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Nucleated red blood cells as marker for an increased risk of unfavorable outcome and mortality in very low birth weight infants

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ABSTRACT

Background: Nucleated red blood cells (NRBC) are normoblastic cells that failed to extrude their nuclei before exiting from bone marrow or liver. While NRBC are frequently found in umbilical cord blood after fetal distress, NRBC counts drop rapidly after birth.
Aims: To determine the predictive value of the NRBC count during the first 120 h after birth as marker for later risk of unfavorable outcome in very preterm infants.
Study design: This cohort study investigated the association between absolute count of NRBC on admission (day 1), the mean NRBC count between day 2 to 5, and outcome (mortality or the composite outcome of mortality and severe morbidity).
Results: 438 infants with a gestational age < 32 weeks and a birth weight < 1500 g were included within a five-year period, of whom 46 patients died and 65 suffered from severe morbidity. Nonsurvivors had significantly higher NRBC counts between day 2 and 5, as compared to survivors. This finding was observed in infants both appropriate and small for gestational age. An increase of 10/nL of the mean NRBC count on postnatal day 2 to 5 had an odds ratio for mortality of 6.95 (95% CI 2.21–21.86) and an odds ratio for the composite outcome mortality and severe morbidity of 3.43 (95% CI 1.43–8.24). The optimal cut-off value for prediction of death was NRBC >2/nL with a sensitivity of 85% and a specificity of 75%.
Conclusions: Elevation of NRBC on postnatal day 2 to 5 is an independent predictor of mortality in preterm infants.

1. Introduction

Nucleated red blood cells (NRBC) are rarely present in the circulating blood of healthy children, adolescents and adults; because the nucleus of a developing normoblast is usually extruded before the cell exits the bone marrow. This process is so efficient that the finding of NRBCs in the circulation raises the suspicion of hemopathology. However, NRBC are often found in preterm and term neonates with fetal distress and intrauterine growth restriction [1–3]. Thus, hypoxia is supposed to be one major factor increasing the number of circulating NRBC due to either stimulated erythropoiesis [4–6] or release from storage pools [2]. Several studies have demonstrated an association between elevated NRBC and poor perinatal outcome, including necrotizing enterocolitis [7], severe intraventricular hemorrhage (IVH) [7–9], cerebral palsy (CP), periventricular leukomalacia (PVL) [10,11], or death [7]. There are conflicting results regarding the association of the NRBC count at birth and the incidence or retinopathy of prematurity (ROP). Lubetzky *et al.* and Christensen *et al.* described an association between high NRBC counts and the incidence of ROP [12,13], whereas Gotru *et al.* could not confirm this association [14].

In this study, we analyzed the NRBC counts at day 1 and during the period from day 2 to 5 after birth and evaluated the association with later mortality and severe morbidity in a large cohort of very low birth weight infants (VLBW).

2. Methods

This study included 438 VLBW infants with a gestational age below 32 weeks who were born within in a five-year period (06/2006–12/2010) and admitted to our tertiary levels NICUs. The exclusion criteria were the lack of blood samples within the first 24 h or between days 2 and 5, or death within the first 24 h. Epidemiologic data, morbidities, and outcome parameters were extracted from electronic records. Surviving was defined as living to be discharged or to be transferred to another hospital. Adverse outcome was defined if one of the following conditions was diagnosed: severe intraventricular hemorrhage

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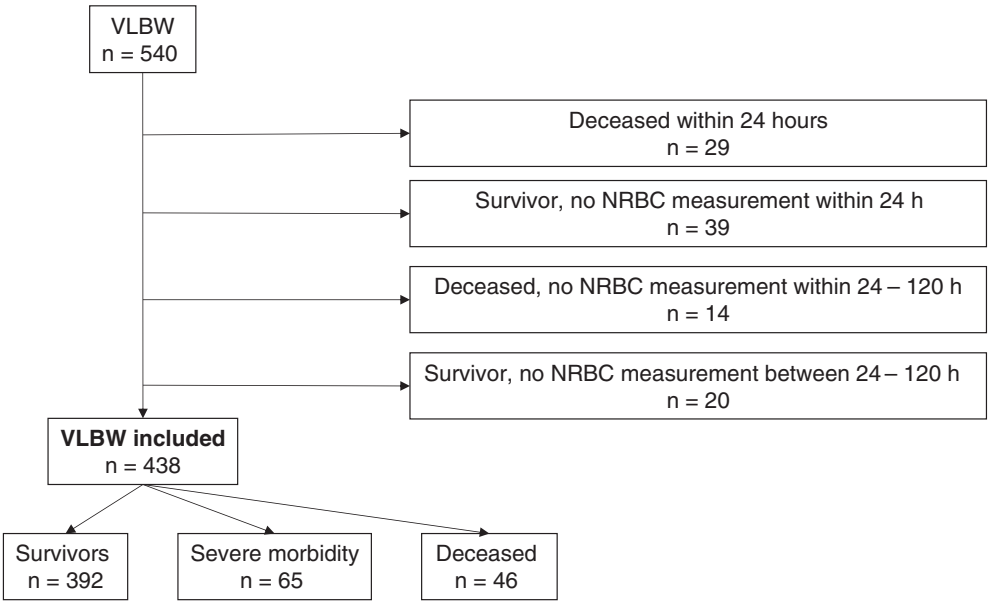

Fig. 1. Study design. The flowchart illustrates the numbers of VLBW infants who were included in this retrospective cohort study.

(grade 3 and 4 bleeding diagnosed by cranial ultrasound or MRI up to day 28 and defined according to the classification of Papile *et al.*), late onset clinical sepsis (according the definition of the center of disease control), intervention for ROP (laser treatment), surgical ligation of a persistent ductus arteriosus (PDA), bronchopulmonary dysplasia (BPD; oxygen dependency at 36 weeks' post-menstrual age), cystic periventricular leukomalacia (PVL)[15], or any urgent laparotomy. Causes of death were determined based on clinical judgment.

All blood count parameters were measured using fluorescence flowcytometry performed with a Sysmex XE-2100 blood analyzer (Sysmex, Kobe, Japan). The NRBC counts were measured after incubation with a combination of a lysing reagent and a nucleic acid staining solution (Stromatolyser-NR, Sysmex), lysing the membranes of red blood cells and leaving only the nuclei of any red blood cell. The blood analyzer detects the absolute NRBC count based on differences in fluorescence intensity and cell volume. For the quantification of the absolute reticulocyte count, the specimen was incubated with polymethine and oxazine dye (Ret Search II, Sysmex) and measured in the reticulocyte channel. In case of multiple blood samples within the first time period (from admittance to the NICU up to 24 h after birth) or the second time period (24–120 h after birth), the mean count of NRBC was calculated and considered for statistical analysis. Gestational age was calculated by obstetrical measures based on the last menstrual period and prenatal ultrasound examination. Being small for gestational age (SGA) was defined by a birth weight below the 3rd percentile for gestational age according to data published by Fenton *et al.* [16]. The study was approved by the local center for clinical studies (Koordinierungszentrum für klinische Studien—KKS, Charité).

Perinatal parameters, morbidities, and blood cell parameters were compared between surviving and deceased VLBW infants by descriptive statistics. To assess the relation between the NRBC count and mortality, multivariable logistic regression was used (yielding adjusted odds ratio (OR) with 95% confidence interval (CI)). Variables for the multivariable model were selected based on univariable findings on their association with mortality and with NRBC counts, as well as published data associated with mortality in VLBW infants, with the aim of a parsimonious model adjusting for potentially confounding factors. To avoid overfitting we additionally applied a reduced logistic regression model excluding the variables: umbilical pH, multiple birth, sex, antenatal steroids and SGA. The association between the NRBC count and mortality was considered to be significant if the two-sided p-value was <0.05. All other p-values were considered exploratory. To assess associations among perinatal parameters or morbidities and NRBC, the NRBC counts of the first and second time periods were compared with epidemiologic parameters using Mann–Whitney-*U*-test for categorical factors and Spearman correlation coefficients for continuous factors. NRBC counts among surviving vs. deceased infants were separately analyzed for being AGA and SGA. ROC curves were used to compare the predictive value for mortality of the NRBC count (24–120 h) with birth weight. The corresponding area under the curve (AUC) values (95% CI) yielded optimal cut-off values.

3. Results

540 VLBW infants were considered for eligibility. Among them, 102 patients were excluded because of lack of blood examination or death within 24 h after birth (Fig. 1). Thus, the study population consisted of

Table 1
Comparison of perinatal parameters and morbidities between surviving and deceased VLBW infants. Data are given as median (range) or counts (%).

Clinical characteristics	Survivors(n = 392)	Deceased(n = 46)	p-Value*
Birth weight [g]	1015 (382–1495)	598 (300–1470)	<0.001
Gestational age [weeks + days]	28 + 1 (23 + 0–31 + 6)	25 + 2 (23 + 2–31 + 6)	<0.001
Small for gestational age	24 (6.0 %)	16 (39.0 %)	<0.001
Female sex	200 (50.4 %)	18 (43.9 %)	0.430
Antenatal steroids	276/370 (74.6 %)	26/36 (72.2 %)	0.756
Multiple birth	185 (46.6 %)	9 (22.0 %)	0.002
Vaginal delivery	56 (14.1 %)	7 (17.1 %)	0.673
Umbilical pH	7.29 (6.78–7.47)	7.26 (6.99–7.42)	0.014
Apgar 5 min	8 (1–10)	7 (1–9)	<0.001
Days to discharge/transfer	61 (10–172)	–	–
Death at postnatal day	–	13 (2–159)	–
Severe IVH	14 (3.5 %)	11 (26.8 %)	<0.001
Any laparotomy	9 (2.3 %)	8 (19.5 %)	<0.001
Late onset sepsis	35 (8.8%)	4 (9.8 %)	0.089
ROP intervention	27 (6.8 %)	13 (31.7 %)	<0.001
PDA ligation	55 (13.9 %)	6 (14.6 %)	0.891
BPD	26 (6.7 %)	2 (4.9 %)	0.630
PVL	1 (<1 %)	0	1.0
Other surgery	79 (19.9 %)	10 (24.4 %)	0.496