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Reference values of blood cell counts in the first days of life

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1. ABSTRACT

The lack of updated neonatal reference values for hematological parameters impacts significantly with clinical management of both healthy and sick newborns. The present pilot study was thus aimed at assessing updated hematological Italian reference values in late preterm and term newborns. From January 2004 to December 2008 hematological laboratory tests were performed in 1175 newborns (820 healthy and 355 sick controls) between 33-41 weeks of gestation, during the first four days after birth. Hematological parameters were sorted for gender and gestational age and statistically analyzed. No gender-related differences were observed at different weeks of gestation and no significant differences were found when study population was sub-grouped for late preterm and term newborns. During the first 4 days of life erythrocytes and platelets remained stable whilst white blood cell counts and differentials were significantly modified. This study shares updated reference values for hematological parameters in the early phases after birth and offers additional support for improving the management of sick infants.

2. INTRODUCTION

It has been recently highlighted that the availability of reference values for laboratory parameters in the first days after birth can constitute a relevant tool for an optimal management of post-natal adaptation phases. In this setting, if a normal population cannot be collected as controls, to define the reference intervals, laboratory results from unselected subjects stratified for age and sex can be recorded and further elaborated. From a statistical point of view, the most heterogeneous is the population, the larger should be the sample. In addition, it is commonly accepted to calculate reference values by identifying the 2.5–97.5% ranges obtained in a cohort of patients addressing to a laboratory facility. This is an adequate approach if the population is heterogeneous but can be significantly improved by using the average values and the multiple of standard deviations whether the population had a normal distribution. Conversely, when distribution is asymmetric, a different approach should be applied, such as the Kairisto algorithm (1). Additional problems in post-natal laboratory management regard: i) ethical problems related with blood

sampling in healthy donors; ii) ethnic differences among different study-populations, and, iii) clinical and laboratory criteria for classifying healthy preterm/late preterm newborns. This latter point is of relevance since in the first hours after birth, post-natal adaptation can require a high/different capacity of transporting oxygen affecting hemoglobin values and blood concentration. Other confounding factors in the first days of life are breathing activity, *perspiratio insensibilis*, water and/or milk assumption and active state behavior that may result in blood cell dilution. Thus, any reference values in this period of investigation is expected to be highly variable and therefore the identification of a particularly accurate reference range is eagerly requested in clinical daily practice. In the last decade, technological improvement in clinical pathology laboratories allowed to measure classic blood cell-related parameters such as hemoglobin (Hb), Red Blood Cells (RBC), White Blood Cells (WBC) and its subpopulations, platelets and calculated parameters with great accuracy and precision. However, reference values in use at present are based on historical data (2-13) largely based on low technology instrumentations and only recently, updated reference values were reported (14).

The aim of the present pilot study was thus to evaluate whether a multicentre study, representative of different regions and organizations in Italy, could share novel reference ranges for hematological parameters. To achieve this goal: i) a feasibility study was carried out in a pediatric Institution in which physiological and high risk deliveries/infants were carried out and, ii) a comparison between healthy and sick newborn hematological parameters was conducted.

3. MATERIAL AND METHODS

The study was approved by Italian Society of Neonatology Board, by Local Ethics Committees and the parents of the subjects gave signed and informed consent.

In the period January 2004-December 2008, 1093 laboratory tests, related to 820 newborns (48,5% females and 51,5% males), were performed. One hundred and twenty eight samples were processed in 2004, 197 in 2005, 187 in 2006, 272 in 2007 and 172 in 2008. In 29% of patients, blood was collected at birth, 25% on day 1, 25% on day 2, 12% on day 3 and 9% on day 4. The gestational age distribution for healthy newborns at sampling was 20% for late preterm (33-36 wks), 47% for 37-39 wks and 33% for 40-42 wks.

Gestational age was determined by clinical data and by a first trimester ultrasound scan. Appropriate growth was defined by the presence of ultrasonographic signs (when biparietal diameter and abdominal circumference were between the 10-90th centiles) according to the normograms of Campbell and Thoms (15) and by postnatal confirmation of a birth-weight between the 10-90th centiles according to our population standards after corrections for the mother's height, weight and parity and the sex of the newborn. All healthy infants admitted to the study fulfilled all the following criteria: no maternal illness, no signs of fetal distress, pH more than 7.2 in cord blood or venous blood, Apgar scores at 1 and 5 minutes more than 7 (16). All control newborns were in normal clinical condition and showed no overt neurological syndrome at the discharge from the hospital (Table 1). Controls subjects were 355 newborns whose neonatal outcomes did not fulfill American Academy of Pediatrics criteria for healthy newborns. In these patients 473 blood samples were collected.

3.1. Evaluation of sample size

Analysis of laboratory data expressing a certain heterogeneity requires the accurate definition of the sample size. On these bases, we decided to calculate the results on a number of subjects that was always wider than that formally required. The N-Query software was then used to calculate how many samples, in each experimental condition, should be needed for the calculation of the lower and upper percentiles (2.5-97.5%).

3.2. Blood cells collection

Blood samples were obtained either from healthy or sick children during the first days from delivery. Blood was collected using venipuncture and immediately transferred to the laboratory facility for the tests. All samples were identified using a unique barcode for that individual, that test and that time. Laboratory results were stored in the Laboratory Information System (LIS), using a specific software (Concerto, Metafora, Milan, Italy). Data from the LIS were collected using another software (Frequenza, Metafora, Milan, Italy) that may store in a export file not only the single laboratory but also personal data of the patient and the time of reception in the laboratory. This is particularly useful to sort samples obtained at different time interval from the birth. Of note, if more than one sample was processed for a single patient, only the earliest was included in this study.

3.3. Laboratory procedure

Routine hematological assays are performed using two different platforms. The Siemens Advia 1210 (Munich, Germany) that uses standard procedures for red blood cell count and peroxidase staining of granulocytes and the Beckman Coulter 750 (Brea, CA, USA) that employs a standard approach for red blood cells but performs differential white blood cell count using volume, scatters and conductivity. The two instruments, according to manufacturers' instructions, were daily aligned in order to obtain identical results. The following parameters were analyzed: total number of circulating RBC, Hb, Hematocrit rate (Ht), Mean Cellular Volume of RBC (MCV), Mean Cellular Hb concentration of Hb (MCH), mean cellular Hb concentration (MCHC), RBC Distribution Width (RDW), total circulating WBC, percentage and absolute number of neutrophilic granulocytes (NEU% and NEU), lymphocytes (LYM% and LYM), monocytes (MON% and MON), eosinophils (EOS% and EOS), basophils (BAS% and BAS), and finally platelets (PLT).

3.4. Quality controls

An internal quality control program was performed to evaluate the day-by day performances of instruments and

Table 1. Neonatal characteristics

	Late Preterm Group (33-36 wks) (n=164)	**Term Group A (37-39 wks) (n=385)**	**Term Group B (40-42 wks) (n=271)**
Maternal age (year)	25.7 +/- 4.1	25.9 ± 3.5.	26.3 ± 2.9
Gestational age at birth (wks)	34 +/- 1*	38 +/- 1	41 +/- 1
Birth weight (g)	2,488 +/- 227*	2,811 +/- 314	3,341 +/- 211
Gender M/F			
Apgar score more than 7 at 5th min (n°/total)	164/164	385/385	271/271
Mother's racial or ethnic group – (n°/total)			271/271
- Black	5 (3)	10 (2.7)	8 (2.9)
- Caucasian	131 (80)	312 (81)	218 (81)
- Other	28 (17)	63 (16.3)	43 (16.1)
Partial venous CO_2 pressure (mmHg)	43.6 +/- 2.2	46.3 +/- 3.5	7.35 +/- 0.3
Partial venous O_2 pressure (mmHg)	39.1 +/- 5.6	41.6 +/- 4.4	43.7 +/- 3.9
Base excess	1.1 +/- 1.1	1.3 +/- 0.3	4172 +/- 5.1

Neonatal characteristics at birth in late preterm (33-36 wks), term A (37-39 wks) and B groups (40-42 wks). Data are given as means +/- SD. *P less than 0.05.

procedure. An external Quality Assurance program was also performed with a standard daily frequency. Both internal and external controls were daily checked by the Quality Control Responsible of the laboratory. In addition, samples from normal donors were also collected to check the stability of the average values of hematological parameters over the months and the years. Data collected for Quality Controls as well as data from healthy adult donors were downloaded from the LIS using the same procedure described for newborn samples. Notably, the Institution has been awarded for excellence by the Joint Commission International in 2006 and the laboratory itself has an ISO 9000 certification since 2003.

3.5. Statistical analysis

Files downloaded from the LIS were analyzed with GraphPad Prism version 3.00 for Windows (GraphPad Software, Inc., San Diego, California, USA), in order to evaluated the following parameters: Average, standard deviation, asymmetry, kurtosis. If the distribution of the single parameter was symmetric, a 2.5-97.5% interval was calculated. On the contrary, in the presence of an asymmetric distribution (usually present for rare events), a different approach (as suggested by Kairisto) was employed. For this, an internal MS-Excel routine was developed, validated and then used. Differences in neonatal outcomes and in hematological parameters among studied groups, after sort by gender, were assessed by using ANOVA on ranks test and *post-hoc* Tukey's test to isolate the statistical differences. Statistical analysis was performed by using Student's *t* test for continuous variables and Mann-Whitney U-two sided test when parameters were not normally distributed. Categorical data were analyzed by means of Fisher's exact test or chi-square analysis as appropriate. Linear regression analysis was used to assess correlation between gestational age at sampling and hematological parameters. A P less than 0.05 was considered statistically significant.

4. RESULTS

4.1. Study populations

Neonatal characteristics of the late preterm and term healthy newborns admitted into the study are shown in Table 1. As expected gestational age and weight at birth was significantly (P less than 0.05, for both) lower in late preterm newborns, whilst, according to recruitment criteria, no significant differences (P more than 0.05, for all) regarding Apgar scores at 1st and 5th minutes, venous blood pH, partial venous CO_2 and O_2 pressures and base excess values were shown. No significant differences (P more than0.05, for all) were found in neonatal outcomes and admission criteria when evaluated for maternal ethnic and racial characteristics.

4.2. Quality controls

During the period of investigation, a number of quality controls were performed in order to demonstrate that stability and reproducibility of the procedures. All internal and external controls resulted within the expected ranges. To further support this evidence, in Figure 1 are presented the mean values of Hb, obtained in the same period and measured in adult control subjects. As shown, the variability of the mean values was always within 7%, thus suggesting that in the whole period the laboratory performances were under control.

4.3. Hematological parameters

Data obtained in newborns were then analyzed. Figure 2 shows hematological parameters distributions: RBC, Hb, Ht, WBC and PLT counts showed a symmetrical pattern, while other parameters did not. Therefore, reference limits for the first four parameters were calculated using the 2.5–97.5 percentiles, whilst reference values for other parameters were assessed by using same percentiles and the Kairisto procedure for asymmetric data.

Table 2 shows the results of this analysis on the whole population, after sort for gender, independently from the gestational age and the time at sampling. Table 3 shows that there were no gender differences (P more than 0.05, for all) among laboratory parameters in both late preterm and term newborns. In Table 4 laboratory data are associated to different weeks of gestation. No significant differences (P more than 0.05, for all) were found when study population was corrected for gestational age at sampling as well as subgrouping for late preterm (33-36 wks) and term newborns (A: 37-39; B: 40-42 wks, respectively). Table 5 reports hematological parameters changes during the first 4 days from birth. Of note, while RBC and PLT remained virtually unmodified (P more than 0.05, for all), Hb concentrations, Ht% and WBC counts were significantly

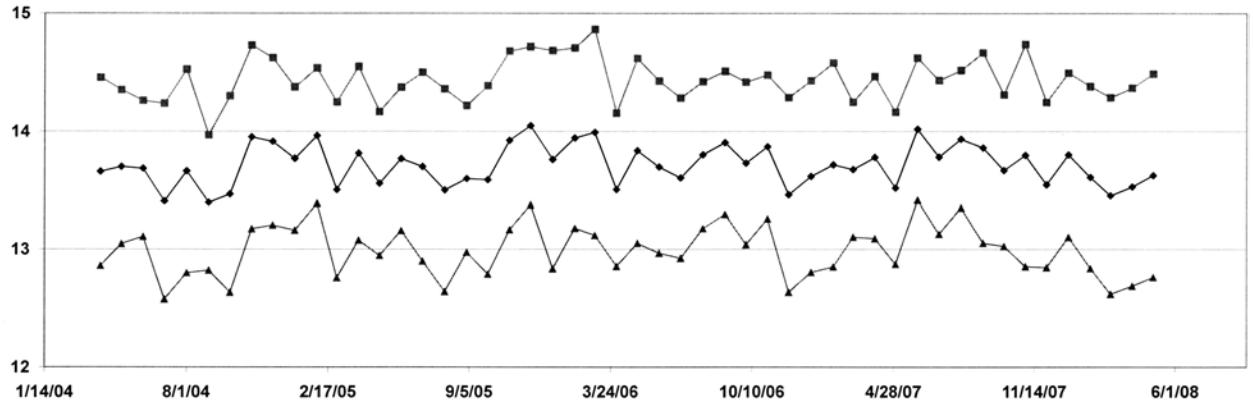

Figure 1. Longitudinal monitoring of hemoglobin blood concentrations (g/dL) in samples collected from healthy donors at different timepoints over the period of collection of newborn data (2004-2008). Data are given in monthly average, lower and upper limits. The high reproducibility of the results is indicative of a stable measurement of hematological parameter during the period of the study.

decreased when compared at birth monitoring time-point. WBC population patterns expressed as percentage values during the first days were of particular interest: neutrophils significantly decreased (P less than 0.05, for all) from 70% to 36%, basophils had a flat trend, while lymphocyte, monocyte and eosinophil percentages showed a slight increase without reaching a statistical significance (P more than 0.05, for all). The finding was noteworthy when WBC populations were expressed as absolute values (Table 6). In details, the mean number of neutrophils was significantly (P less than 0.05) decreased, from about 16000/mmc, at birth, to about 4000/mmc at day 4 (a reduction of about 75%). Lymphocyte, monocyte and eosinophil counts showed a flat pattern, whilst basophil pattern showed a significant decrease from birth to day 4, with an estimated reduction of the 50%.

To further validate the reference values calculated and shown in the abovementioned tables, a representative population of high risk newborns was also studied**.** As expected**,** high risk newborns showed lower Ht and Hb concentrations (estimated difference in percentage: 13%, for both) whilst MCV, MCH and MCHC did not differ. Indeed, significant differences (P less than 0.05, for all) were observed for WBC counts expressed as absolute numbers and as percentages.

5. DISCUSSION

The present pilot study offers hematological reference values in the first days after birth in Italian late preterm and term newborns. The finding is of relevance being reference values and their updates eagerly awaited in clinical daily practice of all disciplines especially in neonatology and neonatal critical care medicine. The request is supported not only by recent technological improvement (manual methods vs. low level automation), that has considerably changed laboratory assessment strategies, but mainly by a series of confounding factors related to population study (i.e. ethnic and socioeconomic differences, immigrations rate etc) that can affect the availability of previous observations. In this respect, it is noteworthy that Caucasians do not represent, nowadays, the totality of the newborns attending to Italian Children' hospitals. Of note, in the present series, no significant differences have been found in neonatal outcomes, admission criteria and finally in laboratory results when corrected for maternal ethnic and racial characteristics.

Despite technological improvement characterized by: i) improvemend of measurement performances with excellent reproducibility; ii) assessment in a variety of biological fluids; iii) possible use in longitudinal monitoring because results can be obtained rapidly and by the use of very small blood volumes, the validation on a large number of cases for standard laboratory parameters reference values is highly required. This issue is of utmost importance when these criteria have to be applied in the perinatal period when several confounding factors, participating in the *so called* post-natal adaptation phase, are still present. To our knowledge, the present results is the first attempt to update laboratory data referring to neonatal period in Italy. Of note, the present findings fit a recent report from a U.S. multicentre study recruiting a cohort of more than 24,000 newborns. In more detail, no significant differences were observed regarding Hb (18.1 g vs 17.5 g) and Ht values (54% vs 51,0%) between the two reports. Further multicenter investigations may be useful in order to verify, in a larger population study, our findings on white blood cells population. However, we did not find any consistent differences with a previous report by our institutes conducted in the late seventies (data not shown).

In the present study we also found no significant differences in laboratory parameters when corrected for gender and gestational age at sampling. This latter issue can be of relevance, bearing in mind the importance of an adequate laboratory monitoring in a such selected population. There is growing evidence that late-preterm population nowadays represent one of the major high-risk newborn population. The main explanation resides in the delivery period (33-36 wks) at a stage when whole organs

Table 2. Distribution of hematological parameters in the whole analyzed population of newborns

Parameter	RBC	HB	HT	MCV	MCH	MCH	RDW	WBC	NEU	LYM	MON	EOS	BAS	PLT
AV/MD	5.2	18.1	54.0	104.2	35.0	33.5	16.7	20.1	65.4	22.4	7.3	2.2	1.0	291.8
2.5%	4.1	14.3	42.7	92.9	30.9	31.7	15.0	8.3	31.4	10.6	3.7	0.5	0.2	96.4
97.5%	6.4	22.6	67.0	114.8	38.3	35.7	19.8	34.8	80.4	46.0	15.0	6.8	4.0	462.2

Red Blood Cells (RBC), Hemoglobin (Hb, g/dL), Hematocrit rate (Ht%), Mean Cellular Volume of RBC (MCV), Mean Cellular Hb concentration (MCH), mean cellular Hb concentration (MCHC), Red Cell Distribution Width (RDW), total circulating white blood cells (WBC), percentage of Neutrophilic granulocytes (NEU%), Lymphocytes (LYM%), Monocytes (MON%), Eosinophils (EOS%), Basophils (BAS%), and Platelets absolute values (PLT) in whole studied population uncorrected for gender, gestational age and the time at sampling. The average (AV) was used when data were normally distributed whilst Median (MD) and 2.5-97.5 percentiles were used when data were asymmetrically distributed.

Table 3. Distribution of hematological parameters in newborns: differences between males and females

Sex	Parameter	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	WBC	NEU	LYM	MON	EOS	BAS	PLT
Male	AV/MD	5.2	18.1	53.9	104.3	35.0	33.6	16.8	19.0	64.6	22.9	7.4	2.4	0.9	281.6
	2,5%	4.1	14.7	43.0	92.1	30.5	31.6	15.2	7.7	31.5	11.7	3.7	0.6	0.2	117.6
	97,5%	6.4	22.4	67.3	115.8	38.3	35.6	20.1	33.3	79.0	46.0	15.2	6.9	4.9	435.5
Female	AV/MD	5.2	18.1	54.0	103.9	35.0	33.5	16.5	21.4	67.1	21.7	7.1	1.9	1.0	302.6
	2,5%	4.1	14.2	42.7	93.6	31.4	31.9	14.9	8.7	31.4	10.6	3.6	0.5	0.2	69.2
	97,5%	6.5	22.7	65.7	113.0	38.3	35.7	19.5	35.5	81.1	45.6	14.6	6.7	3.6	468.4

Red Blood Cells (RBC), Hemoglobin (Hb, g/dL), Hematocrit rate (Ht%), Mean Cellular Volume of RBC (MCV), Mean Cellular Hb concentration (MCH), mean cellular Hb concentration (MCHC), Red Cell Distribution Width (RDW), total circulating white blood cells (WBC), percentage of Neutrophilic granulocytes (NEU%), Lymphocytes (LYM%), Monocytes (MON%), Eosinophils (EOS%), Basophils (BAS%), and Platelets absolute values (PLT) according to gender distribution. There were no differences (P more than 0.05, for all) between groups. The average (AV) was used when data were normally distributed whilst Median (MD) and 2.5-97.5 percentiles were used when data were asymmetrically distributed.

Table 4. Distribution of hematological parameters in newborns: differences related to the weeks of gestation.

wks		RBC	HGB	HCT	MCV	MCH	MCH	RDW	WBC	NEU	LYM	MON	EOS	BAS	PLT
33-35	median	5.0	18.0	53.1	34.2	101.7	33.5	16.6	21.6	65.4	22.0	7.0	1.8	1.1	284.2
	.5 centile	3.9	14.1	42.8	32.1	92.4	31.2	15.2	9.3	41.9	12.1	3.0	0.5	0.3	97.2
	7.5 centile	6.2	21.5	63.5	38.0	110.5	35.3	19.6	28.6	75.4	43.7	13.6	5.0	1.8	396.2
36	median	5.2	18.1	54.1	34.9	103.9	33.5	16.7	20.6	65.0	22.5	7.2	2.3	1.3	290.8
	.5 centile	4.1	14.4	43.3	32.0	94.3	31.7	15.0	8.4	32.8	11.8	3.6	0.6	0.6	97.0
	7.5 centile	6.5	22.8	67.5	38.2	113.6	35.7	20.4	36.7	79.4	44.5	14.8	6.8	2.0	449.3
37	median	5.2	18.3	54.4	35.1	104.6	33.6	16.7	22.4	69.2	20.2	6.9	1.7	1.1	289.3
	.5 centile	4.2	15.0	44.3	31.5	93.0	31.8	14.9	13.1	53.9	10.5	3.7	0.4	0.4	123.2
	7.5 centile	6.3	21.9	66.0	38.4	116.1	35.7	19.5	34.3	80.8	35.9	11.1	4.4	3.3	448.0
38	median	5.1	18.0	53.5	35.2	104.0	33.6	16.7	20.7	64.9	22.9	6.6	2.3	1.0	290.6
	.5 centile	4.1	14.3	43.1	29.9	89.7	31.4	15.3	9.4	43.1	10.5	3.7	0.6	0.2	120.4
	7.5 centile	6.4	22.5	67.4	38.2	116.6	35.3	19.6	34.9	80.6	41.8	11.7	7.1	10.2	450.9
39	median	5.2	17.9	53.2	35.0	103.6	33.8	16.7	13.5	54.9	28.9	9.9	3.9	0.9	319.8
	.5 centile	3.7	13.9	40.3	31.0	93.3	31.7	15.1	6.9	32.7	15.9	4.6	0.7	0.2	175.9
	7.5 centile	6.7	21.0	65.9	38.3	117.2	35.6	21.1	18.2	66.5	52.9	18.4	7.7	3.0	520.2
40	median	5.2	17.4	52.3	34.6	103.2	33.4	16.9	11.7	38.5	39.1	11.3	4.1	1.0	265.3
	.5 centile	3.7	12.8	39.0	27.1	86.4	31.2	15.5	7.2	24.1	18.3	7.2	1.7	0.1	6.6
	7.5 centile	6.8	21.0	66.0	38.4	117.2	35.6	21.2	18.4	66.7	53.1	18.5	7.7	2.7	520.2
41	median	5.2	17.8	53.1	34.3	102.1	33.3	16.9	20.4	62.9	23.3	6.1	2.2	0.9	283.8
	.5 centile	4.3	14.6	42.3	30.9	92.2	31.8	15.1	11.0	40.5	12.7	2.6	0.3	0.2	142.9
	7.5 centile	6.4	21.2	65.5	37.5	110.1	35.1	18.8	31.7	77.8	40.3	15.3	6.7	2.3	403.4

Red Blood Cells (RBC), Hemoglobin (Hb, g/dL), Hematocrit rate (Ht%), Mean Cellular Volume of RBC (MCV), Mean Cellular Hb concentration (MCH), mean cellular Hb concentration (MCHC), Red Cell Distribution Width (RDW), total circulating white blood cells (WBC), percentage of Neutrophilic granulocytes (NEU%), Lymphocytes (LYM%), Monocytes (MON%), Eosinophils (EOS%), Basophils (BAS%), and Platelets absolute values (PLT). Data are given at different weeks of gestation. No differences (P more than 0.05, for all) were observed among late preterm (33-36 wks), and term newborns groups (A: 37-39; B: 40-42 wks), respectively. Data are given as median and 2.5-97.5 percentiles.

and especially the central nervous system growth-process are at their higher levels (17). In particular, CNS growth is estimated in 35% for brain weight, 37% for brain volume, and synaptogenesis, dendritic arborization and axonal elongation processes are at their higher levels of activation. Thus, adequate reference curves for laboratory parameters in a such delicate fetal/neonatal period could be especially useful.

In conclusion, our findings showing changes in laboratory parameters in the first days after birth open a new cue for further multicentre investigations aimed at offering, in a larger population study, reference values of the main laboratory parameters currently used in neonatology and critical care medicine.

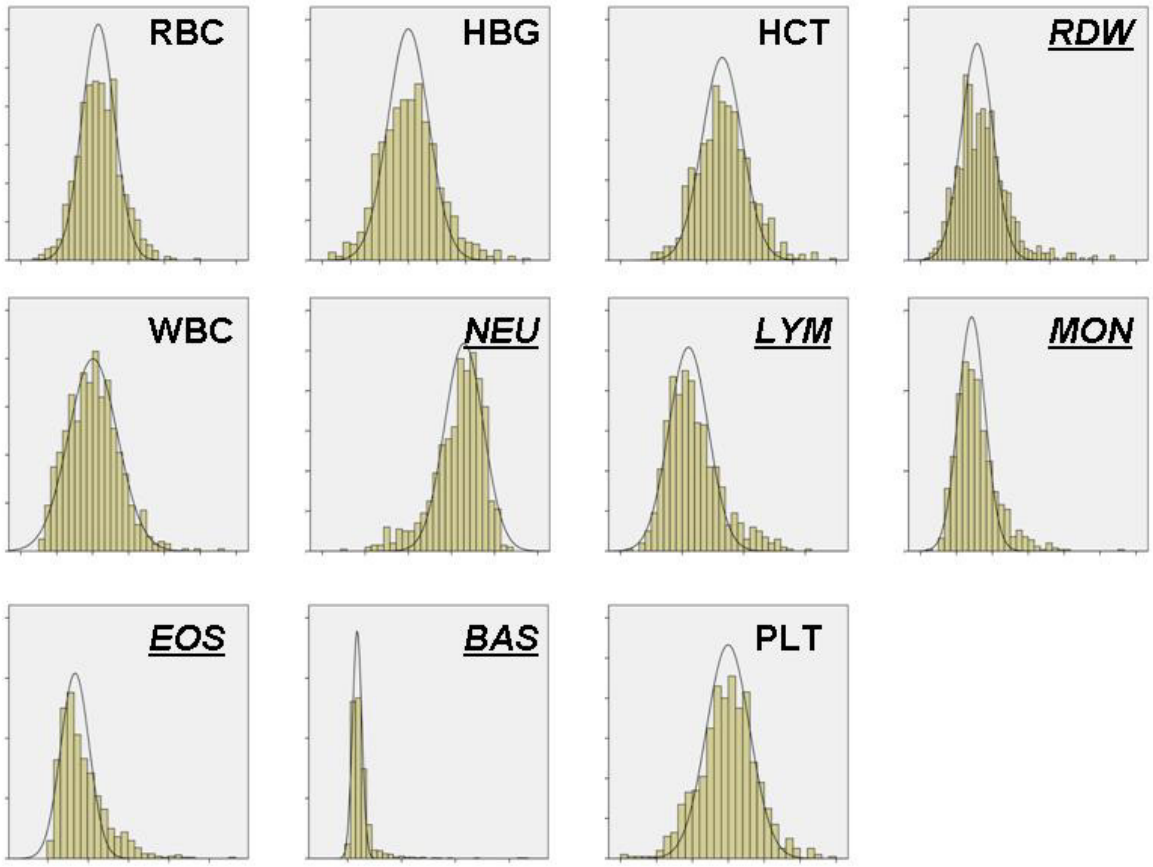

Figure 2. Distribution of laboratory results for different parameters. 1: red blood cells (RBC); 2: hematocrit rate (Ht); 3 hemoglobin concentration g/dl (Hb); 4: Red Cell Distribution Width (RDW); 4: white blood cells (WBC); 6: neutrophils; 7: lymphocytes; 8: monocytes; 9: eosinophils; 10: basophils; 11 platelets. S: symmetrical distribution; *NS*: non symmetrical distribution.

Table 5. Distribution of hematological parameters in newborns: differences related to the day of life of sampling

Timepoint	AV/MD	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	WBC*#	NEU*#	LYM*#	MON	EOS	BAS	PLT
At birth															
median		5.3	18.6	55.4	104.6	35.1	33.4	16.7	23.2	69.4	18.9	6.7	1.7	1.0	288.6
2.5 centile		4.3	15.2	45.3	94.2	31.6	31.8	15.0	13.1	55.1	10.6	3.7	0.5	0.3	116.1
97.5 centile		6.4	22.9	66.8	116.2	38.4	35.3	19.5	36.6	81.1	33.3	11.4	4.4	4.1	445.1
Day 1															
median		5.1	17.8	53.1	104.3	35.0	33.6	16.7	22.0	67.1	21.3	6.5	1.9	0.9	288.8
2.5 centile		4.0	14.1	42.6	92.9	30.9	31.6	15.0	13.0	50.5	10.6	3.5	0.6	0.2	2.8
97.5 centile		6.2	22.0	65.2	113.0	38.2	35.7	19.8	35.1	80.4	36.8	11.2	5.8	3.0	435.2
Day 2															
median		5.1	17.9	53.1	103.5	34.8	33.5	16.9	14.1	55.1	29.1	8.6	3.8	1.0	298.9
2.5 centile		4.0	14.0	40.6	96.0	32.1	31.9	15.2	6.4	34.0	9.8	4.6	1.1	0.2	131.8
97.5 centile		6.4	23.0	68.8	114.5	38.7	35.9	20.1	22.8	75.5	46.8	13.9	9.2	5.4	463.1
Day 3															
median		5.2	17.8	52.7	103.2	35.0	34.1	16.6	12.2	52.2	30.5	10.7	4.0	0.9	309.6
2.5 centile		3.8	13.9	42.5	91.0	30.8	31.9	14.7	6.9	29.8	15.9	5.3	0.7	0.2	141.5
97.5 centile		5.5	19.1	57.2	104.4	34.9	33.4	17.2	18.8	53.7	30.3	9.6	4.6	11.8	391.0
Day 4															
median		5.2	17.5	53.1	103.5	34.2	33.0	16.9	10.9	35.6	42.8	12.5	4.3	1.0	284.1
2.5 centile		3.7	12.8	39.0	86.4	27.1	30.9	15.4	7.3	24.7	18.3	8.1	2.1	0.3	62.7
97.5 centile		7.3	22.8	69.4	117.2	38.4	35.0	21.6	15.9	58.4	51.1	19.4	9.7	3.5	473.0

Red Blood Cells (RBC), Hemoglobin (Hb, g/dL), Hematocrit rate (Ht%) , Mean Cellular Volume of RBC (MCV), Mean Cellular Hb concentration (MCH), mean cellular Hb concentration (MCHC), Red Cell Distribution Width (RDW), total circulating white blood cells (WBC), percentage of Neutrophilic granulocites (NEU%), Lymphocytes (LYM%), Monocytes (MON%), Eosinophils (EOS%), Basophils (BAS%), and Platelets absolute values (PLT) at different monitoring timepoins (birth, day 1, 2, 3 and 4). Data are given as median and 2.5-97.5 percentiles. *P less than 0.05 vs birth timepoint; #P less than 0.05 vs Day 1 timepoint.

Table 6. Distribution of white blood cells in newborns: differences related to the day of life of sampling.

Timepoint	NEU*#	LYM	MON	EOS	BAS*#
At birth					
median	15824	4382	1525	384	237
2.5	7626	2297	703	114	48
97.5	27540	7451	3001	984	967
Day 1					
median	14606	4433	1393	405	184
2.5	6586	2606	649	107	36
97.5	26558	7185	2894	1258	715
Day 2					
median	7616	3810	1215	540	130
2.5	2951	1505	482	156	23
97.5	16531	6216	2235	1292	695
Day 3					
median	5714.6	3464.9	1237.8	465.5	101.0
2.5	2371.0	2025.5	681.1	46.0	24.4
97.5	15526.0	6241.4	2462.2	1149.1	636.7
Day 4					
median	3494	4035	1250	462	109
2.5	2130	2267	753	200	28
97.5	9778	6653	2374	1017	449

Neutrophilic granulocytes (NEU), Lymphocytes (LYM), Monocytes (MON), Eosinophils (EOS), Basophils (BAS) expressed as absolute numbers of white blood cell populations in the first 4 days from birth. Data are given as median and 2.5-97.5 centiles. *P less than 0.05 vs birth timepoint; #P less than 0.05 vs Day 1 timepoint.

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