Serum Concentrations of Granulocyte Colony-Stimulating Factor in Healthy Term and Preterm Neonates and in Those With Various Diseases Including Bacterial Infections

By Peter Gessler, Nicole Kirchmann, Rosemarie Kientsch-Engel, Nikolaus Haas, Peter Lasch, and Walter Kachel

BACTERIAL SEPSIS is a life-threatening event occurring in approximately 1 to 10 neonates per 1,000 live births.1-3 A number of abnormalities have been described in the host defense system of newborn infants.4,5 One of the most important deficits seems to be the quantitative deficiency of the myeloid and phagocytic system.6,7 During bacterial sepsis, neonates tend to develop neutropenia because of reduced mature effector neutrophil storage pools, reduced myeloid progenitor pools (colony-forming unit-granulocyte-macrophage [CFU-GM]), and accelerated steady state myeloid progenitor proliferative rates.8-10 Additionally, neonatal mature neutrophils have qualitative deficiencies, especially with respect to chemotaxis, phagocytosis, C3bi receptor expression, and bactericidal killing compared with adult neutrophils.11-14

Granulocyte colony-stimulating factor (G-CSF)15,16 has been shown to stimulate myeloid progenitor proliferation,17-19 to increase bone marrow neutrophil storage pool (BM NSP), to induce neutrophilia in the peripheral blood,20 and to enhance mature neutrophil effector functions.21-24 Furthermore, in newborn rats with experimental group B streptococcal sepsis, administration of recombinant human G-CSF (rhG-CSF) was synergistic with antibiotic therapy in reducing the mortality rate.25 Similar beneficial results of rhG-CSF treatment were reported in mice with Pseudomonas aeruginosa infection26 and in mice with lipopolysaccharide (LPS)-induced septic shock.27

With these promising results in mind, future therapy of neonatal sepsis or its prevention may include adjuvant prophylactic or therapeutic administration of rhG-CSF.28 However, nothing is known about the endogenous production of G-CSF in neonates during bacterial sepsis. Moreover, except for measurements of G-CSF in cord blood,29 G-CSF levels in healthy term and preterm neonates have not been reported. The present study was undertaken to determine G-CSF levels in healthy term and preterm neonates and during infections. Perinatal risk factors supposed to influence G-CSF levels and the incidence of bacterial infections were evaluated.

MATERIALS AND METHODS

Patients

The studied population included infants admitted to the Department of Pediatrics, University Hospital of Mannheim, during the 6 months from July 1, 1992 through January 1, 1993 (n = 156). Neonates older than 12 hours at the time of admission and infants with severe life-threatening malformations were excluded. Informed written consent was obtained from the parents of each newborn.

Bacterial infection in an ill neonate was confirmed in case of a positive blood culture. Infection was presumed in a clinically symptomatic child in case of Pneumatoasis intestinalis or pneumonia on chest roentgenogram and whenever two or more of the following criteria were fulfilled: positive C-reactive protein, fibrinogen less than 100 mg/dL or greater than 400 mg/dL, platelet count less than 150 X 10^9/L, immature to total (I/T) neutrophil ratio ≥ 0.16, positive skin and gastric culture, maternal history (fever, signs of infection, or rupture of membrane ≥ 24 hours).3 Nasopharyngeal and urinary viral cultures and stool rotavirus tests were performed when clinically indicated.

Antibiotic treatment was performed as thought to be clinically indicated. Cefotaxime and amoxicillin were the first line regime for children weighing less than 1,500 g, amoxicillin and gentamicin for children greater than 1,500 g, which was later on changed according to the results of bacterial culture or clinical course.

The diagnosis of maternal pregnancy-induced hypertension was based on two measurements obtained at least 6 hours apart of a
diastolic blood pressure greater than 90 mm Hg or a systolic blood pressure greater than 140 mm Hg, or an elevation from base line of greater than 30 mm Hg in the systolic pressure or of greater than 15 mm Hg in the diastolic pressure.30

**Blood Cell Counts**

Routinely, a complete blood cell (CBC) count was obtained from all infants on admission and subsequently as thought to be indicated by the clinicians responsible for the infant’s care. The CBC included a nucleated cell count (Sysmex F 800 microcellcounter), platelet count, and a 100-cell differential count. The nucleated cell count represented the total white blood cell (WBC) count, corrected for the presence of nucleated red blood cells. A total neutrophil cell (TNC) count was calculated for each CBC count by multiplying the total WBC by the percentage of polymorphonuclear leukocytes and band forms. An I/T neutrophil ratio was calculated for each CBC count as the combined percentiles of immature neutrophils (band forms, metamyelocytes, and myelocytes) divided by the total percentage of neutrophils in the peripheral blood. Blood samples for CBC count were obtained from heelpricks and peripheral venipunctures.

**Enzyme-Linked Immunosorbent Assay (ELISA) for Detecting Human G-CSF**

Together with clinically indicated blood tests, an additional amount of 0.6 mL blood was taken for G-CSF determination by peripheral venipuncture. Samples were obtained usually upon admission, between days 4 and 6 of life, between weeks 2 and 3 of life, and whenever an infection was suspected. In some cases, cord blood was also available. The blood specimens were immediately centrifuged and the serum was stored at −36.4°F. For determination of G-CSF in human serum, the sandwich enzyme immunoassay technology was used. All incubation steps were performed in irradiated microtiter plates from polystyrol with 96 F-wells (Maxisorp; Nunc) at room temperature with agitation on a shaker table. Microtiter plates, precoated with streptavidine, were coated with 125 µL/well of biotinylated mouse monoclonal anti-G-CSF antibody (clone KM 341; Kyowa) in a concentration of 2 µg/mL and incubated for 1 hour. The plate was then washed three times with 0.9% (wt/vol) NaCl/0.1% (wt/wt) Tween 20. Serum samples or standards were diluted with an equal volume of incubation buffer (Enzymuny LH-assay) and 100 µL/well of this mixture was incubated for 1 hour. Standards were prepared by dissolving known amounts of recombinant human G-CSF in a pool of normal sera, from which G-CSF had been removed by affinity chromatography with polyclonal anti–G-CSF antibodies from rabbit. The next washing was followed by an incubation with 100 µL/well of the second antibody, a serum pool from three rabbits that had been purified by ion-exchange chromatography, cleaved to Fab particles, and conjugated to horseradish peroxidase. The conjugate with 125 mU POD activity/mL in the same incubation buffer as above was incubated for 1 hour and washed afterwards as before. The dye reaction was performed in 40 mmol/L citrate/60 mmol/L phosphate buffer, pH 4.5, with the addition of 3.3 mmol/L sodium perborate and 3.25 mmol/L ABTS [2,2’-azino-di-(3-ethylbenzthiazoline sulfonate)]. The color developed during 30 minutes without shaking and its absorbance was measured in a microtiter plate photometer (SLT 400 AT) at 405 nm, with a reference wavelength of 492 nm. All samples and standards were measured in duplicate and the absorbance means were calculated. From the standard means, a calibration curve was fitted by a least squares linear regression was performed. Results are expressed as rate percent and mean ± standard error (SE) unless otherwise expressed.

**RESULTS**

**Serum Concentrations of G-CSF and TNC Counts of Different Newborns Without Signs of Infection at Birth, at Various Times of Admission, and Later on Until Week 3 of Life**

Mean serum concentrations of G-CSF at birth were higher in neonates older than 37 gestational weeks compared with those in neonates with gestational age 32 to 37 weeks. Cord blood (CB) values of these neonates were 261 ± 23 pg/mL versus 126 ± 68 pg/mL (P = 0.48) (Fig 1). No difference was detectable in CB G-CSF values of neonates with gestational age 32 to 37 weeks compared with those with gestational age 26 to 31 weeks (141 ± 67 pg/mL).

At admission, mean G-CSF levels of neonates with gestational age 32 to 37 weeks and of those older than 37 weeks were highest between 4 and 7 hours of life, with peak levels of 158 ± 22 pg/mL versus 228 ± 40 pg/mL, respectively. Neonates with gestational age 26 to 31 weeks had a mean G-CSF value of 77 ± 30 pg/mL between 4 and 7 hours of life (difference between gestational age groups P = 0.7). In neonates younger than 32 gestational weeks, no data can be shown for the time intervals between 1 through 3 and the 8 through 12 hours of life, because no clinically indicated peripheral venipuncture was performed. The relationship of

**Statistical Analysis**

Statistical analysis was performed using the Mann-Whitney test (for unpaired continuous variables, two-tailed P value) and the χ² test with Yates continuity correction for categorical variables. For multiple comparisons, analysis of variance (ANOVA) with Bonferroni correction. For calculation of correlation coefficient r, least squares linear regression was performed. Results are expressed as rate percent and mean ± standard error (SE) unless otherwise expressed.
G-CSF values between 4 and 7 hours of life to gestational age is shown in Fig 2 (n = 64; correlation coefficient r = .48; P < .0002; n = 64 data points). G-CSF values between 4 and 7 days of life and 2 and 3 weeks of life were lower compared with the time interval between 4 and 7 hours of life and there was no significant difference between the three gestational age groups.

Mean TNC counts (Fig 3) were highest at the time interval between 7 and 12 hours of life in all three gestational age groups. The difference of mean TNC counts between the gestational age groups was significant until 72 hours of life (P < .005), except for direct comparison of neonates younger than 32 gestational weeks of age to neonates 32 to 37 gestational weeks of age (significant difference of these two gestational age groups only in neonates younger than 6 hours of life, Bonferroni P value < .001).

**G-CSF Values and TNC Counts in Twins**

At the time of admission, G-CSF values of twins (dicigotic, n = 9) were lower in the first twin compared with the second born (117 ± 23 pg/mL [n = 9] v 252 ± 71 pg/mL [n = 9]; P = .02). In twins delivered spontaneously (n = 5), the respective values were 122 ± 39 pg/mL versus 307 ± 125 pg/mL; in twins delivered by caesarian section (n = 4), the respective values were 110 ± 25 pg/mL versus 184 ± 40 pg/mL.

After that time, G-CSF values of the second born determined between days 4 and 7 and weeks 2 and 3 of life were slightly higher compared with those of the first born (44 ± 17 v 26 ± 7 pg/mL and 31 ± 17 v 16 ± 4 pg/mL). However, this difference was not statistically significant. The difference in TNC counts of twins at any time until week 3 of life was not significant.

**Effect of Perinatal Events on G-CSF Levels, TNC Counts, and Rate of Infections**

**Maternal hypertension.** Neonates born to hypertensive mothers (n = 21) compared with neonates born to nonhypertensive mothers without signs of infection (n = 100) tended to have lower G-CSF levels (146 ± 42 pg/mL [n = 10] v 182 ± 21 pg/mL [n = 54]) and TNC counts (7.4 ± 0.8 v 10.0 ± 0.6 × 10^9/L) between 4 and 7 hours of life (not significant). Later on, G-CSF levels and TNC counts showed no difference. The rate of presumed infections occurring until day 5 of life was equal in both groups.

**Maternal treatment with glucocorticoids for prevention of respiratory distress syndrome (RDS).** Neonates without signs of infection born to mothers treated with glucocorticoids (n = 26) tended to have lower G-CSF levels between 4 and 7 hours of life than those without maternal glucocorticoid treatment (n = 40, comparable gestational age) (117 ± 18 pg/mL [n = 19] v 174 ± 41 pg/mL [n = 18]; not significant). In contrast, TNC counts at that time were higher in out signs of infection (n = 28) had different G-CSF levels and TNC counts compared with those small for gestational age (SGA) newborns (birth weight <2,500 g, gestational age ≥38 weeks) (n = 13). G-CSF levels determined between 4 and 7 hours of life were higher in AGA neonates (284 ± 54 pg/mL, n = 13) compared with SGA neonates (114 ± 31 pg/mL, n = 8) (P = .05). Later on, there were no significant differences in G-CSF levels between the two groups: G-CSF values between days 4 and 7 of life, 24 ± 6 pg/mL (n = 13) v 21 ± 6 pg/mL (n = 12), and between 2 and 3 weeks of life, 24 ± 14 pg/mL (n = 7) v 8 ± 4 pg/mL (n = 9). Mean TNC counts determined between 4 and 7 hours and between days 4 and 7 of life were higher in AGA neonates (12.7 ± 1.4 v 9.2 ± 1.7 × 10^9/L and 4.2 ± 0.5 v 2.7 ± 0.2 × 10^9/L; P = .04). Of a total number of 34 AGA neonates, 6 infants showed signs of infection compared with 3 of 16 SGA neonates.
neonates of corticoid-treated mothers (8.6 ± 0.9 v 6.8 ± 0.6 x 10^9/L; P = .05). Later on, G-CSF levels and TNC counts showed no difference, which was also true for the rate of presumed infections in both groups.

**RDS.** There was no difference in G-CSF levels of non-infected neonates with (n = 7) and without RDS (n = 60, comparable gestational age) at any time, nor for TNC days 4 and 7 and weeks 2 and 3 of life, TNC counts were higher in neonates with RDS (7.6 ± 1.0 v 3.2 ± 0.2 x 10^9/L; P = .001; and 4.5 ± 0.1 v 3.5 ± 0.5 x 10^9/L; P = .02). The rate of presumed infections was higher in newborns with RDS. Of a total of 12 infants with RDS, 5 had signs of infection (positive blood culture, n = 3) until the day 5 of life, compared with 8 of 68 infants without RDS (positive blood culture, n = 1) (P = .02).

Perinatal asphyxia defined as a 1-minute APGAR score ≤5 had no influence upon G-CSF levels, TNC counts, and rate of presumed infections.

**Maternal infection.** Neonates born to mothers with signs of bacterial infections (positive blood culture, fever >38.5°C, CRP >120 mg/L, premature rupture of membranes, and chorioamnionitis) (n = 17) but without signs and symptoms of neonatal infection (n = 12) had higher levels of G-CSF determined between 4 and 7 hours of life compared with neonates of noninfectious mothers (709 ± 374 pg/mL [n = 5] v 176 ± 19 pg/mL [n = 64]; P = .05). Between days 4 and 7 and weeks 2 and 3 of life, there was no difference in G-CSF levels between the two groups (31 ± 6 v 29 ± 4 pg/mL and 18 ± 8 v 25 ± 5 pg/mL). Mean TNC count of neonates of infectious mothers between 4 and 7 hours was 11.6 ± 2.3 x 10^9/L compared with 9.1 ± 0.6 x 10^9/L of noninfectious mothers (not significant). Later on, TNC counts in both groups were similar. The rate of presumed infections of neonates born of infectious mothers was 29% compared with 14% of noninfectious mothers (P = .09).

**Neonatal Infection: Incidence, G-CSF Values, and TNC Counts**

The overall rate of presumed bacterial infections was 22% (n = 35). Blood culture was positive in 26% of cases of neonatal infection. Positive skin and gastric cultures were found in 29% and 34%, respectively (B streptococcus, n = 6; Staphylococcus epidermidis, n = 6; Escherichia coli, n = 3; Pseudomonas aeruginosa, n = 2; Enterococci, n = 2; others, n = 3). There were 2 cases of NEC (6%), 1 case of pneumonia, and no case of proven viral infection.

The overall rate of infections correlated with the gestational age of the neonates, occurring more often in younger infants. Of 17 neonates younger than 32 gestational weeks, 10 had signs of infection during their hospital stay (59%). Neonates with gestational age 32 to 37 weeks (n = 85), 15 had signs of infection (18%). Of neonates older than 37 gestational weeks of age (n = 54), 10 had signs of infection (19%) (P = .0007). However, younger infants stayed in the hospital for a longer period of time, thus having a greater chance of acquiring a nosocomial infection. Therefore, it is necessary to analyze the rate of infections during the first 5 days of life. Again, there was a relation of the rate of infections to gestational age: 29% (n = 5 of 17) in neonates less than 32 gestational weeks of age, 12% (n = 10 of 85) in neonates 32 to 37 gestational weeks of age, and 19% (n = 8 of 54) in neonates older than 37 gestational weeks of age (P = .03).

With respect to the dynamics of G-CSF levels and TNC counts during the first 5 days of life (Figs 1 and 3), neonates with signs of infection between 4 and 7 hours of life were compared with neonates without signs of infection at that time (Fig 4). G-CSF levels of infected neonates at that time (n = 12) were 1,312 ± 396 pg/mL compared with 176 ± 19 pg/mL in noninfected neonates (n = 64) (P < .0001). TNC count at that time was 11.9 ± 3.9 v 9.2 ± 0.6 x 10^9/L (infected v noninfected; P = NS), with an immature neutrophil cell count of 1.9 ± 0.8 x 10^9/L (I/T ratio = 0.16 ± 0.06). Mean TNC counts as well as the number of immature neutrophil cells were highest between 7 and 12 hours of life.

G-CSF levels determined between days 4 and 7 of life were not significantly different in infected versus noninfected neonates (47 ± 18 v 30 ± 4 pg/mL). However, TNC counts of infected neonates differed significantly: 7.8 ± 2.1 v 3.5 ± 0.2 x 10^9/L; P = .0047).

Between weeks 2 and 3 of life, G-CSF levels and TNC counts of infected and noninfected neonates were equal.

G-CSF levels of neonates determined at the time of first clinical signs of neonatal infection during the first 5 days of life and after that time were not significantly different compared with 4 and 7 hours of life, with a minimum of 216 pg/mL and a maximum of 350,000 pg/mL.

**DISCUSSION**

Serum concentrations of G-CSF in term and preterm neonates without signs of infection reached peak levels during the first 7 hours of life. However, mean TNC counts were highest between 7 and 12 hours of life,31 suggesting a time-effect of the inflammatory response to infection.
increased susceptibility of neonates to bacterial infections. Further studies of G-CSF in infected neonates are needed to assess the role of this cytokine in life-threatening infections during the first days of life.\textsuperscript{20,48,49}

**ACKNOWLEDGMENT**

The authors thank Dagmar Knodel for technical assistance and the nurses of the neonatal care unit for their cooperation.

**REFERENCES**

20. Gillan E, Christensen R, Suen Y, Hunter D, van de Ven C,

C, Modanlou H: Prophylactic or simultaneous administration of control trial of rhG-CSF in newborns with presumed sepsis. Blood


Serum concentrations of granulocyte colony-stimulating factor in healthy term and preterm neonates and in those with various diseases including bacterial infections

P Gessler, N Kirchmann, R Kientsch-Engel, N Haas, P Lasch and W Kachel