Reference Ranges for Lymphocyte Counts of Neonates: Associations Between Abnormal Counts and Outcomes

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WHAT THIS STUDY ADDS: This study provides reference ranges for lymphocytes of neonates. A high count at birth is associated with early onset sepsis and IVH and a low count with early onset sepsis, IVH, and retinopathy of prematurity. Among neonates with birth asphyxia, a low count identifies a high risk for death.

abstract

BACKGROUND AND OBJECTIVE: Both high and low lymphocyte counts at birth have been associated with adverse outcomes. However, the validity of defining a lymphocyte count as “abnormal” depends on having an accurate reference range. We established a reference range for neonatal lymphocyte counts by using multihospital data and used this to assess previously reported relationships between abnormal counts and early onset sepsis (EOS), intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), periventricular leukomalacia, and birth asphyxia.

METHODS: We first created a data set that excluded counts from neonates with diagnoses previously associated with abnormal lymphocyte counts. Then the complete data (counts excluded plus included in the reference range) were used to test associations between abnormal counts and EOS, IVH, ROP, periventricular leukomalacia, and birth asphyxia.

RESULTS: Lymphocyte counts were retrieved from 40,487 neonates, 10,860 of which were excluded from the reference range. A count >95th percentile was associated with EOS (2.07; 95% confidence interval [CI]: 1.80–2.38) and IVH ≥ grade 3 (2.93; 95% CI: 1.83–4.71). A count <5th percentile was associated with EOS (odds ratio:1.24; 95% CI: 1.04–1.48), IVH ≥ grade 3 (3.23; 95% CI: 1.95–5.36), and ROP ≥ stage 3 (4.80; 95% CI: 2.38–9.66). Among 120 meeting criteria for birth asphyxia, those with a low count and a high nucleated red cell count had higher mortality (57% vs 11%, P = .001), more transfusions (P = .000), and more neurology referrals (P < .01).

CONCLUSIONS: A reference range for lymphocytes can identify neonates with abnormal counts, which can be useful because these neonates are at higher risk for certain adverse outcomes. Pediatrics 2012;129:e1165–e1172

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KEY WORDS: reference range, lymphocyte, diagnosis, outcome

ABBREVIATIONS: CBC—complete blood cell CI—confidence interval EOS—early onset sepsis IVH—intraventricular hemorrhage NRBC—nucleated red blood cells OR—odds ratio PVL—periventricular leukomalacia ROP—retinopathy of prematurity

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Previous studies of blood lymphocyte counts in neonates included relatively small numbers of patients.\textsuperscript{1–5} However, a rigorously defined reference range for lymphocytes, based on very large numbers of neonates, is needed to determine whether it is of value to recognize neonates with abnormal counts. Previous reports suggest that low lymphocyte counts in the first hours after birth are associated with early onset sepsis (EOS),\textsuperscript{6–8} whereas high counts are associated with an increased risk for intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), and periventricular leukomalacia (PVL).\textsuperscript{9} Some data suggest that neonates meeting criteria for birth asphyxia who have a low lymphocyte count are likely to have a poor outcome,\textsuperscript{11} whereas a recent study by Shah et al refutes this finding.\textsuperscript{12}

We reasoned that these and other potential associations with abnormal lymphocyte counts can only be validated once reference ranges are constructed by using very large multihospital data sets, in the manner we previously reported for other elements of the complete blood cell (CBC) count.\textsuperscript{13–10} Thus, this study was conducted in 2 parts: (1) constructing such ranges and (2) testing previously reported associations between abnormal lymphocyte counts and EOS, IVH, ROP, PVL, and outcome after birth asphyxia.

**METHODS**

This was a retrospective analysis of clinical and laboratory data existing in Intermountain Healthcare data repositories. The Intermountain Healthcare Institutional Review Board approved the study as a deidentified data-only investigation not requiring written consent of the individuals.

The experimental approach for creating the reference range was to first obtain all lymphocyte counts on all neonates with a date of birth from January 1, 2001, through December 31, 2010. Next we sought specific diagnoses in the electronic records of mothers and/or neonates, which had been reported to be associated with low or high neonatal lymphocyte counts. The purpose of identifying these neonates was to exclude their lymphocyte counts from the reference range data set, in keeping with the reference range concept.\textsuperscript{15} The exclusionary diagnoses we selected are provided in Table 1. The specific data marts used to identify these exclusionary diagnoses were *International Classification of Diseases, Ninth Revision*, Case Mix (the billing, coding, and financial data mart used by Intermountain Healthcare), and EVOX (the extended Vermont-Oxford database). Information was not available regarding the source (venous, arterial, capillary) of the blood tested for each complete blood count. All lymphocyte counts included in the analysis were manual differential cell counts, where the percent lymphocytes identified on the blood film was multiplied by the leukocyte count performed on an automated hematology analyzer. Manual leukocyte differential cell counts were performed in accordance with Intermountain Healthcare Laboratory Services standard operating procedures by certified medical technologists on Wright-stained blood smears enumerating a minimum of 100 leukocytes per test.

Gestational age was determined by obstetrical assignment unless this was changed by the neonatologist on the basis of gestational age assessment (based on physical examination and neurologic/neurodevelopmental findings). The Vermont/Oxford definitions\textsuperscript{20} were used for EOS, IVH grade $\geq 3$, ROP stage $\geq 3$, and PVL. For the purpose of this study, birth asphyxia was defined as a cord blood gas pH $\leq 6.99$, a base deficit $\geq 16$ mm/L, or a 10-minute Apgar score $\leq 5$.

The program used for data collection was a modified subsystem of “clinical workstation.” Clinical workstation is a Web-based electronic medical record application that stores demographic and clinical information, such as history, physical examination results, laboratory data, problem lists, and discharge summaries. 3M Company (Minneapolis, MN) approved the structure and definitions of all data points for use within the program.

Median, 5th, and 95th percentiles were used to express the reference range data. When neonates had multiple lymphocyte counts in the data repository, only the first value from each patient was used. This is to avoid possible skewing.

<table>
<thead>
<tr>
<th>Maternal Diagnosis</th>
<th>No. of Cases Excluded on This Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abruptio placenta</td>
<td>1327</td>
</tr>
<tr>
<td>Immediate neonatal laboratory/clinical data</td>
<td></td>
</tr>
<tr>
<td>Initial pH $\leq 6.99$</td>
<td>149</td>
</tr>
<tr>
<td>5-min Apgar score $\leq 5$</td>
<td>608</td>
</tr>
<tr>
<td>Early onset bacterial infection (proven)</td>
<td>105</td>
</tr>
<tr>
<td>Infection specific to the perinatal period (suspected or proven, ICD-9 771)</td>
<td>4841</td>
</tr>
<tr>
<td>Neonatal outcomes</td>
<td></td>
</tr>
<tr>
<td>Hypoxic-ischemic encephalopathy</td>
<td>120</td>
</tr>
<tr>
<td>Grade 3 or 4 IVH</td>
<td>219</td>
</tr>
<tr>
<td>PVL</td>
<td>27</td>
</tr>
<tr>
<td>ROP stage $\geq 3$</td>
<td>77</td>
</tr>
</tbody>
</table>

From 40,487 neonates with lymphocyte counts in the Intermountain Healthcare database during the period of study, 10,860 were not included in the reference range data set, whereas the remaining 29,627 were. These exclusions were on the basis of findings elements that have been suspected or reported to alter the lymphocyte count, as shown in the table. In addition to the exclusions shown, 4947 cases were excluded from the reference range data set because we were unable to match maternal and neonatal records. ICD-9, *International Classification of Diseases, Ninth Revision*. 

**TABLE 1** Exclusions From the Reference Range Data Set
due to multiple counts on infants with abnormal counts. The relationship between lymphocyte count and cord pH was assessed by logistic regression, and the relationship between lymphocyte count and 5-minute Apgar score was assessed by analysis of variance.

After constructing the reference ranges, we performed 2 studies by using the new ranges. First, all lymphocyte counts, including those excluded from the reference range data set because of an exclusionary diagnosis, were analyzed to identify those with counts above the 95th percentile value and those with counts below the 5th percentile value. We sought associations between abnormal lymphocyte counts and diagnoses previously said to be more common in neonates with abnormal lymphocyte counts; namely EOS, IVH, ROP, and PVL. For each of these diagnoses, the effect size of having an abnormal lymphocyte count in the first 3 hours after birth was estimated by calculating odds ratios (ORs; 95% confidence interval [CI]) for subsequently diagnosing EOS, IVH, ROP, or PVL, by using logistic regression (Statit, Corvallis, OR).

In the second study using the new reference ranges, we identified all neonates in our data set qualifying for a definition of birth asphyxia, and assessed the relationship between an abnormal lymphocyte count and neonatal death. All lymphocyte counts in the first 72 hours after birth were included in this analysis. Means and SDs were used to express values in groups that were normally distributed, and medians and ranges to express values in groups that were not. Differences in categorical variables were assessed by using Fisher’s exact test or χ² test. Student’s t test was used to assess continuous variables. Statistical significance was set as P < .05. A logistic regression analysis was used to account for potential effects of lymphocyte count/nucleated red blood cell (NRBC) count, gestational age, and birth weight on neonatal death.

RESULTS
Reference Ranges for Lymphocyte Counts

The number of live births in our hospitals during the period studied was 302,284. Lymphocyte counts were archived electronically from 40,487 of these neonates, with gestational ages at birth ranging from 23 to 42 weeks. Of these, 10,860 had an exclusionary diagnosis, and thus the remaining 29,627 were used to construct the reference ranges. The number of lymphocyte counts (1 per patient) at each gestational age that were included in the reference range data set is shown in Fig 1. Antenatal steroids were administered to 4.6% of these, and 27.5% were delivered by caesarean section.

The reference range for lymphocyte counts during the first 3 hours after birth, according to gestational age, is shown in Fig 2. The data shown include only 1 value per patient; the first value obtained (no duplicates, even if the patient had several CBC counts...
drawn). Lymphocyte counts over the first 12 hours after birth are shown in Fig 3. No difference was observed between those born >36 vs ≤36 weeks’ gestation. Lymphocyte counts over the first 28 days after birth are shown in Fig 4. During this period, also no difference was observed between those born >36 vs ≤36 weeks’ gestation.

**Abnormal Lymphocyte Counts and Odds of EOS, IVH, ROP, and PVL**

Using the lymphocyte counts from all 40,487 neonates, higher counts correlated with a lower 5-minute Apgar score (slope = -0.0035, *P* = .000), and with a lower cord pH (slope = -0.0715, *P* = .000; data shown in Supplemental Fig 6). Although these relationships were highly significant statistically, the actual differences were very small.

Table 2 shows the odds that EOS, IVH, ROP, and PVL would subsequently be diagnosed if the lymphocyte count at birth (the first 3 hours) were abnormal. Low lymphocyte counts (<5th percentile) were associated with higher odds of EOS, IVH ≥ grade 3, and ROP ≥ stage 3. High lymphocyte counts (>95th percentile) were associated with higher odds of EOS and IVH ≥ grade 3. Similarly, when the analysis was restricted to only those born at ≤33 weeks’ gestation, a low lymphocyte count at birth was associated with higher odds of subsequently developing an IVH ≥ grade 3 (OR: 1.915; 95% CI: 1.107–3.314) and similarly a high lymphocyte count at birth was associated with higher odds of developing an IVH (OR: 1.979; 95% CI: 1.037–3.778).

**Abnormal Lymphocyte Counts and Outcomes From Birth Asphyxia**

One hundred twenty neonates met criteria for birth asphyxia. Fifty-four of these (45%) had a lymphocyte count below the 5th percentile reference range. When leukocyte counts (white blood cell) of these 54 were corrected for NRBC, a fall in white blood cells during the first 72 hours was proportionate to the fall in lymphocytes. No fall in neutrophils or other leukocytes was found during this period. The group with a low lymphocyte count had a mortality rate of 30%, compared with 11% mortality in the remaining 66 that had a normal lymphocyte count (*P* = .008). Sixty-four of the 120 had an elevated NRBC count (>95th percentile using our previously published NRBC reference range14), and this group had a mortality rate of 26% (compared with 11% in the remaining 56, *P* = .028).
Thirty-eight of the neonates with birth asphyxia had the combination of a low lymphocyte count plus an elevated NRBC count, and this group had a mortality rate of 37%, compared with 11% in the remaining 82 (\( P = .001 \)). Figure 5 shows the lymphocyte counts and NRBC counts of the 120 neonates, grouped into 2 categories: group 1, low lymphocytes plus high NRBC, and group 2 all others.

Groups 1 and 2 did not differ in proportion with placental abruption, uterine rupture, prolonged premature rupture of membranes, chorioamnionitis, fetal bradycardia, nuchal cord, meconium at delivery, cord blood pH, cord blood base deficit, gender, or race (Table 3). However, those in group 1 had slightly lower birth weights, gestational ages, and 10-minute Apgar scores, and had a higher mortality rate. Logistic regression indicated the low lymphocyte plus elevated NRBC combination was significantly associated with death (\( P = .002 \)) independent of gestational age (\( P = .294 \)) or birth weight (\( P = .499 \)).

Death occurred on a median of day of life 4 in both groups (\( P = .656 \)). Survivors of group 1 had a higher rate of referral to pediatric neurology follow-up (10 of 24 in group 1 vs 12 of 73 in group 2, \( P = .01 \)). Also more in group 1 received treatment with inhaled nitric oxide (39% vs7%, \( P = .000 \)), and received more red blood cell, platelet, frozen plasma, and cryoprecipitate transfusions (all \( P = .000 \); Table 4).

**DISCUSSION**

Recognizing that a laboratory test result is abnormal is an integral part of medical practice.\(^{21}\) The recognition process is usually straightforward for adult patients because their test results can be directly compared with normal ranges, determined from large groups of healthy adult volunteers. However, the process is imprecise in pediatric medicine because the comparison groups are not healthy controls but are “reference ranges” constructed by using patient data.\(^{12}\) The process is further complicated for children because “normal” laboratory test results can change markedly according to age.\(^{21}\) We performed the current study firstly to construct reference ranges of high validity for lymphocyte counts of neonates because such ranges have been lacking. Secondly, we sought to use these new ranges to test associations previously made between abnormal lymphocyte counts and outcome.

The second part of our study had 2 aspects. For the first we used the entire sample of 40,487 neonates and identified those neonates with counts below the 5th or above the 95th percentile, testing associations with 4 specific diagnoses: EOS, IVH, ROP, and PVL. For the second part, we identified 120 neonates in our system who qualified for a definition of birth asphyxia and then examined for any association between abnormal lymphocyte counts and outcome.

In constructing the lymphocyte reference ranges, we observed that counts normally drop after birth, a finding also reported by Weinberg et al\(^{11}\) and Shah et al.\(^{12}\) Perhaps this fall relates to the corticosteroid surge during labor and delivery because steroids are known to reduce blood lymphocyte concentrations and can do so rapidly.\(^{22,23}\) We
found that about 4 hours after birth, lymphocyte counts level off and thereafter remain stable during the first 12 hours.

The mechanisms causing these kinetic changes in lymphocyte counts after birth are not known with certainty. Lymphocyte counts can rise or fall on the basis of changes in (1) input of lymphocytes into the circulation, (2) margination (including sequestration in the spleen or lymph nodes), and (3) egress from or destruction within the circulation. Although perinatal changes in corticosteroid concentrations may be involved in regulating fetal and neonatal lymphocyte concentrations, other humoral factors such as catecholamines, cytokines, and growth factors might also contribute.22 23

Our findings are consistent with the reports of Sofatzis et al6 and Godula-Stügl et al,7 who reported that abnormal lymphocyte counts in the first 3 hours are associated with increased odds of EOS. Low counts during EOS might be on the basis of corticosteroid release from the “stress” associated with fetal infection. It is unclear whether antenatal maternal corticosteroid treatment might result in lower lymphocyte counts at birth. We did not find this association, but our study predominantly involved late-preterm and term neonates, and only 4.6% received antenatal steroids.

High lymphocyte counts might occur after endogenous catecholamine release, similar to epinephrine-induced neutrophilia.24 26 We found that both high and low lymphocyte counts were associated with EOS, much in the same way Squire et al reported that both high and low neutrophil counts of neonates can be associated with EOS. Newman et al recently used a very large data set to conclusively demonstrate that blood leukocyte counts and absolute neutrophil counts obtained on potentially infected neonates were most informative when low, and that counts obtained at or near birth were less informative than those about 4 hours after birth. We are uncertain whether this also applies to lymphocyte counts and EOS because to get adequate numbers for analysis we combined all counts in the first 3 hours; however, we analyzed these according to our new reference ranges, which reveal a marked fall over this 3-hour period. Clearly, additional investigation is needed to see whether lymphocyte counts add any value to the other elements of the CBC in identifying neonates with EOS.

Our results are also consistent with the reports of Naeye and Shaffer,9 Phelan et al10 and Shah et al,12 in that both low and high lymphocyte counts at birth were associated with the subsequent development of severe IVH, and low counts were associated with the subsequent development of severe ROP.

FIGURE 5
Lymphocyte counts (A), and NRBC counts (B) of 120 neonates meeting criteria for birth asphyxia, categorized as group 1 (low lymphocyte/high NRBC, n = 38), and group 2 (did not have the combination of low lymphocytes/high NRBC, n = 82). The 5th percentile to the 95th percentile reference ranges are shaded for lymphocytes (A) and for NRBC (B).
observe that the combination of a low lymphocyte count and a high NRBC count was associated with a higher rate of neonatal complications and death. The correlation between high NRBC and poor outcome has also been reported in critically ill adult patients.

Limitations of our study, in addition to those already mentioned, include the flaw that we were unable to determine the source (arterial, venous, capillary) of each lymphocyte count because this information was never recorded. Lymphocyte concentrations can be somewhat higher in capillary blood in a manner similar to the concentration of hemoglobin in heel-stick samples. Surely this flaw resulted in a wider reference range than if only venous samples were included. Moreover, all limitations of retrospective studies apply fully to the present report, including unknown factors regarding why the samples were drawn, how the specimens were handled (variable time between sample and run time), and technician error in cell identification. Fortunately, at Intermountain Healthcare, we obtain manual differential counts on all patients less than 28 days old. This consistency improves interpretability of our findings because we do not have to deal with potential differences between fully automated and manual differential cell counts (although such differences are thought to be trivial in samples from adults).

The CBC count is a commonly ordered test for ill newborn infants and is used to recognize abnormal values for hematocrit and hemoglobin, red blood cell indices, leukocyte count, and immature to total neutrophil ratio, platelet count, and eosinophil count. It seems to us that, although not as well studied as other elements of the CBC, recognizing an abnormal lymphocyte count can also be of value. Such recognition can occur by applying the reference ranges displayed in this report. We encourage others to use these ranges to test additional potential associations with abnormal lymphocyte counts in newborn infants.

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