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RESEARCH ARTICLE

Use of granulocyte colony-stimulating factor and risk of relapse in pediatric patients treated for acute myeloid leukemia according to NOPHO-AML 2004 and DB AML-01

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Abstract

Background: Supportive-care use of granulocyte colony-stimulating factor (G-CSF) in pediatric acute myeloid leukemia (AML) remains controversial due to a theoretical increased risk of relapse and limited impact on neutropenic complications. We describe the use of G-CSF in patients treated according to NOPHO-AML 2004 and DB AML-01 and investigated associations with relapse.

Procedure: Patients diagnosed with de novo AML completing the first week of therapy and not treated with hematopoietic stem cell transplantation in the first complete remission were included (n = 367). Information on G-CSF treatment after each course (yes/no) was registered prospectively in the study database and detailed information was gathered retrospectively from each center. Descriptive statistics were used to describe G-CSF use and Cox regression to assess the association between G-CSF and risk of relapse.

Results: G-CSF as supportive care was given to 128 (35%) patients after 268 (39%) courses, with a large variation between centers (0-93%). The use decreased with time—the country-adjusted odds ratio was 0.8/diagnostic year (95% confidence interval [CI] 0.7-0.9). The median daily dose was 5 μg/kg (range 3-12 μg/kg) and the median cumulative dose was 75 μg/kg (range 7-1460 μg/kg). Filgrastim was used in 82% of G-CSF administrations and infection was the indication in 44% of G-CSF administrations. G-CSF was associated with increased risk of relapse—the adjusted hazard ratio was 1.5 (95% CI 1.1-2.2).

Conclusions: G-CSF as supportive care was used in a third of patients, and use decreased with time. Our results indicate that the use of G-CSF may be associated with an increased risk of relapse.

KEYWORDS

AML, G-CSF, pediatric, relapse

1 | INTRODUCTION

Treatment for pediatric acute myeloid leukemia (AML) is highly toxic and 5-10% of patients die as a consequence of treatment.[1–7] Infections during neutropenia are the most frequent cause of treatment-related mortality and a major cause of morbidity.[1,2,4,6–8] Granulocyte colony-stimulating factor (G-CSF) has been used to decrease time with neutropenia, aiming to reduce severe infections and treatment-related mortality. Yet, while G-CSF has been shown to reduce the duration of neutropenia in children treated for cancer,[9] the use of G-CSF in AML remains controversial. This is due to reports showing that myeloid leukemia cells express G-CSF receptors and that G-CSF can stimulate proliferation of leukemic blasts in vitro.[10,11] Furthermore, an increased risk of relapse has been shown in patients receiving G-CSF and overexpressing *G-CSFR* isoform IV.[12] G-CSF does not seem to reduce microbiologically documented infections or treatment-related mortality.[13,14] The general use of G-CSF for prevention of infectious complications in children or adults during treatment is currently not recommended.[15,16]

In the last concluded Nordic and Dutch-Belgian pediatric AML protocols (NOPHO-AML 2004 and DB AML-01), G-CSF was not part of standard recommendations but could be administered according to physician preference. In this study, we aimed to describe supportive-care G-CSF use including indications and cumulative doses in children treated according to NOPHO-AML 2004 and DB AML-01. Secondly, we aimed to investigate associations between G-CSF treatment and risk of relapse.

2 | MATERIALS AND METHODS

2.1 | Patients

Patients treated according to NOPHO-AML 2004 and DB AML-01 were eligible for this study. The protocols were population based in children less than 15 years of age (for some centers, less than 18 years) diagnosed with AML in the five Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) from 2004 to 2013 (NOPHO-AML 2004), in Hong Kong from 2007 to 2013 (NOPHO-AML 2004), in Belgium from 2010 to 2014 (DB AML-01), and in the Netherlands from 2010 to 2013 (DB AML-01). Children with Down syndrome, acute promyelocytic leukemia, isolated granulocytic sarcoma, or secondary AML were excluded. Further exclusion criteria for this study were (a) death within the first week after starting treatment and (b) treatment with hematopoietic stem cell transplantation (HSCT) in the first complete remission (CR1).

2.2 | Protocols

Protocol details for NOPHO-AML 2004 and DB AML-01 including drug doses have been published previously.[17–19] The NOPHO-AML 2004 protocol consisted of two induction courses (AIET and AM) and four consolidation courses (HA$_1$M, HA$_2$E1, HA$_3$, and HA$_2$E2) followed by a randomization to gemtuzumab ozogamicin or no further therapy. Children not in remission after two induction courses were given FLAG as a third induction course, whereas those in remission proceeded to consolidation therapy. G-CSF given as a part of FLAG was not considered supportive care, and is therefore not included in this study. High-risk patients (bone marrow blasts >15% after AIET or no remission after second induction course or the presence of *KMT2A* rearrangements other than t[9;11][p21;q23]) were offered allogeneic HSCT. The following three amendments were made during the protocol period: (1) In 2009, the high-risk criteria were restricted to poor response only, (2) in 2011, *FLT3* with internal tandem duplication (*FLT3*/ITD) without *NPM1* mutations became a high-risk criterion, and (3) in 2011, the second induction course was changed from AM to FLA-Dx in patients with t(8;21) or bone marrow blasts 5-15% on day 15.[20]

The DB AML-01 protocol was similar to NOPHO-AML 2004, and included the same two induction courses, but only three consolidation courses (HA$_2$E1, HA$_3$, and HA$_2$E2, omitting HA$_1$M). Moreover, the protocol did not recommend HSCT in CR1 for any patient and did not include randomization to gemtuzumab ozogamicin. Children not in remission after two induction courses were taken off protocol. The protocols were approved by the national ethics committees in the eight participating countries and written informed consent was obtained from all patients or their parents or guardians.

2.3 | Supportive care

The NOPHO-AML 2004 protocol had recommendations on prophylactic sulfamethoxazole/trimethoprim 2-3 days per week. Prophylactic fluconazole was recommended until 1 month after the last chemotherapy course. No other prophylactic antibacterial or antiviral drugs were recommended. Use of prophylactic G-CSF was not generally recommended, but G-CSF could be considered in patients with neutropenia and life-threatening infection. The DB AML-01 protocol did not contain specific recommendations on prophylactic supportive care and thus prophylactic measures depended on local practice.

2.4 | Data collection

Data on demographics, toxicity, and G-CSF administration (yes/no) after each course and follow-up information were registered prospectively in the joined database for the two protocols by the treating

physician or local data manager. If data on G-CSF administration were missing for a course in the protocol database, it was assumed G-CSF was not given. If G-CSF administration was registered for a patient, an exploratory questionnaire was sent to the center where the patient was treated, retrospectively collecting information on G-CSF preparation, dose, treatment duration, and indication (infection, neutropenia without infection, prophylactic, or other). The indication was defined as prophylactic if G-CSF was administered within the first week after course start. If the daily dose of G-CSF was missing, the patient was assumed to have received the recommended dose for the cumulative dose analyses (filgrastim and lenograstim 5 μg/kg/day, pegfilgrastim 100 μg/kg given once).[21,22]

2.5 | Definitions and statistics

Standard deviation score of body mass index (BMI) for age and sex was calculated according to the World Health Organization growth standards.[23] Chemotherapy dose reductions were defined as more than a 20% reduction of at least one drug dose or chemotherapy interruption of more than 7 days not including intrathecal treatment. Multiple logistic regression was used to assess the association of diagnostic year and G-CSF administration. Cox regression was used to assess the association between G-CSF administration and risk of relapse. For this analysis, follow-up started at the last day with absolute neutrophil count $<0.5 \times 10^9$/L after the last consolidation course (HA$_2$E2) and ended at relapse, death, or end of follow-up. This design excluded treatment-related mortality during therapy. Restricted cubic splines with three knots (at the 50th, 75th, and 90th percentiles)[24] were used to show the continuous relationship of the cumulative dose of G-CSF and risk of relapse. Confounders incorporated into statistical models were defined as factors, which could be a cause of both independent and dependent variables, and were selected a priori. Country was included in the model of diagnostic year and G-CSF administration. Sex, age (continuous), BMI standard deviation score (continuous), t(8;21) (yes/no), inv(16) (yes/no), KMT2A rearrangements (yes/no), FLT3/ITD status (FLT3/ITD yes/no), response to first induction (≥5% blasts between day 15 and start of second induction) (yes/no), and dose reductions (yes/no) were included as covariates in the adjusted model of G-CSF treatment and risk of relapse. Missing values for FLT3 status (24%) were handled by multiple imputation for the analysis of G-CSF treatment and risk of relapse; for details, see Supplementary Material S1. All tests of significance were two sided. Statistical significance was defined as $P < 0.05$.

3 | RESULTS

3.1 | G-CSF treatment in NOPHO-AML 2004 and DB AML-01

Of the 434 patients registered on NOPHO-AML 2004 and DB AML-01, five patients died before day 8 and 62 were treated with HSCT in CR1, and therefore excluded from this study (Figure 1). In total, 367 patients were included and 128 (35%) received G-CSF as

supportive care at least once during treatment (Figure 1). Four of the included patients received FLAG as a third induction course. Baseline characteristics for the cohort are shown in Table 1. Data on G-CSF administration were missing in the protocol database after AIET in six (2%) cases, after AM in four (1%) cases, after HA$_1$M in two (1%) cases, after HA$_2$E1 in 11 (3%) cases, after HA$_3$ in 10 (3%) cases, and after HA$_2$E2 in 12 (4%) cases. In total, 1979 courses were given (not including GO and extra induction courses) and G-CSF was given after 269 (14%) courses. In patients that received G-CSF at least once, 695 courses were given followed by G-CSF administration in 269 (39%). Of the 367 patients in this study, 239 (65%) never received G-CSF, 66 (18%) received it after one course, 17 (5%) after two, 27 (7%) after three, eight (2%) after four, four (1%) after five, and six (2%) received G-CSF after six courses of chemotherapy. The use of G-CSF differed between countries, but even more between centers—in centers that had treated 10 or more protocol patients, the percentage of patients that received G-CSF at least once ranged from 0 to 93%. Of patients treated according to NOPHO-AML 2004, 39% had received G-CSF at least once, and of patients treated according to DB AML-01, 23% had received G-CSF at least once. G-CSF use was more common in patients treated between 2004 and 2009 and in patients with core binding factor leukemia (t[8;21] or inv[16]/t[16;16]), and less common in patients treated between 2010 and 2014, with FLT3/ITD and with KMT2A rearrangements (Table 1). The median cumulative neutrophil recovery time per course did not correlate with cytogenetic abnormalities or FLT3/ITD status (Supplementary Table S2). The odds ratio (OR) of G-CSF administration decreased with increasing diagnostic year (crude OR 0.9/diagnostic year, 95% confidence interval [CI] 0.8-1.0; country-adjusted OR 0.8/diagnostic year, 95% CI 0.7-0.9).

In 19 patients with registered G-CSF use, all or some additional information was missing (Figure 1). The dose of G-CSF was missing after 11 courses. In the remaining cases, the median cumulative G-CSF dose was 75 μg/kg (range: 7-1460 μg/kg). The median total treatment duration was 13 days (range 1-85 days) in patients treated with filgrastim or lenograstim.

Table 2 shows information on G-CSF treatment per course. G-CSF treatment was most common after HA$_1$M and FLA-Dx and the most commonly used drug was filgrastim. The median daily dose of filgrastim or lenograstim was 5 μg/kg (range 3-12 μg/kg). The most common indication for treatment was infection. G-CSF treatment was usually started 2-3 weeks after the start of course if the indication was infection or neutropenia, but immediately after the end of chemotherapy if G-CSF was administered prophylactically (Table 2). The cumulative dose per course was higher if G-CSF was administered prophylactically.

3.2 | G-CSF treatment and risk of relapse

Of the included patients, 46 did not receive all chemotherapy courses due to leaving protocol (n = 4), relapse (n = 26), and death (n = 16; not in CR1 [n = 8]; in CR1 [n = 8]). Of those that received and recovered from the last consolidation course (n = 321), 134 (42%) relapsed. All patients that received the last consolidation course were in complete remission after two induction courses and therefore none had
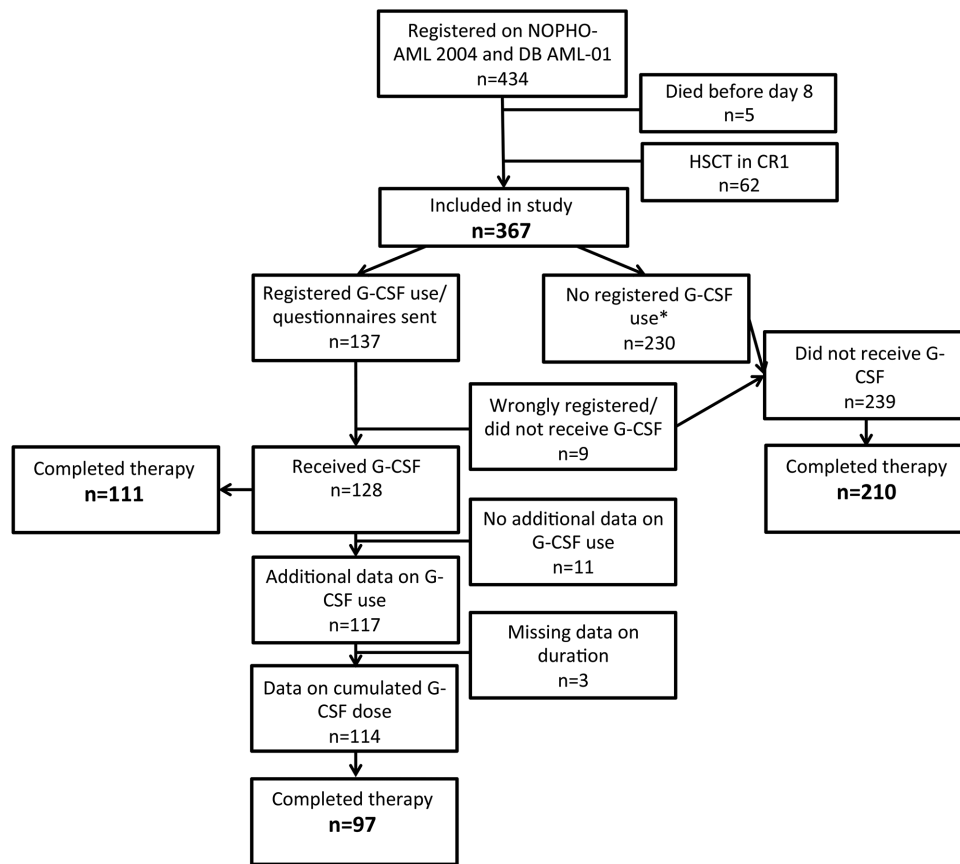
FIGURE 1 Flow diagram of included and G-CSF treated patients; G-CSF, granulocyte colony-stimulating factor; HSCT, hematopoietic stem cell transplantation; CR1, first complete remission. *Missing data on G-CSF administration in NOPHO database: after AIET in six (2%) cases, after AM in four (1%) cases, after HA_1M in two (1%) cases, after HA_2E1 in 11 (3%) cases, after HA_3 in 10 (3%) cases, and after HA_2E2 in 12 (4%) cases

received G-CSF (FLAG) as a part of treatment. The median follow-up time starting at recovery from last consolidation course for patients alive at last follow-up was 5.6 years (range 1.0-12.5 years). Patients that had received G-CSF at least once during treatment had higher risk of relapse compared to those who had not received G-CSF (5-year cumulative incidence of relapse: 51 vs 39%, Table 3). Cumulative dose of G-CSF above the median did not increase the risk of relapse further (Table 3). In the continuous relationship of cumulative G-CSF dose and risk of relapse, it appears that a plateau in the risk of relapse was reached at a cumulative G-CSF dose of about 75-100 μg/kg (Figure 2). Increasing doses above 100 μg/kg appeared to be associated with a slight decrease in risk of relapse, but the CI is wide.

4 | DISCUSSION

In this large, population-based cohort including 367 unselected pediatric patients newly diagnosed with AML and treated in the Nordic countries, Hong Kong, the Netherlands, and Belgium from 2004 to 2013, we found that G-CSF was used as supportive care in about a third of patients, but the use decreased over time. There were large differences in G-CSF use across countries and centers. Filgrastim was the most commonly used drug and infection was the most common indication. Patients who had received G-CSF and finished treatment were

at about 50% higher risk of relapse after adjustment for the most common and well-recognized risk factors.

G-CSF reduces the duration of neutropenia in children and adults treated for AML, but does not seem to reduce the risk of microbiologically documented infections or treatment-related mortality.[13,14] Therefore, prophylactic G-CSF is not recommended in adults or children treated for AML.[15,16] In the NOPHO-AML 2004 protocol, G-CSF was not recommended for prophylactic use, but could be considered for patients with life-threatening bacterial or fungal infections. The DB AML-01 protocol did not include recommendations on G-CSF use. Despite the recommendations, a third of the patients received G-CSF, though in the majority the indication was other than prophylactic. Restricting analysis to Nordic patients treated after 2009, about a fifth had received G-CSF (Table 1). This is comparable to a recent publication from the International Berlin-Frankfürt-Münster group showing that around a third of study groups use G-CSF in case of severe infection or prolonged neutropenia.[25] Infection was the most common indication for G-CSF administration (Table 2), which is compatible with the fact that the frequency of severe infections was high for patients treated according to NOPHO-AML 2004.[7] We have previously shown that AIET and HA_1M were the most toxic courses and neutrophil recovery time was longer after AIET, FLA-Dx, and HA_1M,[7,26] which corresponds well with G-CSF being administered more frequently after these courses. More patients treated according to NOPHO-AML

TABLE 1 Baseline characteristics of the entire cohort and cohort stratified on G-CSF treatment

| | | | G-CSF treatment | |
| | | Entire cohort | Never | At least once |
		n (%)	n (%)	n (%)
Patients		367 (100)	239 (65)	128 (35)
Sex	Male	193 (53)	118 (49)	75 (59)
	Female	174 (47)	121 (51)	53 (41)
Age	Median (range)	6 (0-17)	5 (0-17)	7 (0-17)
BMI standard deviation score*	Median (range)	0.0 (−4.0–3.1)	0.0 (−4.0–3.1)	0.0 (−3.8–3.0)
Country	Sweden	91 (25)	61 (26)	30 (23)
	Denmark	47 (13)	38 (16)	9 (7)
	Norway	39 (11)	28 (12)	11 (9)
	Finland	39 (11)	19 (8)	20 (16)
	Iceland	5 (1)	4 (2)	1 (1)
	Belgium	33 (9)	26 (11)	7 (5)
	The Netherlands	68 (19)	52 (22)	16 (13)
	Hong Kong	45 (12)	11 (5)	34 (27)
Protocol	NOPHO-AML 2004	266 (72)	161 (67)	105 (82)
	DB AML-01	101 (28)	78 (33)	23 (18)
Diagnostic year group	2004-2009	154 (42)	84 (35)	70 (55)
	2010-2014	213 (58)	155 (65)	58 (45)
Diagnostic year group, Nordic countries only	2004-2009	137 (62)	81 (54)	56 (79)
	2010-2013	84 (38)	69 (46)	15 (21)
WBC (10^9/L) at diagnosis*	Median (range)	21 (1-495)	23 (1-495)	18 (1-426)
FLT3	ITD	31 (8)	24 (10)	7 (5)
	Wild type/other	248 (89)	167 (70)	81 (63)
	Unknown	88 (24)	48 (20)	40 (31)
Cytogenetic abnormalities*	t(8;21)	61 (17)	34 (14)	27 (21)
	inv(16)/t(16;16)	38 (10)	20 (8)	18 (14)
	KMT2A	86 (23)	68 (28)	18 (14)
≥5% blast in bone marrow between day 15 and start of second induction*		87 (24)	56 (23)	31 (24)
FLA-Dx as second induction		39 (11)	28 (12)	11 (9)
Treatment duration per course in days for those who completed treatment*	Median (range)	30 (19-47)	29 (19-46)	31 (22-47)
Dose reductions of least one course§		21 (6)	12 (5)	9 (7)

BMI, body mass index; G-CSF, granulocyte colony-stimulating factor; ITD, internal tandem duplication; WBC, white blood count.
*BMI missing in three cases (0.8%), WBC missing in one (0.3%), cytogenetic abnormalities missing in two cases (0.5%), bone marrow blasts after the first induction missing in six cases (2%), and treatment duration missing in five cases.
§More than 20% reduction of at least one drug dose or chemotherapy interruption of more than 7 days, intrathecal treatment not included.

2004 compared to patients treated according to DB AML-01 had received G-CSF (39 vs 23%), which could be due to DB AML-01 being less toxic, but the two groups also differ in median diagnostic year and institutional practices.

Patients with core binding factor leukemia received G-CSF more often. Our group has previously shown that patients with t(8,21) had inferior outcome before the second induction course was changed from AM to FLA-Dx in 2011 for patients with this cytogenetics abnormality.[20] The model of G-CSF and risk of relapse included t(8;21) as a covariate. There was no difference in median cumulative neutrophil recovery time per course according to cytogenetic or FLT3/ITD status (Supplementary Table S2).

We found that G-CSF administration was associated with an increased risk of relapse in our cohort of patients. In adults with AML, a large meta-analysis showed no association of G-CSF use and risk of relapse,[13] but in children the association is not well studied. The pediatric AML protocol AML-BFM 98 included randomization to either no G-CSF or prophylactic G-CSF during induction and showed a trend for increased relapse risk in standard-risk patients treated with G-CSF.[14] In a subsequent analysis of 50 standard risk patients treated according to the AML-BFM 98 protocol, it was found that patients overexpressing the differentiation-defective G-CSFR isoform IV treated with G-CSF had an increased risk of relapse.[12] Our study cohort is comparable to the standard risk group of AML-BFM 98 in that we excluded patients

TABLE 2 G-CSF treatment per course

		AIET	AM	FLA-Dx	HA$_1$M	HA$_2$E1	HA$_3$	HA$_2$E2	In total
Course given, n		367	321	39	254	343	330	325	1979
G-CSF administered, n (%)[a]		57 (16)	41 (13)	8 (21)	51 (20)	45 (13)	33 (10)	34 (10)	269 (14)
Drug received,[b] n (%)	Filgrastim	50 (88)	32 (78)	8 (100)	40 (78)	37 (82)	22 (67)	22 (65)	211 (82)
	Lenograstim	1 (2)	2 (5)	0	3 (6)	1 (2)	0	3 (9)	10 (4)
	Pegfilgrastim	2 (4)	3 (7)	1 (13)	5 (10)	4 (9)	5 (15)	3 (9)	23 (9)
	Missing	6 (11)	4 (10)	0	4 (8)	5 (11)	8 (24)	4 (12)	31 (12)
Cumulative dose in μg/kg, median (range)		51 (10-245)	39 (5-200)	146 (45283)	43 (5-540)	50 (5-220)	53 (5-200)	55 (5-200)	75 (7-1460)
Indication for G-CSF, n (%)	Infection	35 (61)	18 (44)	6 (75)	22 (43)	15 (33)	10 (30)	12 (35)	118 (44)
	Neutropenia	13 (23)	17 (41)	1 (13)	19 (37)	16 (36)	8 (24)	11 (32)	85 (32)
	Prophylactic	3 (5)	4 (10)	1 (13)	6 (12)	8 (18)	7 (21)	5 (15)	34 (13)
	Other[c]	2 (4)	0	0	0	0	0	0	2 (0.7)
	Missing	4 (7)	2 (5)	0	4 (8)	6 (13)	8 (24)	6 (18)	30 (11)
Course start to G-CSF start, median (range)	Infection	17 (6-32)	15 (0-31)	N/A	18 (13-37)	15 (10-26)	15 (3-19)	15 (7-16)	16 (0-37)
	Neutropenia	18 (5-37)	17 (11-27)	N/A	18 (6-35)	15 (4-31)	14 (3-40)	13 (6-35)	16 (2-40)
	Prophylactic	N/A	N/A	N/A	N/A	5 (4-6)	4 (2-6)	N/A	5 (2-7)
Cumulative dose in μg/kg per course, median (range)	Infection	50 (10-145)	43 (5-120)	N/A	35 (5-540)	40 (15-175)	40 (15-125)	30 (5-157)	43 (5-540)
	Neutropenia	63 (20-150)	30 (5-75)	N/A	45 (5-140)	38 (5-150)	53 (5-115)	60 (20-200)	45 (5-202)
	Prophylactic	N/A	N/A	N/A	N/A	107 (80-220)	100 (28-200)	N/A	100 (5-395)

G-CSF, granulocyte colony-stimulating factor; N/A, not applicable (median only stated for seven or more cases).

[a]Data on G-CSF administration were missing in the protocol database after AIET in six (2%) cases, after AM in four (1%) cases, after HA$_1$M in two (1%) cases, after HA$_2$E1 in 11 (3%) cases, after HA$_3$ in 10 (3%) cases, and after HA$_2$E2 in 12 (4%) cases. In case of missing data in the protocol database, it was assumed that the patient did not receive G-CSF.

[b]After seven courses, patients received pegfilgrastim followed by filgrastim (two patients after AIET, one patient after FLA-Dx, one patient after HA$_1$M, two patients after HA$_2$E1, and one patient after HA$_3$).

[c]Other indications cover severe abdominal toxicity and G-CSF given to hasten marrow recovery.

TABLE 3 G-CSF treatment and risk of relapse

	Patients who finished treatment (n)	Five-year cumulative incidence of relapse (95% CI)	Crude HR (95% CI)	P-value	Adjusted[b] HR (95% CI)	P-value
Never G-CSF treated	210	39% (32-45%)	1	-	1	-
G-CSF treated	111	51% (41-60%)	1.4 (1.0-1.9)	0.07	1.5 (1.1-2.2)	0.03
G-CSF ≤75 μg/kg[a]	49	52% (37-66%)	1.5 (0.9-2.3)	0.09	1.5 (1.0-2.5)	0.07
G-CSF >75 μg/kg[a]	48	47% (32-62%)	1.2 (0.8-2.0)	0.4	1.4 (0.8-2.3)	0.2

CI, confidence interval; G-CSF, granulocyte colony-stimulating factor; HR, hazard ratio.

[a]Cumulative G-CSF dose was missing for 14 patients.

[b]Adjusted for sex, age, BMI standard deviation score, *FLT3*/ITD status, *KMT2A* rearrangements, inv(16), t(8;21), response to first induction (≥5% blasts between day 15 and start of the second induction course [yes/no]), and any dose reductions (yes/no).

treated with HSCT. The report of AML-BFM 98 does not address cumulative doses of G-CSF given, but patients received 5 μg/kg/day from day 15 of first and second induction course and continued until the absolute neutrophil count exceeded 0.5×10^9/L on 3 consecutive days. Median time in neutropenia after the first induction course was 18 days and after the second induction course 11 days for G-CSF treated and therefore we estimate the median cumulative dose to be $(18+11) \times 5 = 145$ μg/kg. This is twice as much as the median cumulative dose

of our study, but comparable to that of those patients in our study that received G-CSF prophylactically. Figure 2 indicates that the increasing risk of relapse with increasing median cumulative G-CSF dose plateaus at 75-100 μg/kg. Few patients were treated with doses over this level, hence statistical power to find the plateau threshold is limited. The fact that a plateau is reached corresponds with a study by Inaba et al showing that higher doses of filgrastim (10 vs 5 μg/kg/day) did not further decrease time in neutropenia.[27] If higher doses of G-CSF do not
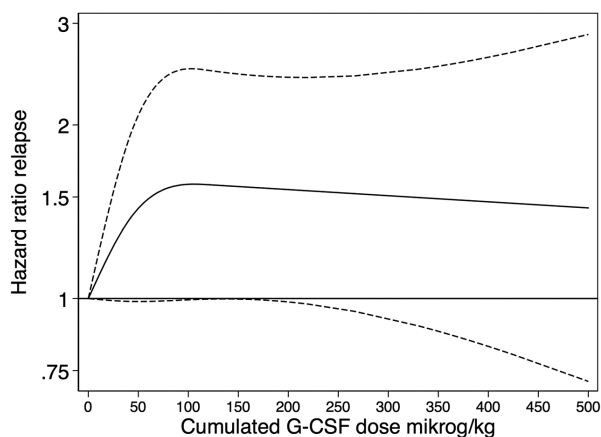
FIGURE 2 Cumulative G-CSF dose and risk of relapse; the figure shows the relationship of cumulative dose of G-CSF in μg/kg and hazard ratio for relapse (solid line) with 95% confidence intervals (dotted lines). Patients that received G-CSF but with missing cumulative dose (n = 14) were excluded from this analysis. Estimates are adjusted for sex, age (continuous), BMI standard deviation score (continuous), *FLT3*/ITD status (*FLT3*/ITD yes/no/missing), response to first induction ($\geq$5% blasts from day 15 to start of second induction yes/no), and any dose reductions (yes/no). Only three patients received cumulative G-CSF dose above 500 μg/kg, and therefore the x-axis is terminated at 500 μg/kg. G-CSF, granulocyte colony-stimulating factor

stimulate neutrophil proliferation further, it is possible that the same is true for AML blasts.

This study needs to be interpreted in light of its limitations. The frequency of G-CSF administration could be underestimated because in case of missing registration in the protocol database, it was assumed that the patient had not received G-CSF. However, since registration was close to complete (<5% missing), we believe this potential bias to be small. Also, with our nonrandomized study design, it was difficult to determine whether patients relapsed due to G-CSF treatment or were treated with G-CSF due to poor general condition, which could be associated with relapse. However, we incorporated the most common, well-established, and potential risk factors for relapse (sex, age, BMI standard deviation score, t(8;21), inv(16), *KMT2A* rearrangements, *FLT3*/ITD status, response to first induction, and dose reductions) in the statistical model and the association remained. Nevertheless, we acknowledge the potential for residual confounding, since it would be impossible to incorporate into a model all the factors that physicians use in the clinical decision of whether G-CSF should be given. Follow-up began at the end of treatment, which separates exposure and outcome in time, avoiding the confounding factor that imminent relapse could result in slow bone marrow regeneration leading to G-CSF administration.

In conclusion, we found that supportive-care use of G-CSF was frequent for patients treated on NOPHO-AML 2004 and DB AML-01 with a large variation between centers. The use of G-CSF decreased over time. Our results indicate that children that received G-CSF as supportive care were at an increased risk of relapse and support the concern that G-CSF use might be harmful in pediatric AML.

AUTHOR CONTRIBUTIONS

D.J.A.L., P.H.A., and H.H. designed the project, interpreted the data, and drafted the manuscript; D.J.A.L. performed statistical analyses; J.A., G.J.L.K., S.-Y.H., O.G.J., M.K., B.L., B.D.M., J.P., and B.Z. collected the data and contributed to the writing of the manuscript.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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