SHORT REPORT

Nucleated red blood cell count in term and preterm newborns: reference values at birth

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The prognostic value of nucleated red blood cell count at birth in relation to neonatal outcome has been established. However, reference values were needed to usefully interpret this variable. The normal range of reference values for absolute nucleated red blood cell count in 695 preterm and term newborns is reported.

Nucleated red blood cells (NRBCs) are immature erythrocytes, commonly found in the peripheral blood of newborns at birth. NRBC count varies widely at birth.¹ Fetal hypoxia, the major risk factor for adverse perinatal outcome, has been claimed to induce a rise in the number of NRBCs.

Distinct NRBC patterns seem to be related to the timing of fetal injury, suggesting that NRBC count may assist in determining the timing of fetal neurological impairment as previously shown by us.²⁻⁴ High numbers of NRBCs reflect the severity and duration of hypoxia, and NRBC count may rise rapidly after brief but severe hypoxia, due to release of stored NRBCs.

Defining normal haematological values for newborns is problematical because the range of values in term or sick infants generally does not apply to preterm or healthy infants. Because of the lack of information on normal NRBC count in infants, we aimed to establish a normal reference range based on evaluation of a large number of neonates without clinical signs of perinatal hypoxia at birth or of any known disease recognised to affect the number of circulating NRBCs.

METHODS AND RESULTS

A total of 695 (455 preterm, consecutively delivered, and 240 term, randomly selected) babies were the subjects of our study.

Gestational age was determined from the last menstrual period and early ultrasonography; if there was any

discrepancy, neonatal Dubowitz evaluation was used. All babies with congenital malformation, haematopoietic anomalies, Rh and/or ABO incompatibility, inborn error of metabolism, sepsis, a mother with diabetes, multiple gestation, smoking, drug abuse, anaemia, placenta previa, abruption, or infarcts were excluded from the study to eliminate risks that could affect the number of circulating NRBCs.

We also prospectively collected specific neonatal data: Apgar score at five minutes, birth weight, cord pH. After excluding neonates documented to have a five minute Apgar score ≤ 6 , cord pH < 7.2 , birth weight more than 2 SDs below the mean, according to the growth chart of Yudkin *et al*,⁵ we identified those whom we designated as "normal". A normal neonate was defined as one without maternal or intrapartum complications, who was also normal on physical examination at the time of blood sampling. The final study population included 695 babies. To exclude any effects of gestational age on NRBC count, the 695 newborns enrolled were divided into four groups according to gestational age (table 1). Informed consent was obtained from the mothers of all normal neonates. The protocol was approved by the human ethics committee of the Medical Faculty, University of Siena.

Heparinised blood samples were obtained from the umbilical vein, immediately after delivery. NRBC was expressed as absolute count (NRBC/mm³), calculated by light microscope examination of May-Grunwald-Giemsa stained blood smears.

Mean, median, and SD were calculated in each group. The Kolmogorov-Smirnov test rejected the hypothesis of normal distribution for all groups. Logarithmic transformation was not tried because zero values of NRBC could not be missed. We drew a right sided asymmetric interval. The 0.975 fractile with its 90% confidence interval was taken as the upper reference value.⁵ Spearman's test was used to evaluate statistical correlations.

RESULTS

Table 1 shows a descriptive statistical analysis of each group. Table 2 reports upper reference value data. Figure 1 shows

Table 1 Birth weight and gestational age of the 695 newborns enrolled

	Mean (SD)	Median	25th-75th centiles
Birth weight (g)			
Group 1 (n = 120)	954 (233.9)	930	780-1105
Group 2 (n = 128)	1413 (397.8)	1360	1100-1720
Group 3 (n = 215)	2189 (472.7)	2235	1940-2520
Group 4 (n = 232)	3201 (669.0)	3210	2905-3590
Gestational age (weeks)			
Group 1 (n = 120)	26.7 (1.0)	27	26-28
Group 2 (n = 128)	30.4 (1.1)	31	29-31
Group 3 (n = 215)	34.7 (1.0)	35	34-36
Group 4 (n = 232)	38.6 (1.3)	38	38-40

Table 2 Upper reference values of nucleated red blood cell (NRBC) count in 695 newborns divided into four groups according to gestational age						
	Upper reference value of NRBC	90% CI	Number of cases above reference value	Mean (SD) of NRBC	Median of NRBC	25th–75th centiles of NRBC
Group 1 (n = 120)	22583	14080 to 31709	4	5643.3 (7228.2)	2601.5	1147–7790
Group 2 (n = 128)	11420	8348 to 14002	6	3327.9 (3576.7)	1901	492–5970
Group 3 (n = 215)	3748	3200 to 4182	9	1099.2 (1274.9)	696	0–1672
Group 4 (n = 232)	2329	1806 to 2580	12	441.6 (807.3)	0	0–638

Figure 1 Upper reference values of nucleated red blood cell (NRBC) count at birth in relation to gestational age.

NRBC count at birth in relation to gestational age by two weekly intervals. As expected, absolute NRBC count decreased with length of gestation and increasing birth weight ($p = -0.559$, $p < 0.0001$ and $p = -0.546$, $p < 0.0001$ respectively).

DISCUSSION

Most of the articles on NRBC count at birth report NRBCs relative to 100 white blood cells. Unfortunately, the variability of leucocyte count after birth results in a wide range of values for NRBCs when expressed in this way. The problem is magnified by the many pathological processes that significantly alter total leucocyte count and lead to a misleadingly low value of NRBCs per 100 white blood cells. The aim of this study was to obtain absolute NRBC reference values based on evaluation of a large number of neonates. Cord blood at birth was used to ensure that the values reasonably represented the effects of the intrauterine environment. As expected, absolute NRBC count decreased with advancing gestation and increasing birth weight. In a previous study, we reported a normal NRBC range for preterm babies.² In this study each neonate was matched with babies of the same birth weight and gestational age, randomly selected from the group of survivors, to exclude any effects of gestational age and birth weight on NRBC count. We understood that to define a reference range for term and preterm subjects it was necessary to study not only subjects who are free of any known disease but also those who may have other abnormalities that could affect laboratory data. We followed these precepts. By excluding babies with pathologies recognised to influence NRBC count at birth, we have defined a clinically useful set of reference ranges for the interpretation of blood counts in the neonatal period. The large number of cases observed in this paper guarantees the accuracy and reliability of the reference as

confirmed by the range of the 90% confidence interval and the small number of cases of our reference population not included in the reference interval. It is important to record and report normal and pathological findings in newborn infants, especially when an abnormally high NRBC count is found in the cord smear and conversely when this count is not raised, which may be just as important. Interesting cases include apparently normal babies with an unusually high NRBC count. In these cases, a normal reference value is useful for both medical and legal purposes. Indeed, a high NRBC count in cord blood strongly suggests an intrauterine hypoxic event several hours before birth. This knowledge is extremely relevant not only as an index of perinatal brain damage for medical use, but also as an indication of the timing of the hypoxic event for legal controversies.

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