Nucleated red blood cells in concordant, appropriate-for-gestational age twins

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KEY WORDS
Fetal hypoxemia
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Objectives: The purpose of this study was to test the hypothesis that neonatal nucleated red blood cell (RBC) counts are elevated in nondiscordant twins compared with singletons.

Study design: We compared absolute nucleated RBC counts taken after birth in 2 groups of term, appropriate-for-gestational age infants; 74 concordant twins, and 29 singleton control infants. We excluded infants with factors associated with a potential increase in absolute nucleated RBC counts.

Results: Birth weight and gestational age were significantly lower in twins than in singletons (P < .01). Hematocrit, absolute nucleated RBC count, and corrected lymphocyte counts were significantly higher in twins (P < .01). In multiple regression, the significantly higher absolute nucleated RBC count in twins remained significantly higher even after taking into account gestational age and Apgar scores.

Conclusion: Concordant, appropriate-for-gestational age twins have increased nucleated RBCs at birth compared with singleton control infants.

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The incidence of twinning has significantly increased in the past 20 years because of the development of assisted reproduction technology. 1,2 Perinatal outcome of twins is significantly worse than that of singleton fetuses, both in terms of perinatal and neonatal morbidity and mortality. 3 Even in the absence of twin-to-twin transfusion, fetal growth is adversely affected by twinning; indeed, until 28 to 32 weeks of gestation, twin fetuses have intrauterine growth patterns indistinguishable from those of singletons. 4 After that time, fetal weight gain slows down in twins compared with singletons, which may be interpreted as an index of relative fetal growth restriction. 4 In fact, if one uses singleton intrauterine growth curves, the incidence of small-for-gestational age twin infants may be as high as 15% to 25%. 5,7 The mechanisms by which the intrauterine growth of a twin fetus may be restricted are not completely

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elucidated, and may involve relative fetal hypoxia. Mori et al\(^8\) found a higher absolute number of circulating nucleated red blood cells (ANRBCs) in the smaller of discordant twins, a difference that increased with the degree of discordance. Because concordant twins grow slower than singletons in the third trimester, we hypothesized that specific markers of fetal hypoxia, the ANRBC and lymphocyte counts,\(^9-11\) measured at birth, are increased in concordant twin infants compared with singleton control infants.

**Material and methods**

We prospectively studied 2 groups of term infants (37 to 41 weeks' gestation by last menstrual period, confirmed by early ultrasound), appropriate-for-gestational age (AGA), delivered vaginally at the Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, between January 1, 1998, and December 31, 2001. Appropriateness for gestational age was determined with the Lubchenco intrauterine growth charts.\(^12\) Infants were recruited consecutively whenever one of the authors (Y.L., D.M., G.S.M) were on call. Group 1 consisted of 74 twin infants (37 pairs) and group 2 of 29 singleton control infants. All infants were Caucasians of Jewish descent. In both groups we excluded infants with other factors associated with a potential increase in ANRBC counts, as described by us and others, such as small-for-gestational age infants, infants born to women with gestational or insulin-dependent diabetes\(^9\); pregnancy-induced hypertension\(^14\); placental abruption or placenta previa; any maternal heart, kidney, lung, or other chronic condition; drug, tobacco, or alcohol abuse\(^10,15\); perinatal infections (eg, fever, leukocytosis, signs of chorioamnionitis)\(^16\); abnormal electronic intrapartum monitoring\(^17\); or infants with low Apgar scores (below 7 at 1 or 5 minutes)\(^8\); infants delivered with the assistance of forceps or vacuum. We also excluded infants with perinatal blood loss, meconium-stained amniotic fluid,\(^19\) hemolysis (blood group incompatibility with positive Coombs’ test),\(^20\) or chromosomal anomalies.\(^21\) The twin group consisted of dichorionic pregnancies only, in order to exclude the potential confounder of twin-to-twin transfusion. They were all concordant in weight, when discordance was defined as a difference in birth weight of more than 20% of the largest infant’s weight.\(^22\)

Capillary blood samples for complete blood cell counts were collected from the infant within 12 hours of birth and analyzed according to laboratory routine using an STK-S counter (Coulter Corporation, Hialeah, Fla). Differential cell counts were performed manually and ANRBC counts were counted per 100 white blood cells (WBC). We showed previously that leukocyte counts and ANRBC numbers are not independent;\(^6\) thus, traditional expression of nucleated RBCs as their number per 100 WBCs might introduce a significant bias.\(^8\) Therefore, we expressed the number of nucleated RBCs as an absolute number rather than per 100 leukocytes, and both WBC and lymphocyte counts were expressed as corrected for the presence of nucleated RBCs.

Our local Institutional Review Board approved the study. Because all newborn infants in our hospital are screened routinely for polycythemia with complete blood count by 12 hours of life, the requirement for informed consent was waived.

This study was a pilot study designed to determine the sample size of a larger study. We aimed to initially study approximately 30 patients in each group because when n ≥30, the distribution of a given variable's mean approximates the normal curve regardless of the distribution of the variable. Statistical analysis included \(\chi^2\) test for discrete variable such as gender, Kruskal-Wallis test for all other continuous variables caused by nonnormal distribution of ANRBCs, and ranked backward stepwise regression analysis. Whenever an overall difference among groups was found by Kruskal-Wallis test, a difference between 2 of 3 subgroups was studied with Tukey family test. Data are reported as mean ± SD or median (range). A P value < .05 was considered significant.

**Results**

Table I depicts the demographic characteristics of our patient population. There were no differences between groups in 1- and 5-minute Apgar scores and infant sex. Birth weight and gestational age were significantly lower in twins than in singletons (\(P < .01\)). The twins’ mothers were significantly older (\(P < .001\)), with a lower parity (\(P = .002\)) than the singletons’ mothers. In the twin group, the ANRBC count of a randomly chosen infant in each set was significantly correlated with the ANRBC count of its mate (\(R^2 = 24.8\%\), \(P = .002\)). Similarly, the ANRBC count of the smallest twin within each set significantly correlated with that of the largest twin of the set (\(R^2 = 24.8\%\), \(P = .002\)).

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higher (Tables II and IV), or nearly significantly higher ($P = .07$) (Table III) in twins than in controls. There were no significant differences in platelet counts and corrected WBC between groups (Tables II, III, and IV). Also, regardless of the way we analyzed the data, there were no significant differences between twins (taken at random, or according to birth weight) for any of the hematologic parameters studied (Tables II and III). Because gestational age and Apgar scores are known as strong confounders of ANRBC counts, we conducted a series of ranked backward stepwise multiple regression analyses with the ANRBC count as the dependent variable, and twinning (smaller vs larger vs control, or random twin vs other twin vs control), gestational age, and Apgar scores (1- or 5-minute) as independent variables. In all analyses, gestational age and twinning...
significantly and independently affected the absolute ANRBC count. In similar analyses, taking the hematocrit or the lymphocyte count as the dependent variable, and gestational age, Apgar scores, and twining as independent variables, the effect of twinning remained statistically significant.

<table>
<thead>
<tr>
<th>Table III</th>
<th>Comparison between twins (larger vs smaller) and control infants</th>
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<tbody>
<tr>
<td></td>
<td>Larger twin (n = 37)</td>
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<tr>
<td>Birth weight (g)*</td>
<td>2787 ± 261</td>
</tr>
<tr>
<td></td>
<td>(2750 (2240-3240))</td>
</tr>
<tr>
<td>Hematocrit*</td>
<td>64.8 ± 5.9</td>
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<tr>
<td></td>
<td>(65 (47-75))</td>
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<tr>
<td>White blood cells (corrected) (×10⁹/L)</td>
<td>24.0 ± 7.6</td>
</tr>
<tr>
<td>Platelets (×10⁹/L)</td>
<td>22.9 (9.7-39.5)</td>
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<tr>
<td></td>
<td>285 ± 85</td>
</tr>
<tr>
<td></td>
<td>280 (98-441)</td>
</tr>
<tr>
<td>Absolute lymphocyte count (corrected)(×10⁹/L)*</td>
<td>7.4 ± 2.6</td>
</tr>
<tr>
<td>Absolute nucleated RBCs ×10⁹/L*</td>
<td>6.6 (2.9-12.9)</td>
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<tr>
<td></td>
<td>380 ± 314</td>
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<td></td>
<td>(291 (0-1239))</td>
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</tbody>
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Data expressed as mean ± 1 SD and median (range). There are no significant differences between the 2 groups of twins for any of the parameters except birth weight (P < .01).

* Control group significantly different (P at least < .05) from any of the 2 twins groups.

<table>
<thead>
<tr>
<th>Table IV</th>
<th>Comparison between mean value for each given set of twins and control infants</th>
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<tbody>
<tr>
<td></td>
<td>Twins (n = 37)</td>
</tr>
<tr>
<td>Birth weight (g)*</td>
<td>2664 ± 244</td>
</tr>
<tr>
<td></td>
<td>(2625 (2220-3204))</td>
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<tr>
<td>Hematocrit*</td>
<td>65 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>(66 (54-74))</td>
</tr>
<tr>
<td>White blood cells (corrected) (×10⁹/L)</td>
<td>23.2 ± 6.0</td>
</tr>
<tr>
<td>Platelets (×10⁹/L)</td>
<td>22.7(13.2-35.8)</td>
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<tr>
<td></td>
<td>272 ± 66</td>
</tr>
<tr>
<td>Absolute lymphocyte count (corrected)(×10⁹/L)*</td>
<td>7.1 ± 2.5</td>
</tr>
<tr>
<td>Absolute nucleated RBCs ×10⁹/L*</td>
<td>6.8 (2.9-14.4)</td>
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<tr>
<td></td>
<td>451 ± 376</td>
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<tr>
<td></td>
<td>(352 (0-1833))</td>
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</tbody>
</table>

Data expressed as mean ± 1 SD and median (range).

* Control group significantly different (P at least < .01) from the twins groups.

Comment

We found that twinning is associated with an increase in hematocrit, nucleated RBCs, and lymphocyte counts (all markers of chronic intrauterine hypoxia⁹,¹¹,²³-²⁵) in term AGA concordant infants. In our study we excluded SGA infants, an important confounding variable. We also excluded infants with other factors associated with potential increase in ANRBC counts, including preterm labor with histologic placental signs of chorioamnionitis, maternal smoking,⁰ meconium-stained amniotic fluid, hemolysis, chromosomal anomalies, maternal diabetes⁹,²³ and potential neurologic insults.¹¹,²⁴

In our patient population, there was, as expected, a lower birth weight in twins than in singletons, in spite of the exclusion of small-for-gestational age infants. Also, mothers of twins were in average, older and of lower parity than mothers of singletons, which is expected in a population seeking and using assisted reproduction technology. We conducted a ranked multiple regression analysis, which confirmed that the ANRBC count was higher in twins even after taking into account gestational age and Apgar scores. Thus, we believe that our study unequivocally shows that twinning is an independent risk factor for an increased newborn ANRBC count.

The mechanism by which twinning increases circulating neonatal ANRBC counts is unknown. A likely explanation is “relative” fetal hypoxia. Obviously, such a “hypoxia” would have to be mild enough to not cause fetal demise, but of sufficient duration and or intensity to cause an increase in hematocrit, as observed in this study. Other previously reported indicators of fetal hypoxia in twins include a decrease in birth weight and
In the present study, corrected lymphocyte smear analysis for this study.

Acknowledgments

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