Nucleated Red Blood Cells in Preterm Infants With Retinopathy of Prematurity

Ronit Lubetzky, MD*‡§; Chaim Stolovitch, MD§; Shaul Dollberg, MD$; Francis B. Mimouni, MD, FAAP$§; Mazal Salomon, RN*; and Dror Mandel, MD, MHA*§

ABSTRACT. Objective. The aim of this retrospective study was to examine hematologic indices of potential intrauterine hypoxia, including circulating nucleated red blood cells, lymphocytes, and platelets in preterm infants who developed retinopathy of prematurity (ROP) compared with suitable controls. We hypothesized that higher neonatal absolute nucleated red blood cell (ANRBC) and lymphocyte counts and lower platelets would be found in infants who developed ROP, compared with control infants.

Methods. Each of 23 infants with ROP was pair matched for gestational age and Apgar scores with a control without ROP. Criteria for exclusion in both groups included factors that may influence the ANRBCs at birth. Venous ANRBC counts were obtained within 1 hour of life. Statistical analyses used paired t tests, a paired Wilcoxon test, and backward stepwise-regression analysis.

Results. Groups did not differ in birth weight, gestational age, Apgar scores, or hematocrit, white blood cell, or platelets counts. The ANRBC counts at birth were significantly higher in infants who developed ROP than in control infants.

Conclusions. Infants who develop ROP have higher ANRBC counts at birth than matched controls. We suggest that increased fetal erythropoiesis exists in preterm infants who later on will develop ROP. If correct, our interpretation supports the theory that long-lasting intrauterine hypoxia is increased compensatory erythropoiesis caused by increased erythropoietin secretion. It is widely considered to be multifactorial. Hypoxia of retinal cells, secondary to any one of a variety of noxious perinatal events, is one of the possible culprits.

In support of an ischemic-hypoxic theory are the facts that an increased rate of severe ROP has been found in infants suffering from fetal growth restriction and neonatal asphyxia, conditions known to potentially compromise blood flow and/or oxygen supply.

One of the well-described consequences of intrauterine hypoxia is increased compensatory erythropoiesis caused by increased erythropoietin secretion. In situations associated with intrauterine hypoxia, such as intrauterine growth restriction, maternal pregnancy-induced hypertension, or maternal diabetes or smoking, there is an elevation of nucleated red blood cell (RBC) counts at birth, presumably caused by increased compensatory erythropoiesis.

The aim of this study was to examine hematologic indices of potential intrauterine hypoxia, including circulating nucleated RBCs, lymphocytes, and platelets in preterm infants who developed ROP compared with suitable controls. We hypothesized that higher neonatal absolute nucleated RBC (ANRBC) and lymphocyte counts and lower platelets would be found in infants who developed ROP, compared with control infants.

PATIENTS AND METHODS

Patients

We retrospectively analyzed the charts of all infants who were admitted to our NICUs, born at the Lis Maternity Hospital, Tel Aviv Sourasky Medical Center between January 1, 2002, and December 31, 2004, and who were diagnosed with ROP. During that period a strict protocol of ROP screening, which was consistent with the 1997 American Academy of Pediatrics guidelines, was followed. Briefly, all infants who were born with a birth weight of ≥1500 g or a gestational age of ≥28 weeks and sick infants (sick enough to require supplemental oxygen therapy, mechanical ventilation, or continuous positive airway pressure or blood pressure support) of ≥1500 g in birth weight underwent a dilated indirect ophthalmoscopic examination to detect ROP. The
examination was conducted in all infants by a single experienced pediatric ophthalmologist (C.S.). The examination was performed in all infants between 4 and 6 weeks’ chronological age or between 31 and 33 weeks’ postmenstrual age.14 Scheduling of follow-up examinations was determined by the findings at the first examination, using the International Classification of Retinopathy of Prematurity.33,14 Follow-up examination was continued until vascularization had proceeded to zone 3. Infants with threshold disease were considered candidates for ablative surgery of at least 1 eye within 72 hours of diagnosis.

Each infant with ROP of any stage was pair matched with the infant admitted immediately after him or her who did not develop ROP and had the same gestational age (±1 week) and 1- and 5-minute Apgar scores (±1). In an attempt to control for the various variables known to affect neonatal nucleated RBC counts, we excluded from the study infants in both groups who were born to women with gestational or insulin-dependent diabetes15; pregnancy-induced hypertension16; intrauterine growth retardation (defined as a birth weight below the 10th percentile using the Lubchenco curves12,17); placental abruption or placenta previa18; any maternal heart, kidney, lung, or other chronic condition; drug, tobacco, or alcohol abuse19; perinatal infections (eg, maternal fever, maternal leukocytosis [white blood cells (WBCs) > 150 x 109/mm³]; clinical signs of chorioamnionitis such as fever and abdominal tenderness)20; any abnormality in electronic intrapartum monitoring (eg, perinatal asphyxia, intracranial hemorrhage [ICH])21; or infants with low Apgar scores (<6 at 1 or 5 minutes).22 We also excluded infants with perinatal blood loss, hemolysis (blood-group incompatibility with positive Coombs test or hematocrit of <45%),22 or chromosomal anomalies.23 Because of these exclusion criteria, we had to exclude 10 potential controls who were each replaced by the appropriate control infant born immediately after it. Follow-up data were available from the medical charts in our pediatric ophthalmology clinic, when available, or by telephone interview with the parents.

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. Venous blood samples for complete blood cell counts were analyzed according to laboratory routine for patent ductus arteriosus closure, and endotracheal administration of surfactant. By design, infants with ROP did not differ from controls in terms of gestational age and Apgar scores. Table 2 shows the highest stage of ROP (most severe stage diagnosed in either of the eyes) of children with ROP, the need for laser therapy, and the outcome. Follow-up data at the age of at least 1 year were available on 18 patients: 10 from the medical charts in our ophthalmology clinic and 8 by tele-

**Table 1.** Demographic and Perinatal Characteristics of Infants With ROP and Matched Controls

<table>
<thead>
<tr>
<th></th>
<th>Infants With ROP (n = 23)</th>
<th>Controls (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk, mean ± SD (range)</td>
<td>28.3 ± 2.3 (24–33)</td>
<td>28.8 ± 2.8 (24–34)</td>
<td>.10</td>
</tr>
<tr>
<td>Birth weight, g, mean ± SD (range)</td>
<td>1097 ± 451 (570–2245)</td>
<td>1186 ± 546 (530–2625)</td>
<td>.16</td>
</tr>
<tr>
<td>Prolonged rupture of membranes (24 h), n (%)</td>
<td>11 (47.8)</td>
<td>11 (47.8)</td>
<td>.77</td>
</tr>
<tr>
<td>Administration of prenatal antibiotics, n (%)</td>
<td>10 (43.5)</td>
<td>10 (43.5)</td>
<td>.72</td>
</tr>
<tr>
<td>Administration of prenatal betamethasone, n (%)</td>
<td>15 (65.2)</td>
<td>14 (60.9)</td>
<td>.30</td>
</tr>
<tr>
<td>1-min Apgar score, median (range)</td>
<td>8 (7–9)</td>
<td>8 (7–9)</td>
<td>.42</td>
</tr>
<tr>
<td>5-min Apgar score, median (range)</td>
<td>8 (6–10)</td>
<td>8 (6–10)</td>
<td>.30</td>
</tr>
<tr>
<td>Presence of respiratory distress syndrome, n (%)</td>
<td>15 (65.2)</td>
<td>16 (69.5)</td>
<td>.33</td>
</tr>
<tr>
<td>Endotracheal surfactant administration, n (%)</td>
<td>13 (56.5)</td>
<td>15 (65.2)</td>
<td>.67</td>
</tr>
<tr>
<td>Duration of ventilation, d, mean ± SD</td>
<td>5.4 ± 8.3</td>
<td>5.3 ± 5.2</td>
<td>.41</td>
</tr>
<tr>
<td>Maximal oxygen delivered, %, mean ± SD</td>
<td>27.5 ± 10.38</td>
<td>24.9 ± 12.15</td>
<td>.56</td>
</tr>
<tr>
<td>Duration of oxygen delivered, d, mean ± SD</td>
<td>10.8 ± 12.8</td>
<td>9.8 ± 10.4</td>
<td>.41</td>
</tr>
<tr>
<td>Patent ductus arteriosus, n (%)</td>
<td>17 (73.9)</td>
<td>18 (78.3)</td>
<td>.19</td>
</tr>
<tr>
<td>Indomethacin for patent ductus arteriosus, n (%)</td>
<td>13 (56.5)</td>
<td>15 (65.2)</td>
<td>.16</td>
</tr>
<tr>
<td>IVH, n (%)</td>
<td>5 (21.7)</td>
<td>6 (26.1)</td>
<td>.43</td>
</tr>
<tr>
<td>Periventricular leukomalacia, n (%)</td>
<td>3 (13.04)</td>
<td>3 (13.04)</td>
<td>.67</td>
</tr>
<tr>
<td>Umbilical artery catheter, n (%)</td>
<td>9 (39.1)</td>
<td>7 (30.4)</td>
<td>.18</td>
</tr>
<tr>
<td>Umbilical vein catheter, n (%)</td>
<td>9 (39.1)</td>
<td>8 (34.8)</td>
<td>.19</td>
</tr>
<tr>
<td>Duration of total parenteral nutrition, d, mean ± SD</td>
<td>7.3 ± 5.8</td>
<td>7.4 ± 4.8</td>
<td>.39</td>
</tr>
</tbody>
</table>

None of the differences were statistically different.

**Statistical Methods**

Data are reported as mean ± SD, n (%), or, for non-normally distributed variables (such as ANRBCs or Apgar scores) as median (range). Statistical analysis included the 2-tailed paired t test for normally distributed variables and paired Wilcoxon test for ANRBCs or Apgar scores. Backward stepwise-regression analysis was used to assess the effect of gestational age (or birth weight), 1- or 5-minute Apgar scores, intraventricular hemorrhage (IVH) status, and ANRBC count (independent variables) on the ROP status (dependent variable). We also used Pearson ranked-regression analysis to study the correlation between ROP severity (defined by its stage from 0 [no ROP] to 4, whichever the zone) and the ANRBC count. P < .05 was considered significant.

Our local institutional review board approved the study. Because all preterm patients in our institution receive a routine complete blood count after birth, including nucleated RBC count, the requirement for informed consent was waived.

**RESULTS**

A total of 23 infants with ROP were retained for analysis and compared with 23 controls. Four additional infants with ROP were excluded because of maternal diabetes (n = 2), neonatal polycythemia (n = 1), and maternal asthma (n = 1). Table 1 depicts some major demographic and clinical characteristics of infants with ROP and controls. There were no significant differences between groups in all clinical or demographic parameters considered, to the inclusion of infant birth weight, gestational age, major diagnoses such as respiratory distress syndrome, patent ductus arteriosus, IVH, and periventricular leukomalacia, and major treatments and procedures such as umbilical artery and vein catheters, mechanical ventilation, antibiotic treatment, indomethacin for patent ductus arteriosus closure, and endotracheal administration of surfactant. By design, infants with ROP did not differ from controls in terms of gestational age and Apgar scores.
phone interview of the parents. All 8 patients had been examined by a pediatric ophthalmologist. All the parents interviewed over the telephone reported that no visual problems had been diagnosed.

Table 3 depicts the hematologic data obtained in both groups. The ANRBC counts at birth were significantly higher in infants with ROP than in control infants (paired Wilcoxon test: \( P = .02 \)). There were no differences between the 2 groups in terms of hematocrit or WBC, lymphocyte, or platelets counts. In backward stepwise logistic-regression analysis, taking into account gestational age (or birth weight), the 1- or 5-minute Apgar scores, the IVH status, and the ANRBC count as independent variables and the ROP status as the dependent variable, only the ANRBC was a predictor of ROP (\( P = .02 \)). Figure 1 depicts in a graphic manner the relationship between ANRBC and ROP stage. Because ANRBCs are not normally distributed, we used the log ANRBC as the predictor for stage of ROP. A best-fit cubic-regression equation was developed: ROP stage = \(-9.74 + 11.23 \times \log(\text{ANRBC}) - 4.14 \times (\log(\text{ANRBC}))^2 + 0.52 \times (\log(\text{ANRBC}))^3 \) (\( R^2 = 0.122; \ P < .001 \)).

**DISCUSSION**

In a retrospective study, we found that the development of ROP was associated with an increase in ANRBCs. In our study we excluded small-for-gestational-age infants, which is an important confounding variable.\(^{26} \) We also excluded infants with other factors associated with potentially increased ANRBC counts, including hemolysis,\(^{23} \) chromosomal anomalies,\(^{15,27} \) maternal diabetes,\(^{15,27} \) and neurologic insults.\(^{28,29} \) It is important to note that the 2 groups in our study (infants with ROP and controls) ended up being very similar in birth weight, gestational age, Apgar scores (by design), and major neonatal complications. Thus, we believe that our study confirms our hypothesis that as a group, preterm infants with ROP have increased neonatal ANRBC counts.

The mechanism by which ROP is associated with increased circulating neonatal ANRBC counts is unknown. A likely explanation is relative fetal hypoxia.\(^{35,25,30} \) In favor of a contribution of hypoxia/ischemia in the pathogenesis of ROP are the facts that an increased rate of ROP has been found in conditions known to potentially compromise retinal blood flow and/or oxygen supply, such as fetal growth restriction\(^7 \) and severe neonatal asphyxia.\(^8 \) In our study, the lymphocyte count, also believed to be an indicator of fetal hypoxia,\(^{25} \) was not elevated, and the platelet count was not decreased, but these hematologic parameters might indicate acute rather than chronic hypoxia.\(^{25} \) In this retrospective study, cord blood gases, which theoretically might have helped in the diagnosis of fetal hypoxemia, were not routinely obtained in all infants. However, cord blood gases are indicative of the acute oxygenation status of the fetus in contrast with ANRBCs, which are indicative of the oxygenation status of the fetus at least a few days before delivery.\(^{31} \) In terms of timing, if the elevation of ANRBC counts in the ROP group is indeed related to fetal hypoxia, as we speculate, this hypoxia must have been of sufficient duration to stimulate erythropoietin secretion. The relationship between hypoxia and ROP is not yet completely investigated but might involve an increase in vascular endothelial growth factor production induced by hypoxia, which in turn may stimulate neovascularization.\(^{5,32,33} \) Another possibility under investigation is hypoxia-induced stimulation of insulin-like growth factor-binding protein-1 production, which in turn may decrease free insulin-like growth factor concentrations, which may prevent normal vessel growth.\(^5,33-35 \)

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**TABLE 2.** Characteristics of the Infants With ROP and Matched Controls

<table>
<thead>
<tr>
<th>Stage</th>
<th>Infants With ROP</th>
<th>Controls</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13 (56.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5 (21.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5 (21.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laser treatment</td>
<td>2 (8.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plus disease</td>
<td>1 (4.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Outcome at \( \geq 1 \) y of age:
- Visual acuity: central, steady, maintained: 14 (61.0)
- Muscle balance: orthophoria: 13 (56.5)
- Refractive error: myopic astigmatism: 4 (17.4)

**DISCUSSION**

The mechanism by which ROP is associated with increased circulating neonatal ANRBC counts is unknown. A likely explanation is relative fetal hypoxia.\(^{35,25,30} \) In favor of a contribution of hypoxia/ischemia in the pathogenesis of ROP are the facts that an increased rate of ROP has been found in conditions known to potentially compromise retinal blood flow and/or oxygen supply, such as fetal growth restriction\(^7 \) and severe neonatal asphyxia.\(^8 \) In our study, the lymphocyte count, also believed to be an indicator of fetal hypoxia,\(^{25} \) was not elevated, and the platelet count was not decreased, but these hematologic parameters might indicate acute rather than chronic hypoxia.\(^{25} \) In this retrospective study, cord blood gases, which theoretically might have helped in the diagnosis of fetal hypoxemia, were not routinely obtained in all infants. However, cord blood gases are indicative of the acute oxygenation status of the fetus in contrast with ANRBCs, which are indicative of the oxygenation status of the fetus at least a few days before delivery.\(^{31} \) In terms of timing, if the elevation of ANRBC counts in the ROP group is indeed related to fetal hypoxia, as we speculate, this hypoxia must have been of sufficient duration to stimulate erythropoietin secretion. The relationship between hypoxia and ROP is not yet completely investigated but might involve an increase in vascular endothelial growth factor production induced by hypoxia, which in turn may stimulate neovascularization.\(^{5,32,33} \) Another possibility under investigation is hypoxia-induced stimulation of insulin-like growth factor-binding protein-1 production, which in turn may decrease free insulin-like growth factor concentrations, which may prevent normal vessel growth.\(^5,33-35 \)
We suggest that increased fetal erythropoiesis exists in preterm infants who later on will develop ROP. If correct, our interpretation supports the theory that fetal hypoxia and/or ischemia may play a role in the pathogenesis of ROP. Although the retrospective aspect of our study requires a replication of results in a prospective manner, we speculate that elevated ANRBCs at birth may help to define a subgroup of preterm infants at increased risk for ROP.

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