Neonatal Absolute Nucleated Red Blood Cell Counts do not Predict the Development of Cystic Periventricular Leukomalacia

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ABSTRACT: **Background:** Infants with severe intraventricular-periventricular hemorrhage (IVH) have higher absolute nucleated red blood cell counts (aNRBC) at birth (a marker of intrauterine hypoxia) than controls. Periventricular leukomalacia (PVL) is known to be associated with prenatal and postnatal events. Whether PVL is also linked to intrauterine hypoxia is unknown.

Objectives: To test the hypothesis that infants with PVL have higher aNRBC counts at birth than controls.

Methods: We studied 14 very low birth weight infants with PVL and compared them with 14 pair-matched controls without PVL. Head ultrasound scans were performed in all infants on days 3–5 and 21–25 of life. Paired tests, Fisher exact tests and stepwise logistic regression were performed for analysis.

Results: The groups were similar for gestational age (GA), birth weight (BW), prolonged rupture of membranes (PROM), Apgar scores, IVH, and aNRBC counts. PVL correlated significantly with low partial pressure of CO_2 (PCO_2) and IVH ($P < 0.01$). In logistic regression, when GA, gender, PROM, antenatal steroid therapy, 1 (or 5) minute Apgar scores, IVH grade, nosocomial sepsis, patent ductus arteriosus, necrotizing enterocolitis (NEC), need for pressors, aNRBC counts and lowest PCO_2 were used as independent variables, PCO_2 ($P = 0.002$), IVH grade ($P = 0.001$), GA ($P = 0.038$), NEC ($P = 0.061$) and use of dopamine ($P = 0.010$) remained in the analysis (total $R^2 = 68.2\%$).

Conclusions: In contrast to severe IVH, aNRBC counts do not predict the development of PVL.

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KEY WORDS: intraventricular hemorrhage (IVH), fetal hypoxia, nucleated red blood cells (NRBC), periventricular leukomalacia (PVL)

Increased numbers of circulating absolute nucleated red blood cells counts are seen in states of active erythropoiesis such as early gestation [1], chronic intrauterine hypoxia [2] and significant blood loss [3]. In a previous study [4] we showed that infants with severe intraventricular-periventricular hemorrhage have higher aNRBC at birth than controls, suggesting that antenatal hypoxia is a significant risk factor for IVH.

The pathogenesis of periventricular leukomalacia is implicated with both cerebral hypoxia ischemia and inflammation [5]. Periventricular leukomalacia has been linked to several prenatal risk factors such as chorioamnionitis [6], male gender [7] and preeclampsia [7], but also with postnatal factors such as hypocarbia [7] (presumably causing brain ischemia) or IVH [7]. The susceptibility of the preterm infant to exhibit a pressure-passive circulation in the "border zone" white matter arteries could lead to hypoxia-ischemia during periods of intrapartum and postnatal hypotension. Whether PVL is also linked to intrauterine hypoxia is unknown. Thus, similar to our previous IVH study [4], we used the aNRBC as a proxy measurement of exposure to intrauterine hypoxia. We tested the hypothesis that infants with PVL have higher aNRBC counts at birth than controls.

PATIENTS AND METHODS

We retrospectively analyzed the charts of all infants born at the Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, who were admitted to our neonatal intensive care unit between 2004 and 2009 and diagnosed with cystic PVL. During that period a strict protocol of PVL screening was followed. Briefly, all infants who were born with a birth weight < 1500 g and all sick infants (sick enough to require supplemental oxygen therapy, mechanical ventilation, or continuous positive airway pressure or blood pressure support) underwent head ultrasonographic examina-

aNRBC = absolute nucleated red blood cells counts
IVH = intraventricular-periventricular hemorrhage
PVL = periventricular leukomalacia

tion on days 3–5 after delivery, again at age 21–25 days, and prior to discharge from the NICU. In addition, in all infants with abnormal HUS on days 3–5 (such as periventricular echodensities or IVH) weekly HUS was performed to follow the diagnosed abnormality. The examination was conducted in all infants by a single experienced pediatric radiologist (L.B.S). Cystic PVL was defined as echolucencies in the white matter dorsolateral to the lateral ventricles, usually bilateral and symmetric. It is sometimes associated with evolving IVH [5]. Every infant identified with PVL was pair-matched with the infant admitted immediately after him or her who did not develop PVL and had the same gestational age (± 1 week) and birth weight (± 100 g). In an attempt to control for some of the variables known to affect neonatal nucleated red blood cell counts, we excluded from the study infants in both groups with perinatal blood loss, hemolysis (blood group incompatibility with positive Coombs' test) or chromosomal anomalies. We also excluded infants born to mothers with clinical chorioamnionitis, a well-known risk factor for PVL, in order to minimize the effect of this major confounding variable. Finally, we excluded infants born in other institutions and transferred to ours, since this is an important source of outcome variability [8]

HEMATOLOGIC METHODS

In our institution all preterm infants admitted to the NICU undergo a routine complete blood count with differential count within the first hour of life. In view of this practice and of the retrospective nature of the study, our local institutional review board approved the study and waived the requirement for informed consent. Venous blood samples for complete blood cell counts were analyzed according to laboratory practice using an STK-S counter (Coulter Corporation, Hialeah, FL, USA). Differential cell counts were performed manually, and nucleated RBC counts were counted per 100 WBC. We showed previously that leukocyte counts and aNRBC numbers are not independent [9]. Thus, traditional expression of nucleated RBC as number per 100 WBC might introduce a significant bias. Therefore, we expressed the number of nucleated RBC as aNRBC rather than per 100 leukocytes, and the WBC count was expressed as corrected for the presence of nucleated RBC. We also corrected the absolute lymphocyte count, another potential index of fetal hypoxia.

CLINICAL DATA

Major demographic, clinical and laboratory variables were recorded. Clinical chorioamnionitis was defined as fever and uterine tenderness. Prolonged rupture of membranes was defined as a period of rupture of membranes exceeding 18 hours. Preeclampsia [10], respiratory distress syndrome [11] and necrotizing enterocolitis [12] were defined as previously reported. Maternal antibiotic therapy during labor, prenatal steroids, gestational age, birth weight, gender and Apgar scores were recorded. We also recorded surfactant therapy, duration of oxygen therapy, maximal fraction of inspired oxygen, low partial pressure of CO_2, patent ductus arteriosus, indomethacin therapy for PDA , nosocomial sepsis (culture-proven), IVH grade as defined by Papile et al. [13], hypotension severe enough to require dopamine therapy, and aNRBC, lymphocyte and platelet counts at age < 1 hour.

STATISTICAL ANALYSIS

Data are presented as mean ± SD, n (%), or for non-normally distributed variables (such as aNRBC or Apgar scores) as median (range). The Minitab 15 software (Minitab, State College, PA, USA) was used. Statistical analysis included the two-tailed paired *t*-test for normally distributed variables and paired Wilcoxon test for aNRBC or Apgar scores. The potential influence of aNRBC and other confounders on PVL was studied by stepwise logistic regression. A P value of < 0.05 was considered significant. This research was designed to be a pilot study that would enable us to make appropriate sample size calculations later on. Thus we elected to enroll all infants who were eligible during the study period,

RESULTS

We identified 14 infants who fulfilled the entry criteria for the PVL group. According to the protocol, each infant with PVL was matched with a control without PVL. Both groups were found to be similar in terms of prolonged rupture of membranes, maternal antibiotic therapy in labor, prenatal steroids, preeclampsia, gestational age, birth weight, gender, Apgar scores, respiratory distress syndrome, surfactant therapy, duration of oxygen therapy, maximal FIO_2, PDA, indomethacin therapy for PDA, nosocomial sepsis, NEC, dopamine therapy, and aNRBC, lymphocyte and platelet counts at age < 1 hour [Table 1]. In contrast, the lowest PCO_2 was significantly lower in the PVL group ($P < 0.01$), while the rate of IVH was significantly higher in the PVL group ($P < 0.01$). In logistic regression, when gestational age (or birth weight), gender, history of PROM (or clinical chorioamnionitis), antenatal steroid therapy, 1 (or 5) minute Apgar scores, IVH grade, nosocomial sepsis, PDA, NEC, infant need for pressors, aNRBC counts and lowest PCO_2 were used as independent variables, lowest PCO_2 ($P = 0.002$), IVH grade ($P = 0.001$), use of dopamine ($P = 0.010$), gesta-

NICU = neonatal intensive care unit
HUS = head ultrasound
WBC = white blood cells
PDA = patent ductus arteriosus
FIO_2 = fraction of inspired oxygen
NEC = necrotizing enterocolitis
PCO_2 = partial pressure of CO_2
PROM = prolonged rupture of membranes

Table 1. Clinical and laboratory data in PVL and controls*

	PVL group (n=14)	No PVL group (n=14)	*P* value
Rupture of membranes > 18 hr	5 (35.7)	4 (28.6)	0.538
Prenatal antibiotics	7 (50)	6 (42.9)	0.50
Prenatal steroids	11 (78.6)	14 (100)	0.436
Preeclampsia	0 (0)	0 (0)	1.0
Gestational age (wks)	28.4 ± 0.5	28.4 ± 0.5	1.0
Birth weight (g)	1174 ± 345	1111 ± 300	0.543
Male:female ratio	6:8	10:4	0.10
1 min Apgar score	6.5 (2–9)	6 (2–8)	0.56
5 min Apgar score	8 (6–10)	8 (6–10)	1.0
Respiratory distress syndrome	14 (100)	14 (100)	1.0
Surfactant therapy	8 (57.1)	8 (57.1)	1.0
Oxygen therapy (days)	11.9 ± 20	14.0 ± 26	0.264
Maximal FIO_2 (%)	42 ± 27	52 ± 33	0.765
Lowest PCO_2 (torrs)	24.9 ± 4.09	30.3 ± 6.3	0.018
PDA	10 (71.4)	5 (35.7)	0.235
Indomethacin for PDA	9 (64.3)	5 (35.7)	0.293
Nosocomial sepsis	9 (64)	7 (50)	0.223
IVH	6 (42.9)	1 (7.1)	< 0.01
NEC	6 (42.9)	3 (21.4)	0.158
Dopamine therapy	2 (14.3)	2 (14.3)	0.404
aNRBC count (/mm^3)	577 (0–13,567)	1479 (318–29,333)	0.286
Lymphocyte count (/mm^3)	7006 ± 2351	9470 ± 11865	0.515
Platelet count (x 1000/mm^3)	284.6 ± 179.3	239.3 ± 100.4	0.471

* Data are expressed as mean ± SD (except for Apgar scores and aNRBC, expressed as median and range) or n (%).

tional age ($P = 0.038$) and NEC ($P = 0.061$) remained in the final analysis, with a total R^2 of 68.2%.

DISCUSSION

In this retrospective pair-matched controlled pilot study we found that infants who developed cystic PVL did not have increased aNRBC counts compared to controls. We excluded infants with hemolysis, chromosomal anomalies and maternal diabetes, all of which have the potential to increase aNRBC counts at birth.

Importantly, both groups of infants were very similar in terms of birth weight, gestational age, gender, Apgar scores, and other major perinatal variables such as clinical chorioamnionitis, prolonged (> 18 hours) rupture of membranes, prenatal antibiotic administration, respiratory distress syndrome, surfactant therapy and oxygen requirements. However, they differed significantly in terms of rate of IVH (higher in the PVL group) and lowest PCO_2 (lower in the PVL group). The two groups were similar in terms of aNRBC counts. Thus, we used logistic regression, which confirmed the significance of a low PCO_2 and the presence of IVH as independent predictors of PVL, even after taking into account other independent variables, particularly the aNRBC count. In multiple regression, the use of dopamine, gestational age ($P < 0.02$) and perhaps NEC as well ($P = 0.061$) also appeared to be significant. Thus, in view of our findings we cannot reject the null hypothesis that aNRBC counts do not differ between PVL patients and their pair-matched controls. However, the range of NRBC counts was extremely wide in both groups, rendering the likelihood of clinical usefulness minimal.

This finding is in striking contrast to what we described in infants with severe IVH [4]. Indeed, in the latter study, infants with severe IVH (grade 3 or above) had increased neonatal aNRBC counts at birth. Since a likely explanation for increased circulating neonatal aNRBC counts is relative fetal hypoxia, we speculated that fetal hypoxia is an important and preventable risk factor for IVH [14]. In contrast, if the current findings are correct, it would appear that fetal hypoxia does not seem to play an important role in the pathophysiology of PVL, even after taking into account the higher rate of IVH in the PVL group. In our study the lymphocyte count, also believed to be an indicator of fetal hypoxia [15], was not elevated but this hematologic parameter might indicate acute rather than chronic hypoxia [16]. Moreover, the platelet count was neither increased nor decreased, while it is known that during the early stages (4–5 days) of acute hypoxia in rats, thrombocytosis is present but switches to thrombocytopenia when the hypoxia is prolonged [17]. In humans, Phelan et al. [18] demonstrated that platelet counts below 100,000/mm^3 at birth are inconsistent with acute birth asphyxia.

The results of our study are in contrast to those of Silva and co-authors [19]; they showed a significant increase in aNRBC counts in 176 preterm infants who later exhibited cerebral white matter injury. However, the methodology of their study is significantly different from ours. The groups in their study were not identical in terms of birth weight, a factor that might influence the aNRBC count. In addition, those authors did not correct the number of circulating aNRBC to the total number of leukocytes.

A limitation of our study is that, in this specific group of patients, none had a history of clinical chorioamnionitis (an exclusion criterion), thus whether or not aNRBC might be increased in a group of patients with PVL born to mothers with chorioamnionitis is unknown. In our study, cord blood gases, which theoretically might have helped in the diagnosis of fetal hypoxemia, were not routinely obtained in all infants. Cord blood gases are indicative of acute oxygenation

status of the fetus. In contrast, aNRBC count is indicative of the oxygenation status of the fetus at least a few days before delivery [20]. We therefore suggest that there is no evidence of increased fetal erythropoiesis in preterm infants who later develop PVL.

As described in the work by Erickson et al. [21], we also found that the lowest PCO_2 was much lower in the PVL than in the control group, and this relationship remained significant in the multiple regression. Although a causal relationship between hypocarbia and brain damage in preterm infants remains unproven, it has been suggested that ischemic brain damage due to cerebral vasoconstriction might be a possible explanation [22].

Similarly, as described earlier, we also found that IVH and IVH grade were significantly associated with PVL, and this relationship remained significant in the multiple regression. This relationship is not novel, and there is a significant overlap between the two conditions, although their pathophysiology seems to be distinct [7].

The use of dopamine, although not significant in univariate analysis, appeared to be significant on multiple regression. Dopamine is usually administered to infants with hypotension, and a known neonatal risk factor for PVL is systemic hypotension [23]. Not surprisingly, gestational age was also found to significantly predict PVL on multiple regression. PVL is indeed more prevalent in infants of younger gestational age [24], although it may be seen in relatively mature preterm infants [25].

NEC was nearly significant as a predictor of PVL in this population of infants ($P = 0.061$). Theoretically, there are multiple ways by which NEC may relate to PVL, including release of inflammatory cytokines during NEC [22] and septic shock with decreased cerebral perfusion. However, most studies of the risk factors for PVL failed to find such a relationship. In the present study we performed many post-hoc comparisons, and a P value of 0.06 may not be accepted as significant.

We conclude from this study that aNRBC counts do not appear to be elevated in infants who develop PVL, as compared to pair-matched controls without PVL. We speculate that, contrary to IVH, chronic intrauterine hypoxia does not appear to play a major role in the pathogenesis of PVL. In contrast, our data suggest that low PCO_2 (a potentially avoidable risk factor), IVH, low gestational age and the use of dopamine in a hypotensive infant may be significant risk factors for PVL.

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