term newborns [2]. Only recently, umbilical cord blood (UCB) has been considered a possible source for performing hematological evaluation in newborn infants at birth. In fact, until the 1980s, the umbilical cord

and placenta were considered useless waste and discarded after birth. UCB drawing has several advantages when compared to venous or capillary blood sampling in the newborn. It avoids the pain and discomfort associated with venipuncture or heel puncture, reduces the time dedicated to blood sampling, and allows the collection of adequate blood samples. UCB values have been demonstrated to reflect newborns' health status and are useful to evaluate clinical conditions such as asphyxia, chorioamnionitis, or meconium staining [6, 7]. Furthermore, hematological values obtained by UCB analysis provide information on the intrauterine environment, in contrast to hematological data obtained after delivery, which can be influenced by postnatal factors. Recent studies have concluded that UCB is a useful alternative source for initial blood tests [8] and, in particular, the neonatal admission CBC can be informative also in preterm newborns [9]. Therefore, UCB could represent a valid alternative choice for CBC evaluation in both healthy and diseased neonates. To our knowledge, few studies have investigated the reference ranges for hematological values of UCB [10-12] as well as their relationship with some variables such as delivery route [13-17], gender [12, 16, 17], gestational age [17-21] and postnatal age [19]. The aim of our preliminary retrospective study was to determine reference values for the main hematological parameters of UCB, stratified by gender, gestational age and delivery route in the Sardinian neonatal population.

Materials and methods

In our retrospective study we reviewed hematological blood counts from UCB samples collected consecutively at the Neonatal and Obstetric Division of San Gavino Hospital, Sardinia, Italy, between January 1, 2013, and June 30, 2014.

UCB collection was not performed in the case of perinatal maternal or neonatal complications. Unsuitable samples (insufficient blood amount, clotted or hemolyzed samples) were excluded from the analysis.

UCB collection was done by trained midwives immediately after delivery and cord clamping, and before the expulsion of the placenta. Four mL of venous UCB were collected in a tube with EDTA K3 as anticoagulant. These samples were immediately transported to the clinical laboratory, where a CBC was performed. Viable samples

were analyzed within 1 hour using the Siemens ADVIA® 2120i Hematology System in order to obtain a CBC, including red blood cells (RBC), hemoglobin (Hb), reticulocytes, platelets, white blood cells (WBC), reticulocyte hemoglobin content (CHr), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and nucleated red blood cells (NRBC). The Siemens ADVIA® 2120i system measures RBC and platelet count using light-scatter analysis. WBC count with differential count is determined by peroxidase cytochemical assay. This assay also allows the determination of erythroblast count. The reticulocyte count is assessed using optical scanning technology following specific staining with oxazine 750. The ADVIA® 2120i analyzer is able to directly measure CHr, which, because of the short half-life of the reticulocytes (1-2 days), provides an early marker of the body iron available for Hb synthesis [22].

All mothers signed a written informed consent to the UCB collection before delivery.

Data analysis

UCB data were stratified by gender, gestational age and delivery route. All analyzed parameter values were expressed as mean ± standard deviation. The mean differences in the variances of CBC by gender (male vs. female), delivery route (vaginal delivery vs. cesarean section), and gestational age (term vs. preterm infants) were compared by using Student's t-test. A p-value < 0.05 was considered statistically significant.

Results

We collected 458 UCB samples at birth between January 1, 2013, and June 30, 2014. Nineteen samples (4.1%) were excluded as coagulated. In total, we analyzed 439 UCB samples obtained from newborn infants with gestational age at birth of 34 to 42 weeks, 225 of whom were male and 214 female. **Fig. 1** shows the distribution of the study population by gestational age. Moreover, of 439 infants deemed eligible for the study, 31 (7%) were late preterm, and 274 (62%) were born by vaginal delivery. **Tab. 1** summarizes the hematological UCB values observed in male and female newborns, expressed as mean ± standard deviation. No significant differences could be found between males and females in RBC, Hb, hematocrit, MCV, MCHC, platelets, WBC. Regarding delivery mode, it seems to significantly influence exclusively the
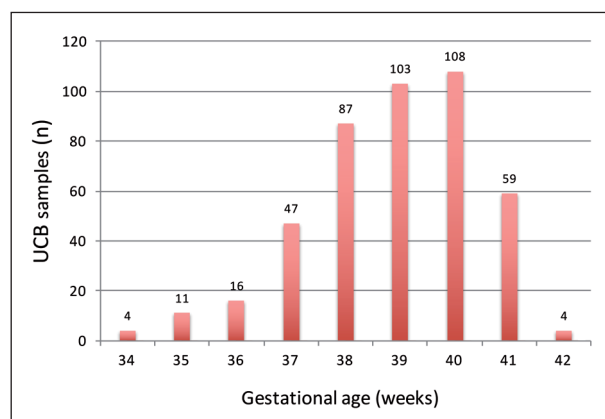
Figure 1. Distribution of the study population by gestational age.

Table 1. Umbilical cord blood (UCB) hematological values of all study population and gender distribution.

Parameters	Total n = 439	Female n = 214 (48.7%)	Male n = 225 (51.3%)	p-value
RBC (x 10¹²/L)	4.74 ± 0.58	4.71 ± 0.55	4.77 ± 0.56	0.2664
Hb (g/dl)	15.4 ± 1.73	15.32 ± 1.78	15.47 ± 1.68	0.3578
MCV (fl)	104.09 ± 7.45	104.33 ± 7.45	103.87 ± 7.45	0.5253
MCH (pg)	32.61 ± 2.74	32.64 ± 2.75	32.57 ± 2.74	0.7922
MCHC (g/L)	313.0 ± 12.3	312.7 ± 11.5	313.3 ± 13.0	0.5842
Reticulocytes (x 10⁹/L)	201.41 ± 40.78	202.74 ± 39.07	200.13 ± 42.42	0.5061
CHr (pg)	32.68 ± 2.37	32.65 ± 2.37	32.71 ± 2.37	0.7863
NRBC (x 10⁹/L)	1.21 ± 1.00	1.12 ± 0.95	1.29 ± 1.05	0.0772
Platelets (x 10⁹/L)	300.89 ± 71.45	305.61 ± 73.00	296.41 ± 69.82	0.1779
WBC (x 10⁹/L)	12.83 ± 3.87	13.09 ± 3.57	12.59 ± 4.12	0.1743

Results are presented as mean ± SD.
RBC: red blood cells; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; CHr: reticulocyte hemoglobin content; NRBC: nucleated red blood cells; WBC: white blood cells.

values of RBC, Hb, platelets and WBC. As reported in **Tab. 2**, the values of these parameters were higher in neonates born vaginally compared to those from cesarean section. Term birth was related to higher values of RBC, Hb, and WBC, to lower values of MCHC, but did not influence other parameter values (**Tab. 3**). CHr and NRBC values were found to be 32.68 ± 2.37 pg and 1.21 ± 1 x 10^9/L, respectively (**Tab. 1**). These parameters' values seem not to be significantly influenced by gender, gestational age, and delivery route.

Discussion

Our retrospective study provides a contribution to determining reference values for the main UCB hematological parameters. In 2009, Katsares et al. [10] conducted a study on a large UCB sample from Greece and proposed reference ranges for the main hematological parameters. In 2015, a large study by Glasser et al. [17] established reference ranges for UCB. In the same year, Suman et al. [11] established the reference intervals for the same UCB parameters highlighting that some of them showed higher values in the Indian cohort than in the previous study. In Italy, reference ranges for hematological UCB parameters have been recently defined by Dima et al. [12]. The present study also analyzed the relationship between UCB values and some variables, such as gestational age, delivery route, and gender. It is well known that UCB hematological parameters values change with gestational age. Noguera et al. [18] have conducted a study on UCB samples collected from newborn

Table 2. Umbilical cord blood (UCB) data stratified by delivery route.

Parameters	Cesarean delivery n = 165 (37.6%)	Vaginal delivery n = 274 (62.4%)	p-value
RBC (x 10^{12}/L)	4.62 ± 0.55	4.82 ± 0.55	**0.0003**
Hb (g/dl)	15.14 ± 1.65	15.55 ± 1.76	**0.0150**
MCV (fl)	104.82 ± 7.60	103.69 ± 7.35	0.1261
MCH (pg)	32.94 ± 2.80	32.42 ± 2.69	0.0537
MCHC (g/L)	314.2 ± 11.9	312.4 ± 12.5	0.1447
Reticulocytes (x 10^9/L)	204.29 ± 42.74	199.69 ± 39.55	0.2552
CHr (pg)	32.97 ± 2.47	32.51 ± 2.30	0.0513
NRBC (x 10^9/L)	1.13 ± 1.07	1.26 ± 0.96	0.2048
Platelets (x 10^9/L)	285.48 ± 62.02	310.05 ± 74.96	**0.0005**
WBC (x 10^9/L)	11.21 ± 3.04	13.82 ± 3.99	**< 0.001**

Results are presented as mean ± SD.
RBC: red blood cells; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; CHr: reticulocyte hemoglobin content; NRBC: nucleated red blood cells; WBC: white blood cells.

Table 3. Umbilical cord blood (UCB) data stratified by gestational age.

Parameters	Late preterm infants n = 31 (7.1%)	Term infants n = 408 (92.9%)	p-value
RBC (x 10^{12}/L)	4.46 ± 0.70	4.76 ± 0.54	**0.0033**
Hb (g/dl)	14.61 ± 2.09	15.46 ± 1.69	**0.0085**
MCV (fl)	103.91 ± 7.99	104.13 ± 7.42	0.8755
MCH (pg)	32.97 ± 3.16	32.59 ± 2.71	0.4485
MCHC (g/L)	317.4 ± 16.8	312.7 ± 11.8	**0.0422**
Reticulocytes (x 10^9/L)	206.00 ± 45.78	201.06 ± 40.42	0.5167
CHr (pg)	33.28 ± 2.92	32.64 ± 2.32	0.1448
NRBC (x 10^9/L)	1.05 ± 0.90	1.22 ± 1.01	0.3586
Platelets (x 10^9/L)	289.90 ± 58.21	301.70 ± 72.23	0.3753
WBC (x 10^9/L)	9.34 ± 3.29	13.11 ± 3.79	**< 0.001**

Results are presented as mean ± SD.
RBC: red blood cells; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; CHr: reticulocyte hemoglobin content; NRBC: nucleated red blood cells; WBC: white blood cells.

infants with gestational age ranging from 30 to 37 weeks. These authors have documented that Hb values were lower and MCV values were higher in preterm rather than in full-term infants. More recently, a similar pattern was shown in two studies conducted on newborn infants with a gestational age of 22/23-40 weeks [19, 21]. Glasser et al. have found the same Hb and MCV trend in a different study population with gestational ages ranging from 30 to 44 weeks. Furthermore, the authors have documented that WBC count increased with gestational age and platelet count increased from 30 to 35 weeks [17]. Our study documented that RBC, Hb, and WBC were higher in full-term than late preterm infants. Considering the different gestational ages of the study population, our study results are not fully comparable with those of the above-mentioned studies. Maconi et al. [20] have enrolled newborn infants of 34-42 weeks' gestational age to investigate the differences in reticulocyte parameters, showing that reticulocyte count and CHr were inversely proportional to gestational age. By contrast, our data did not show any significant difference in these parameters between preterm and full-term infants, although the relatively small sample size of preterm infants and the fact that we enrolled only late preterms should be considered. Few studies have shown that the delivery route might influence UCB parameter values. Two recent studies have documented that RBC and WBC values are higher after vaginal delivery than after cesarean section [16, 17]. Higher WBC values after vaginal delivery were found by Sparrow et al. [14]. Chang et al. [16] documented that Hb and platelets were higher after vaginal delivery than cesarean section. On the other hand, another study reported no difference in RBC and platelet values with regard to the delivery route but higher WBC values in vaginal delivery [13]. In our study population, the delivery route was shown to influence RBC, WBC, platelet counts and Hb values as all these parameters were higher after vaginal delivery than after cesarean section. We may hypothesize that labor and its physiological changes or hemoconcentration could explain the differences in these parameter values. Conflicting data have been reported about the influence of gender on UCB hematological values. Chang et al. [16] and Dima et al. [12] have found lower RBC and Hb values in females than in males. Furthermore, two studies have documented higher platelet and WBC values in females than in males [10, 16]. These data have

not been confirmed in other studies that found no differences between males and females [11, 23]. Our study found no significant differences by gender in analyzed UCB parameters. Little is known about CHr and NRBC values in UCB. NRBC are produced in the bone marrow during fetal life. They are the most immature RBC that can be observed in newborn blood and represent the precursor cells of reticulocytes. Reticulocytes remain in circulation for only one or two days before becoming mature RBC. CHr has been defined as the amount of Hb in reticulocytes and might provide an early marker of the body iron available for Hb synthesis, because of the short half-life of reticulocytes. Our study results show that CHr values seem not to be influenced by gender, gestational age, and delivery route. These results are partly in contrast with those reported in the study by Maconi et al. [20], which observed that CHr values were inversely proportional to gestational age. Three years later, Lorenz et al. [21] published a review reporting that the CHr values found in term infants were higher than those observed in adults; the authors, however, highlighted the lack of studies about CHr values in UCB of preterm infants.

Conclusions

UCB hematological parameters may be a useful tool for clinical evaluation of newborn infants, but little is known about their normal values.

Our preliminary findings may contribute to define reference values for UCB hematological parameters in term and late preterm infants from the Mediterranean island of Sardinia, thus favoring a wider use of these parameters in the clinical management of the newborn.

Larger studies across diverse neonatal populations are needed to establish hematological reference values and their variations according to gender, gestational age, and delivery route.

Abbreviations

CBC: complete blood count

CHr: reticulocyte hemoglobin content

Hb: hemoglobin

MCH: mean corpuscular hemoglobin

MCHC: mean corpuscular hemoglobin concentration

MCV: mean corpuscular volume

NRBC: nucleated red blood cells

QC: quality control

RBC: red blood cells

UCB: umbilical cord blood

WBC: white blood cells

Declaration of interest

The Authors have no competing interests.

References

1. Sheeran M. Pain in infants: a literature review. J Neonatal Nurs. 1997;3:13-8.

2. Shah VS, Ohlsson A. Venepuncture versus heel lance for blood sampling in term neonates. Cochrane Database Syst Rev. 2011;(10):CD001452.

3. Meehan RM. Heelsticks in neonates for capillary blood sampling. Neonatal Netw. 1998;17(1):17-24.

4. Abril JC, Aguilar L, Albinana J. Flatfoot and calcaneal deformity secondary to osteomyelitis after neonatal heel puncture. J Pediatr Orthop B. 1999;8(2):122-4.

5. Fleischman AR, Borris LC, Bender D, Matthews D. Clinical considerations for infant heel blood sampling. Neonatal Intensive Care. 1992;5(3):62-8.

6. Tungalag L, Gerelmaa Z. Nucleated red blood cell counts in asphyxiated newborns. Open Sci J Clin Med. 2014;2(1):33-8.

7. Lee JC, Ahern TP, Chaves FP, Quillen K. Utility of hematologic and volume, conductivity, and scatter parameters from umbilical cord blood in predicting chorioamnionitis. Int J Lab Hematol. 2010;32(3):351-9.

8. Baer VL, Lambert DK, Carroll PD, Gerday E, Christensen RD. Using umbilical cord blood for the initial blood tests of VLBW neonates results in higher hemoglobin and fewer RBC transfusions. J Perinatol. 2013;33(5):363-5.

9. Carroll PD, Nankervis CA, Iams J, Kelleher K. Umbilical cord blood as a replacement source for admission complete blood count in premature infants. J Perinatol. 2012;32(2):97-102.

10. Katsares V, Paparidis Z, Nikolaidou E, Karvounidou I, Ardelean K, Drossas N, Grigoriadis N, Grigoriadis J. Reference ranges for umbilical cord blood hematological values. Lab Med. 2009;40(7):437-9.

11. Suman FR, Raj RS, Priyathersini N, Rajendran R, Rajendran R, Ramadoss U. Biological reference interval for hematological profile of umbilical cord blood: a study conducted at a tertiary care centre in South India. J Clin Diagn Res. 2015;9(10):SC07-9.

12. Dima F, Montagnana M, Raffaelli R, Cascella S, Bovo C, Franchi M, Lippi G. Intervalli di riferimento dell'esame emocromocitometrico nel sangue di cordone ombelicale. Biochim Clin. 2016;40(3):204-7.

13. Nikischin W, Peter M, Oldigs HD. The influence of mode of delivery on hematologic values in the umbilical vein. Gynecol Obstet Invest. 1997;43(2):104-7.

14. Sparrow RL, Cauchi JA, Ramadi LT, Waugh CM, Kirkland MA. Influence of mode of birth and collection on WBC yields of umbilical cord blood units. Transfusion. 2002;42(2):210-5.

15. Redźko S, Przepieść J, Żak J, Urban J, Wysocka J. Influence of perinatal factors on hematological variables in umbilical cord blood. J Perinat Med. 2005;33(1):42-5.

16. Chang YH, Yang SH, Wang TF, Lin TY, Yang KL, Chen SH. Complete blood count reference values of cord blood in Taiwan and the influence of gender and delivery route on them. Pediatr Neonatol. 2011;52(3):155-60.

17. Glasser L, Sutton N, Schmeling M, Machan JT. A comprehensive study of umbilical cord blood cell developmental changes and reference ranges by gestation, gender and mode of delivery. J Perinatol. 2015;35(7):469-75.

18. Noguera NI, Detarsio G, Perez SM, Bragos IM, Lanza O, Rodriguez JH, Acosta I, Davoli R, Milani AC. Hematologic study of newborn umbilical cord blood. Medicina (B Aires). 1999;59(5 Pt 1):446-8.

19. Jopling J, Henry E, Wiedmeier SE, Christensen RD. Reference ranges for hematocrit and blood hemoglobin concentration during the neonatal period: data from a multihospital health care system. Pediatrics. 2009;123(2):e333-7.

20. Maconi M, Formisano D, Cavalca L, Rolfo A, Cardaropoli S, Danise P. Reticulocyte count and reticulocyte maturation profile in human umbilical cord blood from healthy newborns. Lab Hematol. 2010;16(1):3-7.

21. Lorenz L, Peter A, Poets CF, Franz AR. A review of cord blood concentrations of iron status parameters to define reference ranges for preterm infants. Neonatology. 2013;104(3): 194-202.

22. Harris N, Jou JM, Devoto G, Lotz J, Pappas J, Wranovics D, Wilkinson M, Fletcher SR, Kratz A. Performance evaluation of the ADVIA 2120 hematology analyzer: an international multicenter clinical trial. Lab Hematol. 2005;11(1):62-70.

23. Adewumi A, Titilope AA, Akinsegun AA, Abidoye G, Ebele U, Sulaimon AA. Cord blood full blood count parameters in Lagos, Nigeria. Pan Afr Med J. 2014;17(1):192.