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## ORIGINAL ARTICLE

# *Assessing the efficacy of the recombinant human granulocyte colony-stimulating factor in the treatment of early neonatal sepsis in premature neonates*

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### Abstract

**Objective:** to evaluate the efficacy of the recombinant human granulocyte colony-stimulating factor (rhG-CSF) in the treatment of early-onset neonatal sepsis among premature infants.

**Materials and Methods:** a double-blind, randomized, placebo-controlled trial was performed among forty-four preterm neonates who had "clinical diagnosis" of early-onset sepsis. The treatment group (n=22) received 10µg/kg/d of rhG-CSF, IV once daily for three consecutive days, and the placebo group (n=22) received the same volume of a visually-indistinguishable vehicle. Prior to the first dose, and prior to the second and third doses, and again 10 days after the first dose, we measured tumor necrosis factor-a, interleukin-6, granulocyte-macrophagocyte colony-stimulating factor, G-CSF, leukocyte count, absolute neutrophil count, immature/total neutrophil ratio, platelet count, and hemoglobin concentration. A bone marrow aspiration was performed seven days after the first dose, and both the neutrophil storage pool (NSP) percent and the NSP/NPP (neutrophil proliferative pool) ratios were tabulated.

**Results:** the treatment and placebo groups were of similar gestational age (29± 3 vs 31± 3 weeks) and birth weight (1376 ± 491 vs 1404 ± 508 grams). They had similar Apgar scores and 24 hour SNAP scores. No deaths occurred during the first week of life among the treatment group while three deaths occurred in the placebo group. RhG-CSF treatment did not alter the serum concentrations of the cytokines measured (except for G-CSF). Serum G-CSF levels, blood leukocyte counts, absolute neutrophil counts, NSP percentages, and NSP/NPP ratios were higher in the treatment group 24 hours and 72 hours after dosing. The occurrence of a subsequent infection over the two week period following dosing was significantly lower in the treatment group (n=2) than in the placebo group (n=9; $p<0.02$, RR 0.19 [0.05-0.78]). The overall mortality rate during the entire hospitalization was not different between treatment and placebo groups.

**Conclusions:** administration of rhG-CSF to premature neonates with the clinical diagnosis of early-onset sepsis was associated with lower incidence of nosocomial infection over the ensuing three weeks period, but it did not change the overall mortality rate.

*J Pediatr (Rio J) 2000; 76(3): 193-9:* recombinant human granulocyte colony-stimulating factor, neutropenia, sepsis.

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### Introduction

The neonatal anti-infective defense system is characterized by imature humoral and cellular development. Neutrophil qualitative and quantitative deficiencies are reproducible and include a depleted neutrophil storage pool (NSP = sum of polymorphonuclear leukocytes, basophils,

and metamyelocytes, deficient production of neutrophils after bacterial inoculation, and decreased neutrophil functions.[1,2] Neutropenia during sepsis, resulting from reduced NSP, is associated with a high rate of deaths and with the involvement of several organs by fulminant bacterial sepsis and nosocomial infection.[3-5]

G-CSF is the physiologic regulator of neutrophil production and function; its biological action consists of stimulating the proliferation of neutrophil precursors and increasing chemotaxis, phagocytosis, superoxide production, and bactericide activity.[6,7] The production of G-CSF is relatively poor in the monocytes from newborns if compared to adults.[8-10] These data suggest that the administration of rhG-CSF could improve the prognosis of infected newborns or decrease the incidence of nosocomial infection.[8-11] Besides, the administration of rhG-CSF in animal models with neonatal sepsis has been associated with increased survival.[12-14] Two phase I/II trials concerning the administration of rhG-CSF in newborns with suspicion of sepsis have shown a dose-dependent increase in the neutrophil count in the peripheral blood and in the bone marrow;[15] among three clinical studies,[16-18] two have observed that the effects of rhG-CSF on survival are generally positive.[16,17] However, those studies included a small group of patients, and two of them used history controls. Schibler et al.[18] carried out a randomized, double-blinded, placebo-controlled trial, and did not observe an increase in either circulating neutrophils or the neutrophil pool.

Our hypothesis was that the administration of rhG-CSF in preterm neonates with a clinical diagnosis of early-onset sepsis could reduce the mortality rate due to early-onset sepsis, as well as the incidence of nosocomial infection during the 3-week period after administration of the last dose of the drug. An experimental, randomized, double-blinded and placebo-controlled clinical design was used.

## Methods

*Patient selection criteria*

The selection included all newborns with birth weight between 500 and 2000 g, less than 37 weeks of gestational age, less than 5 days of life, clinical diagnosis of sepsis, and in-patients at the Neonatal Unity of the Hospital de Clínicas de Porto Alegre, in the period of July, 1st, 1996, to June, 30th, 1997. The presence of at least three of the following neonatal items was standardized for the diagnosis of clinical sepsis, with one or more maternal risk factors for the development of neonatal sepsis, associated with at least one of the infection acute phase reactants altered.[15] The neonatal items were: 1) fever (above 38 ºC) or hypothermia (below 36.5 ºC); 2) respiratory dysfunction (apnea and tachypnea); 3) neurological dysfunction (convulsion or hypotonia); 4) gastrointestinal signs (vomits or abdominal distension); 5) idiopathic hyperbilirubinemia (with no other possible cause but sepsis); 6) chock; and 7) unexplainable bleeding. Maternal risk factors for neonatal infection were: 1) fever; 2) intra-amniotic infection; 3) premature rupture of the membranes; 4) prolonged rupture of the membranes >24 hours; and 5) urinary infection. Laboratory data considered were: 1) neutropenia, defined as an absolute neutrophil count < 1500/uL; 2) reference of immature neutrophils in relation to total neutrophils higher than 0.2;[19] 3) plasmatic IL-6 higher than 32 pg/mL;[20] 4) plasmatic TNF–$\alpha$ higher than 12 pg/ml.[21] Criteria for exclusion were: mother having received two or more doses of antibiotics before labor; newborn with serious malformation and severe asphyxia, that is, with no spontaneous, hypoactive ventilation; bradycardia after reanimation maneuvers. Preterm neonates were considered to have nosocomial infection when, after the 7th day of life, the newborn presented clinical signs of sepsis associated with positive hemoculture or necrotizing enterocolitis, and radiographic signs of intestinal pneumatosis or its confirmation by the anatomic pathology examination. This project was approved by the Group of Research and Post-Graduation and by the Ethics Committee of the hospital. Written consent was obtained from the newborns' parents for each case.

*Treatment protocol*

In the protocol, the newborns in the treatment group (TG) received rhG-CSF (Granulokine, Roche-Amgen, Thousand Oaks, CA) intravenously, in the dose of 10 $\mu$g/kg diluted in 10 ml of glucosic serum at 5% + 0.2% albumin (2 mg/ml), and infused in an infusion pump for 30 minutes, once a day, during 3 consecutive days. Newborns in the placebo group (PG) received an identical volume intravenously without rhG-CSF; both the solutions were indistinguishable. All the selected newborns were randomly distributed to receive G-CSF or placebo through a simple raffle criterion (44 envelopes with 22 TG and 22 PG), which was only known by the pharmaceutical of the Chemotherapy Unity. The participants, investigators, and the team responsible for the newborns were blinded, and, consequently, did not know which the employed treatment was. The criteria of suspension of the study were absolute neutrophil count superior to 60,000 cells, immediate side effects to the drug administration, or, still, withdrawal of parents' consent.

*Hematological laboratory assessment*

For this research, leukocyte count, absolute neutrophil count, monocyte, eosinophil and lymphocyte count, immature neutrophil/total neutrophil ratio, hemoglobin concentration, and plaque count were performed at the moment of admission, before the administration of the first dose of rhG-CSF or placebo, 24 hours, 72 hours, and 10 days after the first dose of the treatment. This hematological assessment of the peripheral blood was performed with Cobas Minus electronic counter, and the differential

leukocyte count was manually performed in red blade by the May-Grunwald-Giemsa method. Seven days after the administration of the first dose of the rhG-CSF or placebo, the patients were submitted to medullary aspiration in the tibia. The total count was also manually performed with Cobas Minus, and the differential cell count was done in red blade by the May-Grunwald-Giemsa method. They evaluated the percental sum of the nucleated cells identified as myeloblasts percentage. Promyelocytes and myelocytes were called NPP (neutrophil proliferative pool). The percental sum of nucleated cells identified as metamyelocytes, stab neutrophils, and segmented neutrophils was called NSP (neutrophil storage pool). The medullary myeloid/erythroid ratio was also assessed. All the results of circulatory and medullary hematological components were executed by the same hematologist.

*Cytokines laboratory assessment*

Blood samples were obtained in order to determine hematopoietic cytokines G-CSF, GM-CSF, IL-6, and TNF–α immediately before the administration of the first dose of rhG-CSF or placebo, 24 hours after and immediately before the administration of the second dose of rhG-CSF or placebo, 72 hours after the administration of the first dose of rhG-CSF or placebo, and 10 days after the first administration of rhG-CSF or placebo. These cytokines were measured in the plasma through the sandwich ELISA method (enzyme-linked immunosorbent assay, Quantikine, R&D Systems, Minneapolis,MN), according to the manufacturer's orientation. The samples were stored at -70 ºC, and afterwards they were simultaneously analyzed. Minimum and maximum values were, respectively, 11 pg/ml and 6000 pg/ml for G-CSF, 3 pg/ml and 13 pg/ml for GM-CSF, 1 pg/l and 1437 pg/ml for IL-6, and 11 pg/ml and 62 pg/ml for the TNF–α.

*Disease severity assessment*

The severity of the studied cases was evaluated through the method of the Score for Neonatal Acute Physiology (SNAP) at the moment of the patients' admission.[22] Mortality and morbidity were analyzed as outcome measures. Complications related to the sepsis and to the prematurity were intraventricular hemorrhage, period of time in mechanical ventilation, period of time in oxygen, necrotizing enterocolitis, meningitis, and the occurrence of an episode of subsequent sepsis during hospitalization.

*Sample size and statistical analysis*

The study was developed to test the hypothesis that the use of rhG-CSF could reduce mortality by sepsis and the incidence of nosocomial infection in 50%. In the first phase, we studied 26 preterm neonates, when three deaths occurred out of 13 cases (23%) in the TG, and six deaths out of 13 cases (46%) in the PG. The temporary statistical analysis showed that 42 patients being treated in these conditions would be necessary to prove a statistically significant difference of the protective effect of rhG-CSF ($P<0.05$). We used, as the study power, a 90% CI, which resulted in a relative risk of 0.5 (0.2-0.99). The demographic data were compared through Student's parametric test, Fisher's exact test (mortality and nosocomial infection), and Kruskal-Wallis's non-parametric test. The median cytokine level (G-CSF, GM-CSF, IL-6, TNF–α ), complete blood count (leukocyte count, absolute neutrophil count, immature neutrophil/total neutrophil ratio, lymphocyte, monocyte and eosinophil count, plaque count, hemoglobin), and the neutrophilic count of the bone marrow (NSP, NPP, medullary myeloid/erythroid ratio) were compared in each moment by analysis of variance, using the Kruskal-Wallis non-parametric test. Values of $P< 0.05$ were considered important.

**Results**

Forty four premature newborns were included in the study, divided in two groups: 22 in the TG, and 22 in the PG. Two cases were excluded because the parents' consent was not available. Demographic clinical features of the assessed sample did not show significant differences in relation to average birth weight, average gestational age, average Apgar score at the 5th minute of life, average SNAP scores on the day of admission, number of neutropenic newborns (absolute neutrophil count < 1500/c), time of hospitalization, time of oxygen administration and mechanical ventilation (Table 1). Thirty cases were admitted on the 1st day of life, ten cases on the 2nd day of life, and other four cases up to the 4th day of life.

At the beginning of the study, the following pathogenic agents were isolated: Group B *Streptococcus* (one case, TG), *Staphylococcus aureus* (two cases, TG and PG), *Staphylococcus epidermis* (one case, PG), *E. coli* (one case, TG) and a non-identified gram-negative bacillus (one case, TG).

Before the treatment, the levels of G-CSF varied from a slight to a striking increase in the PG (average 141 pg/ml; variation 18-4985), as well as in the TG (average 154 pg/ml; variation 23-4047). Twenty-four hours after the first dose (immediately before the second dose), the levels of G-CSF were significantly higher in the TG (average 2568 pg/mL; variation 30-6000) than in the PG (average 56 pg/mL; variation 19-2289, $P<0.00001$). Seventy-two hours after the first dose of rhG-CSF (immediately before the third dose), the levels of G-CSF remained significantly higher in the TG (average 129 pg/mL; variation 28-6000) than in the PG (average 37 pg/mL; variation 19-1608, $P<0.007$). Ten days after the beginning of the treatment, the levels of G-SF were similar in both groups (Table 2). The plasmatic levels of GM-CSF, TNF–α and IL-6 were not different after the administration of rhG-CSF to the TG and the PG.

Before the treatment, leukocyte count and absolute neutrophil count were similar in both groups. However, 24

**Table 1 -** Clinical data and results for 44 premature newborns with clinical diagnosis of early-onset sepsis

| | Placebo (n=22) | rhG-CSF (n=22) |
|---|---|---|
| Weight (g, average ± SD) | 1404±508 | 1376±491 |
| Gestational Age (without, average ± SD) | 31±3 | 29±3 |
| Male | 14 | 9 |
| Female | 8 | 13 |
| Apgar at 5th min (average, variation) | 8 (1-10) | 7 (3-10) |
| SNAP (average, variation) | 9 (0-23) | 8 (3-28) |
| Deaths <7 days of life | 3 | 0 |
| Deaths between 8-28 days of life | 3 | 5 |
| Nosocomial infection* | 9 | 2 |

***** P=0.02; RR=0.19; CI 95% 0.05-0.78.

and 72 hours after, both were significantly more elevated in the TG than in the PG (Table 3). Ten days after the beginning of the treatment, there were no differences between the groups anymore. No differences were observed between the two groups in relation to the difference in the immature/total neutrophil ration (TG: 0.18 at admission, 0.19 after 24 hours, 0.13 after 72 hours, and 0.09 after 10 days; PG: 0.18 at admission, 0.14 after 24 hours, 0.13 after 72 hours, and 0.09 after 10 days). No changes occurred in the absolute eosinophil, lymphocyte and plaque count. There was an important increase in the absolute monocyte count 72 hours after the beginning of the treatment in the TG (1178/$mm^3$, variation 0-5291), more than in the PG (481/$mm^3$, variation 86-1158, P<0.0002). Bone marrow aspiration was performed 7 days after the beginning of the treatment in 36 patients (three died before the 7th day, and five were not in good conditions). The NSP and the NSP/NPP ratio were significantly higher in the TG than in the PG (Table 4).

During the 1st week of life, there were no deaths in the TG, while in the PG three deaths occurred. Necropsies have been performed in the three cases, and intraventricular hemorrhage of intravenous degree was the death cause.

Eleven cases of nosocomial sepsis occurred during the ensuing 3-week period after the treatment: 9/19 (47.3%) in the placebo group, and 2/22 (9.1%) in the rhG-CSF group (P<0.02, RR 0.19, CI 95% 0.05-0.78). Nine out of these eleven cases were identified through hemoculture, and two cases of necrotizing enterocolitis were diagnosed by radiographic and anatomic pathology studies (PG). The following bacterial agents were isolated: *Staphylococcus aureus* (four cases, two in TG and two in PG), *Staphylococcus epidermis* (four cases, PG), and *Staphylococcus hemolyticus* (one case, PG).

Three deaths occurred in the PG in the late phase; on the 24th day of life, one patient died in the postoperative period of intestinal obstruction due to annular pancreas, and two deaths happened due to necrotizing enterocolitis, respectively on the 22nd and 24th day of life. Five deaths occurred in the TG: two had intraventricular hemorrhage of third degree, respectively on the 8th and 13th day of life; two died due to pulmonary hemorrhage and hyaline membrane disease, both with 14 days of life; the fifth death occurred on the 25th day of life due to acute renal insufficiency.

The main measured considered as complications were similarly distributed between the two groups. Average hospitalization time: 35 versus 40 days (P=0.5); oxygentherapy: 11.5 versus 12.8 days (P=0.8); CPAP: 5.8 versus 4.3 days (P=0.3); mechanical ventilation: 10.6 versus 8.1 days (P=0.4).

## Discussion

Bacterial sepsis continues to be a very important problem in clinical neonatology. Early-onset sepsis and nosocomial infection are the main causes of mortality, morbidity, hospitalization time, and high costs in intensive care unities.[23] The immaturity of certain antibacterial defenses, particularly in the neutrophilic system, is one of the reasons for this problem.[1,2] With the availability of rhG-CSF and

**Table 2 -** Plasmatic concentration of G-CSF, GM-CSF, TNF–α, and IL-6 in studied neonates before (0 hour), 24 hours (immediately before the second dose), 72 hours (immediately before the final dose) and 10 days after the administration of rhG-CSF or placebo

| CITOKINES (average, variation) | 0 hour | | 24 hours | | 72 hours | | 10 days | |
|---|---|---|---|---|---|---|---|---|
| | Placebo | rhG-CSF | Placebo | rhG-CSF | Placebo | rhG-CSF | Placebo | rhG-CSF |
| G-CSF pg/mL | 141 (18-4985) | 154 (23-4047) | 56 (19-2289) | 2568 (30-6000) * | 37 (19-1608) | 129 (28-6000) ** | 29 (23-355) | 39 (20-4258) |
| GM-CSF pg/mL | 5.5 (4-12) | 5.5 (4-13) | 5 (4-10) | 5 (4-13) | 5 (3-8) | 5 (4-8) | 5 (3-11) | 5 (4-9) |
| TNF-α pg/mL | 14 (11-23) | 13 (11-29) | 14 (12-18) | 14 (12-23) | 14 (12-17) | 14 (12-20) | 14.5 (12-21) | 14 (12-62) |
| IL-6 pg/mL | 27.5 (1-1453) | 20.5 (6-1500) | 12 (5-1295) | 15 (5-363) | 11 (3-867) | 11 (3-104) | 11.5 (3-620) | 6 (4-1034) |

* P<0.00001 *versus* placebo ** P<0.007 *versus* placebo

**Table 3 -** Hematological values before (0 hour), 24 hours (immediately before the second dose), 72 hours (immediately before the final dose), and 10 days after the administration of rhG-CSF or placebo

| Hematological values | Placebo (n= 22) | | | | rhG-CSF (n= 22) | | | |
|---|---|---|---|---|---|---|---|---|
| | Before | 24 hours | 72 hours | 10 days | Before | 24 hours | 72 hours | 10 days |
| Leukocytes/uL | 8750 (1900-34000) | 8100 (1400-27100) | 9250 (2000-21000) | 12000 (7000-32900) | 11300 (2700-29600) | 15200 (4400-65300) * | 23100 (9500-48100) † | 13050 (9100-30200) |
| Absolute neutrophils/uL | 5316 (748-22440) | 4526 (918-24932) | 4703 (1537-15540) | 5049 (2856-23688) | 7076 (1458-20424) | 9522 (2948-50281)‡ | 16843 (6270-35113) § | 8446 (1818-17818) |
| Lymphocytes/uL | 2571 (114-3288) | 2349 (42-7560) | 3272 (220-6364) | 4002 (1602-8721) | 2604 (616-9360) | 3886 (540-13713) | 4037 (984-7600) | 4320 (900-10268) |
| Monocytes/uL | 386 (38-1450) | 405 (58-1494) | 481 (86-1158) | 615 (70-2632) | 387 (116-2664) | 665 (88-3140) | 1178 (0-5291) ¶ | 720 (182-2250) |
| Platelets $X10^3$/uL | 207(58-332) | 126(35-281) | 160(41-331) | 306(28-611) | 217(126-380) | 181(118-359) | 187(65-428) | 315(75-828) |
| Hemoglobin g/dL | 14.7± 2.2 | 14.7± 2.4 | 13.9± 2.8 | 12.2± 1.8 | 13.8± 2.8 | 14.5± 2.2 | 15.1± 2.0 | 13.6± 1.6 |

*P<0.006 *versus* placebo † P<0.00002 *versus* placebo ‡ P<0.005 *versus* placebo § P<0.00002 *versus* placebo ¶ P<0.0002 *versus* placebo

the finding of its role in neutrophil production and functioning, and with the understanding that G-CSF production is relatively limited in premature newborns, we can speculate that the administration of rhG-CSF may be a precious adjuvant in antibioticotherapy and in intensive care.[8,11]

It has been clearly demonstrated, since the first study published by Gillan, et al.,[15] that the administration of rhG-CSF has essentially the same biological effects in newborns and in adults. This means that it induces the production of neutrophils coming from the NSP, and, subsequently, causes a dose-dependant increase in neutrophil production, circulation and medullary storage. Gillan, et al.[15] studied 42 newborns who presented presumable sepsis, treated with placebo (n=9) or in doses that vary from 1.0 to 20.0µg/kg/day for 3 consecutive days. Although this study did not evaluate the effect of rhG-CSF on mortality, none of the 42 neonates died, reflecting a sample composed predominantly by noncritical patients.

**Table 4 -** Hematological values (average and variation) in the bone marrow of the neutrophil storage in the studied neonates 7 days after the administration of placebo or rhG-CSF

| | Placebo (n = 18) | rhG-CSF (n = 18) | Kruskal-Wallis |
|---|---|---|---|
| NSP (%) | 51.5 (19 – 62) | 61 (43 – 70) | 0.003 |
| NPP (%) | 17.5 (12 – 57) | 16 (7 – 48) | 0.3 |
| NSP/NPP | 2.9 (0.3 – 4.5) | 3.7 (0.9 – 9.4) | 0.05 |
| M/E* | 11 (2 – 44) | 19.5 (7 – 80) | 0.06 |

* Medullary myeloid/erythroid ratio

Later on, Kocherlakota, et al.[16] described the results of 27 neonates, 14 receiving rhG-CSF, and 13 concomitant controls. Only one premature death occurred in the rhG-CSF group, compared to three deaths in the control group. During the whole hospitalization time, there was one more death in the rhG-CSF group, and six more in the control group. Schibler et al.[17] made a randomized study with 10 premature newborns with early-onset sepsis receiving rhG-CSF, and 10 receiving placebo. There were two deaths during the study in each group. Complications like intraventricular hemorrhage, bronchopulmonary dysplasia, and nosocomial infection were similarly distributed between the two groups. Barak et al.[18] treated 14 premature newborns who presented presumable sepsis with rhG-CSF, and reported only one premature death, compared to nine deaths among 24 history-cases that were not treated with rhG-CSF. Similarly to data collected by Kocherlakota, Schibler, and Barak, we did not observe any premature death among the rhG-CSF receptors, against three deaths that occurred in the placebo group. This fact is possibly relevant, once Klein described that 72% of the deaths due to early-onset sepsis occurred in the first 48 hours following the diagnosis.[29] When data about the premature deaths of the present study were added to those described by Gillan, Kocherlakota, Schibler, and Barak, we observed that there were only two deaths in 93 rhG-CSF receptors (2.2%), against 18 deaths in 76 controls (23.7%). When the total number of deaths (early and late deaths) was consolidated in the four studies, we could observe that 11 deaths out of 93 rhG-CSF receptors

(11.7%) occurred, against 27 deaths out of 76 control neonates (36.8%). We stress that the five studies come from different patient samples, and that the results cannot be safely interpreted. For instance, in the present study, the previous relatively low levels of G-CSF, TNF–α, IL-6, as well as the immature/total neutrophil ratio, indicate that our patients were less critically sick than those studied by Schibler.[18] The studies described by Gessler[24] and Kennon[25] indicate that neonates with serious sepsis generally have high levels of endogenous G-CSF, while our patients only had normal to moderately high previous levels of endogenous G-CSF. It is necessary to wait for other studies methodologically similar to undergo a meta-analytical study.

None of the three works mentioned studied the effect of rhG-CSF application on the incidence of nosocomial infection in a prospective way. Gillan et al.[15] cited a potentially salutary effect, that is, that the treatment with rhG-CSF resulted in a considerable increase in the premature newborn size. We observed the same effect in our study, and also a smaller nosocomial infection incidence rate among the group treated with rhG-CSF. We speculated that the smaller nosocomial infection incidence rate may be due to the higher NSP storage, which persists for an undetermined period of time, following the surcease of rhG-CSF administration, or, still, that it may be due to the improvement of neutrophil functioning (chemotaxis and phagocytosis), persisting by a given period of time, once the application of rhG-CSF or the combination of quantitative and qualitative effects was ceased in the neutrophils.

Recently, Cairo et al.[26] published a randomized, placebo-controlled trial about nosocomial infection incidence after prophylactic application of rhGM-CSF in RNMBP. They studied 264 neonates with less than 72 hours of life; 134 cases received rhGM-CSF in the dose of 8 μg/kg/day, intravenously, for 7 days, and after that, an alternated dose every other day up to the 21st day; 130 received placebo. They found no differences in the incidence of nosocomial infection in the groups. However, an increase in the ANC was observed on days 7, 14 and 21; an increase in the expression of the C3bi receptors and an elevated concentration of progenitor cells in the peripheral blood was observed on the 8th day in the rhGM-CSF group. Car et al.[27] using the same rhGM-CSF prophylactically for 5 days in a randomized, placebo-controlled trial with 72 premature neonates in their first 72 hours of life, observed that neutropenia disappeared in the treated group, compared with an incidence of 40% of neutropenia in the control group. There were no differences in mortality in both groups, but there was a tendency to a smaller number of cases of nosocomial infection in the treated group: 11/36 versus 18/39 (OR 0.51, CI 95% 0.20-1.31).

We observed that the TNF–α and IL-6 levels were slightly elevated during the trial, and both may be useful markers of neonatal sepsis.[20,21,28,29] Although GM-CSF, TNF–α and IL-6 levels did not show differences after the rhG-CSF administration, Görgen et al.[30] demonstrated a reduction in mortality and a suppression of the TNF–α activity after the application of high doses of rhG-CSF (250 mg/kg) in rats with sepsis due to gram-negative. Similarly, in rats with experimental sepsis, Lundbland et al.[29] showed a reduction in mortality, bacteria count, TNF–α and endothelin-1 after the application of high doses of rhG-CSF.

None of the previous studies with the administration of rhG-CSF in infected human newborns showed adverse short and long-term effects. In the same way, we did not observe any adverse effect in our patients. Potentially, a pulmonary endothelial lesion produced by the administration of rhG-CSF may occur; it can be manifested by increasing oxygen needs and by a higher period of mechanical ventilation.[31,32] We did not observe any of these effects, and we may conclude that no pulmonary damaging effects were observed. We detected an increase in monocyte concentration, similar to that described by Gillan,[15] Schibler,[17] Kocherlakota,[16] and Bedford-Russell;[33] however, thrombocytopenia, described by Bedford-Russell, but not by Gillan, Schibler, and Kocherlakota, was not observed in our study either.

In conclusion, we observed that the administration of rhG-CSF in premature neonates with clinical diagnosis of early-onset sepsis did not alter mortality rate. Yet, it produced an increase of neutrophil concentration in peripheral blood and bone marrow, in addition to a significant reduction in nosocomial infection rates during the subsequent 3-week period.

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