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Effect of age and body weight on toxicity and survival in pediatric acute myeloid leukemia: results from NOPHO-AML 2004

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Haematologica 2016
Volume 101(11):1359-1367

ABSTRACT

Treatment for pediatric acute myeloid leukemia is very toxic and the association between outcome and age and Body Mass Index is unclear. We investigated effect of age and Body Mass Index on toxicity and survival in pediatric acute myeloid leukemia. We studied all patients who completed first induction course of NOPHO-AML 2004 (n=318). Toxicity following induction and consolidation courses (n=6) was analyzed. The probabilities of toxicity and death were determined using time-to-event analyses with Cox multivariate proportional hazard regression for comparative analyses. Age 10-17 years was associated with sepsis with hypotension [hazard ratio 2.3 (95% confidence interval 1.1-4.6)]. Being overweight (>1 standard deviation) was associated with requiring supplemental oxygen [1.9 (1.0-3.5)]. The 5-year event-free and overall survival were 47% and 71%. Children aged 10-17 years showed a trend for inferior 5-year overall survival compared to children aged 2-9 (64% *vs.* 76%; *P*=0.07). Infants showed a trend for superior 5-year event-free survival (66% *vs.* 43%; *P*=0.06). Overweight children aged 10-17 years showed a trend for superior survival [5-year event-free survival 59% *vs.* 40% (*P*=0.09) and 5-year overall survival 78% *vs.* 56% (*P*=0.06)] compared to healthy weight children aged 10-17 years. In conclusion, children aged 10-17 years and overweight children had a higher risk of grade 3-4 toxicity. Children aged 10-17 years showed inferior survival, but, unexpectedly, in this age group overweight children tended to have increased survival. This suggests different pharmacokinetics of chemotherapeutic drugs in adolescents and warrants further studies.

Introduction

Despite good overall treatment results for childhood acute myeloid leukemia (AML), 30%-35% of patients die from resistant disease, relapse, or treatment-related toxicity.[1-3] Due to the high-intensity treatment of pediatric AML, most patients develop severe toxicity, and about 10% die from treatment-related toxicity.[2,4-7] The most common toxicity is infection, often leading to life-threatening sepsis. Almost all patients with AML experience an infection during the first induction course.[8,9] Older age and being overweight at diagnosis have been associated with inferior survival in children with AML[10-15] and older age has been associated with treatment-related mortality (TRM).[14,16-18] Infants and older children treated for AML have more severe infections[18,19] and being overweight has been associated with more severe

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Received: March 17, 2016.
Accepted: July 25, 2016.
Pre-published: July 28, 2016.
doi:10.3324/haematol.2016.146175

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/101/11/1359

abdominal pain, hypotension, pulmonary toxicity and coagulopathy.[13]

Despite pediatric AML treatment causing significant morbidity and mortality, no previous studies have thoroughly reviewed the numerous treatment-related grade 3-4 toxicities or investigated if age and body weight at diagnosis is associated with risk of toxicity.

We aimed to describe all grade 3-4 toxicities in the Nordic Society of Pediatric Hematology and Oncology (NOPHO) AML 2004 protocol and investigate associations between toxicity, survival, age and body weight at diagnosis.

Methods

Patients

The NOPHO-AML 2004 protocol (*clinicaltrials.gov identifier: 00476541*) included all children below 15 years of age (for some centers all children below 18 years) diagnosed with AML in the five Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) from 2004-2013 and in Hong Kong from 2007-2013. Children with Down syndrome, acute promyelocytic leukemia, isolated granulocytic sarcoma, or secondary AML were excluded. The number of newly diagnosed pediatric patients with AML during the study period determined the sample size of this study. The national ethics committees in the six participating countries approved the protocol. Children who did not complete first induction course were excluded from these analyses.

Treatment plan

Figure 1 illustrates the treatment of NOPHO-AML 2004 including number of patients receiving each course. Protocol details including drug doses have been published previously.[20] Children below one year of age or with body weight below 10 kg received doses calculated according to body weight instead of body surface with 1 m^2 = 30 kg. No dose adjustment was recommended in overweight patients.

The randomization to gemtuzumab ozogamicin (GO) or no further therapy after completion of consolidation has been reported previously.[21]

Patients received prophylactic sulfamethoxazole/trimethoprim 2-3 days per week. Prophylactic fluconazole was recommended until one month after the last course. No other prophylactic antibacterial or antiviral drugs were recommended. Use of prophylactic granulocyte colony-stimulating factor was not recommended. Patients were discharged to their own homes when clinically stable regardless of neutrophil count.

Toxicity registration

Fourteen grade 3-4 toxicities were collected and graded after each treatment block by the treating physician or the local data manager. The toxicities were defined according to the World Health Organization (WHO).[22]

Definitions and statistics

Patients were divided into age and Body Mass Index (BMI) z-score groups. Children below two years of age were excluded from the weight analyses. Standard deviations (SD) of BMI for age and sex were calculated according to WHO criteria.[23] Being underweight was defined as BMI below -2 SD, overweight as BMI above +1 SD [pooling overweight (+1 to +2 SD, n=40) and obesity (>+2 SD, n=16)], and healthy weight as BMI between -2 and +1 SD.[23]

Cumulative incidence for first episode of a grade 3-4 toxicity or TRM during therapy was calculated; and for three toxicities (general condition, infection and hypoxia) a separate cumulative incidence of grade 4 toxicity was calculated. The seven most common grade 3-4 toxicities were used for the comparative analyses. Missing data (<1%) were coded as no grade 3-4 toxicity.

Interaction of age and body weight group as risk factors for toxicity was calculated by relative excess risk caused by interaction (RERI), using the algorithm of Andersson.[24]

Treatment-related mortality, event-free survival (EFS) and overall survival (OS) are defined in *Online Supplementary Table S1*.

Differences in toxicity and survival in age and body weight

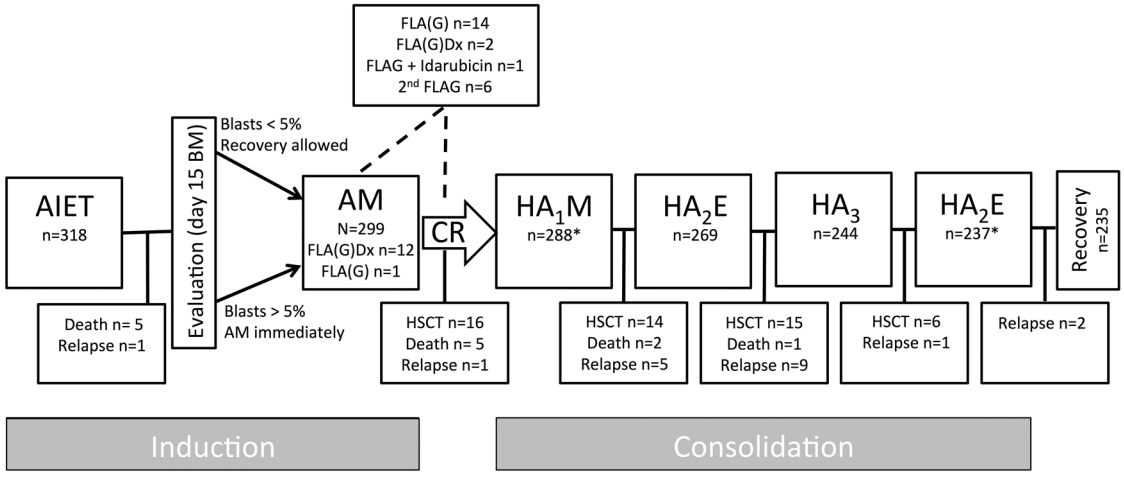

Figure 1. Flow chart of the 318 patients who completed the first course of the NOPHO-AML 2004 protocol. AIET: cytarabine, 6-thioguanine, etoposide, and idarubicin; AM: cytarabine and mitoxantrone; HA_1M: high-dose cytarabine and mitoxantrone; HA_2E: high-dose cytarabine and etoposide; HA_3: high-dose cytarabine; FLA: fludarabine 30 mg/m^2 day 1-5 and cytarabine 2000 mg mg/m^2 day 1-5; G: G-CSF 200 μg/m^2 day 0-5; Dx: liposomal daunorubicin 60 mg/m^2 day 2, 4 and 6. *Protocol deviations: one patient received HA_2E-HA_1M- HA_2E after induction, one patient skipped HA_1M and had an extra HA_3 after the second HA_2E, one patient skipped HA_1M, and one patient had etoposide, idarubicin and 6-thioguanine as the fourth consolidation.

Table 1. Baseline characteristics according to age group (n=318) and weight group (n=239).

		Age group (n=318)				Weight group (n=239)		
		0 yr. N (%)	1 yr. N (%)	2-9 yr. N (%)	10-17 yr. N (%)	<-2 SD N (%)	-2-+1 SD N (%)	>+1 SD N (%)
Patients		34 (11)	44 (14)	126 (40)	114 (36)	12 (5)	171 (72)	56 (23)
Sex	Male	9 (26)	23 (52)	68 (54)	79 (69)	10 (83)	92 (54)	44 (79)
	Female	25 (74)	21 (48)	58 (46)	35 (31)	2 (17)	79 (46)	12 (21)
Age group	2-9 years	–	–	–	–	4 (33)	103 (60)	19 (34)
	10-17 years	–	–	–	–	8 (67)	68 (40)	37 (66)
Weight group	Underweight (<-2 SD)	–	–	4 (3)	8 (7)	–	–	–
	Healthy (-2-+1 SD)	–	–	103 (82)	68 (60)	–	–	–
	Overweight (>+1 SD)	–	–	19 (15)	37 (33)	–	–	–
Ethnicity	White	27 (79)	35 (80)	87 (69)	75 (66)	5 (42)	118 (69)	38 (68)
	Asian	5 (15)	8 (18)	22 (17)	29 (25)	6 (50)	33 (19)	12 (21)
	Other*	2 (6)	1 (2)	17 (13)	10 (9)	1 (8)	20 (12)	6 (11)
WBC (10^9/L)	0-9.9	11 (32)	11 (25)	48 (38)	40 (35)	4 (33)	61 (36)	23 (41)
	10-99.9	16 (47)	28 (64)	58 (46)	57 (50)	6 (50)	82 (48)	26 (46)
	≥100	7 (21)	5 (11)	20 (16)	17 (15)	2 (17)	28 (16)	7 (13)
FAB subtype	M0	4 (12)	3 (7)	9 (7)	5 (4)	0 (0)	11 (6)	3 (5)
	M1	3 (9)	1 (2)	15 (12)	22 (19)	1 (8)	27 (16)	9 (16)
	M2	5 (15)	2 (5)	38 (30)	33 (29)	3 (25)	49 (29)	19 (34)
	M4	7 (21)	8 (18)	20 (16)	19 (17)	1 (8)	29 (17)	8 (14)
	M5	10 (29)	13 (30)	21 (17)	25 (22)	3 (25)	31 (18)	12 (21)
	M6	0 (0)	3 (7)	2 (2)	0 (0)	1 (8)	1 (1)	0 (0)
	M7	4 (12)	11 (25)	11 (9)	0 (0)	0 (0)	9 (5)	2 (4)
	Unclassified/unknown	1 (3)	3 (7)	10 (8)	10 (9)	3 (25)	14 (8)	3 (5)
FLT3	ITD	0 (0)	0 (0)	9 (7)	14 (12)	1 (8)	19 (11)	3 (5)
	Wild-type/other	23 (68)	28 (64)	87 (69)	59 (52)	6 (50)	102 (60)	37 (66)
	Unknown	11 (32)	16 (36)	30 (24)	41 (36)	5 (42)	50 (29)	16 (29)
Cytogenetics	t(8;21)	0 (0)	0 (0)	27 (21)	20 (18)	0 (0)	32 (19)	15 (27)
	inv(16)	2 (6)	2 (5)	12 (10)	12 (11)	0 (0)	15 (9)	9 (16)
	MLL rearrangements	15 (44)	16 (36)	27 (21)	16 (14)	4 (33)	27 (16)	12 (21)
	None of the above	17 (50)	26 (59)	60 (48)	66 (58)	8 (67)	97 (57)	20 (36)
Achieved CR1	No	1 (3)	3 (7)	5 (4)	5 (4)	2 (17)	7 (4)	1 (2)
	After one induction	27 (79)	35 (80)	89 (71)	70 (61)	4 (33)	115 (67)	40 (71)
	After two inductions	6 (18)	6 (14)	29 (23)	32 (28)	5 (42)	42 (25)	13 (23)
	After three inductions	0 (0)	0 (0)	3 (2)	7 (6)	1 (8)	7 (4)	2 (4)
HSCT in CR1		4 (12)	6 (14)	21 (17)	24 (21)	2 (17)	33 (19)	10 (18)

**The other category consists of children with African, Arab and mixed ancestry and 2 children where only the ethnicity of one of the parents was known. yr.: year; SD: standard deviation; N: number; WBC: white blood cell count; FAB: French-American-British classification; CR1: first complete remission; HSCT: hematopoietic stem cell transplantation; WBC: white blood count.*

groups were analyzed using Cox regression and adjusted for potential confounders. OS and EFS were estimated using the Kaplan-Meier method. Differences in survival were compared using log rank tests. All tests of significance were two-sided. Statistical significance was defined as $P<0.05$.

Results

Patients' characteristics

In total, 323 patients from the six participating countries were treated according to the NOPHO-AML 2004 protocol. Five patients who died within the first seven days after diagnosis due to aggressive/progressive AML were excluded, leaving 318 for analysis. The five excluded patients were 0, 2, 2, 2, and 7 years old; 3 were underweight, one of healthy weight and in one BMI was not calculated due to the patient being under 2 years old.

The median age at diagnosis was 6.4 years (range 0.1-17.9) and 37 patients were aged between 15-17 years.

There were more females in the infant group and more males in the older and overweight groups. Older children were more overweight, had more FAB type M1 and M2, needed more induction courses to achieve remission, had more *FLT3*-ITD mutations, inv(16), or t(8;21) and more were of Asian ancestry. Younger children had more FAB type M5 and M7 and *MLL* arrangements. Overweight patients were older, had less frequently *FLT3*-ITD mutations, and more often inv(16) and t(8;21) (Table 1). The higher rate of inv(16) and t(8;21) in the overweight group was not due to older age; the frequencies of inv(16) or t(8;21) were higher in overweight children age 2-9 years (10 of 19, 53%) than in overweight children age 10-17 years (14 of 37, 38%).

Very few patients were underweight (n=12) and this group was thus excluded from the comparative toxicity and survival analyses.

The two induction and four consolidation courses were completed by 237 (75%) of the 318 patients; Figure 1 shows the therapy courses completed. The median duration of therapy (time from start of AIET to last day with ANC below $0.5x10^9$/L after HA_2E2) for those who completed six courses was 182 days (range 128-281 days).

Toxicity

The 318 patients completed 1670 courses and 14 different toxicities were requested for registration after each course. Toxicities were registered 23,206 times (99.3% complete).

Table 2. The 14 toxicities registered after each treatment course, the definitions of grade 3 and 4 toxicities from the toxicity registration form, number of first grade 3-4 toxicities and the cumulative incidence of grade 3-4 toxicity.

Toxicity	Definition of grade 3 and 4	N. of first events	Cumulative incidence % (95%CI)
Any grade 3 or 4 toxicity		283	90 (87-94)
General condition	3: Bedridden, in need of care	204*	65 (59-70)*
	4: Intensive care, very sick	58	19 (14-23)
Infection	3: Pathogen identified, intravenous antibiotics given	246*	79 (74-84)*
	4: Septic shock/hypotension	41	13 (10-17)
Hypoxia	3: Decreased O_2 sat at rest, requiring supplemental oxygen	70*	23 (18-27)*
	4: Decreased O_2 sat requiring CPAP or assisted ventilation	33	11 (7-14)
Abdominal pain/constipation	3: Severe pain or analgesics severely interfering with activities of daily life 4: Paralytic ileus or intestinal obstruction	86*	28 (23-33)*
Abdominal symptom	3: No registration		
	4: Leading to laparotomy	8	2.5 (0.8-4.3)
Renal	3: Creatinine > 3.0-6.0 x UNL 4: Creatinine > 6.0 x UNL	8*	2.6 (0.8-4.4)*
Allergic reaction	3: Bronchospasm, requiring parenteral medication 4: Anaphylaxis	5*	1.6 (0.2-3.0)*
Hyperglycemia	3: No registration		
	4: Need of insulin	4	1.3 (0.03-2.5)
Bilirubin	3: 3-10 x UNL	13*	4.2 (1.9-6.4)*
	4: >10 x UNL		
Thrombosis	3: Requiring systemic anticoagulation 4: Severe thrombosis causing organ dysfunction	7*	2.2 (0.6-3.8)*
Hemorrhage	3: No registration		
	4: Catastrophic bleeding requiring non-elective intervention	9	2.9 (1.0-4.8)
Cardiac function	3: Mild congestive heart failure, therapeutically compensated 4: Severe/refractory congestive heart failure	11*	3.8 (1.6-6.1)*
Central neurotoxicity	3: Somnolence > 50%/day or severe disorientation or hallucinations 4: Coma or seizures	15*	4.9 (2.5-7.3)*
Peripheral neurotoxicity/ myopathy	3: Unbearable paresthesia or pronounced deficit in motor functions 4: Paralysis	6*	2.0 (0.4-3.5)*

**Combination of grade 3 and 4 toxicity; UNL: upper normal limit. CPAP: continuous positive airway pressure.*

The first induction (AIET) and the first consolidation (HA_1M) were most toxic with longer median time to ANC recovery, more infections requiring intravenous administration of antibiotics for AIET (including fever with both known and unknown pathogen), and more grade 3-4 toxicity (*Online Supplementary Table S2*). All 318 children received antibiotics due to a febrile episode at least once during treatment.

The treatment caused a high degree of toxicity (Table 2). Almost all patients (90%) had at least one grade 3 or 4 toxicity with infection with a verified pathogen (infection grade 3 or 4) being the most common. Moreover, severe abdominal pain, being bedridden/needing care (general condition grade 3), admission to the intensive care unit (general condition grade 4), need of supplemental oxygen, assisted ventilation, and sepsis with hypotension were frequent (cumulative incidence above 10%). Abdominal symptoms leading to laparotomy, creatinine above three times the upper normal limit (UNL), allergic reactions, hyperglycemia, bilirubin above three times the UNL, thrombosis, hemorrhage, cardiac failure, central and peripheral neurotoxicity were rare (cumulative incidence below 10%) and were not analyzed further [except for being included in any grade 3 or 4 toxicity (Table 3)].

The cumulative incidence of TRM was 4.6% (95%CI: 2.2%-7.0%). Nine of the 14 TRM cases occurred during induction (AIET, AM or FLAG). Nine patients died from or with an infection (seven bacterial, one viral and one fungal). Further details on TRM are provided in *Online Supplementary Table S3*.

The risk of grade 3-4 toxicity correlated with age and body weight groups (Table 3). Confounders were defined as factors, which could influence both independent variables (age and weight at diagnosis) and dependent variables (toxicity and survival) and were selected *a priori*. Consequently, age analyses were adjusted for ethnicity and sex. The age analyses were not adjusted for body weight group, since this does not influence age at diagnosis. The body weight analyses were adjusted for age group, sex and ethnicity. Children aged 10-17 years had an increased risk of sepsis with hypotension compared to children aged 2-9 years. Children aged 10-17 years also showed a trend for increased risk of a number of toxicities, e.g. admission to the intensive care unit and severe abdominal pain. Infants did not seem to experience increased toxicity during chemotherapy. In the 30 infants and 99 children aged 2-9 years who completed the four high-dose cytarabine consolidation courses, 16 infants compared to 59 children aged 2-9 years (53 *vs.* 59%) had at least one infection with a verified pathogen; no infant was admitted to the intensive care unit or needed assisted ventilation compared to 9 (9%) and 3 (3%) of the children aged 2-9 years; one infant (3%) had liver toxicity compared to no children aged 2-9 years and no infant had central neurotoxicity compared to 5 children aged 2-9 years (5%).

Overweight children had higher risk of being bedridden

and requiring supplemental oxygen. This group also showed a trend for higher risk of several other toxicities, e.g. sepsis with hypotension and severe abdominal pain. Of the 56 overweight patients (298 courses), only 5 (9%) had dose reductions of one or two courses and none had dose reductions of more than two courses. Because we did not have information on change in body weight during treatment, and therefore do not know if patients who were overweight at diagnosis remained overweight during treatment, we performed the toxicity analyses again including toxicity after the first course (AIET) only (*Online Supplementary Table S4*). After the first course, overweight children had an increased risk of requiring supplemental oxygen and severe abdominal pain.

The subgroup RERI analysis for interaction of body weight and age rendered the groups small and confidence intervals were thus wide (*Online Supplementary Table S5*). No conclusions can be made on the basis of these calculations, but being both older and overweight seemed to markedly increase the risk of sepsis.

Outcome

The median follow up time for patients alive at last follow up was 5.0 years (range 0.8-10.7 years). The 5-year EFS and OS for the 318 patients were 47% (95%CI: 42%-53%) and 71% (95%CI: 65%-76%), respectively. The main cause of decreased EFS was relapse; 125 (78%) of 161 events were due to relapse and 16 (10%) were due to TRM in first complete remission (2 after HSCT). Outcome was associated with age (Figure 2A and B). Infants (<1 year) showed a trend for superior EFS compared with children aged 2-9 years (5-year EFS 66% *vs.* 43%, sex and ethnicity adjusted HR 0.5, 95%CI: 0.3-1.0). OS differed between children aged 2-9 and aged 10-17 years with the older children showing a trend for inferior OS (5-year OS 64% *vs.* 76%, adjusted HR 1.5, 95%CI: 0.9-2.4). Children aged 1 year also had a trend for inferior OS (5-year OS 65% *vs.* 76%, adjusted HR 1.7, 95%CI: 0.9-3.2).

For children aged 2-9 years, being overweight (BMI> +1 SD for age) at diagnosis did not appear to influence prognosis (Figure 3A and C). In children aged 10-17 years, there was a trend for improved outcome in overweight patients, EFS (5-year EFS 59% *vs.* 40%, adjusted HR 0.6, 95%CI: 0.3-1.1) and OS (5-year OS 78% *vs.* 56%, adjusted HR 0.5 95%CI: 0.2-1.0) (Figure 3B and D). To test if this difference was influenced by the high frequency of t(8;21) in overweight patients, the weight-group analysis of children aged 10-17 years was stratified by t(8;21). A similar trend of superior survival in overweight patients was found in patients with t(8;21) (n=20, 5-year EFS 56% *vs.* 45% and 5-year OS 67% *vs.* 48%) and without t(8;21) (n=94, 5-year EFS 60% *vs.* 39% and 5-year OS 82% *vs.* 56%).

As a sensitivity analysis, we performed the survival analysis using the 2000 Center for Disease Control (CDC) Growth charts[25] to investigate if differences in results between our and previous studies[12,13] could be explained by difference in growth standard. Sixteen children were reclassified (11 overweight as healthy weight and 5 healthy weight as underweight) using this approach, but the tendency for overweight children aged 10-17 years to have a better outcome remained (5-year EFS 57% *vs.* 43%, adjusted HR 0.6 95%CI: 0.3-1.2 and 5-year OS 77% *vs.* 58%, adjusted HR 0.5 95%CI: 0.2-1.2).

Discussion

The aim of this study of 318 children treated according to the NOPHO-AML 2004 protocol during a 10-year period was to describe grade 3-4 toxicity and investigate asso-

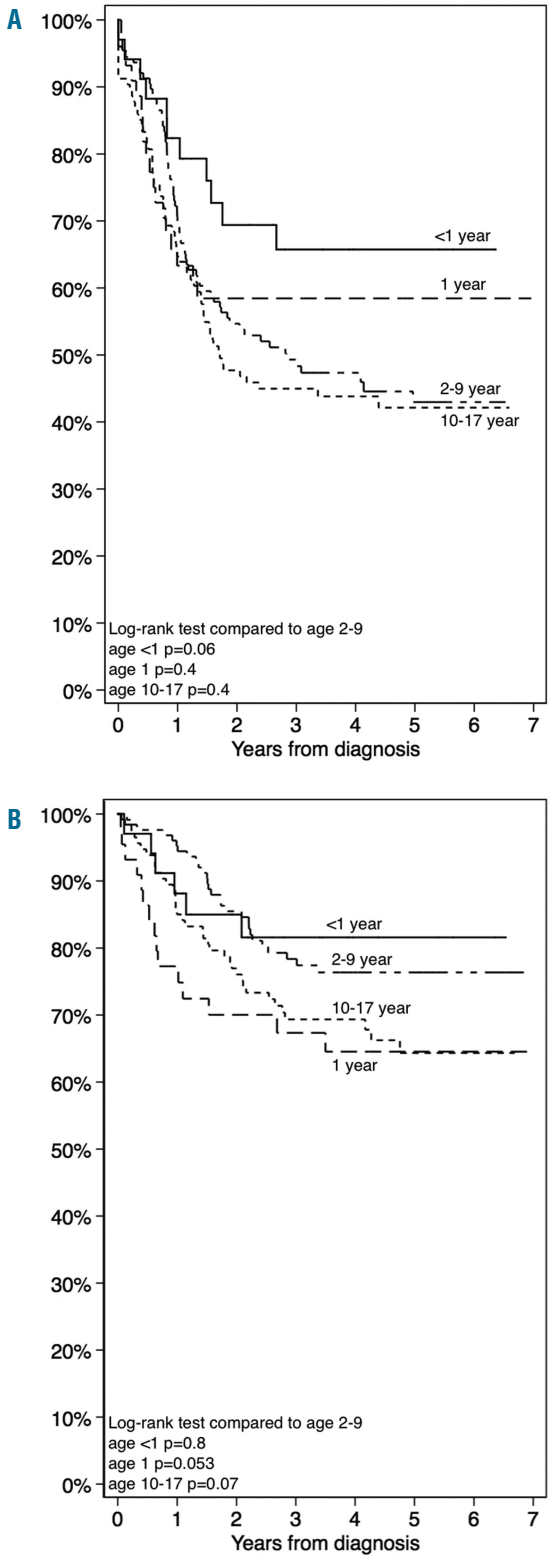

Figure 2. Event-free (A) and overall survival (B) according to age groups (n=318).

ciations between age and body weight at diagnosis and severe toxicity and survival. The cumulative incidence of grade 3-4 toxicity was high (90%). Children aged 10-17 years and overweight children were at higher risk of several grade 3-4 toxicities. To our knowledge, no previous studies have reported such a thorough review of toxicities and associations with age and body weight at diagnosis, though many studies suggest similar associations.[12,13,18,19,26,27]

We found a trend for age 10-17 years being associated with poorer survival. In contrast to previous studies,[12,13] we found a trend for being overweight being associated with improved survival in children aged 10-17 years.

Our patient cohort is complete and unselected including all newly diagnosed pediatric AML cases from the Nordic countries between 2004 and 2013, and Hong Kong between 2007 and 2013. All patients were treated according to the same protocol during a relatively short time

Table 3. Analysis of differences in toxicities according to age and weight groups.

Toxicity	Age/body weight group	N. of first events	Crude hazard ratio (95%CI)	P	Adjusted* hazard ratio (95%CI)	P
Any grade 3 or 4 toxicity	0	28	0.9 (0.6-1.3)	0.5	0.9 (0.6-1.3)	0.5
	1	41	1.1 (0.8-1.6)	0.6	1.1 (0.7-1.5)	0.7
	2-9	114	1	–	1	–
	10-17	100	0.9 (0.7-1.2)	0.7	0.9 (0.7-1.2)	0.6
	Healthy weight	152	1	-	1	–
	Overweight	51	1.1 (0.8-1.6)	0.4	1.2 (0.9-1.7)	0.3
Bedridden and in need of care (general condition grade 3 and 4)	0	20	1.0 (0.6-1.6)	0.9	1.0 (0.6-1.6)	0.9
	1	31	1.3 (0.8-1.9)	0.3	1.3 (0.8-1.9)	0.3
	2-9	75	1	–	1	–
	10-17	78	1.2 (0.9-1.6)	0.3	1.2 (0.9-1.7)	0.2
	Healthy weight	99	1	–	1	-
	Overweight	45	1.6 (1.1-2.2)	0.01	1.6 (1.1-2.3)	0.02
Intensive care unit (general condition grade 4)	0	8	1.8 (0.8-4.0)	0.2	1.6 (0.7-3.8)	0.3
	1	6	1.0 (0.4-2.5)	1.0	1.0 (0.4-2.5)	1.0
	2-9	18	1	–	1	–
	10-17	26	1.7 (0.9-3.1)	0.09	1.8 (1.0-3.3)	0.06
	Healthy weight	26	1	–	1	–
	Overweight	15	1.9 (1.0-3.5)	0.06	1.9 (0.9-3.6)	0.07
Requiring supplemental oxygen (hypoxia grade 3 and 4)	0	9	1.3 (0.6-2.8)	0.5	1.2 (0.6-2.6)	0.6
	1	4	0.4 (0.2-1.2)	0.1	0.4 (0.1-1.2)	0.1
	2-9	26	1	–	1	–
	10-17	31	1.4 (0.8-2.3)	0.2	1.4 (0.8-2.4)	0.2
	Healthy weight	34	1	-	1	-
	Overweight	19	1.8 (1.0-3.2)	0.04	1.9 (1.0-3.5)	0.04
Continuous positive airway pressure or assisted ventilation (hypoxia grade 4)	0	4	1.7 (0.5-5.5)	0.4	1.4 (0.4-4.7)	0.6
	1	4	1.3 (0.4-4.3)	0.6	1.2 (0.4-4.1)	0.7
	2-9	9	1	–	1	–
	10-17	16	2.0 (0.9-4.6)	0.09	2.2 (1.0-5.0)	0.07
	Healthy weight	17	1	–	1	–
	Overweight	6	1.1 (0.4-2.8)	0.9	1.1 (0.4-2.8)	0.9
Infection with an identified pathogen (infection grade 3 and 4)	0	26	1.1 (0.7-1.7)	0.7	1.1 (0.7-1.7)	0.7
	1	36	1.1 (0.8-1.7)	0.5	1.1 (0.8-1.6)	0.6
	2-9	98	1	–	1	–
	10-17	86	1.0 (0.7-1.3)	0.8	1.0 (0.7-1.3)	0.8
	Healthy weight	130	1	–	1	–
	Overweight	45	1.2 (0.9-1.7)	0.3	1.2 (0.9-1.8)	0.3
Sepsis/hypotension (infection grade 4)	0	3	0.9 (0.3-3.3)	0.9	1.0 (0.3-3.5)	1.0
	1	3	0.7 (0.2-2.6)	0.6	0.7 (0.2-2.7)	0.6
	2-9	12	1	–	1	–
	10-17	23	2.3 (1.1-4.6)	0.02	2.3 (1.1-4.6)	0.02
	Healthy weight	19	1	–	1	–
	Overweight	14	2.4 (1.2-4.8)	0.01	2.1 (1.0-4.3)	0.052
Abdominal pain severely interfering with activities of daily life (abdominal pain grade 3 and 4)	0	9	1.1 (0.5-2.4)	0.8	1.0 (0.5-2.2)	0.9
	1	9	0.9 (0.4-1.9)	0.8	0.9 (0.4-1.8)	0.7
	2-9	30	1	–	1	–
	10-17	38	1.5 (0.9-2.4)	0.1	1.6 (1.0-2.6)	0.07
	Healthy weight	44	1	–	1	–
	Overweight	23	1.7 (1.0-2.8)	0.04	1.7 (1.0-2.9)	0.06

**The age analysis was adjusted for sex and ethnicity. The Body Mass Index analysis was adjusted for sex, age group and ethnicity and only included children 2 years or older. The underweight group was very small (n=12) and was therefore not included in these analyses. N: number.*

period and toxicity registration was more than 99% complete. The grade 3 and 4 toxicities were defined by the WHO, but we cannot exclude minor institutional difference in registration practices. Another limitation to our study is an inadequate number of patients to provide confident estimates for the more rare toxicities. Furthermore, weight change during treatment has been shown to have prognostic value in pediatric ALL,[28,29] but we did not have information on weight change in our cohort and could thus not examine this further.

Under-reporting of toxicities in pediatric AML studies is a problem,[30] so the cumulative incidences of toxicities might be under-estimated, but we have no reason to believe that this should differ across age or body weight groups.

Not all the toxicity end points were ideal. The toxicities were selected at conception of the protocol, but it became evident that not all were AML-relevant based on the low prevalence. Some of the end points were not specific enough (e.g. abdominal pain which could be caused by a number of underlying conditions). The knowledge gained from this study can guide the planning of future toxicity registrations in pediatric AML protocols.

The baseline characteristics of the cohort were as expected. The age distribution of FAB groups, cytogenetics, and *FLT3*-ITD mutations were comparable to previous rapports.[11,26,31] The higher frequency of inv(16) and t(8;21) in overweight patients was not the result of older age, and higher frequency of t(8;21), t(9;11) and inv(16) in overweight patients had also been found in a previous study,[13] suggesting that being overweight may be associated with certain AML subtypes.

The cumulative incidence of grade 3-4 toxicity, especially verified infection, was excessive, similar to the St Jude AML02 trial,[3] reflecting the acute toxicity to be expected from modern pediatric AML protocols. The St Jude group have introduced antibiotic prophylaxis with vancomycin and ciprofloxacin for children treated for AML,[9] but there is no international consensus on prophylactic antibiotics for children treated for AML.[32] Specific microbiological organisms were not required for registration in the NOPHO-AML 2004 study, but have been collected from Danish patients treated on the protocol[8] showing that viridians group streptococci was the most common cause of bloodstream infections and fungal infections were rare.

Toxicity did not increase during the course of treatment (*Online Supplementary Table S2*). The second and fourth consolidation courses were identical (HA_2E) and no more toxicity followed the second HA_2E compared to the first, indicating that the bone marrow does not become exhausted during treatment. The course with the highest dose of cytarabine (HA_3) given as monotherapy was the least toxic.

Infants have been reported to have lower cytarabine clearance[33] and other collaborative groups have considerably reduced cytarabine doses for infants.[19,34] Infants did

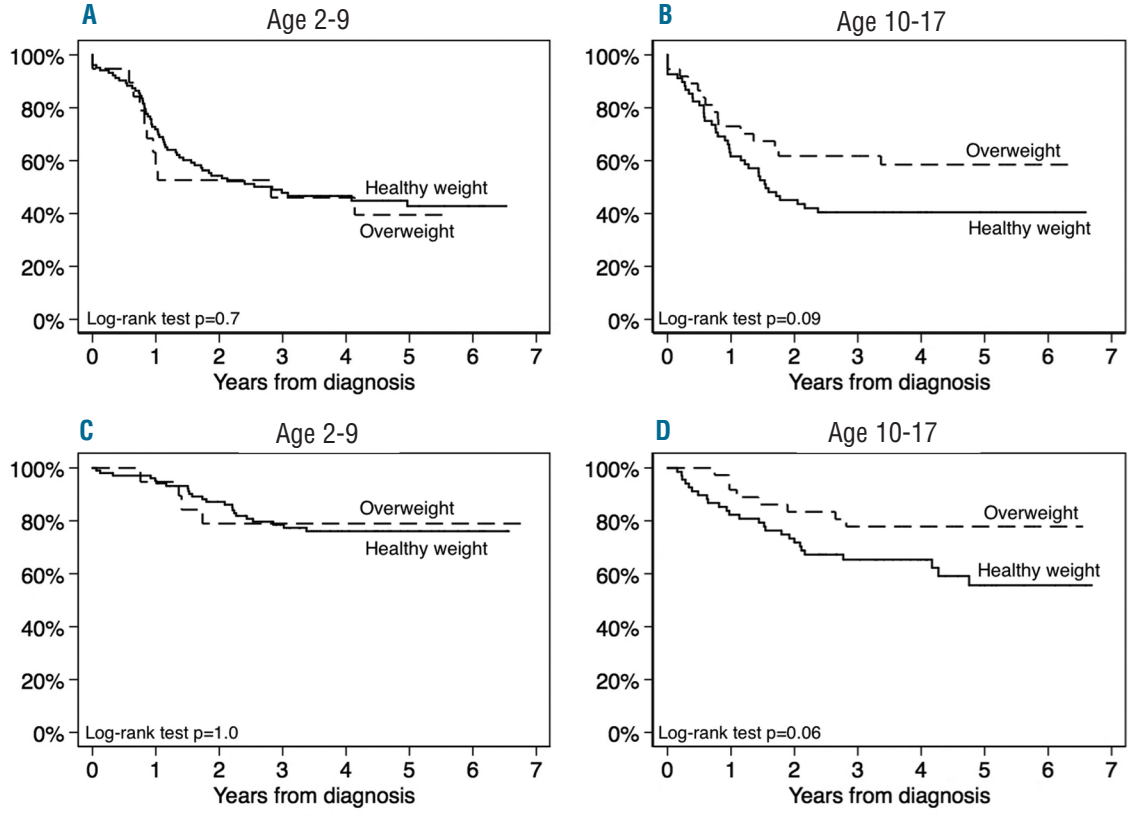

Figure 3. Event-free (A and B) and overall survival (C and D) according to body weight group and stratified for age group (n=239). The underweight group was very small (n=12) and was therefore not included in these analyses.

not seem to experience more toxicity compared to 2-9-year-olds, but the infant group in our study was small (n=34) so we might have missed small differences. The NOPHO-AML 2004 protocol did not seem to be more toxic for infants compared to the three previous BFM protocols (AML-BFM-98, -98-Interim and -2004),[19] despite the higher doses of cytarabine. In particular, the rate of central neurotoxicity (a known cytarabine toxicity[35]) following the high-dose cytarabine consolidation courses was low, and during consolidation infants did not have more toxicity than children aged 2-9 years. The outcome for infants treated according to NOPHO-AML 2004 was excellent (5-year EFS: 66%, 95%CI: 46%-79%, 5-year OS: 82%, 95%CI: 63%-91%) and confirm previous reports showing favorable outcome for infants.[19,34] High-dose cytarabine seems safe and effective in treating infants with AML, and our results do not support further dose reductions in infants as recommended by others.[14]

Children aged 10-17 years were at higher risk of toxicity. In particular, the risk of sepsis with hypotension was higher in children aged 10-17 years in agreement with previous findings.[18] The older children also had higher risk of severe abdominal pain, which we speculate could be due to increased mucosal barrier injury in this group; mucosal barrier injury leads to infection with more virulent pathogens and a stronger host immune response.[36]

A review of pharmacology in adolescent cancer patients showed slower clearance of etoposide in adolescents compared to younger children.[37] If older children have decreased clearance of antineoplastic drugs used in AML, increased exposure to toxic metabolites could lead to increased toxicity.

We found a trend for OS was better for children aged 2-9 years. The 14 TRM cases on this protocol meant the study was not sufficiently powered to demonstrate if the increased cumulative incidence of toxicity in children aged 10-17 years translated into increased TRM, as shown by others.[14,16-18] Children overweight at diagnosis were also at higher risk of several grade 3-4 toxicities both after the first course and during the entire course of treatment. Chemotherapy dose reduction in overweight children was not recommended and very few received reduced doses. In contrast to this, studies in adults show that, despite dose reductions not being recommended, overweight patients often receive reduced doses.[38] In our study, overweight children received chemotherapy doses based on actual weight (and not "ideal weight"), which could lead to increased toxicity. This speculation is in part contrasted by Hijiya *et al.*[39] who have shown that in pediatric ALL, there is no statistical difference in pharmacokinetics of cytarabine between normal and overweight patients. Previous studies have found increased TRM in overweight[12] and obese[13] patients with AML resulting in poorer outcome for this group; in this cohort, however, none of 9 TRM cases in children over one year of age were overweight. In contrast, we surprisingly found a trend for being overweight at diagnosis being associated with superior outcome in children aged 10-17 years [unrelated to t(8;21) status], similar to what has been reported in adults with AML.[40-42] The effect of being overweight in the oldest children with AML may be more similar to the effect of being overweight in adults. In addition, the overweight group may have benefitted from the therapy without dose reductions. In our cohort, few were obese (n=16, 29% of the overweight group) compared to all and 57% of the patients in the overweight group in two American studies.[12,13] This difference might partly explain the difference in results.

In conclusion, we found the toxicity of the NOPHO-AML 2004 protocol to be considerable. The high doses of cytarabine given were safe for infants in our setting and resulted in excellent outcome for the youngest patients. Age 10-17 years was associated with increased toxicity and a trend for poorer survival. Further studies on the pharmacology of AML drugs in adolescence are needed. Being overweight at diagnosis was associated with increased toxicity, but also with a trend for improved survival in children aged 10-17 years. Dose reduction in overweight patients does not seem justified in those patients with appropriate supportive care.

Acknowledgments

The authors would like to thank all the participating patients and their parents, clinicians and research staff at the NOPHO institutions for reporting data, and Peter Haubjerg Asdahl for statistical assistance.

Funding

This study was supported by grants from the Danish Childhood Cancer Foundation, the Danish Cancer Society and the Novo Nordisk Foundation.

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