Early-Onset Neutropenia in Small-for-Gestational-Age Infants

Robert D. Christensen, MD, Bradley A. Yoder, MD, Vickie L. Baer, RN, Gregory L. Snow, PhD, Allison Butler, MS

BACKGROUND: Early neutropenia is more common in small for gestational age (SGA) neonates (birth weight <10th percentile) than in appropriately grown neonates. However, several aspects of this variety of neutropenia are unknown, including the duration, kinetic mechanism, and outcomes.

METHODS: Using 10 years of multihospital records, we studied SGA neonates who, during the first week after birth, had neutrophil counts <1000/μL.

RESULTS: This degree of neutropenia was more common in SGA neonates (6%, 207/3650) than in non-SGA matched controls (1%, 46/3650; P < .001). Neutrophil counts stayed below the lower reference interval for 7 days. Ratios of immature to total neutrophils were within the reference interval, suggesting reduced neutrophil production, not accelerated neutrophil use or destruction. Increased nucleated red cells at birth correlated with decreased neutrophils (P < .001). Neutropenia was not independently associated with maternal hypertensive disorders, over and above the effect of SGA. Of 201 neutropenic SGA neonates, 129 (64%) also had thrombocytopenia. Sixteen percent of neutropenic neonates were treated with recombinant granulocyte colony-stimulating factor (rG-CSF) or intravenous immunoglobulin (IVIG), with no reduction in late-onset sepsis or necrotizing enterocolitis (NEC). Regression analysis showed that neutropenia (but not thrombocytopenia in the absence of neutropenia) was independently associated with increased odds of developing necrotizing enterocolitis (odds ratio 4.01, 90% confidence interval 2.08 to 7.35, P < .001).

CONCLUSIONS: Neutropenia of SGA is a condition of 1-week duration. It is more closely associated with SGA than maternal hypertension (likely owing to neutrophil hypoproduction associated with intrauterine hypoxia), often accompanied by thrombocytopenia, not obviously improved by rG-CSF or IVIG, and associated with an increased risk for NEC.

WHAT'S KNOWN ON THIS SUBJECT: Small for gestational age neonates (weight <10th percentile) are at risk for neutropenia during the first days after birth. However, the duration, responsible mechanism, and outcomes of this variety of neonatal neutropenia are not precisely known.

WHAT THIS STUDY ADDS: Six percent of small for gestational age neonates had neutrophils <1000/μL, with an average neutropenia duration of 7 days. Neutropenia was more closely linked with small for gestational age status than maternal hypertension. This neutropenia is associated with elevated nucleated red blood cell count and increased odds of necrotizing enterocolitis.
METHODS

Patient Information
Data were collected retrospectively as deidentified limited data sets from archived Intermountain Healthcare records. Intermountain Healthcare is a not-for-profit healthcare system that owns and operates 19 hospitals with labor and delivery units in Utah and Idaho. The information collected was limited to the information in this report. Patient records were accessed if the neonate had a date of birth from January 1, 2004, through December 31, 2013. The Intermountain Healthcare Institutional Review Board approved this deidentified data-only study as not requiring the consent of individual subjects.

Blood Cell Counts
Leukocyte counts, neutrophil counts, and ratios of immature to total neutrophils (I/T ratios) were determined in all hospitals with the Beckman Coulter LH750 Hematology Analyzer (Fullerton, CA) from 2004 through mid-2012. After mid-2012, blood cell counts were determined using Sysmex counters (Sysmex America, Lincolnshire, IL). It is standard operating procedure in all Intermountain Healthcare Clinical Laboratories to include a manual differential cell count on all complete blood counts (CBCs) of infants in the first 90 days after birth. All blood tests were performed in accordance with Intermountain Healthcare Laboratory Services standard operating procedures. Leukocyte differential counts were performed by enumeration by certified medical technologists on Wright-stained blood smears, counting a minimum of 100 nucleated cells per test. The reference intervals for CBC parameters are those we published from Intermountain Healthcare databases.14

Gestational age was determined by obstetrical assignment unless this was changed by the neonatologist on the basis of gestational age assessment (physical examination and neurologic-neurodevelopmental findings). Neonates were classified as SGA if their weight at birth was <10th percentile for gestational age, using normative values from our Intermountain Healthcare population.15 Severity of SGA was classified according to 3 categories: severe, less than first percentile; moderate, first to fifth percentile; and mild, sixth to 10th percentile. Cases of maternal hypertension were identified from case-mix records using International Classification of Diseases, Revision 9 definitions 6425 (severe preeclampsia), 6426 (eclampsia), or 6427 (preeclampsia or eclampsia superimposed on existing hypertension). We did not include women with the International Classification of Diseases, Revision 9 code 6424 (mild or nongeneric), because in our previous studies we found that this definition includes transient, resolved, or mild hypertension and that eliminating this code reduces heterogeneity.16 No specific guidelines for administering intravenous immunoglobulin (IVIG) and recombinant granulocyte colony-stimulating factor (rG-CSF) were sanctioned by the Intermountain Healthcare NICUs during this period, but general guidelines were available.7 Early thrombocytopenia was defined as ≤2 platelet counts <150 000/μL during the first week after birth.16

SGA neonates were matched 1:1 with neonates from the same hospitals born during the same period of time who were not SGA. Matching was performed on the basis of gestational age (±1 week) and birth month (±1 month). “Early neutropenia” was defined as an absolute neutrophil count <1000 neutrophils/μL during the first week after birth. Patients whose data would otherwise qualify for inclusion in the neutropenia of SGA group were excluded if they were recognized to have another variety of
neutropenia, such as early-onset sepsis or immune-mediated neutropenia.

A diagnosis of necrotizing enterocolitis (NEC) was recorded if it qualified as Bell stage ≥2.17 Spontaneous intestinal perforation was not included if this entity was recognized as the cause of the bowel dysfunction.18 Late-onset bacterial sepsis (LOS) was recorded if, after day of life 3, 1 blood cultures were positive in concert with clinical signs interpreted by the attending clinician as clinical sepsis and entered into the clinical record as a case of late-onset culture-positive sepsis.19

Data Collection and Statistical Analysis
The program used for data collection was a modified subsystem of Clinical Workstation (3M, Minneapolis, MN); 3M approved the structure and definitions of all data points for use within the program. Data were managed and accessed by authorized data analysts. Means and SDs were used to express values in groups that were normally distributed, and medians and interquartile ranges to express values in groups that were not. Differences in categorical variables were assessed by using the Fisher exact test or χ² test for normally distributed data and the Tukey biweight estimator for groups that were not. Statistical analysis used Statit (Midas, Tucson, AZ) or the R Foundation package (Statistical Computing, Vienna, Austria). The mixed-effects model used NIME, version 3.1-105, also from the R package. Statistical significance was set as P < .05.

RESULTS
Severity and Duration of Neutropenia
During the 10-year period studied, 24,036 neonates were admitted to an Intermountain Healthcare NICU, 3964 of whom were SGA (Fig 1). Of these, 3650 had ≥1 neutrophil counts measured in the first week, and 207 (6%) of those had ≥1 counts <1000/μL, thereby qualifying for the definition of “early” (first-week) neutropenia. Neutropenia was more common among SGA neonates than gestational age–matched control neonates who were not SGA (P < .001) (Fig 1). The 3650 non-SGA controls were well matched with the 3650 SGA neonates on the basis of gestational age (Table 1), but the non-SGA control group had a higher birth weight (as expected) as well as lower rates of Cesarean delivery, eclampsia or preeclampsia, and death in the NICU compared with the SGA neonates.

Four of the 207 neutropenic SGA neonates had early-onset culture-positive bacterial sepsis. In addition, 1 had alloimmune neutropenia, and 1 had Barth syndrome. These 6 neonates, who had alternative explanations for their neutropenia, were excluded from further analyses, leaving 201 with a condition we termed neutropenia of SGA.

Neutrophil counts of the 201 neutropenic SGA neonates are shown in Fig 2. These 201 had a gestational age of 30 ± 4 weeks and a birth weight of 1004 ± 578 g. The reference intervals for blood neutrophils during their first 9 days (5th to 95th percentile limits) for neonates of 28 to 36 weeks’ gestational age range, which we published previously,14 are shown for comparison in the shaded area. The median value of all neutrophil counts in this group, during the first 4 days, was 1400/μL (first quartile 700/μL, third quartile 2500/μL). The lowest neutrophil counts (median nadir of 570/μL, first quartile 400/μL, third quartile 800/μL) was also during the first 4 days. By day of life 7, the neutrophil counts of those with the neutropenia of SGA (95% confidence interval [CI] 1800 to 10,100/μL) were approximately within the reference interval of the gestational age–matched controls (CI 2000 to 12,000/μL14). Neonates with severe SGA (weight less than first percentile) had neutrophil counts in the same range as those...
with moderate (first to fifth percentile) or mild (sixth to 10th percentile) SGA (Fig 3).

**SGA Versus Maternal Hypertension**

Associations of neutropenia with SGA status versus maternal hypertensive disorders were sought by comparing the lowest neutrophil counts during the first 4 days of life among 4 groups of NICU patients matched for gestational age and month of birth (Fig 4). Group 1 consisted of neonates who were SGA but had no record of a maternal hypertensive disorder. These neonates had lower neutrophil counts than did those of Group 2, which consisted of neonates who were not SGA but were born to women with hypertensive disorders during pregnancy (P < .005). Among SGA neonates, the presence or absence of hypertension during pregnancy made no difference in the neutrophil count. Thus maternal hypertension may not be associated with a risk of neonatal neutropenia over and above the risk associated with being SGA. Of 201 infants with neutropenia of SGA, 129 (64%) also had early thrombocytopenia, defined as ≥2 platelet counts <150 000/μL during the first week.

**Nucleated Red Blood Cells, I/T Ratios, IVIG, and rG-CSF**

Among the 3650 SGA neonates, 2981 had a nucleated red blood cell count (NRBC/μL) recorded on the day of birth. The NRBC count inversely correlated, somewhat, with the neutrophil count during the first days after birth (Fig 5) (r = 0.262; P < .001). The subgroup of the most severely weight-restricted (less than first percentile) SGA neutropenic neonates had a trend toward higher NRBC counts (mean ± SEM 2200 ± 420/μL) than did those in the first to 10th percentile (1700 ± 160/μL, P = .06). The weak to moderate association between fetal hypoxia and neonatal neutropenia is similar to the relationship we reported between NRBC and platelet counts in the first week, which we speculated was an effect of intrauterine hypoxia on increased red blood cell production with a subsequent reduction in platelet and neutrophil production.15,20

The 201 neonates with neutropenia of SGA had I/T ratios, as a measure of the leukocyte "left shift," within the normal reference range of 0.00 to 0.25. Moreover, the I/T ratios of these 201 were not different from those of the group of 3443 SGA neonates who did not have neutropenia.

Thirty-four of the 201 neutropenic neonates were treated, during their first days of life, with either IVIG or rG-CSF. These treatments were not by protocol, but rather at the discretion of the various attending neonatologists. Eighteen neonates were treated with IVIG alone (500 to 1000 mg/kg), 11 were treated with rG-CSF alone (1 to 3 doses of 10 μg/kg), and 5 were treated with both medications. Neither IVIG nor rG-CSF treatment appeared to improve outcomes (see Supplemental Table 3). In fact, the 167 neutropenic neonates who received IVIG or rG-CSF were more likely to subsequently develop LOS or NEC (P = .014). Treatment of this neutropenia using IVIG or rG-CSF became progressively less common over the 10-year period studied: of the 34 treated with these medications, 11 were treated in 2004 and 2005, 10 were treated in 2006 and 2007, 7 in 2008 and 2009, 5 in 2010 and 2011, and only 1 in 2012 and 2013.

**Outcomes**

NEC and LOS were diagnosed more commonly among the 201 neonates with neutropenia of SGA than among the 3443 SGA neonates who did not have neutropenia (Table 2). Because of the well-described associations between gestational age and NEC and between gestational age and LOS, we performed regression analysis focusing on the independent contribution of

| TABLE 1: Associations Between SGA Status and Early (First-Week) Neutropenia |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Group** | **n** | **Gestational Age (wks)** | **Birth Weight (g)** | **Gender (%Male)** | **Cesarean Delivery** | **Mechanical Ventilation** | **Surgery** | **Neutropeniaa** | **Gestational Hypertension** | **Mortality Rate c** |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| SGA | 3650 | 36 ± 3 | 2848 ± 360 | 63% | 1034 (44) | 1034 (44) | 1034 (44) | 1034 (44) | 1034 (44) | 1034 (44) | 1034 (44) | 1034 (44) |
| Not SGA | 3650 | 36 ± 3 | 2848 ± 360 | 63% | 1034 (44) | 1034 (44) | 1034 (44) | 1034 (44) | 1034 (44) | 1034 (44) | 1034 (44) | 1034 (44) |

Data are expressed as mean ± SD or n (%). SGA neonates who had 1 CBCs in the first week after birth (n = 3650) were matched 1:1 by gestational age (± 1 wk) and date of birth (± same health system who also had 1 CBCs in the first week.}

b Neutrophil count, 1000/μL during the first week after birth.

c Death in NICU.
neutropenia to these outcomes. As expected, among SGA neonates, higher gestational age correlated with lower odds of acquiring NEC (estimate $-0.15$, OR $0.86$, $P < .001$) and LOS (estimate $-0.16$, OR $0.87$, $P < .001$). Neutropenia was independently associated with higher odds of acquiring NEC (odds ratio [OR] $4.01$, 95% CI $2.08$–$7.35$, $P < .001$). Thrombocytopenia alone (without neutropenia) was not associated with higher odds of acquiring NEC (OR $1.16$, 95% CI $0.72$–$1.79$, $P = .60$). Neutropenia was not independently associated with higher odds of acquiring LOS (OR $1.44$, 95% CI $0.73$–$2.61$, $P = .342$).

FIGURE 2
Blood neutrophil concentrations of 201 neonates with neutropenia of SGA during the first 9 days after birth (mean neutrophil count and SD). The shaded area shows the normal reference interval based on $>100,000$ neutrophil counts of neonates during the first 9 days after birth (modified from Henry and Christensen$^{14}$).

FIGURE 3
Blood neutrophil concentrations (mean and SD) during the first 4 days after birth among 4 groups of NICU admissions: (1) SGA, birth weight less than first percentile; (2) SGA, birth weight first to fifth percentile; (3) SGA, birth weight sixth to 10th percentile; and (4) matched controls who were not SGA (birth weight $>10$th percentile).
DISCUSSION

Neutropenia is classically defined as a blood neutrophil concentration <2 SDs of the appropriate reference-population mean. However, problems arise in applying this definition to newborn infants. First, the normal rise in neutrophil counts during the first day after birth would indicate that neonates who are 6 to 12 hours old are neutropenic with counts as high as 5000/μL, and between the second and fourth days after birth, counts <3000/μL would be termed neutropenic. It seems very unlikely to us that neutrophil counts as high as 3000 to 5000/μL would constitute a host defense deficiency, even if those counts are statistically abnormally low. Perhaps a more clinically meaningful definition of neonatal neutropenia would be a neutrophil count sufficiently low to cause an antibacterial host-defense deficiency, but that condition is difficult to recognize precisely. In fact, the risk of infection imparted by neutropenia is a complex issue, being influenced not only by the neutrophil count, but also by the duration of the low count, the size of the marrow neutrophil storage pool, neutrophil function, and the underlying cause of the neutropenia.

Because of these complexities, rather arbitrary definitions for neonatal neutropenia have been used. One such definition is a count <1500/μL, but it is not clear that counts in the 1000 to 1500/μL range impart any significant host defense deficiency. We desired, in the present analysis, to select a biologically meaningful definition of neutropenia, and chose the definition of a count <1000/μL typically used in older children and adults. We realized before beginning our study that by selecting this definition, our proportion of neonates with neutropenia would be much lower than in all previous reports that defined neutropenia using higher cutoff values. Using this definition, we...
found neutropenia in 6% of SGA neonates versus 1% of matched non-SGA controls. Six of the 207 neutropenic SGA neonates had other conditions that likely could have generated early neutropenia. Excluding these 6 neonates from further analysis, we labeled 201 as having the neutropenia of SGA. We found that their low neutrophil counts remained rather unchanged during the first 4 days after birth and then increased into the normal reference range by day 7 to 9. We recognize a potential problem in applying our neutrophil counts to other published values. Our hospitals are at altitudes ranging from 2661 to 4649 feet (881 to 1417 m) above sea level. We previously reported that our reference intervals have a lower border (95th percentile) than in those with moderate or mild growth restriction (first to 10th percentile). However, we did not see that same pattern in neutrophil counts in the current study.

We speculate that the neutropenia of SGA is due to reduced neutrophil production, not accelerated neutrophil utilization or destruction. This speculation is based on the normal I/T ratios. The neutropenia resulting from accelerated neutrophil usage or destruction (eg, sepsis, alloimmune) is typically accompanied by a left shift,\(^8\,9\,23\,27\,29\)

Several previous studies reported that neutropenia associated with either maternal hypertension or SGA resulted in an elevated risk for subsequently developing LOS.\(^1\,28\,30\,33\) However, other studies did not find that association.\(^25\,26\)

Using a regression analysis focused on those with a neutrophil count <1000/μL, our data suggest that this variety of early neutropenia is associated with a 4-fold increased risk for the development of NEC, and this was the case for the subgroups weighing ≥1500 g and <1500 g at birth. However, although univariate analysis also suggested a higher risk of LOS, this association was explained by other variables, particularly gestational age. In concordance with our findings, Ree et al reported that SGA neonates were 2-fold more likely to develop NEC than appropriate for gestational age–matched controls.\(^34\) Luig et al also found a relationship between SGA and a risk of NEC, but reported that maternal hypertensive disorders of pregnancy were associated with a reduced risk for NEC.\(^35\) Our findings suggest that the subset of SGA infants with neutropenia are at greater risk for NEC than the entire group of SGA neonates. The mechanism by which the NEC risk occurs is not known. It might not be due to the early neutropenia per se, but could result from other immunologic or morphologic abnormalities that accompany the neutropenia of SGA.\(^36\,37\) For instance, perhaps the intestine is more severely injured among the subset of SGA neonates who develop neutropenia than among the SGA neonates who maintain normal neutrophil counts. Additional work is needed to clarify this issue.

Our studies >20 years ago suggested to us that an inhibitor of neutrophil production was present in the placentas of women with preeclampsia, and the resolution of neonatal neutropenia was the result of removing that source of inhibitor at birth.\(^30\) This issue remains unresolved, but the fairly consistent resolution of neutropenia in 6 to 7 days seems consistent with the elimination of an inhibitor at birth. Tsao et al posed the alternative hypothesis that neutropenia of SGA could be the result of inadequate fetal G-CSF production.\(^38\) This is a rational alternative explanation, but it seems to us that if G-CSF production were defective in utero and then reestablished after birth, it might take much longer to generate neutrophils than would the immediate release of an inhibitor from an otherwise ready-to-go system.

### Table 2: Associations Between Early Neutropenia (<1000/μL) and Subsequent Development of Either NEC or LOS in 3644 SGA Neonates

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>NEC*</th>
<th>LOS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGA and neutropenia</td>
<td>201</td>
<td>19 (9.5)</td>
<td>56 (27.9)</td>
</tr>
<tr>
<td>SGA and neutropenia &lt;1500 g</td>
<td>170</td>
<td>15 (8.8)</td>
<td>53 (49.5)</td>
</tr>
<tr>
<td>SGA and no neutropenia</td>
<td>3443</td>
<td>69 (2.0)</td>
<td>161 (4.7)</td>
</tr>
<tr>
<td>SGA and no neutropenia &lt;1500 g</td>
<td>501</td>
<td>23 (4.6)</td>
<td>43 (8.5)</td>
</tr>
</tbody>
</table>

Univariate analysis, SGA and neutropenia vs SGA and no neutropenia, \(P\) = 0.039, \(<0.001\).

Regression analysis, SGA neutropenia vs SGA no neutropenia:

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>(P)</th>
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<tbody>
<tr>
<td>4.01 (2.08–7.35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1.44 (0.73–2.61)</td>
<td>0.342</td>
</tr>
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Data are expressed as \(n\) (%) unless noted otherwise.

* Bell stage ≥2

** Blood culture–positive bacterial sepsis diagnosed >3 days after birth.
Our previous study on thrombocytopenia in SGA neonates and the present work suggest to us that the neutropenia and thrombocytopenia associated with SGA are more directly linked with fetal growth restriction than with maternal hypertension. This conclusion is also the one reached by Wirbelauer et al, namely that it is SGA status, not high maternal blood pressure, that is associated with neonatal neutropenia. In fact, we suggest that what has been termed neutropenia of pregnancy-induced hypertension and thrombocytopenia of pregnancy-induced hypertension might more properly be called neutropenia of SGA and thrombocytopenia of SGA.

The value of administering IVIG and/or rG-CSF to neonates with neutropenia of SGA remains unproven, but from our present analysis, a substantial benefit is unlikely. Although IVIG was given to 9% of our neonates, rG-CSF was given to 5%, and both were given to 2%, we were unable to identify a benefit. A review by Ohlsson and Lacy concluded that administering IVIG to low birth weight neonates reduces their odds of developing LOS, but only by about 3%. LaGamma and colleagues convincingly demonstrated that administering rG-CSF to neutopenic neonates increases their circulating neutrophil counts, but Carr et al found no evidence that prophylactic administration of rG-CSF or rGM-CSF to very low birth weight neonates reduces their odds of subsequently developing a late-onset infection. Whether neonates with neutropenia of SGA would derive any protection from NEC from treatment with IVIG or rG-CSF is unknown and cannot be concluded from these studies, because the numbers treated with IVIG or rG-CSF were so small. However, even if no medications can currently be identified as preventing NEC in these patients, it seems conceivable to us that just the awareness that these neonates are at increased risk could be of some value in their daily care and expectant management.

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ABBREVIATIONS
CBC: complete blood count
CI: confidence interval
I/T ratio: immature to total neutrophil ratio
IVIG: intravenous immunoglobulin
LOS: late-onset sepsis
NEC: necrotizing enterocolitis
NRBC: nucleated red blood cell count
OR: odds ratio
rG-CSF: recombinant granulocyte colony-stimulating factor
SGA: small for gestational age

REFERENCES


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