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Reference values for nucleated red blood cells and serum lactate in very and extremely low birth weight infants in the first week of life

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ABSTRACT

Study purpose: To provide reference values for nucleated red blood cells and serum lactate concentrations in very and extremely low birth weight (VLBW/ELBW) infants in the first week of life.
Patients and methods: Retrospective data analysis of serial, daily measurements of NRBC counts and serum lactate during the first 6 days of life in VLBW and ELBW infants.
Results: In total, 250 infants < 1500 g were included in this study. Intrauterine growth retardation (IUGR) was seen in 87 (34.8%) patients. Gestational age (GA) ranged from 23 to 35 weeks (mean 29.0 weeks) and birth weight (BW) was 320– 1499 g (mean 1047.9 g). During hospital stay, 55 (22%) patients developed IVH and 55 children (22%) BPD. PVL was seen in 12 (4.8%) cases, ROP in 93 (37.2%) and NEC in only 1 (0.4%) patient.
NRBC counts as well as serum lactate concentrations depended significantly on birth weight ($p < 0.01$) and presence respectively absence of IUGR ($p < 0.01$). Both NRBC counts and serum lactate concentrations declined constantly during the 6-day period ($p < 0.01$), and both were highly inter-correlated ($p < 0.01$).
Conclusions: This is one of only a very few studies that systematically and serially evaluated both NRBC counts and serum lactate concentration in VLBW and ELBW infants in the first 6 days of life. Both parameters were significantly dependent on birth weight and the presence of IUGR. Moreover, a significant correlation between NRBC counts and serum lactate concentrations in this early period of life could be demonstrated. In future studies, the role of these readily available biomarkers in predicting important neonatal outcome parameters needs to be evaluated in a prospective manner.

1. Introduction

Nucleated red blood cells (NRBC) are progenitor cells of red blood cells physiologically seen in the bone marrow. Normally, in healthy infants, adolescents and adults NRBCs are rarely present in the peripheral blood because the nucleus is ejected in the process of cellular differentiation prior to its release into the bloodstream [1]. In contrast, NRBCs are physiological in the peripheral blood of the fetus and newborn in the early postnatal period – most importantly in premature infants and in neonates with fetal distress and intrauterine growth restriction (IUGR). The number of NRBC correlates with gestational age [2,3].

The appearance of NRBC in the peripheral blood is also an indicator of hypoxia in utero [2–7]. Hypoxia induces an elevated erythropoiesis because of an increased erythropoietin secretion [8]. As a consequence, situations with intrauterine hypoxia like placental insufficiency, pregnancy-induced hypertension, gestational diabetes, rhesus immunization, chorioamnionitis or maternal nicotine consumption result in elevated NRBCs in the peripheral blood.

Moreover several studies proved the association between elevated NRBCs in the peripheral blood and important parameters of neonatal

Abbreviations: BPD, bronchopulmonary dysplasia; BW, birth weight; ECMO, extracorporeal membrane oxygenation; EFW, estimated fetal weight; ELBW, extremely low birth weight; GA, gestational age; HIE, hypoxic ischemic encephalopathy; IUGR, intrauterine growth restriction; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; PVL, periventricular leukomalacia; NICU, Neonatal Intensive Care Unit; NRBC, nucleated red blood cell; ROP, retinopathy of prematurity; VLBW, very low birth weight.

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outcome (i.e. intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), cerebral palsy, periventricular leukomalacia (PVL), retinopathy of prematurity (ROP) and mortality) [1,3,4,9–13].

Another important biomarker for poor neonatal outcome constitutes serum lactate in the newborn infant. Lactate is produced in every organ tissue but its main site of production is the liver and it accumulates under anaerobic conditions. Reference values for newborns are scarce [14,15]. Several studies found associations between elevated serum lactate levels and poor neurologic outcome, poor outcome after surgery for congenital heart defects or after extracorporeal membrane oxygenation (ECMO), NEC and asphyxia [16–24]. However and in contrast to NRBC counts, serum lactate concentrations appear not to be associated with PVL [25].

The aim of our study was to define reference values for NRBC counts and serum lactate concentrations during the first week of life in very low birth weight (VLBW) and extremely low birth weight (ELBW) infants and to assess a possible correlation between NRBC counts and serum lactate concentrations.

2. Patients and methods

This retrospective study was performed at the Neonatal Intensive Care Unit (NICU) at Saarland University Hospital, Homburg/Saar, Germany, after institutional approval by our local ethics committee. Our NICU has an annual admission rate of VLBW infants of about 55–60.

Between January 1st, 2009 and December 31st, 2012 we included 250 VLBW and ELBW infants into this study. For all patients, we screened the electronical and hand- written patients' medical charts for selected data including all relevant parameters with regard to ante- and postnatal variables. Specifically, the following items were collected: administration of antenatal steroids, gender, multiparity, gestational age, birth weight (BW), IUGR, Apgar score at 1 min, 5 min and 10 min, and NRBC as well as serum lactate values during the first 6 days of life. Furthermore relevant neonatal complications of prematurity (IVH, BPD, PVL, NEC and ROP) were retrieved as well as patient survival rate.

IUGR was defined as an estimated fetal weight (EFW), abdominal circumference or birth weight < 10th percentile with oligohydramnios or with abnormal umbilical arterial Doppler (pulsatility index >95th percentile, absent or revered end-diastolic flow) [26] using percentile values for the anthropometric dimensions of neonates in Germany [27].

Table 1
Patients' characteristics according different birth weight categories.

	BW < 749 g	BW 750–999 g	BW 1000–1249 g	BW 1250–1499 g
Gender	21 male (40.4%) 31 female (59.6%)	35 male (60.3%) 23 female (39.7%)	31 male (45.6%) 37 female (54.4%)	36 male (50%) 36 female (50%)
Multiparity	6 twins (11.5%) 3 triplets (5.8%)	12 twins (20.7%) 4 triplets (6.9%)	21 twins (30.9%) 3 triplets (4.4%)	25 twins (30.9%) 7 triplets (4.4%)
GA [days]	164–229 (mean 183.3)	169–225 (mean 194.6)	183–242 (mean 209.0)	199–245 (mean 219.2)
BW [g]	320–740 (mean 603.5)	750–990 (mean 886.3)	1000–1245 (mean 1151.1)	1250–1499 (mean 1401.5)
IUGR	30 (57.7%)	18 (31.0%)	21 (30.9%)	18 (25.0%)
Apgar 10	4–9 (median 8)	5–10 (median 9)	6–10 (median 9)	7–10 (median 9)
IVH	17 (32.7%)	19 (32.8%)	11 (16.2%)	8 (11.1%)
BPD	22 (42.3%)	24 (41.4%)	6 (8.8%)	3 (4.2%)
PVL	1 (1.9%)	4 (6.9%)	4 (5.9%)	3 (4.2%)
ROP	31 (59.6%)	29 (50.0%)	22 (32.4%)	11 (15.3%)
NEC	0	1 (1.7%)	0	0
Antenatal steroids	50 (96.2%)	48 (84.2%)	52 (76.5%)	51 (71.8%)
Mortality	17 (32.7%)	6 (10.5%)	0	2 (2.8%)

Table 2
Patients characteristics – IUGR vs. non-IUGR.

	IUGR	Non-IUGR	*p*-Value
Gender	38 male (43.7%) 49 female (56.3%)	85 male (52.5%) 77 female (47.5%)	0.19
Multiparity	15 twins (17.2%) 9 triplets (10.3%)	49 twins (30.2%) 8 triplets (4.9%)	0.4
GA [days]	169–245 (mean 209.5)	164–230 (mean 200.0)	0.000
BW [g]	320–1490 (mean 945.0)	540–1499 (mean 1013.7)	0.000
Apgar 10	5–10 (median 9)	4–10 (median 9)	0.68
IVH	10 (11.5%)	45 (27.8%)	0.003
BPD	17 (19.5%)	38 (23.5%)	0.46
PVL	1 (1.1%)	11 (6.8%)	0.47
ROP	34 (39.1%)	58 (35.8%)	0.81
NEC	1 (1.1%)	0	0.17
Antenatal steroids	68 (78.1%)	133 (82.1%)	0.01
Mortality	7 (8.0%)	18 (11.1%)	0.45

During their stay, all patients were managed based on standard protocols and guidelines developed by our NICU as described previously [28].

Blood samples were collected using micro tubes containing 1.6 mg EDTA/ml blood (Micro tube 1.3 ml K3E, Sarstedt Aktiengesellschaft & Co., Nümbrecht, Germany). Analyzation of the blood samples was performed using Sysmex XE-5000 (Sysmex Corporation, Japan). Serum lactate concentrations were determined by blood gas analysis using ABL 800, Flex blood gas analyzer, Radiometer, Copenhagen, Denmark.

Statistics: Statistical analysis was performed by entering the data into an electronic database using IBM SPSS Statistics (IBM® Corp., Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp., USA). Data are presented as mean, median, 25th and 75th percentile and range. Comparison of variables was performed using *Mann-Whitney-U Test for two independent samples* and correlation between the variables by using *Spearman-ranksum-correlation coefficient*. *p*-values are two-sided and subject to a local significance level of 0.05. Because of only comparing patient characteristics between the IUGR and non-IUGR groups, *p*-values were not adjusted for the issue of multiple testing. All patients were pseudonymized to guarantee data privacy.

3. Results

In total, 250 patients were included whereof 123 (49.2%) were male and 127 (50.8%) female. Out of this cohort 64 (25.6%) were twins and 17 (6.8%) triplets. Intrauterine growth retardation (IUGR) was seen in 87 (34.8%) patients. Gestational age (GA) ranged from 23 weeks to 35 weeks (mean 29.0 weeks) and birth weight (BW) was 320–1499 g (mean 1047.9 g). Apgar scores at 10 min were categorized into 3

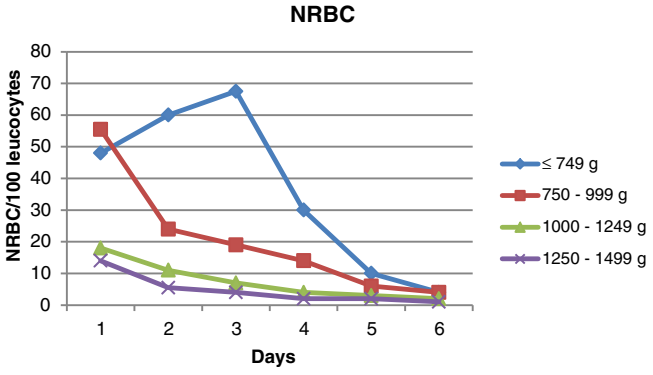

Fig. 1. Number of NRBC/100 leucocytes (median) for group 1 (≤749 g), group 2 (750–999 g), group 3 (1000–1249 g) and group 4 (1250–1499 g) over the first 6 days of life.

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