Leukemia (2005) 19, 2090–2100

www.nature.com/leu

Long-term results in children with AML: NOPHO-AML Study Group – report of three consecutive trials

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In all, 447 children with acute myeloid leukaemia (AML) have been treated on three consecutive NOPHO studies from July 1984 to December 2001. NOPHO-AML 84 was of moderate intensity with an induction of three courses of cytarabine, 6-thioguanine and doxorubicin followed by four consolidation courses with high-dose cytarabine. The 5-year event-free survival (EFS), disease free survival (DFS) and overall survival (OS) were 29, 37 and 38%. NOPHO-AML 88 was of high intensity with the addition of etoposide and mitoxantrone in selected courses during induction and consolidation. The interval between the induction courses should be as short as possible, that is, time intensity was introduced. The 5-year EFS, DFS and OS were 41, 48 and 46%. In NOPHO-AML 93, the treatment was stratified according to response to first induction course. The protocol utilised the same induction blocks as NOPHO-AML 88, but after the first block, children with a hypoplastic, nonleukaemic bone marrow were allowed to recover before the second block. Consolidation was identical with NOPHO-AML 88. The 5-year EFS, DFS and OS in NOPHO-AML 93 were 48, 52 and 65%. The new NOPHO-AML protocol has been based on experiences from previous protocols with stratification of patients with regard to *in vivo* response and specific cytogenetic aberrations.
Leukemia (2005) **19,** 2090–2100. doi:10.1038/sj.leu.2403962
Keywords: acute myeloid leukaemia; children; cytogenetic; dose intensity; stem cell transplantation

Introduction

In recent years, therapeutic trials have shown that intensified induction and consolidation chemotherapy improves outcome in acute myeloid leukaemia (AML). According to the best results published, event-free survival (EFS) will end up at 50% of children with AML[1–8] and overall survival (OS) figures of 60–65% have been obtained.[4,5,7]

The child population (<15 years) is 4.5 million in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) and the annual number of new paediatric AML cases is 25–30. The children have been treated in 21 centres (Table 1). Since 1984, three consecutive AML trials have been conducted by the NOPHO-AML Study Group.

Background of the NOPHO-AML trials

Before 1984, children with AML were treated according to regional or national protocols in the Nordic countries. A pilot study on AML therapy was started by Lie and Slördahl[9] in Oslo in 1981. The induction therapy was a modification of the ongoing MRC Trial in UK with the combination of doxorubicin, cytarabine and 6-thioguanine. For the first time in children, the consolidation therapy was based on high-dose cytarabine, 2 g/m^2 twice daily for 3 days repeated four times. Maintenance was used with monthly courses of cytarabine and 6-thioguanine during the first year. The results were promising with the first eight patients remaining in remission 5–29 months after diagnosis.

Based on the results of the pilot study, the first cooperative NOPHO-AML trial for treatment of childhood AML in the five Nordic countries was initiated in 1984 (NOPHO-AML 1984). The protocols were modified in 1988 (NOPHO-AML 1988) and 1993 (NOPHO-AML 1993), mainly based on the outcome and experiences achieved from the previous protocols. The treatment of the three trials is shown schematically in Figure 1 and the treatment courses including cumulative drug dosages are summarised in Table 2.

Study NOPHO-AML 84: The induction consisted of three courses of cytarabine, 6-thioguanine and doxorubicin (ATDox × 3) and was followed by a consolidation of four courses with cytarabine 2 g/m^2 twice daily for 3 days ($HA_2 \times 4$). One intrathecal dose of methotrexate was given on the first day of all courses. For children less than 2 years of age, drug doses were calculated per body weight with 1 m^2 equalling 30 kg. Intrathecal methotrexate was given at age-adjusted doses as follows: below 1 year, 6 mg; 1 year old, 8 mg; 2 years old, 10 mg; and 3 years and older, 12 mg. Allogeneic stem cell transplantation (SCT) was recommended to all patients in first remission with an HLA-matched family donor (MFD). No maintenance therapy was given. The results from this trial showed EFS of 29%, that is, a significant number of children were cured but the frequencies of nonresponders (NR) and relapse were high.

Study NOPHO-AML 88: In order to reduce the rate of NR and relapse, the treatment was intensified by the addition of mitoxantrone and etoposide (VP-16) to induction and consolidation phases. All patients were treated uniformly with three induction courses ATEDox-AM-ATEDox.

The interval between the first and second induction courses was intended to be 14–16 days if the condition of the patient allowed it. The three induction courses were followed by four courses of a high-dose cytarabine-based consolidation. The consolidation elements were based on combinations

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Received 14 December 2004; accepted 19 July 2005

Table 1 Patient accrual and follow-up

Trial	*Accrual of patients: time period*	*Total number of patients (n)*	*Centres (n)*	*Number of patients/ centre (mean/range)*	*Number of patients/year*	*Follow-up (median, range, years)*[a]
NOPHO-AML 84	7/1984–6/1988	96	21	5 (1–15)	24	16 (2–18)
NOPHO-AML 88	7/1988–12/1992	108	21	5 (1–13)	24	12 (9–14)
NOPHO-AML 93	1/1993–12/2001	243	21	11 (1–24)	27	5 (1–10)

[a]Follow-up: patients alive.

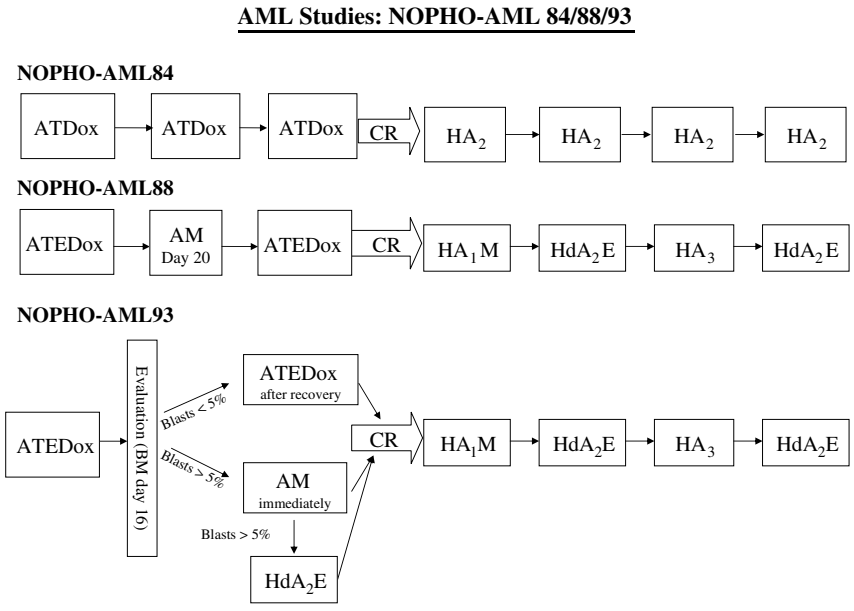


Figure 1 Flow diagram of studies NOPHO-AML 84/88/93.

of cytarabine $1\,g/m^2$ with mitoxantrone (HA_1M) or cytarabine $2\,g/m^2$ with etoposide ($HA_2E \times 2$) or cytarabine $3\,g/m^2$ as a single drug (HA_3). SCT was recommended to all patients in first remission with an HLA MFD. No maintenance therapy was given. Treatment with autologous SCT (ASCT) was optional during NOPHO-AML 88 and was performed on a nonrandomised basis decided by the responsible physician. In the present analysis, ASCT has been included in the chemotherapy-only group. This strategy led to a decrease in both NR and relapse rates. The EFS increased to 41%, but the protocol suffered from an unacceptably high frequency of toxic deaths during induction and early in consolidation.

Study NOPHO-AML 93: Since the previous study had significant antileukaemic effect, NOPHO-AML 93 utilised the same treatment courses. In order to reduce the high toxicity, dose intensity was modified by abandoning the extreme upfront loading concept. Following the first course of induction, children without evidence of residual disease were allowed to recover before a second identical induction course was given. Children with persistent disease were recommended to receive sequentially different courses until remission was obtained. The time between the first and second courses was increased in order to allow patients to show the degree of response to first course and to allow regeneration of the bone marrow and thereby reduce toxicity. In good response patients, the second course was not given until the patient had recovered from the first course. Patient who had >5% blast after first course should have the second course as soon as possible. It was intended that these patients should tolerate the more time-intensive treatment as they had a resistant tumour burden. All children received course ATEDox as first course. Following bone marrow evaluation, good responders received a second ATEDox course, whereas poor responders received an AM course. Patients with >5% blasts in the bone marrow after the AM course were given a HA_2E course. If leukaemia still persisted, the child was classified as NR (see definitions). All patients in remission were treated with courses of high-dose cytarabine-based consolidation as in NOPHO-AML 88. The total number of courses was reduced from seven in NOPHO-AML 88 to six in NOPHO-AML 93, with the exception of the few patients who required three induction courses to enter remission. The maximum cumulative dose of anthracycline in NOPHO-AML 93 was $375\,mg/m^2$. SCT was recommended to all patients in first remission with an HLA MFD. At the discretion of the treating physicians, matched unrelated donor (MUD) transplantation was performed in a few patients. ASCT was optional during the first part of NOPHO-AML 93 and performed on a nonrandomised basis as decided by the responsible physician. In the present analysis, patients with ASCT have been included in the chemotherapy group.

Table 2 Treatment elements in the three NOPHO-AML studies 1984–1993

Courses in NOPHO-AML studies 84/88/93		*Cumulative dose (mg/m²)*		
		Ara-C (g/m²)	*Doxo/mitoxan (mg/m²)*	*Etoposide (mg/m²)*
1984-Induction ATDox × 3	*ATDox*: Ara-C 100 mg/m²/day every 12 h on days 1–4; 6-TG 100 mg/m² every 12 h days 1–4; doxorubicin 75 mg/m² as 8 h inf. day 5; methotrexate age-adjusted 6–12 mg i.th. day 1.	2.4	225/0	—
1984-Consolidation $HA_2 \times 4$	*HA_2*: Ara-C 2 g/m² as 2 h inf. every 12 h days 1–3; methotrexate age-adjusted 6–12 mg i.th. day 1.	48.0	—	—
Total dose AML-84		50.4	225/0	—
1988-Induction ATEDox- AM- ATEDox	*ATEDox*: Ara-C 200 mg/m²/day continuous inf. on days 1–4; 6-TG 100 mg/m² every 12 h days 1–4; etoposide (VP-16) 100 mg/m²/day continuous inf. on days 1–4; doxorubicin 75 mg/m² as 8 h inf. day 5; methotrexate 6–12 mg i.th. day 1. *AM*: Ara-C 100 mg/m²/day continuous inf. days 1–5; mitoxantrone 10 mg/m² as 30 min inf. days 1–3; methotrexate 6–12 mg i.th. day 1.	2.1	150/30	800
1988-Consolidation HA_1M- HA_2E- HA_3- HA_2E	*HA_1M*: Ara-C 1 g/m² as 2 h inf. every 12 h days 1–3; mitoxantrone 10 mg/m² as 30 min inf. days 3–5; methotrexate 6–12 mg i.th. day 1. *HA_2E*: Ara-C 2 g/m² as 2 h inf. every 12 h days 1–3; etoposide (VP-16) 100 mg/m²/day as 1 h inf. days 2–5; methotrexate 6–12 mg i.th. day 1. *HA_3*: Ara-C 3 g/m² as 2 h inf. every 12 h days 1–3; methotrexate 6–12 mg i.th. day 1.	48.0	0/30	800
Total dose AML-88		50.1	150/60	1.600
1993-Induction Good resp: ATEDox × 2 Poor resp: ATEDox+AM ± HA_2E	*ATEDox*: Ara-C 200 mg/m²/day continuous inf. on days 1–4; 6-TG 100 mg/m² every 12 h days 1–4; etoposide (VP-16) 100 mg/m²/day continuous inf. on days 1–4; doxorubicin 75 mg/m² as 8 h inf. day 5; methotrexate 6–12 mg i.th. day 1. *AM*: Ara-C 100 mg/m²/day continuous inf. days 1–5; mitoxantrone 10 mg/m² as 30 min inf. days 1–3; methotrexate 6–12 mg i.th. day 1. *HA_2E*: Ara-C 2 g/m² as 2 h inf. every 12 h days 1–3; etoposide (VP-16) 100 mg/m²/day as 1 h inf. days 2–5; methotrexate 6–12 mg i.th. day 1.	GR: 1.6 PR: 13.3	GR: 150/0 PR: 75/30	GR: 800 PR: 800
1993-Consolidation HA_1M- HA_2E- HA_3- HA_2E	*HA_1M*: Ara-C 1 g/m² as 2 h inf. every 12 h days 1–3; mitoxantrone 10 mg/m² as 30 min inf. days 3–5; methotrexate 6–12 mg i.th. day 1. *HA_2E*: Ara-C 2 g/m² as 2 h inf. every 12 h days 1–3; etoposide (VP-16) 100 mg/m²/day as 1 h inf. days 2–5; methotrexate 6–12 mg i.th. day 1. *HA_3*: Ara-C 3 g/m² as 2 h inf. every 12 h days 1–3; methotrexate 6–12 mg i.th. day 1.	48.0	0/30	800
Total dose AML-93		GR: 49.6 PR: 61.3	GR: 150/30 PR: 75/60	GR: 1.600 PR: 1.600

Patients and methods

Eligibility

The entry criteria for studies NOPHO-AML 84/88/93 included newly diagnosed AML patients, age below 17 years. Patients with isolated myelosarcoma ($n=2$), secondary AML ($n=20$), myelodysplastic syndrome ($n=10$), Down's syndrome (DS) ($n=79$), patients treated on other protocols ($n=15$) and 11 patients with other serious diseases (malformations, brain damages, complex heart diseases) who were not treated were excluded from the study. There were 447 patients who fulfilled the inclusion criteria.

Diagnosis

The diagnosis and therapy were centralised to the 21 University Hospitals in the five countries. Informed consent and ethical approval were obtained according to national regulations. The diagnosis was established by morphological analysis of bone marrow aspirates according to the FAB and WHO classifications.[10–12] Central nervous system (CNS) involvement was diagnosed if more than five leucocytes per microlitre were identified in the CSF in combination with detectable leukaemia cells in the cytospin and/or with presence of neurological symptoms such as, for example, cranial nerve palsy.[13]

Treatment

See Introduction for details.

Definitions and statistics

Complete remission (*CR*): CR was defined according to the CALGB criteria[14] with minor deviations as follows: CR, $\leqslant 5\%$ leukaemia blasts in the bone marrow with signs of normal haematopoiesis in the bone marrow and with clear signs of regeneration of normal blood cell production in the peripheral

blood (platelets $>80\times10^9$/l without transfusions, neutrophils $>1.0\times10^9$/l), and no leukaemia cells in the PB or in the CSF. Response after induction was evaluated close to day 15 by the blast cell count in the bone marrow $\leqslant/>5\%$.

Early death (*ED*): ED patients were defined as those dying within the first 6 weeks of treatment. Since the main cause of death varies in different phases of initial treatment, ED was divided into (a) ED before starting treatment, (b) ED during and after the first therapy block (<15 days of treatment) and (c) ED in aplasia between days 15 and 42 before remission. This subdivision reflects the early death rate due to initial complications (hyperleucocytosis, leucostasis) or due to aplasia after induction therapy.

NR: All patients not achieving remission (CR) and surviving the first 6 weeks of treatment were classified as NRs. Patients with CR criteria lasting <4 weeks were also classified as NR (type V failures according to CALGB criteria).[14]

Toxicity was not analysed due to incomplete reporting from the centres. However, all severe toxicity and treatment-related mortality was reported.

Statistical analysis: SPSS Analysis System Version 11.5 software was used for the statistical analysis.[15] EFS was calculated from the date of diagnosis to the last follow-up or first event (failure to achieve remission, NRs, relapse, second malignancy or death of any cause). Patients who did not attain a CR were considered failures at time zero. OS was calculated from the time of diagnosis to death of any cause or to last follow-up. DFS of patients achieving remission was calculated as time from remission to first event (relapse, second malignancy, death of any cause). The probabilities of EFS, DFS and OS were calculated using the Kaplan–Meier method, and the different subgroups were compared using the log-rank test. A stepwise, multiregression analysis according to Cox was used to identify prognostic risk factors. Differences between mean periods

Table 3 Initial patient data of studies NOPHO-AML 84, 88 and 93

	NOPHO-AML 84		*NOPHO-AML 88*		*NOPHO-AML 93*		*AML – all patients*	
	n	%	*n*	%	*n*	%	*n*	%
Number eligible patients	96		108		243		447	
Gender (male)	41	42.7	54	50.0	124	51.0	219	49.0
Age (years)								
<2	25	26.0	35	32.4	57	23.5	117	26.2
2–9	43	44.8	38	35.2	107	44.0	188	42.1
>10	28	29.2	35	32.4	79	32.5	142	31.8
WBC $\times10^9$/l								
<20	52	54.2	62	57.4	137	56.4	251	56.2
20–<100	30	30.2	29	26.9	81	33.3	140	31.1
>100	14	15.6	17	15.7	25	10.3	56	12.8
CNS leukaemia (yes)	4	4.2	4	3.7	16	6.6	24	5.4
(ND or questionable)	(6)		(4)		(3)		(13)	
Blasts after A >5%					77	31.7		
(ND)					0			
FAB types								
M0					16	6.6	16	3.6
M1	15	15.6	23	21.3	36	14.8	74	16.3
M2	29	30.2	28	25.9	51	21.0	108	24.2
M3	8	8.3	6	5.6	11	4.5	25	5.6
M4	19	19.8	17	15.7	49	20.2	85	19.0
M5	11	11.5	20	18.5	46	18.9	77	17.2
M6	5	5.2	1	0.9	6	2.5	12	2.7
M7	0	0	3	2.8	16	6.6	19	4.3
Others/ND	9	9.4	19	3	13	4.5	31	7.2
Karyotypes ND	82	85	68	63	9	3.7		
Cytogenetic favourable[a]								
t(8;21)	1		7		19	7.8		
inv(16)			2		14	5.8		
t(15;17			1		9	3.7		
Normal	6		15		62	25.5		
Other	6		12		93	38.3		
t(9;11)	1		3		19	7.8		
11q23 – not t(9;11)	0		0		18	7.4		

ND = no data.
[a]Definition of favourable cytogenetics: t(8;21), t(15;17), inv(16).

between courses and protocols were tested by analysis of variance.

Patient characteristics

During the period July 1984–December 2001, 447 patients were enrolled in studies NOPHO-AML 84/88/93; the clinical characteristics are presented in Table 3. The male:female ratio was 0.96. The median age at presentation was 6.0 years. Of the patients, 26% were less than 2 years of age and 32% were 10 years old or more at diagnosis. CNS involvement was diagnosed in 5%. The median white blood counts (WBC) at diagnosis was 14.2×10^9/l and 13% of the children had WBC $\geqslant 100 \times 10^9$/l. Comparison of initial patient data of the studies NOPHO-AML 84, 88 and 93 revealed no clinically important differences (Table 3). An exception was a decrease in FAB types M1/M2 in the NOPHO-AML 93 with a corresponding increase in FAB types M0 and M7. This was caused by a more precise definition of these subtypes in the last study period. Cytogenetic analyses were successfully performed in an increasing proportion of the patients from 15% in NOPHO-AML 84 to 96% in NOPHO-AML 93.

Results

Overall outcome in studies NOPHO-AML 84, 88 and 93: The results of the three studies are shown in Table 4. The results in patients <15 years of age are presented separately in order to facilitate the comparisons with other studies (Table 4b). Overall, the probabilities of OS, EFS and DFS have improved in the three consecutive studies. The early death rate (days 0–42) was higher in the NOPHO-AML 88 study (9.3%) as compared to both NOPHO-AML 84 (5.2%) and NOPHO-AML 93 (2.0%) (Table 4a). Death rates between days 15 and 150 showed a decrease between NOPHO-AML 88 (15.7%) and NOPHO-AML 93 (2.9%) ($P<0.01$). In contrast, the percentage of NRs was lowest in NOPHO-AML 88 (3.7%) as compared to both NOPHO-AML 84 (14.6%) and NOPHO-AML 93 (5.8%) (Table 4a). The cumulative risk of relapse decreased from 46% in NOPHO-AML 84 to 36% in NOPHO-AML 88 and 39% in NOPHO-AML 93. The frequency of NRs and relapse rate was reduced in NOPHO-AML 88 as compared to NOPHO-AML 84, but with an increase in both early and late treatment-related toxic deaths in NOPHO-AML 88 (Table 4b). We observed a reduction of early/late toxic deaths in NOPHO-AML 93 as compared to NOPHO-AML 88, but no change in the frequencies of NRs and relapses. Table 5 shows the outcome for the protocols with regard to specific presenting features at diagnosis and after first course of treatment.

Results of study NOPHO-AML 84

Response to induction treatment: Of the 96 children, 75 obtained CR (78%), seven (7%) died in aplasia and 14 (15%) were classified as NRs (Table 4).

Postremission therapy/outcome: Of the 75 children, 12 (16%) received SCT in the first remission, of whom five relapsed and two died in CCR. The chemotherapy group ($n=63$) included eight patients who received ASCT during consolidation. Among these children, there were 39 relapses and one child who died in CCR. There was no difference in DFS between the SCT and chemotherapy groups. (DFS 42 and 36%; $P=0.5$). In total, 40 children had BM relapses, two isolated CNS and two relapse with other sites. The cumulative risk of relapse was 46%. The 5-year OS, EFS and DFS of the first NOPHO-AML study were 38 ± 5, 29 ± 5 and 37 ± 6% (Figures 2–4)

Results of study NOPHO-AML 88

Response to induction treatment: CR was obtained in 90/108 (83%) of the children, 14 (13%) died in aplasia and 4 (4%) were classified as NRs (Table 4). As a marker of dose intensity, the number of days between the courses was analysed. The mean/median numbers of days between the start of the first and second course in NOPHO-AML 88 were 22/21 days.

Postremission therapy/outcome: SCT in first remission was performed in 19/90 patients (21%; 17 MFD, two MUD), of whom seven relapsed. The chemotherapy group ($n=71$) included 24 children who received ASCT during consolidation. Among these children, there were 32 relapses and 10 children who died in CCR, most of them within 6 months from remission, but two patients died late in remission after 91 and 153 months. There was no significant difference in 5-year DFS between the SCT and chemotherapy groups (DFS 58 and 45%; $P=0.2$). In all, 32 children had BM relapse, five isolated CNS relapse and one relapse with other site, whereas one child developed MDS. Owing to the intensification of therapy in NOPHO-AML 88 as compared to NOPHO-AML 84, the frequencies of NRs and relapses were reduced, but there was a significant increase in toxic deaths during early and late induction phases. These deaths occurred more frequently in females, in children below 2 years of age at diagnosis and in patients with WBC $<100 \times 10^9$/l at diagnosis. The cumulative incidences of death in CCR and relapse were 9 and 36%, respectively (Table 4). The 5-year OS, EFS and DFS of the NOPHO-AML 88 study were 46 ± 5, 41 ± 5 and 48 ± 5%, respectively (Figures 2–4).

Results of study NOPHO-AML 93

Response to induction treatment: After the first induction course, CR was achieved in 162 of the 243 patients (67%) in NOPHO-AML 93. Four children died before achieving remission (three early deaths, one death in aplasia). The 77 poor responders were treated with AM course. In all, 47 (61%) achieved remission after AM course; two children died in aplasia, four NRs were taken off protocol, while 24 patients still not in remission were treated with course HA_2E. Of these patients, 14 (58%) achieved remission, while 10 patients had resistant disease and were classified as NRs. In total, six patients died in aplasia and 14 were NRs. The CR rate was 92% (223/243; Table 4). The mean/median interval in NOPHO-AML 93 between the start of the two ATEDox courses was 32/31 days and between ATEDox and AM 33/27 days. The interval between the first two induction courses was significantly longer in NOPHO-AML 93 than in NOPHO-AML 88 ($P<0.01$). The time periods between the subsequent courses did not differ between the protocols.

Postremission therapy/outcome: SCT in first remission was performed in 55 patients (27%): 49 MFD and six MUD. ASCT

Table 4 (a) Results in studies NOPHO-AML 84, 88 and 93 and (b) results in studies NOPHO-AML 84, 88 and 93 (only patients <15 years at diagnosis)

	NOPHO-AML 84		*NOPHO-AML 88*		*NOPHO-AML 93*	
	n	*% (s.e.)*	*n*	*% (s.e.)*	*n*	*% (s.e.)*
(a)						
Number of patients	96		108		243	
Median follow-up of pts. in CCR (years, range)	15.6	(2.2–18.3)	11.8	(9.3–14.4)	4.9	(0.9–9.8)
EDs (total)[a]	5	5.2	10	9.3	5	2.0
Deaths days 42–150	2	2.1	4	3.7	1	0.4
NR	14	14.6	4	3.7	14	5.8
CR achieved	75	78	90	83	223	92
Death in CCR (5 years) (cumulative incidence)	3	3.1	10	9.3	8	3.3
Relapse (cumulative incidence)	44	46	39	36	96	39
(With CNS involvement)	2	2	5	4	6	2
Lfu in CCR	4	11 (2–16)	0		0	
P survival						
At 5 years	36	38 (5)	50	46 (5)	80	65 (3)
At 10 years	34	36 (5)	46	45 (5)		
pEFS						
At 5 years	27	29 (5)	44	41 (5)	59	48 (3)
At 10 years	27	29 (5)	39	39 (5)		
pDFS						
At 5 years	27	37 (6)	44	48 (5)	59	52 (3)
At 10 years	27	37 (6)	39	46 (5)		
Allogeneic/MUD BMT in first CR	12	12	19	17.0	55	23
(b)						
Number of patients	91		101		223	
Median follow-up of pts. in CCR (years, range)	15.6	(13.5–18.3)	11.8	(9.3–14.4)	4.8	(1.1–9.8)
Early deaths	5	5.5	9	7.0	4	1.7
Deaths days 42–150	1	1.1	4	4.0	1	0.4
Nonresponse	14	15.4	3	3.0	12	5.4
CR achieved	71	78.0	85	84.0	206	92.3
Death in CCR cumulative incidence	3	3	8	8	5	2
Relapse (cumulative incidence)	42	46	36	36	89	39
(With CNS involvement)	2	2	4	4	6	3
Lfu in CCR	4	11 (2–18)				
P survival						
At 5 years	34	37 (5)	50	50 (5)	74	66 (3)
At 10 years	32	35 (5)	46	49 (5)		
pEFS						
At 5 years	26	29 (5)	44	44 (5)	55	50 (3)
At 10 years	26	29 (5)	39	42 (5)		
pDFS						
At 5 years	26	37 (6)	44	51 (5)	55	54 (3)
At 10 years	26	37 (6)	39	48 (5)		

CR = complete remission; CCR = continuous complete remission; lfu = lost to follow-up.
[a]EDs are defined as death until day 42 and reported in detail (a) before starting therapy and (b) during the first 14 days of treatment.

was performed in 16 patients included in the chemotherapy group. The frequency of poor responders was significantly higher in the SCT group (23/55 = 42%) as compared to the chemotherapy group (38/168 = 23%, $P<0.01$). We observed a borderline significant difference in DFS between patients receiving SCT in first remission compared to the chemotherapy group (61 *vs* 49%; $P=0.08$). However, the OS in patients who achieved remission was similar in those who received chemotherapy only in first remission (68 ± 4%) as compared to those who had a SCT in first remission (68 ± 7%; $P=0.9$). This was explained by a significant superior prognosis in second remission among the children treated with chemotherapy in first remission and SCT in second remission (*p*-2.EFS = 64 ± 8%; $n=43$) as compared to 18 ± 15% ($n=13$) in children who relapsed after SCT in first remission and 6 ± 4% ($n=39$) for children treated with chemotherapy in both first and second remission. Subgroup analyses of OS in NOPHO-AML 93 according to a poor or good response to the first induction course showed no difference between SCT and chemotherapy only. Survival in children treated with SCT-1 showed no difference between NOPHO-AML 88 (63 ± 11%) and NOPHO-AML 93 (68 ± 6%; $P=0.6$).

Table 5 Results according to different risk parameters in studies NOPHO-AML 84, 88 and 93 (5-year pEFS (%), only for subgroups $n \geqslant 10$)

Presenting feature	*NOPHO-AML 84*		*NOPHO-AML 88*		*NOPHO-AML 93*	
	Total number of patients	*pEFS (s.e.)*	*Total number of patients*	*pEFS (s.e.)*	*Total number of patients*	*pEFS (s.e.)*
n	96	29 (5)	108	41 (5)	243	48 (3)
Male	41	22 (6)	54	30 (6)	124	54 (5)
Female	55	34 (6)	54	52 (7)	119	42 (5)
Karyotypes ND	82		68		9	
Cytogenetics favourable[a]						
t(8;21)	1		7		19	59 (12)
inv(16)			2		14	71 (12)
t(15;17)			1		9	
Cytogenetics normal	6		15		62	35 (6)
Cytogenetics other	6		12		93	48 (5)
t(9;11)	1		3		19	77 (10)
11q23 – not t(9;11)	0		0		18	32 (12)
FAB						
M0					16	29 (12)
M1	15	13 (9)	23	35 (10)	37	36 (8)
M2	29	27 (8)	28	50 (9)	51	52 (7)
M3	8	—	6	—	11	55 (15)
M4	19	37 (11)	17	59 (12)	49	50 (7)
M5	11	27 (13)	20	30 (10)	46	56 (8)
M6	5	—	1	—	6	—
M7	ND	—	3	—	16	35 (13)
Others/ND	9	—	10	—	11	48 (14)
Blasts						
Day 15 $<5\%$	ND	ND	ND	ND	166	55 (4)
Day 15 $>5\%$	ND	ND	ND	ND	77	34 (6)
WBC						
<100	82	29 (5)	91	38 (5)	218	49 (4)
$\geqslant 100$	14	27 (11)	17	53 (12)	25	40 (9)
Age (years)						
<2	25	44 (10)	35	34 (8)	57	54 (7)
$\geqslant 2$	71	24 (5)	73	44 (6)	186	46 (4)
CNS						
Positive	4		4		16	44 (12)
Negative	86	28 (5)	100	40 (5)	224	48 (4)

ND = not determined.
[a]Definition of favourable cytogenetics: t(8;21), t(15;17), inv(16).

Deaths in remission: Six out of eight deaths in CR-1 were in patients treated with SCT. All deaths occurred within 9 months from diagnosis and the causes were infections or multiorgan failure. In the chemotherapy group, one death (sepsis/meningitis) occurred after the second consolidation course and one child died from aspergillus 9 months from diagnosis. The cumulative incidence of death in CR-1 was 3% (Table 4).

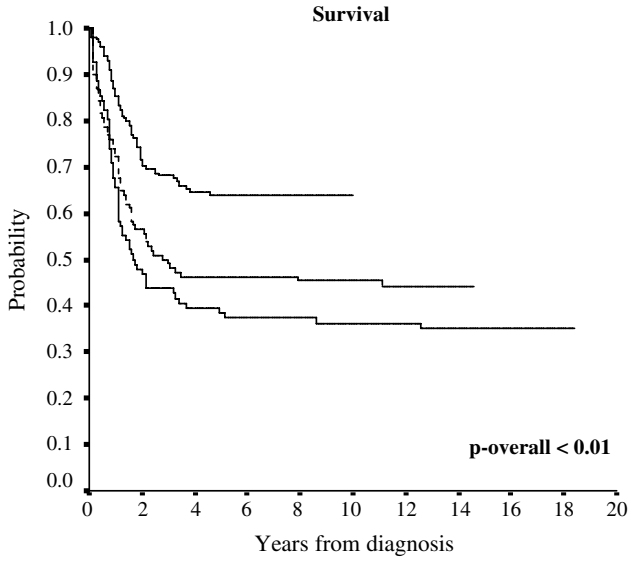
Relapses: There were 96 relapses, 14 out of 55 (25%) in the SCT-1 group and 82 out of 168 (48%) in the chemotherapy only group. In all, 84 children had BM relapse, six CNS relapse, five had other site, whereas one child developed MDS. The cumulative risk of relapse was 39%.

Prognostic factors in NOPHO-AML 93

Cox analyses for EFS/DFS showed that the strongest prognostic factors were the response to first induction course, WBC and age at diagnosis. Also, SCT in CR-1 was significantly associated with increased DFS.

Response to chemotherapy: The strongest prognostic factor in NOPHO-AML 93 was the response to the first induction course. Children who achieved remission after the first ATEDox course (67% of the patients) had a better outcome as compared to poor responders (EFS 54 ± 4 *vs* $34 \pm 6\%$, $P<0.01$) (Figure 5). The difference persisted even if induction failures were excluded from the analysis (good responders, DFS $56 \pm 4\%$, poor responders $42 \pm 7\%$, $P=0.02$).

WBC at diagnosis: WBC $\geqslant 50 \times 10^9$/l at diagnosis was associated with a higher rate of induction failure (18 *vs* 5%) with EFS of $40 \pm 9\%$ as compared to $49 \pm 4\%$ for children with WBC $<50 \times 10^9$/l ($P=0.02$) (Table 5). WBC lost its prognostic impact for children achieving CR.



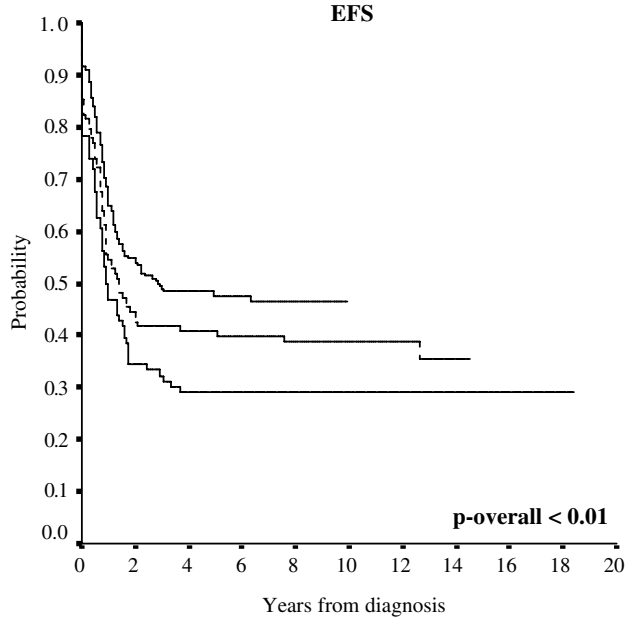
NOPHO-AML 84/88/93 Survival (5 yrs)

0.65 ± 0.03 (n=243) NOPHO-93

0.46 ± 0.05 (n=108) NOPHO-88

0.38 ± 0.05 (n= 96) NOPHO-84

Figure 2 Estimated probability of survival in patients of studies NOPHO-AML 84/88/93.



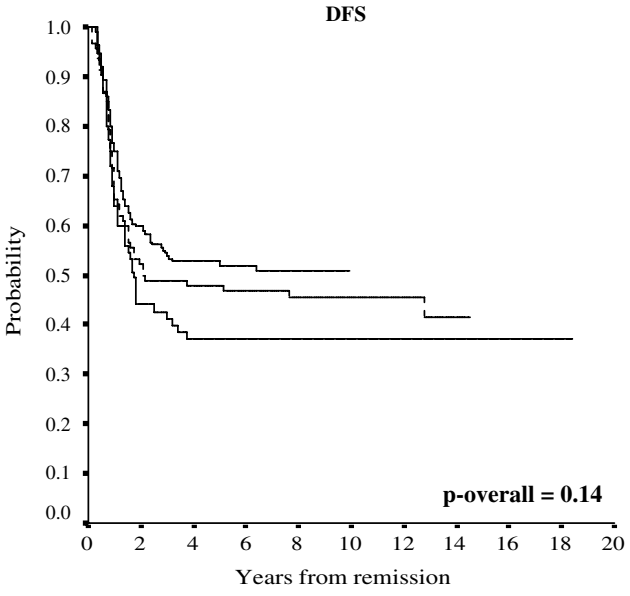
NOPHO-AML 84/88/93 EFS (5 yrs)

0.48 ± 0.03 (n=243) NOPHO-93

0.41 ± 0.05 (n=108) NOPHO-88

0.29 ± 0.05 (n= 96) NOPHO-84

Figure 3 Estimated probability of EFS in patients of studies NOPHO-AML 84/88/93.



NOPHO-AML 84/88/93 DFS (5 yrs)

0.52 ± 0.03 (n=223) NOPHO-93
0.48 ± 0.05 (n= 92) NOPHO-88
0.37 ± 0.06 (n= 75) NOPHO-84

Figure 4 Estimated probability of DFS in patients of studies NOPHO-AML 84/88/93.

Age at diagnosis: Children 10 years of age or older ($n=79$) had an inferior prognosis as compared to younger children ($n=164$); EFS 41 ± 6 *vs* 51 ± 4%, $P=0.05$. Of the 79 children over 10 years, 12 were induction failures (15%) as compared to 8/164 (5%) of children below 10 years of age at diagnosis. Six of nine children who died during remission were over 10 years of age at diagnosis. The relapse rate did not differ between younger and older children. EFS were similar in patients below 2 years as compared to children over 2 years of age at diagnosis (54 ± 7 *vs* 46 ± 4%; $P=0.2$).

SCT: The DFS was higher in patients treated with SCT in CR-1 as compared to those treated with conventional chemotherapy. However, analysis of survival showed no difference (68 ± 7 *vs* 68 ± 4%; $P=0.9$), explained by a good prognosis in second remission among the 43 children treated with SCT in second remission (*p*-2.EFS 64 ± 8%).

Outcome in relation to other criteria at diagnosis

Gender: The EFS were similar in boys and girls in the NOPHO-AML 93 study (54 ± 5 *vs* 42 ± 5%, $P=0.2$).

FAB: The impact of FAB classification was difficult to evaluate as most groups were too small for analyses. EFS varied considerably between the FAB groups. The M1 patients did poorly in all three NOPHO-AML trials. There was a significantly better prognosis in M5 patients treated on NOPHO-AML 93 as compared to NOPHO-AML 84/88 (EFS 56 *vs* 27%, $P<0.01$).

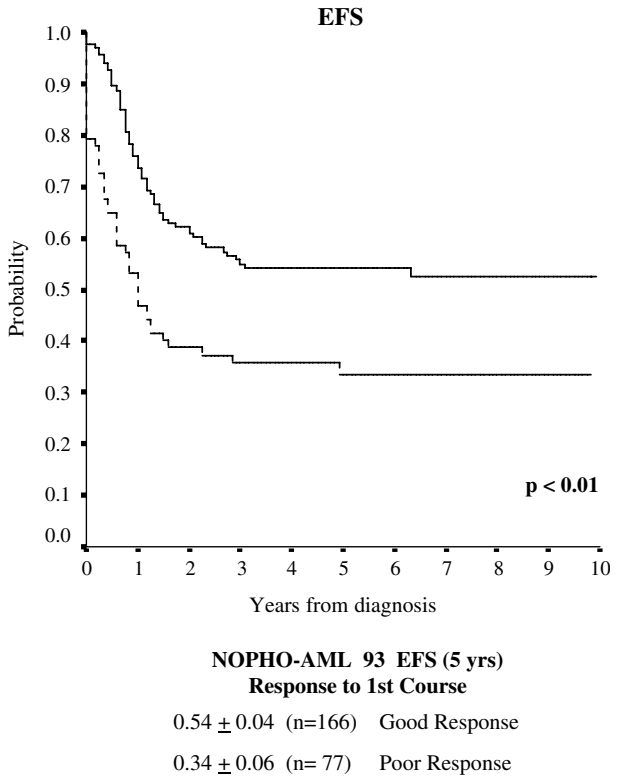


Figure 5 Estimated probability of EFS in NOPHO-AML 93 patients with good or poor response to first induction course.

Cytogenetics

Successful karyotype analysis was performed in 234/243 (95%) of the patients enrolled in the NOPHO-AML 93 study. Clonal aberrations were found in 70% (Table 3). The largest specific subgroup was children with 11q23/MLL rearrangements ($n=37$), the most common being t(9;11)(p22;q23) ($n=19$). Despite the number of patients in each specific subgroups was small, two subgroups were associated with better outcome, namely those with t(9;11)(p22;q23), with an EFS of $77\pm10\%$ and the 14 patients with chromosome 16 aberrations – inv(16;16)(p13q22) or t(16;16)(p13;q22) – with an EFS of $71\pm12\%$. The 17 patients with t(8;21)(q22;q22) had an intermediate prognosis with EFS of $59\pm12\%$. Children with normal karyotype and those with other 11q23/MLL aberrations than t(9;11) seemed to do worse, but the differences were not statistically significant (Table 5).

Promyelocytic leukaemia (FAB M3)

There were 25 study patients diagnosed with M3 during the period (21 <15 years, four patients $\geqslant15$ years of age at diagnosis). The prognosis has been stable during the study period with a 5-year OS of $58\pm10\%$ and EFS of $43\pm10\%$. We found 14 events (five early deaths/aplasia deaths, two deaths in CCR and seven relapses) among these children, that is, the risk for treatment-related deaths was at the same level as the risk of relapse of the disease. ATRA was introduced in the 1990s, but the low number of patients does not allow any analysis on the influence on outcome. The prognosis for M3 patients was not superior as compared to other FAB groups, probably explained by the high frequency of treatment-related deaths in M3.

Down's syndrome

One characteristic of the Nordic AML material has been the high frequency of patients with DS.[2] Myeloid leukaemia in children with DS has during recent years been recognised as a unique biological entity[11] with better prognosis than AML in non-DS,[1] and therefore DS should be excluded from the analysis of AML series. There were 71 children with DS diagnosed with *de novo* AML during the study period. Eight of these were left without cytostatic treatment (six during NOPHO-AML 84 period, two during NOPHO-AML 88 period) and two children were treated on other protocols. Thus, 61 patients were treated on NOPHO-AML protocols (24 on NOPHO-AML 84/88, 37 on NOPHO-AML 93). In the total patient group diagnosed during the NOPHO-AML 84/88 period ($n=34$), the 5-year survival probability was $41\pm8\%$ and was $54\pm9\%$ for children treated on NOPHO protocol ($n=24$). DS children during the NOPHO-AML 93 period ($n=37$) were treated on protocol and the 5-year OS was $86\pm6\%$ and the 5-year EFS was $81\pm6\%$. These figures for DS patients were significantly better than for non-DS treated on NOPHO-AML 93 protocol ($P<0.01$).

Discussion

The three consecutive NOPHO-AML protocols for the treatment of childhood AML have shown a successive improvement in overall outcome. The NOPHO-AML 84 protocol resulted in a relative high survival with acceptable toxicity but high rates of NRs and relapse.

In order to reduce the frequency of NRs and relapse, the NOPHO-AML 88 protocol adopted the concept of increased dose intensity during induction.[8,16] Accordingly, the first two courses in NOPHO-AML 88 were given as close together as possible. As hoped for, the proportion of NRs decreased from 14% in NOPHO-AML 84 to 4% in NOPHO-AML 88, and the relapse rates decreased from 46 to 36%. However, these improvements came at the price of an increased frequency of both early (7–13%) and late toxic deaths (4–9%). We concluded that the increased morbidity and mortality outweighed the undoubtedly enhanced antileukaemic effect of the intensified therapy.[3,17] Furthermore, whereas the German and British studies in paediatric AML produced favourable results without such intensively timed induction, even the best arm in the CCG study had no superior outcome than the NOPHO-AML 88 study.[5,6,8] The NOPHO-AML 84/88 protocols did not include any stratification because analyses of the NOPHO-AML 84 patients failed to identify significant prognostic factors. Owing to the high rates of early and late toxic deaths in NOPHO-AML 88, the concept of intensively timed induction was abandoned for patients with good response. Thus, in NOPHO-AML 93 the 67% who entered remission after the first course were given their second course only following haematological recovery. This resulted in an increased median interval between the first and the second induction course from 21 to 31 days with an associated reduction of toxic deaths. Somewhat surprisingly, since the protocol stated that AM should be given immediately if there was evidence of persistent disease on first BM examination, the median interval between ATEDox and AM was 27 days. This was mainly caused by the fact that many early BM examinations following ATEDox were too hypoplastic for

definite evaluation even in cases where repeat examination later showed persistent disease. Nevertheless, this strategy significantly decreased the rate of early and late deaths. The most important prognostic factor according to NOPHO-AML 93 was the response to the first course of therapy as also reported by others.[18,19] Children with persistent disease after the first course received a different and more toxic induction therapy, while responding children were spared of such intensification. Although they received less intensive treatment, the EFS and DFS values for good responders were significantly superior as compared to the poor responders. Although values of WBC $\geqslant 50 \times 10^9$/l at diagnosis were associated with a higher rate of induction failures (18 *vs* 5%), this criterion had no prognostic impact in patients who achieved remission. Children 10 years of age or older had an inferior prognosis as compared to younger children. This was caused by an increased frequency of induction failure and death in remission, whereas the relapse rate was similar in younger and older patients. In the NOPHO-AML 84/88 studies, outcome for girls was significantly better than for boys.[3] In NOPHO-AML 93, this difference has disappeared. The main reason is a decrease of relapse rate in boys treated on NOPHO-AML 93 as compared to the earlier protocols. There was no significant difference between gender with regard to CR rates, the type of therapy received or frequency of toxic deaths. The patients were genetically randomised to SCT-MFD in CR-1. Patients receiving SCT had fewer relapses, but due to a high salvage rate in those relapsing following chemotherapy only, the OS between the two groups did not differ. This is in contrast to results from other groups.[16,20] The OS of 68% in NOPHO-AML 93 in patients not transplanted in CR-1 is comparable to the best results in patients receiving SCT.[16] The good OS in the chemotherapy group is explained by a good result in relapsed patients treated with SCT in CR-2. Thus, it may be prudent to use more restrictive indications for SCT in first remission, reserving this procedure for poor risk and relapsed patients.[4,20,21] The 5-year EFS of 48%, DFS of 52% and OS of 65% in the NOPHO-AML 93 compares favourably with other AML studies. CCG-2891 showed DFS of 55% and survival of 63% in the intensive-timing group.[8] The MRC AML10 and 12 showed EFS and survival in children of 52 and 61%, respectively,[7] and the BFM-AML 93 protocol had an EFS and OS of 51 and 60%, respectively.[5] However, the MRC protocol included a high cumulated dose of anthracyclines and the BFM protocol contained cranial irradiation, both of which rising the concern for long-term side effects. The NOPHO protocols contain moderate amount of anthracyclines and a high cumulative dose of cytarabine. The courses of cytarabine were well tolerated. Although no systematic long-term evaluation has been performed, we are not aware of any cytarabine-associated long-term side effects in our patients. The frequency of various cytogenetic aberrations in this study is in accordance with other large series.[22,23] The most common aberrations in our material were 11q23/MLL rearrangements. A significantly better survival was observed in children with t(9;11)(p22;q23) (EFS 77%), whereas other 11q23 translocations were associated with a poor outcome. Data from St Jude Children's Research Hospital has also shown an improved survival in t(9;11)(p22;q23).[24,25] *In vitro* sensitivity studies have shown an increased cytotoxicity of cytarabine and doxorubicin in patients with t(9;11)(p22;q23).[26] None of the other cytogenetic groups showed any significant differences in survival. The survival of patients with t(8;21)(q22;q22) and inv(16)/t(16;16)(p13;q22) showed a non-significant trend for a better outcome as has been found in other studies.[23] The current protocol builds on our previous findings and stratifies the patients according to the *in vivo* response (first and second induction courses) and cytogenetic subgroups at diagnosis. The protocol will prospectively evaluate the prognostic importance of *in vitro* drug resistance and MRD levels at different time points.[27–29] This may help us to identify new subgroups in AML, which may benefit from different therapeutic strategies.

Acknowledgements

Investigators in Denmark: S Rosthoej/E Oestergaard, University Hospital, Aalborg; N Clausen/H Hasle/H Schroeder, Skejby Hospital, Aarhus; K Schmiegelow/M Yssing/C Reichnitzer, Rigshospitalet, Copenhagen, N Carlsen/M Hejl, University Hospital, Odense. *Investigators in Finland:* L Hovi/MA Siimes/K Vettenranta /U Pihkala, University Hospital, Helsinki; M Perkkiö/P Riikonen, University Hospital, Kuopio; A Mäkipernaa/M Arrola/K Parto, University Hospital, Tampere; T Salmi, University Hospital, Turku; M Lanning/M Möttönen, University Hospital, Oulu. *Investigators in Iceland:* G Jonmundsson/J Kristinsson, University Hospital, Reykjavik. *Investigators in Norway:* S Lie/A Glomstein/B Zeller, Rikshospitalet, Oslo; M Hellebostad, University Hospital, Ullervaal; J Helgestad/B Moström, University Hospital, Bergen; S Kolmannskog/R Nygaard, University Hospital, Trondheim; T Stockland/T Flaegstad, University Hospital, Tromsoe. *Investigators in Sweden:* L Mellander/J Abrahamsson, Queen Silvia Children's Hospital, Gothenburg; M Behrentz, University Hospital, Linkoping; J Heldrup/S Garwicz, University Hospital, Lund; G Gustafsson/S Soderhall, Karolinska Hospital, Stockholm; E Forestier/K Johansson/P Sandström, University Hospital, Umea; A Kreuger/G Lönnerholm/BM Frost, Akademiska Hospital, Uppsala.

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