Pediatr Blood Cancer 2011;56:551–559

Risk Factors for Treatment Related Mortality in Childhood Acute Lymphoblastic Leukaemia

Bendik Lund, MD,[1,2,*] Ann Åsberg, MD, PhD,[1] Mats Heyman, MD, PhD,[3] Jukka Kanerva, MD, PhD,[4] Arja Harila-Saari, MD, PhD,[5] Henrik Hasle, MD, PhD,[6] Stefan Söderhäll, MD, PhD,[3] Ólafur Gisli Jónsson, MD,[7] Stian Lydersen, PhD,[8] and Kjeld Schmiegelow, MD, PhD[2,9] On behalf of the Nordic Society of Paediatric Haematology and Oncology (NOPHO)

Background. In spite of major improvements in the cure rate of childhood acute lymphoblastic leukaemia (ALL), 2–4% of patients still die from treatment related complications. ***Procedure.*** We investigated the pattern of treatment related deaths (TRDs) and possible risk factors in the NOPHO ALL-92 and ALL-2000 protocols. Fifty-five TRDs were identified among the 1,645 ALL-92 patients and 33 among the 1,090 ALL-2000 patients. ***Results.*** There was no significant difference in the incidence of TRDs between the two protocols (3.4% vs. 3.2%). Five patients died before initiation of therapy (0.2%), and the overall subsequent risk of induction death and death in first complete remission (CR1) was 1.2% and 1.8%, respectively. Infections were the major cause of death comprising 72% of all cases including 9 deaths from *Pseudomonas aeruginosa* and 11 deaths from fungal infections. Other causes of death included bleeding or thrombosis (eight patients), tumour burden related toxicities (seven patients) and organ toxicity (seven patients). Female gender (hazard ratio (HR): 2.2, 95% confidence interval (95% CI): 1.4–3.4), high white blood cell count ($\geq 200 \times 10^9$/L) at diagnosis (HR: 3.5, 95% CI: 1.7–7.1), T-cell disease (HR: 1.9, 95% CI: 1.01–3.7), Down syndrome (HR: 7.3, 95% CI: 3.6–14.9) and haematopoietic stem cell transplantation in CR1 (HR: 8.0, 95% CI: 3.3–19.5) were identified as independent risk factors for TRD. ***Conclusion.*** Several TRDs were potentially preventable and future efforts should be directed towards patients at risk. Pediatr Blood Cancer. 2011;56:551–559.

Key words: acute lymphoblastic leukaemia; paediatric oncology; risk factors; toxicity; treatment-related death

INTRODUCTION

Improved risk grouping and intensification of chemotherapy have significantly reduced the relapse rate of childhood acute lymphoblastic leukaemia (ALL) [1–3]. In contrast, and in spite of improved supportive care, treatment related deaths (TRDs) continue to occur in 2–4% of the patients (Table I) [4–11]. Thus, the relative significance of TRDs among all events has increased because of the decreasing relapse-rate in current treatment protocols. TRD represents the 'tip of the iceberg' of the total toxicity related to modern treatment of childhood ALL.

Infections, bleeding or thrombosis, tumour burden complications, and therapy induced organ toxicities are the most common causes of TRD [4,7,12,13]. Four major factors influence the risk of these and other severe, although non-fatal, toxicities: the leukaemia itself (e.g., the tumour burden and specific organ involvement), the treatment intensity, the supportive care (including specific guidelines and physician and patient compliance to these) and host factors (including inherited genetic polymorphisms that influence drug disposition and immune function) [2,14–16].

To identify potentially preventable risk factors for specific TRDs, we explored all 88 TRDs among 2,735 ALL patients treated on two consecutive Nordic protocols from 1992 to 2008.

MATERIALS AND METHODS

Since 1992 all children with ALL in the five Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) have been treated according to common Nordic protocols [1]. Long-term results have recently been published for the NOPHO ALL-92 study showing a 10-year event-free survival (EFS) of $74.6 \pm 1.1\%$ and an overall survival of $84.7 \pm 0.9\%$. For the NOPHO ALL-2000 protocol, the 5-year EFS was $79.4 \pm 1.5\%$ and the overall survival was $89.1 \pm 1.1\%$ [17]. Between January 1992 and June 2008, 2,882 children 1.0–14.9 years of age with B-cell precursor or T-cell ALL were diagnosed within the Nordic countries. We excluded the following patients from this study: 2 Down syndrome patients who received no anti-leukaemic therapy (1 with significant co-morbidity and 1 diagnosed post-mortem), 14 patients not treated according to NOPHO ALL-92 or ALL-2000 protocols, 41 patients treated according to the NOPHO ALL-2000 protocol before it was officially opened, 20 patients treated according to the international protocol for Ph+ (t(9;22)(q34;q11)/*BCR-ABL* fusion) ALL, 14 patients who were

[1]Department of Pediatrics, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway; [2]Department of Pediatrics, The University Hospital Rigshospitalet, Copenhagen, Denmark; [3]Department of Pediatrics, Astrid Lindgrens Barnsjukhus, Stockholm, Sweden; [4]Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland; [5]Department of Paediatrics and Adolescence, Oulu University Hospital, Oulu, Finland; [6]Department of Pediatrics, Aarhus University Hospital, Skejby, Denmark; [7]Department of Pediatrics, University Hospital, Reykjavik, Iceland; [8]Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, Norway; [9]The Faculty of Medicine, Institute of Gynaecology, Obstetrics and Paediatrics, University of Copenhagen, Copenhagen, Denmark

Grant sponsor: The Danish Childhood Cancer Foundation; Grant sponsor: Childrens Cancer Foundation, Department of Pediatrics, St. Olavs Hospital, Trondheim University Hospital; Grant sponsor: The Swedish Childhood Cancer Foundation.

Conflict of interest: Nothing to declare.

*Correspondence to: Bendik Lund, Department of Pediatrics, St. Olavs Hospital HF, Trondheim University Hospital, N-7006, Trondheim, Norway. E-mail: bendik.lund@ntnu.no

Received 10 January 2010; Accepted 25 May 2010

DOI 10.1002/pbc.22719
Published online 8 December 2010 in Wiley Online Library (wileyonlinelibrary.com).

TABLE I. Treatment Related Death (TRD) in Childhood Acute Lymphoblastic Leukaemia (ALL) and Study Groups

Study group	Years of study	Age range (years)	No. of patients included	Total no of TRDs, no. of pat. (%)	Pre-treatment deaths, [a] no. of pat. (%)	Induction deaths, no. of pat. (%)	Death in CR1, no. of pat. (%)	Death post-HSCT in CR1, no. of pat. (%)
Present study	1992–2008	1–15	2,735	88 (3.2)	5 (0.2)	34 (1.2)	49 (1.8)	10 (0.4)
Moricke et al. [6], BFM-95	1995–2000	0–18	2,169	62 (2.9)	Excluded	16 (0.7)	33 (1.5)	13 (0.6)
Hargrave et al. [9], MRC UKALL XI	1991–1997	1–15	2,090	56 (2.7)	Excluded	25 (1.2)	27 (1.3)	4 (0.2)
Vilmer et al. [8], CLCG-EORTC 58881	1989–1998	0–18	2,065	76 (3.7)	10 (0.5)	9 (0.4)	57 (2.8)	Not reported
Conter et al. [5], AIEOP-91 *Study 91*	1991–1995	0–15	1,194	[b](3.2)	Excluded	[b](1.4)	[b](1.8)	Not reported
Rubnitz et al. [7], SJCRH	1984–1999	0–18	1,011	36 (3.6)	Not reported	14 (1.4)	16 (1.7)	6 (0.6)
Slats et al. [11], DCOG	1984–1996	0–15	875	29 (3.3)	3 (0.3)	6 (0.7)	14 (1.6)	5 (0.7)
Prucker et al. [4], A-BFM	1981–1999	0–21	896	31 (3.5)	Excluded	7 (0.8)	24 (2.7)	Excluded
Moghrabi et al. [10], DFCI 95-01	1996–2000	0–18	491	7 (1.4)	Not reported	4 (0.8)	3 (0.6)	Not reported

AIEOP, Associazione Italiana Ematologia Oncologia Pediatrica; BFM, Berlin-Frankfurt-Münster Study Group; SJCRH, St. Jude Children's Research Hospital; CLCG-EORTC, Children Leukemia Cooperative Group—European Organisation for Research and Treatment of Cancer; DFCI, The Dana-Farber Cancer institute; MRC UKALL, Medical Research Council United Kingdom Acute Lymphoblastic Leukaemia; NOPHO, Nordic Society of Paediatric Haematology and Oncology; DCOG, Dutch Childhood Oncology Group; A-BFM, Austrian Berlin-Frankfurt-Münster Study Group; CR1, first complete remission. Included is death post-HSCT when reported; HSCT, haematopoietic stem cell transplantation. [a]Death before start of treatment; [b]Numbers not available.

diagnosed and started on anti-leukaemic therapy outside the Nordic countries, 52 patients who changed protocol during therapy (from ALL-92 to ALL-2000, or from ALL-2000 to ALL-2008), 1 patient who died from Leighs encephalopathy day 6 during induction therapy and three patients with bilineage leukaemia. Thus, 2,735 patients were included in the present study (Fig. 1). Patients with resistant disease to NOPHO treatment were included until the day resistant disease was recognised. Patients dying from a second malignant neoplasm or after relapse were not considered as TRD in this study. Since the purpose of this study was to explore the risk of TRD among children 1.0–14.9 years of age in order to improve the treatment and supportive care for non-infants and non-Ph + ALL patients, we excluded patients treated according to other protocols, including the international Interfant and EsPhALL protocols. Of the 1,231 females and 1,504 males, 1,645 patients were treated according to the NOPHO ALL-92 protocol (open from January 1992 until October 2001), and 1,090 patients were treated according to the NOPHO ALL-2000 protocol (open from January 2002 until June 2008). There were 2,431 (89%) B-cell precursor and 277 (10%) T-cell ALL patients (no information on lineage was available for 27 patients). Fifty-nine had Down syndrome and 68 patients were registered with CNS leukaemia at diagnosis. Nine hundred and fifty-five (35%) were standard risk, 942 (34%) intermediate risk and 838 (31%) were high-risk patients according to the ALL-92 and ALL-2000 criteria. Patients who stayed in first complete remission (CR1) were followed until April 2009. Data were obtained from the prospective registration of all patients in the NOPHO leukaemia registry. Additional clinical data concerning cause of death from the patient files for the 88 TRDs were obtained through a questionnaire sent out to the treating centres. Fifty-five of the 88 TRDs in this study have previously been published [12].

Risk Grouping

Patients were stratified into three risk groups: standard risk (SR), intermediate risk (IR) and high-risk (HR) ALL. In this study, SR and IR ALL are combined as low-risk ALL. Stratification criteria for the low-risk groups were (all criteria needed): WBC $<50.0 \times 10^9$/L, B-cell precursor ALL, no CNS or testicular leukaemia, no unfavourable cytogenetic alterations (i.e. 11q23/*MLL*-rearrangements, t(9;22)(q34;q11)/*BCR-ABL* fusion, t(1;19)(q23;p13)/*E2A-PBX1* fusion, hypodiploidy (<45 chromosomes)), and good response to initial therapy defined as M1 or M2 bone marrow at day 15 and M1 bone marrow at day 29. The HR patients fulfilled at least one of the high-risk criteria listed above. In the ALL-2000 protocol, HR patients with at least one of the following criteria were allocated to haematopoietic stem cell transplantation (HSCT) in CR1: M3 bone marrow on day 29, 11q23/*MLL* rearrangements and age ≥10 years, t(9;22)(q34;q11)/*BCR-ABL* fusion, a karyotype with a modal chromosome number <34, or initial WBC $\geq 200 \times 10^9$/L. There were no uniform Nordic criteria for HSCT in the ALL-92 protocol.

Induction Remission Treatment, Both Protocols

The first 50 days of therapy included only small differences between the two protocols and consisted of the following: induction included prednisolone (60 mg/m^2/day) day 1–36 followed by 10 days tapering. Pre-treatment for 1–6 days with prednisolone dose increments was used in cases with large leukaemic cell burden at

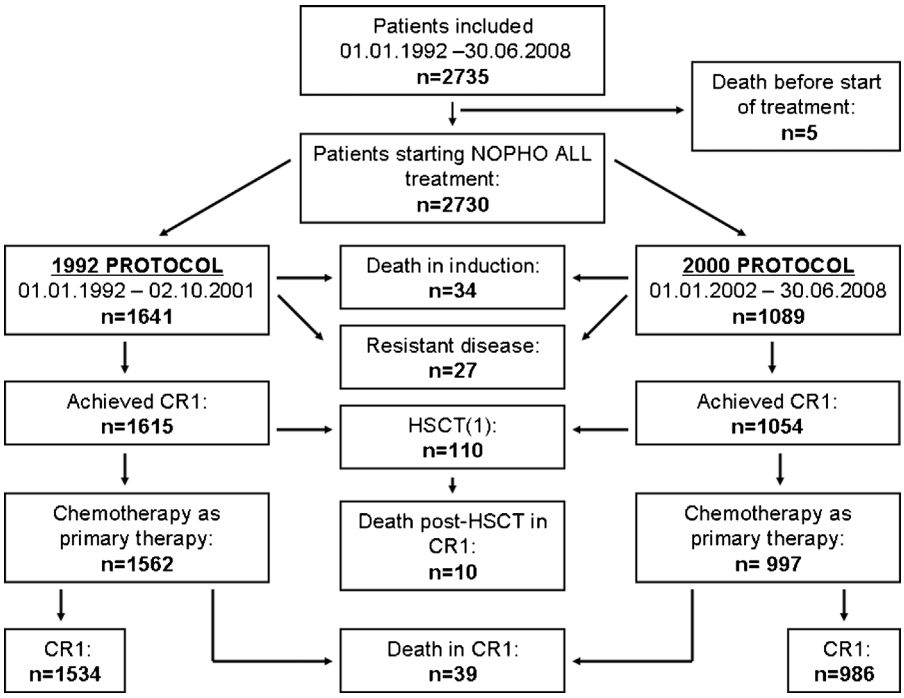

Fig. 1. Flow chart of patients included in the study.

diagnosis to decrease the risk of tumour lysis syndrome (TLS). Vincristine (Vcr) was given weekly for 6 weeks in doses of 2.0 mg/m^2; the maximum dose was increased from 2.0 mg in the ALL-92 protocol to 2.5 mg in the ALL-2000 protocol. In the ALL-92 protocol doxorubicin at doses of 40 mg/m^2 was given three and four times in the low-risk and high-risk groups, respectively. In the ALL-2000 protocol the corresponding number was reduced to two or three doses for the low- and high-risk groups, respectively. Methotrexate (MTX) was given intrathecally (i.t.) at days 1, 8, 15 and 29 in age-adjusted doses. For the ALL-92 patients Erwinia asparaginase was used (dose: 30,000 IU/m^2 daily on days 37–46), and for the ALL-2000 patients, *Escherichia coli* asparaginase was used (dose: 6,500 IU/m^2 at 3- to 4-day intervals up to a total of four doses starting treatment day 36).

Further Treatment

NOPHO ALL-92 protocol: Detailed information concerning therapy for the NOPHO ALL-92 protocol has been published earlier [1,18]. Treatment duration from the day of diagnosis was 2.5 years for the SR group and 2.0 years for the other groups. There were no specific guidelines for supportive care in the ALL-92 protocol.

NOPHO ALL-2000 protocol: Following induction, the low-risk groups received identical consolidation therapy consisting of 6-MP (25 mg/m^2/day) and alternating blocks with high-dose MTX (5 g/m^2/24 hr with i.t. MTX and Leucovorin rescue) and low-dose cytarabine (75 mg/m^2/day for 4 days, two times). After induction, early intensification therapy followed for the high-risk groups, which included two doses of cyclophosphamide (1,000 mg/m^2) 4 weeks apart with low-dose cytarabine (75 mg/m^2 daily for two 4 days periods), oral 6-thioguanine (6-TG) and two doses of i.t. MTX. Consolidation therapy for the high-risk groups included alternating courses of high-dose MTX (8 g/m^2/24 hr with i.t. MTX and Leucovorin rescue) and high-dose cytarabine (12 g/m^2) times two or four, with two 2 months intervening periods of oral weekly MTX and daily 6-MP with two Vcr/prednisolone reinductions per period. In the low-risk groups the interval between high-dose MTX courses was increased from 2 to 4 weeks compared to the ALL-92 protocol, and the start of Leucovorin rescue was delayed 6 hours to "hour 42" from start of the MTX infusion. Patients with IR- or HR-ALL received delayed intensification therapy with dexamethasone (doses: IR patients 6 mg/m^2/day, HR patients 10 mg/m^2/day) for 2 weeks, weekly Vcr (2.0 mg/m^2) for 4 weeks, weekly doxorubicin (HR) or daunorubicin (IR), at a dose of 30 mg/m^2/day, three (HR) or four (IR) times. In addition, *E. coli* asparaginase (dose: 6,500 IU/m^2) was given four times followed by cyclophosphamide (dose: 1,000 mg/m^2), low-dose cytarabine and 6-TG. Maintenance therapy consisted of weekly oral MTX (starting dose: 20 mg/m^2) and daily oral 6-MP, dose adjusted according to TPMT activity (starting doses, wild-type patients: 75 mg/m^2/day, heterozygous patients: 50 mg/m^2/day, TPMT deficient patients: 5–10 mg/m^2/day). In addition, low-risk patients received alternate pulses at four-week intervals Vcr (2.0 mg/m^2, one dose)/dexamethasone (6 mg/m^2/day for 5 days) and high-dose MTX (5 g/m^2/24 hr) times five during the first year of maintenance therapy. HR patients received reinductions every four weeks throughout maintenance therapy consisting of Vcr (2.0 mg/m^2) and dexamethasone (6 mg/m^2/day for 5 days). After 1 year in maintenance patients in the low-risk groups were randomised into two arms: one arm with only 6-MP/MTX, and one arm with 6-MP/MTX plus an additional eight pulses every 6 weeks of dexamethasone (dose: orally 6 mg/m^2/day) and Vcr (dose: i.v. 2.0 mg/m^2, max. dose 2.5 mg) for 5 days. For HR patients who were not treated with HSCT in CR1, two cycles of the LSA_2L_2 regimen [19] was inserted at the beginning of maintenance therapy. Children above 5 years of age with T-cell ALL and mediastinal mass, and/or WBC at diagnosis $100 < 200 \times 10^9$/L, and/or CNS leukaemia at diagnosis

received cranial irradiation (24 or 18 Gy depending on whether or not CNS leukaemia was present at diagnosis). The treatment duration was 2.5 years for low-risk patients and 2.0 years for high-risk patients.

The ALL-2000 protocol included guidelines for supportive care concerning TLS, hyperleukocytosis, superior vena cava syndrome and superior mediastinal syndrome. There were no general recommendations for prevention of infection with *Pneumocystis jiroveci* (PJ), fungal infections or management of febrile neutropenia. The use of myeloid growth factors was optional after courses with high-dose cytarabine (2 g/m^2).

Causes of Death

The causes of death were grouped as (i) related to tumour burden (i.e. TLS or leukostasis with compromised organ function due to infiltrating blast cells), (ii) bleeding or thrombosis, (iii) infections, (iv) therapy-induced organ toxicity and (v) other or uncertain cause. The criteria for infectious deaths were clinical signs of infections in combination with fever and/or raised C-reactive protein and/or microbiologically proven infection, and no other obvious cause of death. An autopsy was performed in 34 (39%) cases.

Statistical Analysis and Definitions

Proportions were compared by Chi-square tests. Kaplan–Meier plots and survival tables were used for survival analysis including estimation of cumulative incidence of TRD and subgroups were compared using Log-rank tests. The main event in the analysis was TRD including (i) pre-treatment death (death before any anti-leukaemic therapy), (ii) induction death (death after start of treatment, but before achieved remission) and (iii) death in CR1 (included deaths happening up to 6 months after end of treatment). Patients who experienced other events (resistant disease, relapse or second malignant neoplasms) were censored at the time of these events. Time to TRD was defined as time between diagnosis of ALL and date of death. Patients who experienced no events were censored at the time of last follow-up. Cox proportional hazard regression analysis was performed with time to TRD (or time to infectious TRD) as only event, and the following covariates were included: sex, age ($<$ or $\geq$10 years), WBC ($<$ or $\geq 200 \times 10^9$/L), B-cell precursor versus T-cell disease, protocol (ALL-92 vs. ALL-2000), presence or absence of CNS-disease, Down syndrome and HSCT in CR1. Age was dichotomised at 10 years since this was used as stratification criteria for the intermediate risk group in both protocols. For patients who underwent HSCT in CR1, a time-dependent covariate was defined as zero before, and one after the date of HSCT, and for all other patients, this time-dependent covariate was defined as zero. We checked for the possible 10 two-way interactions among the 5 significant covariates, using a stepwise approach with Bonferroni adjusted significance level $0.05/10 = 0.005$. In all other analyses, P-values <0.05 were considered significant. All tests were two-sided. The statistical analyses were performed using the SPSS 16.0 statistical software.

Ethical Considerations

The protocols were approved by the national or regional ethics committees in the five Nordic countries, and the study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Of the total of 2,735 patients, 51 females and 37 males (3.2%) with a median age of 4 years (75%, range 2.0–11.0 years) at diagnosis experienced a TRD. The TRDs constituted 25% of all 354 deaths in the study population with 240 deaths after relapse as the largest group. According to risk groups (stratification based on up-front criteria) the cumulative incidence of TRD was $1.7 \pm 0.4\%$, $2.4 \pm 0.5\%$ and $6.7 \pm 0.9\%$ for the SR, IR and HR group, respectively ($P < 0.001$). Of all patients, five deaths (0.2%) occurred before initiation of treatment (pre-treatment deaths), 34 deaths (1.2%) occurred during remission induction and 49 deaths (1.8%) happened during post-induction treatment in CR1 including 10 deaths after HSCT (Fig. 1). Of the 88 TRDs, 63 (72%) patients died from infections, 8 (9%) died from bleeding or thrombosis, 7 (8%) died from organ toxicity and 7 (8%) died from tumour burden complications. In addition, two HSCT patients died from severe graft-versus-host disease (GVHD) of which one of them had additional respiratory failure, and one patient died from an erroneous procedure (intrathecal injection of Vcr). The cumulative incidence of TRD in the ALL-92 and ALL-2000 protocol did not differ significantly, and was $3.4 \pm 0.5\%$ and $3.2 \pm 0.6\%$, respectively ($P = 0.85$).

Tumour Burden Related Early Deaths

Of the seven patients (six boys) who died from tumour burden related problems (of which five died pre-treatment), six patients had a WBC $\geq 350 \times 10^9$/L at diagnosis. All, except one patient, died from intracerebral infiltration of leukaemic blast cells with or without intracerebral bleeding. One patient with a large mediastinal tumour (WBC at diagnosis: 23×10^9/L) was resuscitated because of cardiac arrest during anaesthesia for diagnostic bone marrow aspiration and died after 11 days due to secondary brain damage. No patients died from TLS complications.

Treatment Related Death in Relation to Time and Phase of Therapy

The TRDs occurred at a median of 6 weeks from diagnosis (Fig. 2) and 76% of the non-HSCT related deaths occurred within the first 80 days of treatment. The annual proportion of TRD before remission (pre-treatment deaths and induction deaths) ranged from 0.0–2.7% (calendar years 1992–2007). Of the 2,730 patients who started anti-leukaemic treatment, 34 patients died during remission induction (Fig. 1) yielding a proportion of induction death of 1.2% (0.7% in the low-risk groups and 2.4% in the high-risk group, $P < 0.001$). The causes of induction deaths were infections (n = 26), tumour burden (n = 2) and bleeding or thrombosis (n = 6).

Of the 2,669 patients who achieved remission, 49 died in CR1 (including 10 post-HSCT patients) giving a proportion of death in CR1 of 1.8% (1.3% in the low-risk groups and 3.2% in the high-risk group, $P = 0.001$). Of these, 17 died during the last part of the induction phase, 3 died during early intensification, 2 during consolidation, 3 during late intensification, 12 during maintenance (none of which during the LSA_2L_2 regimen) and 1 during cranial irradiation. For one patient, exact information concerning treatment phase was lacking. Of the 12 patients who died during maintenance, only 2 infectious TRDs occurred within 6 weeks from administration of pulses of Vcr/dexamethasone. Of the 39 non-HSCT TRDs, 30 (77%) patients died from infections and 6 (15%) from organ toxicity includ-

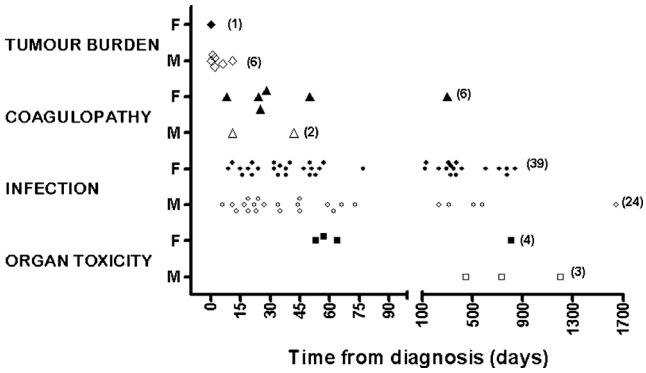

Fig. 2. Causes of death in relation to time from diagnosis for 85 out of 88 treatment related deaths (TRDs) in the NOPHO ALL-92 and NOPHO ALL-2000 protocol (TRDs post-HSCT included). Not shown are one patient who died from an accidental intrathecal injection of vincristine, and two post-haematopoietic stem cell transplantation patients who died from severe GVHD. F: Female (n = 1,231). M: Male (n = 1,504).

ing two deaths from acute pancreatitis after the second and third dose of asparaginase, respectively. Other deaths from organ toxicity included one MTX-related and one hypertensive encephalopathy, one toxic hepatitis following a high-dose MTX course and one patient who died shortly after cessation of therapy of haemolytic uremic syndrome of unknown origin. One patient died 6 weeks after diagnosis from an intestinal bleeding, one Down syndrome patient died after 10 months from an intracerebral bleeding and one patient died after an erroneously administered intrathecal dose of Vcr.

Of the 110 patients who underwent HSCT in CR1, 10 patients died from treatment related complications; 5 died from infections, 1 from leukoencephalopathy and 2 from GVHD. In two HSCT patients who died, CMV infection was suspected, but not proven.

Infectious Deaths

Of the 63 infectious deaths (Table III), 7 cases were polybacterial or polymicrobial, and in 16 cases no microorganism was found. Chemotherapy induced neutropenia (defined as neutrophils $\leq 0.5 \times 10^9$/L) during the last week of life was found in relation to 29 (48%) of the infectious deaths.

Bleeding and Thrombosis

Of the eight TRDs caused by bleeding or thrombosis (leukostasis-associated TRDs excluded), six deaths occurred within the first 50 days from diagnosis including four patients who died before the first dose of asparaginase. Of the bleeding deaths, two of the haemorrhages were located to the brain, one to the intestines and one haemorrhage was diffuse involving multiple organs. Of the four who died from thrombosis/infarction, three occurred in the brain and one in the intestines.

Risk Factors

In simple Cox regression analyses several closely associated clinical features were linked to an increased risk of TRDs: T-cell disease, WBC $\geq 200 \times 10^9$/L at diagnosis, presence of CNS disease and HSCT in CR1 (Table II). Kaplan–Meier plot for WBC is shown in Figure 3. In multiple Cox regression analysis, female gender, WBC $\geq 200 \times 10^9$/L at diagnosis, T-cell disease, Down syndrome, and HSCT in CR1 were identified as independent risk factors for

TABLE II. Risk Factors for Treatment Related Death (TRD), NOPHO ALL-1992 and NOPHO ALL-2000 Protocol

Risk factors	TRD (n = 88)	All patients (n = 2735)	HR (95% CI), simple regression	Adjusted HR (95% CI), multiple regression
Sex				
Female	51	1,231	1.7 (1.1–2.6)	2.2 (1.4–3.4)
Male	37	1,504	1.0	1.0
Age (years)				
1–9	71	2,274	1.0	1.0
10–14	17	461	1.2 (0.7–2.1)	0.9 (0.51–1.5)
WBC ($\times 10^9$/L)				
<200	69	2,603	1.0	1.0
≥200	19	132	6.5 (3.9–10.8)	3.5 (1.7–7.1)
Immunophenotype				
T-cell	22	277	3.2 (2.0–5.3)	1.9 (1.01–3.7)
Not T-cell	66	2,458	1.0	1.0
Protocol				
1992	55	1,645	1.0 (0.7–1.6)	1.2 (0.76–1.8)
2000	33	1,090	1.0	1.0
Down syndrome				
Yes	9	59	5.6 (2.8–11.2)	7.3 (3.6–14.9)
No	79	2,676	1.0	1.0
CNS				
Yes	7	68	3.8 (1.7–8.2)	2.1 (0.93–4.6)
No	81	2667	1.0	1.0
HSCT in CR1				
Yes	10	109	17.7 (8.1–38.6)	8.0 (3.3–19.5)
No	78	2,626	1.0	1.0

HR = hazard ratio; 95% CI = 95% confidence interval; WBC = white blood cell count at diagnosis; HSCT in CR1 = haematopoietic stem cell transplantation in first complete remission. A time-dependent covariate was constructed for HSCT patients (see text).

TABLE III. Causative Organisms in the 63 Infectious Deaths in the NOPHO ALL-1992 and ALL-2000 Protocol

		Number of patients	
Major group	Organism	ALL-1992	ALL-2000
Bacteria	*Coagulase negative staphylococcus*	1	
	Bacillus cereus[a]		1
	Pseudomonas aeruginosa[†]	8	1
	Eschericia coli	3	1
	Klebsiella	1	
	Listeria[a]		1
	Stenotrophomonas (Xanthomonas) *maltophilia*	1	
	Enterobacter	1	
	Stomatococcus	1	
	Micrococcus	1	
	Unspecified		2
Virus	*Cytomegalovirus***	1	2
	Adenovirus	1	
	Influenza B	1	
	Respiratory syncytial virus		1
Fungi	*Candida**	2	3
	Geotrichum capitatum		1
	Pneumocystis jirovechi	1	
	Unspecified[†]	3	1
Polybacterial or microbial**,[†]	6	1	
Unspecified microorganism**,[††]	9	7	
Total	41	22	

The number of daggers indicate the number of Down syndrome cases (n = 5) in respective groups. The number of asterisks indicate the number of deaths in respective groups post-haematopoietic stem cell transplantation (HSCT) (n = 7). [a]Central nervous system infections (n = 2).

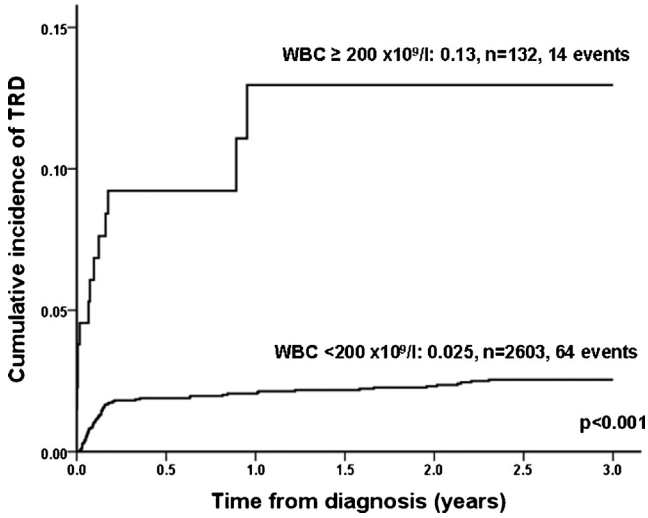

Fig. 3. Cumulative incidence of treatment related death (TRD) by white blood cell count at diagnosis (WBC). Patients with WBC $<200 \times 10^9$/L ($2.5 \pm 0.3\%$ (standard error)) versus patients with WBC $\geq 200 \times 10^9$/L ($13.0 \pm 3.6\%$) at diagnosis (Kaplan–Meier estimator, $P < 0.001$, Log-rank test). Patients who underwent HSCT in CR1 were censored at the time of HSCT.

TRD (Table II). The only significant interaction was Down syndrome and WBC $\geq 200 \times 10^9$/L ($P < 0.001$), where the presence of both these factors attenuated the hazard. However, this did not significantly affect the effects of the other risk factors in Table II. The haemoglobin level at diagnosis was not found to be a risk factor for TRD. Analysing infectious TRDs separately, females more often died from infections compared to males (adjusted HR: 2.4, 95% CI: 1.4–4.0). Of the nine Down syndrome TRDs, seven occurred before achieved remission.

DISCUSSION

Despite a steady improvement in supportive care over the last 30 years, treatment related toxicity remains a major challenge in childhood ALL therapy, and the risk of treatment related mortality has not decreased substantially in the last 20 years [4,7,12,20]. Furthermore, in parallel to the decreasing number of patients dying from the leukaemia itself (i.e. tumour burden related death, resistant disease and relapse), TRDs comprise an increasing proportion of the overall mortality (25% in our study). In addition, TRD represents the most severe form of overall treatment related toxicity, and preventive efforts directed towards factors influencing TRD will also affect toxicity in general.

The TRDs can be subdivided into infections, tumour burden complications, organ toxicity and bleeding/thrombosis with infections as the most frequent cause. To reduce toxicity and prevent TRD, interventions are needed to address the risk factors, that is the tumour burden, the treatment intensity and the supportive care. In addition, there is a need of increased knowledge of relevant genetic host factors.

Concerning the seven deaths from tumour burden related problems within the first days from diagnosis, the outcome for patients like these is clearly related to the rapidity of the diagnostic work-up and initiation of therapy. Traditionally, patients with a high tumour burden have been treated with intravenous alkaline fluids, a xanthine oxidase inhibitor (allopurinol), and gradually increasing doses of a corticosteroid, delaying more intensive chemotherapy until the blast

count has fallen and thereby lowering the risk of TLS. Modern treatment with recombinant urate oxidase (rasburicase) has been shown to be safe and effective for prophylaxis or treatment of the urate related problems of TLS in childhood malignancies [21]. Rasburicase produces a rapid decrease in plasma uric acid concentration and makes it possible to start with tumour reducing therapy within hours [21,22]. Since a mononuclear white blood cell count above 200 is virtually pathognomonic for leukaemia, rapid initiation of full dose corticosteroid therapy should be considered after administration of urate oxidase at the presentation of such patients and sampling of peripheral blood for the diagnostic leukaemia work-up. This approach could potentially prevent the tumour burden related TRDs seen in this study. In case of significant electrolyte disturbances, for example hyperphosphatemia, these should be corrected before start of anti-leukaemic therapy.

The treatment-induced immunosuppression includes neutropenia, impaired humoral antibody response, impaired cell-mediated immunity, phagocytic defects and disturbed cytokine function [23,24]. Furthermore, Eyrich et al. [25] recently showed that B-cells were most severely affected throughout therapy and did not recover before the end of therapy. T-cells and natural killer cells partially recovered at the end of induction therapy and are the dominating lymphocyte subset during maintenance therapy. We have shown that infections remain the most common cause of TRD comprising 72% of all cases, which is in line with the findings of most other groups [4,9,13,26].

It is noteworthy that 76% of all TRDs occurred during the first 80 days of treatment when the immune deficiency is most pronounced due to the tumour load itself (i.e. infiltrating blast cells in bone marrow and other lymphoid tissue) and very intensive chemotherapy including corticosteroids.

Of the patients dying from infections, 48% had chemotherapy-induced neutropenia during the week preceding death. However, the choice of empirical anti-microbial therapy for febrile neutropenia varies widely between treating centres even within the same collaborative group. In a British survey of 21 United Kingdom Children's Cancer Study Group (UKCCSG) centres treating children with febrile neutropenia, the management varied both concerning the definition of fever, the definition of neutropenia, and in the choice of empirical antibiotic therapy [27]. Studies have shown that combination therapy of a *Pseudomonas*-covering beta-lactam (e.g. ceftazidime) and an aminoglycoside is superior to monotherapy in case of *Pseudomonas* infections [28,29]. Out of the nine *Pseudomonas* TRDs in our study, only one occurred in the ALL-2000 protocol. A likely explanation for this is an increased use of empiric *Pseudomonas*-covering antibiotic therapy in case of febrile neutropenia, but no data on routine supportive care have been registered as part of this study.

There was no uniform approach to PJ infection prophylaxis during the study period and no patient-specific data on the use of PJ-prophylaxis is available. Thus, it is unknown to what extent local strategies for PJ-prophylaxis have contributed to the low frequency of mortality from PJ (Table III).

Concerning antibacterial prophylaxis, there is no uniform approach in the Nordic region. The use of prophylactic TMP-SMX has earlier been shown to reduce the incidence of both PCP infections and other infections and bacteraemia in ALL patients [30,31]. A retrospective Danish study comparing two different patients groups, one receiving TMP-SMX prophylaxis during induction treatment, and one without, showed that the TMP-SMX group had significantly fewer episodes of fever and fewer fever-related positive blood cultures during induction therapy [32]. In a review article on efficacy of oral prophylactic antibiotics in neutropenic afebrile oncology patients (including 22 clinical trials) van de Wetering et al. [33] concluded that oral prophylactic antibiotics decreased Gram-negative bacteraemia and infection related mortality. However, only 3 of the 22 reviewed trials included paediatric patients. Larger prospective studies are needed to explore if antibacterial prophylaxis during the neutropenic phases of the first 3 months of anti-leukaemic therapy can reduce the risk of TRDs in childhood ALL.

Of the 11 deaths due to fungal infections, 9 occurred within the first 9 weeks of treatment. Early death from fungal infections has also been found by others [7], pointing at the potential advantage of anti-fungal antibiotic prophylaxis at least during the early part of treatment. One possible disadvantage when using azoles as anti-fungal prophylaxis in combination with weekly administration of Vcr (as during induction therapy) is increased Vcr-related neurotoxicity. Vcr is metabolised by CYP3A enzymes and azoles are potent inhibitors of CYP3A isoenzymes resulting in higher Vcr concentrations [34,35]. Fluconazole is a relatively weaker inhibitor of CYP3A compared to other azoles (e.g. itraconazole) [35] leaving fluconazole as a reasonable drug of choice, although it has a limited effect on invasive aspergillosis and fluconazole-resistant candida strains [36,37]. In the British UKCCSG study on febrile neutropenia, the strategy for empirical anti-fungal treatment was not described in detail [27], and further studies are needed to address this issue.

Some of the patients in our study died of infections despite seemingly adequate antibiotic treatment according to the resistance pattern of the microorganism in question. This raises the question of the role of critical host factors including pharmacogenetics and immunogenetics. Studies on genetic polymorphisms in immunoregulating mediators have shown an association with outcome during childhood leukaemia induction therapy [14], in childhood AML patients [38], in childhood malignancies in general [16] and in post-HSCT patients [39,40]. However, most studies are candidate gene based involving only a few genes, and genome-wide studies of variations within the immune response involving multiple genes and haplotypes are lacking. Identification of possible immunogenetic risk profiles at the start of therapy could potentially be helpful for risk adapted and individualised supportive care. Possible preventive measures for patients at significantly increased risk of infectious TRD could include: (i) reduced or modified anti-leukaemic therapy intensity, (ii) prophylactic antibiotic therapy, (iii) immunological reconstitution (e.g. immunoglobuline substitution) and (iv) granulocyte colony-stimulating factor therapy.

Subgroups of patients dying from specific organ toxicities (bleeding or thrombosis included) constitute a relatively small fraction of TRDs. The two deaths from asparaginase induced acute pancreatitis are rare events and most patients experiencing acute pancreatitis during treatment are successfully treated [41]. Another frequent complication from asparaginase treatment is thrombotic events occurring in up to 36% of patients [42]. In our study, the only patient possibly dying from an asparaginase related thrombotic event was a Down syndrome patient who died on treatment day 50 from a thrombosis in the right common carotid artery resulting in massive cerebral infarction. Of the four patients who died from bleeding complications (tumour burden associated bleedings excluded), three had accompanying severe thrombocytopenia (platelets $<20 \times 10^9$/L). Three of the patients also had an ongoing

infection, illustrating the additive risk of infection and coagulation disturbances. Very large, probably international, genetic studies such as those performed by the Ponte di Legno group (see Biondi et al. [43]) are needed to identify patients at excessive risk of very rare fatal events such as MTX-induced encephalopathy and liver failure.

In conclusion, TRD remains a major challenge and constitutes an increasing fraction of all deaths in childhood ALL. Accordingly, studies addressing the prevention of TRDs have become as important as efforts to overcome the resistance to the anti-leukaemic therapy.

ACKNOWLEDGMENTS

This study has received financial support from The Danish Childhood Cancer Foundation; Childrens Cancer Foundation, Department of Pediatrics, St. Olavs Hospital, Trondheim University Hospital; and The Swedish Childhood Cancer Foundation. Some results from this study (data from the NOPHO ALL-1992 protocol) have partly been published earlier [12]. Kjeld Schmiegelow holds the Danish Childhood Cancer Foundation professorship in Paediatric Oncology. *Authorship*: Bendik Lund has written the paper and, together with Kjeld Schmiegelow, designed the study which was based on the design of the study by Christensen et al. [12]. It has been written in close collaboration with the NOPHO Event group where Jukka Kanerva is the present chair. All co-authors have participated in the writing process and interpretation of the data. Mats Heyman has compiled and scrutinised the data in the NOPHO leukaemia registry. Ann Åsberg, Arja Harila-Saari, Henrik Hasle, Stefan Söderhäll (members of NOPHO Event-group) and Ólafur Gisli Jónsson provided clinical data through their roles as national coordinators for this study. Statistical analyses were performed by Bendik Lund and Stian Lydersen.

REFERENCES

1. Gustafsson G, Schmiegelow K, Forestier E, et al. Improving outcome through two decades in childhood ALL in the Nordic countries: The impact of high-dose methotrexate in the reduction of CNS irradiation. Nordic Society of Pediatric Haematology and Oncology (NOPHO). Leukemia 2000;14:2267–2275.
2. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med 2006;354:166–178.
3. Stanulla M, Schrappe M. Treatment of childhood acute lymphoblastic leukemia. Semin Hematol 2009;46:52–63.
4. Prucker C, Attarbaschi A, Peters C, et al. Induction death and treatment-related mortality in first remission of children with acute lymphoblastic leukemia: A population-based analysis of the Austrian Berlin-Frankfurt-Munster study group. Leukemia 2009;23:1264–1269.
5. Conter V, Arico M, Valsecchi MG, et al. Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) acute lymphoblastic leukemia studies, 1982–1995. Leukemia 2000;14:2196–2204.
6. Moricke A, Reiter A, Zimmermann M, et al. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: Treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood 2008;111:4477–4489.
7. Rubnitz JE, Lensing S, Zhou Y, et al. Death during induction therapy and first remission of acute leukemia in childhood: The St. Jude experience. Cancer 2004;101:1677–1684.
8. Vilmer E, Suciu S, Ferster A, et al. Long-term results of three randomized trials (58831, 58832, 58881) in childhood acute lymphoblastic leukemia: A CLCG-EORTC report. Children Leukemia Cooperative Group. Leukemia 2000;14:2257–2266.
9. Hargrave DR, Hann II, Richards SM, et al. Progressive reduction in treatment-related deaths in Medical Research Council childhood lymphoblastic leukaemia trials from 1980 to 1997 (UKALL VIII, X and XI). Br J Haematol 2001;112:293–299.
10. Moghrabi A, Levy DE, Asselin B, et al. Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia. Blood 2007;109:896–904.
11. Slats AM, Egeler RM, van der Does-van den Berg A, et al. Causes of death—other than progressive leukemia—in childhood acute lymphoblastic (ALL) and myeloid leukemia (AML): The Dutch Childhood Oncology Group experience. Leukemia 2005;19:537–544.
12. Christensen MS, Heyman M, Mottonen M, et al. Treatment-related death in childhood acute lymphoblastic leukaemia in the Nordic countries: 1992–2001. Br J Haematol 2005;131:50–58.
13. Wheeler K, Chessells JM, Bailey CC, et al. Treatment related deaths during induction and in first remission in acute lymphoblastic leukaemia: MRC UKALL X. Arch Dis Child 1996;74:101–107.
14. Kidas E, Moricke A, Beier R, et al. Genetic polymorphisms of the lymphotoxin alpha gene are associated with increased risk for lethal infections during induction therapy for childhood acute leukemia: A case-control study. Int J Hematol 2009;89:584–591.
15. Davidsen ML, Dalhoff K, Schmiegelow K. Pharmacogenetics influence treatment efficacy in childhood acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2008;30:831–849.
16. Neth O, Hann I, Turner MW, et al. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: A prospective study. Lancet 2001;358:614–618.
17. Schmiegelow K, Forestier E, Hellebostad M, et al. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia 2010;24:345–354.
18. Schmiegelow K, Al-Modhwahi I, Andersen MK, et al. Methotrexate/6-mercaptopurine maintenance therapy influences the risk of a second malignant neoplasm after childhood acute lymphoblastic leukemia: Results from the NOPHO ALL-92 study. Blood 2009;113:6077–6084.
19. Wollner N, Exelby PR, Lieberman PH. Non-Hodgkin's lymphoma in children: A progress report on the original patients treated with the LSA2-L2 protocol. Cancer 1979;44:1990–1999.
20. Hill FG, Richards S, Gibson B, et al. Successful treatment without cranial radiotherapy of children receiving intensified chemotherapy for acute lymphoblastic leukaemia: Results of the risk-stratified randomized central nervous system treatment trial MRC UKALL XI (ISRC TN 16757172). Br J Haematol 2004;124:33–46.
21. Pui CH, Mahmoud HH, Wiley JM, et al. Recombinant urate oxidase for the prophylaxis or treatment of hyperuricemia in patients With leukemia or lymphoma. J Clin Oncol 2001;19:697–704.
22. Coiffier B, Altman A, Pui CH, et al. Guidelines for the management of pediatric and adult tumor lysis syndrome: An evidence-based review. J Clin Oncol 2008;26:2767–2778.
23. Pui CH. Childhood leukemias. New York: Cambridge University Press; 2006.
24. Hann I, Viscoli C, Paesmans M, et al. A comparison of outcome from febrile neutropenic episodes in children compared with adults: Results from four EORTC studies. International Antimicrobial Therapy Cooperative Group (IATCG) of the European Organization for Research and Treatment of Cancer (EORTC). Br J Haematol 1997;99:580–588.
25. Eyrich M, Wiegering V, Lim A, et al. Immune function in children under chemotherapy for standard risk acute lymphoblastic

leukaemia—A prospective study of 20 paediatric patients. Br J Haematol 2009;147:360–370.
26. Conter V, Arico M, Valsecchi MG, et al. Intensive BFM chemotherapy for childhood ALL: Interim analysis of the AIEOP-ALL 91 study. Associazione Italiana Ematologia Oncologia Pediatrica. Haematologica 1998;83:791–799.
27. Phillips B, Selwood K, Lane SM, et al. Variation in policies for the management of febrile neutropenia in United Kingdom Children's Cancer Study Group centres. Arch Dis Child 2007;92:495–498.
28. Hilf M, Yu VL, Sharp J, et al. Antibiotic therapy for Pseudomonas aeruginosa bacteremia: Outcome correlations in a prospective study of 200 patients. Am J Med 1989;87:540–546.
29. Micek ST, Lloyd AE, Ritchie DJ, et al. Pseudomonas aeruginosa bloodstream infection: Importance of appropriate initial antimicrobial treatment. Antimicrob Agents Chemother 2005;49:1306–1311.
30. Goorin AM, Hershey BJ, Levin MJ, et al. Use of trimethoprim-sulfamethoxazole to prevent bacterial infections in children with acute lymphoblastic leukemia. Pediatr Infect Dis 1985;4:265–269.
31. Hughes WT, Rivera GK, Schell MJ, et al. Successful intermittent chemoprophylaxis for Pneumocystis carinii pneumonitis. N Engl J Med 1987;316:1627–1632.
32. Schroder H, Agger KE, Rosthoj S, et al. Antibacterial prophylaxis with trimethoprim-sulfamethoxazole during induction treatment for acute lymphoblastic leukemia. Dan Med Bull 2001;48:275–277.
33. van de Wetering MD, de Witte MA, Kremer LC, et al. Efficacy of oral prophylactic antibiotics in neutropenic afebrile oncology patients: A systematic review of randomised controlled trials. Eur J Cancer 2005;41:1372–1382.
34. Takahashi N, Kameoka Y, Yamanaka Y, et al. Itraconazole oral solution enhanced vincristine neurotoxicity in five patients with malignant lymphoma. Intern Med 2008;47:651–653.
35. Venkatakrishnan K, von Moltke LL, Greenblatt DJ. Effects of the antifungal agents on oxidative drug metabolism: Clinical relevance. Clin Pharmacokinet 2000;38:111–180.
36. Leventakos K, Lewis RE, Kontoyiannis DP. Fungal infections in leukemia patients: How do we prevent and treat them? Clin Infect Dis 2010;50:405–415.
37. Marr KA, Carter RA, Crippa F, et al. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis 2002;34:909–917.
38. Lehrnbecher T, Bernig T, Hanisch M, et al. Common genetic variants in the interleukin-6 and chitotriosidase genes are associated with the risk for serious infection in children undergoing therapy for acute myeloid leukemia. Leukemia 2005;19:1745–1750.
39. Middleton PG, Cullup H, Dickinson AM, et al. Vitamin D receptor gene polymorphism associates with graft-versus-host disease and survival in HLA-matched sibling allogeneic bone marrow transplantation. Bone Marrow Transplant 2002;30:223–228.
40. Shamim Z, Ryder LP, Heilmann C, et al. Genetic polymorphisms in the genes encoding human interleukin-7 receptor-alpha: Prognostic significance in allogeneic stem cell transplantation. Bone Marrow Transplant 2006;37:485–491.
41. Kearney SL, Dahlberg SE, Levy DE, et al. Clinical course and outcome in children with acute lymphoblastic leukemia and asparaginase-associated pancreatitis. Pediatr Blood Cancer 2009;53:162–167.
42. Mitchell LG, Andrew M, Hanna K, et al. A prospective cohort study determining the prevalence of thrombotic events in children with acute lymphoblastic leukemia and a central venous line who are treated with L-asparaginase: Results of the Prophylactic Antithrombin Replacement in Kids with Acute Lymphoblastic Leukemia Treated with Asparaginase (PARKAA) Study. Cancer 2003;97:508–516.
43. Biondi A, Baruchel A, Hunger S, et al. The Eleventh International Childhood Acute Lymphoblastic Leukemia Workshop Report: Ponte di Legno, Italy, 6–7 May 2009. Leukemia 2009.