

# Nucleated red blood cell counts in the first week of life: a critical appraisal of relationships with perinatal outcome in preterm growth-restricted neonates

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**OBJECTIVE:** Nucleated red blood cells (NRBC) are fetal hematologic markers for placental dysfunction, hypoxemia, and asphyxia. NRBC count elevation at birth or persistence is linked statistically to adverse outcome, but clinical predictive value is variable. We studied novel indices to better define overall magnitude of NRBC response.

**STUDY DESIGN:** Peripheral NRBC count was obtained from preterm (<34 weeks of gestation) growth-restricted neonates within 2 hours of life. Daily counts and duration of NRBC count >30/100 white blood cells were determined. Mean counts (NRBC-mean), area under the curve (NRBC-AUC), and declination (NRBC-slope) were analyzed over week 1. NRBC parameters were related to major morbidity (bronchopulmonary dysplasia, grade III/IV intraventricular hemorrhage, necrotizing enterocolitis included) and neonatal death (NND).

**RESULTS:** Twenty-two of 176 patients (12.5%) had acidosis. Complications included bronchopulmonary dysplasia ( $n = 36$ ; 20.5%), intraventricular hemorrhage ( $n = 18$ ; 10.2%), necrotizing enterocolitis ( $n = 18$ ; 10.2%), NND ( $n = 18$ ; 10.2%). NRBC-AUC and NRBC-mean

correlated most strongly with pH, birthweight, and gestational age (Pearson coefficient,  $r = -0.45$  to  $-0.18$ ; all  $P < .001$ ). NRBC-AUC varied most between nonmorbid and morbid; NRBC-mean varied most between survivors and NND (all  $P < .001$ ). NRBC persistence strongly predicted NND: clearance by day 4 was achieved by 80% of survivors and only 35% of NNDs. Logistic regression identified prematurity and persistent NRBC counts as primary morbidity determinants ( $r^2 = 0.56$ ;  $P < .01$ ). Although the importance of individual NRBC counts varied, day-4 NRBC counts of >70 predicted morbidity best (sensitivity, 82%; specificity, 96%). Presence of morbidity and birthweight were prime determinants of death ( $r^2 = 0.42$ ;  $P < .01$ ).

**CONCLUSION:** Simple daily NRBC counts provide clinical information that is equivalent to more complicated methods. The importance of prematurity and growth are emphasized, but elevated NRBC counts beyond day 3 are relevant independent predictors of adverse outcome.

**Key words:** fetus, growth restriction, nucleated red blood cells, perinatal outcome

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The identification of prognostic markers for adverse perinatal outcome is a major focus of modern fetal and neonatal medicine. Among the markers that have been studied, those that reflect compromised metabolic status during the transition from fetal to neonatal life have received special attention. Obstetric intervention and neonatal resuscitation in this important period

are especially critical in the management of fetal growth restriction. The perinatal and long-term liabilities of fetal growth restriction are due to the combination of adverse intrauterine environment, peripartum events, and postdelivery complications that are manifested in many organ systems.<sup>1-4</sup> In this context, fetal hematologic responses have received great interest because they reflect

the chronic nature of the fetal condition and the acutely superimposed disturbances in the transition to extrauterine life.<sup>5-10</sup>

Fetal hypoxemia can trigger erythropoietin release that causes stimulation of red blood cells, both at intramedullary and extramedullary sites. In the human fetus, erythropoiesis typically progresses from erythroid commitment of colony-forming stem cells to extrusion of nuclear material with concomitant reduction in cell size.<sup>11,12</sup> This process yields a mature red blood cell without a nucleus that contains the highest concentration of hemoglobin. Early stages of mature erythropoiesis typically are confined to the bone marrow, where capillary fenestrations limit the passage of large nucleated red blood cell (NRBC) precursors into the peripheral circulation.<sup>13</sup> Conversely, extramedullary sites are believed to have larger capillary fenestrations that

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**TABLE 1**  
**Calculation of various NRBC parameters**

NRBC parameter	Calculation
Peak NRBC count	First NRBC count/100 WBC at birth
NRBC count persistence	Persistence of NRBC count elevation of >30 NRBC/100 WBC in days
Percentage NRBC count elevation	No. of days with NRBC count of >30 NRBC/100 WBC/days alive during week 1
Mean NRBC count	Sum of daily NRBC counts/7
NRBC AUC	Sum (NRBC day 1-7 × day 1-7)/7
NRBC slope of decline	(First NRBC count <30/100 WBC – NRBC count at birth)/days of NRBC count of >30/100 WBC

permit the release of large NRBCs. During periods of high extramedullary production, NRBC counts of up to 30/100 white blood cells (WBC) are physiologic at <30 weeks of gestation, although levels of 5-10/100 WBC are normal thereafter.<sup>12</sup> Isolated polycythemia in intrauterine growth-restricted fetuses suggests enhanced intramedullary erythropoiesis; polycythemia with elevated NRBC counts is suggestive of chronic extramedullary hematopoiesis.<sup>8,10-13</sup>

The NRBC count in the peripheral circulation of growth-restricted neonates has received special interest as a surrogate marker for the severity and chronicity of fetal acid-base disturbance and a prognostic factor for adverse outcome.<sup>5,6</sup> Peak NRBC count at delivery and persistence of NRBC count elevation after delivery have been studied, but prognostic value is limited because of their wide variability and the overriding effect of gestational age.<sup>6</sup> Another limitation of such an approach is that it emphasizes 2 isolated time points without accounting for the pattern of NRBC decline. The impacts of disease in the intensive care setting on NRBC counts and the associated prognostic value have been documented in critically ill adults.<sup>27</sup> In these patients, worsening of the clinical condition is associated with increasing NRBC counts. Similarly, growth-restricted neonates may react to adverse events in the neonatal intensive care unit with their large extramedullary potential for NRBC release. Therefore, neonatal NRBC counts may show a rapid drop, sustained elevation, or a delayed decline

on the basis of the clinical condition. Evaluating the NRBC response by the initial count at birth and the persistence in days therefore may be inadequate to define this response accurately. This study was designed to quantify the NRBC response with more comprehensive parameters. It was our hypothesis that these parameters describe the NRBC response more accurately and provide an accurate prediction of outcome in preterm growth-restricted neonates.

## PATIENTS AND METHODS

We performed a prospective observational study at 2 tertiary referral centers for fetal medicine from 2000-2005. Patients carrying a fetus with a sonographically measured abdominal circumference of <5th percentile for gestational age and an elevated umbilical artery Doppler index as evidence of placental dysfunction were asked to participate. The study was approved by the institutional review boards, and all patients gave informed written consent at enrollment. Only patients with accurate gestational dating were enrolled. The final analysis was restricted to neonates delivered at <34.0 weeks of gestation. Exclusion criteria were maternal diabetes mellitus, fetal anomalies, abnormal karyotype, chorioamnionitis, and twin gestation.

Birthweight, gestational age at delivery, Apgar scores at 1 and 5 minutes, and arterial cord blood gas were recorded. A peripheral blood sample from the neonate was obtained in an EDTA-tube

within 2 hours of delivery. A manual count of NRBCs per 100 WBC after Pappenheim staining of the blood smear was performed in the hospital laboratory; this constituted the delivery NRBC count. Peripheral blood samples were analyzed daily until day 7 of life.

Several NRBC parameters were ascertained for the final analysis. These included the first NRBC count ( $y_1$ ) on the day of delivery ( $x_1$ ), persistence of NRBC count elevation, the percentage of days with NRBC count elevation corrected for days alive, the mean NRBC count in the first week of life, the area under the curve (AUC) for the pattern of NRBC decline and the calculated slope of the NRBC count decline. *Persistence* was defined as the number of days the NRBC count was >30/100 WBC, with  $y_2$  being the first value below 30 and  $x_2$  being the day of life that this occurred. For neonates with several daily blood draws, mean NRBC count for each individual day was used in the analysis. In addition to the persistence in days, the percentage of days with elevated NRBC count was calculated for the first week. This was done to account for early neonatal deaths. For example, if a neonate had elevated NRBC counts for 2 days but died on the second day of life, then the persistence would be only 2 days. However, the NRBC count was elevated 100% of the days that the neonate was alive. The AUC was calculated with the trapezoid formula as the average of the daily product of NRBC count times the day of life. The slope was calculated as  $= y_2 - y_1/x_2 - x_1$ . For neonates who died within the first week of life, the number of days alive was taken as the denominator. The parameters used in the calculation are summarized in Table 1.

On completion of the neonatal course development of bronchopulmonary dysplasia (BPD), severe intraventricular hemorrhage (>grade 2, according to Papile et al<sup>15</sup>), and necrotizing enterocolitis were noted. The diagnosis of BPD was based on radiologic criteria. Intraventricular hemorrhage was diagnosed by cranial ultrasound scan that was performed routinely on postpartum days 2 and 7, or more often if clinically indicated. Presences of any of the neonatal

complications and/or neonatal death were grouped as the composite variable "poor outcome."

We restricted our study population to very preterm growth-restricted neonates for several reasons. This population is at highest risk for adverse outcomes and is treated in the neonatal intensive care setting. Daily blood counts typically are performed as part of neonatal monitoring and do not constitute a significant deviation from the standard of care. Conversely, term- or near-term growth-restricted neonates are at relatively low-risk for major morbidities and typically are treated in the nursery. Examination of this subset of patients was not at the core of our research question, and the institution of daily blood draws that would have been necessitated by study inclusion appeared unjustified.

The results were analyzed with SPSS software (version 10.0; SPSS Inc, Chicago, IL). Distribution of gestational age, birthweight, cord artery blood gases, and NRBC parameters were related to individual complications and neonatal death with the use of the Mann-Whitney *U* test after the results failed tests of normality. Proportional distribution of Apgar score <7 at 5 minutes and cord artery pH <7.20 were also related to these outcome variables with the use of the chi-square test. Parameters significantly different among neonates with individual adverse outcomes were selected for further analysis by logistic regression. In this regression analysis, the individual outcomes were selected as dependent variables. A probability value of <.05 was considered statistically significant.

## RESULTS

A total of 186 patients agreed to participate in the study. Following 10 stillbirths, 176 neonates remained for final analysis. Placental dysfunction was documented in all cases by abnormal umbilical artery Doppler findings in the form of Doppler index elevation with preserved end-diastolic velocity ( $n = 98$ ; 55.7%), absent ( $n = 27$ ; 15.3%), or reversed end-diastolic velocity ( $n = 51$ ; 29%). One hundred sixty-four (93.2%) fetuses received a complete course of be-

tamethasone before delivery. In the remaining patients, the timing of the delivery decision allowed for only the administration of a single course of steroids.

In this predominantly white population, delivery at a median of 29.6 weeks of gestation was mainly by cesarean section for fetal indications (Table 2). Umbilical cord artery pH was <7.20 in 12.5%, and difficult resuscitation with a low 5-minute Apgar score occurred in 10.8% (Table 2). BPD was the most frequently observed complication (36; 20.5%). Major morbidities were observed in 50 neonates (28.4%). Although the neonatal mortality rate was 10.2% overall, it rose to 26% (13/50) in the presence of major neonatal morbidities (chi-square,  $P < .001$ ). Five of the neonates who died within the first week of life did not have any of the aforementioned complications but experienced severe cardiorespiratory instability that required high-frequency ventilation and pressor support.

A wide range of distribution in NRBC counts was observed from the day of delivery through the end of the first week of life. Although a decline in NRBC counts after delivery was observed generally, some neonates had a rise reflected in a positive slope (Table 3). All NRBC parameters correlated most strongly with the umbilical artery, pulsatility index, umbilical artery base excess, birthweight, and gestational age at delivery (Table 4). More marked elevations of the Doppler index were associated with higher NRBC parameters; earlier gestational ages, smaller birthweights, and more decline in the base excess were related to an increase in NRBC parameters. These relationships remained significant throughout the first week of life. Overall correlations were strongest for the AUC and mean NRBC count elevation in the first week of life (Table 4). Similarly, an abnormal biophysical profile score, delivery for nonreassuring fetal status, a cord artery pH <7.20, and a 5-minute Apgar score of <7 were all associated with significantly higher daily NRBC counts, longer persistence of NRBC count elevation, and higher calculated

NRBC parameters (Mann-Whitney *U*, all  $P < .005$ ).

All NRBC parameters were elevated significantly in neonates who experienced BPD and severe intraventricular hemorrhage, who died, and who had composite poor outcome. (Table 5). Necrotizing enterocolitis was the only complication that was not associated consistently with in elevation of NRBC parameters. Differences in the magnitude of NRBC response in neonates with postpartum complications were best illustrated by the AUC (Figure 1). Mean NRBC count was the variable that best separated neonatal survivors from those who died (Table 5; Figure 2). Although >80% of survivors cleared their NRBC count elevation by the fourth day of life, 65% of neonates who died had a persistent elevation beyond this point.

Next, the contribution of NRBC parameters to adverse outcome was analyzed by logistic regression analysis. Despite significantly altered distributions of NRBC parameters with individual complications, none of these were selected by the regression model as independent predictors of adverse outcomes. Overall, gestational age at delivery was the primary determinant for the development of BPD ( $P = .005$ ;  $r^2 = 0.54$ ) and intraventricular hemorrhage ( $P = .014$ ;  $r^2 = 0.21$ ). Birthweight was identified as the primary determinant for necrotizing enterocolitis ( $P = .05$ ;  $r^2 = 0.17$ ). Gestational age and birthweight were significant independent predictors of neonatal death ( $P < .005$ ;  $r^2 = 0.53$ ). Receiver operator curve statistics identified gestational age of 28 weeks and birth weight of 600 g as the best combination of sensitivity and specificity for the prediction of death. Accordingly, a subanalysis was performed to identify the contribution of NRBC parameters to the mortality risk after these cutoffs. This subanalysis showed that, in neonates delivered at <28 completed weeks of gestation with a birthweight of <600 g, the gestational age at delivery remained the primary determinant of death, although the slope of NRBC decline was an independent predictor ( $P < .05$ ;  $r^2 = 0.35$ ). After this gestational age, persistence of NRBC count elevation beyond day 3 of life was the

**TABLE 2**  
**Perinatal characteristics**

Characteristic	N (%)	Mean ± SD (range)
Maternal age (y)		27.3 (14-40)
Maternal race		
White	127 (72.2)	
African American	49 (27.8)	
Parity		
0	124 (70.5)	
1	36 (20.5)	
2	13 (7.4)	
3	3 (1.7)	
Gestational age at delivery (wk)		29.6 (24.0-33.6)
Birthweight (g)		918 (360-1520)
Mode of delivery		
Spontaneous vaginal	12 (6.8)	
Cesarean section	164 (93.2)	
Delivery indication		
Nonreassuring fetal heart rate	55 (31.5)	
Nonreassuring Doppler scan	30 (17.0)	
Nonreassuring biophysical profile score of <6	10 (5.7)	
Fetal distress*	6 (3.4)	
Placental abruption	4 (2.3)	
Severe preeclampsia/ hemolysis, elevated liver enzymes, and low platelet count syndrome (HELLP)	46 (26.1)	
Spontaneous onset of labor	5 (2.9)	
Other	20 (11.4)	
Cord artery blood gas (mm Hg)		
pH		7.23 ± 0.05
pO <sub>2</sub>		16.1 ± 5.5
pCO <sub>2</sub>		54.5 ± 7.4
HCO <sub>3</sub>		22.3 ± 2.0
Base excess		-5.1 ± 2.6
pH < 7.20	22 (12.5)	
5-Minute Apgar <7	19 (10.8)	
BPD	36 (20.5)	
Necrotizing enterocolitis	18 (10.2)	
Severe intraventricular hemorrhage	13 (7.4)	
Major morbidity	50 (28.4)	
Postnatal death	18 (10.2)	
Poor outcome	55 (31.3)	

\* Bradycardia of the fetal heart rate or spontaneous repetitive late decelerations.

only NRBC parameter that remained as an independent predictor in addition to gestational age ( $r^2 = 0.26$ ). On day 4 of life, an NRBC count of >70/100 WBC predicted major morbidity, with a sensitivity of 82% and a specificity of 96% (odds ratio, 7.33; 95% CI, 2.4-22.4; chi-square,  $P < .001$ ).

### COMMENT

NRBC counts at birth and persistence of NRBC count elevation have been studied as prognostic indicators for poor neonatal outcome and risk factors for poor neurodevelopment. This interest is based on the assumption that the expansion of hematopoiesis to extramedullary sites is a prerequisite for the appreciable peripheral release of these cells. Because stimulation of extramedullary sites and release of their less mature cells requires a period of time, an increase in peripheral NRBC counts implies chronic acid-base disturbance.<sup>6,13</sup> Such chronic deterioration of fetal acid-base status is considered an important antecedent for poor neurodevelopment.<sup>17-19</sup> Cord artery pH at birth is an acute marker; it does not provide a good estimate of the chronicity of acid-base derangement. On the other hand, fetal/neonatal NRBC responses reflect both chronicity and severity of acid-base disturbance and may hold promise as an independent prognostic marker for short- and long-term outcome. Previous studies have used the NRBC count at birth and the persistence of NRBC count elevation with variable results. Because neonatal events may have additional impacts on the complicated dynamics of the NRBC response, this study used several novel indices to evaluate relationships with short-term outcome in preterm growth-restricted neonates.

We studied a selected population of preterm neonates who had growth restriction because of early onset placental dysfunction. Metabolic acidemia, deterioration of biophysical parameters, elevation in placental blood flow resistance, birthweight, and gestational age at delivery were the primary factors that determined the magnitude of NRBC response in this group of otherwise normal

**TABLE 3**  
**NRBC parameters**

NRBC parameter	Median	Range
NRBC count		
At birth	67	0-2930
Day 1	27	0-2380
Day 2	9	0-1723
Day 3	2	0-1730
Day 4	0	0-851
Day 5	0	0-980
Day 6	0	0-372
Day 7	0	0-87
Days of NRBC persistence > 30/100 WBC (n)	1	0-7
Percentage of days with NRBC persistence (%)	0	0-100%
Mean first week NRBC count	16.4	14-2244
NRBC-AUC	82	1-6167
NRBC slope of decline	-15	-629 to 140

growth-restricted neonates. Although all NRBC parameters showed wide variability their primary correlation with acid-base balance, placental blood flow resistance, birthweight, and gestational age at delivery were maintained over the first

week of life. Composite neonatal morbidity and death were associated with higher absolute NRBC counts and the derived parameters. Necrotizing enterocolitis was the only complication that was not associated consistently with ele-

vated NRBC parameters. Although the magnitude of NRBC response is best depicted mathematically by the AUC, simple daily draws provide identical prognostic information. Although gestational age remains the primary determinant of adverse outcome, an NRBC count elevation of >70/100 WBC on day 4 of life is a clinically useful independent prognostic parameter.

Although multiple previous studies have reported associations between elevated NRBC count and adverse perinatal outcome and neurologic impairment, the magnitude of the neonatal NRBC response has not been studied in much detail. Buonecure et al<sup>18</sup> reported that the NRBC count was significantly higher in infants with abnormal cerebral artery Doppler scans at 48-72 hours after birth, compared with healthy neonates. Similar observations were made for 6-month-old infants with sequelae of hypoxic-ischemic encephalopathy and in 3-year-old children with abnormal developmental status. The authors concluded that the NRBC count at birth reflects not only a response of the infant to

**TABLE 4**  
**Correlations between NRBC and perinatal parameters**

NRBC parameter	UA PI	Gestational age at delivery	Birthweight	pH	pO <sub>2</sub>	pCO <sub>2</sub>	HCO <sub>3</sub>	Base excess
NRBC count								
At birth	0.38*	-0.22*	-0.36*	-0.18*	-0.14	-0.01	-0.41*	-0.39*
Day 1	0.43*	-0.20*	-0.31*	-0.27*	-0.20*	0.06	-0.32*	-0.38*
Day 2	0.46*	-0.26*	-0.26*	-0.25*	-0.12	-0.02	-0.34*	-0.36*
Day 3	0.45*	-0.20*	-0.29*	-0.30*	-0.14	0.03	-0.33*	-0.36*
Day 4	0.48*	-0.23*	-0.27*	-0.25*	-0.09	0.02	-0.28*	-0.30*
Day 5	0.32*	-0.16†	-0.21*	-0.23*	-0.11	0.03	-0.23*	-0.28*
Day 6	0.47*	-0.17†	-0.21*	-0.16†	-0.12	0.02	-0.19†	-0.20†
Day 7	0.39*	-0.10	-0.18†	-0.19†	-0.15	0.07	-0.14	-0.19†
Days of NRBC persistence > 10/100 WBC	0.56*	-0.40*	-0.48*	-0.39*	-0.23*	0.11	-0.33*	-0.43*
Percentage of days with NRBC persistence	0.52*	-0.45*	-0.45*	-0.37*	-0.13	0.04	0.36*	-0.44*
Mean first week NRBC count	0.37*	-0.22*	-0.33*	-0.28*	-0.08	0.01	-0.40*	-0.41*
NRBC-AUC	0.51*	-0.25*	-0.35*	-0.30*	-0.017†	0.03	-0.37*	-0.41*
NRBC slope of decline	-0.27*	0.17†	0.25*	0.05	0.19†	-0.02	0.24*	0.24*

Data are presented with the Pearson correlation coefficients for NRBC and perinatal parameters. UA PI, umbilical artery Doppler index z-score, pH, pCO<sub>2</sub>, pO<sub>2</sub>, HCO<sub>3</sub>, and BE are components of the umbilical artery blood gas at birth.

\* Two-tailed,  $P < .001$ .

† Two-tailed,  $P < .05$ .

**TABLE 5**  
**NRBC parameters and postpartum complications**

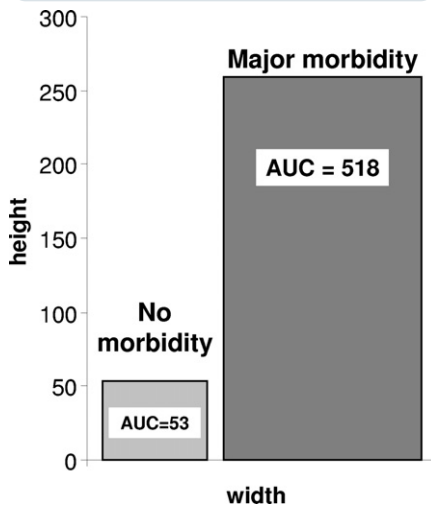
Variable	No BPD	BPD	No intraventricular hemorrhage	Intraventricular hemorrhage	No necrotizing enterocolitis	Necrotizing enterocolitis	No morbidity	Neonatal morbidity	Alive	Neonatal death
NRBC count										
At birth	54 (0-1600)	184 (0-2930)*	66 (0-2930)	269 (0-1660)†	69 (0-1660)	99 (2-2930)	49 (0-1600)	182 (0-2980)*	57 (0-1660)	265 (0-2930)*
Day 1	21 (0-2380)	95 (0-1800)†	24 (0-1400)	247 (0-2380)*	25 (0-2380)	52 (0-1394)	19 (0-1400)	102 (0-2380)*	22 (0-1800)	247 (8-2380)*
Day 2	8 (0-1500)	79 (0-1723)*	9 (0-1723)	135 (0-1452)*	10 (0-1723)	121 (0-1022)	5 (0-1500)	90 (0-1723)*	6 (0-1723)	144 (4-1452)*
Day 3	1 (0-1730)	17 (0-1502)*	2 (0-1502)	16 (0-1730)*	2 (0-1730)	21 (0-701)†	0 (0-1389)	17 (0-1730)*	2 (0-1502)	110 (3-1730)*
Day 4	0 (0-851)	5 (0-779)*	0 (0-850)	4 (0-851)†	0 (0-851)	10 (0-107)*	0 (0-850)	9 (0-851)*	0 (0-850)	51 (0-851)*
Day 5	0 (0-980)	3 (0-628)*	0 (0-648)	4 (0-980)†	0 (0-980)	6 (0-150)*	0 (0-648)	4 (0-980)*	0 (0-648)	10 (0-980)*
Day 6	0 (0-372)	1 (0-300)*	0 (0-372)	2 (0-300)*	0 (0-372)	2 (0-23)*	0 (0-372)	2 (0-300)*	0 (0-372)	7 (0-300)*
Day 7	0 (0-87)	0 (0-69)*	0 (0-87)	2 (0-69)*	0 (0-87)	0 (0-10)	0 (0-87)	0 (0-69)*	0 (0-87)	2 (0-69)*
NRBC persistence	0 (0-7)	2 (0-7)*	0 (0-7)	2 (0-7)†	0 (0-7)	2 (0-5)	0 (0-7)	2 (0-7)*	0 (0-7)	4 (0-7)*
NRBC persistence (%)	0 (0-100)	29 (0-100)*	0 (0-100)	29 (0-100)*	0 (0-100)	29 (0-71)	0 (0-100)	43 (0-100)*	0 (0-100)	100 (0-100)*
Mean first week NRBC count	13 (0-2245)	64 (43-994)*	16 (0-995)	117 (43-2245)*	17 (0-2245)	59 (29-570)	11 (0-896)	107 (1-2245)*	14 (0-995)	211 (8-2245)*
NRBC-AUC	60 (1-6148)	357 (2-6167)*	76 (1-6167)	658 (29-6148)*	63 (1-6167)	331 (1-3586)	50 (1-5683)	595 (1-6167)*	70 (1-6167)	679 (38-6148)*
NRBC slope of decline	-30.5 (-618 to 186)	-71 (-824 to 19)*	-31 (-743 to 102)	-61 (-824 to 186)	-33 (-824 to 186)	-37 (-743 to 0)	-27 (-618 to 103)	-61 (-824 to 186)†	-32 (-824 to 0)	-48.7 (-743 to 186)

Data are presented as median and range (in brackets); all are Mann Whitney *U* tests.

\* *P* < .001, compared with neonates without the respective complication.

† *P* < .05, compared with neonates without the respective complication.

**FIGURE 1**  
NRBC area under the curve and major morbidity

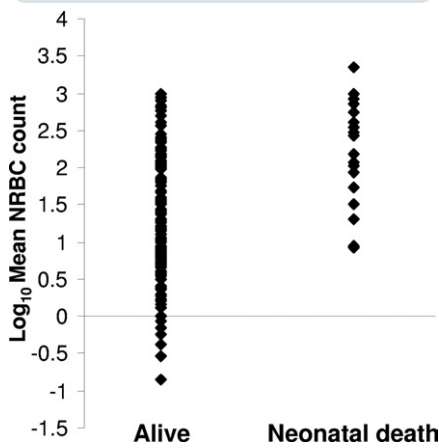


A display of the AUC of the NRBC response in the first week of life in relationship to major morbidity.

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perinatal hypoxia but also is the risk for perinatal brain damage. Growth-restricted neonates have significantly higher NRBC counts than adequately grown counterparts,<sup>18,21,22</sup> but elevated

**FIGURE 2**  
Mean NRBC counts and morbidity



A display of the distribution of mean NRBC counts in survivors and neonates that died in the first 28 days of life.

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NRBC counts and NRBC persistence also correlated with short- and long-term outcomes.<sup>6,7,23,24</sup> The wide variability in these parameters has precluded the identification of a discrete prognostic cutoff. Although this variability could be explained by variations in the severity of the fetal condition, there is evidence to suggest that neonatal factors may modulate NRBC dynamics equally.

Studies on adults in the intensive care unit have shown that development of complications is associated with an increase in NRBC counts in the peripheral circulation.<sup>14</sup> Although individual triggers for NRBC release have not been elucidated fully, these findings suggest that postpartum events may be capable of triggering additional NRBC release. Therefore, the wide variability of NRBC count and persistence in previous neonatal studies is more likely to be due to the combination of fetal and neonatal factors. In this setting, the magnitude of NRBC release will depend on the severity of insult and the extent of extramedullary hematopoietic sites. Because these are already stimulated in growth-restricted fetuses, significant new NRBC release with prolonged persistence of NRBC count elevation follows. This is the first study that evaluates these NRBC dynamics in greater detail. We have shown that the AUC gives a better mathematical description of the NRBC response than delivery count and persistence alone. This technique emphasizes failure to clear their NRBC counts <70/100 WBC as a significant risk for adverse outcome. Our analysis does not allow us to conclude whether this prolonged persistence is due to delayed clearance or new release of NRBCs. If prolonged persistence is due to new release, it remains to be determined whether continued effects of fetal metabolic disturbance or neonatal neonatal complications are the principal triggers.

The strengths of this study are a large population of carefully defined neonates who were closely monitored with multiple antenatal and postnatal parameters. The limitations of our findings arise from the complexity of red blood cell production. The various previous observations on NRBC parameters and short-

and long-term sequelae offer no clear explanation. This study clarifies the best way to depict NRBC characteristics, but further investigations are needed to define the dynamics of NRBC regulation as neonatal life progresses. It may well be that both NRBC peak at delivery (as a marker of chronic fetal impact) and NRBC persistence (as a marker of continued and/or added neonatal impacts) are separate predictors. When NRBCs persist at >70 beyond day 3 of life, new attention may need to be focused on measures that are specific to growth-restricted neonates, and a diagnostic workup for subclinical or clinical deterioration may be initiated. Wide variability and the complexity of the interactions mean that NRBC count cannot be used not only in isolation but also as a part of a consortium of hypoxemic markers to optimize predictive accuracy. Previously identified risk factors for postpartum complications play a predominant role, particularly in preterm growth-restricted neonates. Further studies that will expand to other hypoxemia markers are needed to clarify the associations with perinatal outcome in growth-restricted and appropriately grown neonates.<sup>16,17,19-24</sup> ■

## REFERENCES

1. Barker DJ. Fetal growth and adult disease. *BJOG* 1992;99:275-6.
2. Battaglia FC, Lubchenco LO. A practical classification of newborn infants by weight and gestational age. *J Pediatr* 1967;71:159-63.
3. Bernstein IM, Horbar JD, Badger GJ, Ohlsson A, Golan A. Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction: the Vermont Oxford Network. *Am J Obstet Gynecol* 2000;182:198-206.
4. Baschat AA. Fetal responses to placental insufficiency: an update. *BJOG* 2004;111:1031-41.
5. Blackwell SC, Refuerzo JS, Wolfe HM, et al. The relationship between nucleated red blood cell counts and early-onset neonatal seizures. *Am J Obstet Gynecol* 2000;182:1452-7.
6. Baschat AA, Gembruch U, Reiss I, Gortner L, Harman CR. Neonatal nucleated red blood cell count and postpartum complications in growth restricted fetuses. *J Perinat Med* 2003;31:323-9.
7. Leikin E, Verma U, Klein S, Tejani N. Relationship between neonatal nucleated red blood cell counts and hypoxemic-ischemic injury. *Obstet Gynecol* 1996;87:439-43.

- 8.** Weiner CP, Williamson RA. Evaluation of severe growth retardation using cordocentesis: hematologic and metabolic alterations by etiology. *Obstet Gynecol* 1989;73:225-9.
- 9.** Soothill PW, Nicolaides KH, Campbell S. Prenatal asphyxia, hyperlacticaemia, hypoglycaemia, and erythroblastosis in growth retarded fetuses. *BMJ* 1987;25:1051-3.
- 10.** Thilaganathan B, Athanasiou S, Ozmen S, Creighton S, Watson NR, Nicolaides KH. Umbilical cord blood erythroblast count as an index of intrauterine hypoxia. *Arch Dis Child Fetal Neonatal Ed* 1994;70:F192-4.
- 11.** Maier RF, Gunther A, Vogel M, Dudenhausen JW, Obladen M. Umbilical venous erythropoietin and umbilical arterial pH in relation to morphologic placental abnormalities. *Obstet Gynecol* 1994;84:81-7.
- 12.** Moritz KM, Lim GB, Wintour EM. Developmental regulation of erythropoietin and erythropoiesis. *Am J Physiol* 1997;273:1829-44.
- 13.** Snijders RJ, Abbas A, Melby O, Ireland RM, Nicolaides KH. Fetal plasma erythropoietin concentration in severe growth retardation. *Am J Obstet Gynecol* 1993;168:615-9.
- 14.** Stachon A, Kempf R, Holland-Letz T, Friese J, Becker A, Krieg M. Daily monitoring of nucleated red blood cells in the blood of surgical intensive care patients. *Clin Chim Acta* 2006;366:329-35.
- 15.** Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1500 gm. *J Pediatr* 1978;92:529-32.
- 16.** Hanlon-Lundberg KM, Kirby RS. Nucleated red blood cells as a marker of acidemia in term neonates. *Am J Obstet Gynecol* 1999;181:196-201.
- 17.** Soothill PW, Aiayi RA, Campbell S, et al. Relationship between fetal acidemia at cordocentesis and subsequent neurodevelopment. *Ultrasound Obstet Gynecol* 2000;1:80-3.
- 18.** Naeye RL, Localio AR. Determining the time before birth when ischemia and hypoxemia initiated cerebral palsy. *Obstet Gynecol* 1995;86:713-9.
- 19.** Buonocore G, Perrone S, Gioia D, et al. Nucleated red blood cell count at birth as an index of perinatal brain damage. *Am J Obstet Gynecol* 1999;181:1500-5.
- 20.** Phelan JP, Ahn MO, Korst LM, Martin GI. Nucleated red blood cells: a marker for fetal asphyxia? *Am J Obstet Gynecol* 1995;173:1380-4.
- 21.** Minior VK, Shatzkin E, Divon MY. Nucleated red blood cell count in the differentiation of fetuses with pathologic growth restriction from healthy small-for-gestational-age fetuses. *Am J Obstet Gynecol* 2000;182:1107-9.
- 22.** Philip AG, Tito AM. Increased nucleated red blood cell counts in small for gestational age infants with very low birthweight. *Am J Dis Childhood* 1989;143:164-9.
- 23.** Minior VK, Bernstein PS, Divon MY. Nucleated red blood cells in growth-restricted fetuses: associations with short-term neonatal outcome. *Fetal Diagn Ther* 2000;15:165-9.
- 24.** Green DW, Hendon B, Mimouni FB. Nucleated erythrocytes and intraventricular hemorrhage in preterm neonates. *Pediatrics* 1995;96:475-8.