Late-Onset Neutropenia in Very Low Birth Weight Infants

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ABSTRACT. Background. Neutropenia, defined as absolute neutrophil count (ANC) <1500/mm³, affects 6% to 58% of premature infants in the first week of life. This early-onset neutropenia in premature infants has previously been correlated with sepsis, maternal hypertension, severe asphyxia, and periventricular hemorrhage. Late-onset neutropenia, defined as ANC <1500/mm³ at a postnatal age of ≥3 weeks, has not been previously reported.

Objectives. The purposes of this study were to determine the prevalence of late-onset neutropenia in very low birth weight (VLBW) infants and to examine the factors that may be associated with this phenomenon.

Methods. A weekly complete blood cell count (CBC) was performed routinely in all premature infants with birth weight ≤1500 g (n = 225) admitted to the neonatal intensive care in a 3-year period who survived until discharge. CBC and differentials were recorded at day 1, day 3, and then weekly until discharge. The clinical data of the study infants were collected by reviewing the medical records retrospectively.

Results. Late-onset neutropenia was detected in 51 infants (22%). In both neutropenic (n = 51) and nonneutropenic infants (n = 174), ANC increased postnatally, remained above 5000/mm³ for the first 3 weeks of life, and had a marked decrease at ~4 weeks of age. Thereafter, ANC decreased to a level of ~1400/mm³ in the neutropenic infants and 4000/mm³ in the nonneutropenic infants. The neutropenic infants had a significantly lower nadir ANC, lower hemoglobin, and higher reticulocyte count than did the nonneutropenic infants with similar platelet counts. None of the study infants received erythropoietin during their hospitalization. This late-onset neutropenia occurred at postnatal age of 6 ± 2 weeks (range: 3–10 weeks). The duration of neutropenia was 1.7 ± .7 weeks (range: 1–3 weeks). All of the neutropenic infants had anemia of prematurity with high reticulocyte count and normal platelet count. The neutropenic infants were stable, growing on full oral feedings, and had no signs or symptoms of sepsis. No adverse effects of late-onset neutropenia were apparent in these infants.

Conclusion. Late-onset neutropenia is a common incidental finding in stable, growing VLBW infants that has not been previously reported. Late-onset neutropenia is a phenomenon that occurs in anemic premature infants who have marked reticulocytosis. Normal regulation of hematopoiesis is accompanied by a balance between colony-stimulating factors, such as erythropoietin and granulocyte colony-stimulating factor, which regulate erythropoiesis and granulopoiesis. We speculate that imbalance of these factors with increased reticulocytopenia in response to anemia of prematurity may explain this phenomenon. We recommend avoiding institution of aggressive, potentially harmful therapy for this phenomenon in healthy, growing VLBW infants.

ABBREVIATIONS. CBC, complete blood cell count; ANC, absolute neutrophil count; VLBW, very low birth weight; SGA, small for gestational age; RDS, respiratory distress syndrome; IVH, intraventricular hemorrhage; WBC, white blood cell count; SEM, standard error of the mean; G-CSF, granulocyte colony-stimulating factor.

The complete blood cell count (CBC) with differential is the most common screening test performed in the evaluation of infants admitted to the neonatal intensive care unit. Recognition of significantly abnormal CBC and differential values requires adequate normative data to be available for comparison. The use of absolute neutrophil count (ANC) has improved early recognition and sensitivity in screening for neonatal bacterial sepsis. Several investigators have suggested reference values for neutrophil cell counts in premature infants during the first 28 days of life. This published data may be inadequate when dealing with stable, growing premature infants >28 days of age. Neutrophils are important as a host defense against bacterial infection. When the neutrophil supply is inadequate, neutropenia can develop and the possibility of surviving an infection decreases. Neutropenia, defined as ANC <1500/mm³, affects 6% to 58% of premature infants in the first week of life. This early-onset neutropenia has been reported to occur within the first week of life, with one half of the total episodes starting on the first day of life and approximately two thirds of all episodes lasting <1 week. Christensen, Manroe, and others had proposed several explanations for this early-onset neutropenia. Premature infants may have a decreased neutrophil storage pool and may exhaust their reserve of neutrophils quickly. Neutropenia can be a sign of inadequate neutrophil production or increased consumption. In addition, neutropenia can occur even in the presence of normal neutrophil supply, as during margination of circulating neutrophils. Thus, the detection of neutropenia does not signify...
that the host defense is compromised or that the neutrophil supply is diminished.

Early-onset neutropenia in premature infants has previously been correlated with sepsis, maternal hypertensive, severe asphyxia, and periventricular hemorrhage.\textsuperscript{1,8,13,14,17–19,22–25} Manroe et al\textsuperscript{1} identified neutropenia as the single most accurate predictor of infection in newborn infants. Early-onset neutropenia in premature infants may be associated with an increase in the incidence of early-onset sepsis\textsuperscript{26} and nosocomial infection.\textsuperscript{8,19}

Late-onset neutropenia in stable, growing premature infants, defined as ANC <1500/mm\textsuperscript{3} at a postnatal age of ≥3 weeks, has not been previously reported. The purposes of this study were to determine the prevalence of late-onset neutropenia in very low birth weight (VLBW) infants (≤1500 g) and to examine the factors that may be associated with this phenomenon.

METHODS

All premature infants (≤1500 g) who were admitted consecutively to the neonatal intensive care unit at Sparrow Hospital, Lansing, Michigan, between July 1995 and August 1998 were enrolled in this study. The institutional review board of the hospital approved the study. A weekly CBC and differential was performed routinely in all of these infants during their hospitalization. Infants who expired during hospitalization or were transported to other centers or had congenital anomalies were excluded from the study. Medical records of the remaining premature infants were reviewed retrospectively for birth weight, gestational age, Apgar scores, sex, race, small for gestational age (SGA), respiratory distress syndrome (RDS), ventilator support, oxygen therapy, intraventricular hemorrhage (IVH), history of sepsis, prenatal and postnatal steroids, blood transfusions, age to reach full oral feeding, duration of iron therapy, discharge weight, and length of hospitalization. CBC and differentials were recorded at day 1, day 3, and then weekly until discharge. All blood samples for CBC were drawn either from an umbilical catheter or from a peripheral puncture if an umbilical catheter was no longer present. The majority of the CBC values in the study infants after the third week of life were obtained from heel sticks. White blood cell counts (WBCs) were performed using a Coulter-Stks Analyzer (Beckman Coulter Inc, Miami, FL) then the differentials were analyzed automatically. The laboratory technician then individually reviewed the smears and counted the differential manually.

The ANC was determined by multiplying the total WBC by the percentage of neutrophils. Neutrophils were corrected for the nucleated RBCs. Late-onset neutropenia was defined as ANC <1500/mm\textsuperscript{3} at a postnatal age of ≥3 weeks. Other hematologic data (such as hematocrit, platelet, and reticulocyte count) were recorded. During discharge planning and in coordination with the pediatricians, infants who had late-onset neutropenia at the time of hospital discharge had follow-up CBCs performed until their neutropenia was resolved. In addition, if any of the neutropenic infants were rehospitalized within 6 months after discharge, the medical records were reviewed to identify the cause of their hospitalization.

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM) unless stated otherwise. Student’s t test, x\textsuperscript{2} test, Mann-Whitney rank-sum test, and 2-way analysis of variance with repeated-measures of 1 factor were used for statistical analysis. A P value of <.05 was considered to indicate statistical significance.

RESULTS

During the study, 277 infants with a birth weight ≤1500 g were admitted to the neonatal intensive care unit. Fifty-two infants were ineligible: 36 expired, 10 were transported to other centers, and 6 had congenital anomalies. The number of infants who were eligible and who were included in the study was 225 infants. After collecting the data, the infants were divided into 2 groups: infants who developed neutropenia (n = 51; 22%) and infants who did not develop neutropenia (n = 174; 78%).

ANC increased postnatally in both neutropenic and nonneutropenic infants and remained above 5000/mm\textsuperscript{3} for the first 3 weeks of life and had a marked decrease at ~4 weeks of age (Fig 1). Subsequently, the neutrophil counts decreased to a level of ~1400/mm\textsuperscript{3} in the neutropenic infants and 4000/mm\textsuperscript{3} in the nonneutropenic infants. The hematocrit was high initially and started to decrease gradually in both groups. The hematocrit tended to be lower in the neutropenic infants (Fig 2). Reticulocyte count was high initially in both groups and decreased by week 2 of life. Reticulocyte count started to increase in both groups by week 4 of life and was significantly

Fig 1. ANC (linear) of the neutropenic and nonneutropenic infants. Numbers in parenthesis represent the number of infants that had CBC at that data point. Error bars represent mean ± SEM. *P < .05 versus nonneutropenic infants.
higher in the neutropenic infants by week 7 of life (Fig 3). There was no difference in platelet count between the 2 groups (Fig 4). Late-onset neutropenia occurred at a postnatal age of $6 \pm 2$ weeks (range: 3–10 weeks), and the duration of neutropenia was $1.7 \pm .7$ weeks (range: 1–3 weeks). Our ability to detect the exact duration is limited because of early hospital discharge. Some infants were discharged as early as the third week of life in both groups. So, the incidence of late-onset neutropenia may have been higher if the nonneutropenic infants had been followed beyond hospital discharge. All the neutropenic infants had anemia of prematurity with hemoglobin of $9.7 \pm 1.1$ g/dL (range: 6.7–11.8 g/dL), high reticulocyte count of $5.4\% \pm 2.1\%$ (range: 3%–10.4%), and normal platelet count ($391 \pm 128 \times 10^3$/mm$^3$). None of the study infants received erythropoietin during their hospitalization. The neutropenic infants were stable, growing on full oral feedings, and had no signs or symptoms of sepsis. No adverse effects of late-onset neutropenia were apparent in these infants. There was no difference between the infants who developed late-onset neutropenia ($n = 51$) and those who did not ($n = 174$) in gestational age ($28 \pm 2$ weeks vs $28 \pm 2$ weeks), birth weight ($1011 \pm 265$ g vs $1088 \pm 270$ g), Apgar scores, race, sex, and the number of infants who were SGA (Table 1). Also, there was no difference between the groups in incidence of RDS, duration of mechanical ventilation, oxygen therapy, incidence of patent ductus arteriosus, IVH, sepsis, exposure to antenatal and postnatal steroids, the need for blood transfusion, age to reach full oral feedings, duration of iron therapy, the incidence of early-onset neutropenia, discharge weight, and the length of hospitalization. Neutropenic infants had a significantly lower nadir ANC than did nonneutropenic infants ($1067 \pm 44$/mm$^3$ vs $4062 \pm 113$/mm$^3$; $P < .001$), lower hemoglobin ($9.7 \pm .2$ g/dL vs $21.2 \pm .1$ g/dL; $P < .001$), higher reticulocyte count ($6.1\% \pm .4\%$ vs $4.9\% \pm .3\%$; $P < .01$) but similar platelet counts ($391 \pm 19$/mm$^3$ vs $944 \pm 18$/mm$^3$; $P = $ not significant).

Fig 2. The hematocrit (%) of the neutropenic and nonneutropenic infants. Error bars represent mean ± SEM. *$P < .05$ versus nonneutropenic infants.

Fig 3. The reticulocyte count (%) of the neutropenic and nonneutropenic infants. Error bars represent mean ± SEM. *$P < .05$ versus nonneutropenic infants.
Fifty percent of the infants with late-onset neutropenia (26/51) had their ANC increased to a level above 1500/mm³ before their hospital discharge, while 24 infants had their ANC increased to that level within 1 month after discharge. One infant had intermittent neutropenia that recovered by 2 months after discharge. Seventeen percent of the infants with late-onset neutropenia (9/51) were rehospitalized within 6 months after discharge. Seven infants were hospitalized for hernia repair, 2 infants for apnea, and none of them had sepsis or neutropenia. All of these infants were rehospitalized to our regional children’s medical center.

**DISCUSSION**

Late-onset neutropenia that occurs in stable, growing premature infants has not been previously reported. In this review, late-onset neutropenia occurred in 22% of VLBW infants. Even when neutropenia was defined as ANC <1000/mm³, the incidence was still as high as 10% of the study infants (22/225 infants). This late-onset neutropenia occurred at ≥3 weeks of age, in the absence of the known risk factors for this phenomenon, and lasted for 1 to 3 weeks. Our ability to detect the exact incidence and duration of this type of neutropenia is limited because of the early hospital discharge of some infants. The incidence and duration may even be higher if the CBCs were followed for a longer time in those infants who were discharged from the hospital early.

Koenig and Christensen¹⁹ and Xanthou² have shown significant relationship between gestational age, birth weight, and increased susceptibility of VLBW infants to neutropenia. There was no difference between the neutropenic and nonneutropenic infants in gestational age and birth weight as shown in Table 1. Additionally, none of these neutropenic episodes were attributable to isoimmune neutropenia or postexchange transfusion neutropenia as has been shown in early-onset neutropenia.¹³,¹⁴ Gessler et al¹⁵ have previously shown that there was a high incidence of neutropenia in premature infants after 1 week of therapy with certain antibiotics. The number of infants who had sepsis that required a full course of antibiotics was similar in both neutropenic and nonneutropenic infants. In addition, none of the neutropenic infants in our study were on antibiotics for at least 2 to 3 weeks before detection of late-onset neutropenia. Halperin et al²⁷ have shown that erythropoietin therapy in premature infants can be complicated by development of neutropenia. None of the infants in our study were treated with erythropoietin.

The exact mechanism responsible for the reduced ANC in our patients is not known. The neutropenic infants in our study were anemic with significantly lower hemoglobin and higher reticulocyte count, compared with nonneutropenic infants. Previously, Manroe et al¹ observed a similar phenomenon and found that newborn infants who developed neutropenia had reticulocytosis occurring at 14 days of postnatal age. In addition, Shannon et al²⁸ observed an inverse relationship between reticulocytic and neutrophil counts in premature infants. Normal regulation of hematopoiesis is accompanied by a balance between colony-stimulating factors, such as erythropoietin and granulocyte colony-stimulating factor (G-CSF), which regulate erythropoiesis and granulopoiesis.²⁹–³¹ We postulate that late-onset neutropenia may be the result of a decrease in neutrophil-specific differentiation or growth factors imbalance or combinations of the 2.

The studies of Chen, Nicola, and others³²–³⁵ have suggested a mechanism for down-modulation of neutrophil production in newborns with neutropenia. These investigators observed that when 1 type of hematopoietic growth factor becomes bound to its cell-surface receptors, receptors for other hematopoietic growth factors may be down-regulated. Thus, the receptors on hematopoietic progenitors that are specific for neutrophilic growth factors may undergo down-modulation by the previous interaction of the
receptors with other hematopoietic growth factors such as erythropoietin.

One other possible mechanism for the development of late-onset neutropenia is imbalance of hematopoietic growth factors that regulate hematopoiesis. Christensen et al. previously observed that human fetal progenitor cells exposed to extraordinarily high concentrations of erythropoietin had a down-modulation of neutrophil production. Koenig and Christensen have also observed that newborns with concurrent Rh hemolytic disease and neutropenia have a very high level of erythropoietin and a marked increase in erythropoiesis. These investigators speculated that erythropoiesis in these neutropenic infants may be accompanied by a down-modulation of neutrophil production. Other investigators have shown spontaneous increase in endogenous production of erythropoietin associated with reticulocytosis in premature infants. Therefore, we speculate that the majority of progenitor cells that are available for hematopoiesis may be consumed during the enhanced erythropoiesis secondary to anemia of prematurity and may limit their availability for granulopoiesis. This may lead to a decrease in neutrophil production, failure of neutrophil storage pool mechanisms to replace the consumed neutrophils, and ultimately a lower ANC and neutropenia. We did not measure erythropoietin or other cytokine concentrations in the present study. Whatever the causative mechanism, we propose that this type of neonatal neutropenia may be the result of a transient decrease in neutrophil production during erythropoiesis.

The ANCs in our study infants (Fig 1) have a similar trend to neutrophil counts in previous studies. The postnatal changes in hematocrit and reticulocyte counts may help to explain the mechanism of this trend. The high hematocrit and reticulocyte counts in premature infants at birth are manifestations of the high erythropoiesis in utero. Subsequently, during the first 3 to 4 weeks of life, premature infants received multiple blood transfusions to maintain their hematocrit. Blood transfusions have been shown to be associated with a decrease in erythropoiesis. This may lead to an increase in the availability of progenitor cells for granulopoiesis and ultimately to a postnatal increase in the ANC in both neutropenic and nonneutropenic infants (Fig 1). The decrease in ANC at ~4 weeks of age was probably attributable to the decrease in blood transfusion requirements, lower hematocrit,
and enhancement of erythropoiesis with subsequent decrease in granulopoiesis as discussed earlier.

ANCs tended to be lower in the neutropenic than in the nonneutropenic infants from day 1 of life. There is no clear explanation for this tendency and it seems that this tendency could be a variance of normal, because both groups of infants were similar in the severity of their illness (Table 1). In addition, birth weight, number of infants who were SGA, age to reach full oral feeding, and discharge weight were similar in both groups (Table 1).

During the first 3 to 4 weeks of life, premature infants are critically ill with multiple foreign bodies, such as endotracheal tubes, central or peripheral lines that increase their susceptibility to infection, especially if they have early-onset neutropenia. G-CSF increases proliferation of progenitor cells and enhances the cell function of neutrophils.40,41 Multiple studies have shown a possible beneficial effect of the use of G-CSF in premature infants, especially those with early-onset neutropenia.42–48 We doubt that such a therapy would be of any benefit for premature infants with late-onset neutropenia. These infants are usually stable without the risk factors that may increase their susceptibility to infection. In addition, 50% of the infants with late-onset neutropenia had their ANC increased to a level above 1500/mm³ before discharge, while the remaining neutropenic infants had their ANC increased to that level within 1 month after hospital discharge, with the exception of 1 infant who had intermittent neutropenia that recovered by 2 months after discharge. All of these neutropenic infants were stable, growing, and had no signs or symptoms of sepsis. Late-onset neutropenia is probably a benign developmental phenomenon in VLBW infants with anemia of prematurity and reticulocytosis. The number of infants with late-onset neutropenia was 51, which is too small to statistically power the possibility that nosocomial infection is absent in these infants. We recommend avoiding the institution of aggressive therapy such as G-CSF for this phenomenon until further studies are performed to examine the incidence of nosocomial infection in stable, growing VLBW infants with late-onset neutropenia.

The ANC in our study infants, especially in the neutropenic infants, were below the lower limit of the reference values established by Manrooe et al1 and Xanthou2 but were similar to the reference values established by Mouzinho,3 These reference values were based on data obtained during the first 28 days of life.1–3 The high incidence of late-onset neutropenia suggests the need for development of reference ranges for neutrophil counts among VLBW infants >28 days of age. In addition, further investigation will be needed to clarify the exact mechanism responsible for the development of late-onset neutropenia in stable, growing VLBW infants.

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