Medical and Pediatric Oncology 25:365–371 (1995)

Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor in Septic Neutropenic Pediatric Cancer Patients: Detection of Circulating Hematopoietic Precursor Cells Correlates With Rapid Granulocyte Recovery

Franz-Martin Fink, MD, Kathrin Maurer-Dengg, MD, Gerhard Fritsch, PhD, Georg Mann, MD, Andreas Zoubek, MD, Markus Falk, MSc, and Helmut Gadner, MD

Cycling intensive chemotherapy currently used to treat pediatric solid tumors induces severe neutropenia. Prolonged neutropenia is a major risk factor for septic death which occurs in up to 5% of febrile or septic neutropenic episodes. We treated 18 neutropenic pediatric cancer patients (eight females, 10 males) during 30 febrile and/or septic episodes with single daily doses of *E. coli*-derived non-glycosylated recombinant human granulocyte-macrophage colony-stimulating factor (rh-GM-CSF, 5 μg per kg of body weight). The cytokine was administered for a median period of 6.5 days (2–12 days). Analysis of circulating hematopoietic progenitor cells was performed at day 1 (baseline) and day 5 of rh-GM-CSF treatment and included flow cytometric CD34 analysis as well as the methylcellulose-based clonogenic assay.

Prompt hematopoietic recovery and resolution of septic problems was observed in all children. The counts of leukocytes (WBC), absolute neutrophils (ANC), and platelets (PLT) rose above 1,000/μL, 1,000/μL, and 50,000/μL within 4 days (0–9), 5.5 days (2–13), and 6 days (0–14), respectively. Faster granulocyte recovery and improved recruitment of circulating hemopoietic precursors was observed in children with detectable amounts (>0.1%) of CD34-positive mononuclear cells prior to rh-GM-CSF treatment.

We conclude that, to some extent, the efficacy of rh-GM-CSF treatment in neutropenic cancer patients is influenced by the hematopoietic recovery status on the progenitor cell level. Although they respond more slowly to the treatment, patients without circulating CD34-positive progenitor cells may gain most from growth factor therapy. Rh-GM-CSF can be safely administered to febrile and/or septic neutropenic children treated for cancer.

Key words: hematopoietic progenitor cells, rh-GM-CSF, neutropenia, septicemia, antineoplastic therapy, children

INTRODUCTION

Pediatric oncology has achieved major advances in the treatment of pediatric solid tumors. High cure rates are obtained—basically by the use of intensive multi-agent chemotherapy in addition to surgery and radiotherapy. Hematologic toxicities of cancer chemotherapy, namely the induction of severe neutropenias, are the cause of septic complications in a substantial fraction of patients. Despite modern antimicrobial treatment, septic deaths occur in up to 5% of septic episodes in severely neutropenic cancer patients. Prolonged and persistent neutropenia is the major risk factor for death in these patients [1–6]. Recombinant human colony-stimulating factors have been shown to accelerate hematologic recovery after cytotoxic chemotherapy and may thus reduce the risk of septic complications [7–11].

While data on the clinical effects of prophylactic therapy with hematopoietic growth factors in children and adults are rapidly accumulating [8–16], only a few studies on the interventional administration of these factors in the febrile or septic patient are available [17–21].

We report the successful use of *E. coli*-derived non-glycosylated recombinant human granulocyte-macro-

From the Departments of Pediatrics (F.-M.F., K.M.-D.), Biostatistics and Documentation (M.F., H.G.), University of Innsbruck, Innsbruck; St. Anna Children's Hospital and Children's Cancer Research Institute, Vienna (G.F., G.M., A.Z.), Austria.

Received February 28, 1994; accepted November 9, 1994.

Address reprint requests to Franz-Martin Fink, M.D., Department of Pediatrics, University of Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria.

TABLE I. Hematological Values Before rh-GM-CSF Treatment

Parameter	Median	Minimum	Maximum
WBC/μL	300	100	1,400
ANC/μL	20	0	312
PLT/μL	28,500	6,000	165,000

phage colony-stimulating factor (rh-GM-CSF) in neutropenic pediatric cancer patients who suffered from septic complications. In addition we correlated the pretreatment hematopoietic recovery status at the progenitor cell level with the effects of rh-GM-CSF treatment in these patients.

MATERIALS AND METHODS

We treated 30 episodes of septic fever (38.5°C or higher) in 18 severely neutropenic pediatric cancer patients. The children (eight girls and 10 boys) with a median age of 7.9 years (range 1.15 to 21.0 years) suffered from various solid tumors: soft tissue sarcoma (n = 5), Ewing's sarcoma (n = 4), neuroblastoma (n = 3), malignant brain tumor (n = 2), osteosarcoma (n = 2), hepatoblastoma (n = 1), and pulmonary blastoma (n = 1). They had been on cytotoxic chemotherapy for a median of 4.21 months (range 0.36 to 28.8 months) before their first rh-GM-CSF treatment course. Chemotherapy-induced bone marrow aplasia was defined as an absolute neutrophil count (ANC) below 500/μL and, at the time of initiation of rh-GM-CSF treatment, all children were severely leukocytopenic and neutropenic, most patients were also thrombocytopenic (Table I).

The chemotherapy courses preceding the episode of febrile neutropenia were classified according to their relative intensity: group A (n = 5, moderate intensity: high-dose methotrexate 5 g/m^2 [n = 4]; doxorubicin 90 mg/m^2 [n = 1]); group B (n = 16, high intensity without cisplatinum: EVAIA/EVACA [n =12]: etoposide 600 mg/m^2, vincristine 1.5 mg/m^2, doxorubicin 60 mg/m^2 or actinomycin-D 1.5 mg/m^2, ifosfamide 6 g/m^2 or cyclophosphamide 100 mg/kg, N5 [n = 3]: cyclophosphamide 140 mg/m^2, doxorubicin 45 mg/m^2, vincristine 0.1 mg/kg; others: n = 3); group C (n = 6, high intensity including cisplatinum: PA: cisplatinum 120 mg/m^2, doxorubicin 60 or 80 mg/m^2 [n = 3]; IPE: ifosfamide 6 g/m^2, cisplatinum 200 mg/m^2, etoposide 450 mg/m^2 [n = 1]; others: n = 2).

Bacteremia was detected in seven instances (staphylococci, four times; E. coli, two times; unclassified micrococci, once). One boy suffered from life-threatening *Clostridium difficile* enterocolitis. Additional five septic episodes were associated with mucositis, two with a perigenital cellulitis, two with vulvitis, one with radiogenic dermatitis, and one with varicella infection.

In addition to appropriate antimicrobial treatment (aminoglycoside + β-lactam-antibiotics), rh-GM-CSF treatment was started within 24 hours and was continued until a WBC of 3,000/μL or an ANC of 1,500/μL was reached.

Rh-GM-CSF (Molgramostim, Leucomax™) in vials of 150 μg/ml (1.66*10^6 units) was kindly supplied by Aesca Ges. m. b. H (Traiskirchen, Austria). Single daily doses of 5 μg rh-GM-CSF per kg body weight were given subcutaneously. The protocol had been approved by the institutional ethical review boards and appropriate informed consent was obtained from the patients and/or their parents.

The diagnostic procedures included a daily physical examination of the patients, a complete bacteriological workup, complete daily blood counts, as well as extensive blood chemistry at baseline and on day 5 of the rh-GM-CSF treatment. Chest x-rays and other special diagnostic procedures were performed as clinically indicated.

Analysis of the hematopoietic stem cells was performed at days 1 and 5 of rh-GM-CSF treatment and included the flow cytometric determination of CD34-positive cells (FACS-analysis) and the culture of hematopoietic precursor cells in the clonogenic assay. Mononuclear cells (MNC) prepared by density centrifugation on Ficoll-Paque (specific gravity 1.077 g/cm^3, Pharmacia, Uppsala, Sweden) were stained with FITC-labeled anti CD34 MoAb (HPCA-2, clone 8G12, Becton-Dickinson, Sunnyvale, CA) and analysed on a FACStar Plus (Becton-Dickinson) [22,23]. Depending on the proportion of CD34 positive MNC, between 1×10^3 and 1×10^5 MNC per ml were cultured in the methylcellulose-based clonogenic assay, which contained 2.5 U rh-EPO (Cilag, Schaffhausen, Switzerland), 100 U rh-GM-CSF, and 10 U rh-IL-3 (both from Genzyme, Cambridge, MA). The colony-forming units (CFU, >50 cells per colony) which were counted after 14 days of incubation included mixed-lineage-CFU [CFU-GEMM], granulocyte-macrophage-CFU [CFU-GM], and erythroid burst-forming units [BFU-E, >50 cells per colony]) as described previously [23].

For comparison, the rh-GM-CSF treatment episodes were grouped into those with early (median on day 10 after the first day of the preceeding chemotherapy, range day 4 to 12, n = 14) of late commencement (median day 14, range day 13 to 20, n = 16). In addition, episodes with a low or undetectable baseline percentage of CD34-positive peripheral blood MNC (n = 12) were compared with those in which a higher proportion of these hematopoietic precursor cells was observed (n = 11).

Statistical Methods

Statistical analysis was performed based on the assumption that the rh-GM-CSF courses were independent

TABLE II. Comparison of Baseline Characteristics in CD34-Low vs. CD34-High Subgroups Before Start of rh-GM-CSF Treatment

	Median (range)		
Values on day 1	CD34 + low (n = 12)	CD34 + high (n = 11)	*P*-value (T-test)
CD34 + MNC	0.1% (0–0.1%)	0.3% (0.2–6.5%)	—
WBC/μL	250 (100–1,400)	464 (200–900)	.53
ANC/μL	17 (0–312)	26 (0–126)	.73
PLT/μL	29 (6–67)	28 (7–124)	.29
Days after start of chemotherapy	13 (4–17)	13 (8–20)	.41
	Numbers	Numbers	*P*-value (Chi-square)
First/second episodes	7/5	7/4	.80
Chemotherapy intensities—group A/B/C	2/6/4	2/7/2	.70

from each other, irrespective of the fact that 10 patients were treated twice and one patient three times within the study. This seemed justified since separate analysis of first courses only gave similar statistical results with somewhat lower statistical significance for leucocytes (n = 18, ANOVA *P*-values for leucocytes and neutrophils 0.07 and 0.001, respectively, data not shown). Furthermore, first and subsequent episodes were evenly distributed between the subgroups with low and high proportion of CD34-positive precursor cells in peripheral blood MNC (Table II), respectively, and eight of these 11 multiple-studied patients contributed to the CD34-low subgroup in one and to the CD34-high subgroup in the other episode. All these circumstances made it unlikely that the comparisons would be biased by multiple measurements of individual patients.

Differences in Kaplan-Meier life-table analyses were compared by the log-rank test, the T-test was applied for comparison of continuous variables, and the Chi-square test for categorial variables [24,25]. Differential effects of continuous variables over time betwen groups were studied by an analysis of variance (ANOVA) with repeated measures [26]. To account for the approximate log-normal distribution seen in all variables, the data were log-transformed before confirmatory statistical analysis was done. Statistical analyses were performed with SPSS for Windows 6.0™ (T-test, Chi-square) and BMDP 5V™ (ANOVA).

Box-and-Whiskers-Plots are used for graphic demonstration of the data. The box gives the interquartile range (50% of all values lie within this range), the whiskers show the range from the smallest to the greatest value, which was not outlying or extreme. Outlying values (1.5 to 3 times the interquartile range above the 75th percentile, symbol: ○) and extreme values (more than three times the interquartile range above the 75th percentile, symbol: *) are displayed separately.

TABLE III. Hematopoietic Recovery Data*

Parameter	Median	Minimum	Maximum
WBC >1,000/μL	4	0	9
ANC >500/μL	5	1	11
>1,000/μL	5.5	2	13
PLT >50,000/μL	5.5	0	10
>100,000/μL	7	0	15

*The number of days which were required after initiation of rh-GM-CSF to achieve the indicated cell counts are given.

RESULTS

In the 30 episodes of febrile or septic neutropenia studied, the rh-GM-CSF treatment was started after a median of 13 days counted from the beginning of the preceeding intensive cytotoxic chemotherapy (range 4 to 20 days). The median rh-GM-CSF treatment duration was 6.5 days (range 2 to 12 days).

Prompt hematopoietic recovery occurred in all children (Table III). The WBC rose above 1,000/μL after 4 days (median) of rh-GM-CSF treatment (range 0 to 9 days; two patients never had a WBC below 1,000/μL). An ANC above 500/μL was reached after 5 days (range 1 to 11 days), and above 1,000/μL after 5.5 days (range 2 to 13 days). Platelet counts rose above 50,000/μL after 6 days (range 0 to 14 days; three patients never had PLT below 50,000/μL), and above 100,000/μL after 7 days (range 0 to 15 days; one patient never had PLT below 100,000/μL).

Together with hematopoietic recovery, septic problems resolved. Seven children with proven bacteremia were antimicrobially treated for a median of 7 days (5 to 10 days). Only one of them had a second positive blood culture one day after the start of rh-GM-CSF. A 4 to 8 day antimycotic treatment with amphotericin-B (i.v.) was necessary in eight children (median 5 days). The boy with life-threatening enterocolitis caused by *Clostridium difficile* recovered within one week under continued rh-GM-CSF therapy.

No significant differences were observed, with regard to the recovery patterns of the different cell types counted, between early and late (as defined earlier) commencement of cytokine treatment. For both groups, the Kaplan-Meier analysis revealed identical median time periods required for WBC, ANC, and PLT counts to increase above 1,000/μL, 500/μL or 1,000/μL, and 50,000/μL, respectively. Baseline characteristics including initial WBC, ANC, and PLT, as well as the percentage of CD34-positive cells and the duration and type of preceeding chemotherapy did not differ between the two groups (data not shown).

Results from stem cell determinations (FACS-analysis and clonogenic assay) were available at baseline in 23 patients, at day 5 in 25 patients. Baseline peripheral blood MNC contained 0.1% (median, range 0.0–6.5%) CD34-positive cells (Table II). By day 5, the percentage of CD34-positive MNC had increased to 0.7% (median, range 0 to 12%). This increase of mobilized hematopoietic progenitor cells was also observed in the clonogenic assay in which the number of CFU per 10^5 MNC (including CFU-GM, CFU-GEMM, and BFU-E) rose from 15.5 (median, range 1 to 70) to 240 (median, range 19–2,160).

The speed of hematopoietic recovery was predicted by the amount of CD34-positive cells present in the peripheral blood MNC at the first day of rh-GM-CSF treatment: patients with higher baseline values (>0.1% CD34-positive cells, median 0.3%, range 0.2 to 6.5%, n = 12) responded significantly faster to rh-GM-CSF compared to those with very low (≤0.1%) or undetectable CD34-positive cells (median 0.1%, range 0 to 0.1%; n = 11). This could be demonstrated for leucocyte ($P = 0.007$, Fig. 1a) as well as for granulocyte recovery ($P = 0.002$, Fig. 1b), whereas the platelet response was not affected (data not shown). Other baseline characteristics including initial WBC, ANC, and PLT, as well as the type and timing of preceeding chemotherapy did not differ between the two groups (Table II).

In addition to the faster rise in peripheral blood cell counts, patients with demonstrable peripheral blood CD34-positive cells (>0.1%) showed a better recruitment of peripheral blood precursor cells by day 5 of rh-GM-CSF-treatment. In these children, CD34-positive cells increased to a median of 3.5% (range 0.2 to 12%) compared to only 0.25% (range 0 to 4.2%) in those without significant amounts of baseline CD34-positive cells. Clonogenic assays revealed 775 CFU per 10^5 MNC (range 110–1,610) in children with the higher proportion of CD34-positive cells compared to only 122 CFU per 10^5 MNC (range 19–300, and one additional extreme value of 2,110) in the complementary group ($P = 0.04$, Fig. 2).

Rh-GM-CSF was well tolerated. Five patients complained of temporary minor local irritation at the site of injection. No case of bone pain was documented. Fever caused by the septic complication subsided in all instances and no case of rh-GM-CSF-induced fever was observed. Elevated levels of C-reactive protein improved in all patients within the period of rh-GM-CSF treatment, serum levels of bilirubine, asparat-aminotransferase (AST), proteine, creatinine, as well as urine analyses did not indicate inadvertent side effects at the respective organs (Table IV).

DISCUSSION

Clinical effects and side effects of rh-GM-CSF, particularly in the prophylactic setting without a septic complication, are well documented in adult cancer patients [7–11], but the experience in the respective pediatric age group is still limited [12–17,24]. Riikonen et al. have recently shown that intravenous rh-GM-CSF is safe and effective in the treatment of febrile neutropenia in pediatric cancer patients [17]. Compared to a placebo group, duration of neutropenia and the duration of antibiotic therapy was shortened. We also have demonstrated the safety of daily subcutaneous doses of *E. coli*-derived non-glycosylated rh-GM-CSF administered to severely neutropenic and febrile children suffering from various solid tumors. Furthermore, this is the first study that correlates the growth factor-induced kinetics of hematopoietic recovery with circulating hematopoietic precursor cells present before the start of cytokine treatment.

Neutropenic patients who, at the commencement of rh-GM-CSF treatment, have detectable amounts of circulating hematopoietic precursor cells respond faster to the cytokine treatment. This seems reasonable because circulating hematopoietic precursor cells may reflect a larger pool of determined stem cells ready to respond to the growth factor. The leukocyte and granulocyte counts rose more rapidly compared to patients without detectable CD34-positive cells. In addition, the mobilization of progenitor cells to the peripheral blood was more pronounced in these children. This recruitment of peripheral blood progenitor cells after intensive cytotoxic chemotherapy is a well known phenomenon enhanced by the administration GM-CSF or G-CSF, respectively [27–33] In contrast to the results of the baseline precursor cell analysis, the variable time interval between cytotoxic chemotherapy and the initiation of rh-GM-CSF did not significantly influence the growth factor-induced kinetics of hematopoietic recovery.

Under the cytokine treatment, all patients improved clinically, and septic problems resolved within a short period. In proven bacteremias, cultures became negative within 48 hours and the episode of life-threatening enterocolitis caused by *Clostridium difficile* resolved within 1 week under continued rh-GM-CSF therapy. The examined serum parameters improved or remained unchanged compared to baseline values. Serious side effects due to

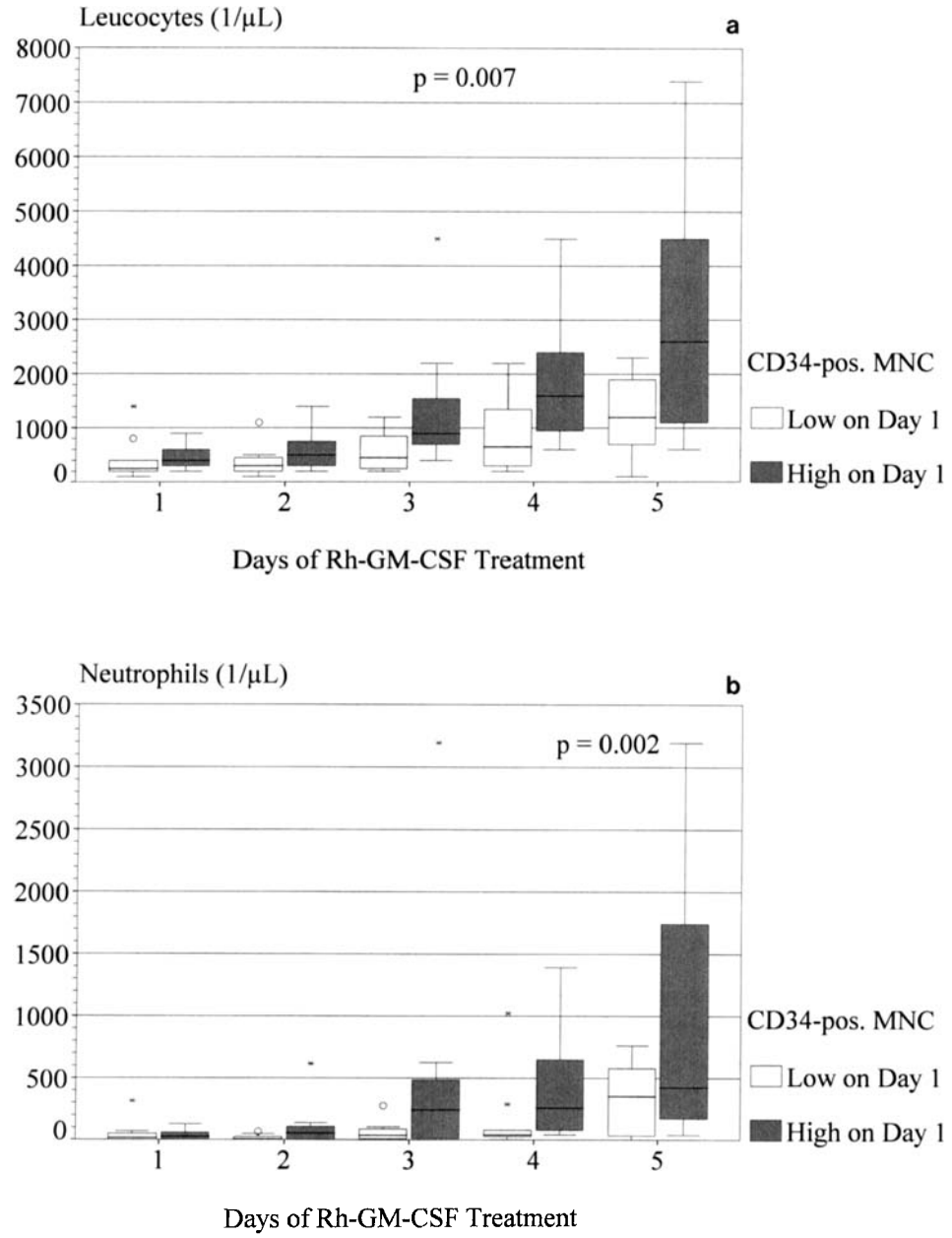

Fig. 1. Recovery of leucocytes (**a:** WBC) and absolute neutrophils (**b:** ANC) in patients with an initially low percentage of CD34-positive cells (□ , median 0.1%, range 0 to 0.1%) and a higher proportion of CD34-positive cells (■ , median 0.3%, range 0.2 to 6.5%) of the MNC (Box-and-Whiskers-Plot, for description see "Statistical Methods").

the rh-GM-CSF treatment were not encountered among these 30 septic episodes. In particular, respiratory disturbances, excessive weight gain or hypoproteinemia were not observed and cardiovascular, liver, and kidney function were not impaired. Our observation fits into the general consensus that relevant toxicities are almost exclusively seen with the use of daily doses well above 10 µg/kg body weight or 250 µg/m^2 body surface rh-GM-CSF daily. Both glycosylated and non-glycosylated rh-GM-CSF have been effective and well tolerated in previous prophylactic pediatric studies following intensive chemotherapy or bone marrow transplantation, respectively [12–16]. Partially glycosylated yeast-derived rh-GM-CSF has in children even been tolerated at dosages of up to 1,500 µg/m^2, which may indicate a greater tolerance of this patient group compared to adults [9,13,14]. Our study demonstrates that *E. coli*-derived rh-GM-CSF can be safely applied in febrile or septic neutropenic children. The absence of adverse events may, in part, be explained by the low-dose regimen together with the subcutaneous route of administration [27].

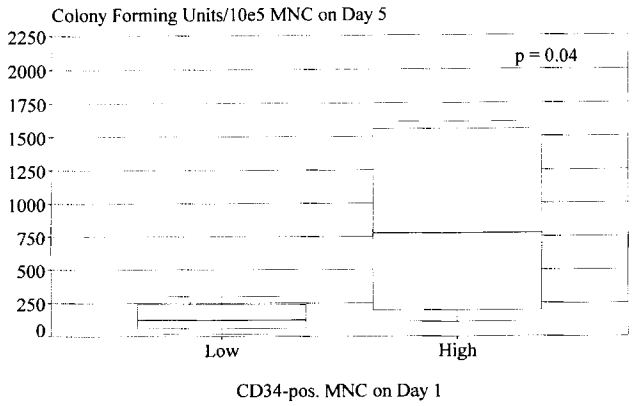

Fig. 2. Recruitment of peripheral blood precursor cells (CFU = CFU-GM, CFU-GEMM, and BFU-E) on day 5 of rh-GM-CSF therapy from patients with an initially low or higher percentage of CD34-positive MNC (for the definition of "low" and "high" see Fig. 1) (Box-and-Whiskers-Plot, for description see "Statistical Methods").

TABLE IV. Serum Parameters*

Parameter	Day 1	Day 5	*P*-value
CRP (mg/dL)	5.71 (± 0.89)	3.90 (± 0.81)	n.s.
Bilirubine (mg/dL)	0.68 (± 0.06)	0.47 (± 0.04)	< 0.01
AST (U/L)	27.9 (± 13.1)	17.8 (± 6.4)	< 0.01
LDH (U/L)	175.4 (± 9.1)	191.2 (± 15.6)	n.s.
Proteine (g/L)	64.9 (± 1.8)	67.3 (± 1.4)	< 0.10
Creatinine (mg/dL)	0.55 (± 0.05)	0.54 (± 0.05)	n.s.

*Baseline values on day 1 and values on day 5 of rh-GM-CSF treatment are given, means ± standard error.

Previous studies in children [17,18] and adults [19,20,34] indicated that hematopoietic growth factor therapy is beneficial for a subgroup of severely neutropenic febrile or septic cancer patients: prolonged neutropenia has effectively been eliminated in these studies [17–19]. However, at the onset of febrile neutropenia the duration of granulopoietic insufficiency may be difficult to foresee. Though responding more slowly to the treatment, patients without detectable circulating CD34-positive precursor cells may gain most from growth factor therapy.

ACKNOWLEDGMENTS

We thank Petra Buchinger and Dieter Printz for their assistance with immunological cell staining, flow cytometric analysis, and clonogenic assay. Part of this work has been supported by the "Österreichische Kinderkrebshilfe."

REFERENCES

1. Bodey GP, Buckley M, Sathe YS, Freireich EJ: Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. Ann Intern Med 64:328–340, 1966.
2. Bodey G: Infection in cancer patients: a continuing association. Am J Med 81 [suppl A]:11–26, 1986.
3. Pizzo PA: Granulocytopenia and cancer therapy: past problems, current solutions, future challenges. Cancer 54:2649–2661, 1984.
4. Sculier JP, Weerts D, Klastersky J: Causes of death in febrile granulocytopenic cancer patients receiving empiric antibiotic therapy. Eur J Cancer Clin Oncol 20:55–60, 1984.
5. Pizzo PA, Hathorn JW, Hiemenz J, Browne M, Commers J, Cotton D, Gress J, Longo D, Marshall D, McKnight J, et al: A randomized trial comparing ceftazidime alone with combination antibiotic therapy in cancer patients with fever and neutropenia. N Engl J Med 315:552–558, 1986.
6. Fink FM, Frischhut-Schiffer EM, Ausserer B, Allerberger F, Guggenbichler JP: Empirical antimicrobial therapy of septicemia or fever in children and adolescents with neutropenia following cytotoxic chemotherapy. Paediatr Paedol 24:33–42, 1989.
7. Lieschke GJ, Burgess AW: Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (first of two parts). N Engl J Med 327:28–35, 1992.
8. Lieschke GJ, Burgess AW: Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (second of two parts). N Engl J Med 327:99–106, 1992.
9. Nemunaitis J: Granulocyte-macrophage-colony-stimulating factor: a review from preclinical development to clinical application. Transfusion 33:70–83, 1993.
10. Blackwell S, Crawford J: Colony-stimulating factors: clinical applications. Pharmacother 12:20S–31S, 1992.
11. Shadduck RK: Granulocyte-macrophage colony-stimulating factor: present use and future directions. Semin Hematol 29 [suppl 3]:38–42, 1992.
12. Furman WL, Pratt CB, Fairclough D, Arnold B, Bowman L, Santana V, Meyer W, Crist WM: Phase I/II trial of granulocyte-macrophage colony-stimulating factor (rh-GM-CSF) after intensive chemotherapy for patients with relapsed neuroblastoma [abstr 76, SIOP XXII]. Med Pediatr Oncol 18:383–384, 1990.
13. Furman WL, Fairclough D, Huhn RD, Pratt CB, Stute N, Petros WP, Evans WE, Bowman LC, Douglass EC, Santana VM, et al: Therapeutic effects and pharmacokinetics of recombinant human granulocyte-macrophage colony-stimulating factor in childhood cancer patients receiving myelosuppressive chemotherapy. J Clin Oncol 9:1022–1028, 1991.
14. Furman WL, Fairclough D, Cain AM, Arnold BA, Pratt CB, Meyer WH, Crist WM: Use of GM-CSF in children after high-dose chemotherapy. Med Pediatr Oncol [suppl]2:26–30, 1992.
15. Saarinen UM, Hovi L, Riikonen P, Pihkala J, Juvonen E: Recombinant human granulocyte-macrophage colony-stimulating factor in children with chemotherapy-induced neutropenia. Med Pediatr Oncol 20:489–496, 1992.
16. Nemunaitis JJ: Rh-GM-CSF in bone-marrow transplantation: experience in pediatric patients. Med Pediatr Oncol [suppl] 2:31–33, 1992.
17. Riikonen P, Saarinen UM, Mäkipernaa A, Hovi L, Komulainen A, Pihkala J, Jalanko H: Rh-granulocyte-macrophage colony-stimulating factor in the treatment of febrile neutropenia: a double-blind placebo-controlled study in children. Pediatr Infect Dis J 13:197–202, 1994.
18. Mayordomo JI, Rivera F, Díaz-Puente MT, Lianes MP, López-Brea M, López E, Paz-Area L, Hitt R, Alonso S, Sevilla I, Rivas I, Cortés-Funes H: Decreasing morbidity and cost of treating febrile neutropenia by adding G-CSF and GM-CSF to standard antibiotic therapy: results of a randomized trial [abstr 1510]. Proc Amer Soc Clin Oncol 12:437, 1993.
19. Maher D, Green M, Bishop J, Stuart-Harris R, Lieschke G, Wolf M, Sheridan W, Kefford R, Cebon J, Olver I, McKendrick J, Toner G, Lieschke M, Bradstock K, Cruickshank S, Hoffman E, Fox R, Morstyn G: Randomized, placebo-controlled trial of filgrastim (r-metHuG-CSF) in patients with febrile neutropenia fol-

lowing chemotherapy [abstr 1498]. Proc Amer Soc Clin Oncol 12:434, 1993.
20. Biesma B, de Vries EGE, Willemse PHB, Sluiter WJ, Postmus PE, Limburg PC, Stern AC, Vellenga E: Efficacy and tolerability of recombinant human granulocyte-macrophage colony-stimulating factor in patients with chemotherapy-related leukopenia and fever. Eur J Cancer 26:932–936, 1990.
21. Gerhartz HH, Stern AC, Wolf-Hornung B, Kazempour M, Schmetzer H, Gugerli U, Jones TC, Wilmanns W: Intervention treatment of established neutropenia with human recombinant granulocyte-macrophage colony-stimulating factor (rhGM-CSF) in patients undergoing cancer chemotherapy. Leuk Res 17:175–185, 1993.
22. Fritsch G, Emminger W, Buchinger P, Printz D, Gadner H: CD34-positive cell proportions in peripheral blood correlate with colony-forming capacity. Exp Hematol 19:1079–1083, 1991.
23. Fritsch G, Buchinger P, Printz D: Use of flow cytometric CD34 analysis to quantify hematopoietic progenitor cells. Leukemia Lymphoma 10:443–451, 1993.
24. Kaplan E, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958.
25. Lee ET: "Statistical methods for survival data analysis." Belmont, CA: Wadsworth, 1980.
26. Dixon WJ, Merdian K. "ANOVA and regression with BMDP 5V." Los Angeles, CA: Dixon Statistical Associates, 1992.
27. Holldack J, Burdach S, Eisberg A, Frisch J, Schulz G: Biology and pharmacology of hematopoietic growth factors. Med Pediatr Oncol [suppl] 2:2–9, 1992.
28. Cebon J, Lieschke G, Bury R, Morstyn G: The dissociation of GM-CSF efficacy from toxicity according to the route of administration: a pharmacodynamic study. Br J Hematol 80:144–150, 1992.
29. Siena S, Bregni M, Brando B, Ravagnani F, Bonadonna G, Gianni AM: Circulation of CD34-positive hematopoietic stem cells in the peripheral blood of high-dose cyclophosphamide-treated patients: enhancement by intravenous recombinant human granulocyte-macrophage colony-stimulating factor. Blood 74:1905–1914, 1989.
30. Fritsch G, Emminger W, Buchinger P, Printz D, Gadner H. CD34 analysis in peripheral blood correlates with colony forming capacity—an update. In Gross GS, Gee A, Worthington-White D (eds): "Advances in Bone Marrow Purging and Processing. Progress in Clinical and Biological Research," Vol. 377. New York: Wiley-Liss, Inc., 1992, pp. 531–536.
31. Emminger W, Fritsch G, Emminger-Schmidmeier W, Buchinger P, Printz D, Kundi M, Gadner H: Recovery kinetics after chemotherapy and circulating mononuclear cells expressing the CD34 antigen in pediatric cancer patients. Ann Hematol 64:181–184, 1992.
32. Peters WP, Rosner G, Ross M, Vredenburg J, Meisenberg B, Gilbert C, Krutzberg J: Comparative effects of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) on priming peripheral blood progenitor cells for the use with autologous bone marrow after high-dose chemotherapy. Blood 81:1709–1719, 1993.
33. Fritsch G, Buchinger P, Printz D, Fink FM, Mann G, Peters C, Wagner T, Adler A, Gadner H: Rapid discrimination of early CD34+ myeloid progenitors using CD45-RA analysis. Blood 81: 2301–2309, 1993.
34. Gattringer C, Thaler J, Drach J, Miksche M, Huber H: GM-CSF treatment in aplasia after cytotoxic therapy. Onkologie 12:16–18, 1989.