Risk Factors for Treatment Related Mortality in Childhood Acute Lymphoblastic Leukaemia

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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 ± 1.1% and an overall survival of 84.7 ± 0.9% [4]. For the NOPHO ALL-2000 protocol, the 5-year EFS was 79.4 ± 1.5% and the overall survival was 89.1 ± 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-leukemic 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(+)-positive acute lymphoblastic leukaemia (Ph(+)-ALL; fusion) ALL, 14 patients who were...
<table>
<thead>
<tr>
<th>Study group</th>
<th>Study (years included)</th>
<th>No. of patients (range)</th>
<th>Total no of TRDs (%)</th>
<th>Pre-treatment deaths (%)</th>
<th>Induction deaths (%)</th>
<th>Death in CR1 (%)</th>
<th>Death post-HSCT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>1992–2008 1–15 2,735</td>
<td>88 (3.2)</td>
<td>5 (0.2)</td>
<td>34 (1.2)</td>
<td>49 (1.8)</td>
<td>10 (0.4)</td>
<td></td>
</tr>
<tr>
<td>Moricke et al. [6], BFM-95</td>
<td>1995–2000 0–18</td>
<td>76 (4.4)</td>
<td>14 (0.8)</td>
<td>7 (0.4)</td>
<td>67 (4.0)</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Vilmer et al. [8], CLCG-EORTC 58881</td>
<td>1989–1998 0–18 2,065</td>
<td>76 (3.7)</td>
<td>10 (0.5)</td>
<td>9 (0.4)</td>
<td>57 (2.8)</td>
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<td></td>
</tr>
<tr>
<td>Conter et al. [5], AIEOP-91</td>
<td>1991–1995 0–15 1,194</td>
<td>34 (2.8)</td>
<td>Not reported</td>
<td>14 (1.2)</td>
<td>16 (1.4)</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Rubnitz et al. [7], SJCRH</td>
<td>1984–1999 0–18 1,011</td>
<td>34 (3.3)</td>
<td>Not reported</td>
<td>14 (1.4)</td>
<td>16 (1.6)</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Slats et al. [11], DCOG</td>
<td>1996–2000 0–18 491</td>
<td>7 (1.4)</td>
<td>Not reported</td>
<td>4 (0.8)</td>
<td>3 (0.6)</td>
<td>Not reported</td>
<td></td>
</tr>
</tbody>
</table>

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.

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 $\geq 10$ years, t(9;22)(q34;q11)/BCR-ABL fusion, a karyotype with a modal chromosome number $<43$, and 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/m²/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

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diagnosis to decrease the risk of tumour lysis syndrome (TLS). Vin-
cristine (Vcr) was given weekly for 6 weeks in doses of 2.0 mg/m²; the maximum dose was increased from 2.0 mg in the ALL-92 pro-
tocol to 2.5 mg in the ALL-2000 protocol. In the ALL-92 protocol doxorubicin at doses of 40 mg/m² 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. For the ALL-92 patients Erwinia asparaginase was used (dose: 30,000 IU/m² daily on days 37–46), and for the
ALL-2000 patients Escherichia coli asparaginase was used (dose: 6,500 IU/m² 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/m²/day) and alternating blocks with high-dose MTX
(5 g/m²/24 hr with i.t. MTX and Leucovorin rescue) and low-dose
cytarabine (75 mg/m²/day for 4 days, two times). After induc-
tion, early intensification therapy followed for the high-risk groups,
which included two doses of cyclophosphamide (1,000 mg/m²) 4
weeks apart with low-dose cytaraibine (75 mg/m² 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/m²/24 hr with i.t. MTX and Leucov-
orin rescue) and high-dose cytarabine (12 g/m²) 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 pro-
tocol, 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 dexametha-
sone (doses: IR patients 6 mg/m²/day, HR patients 10 mg/m²/day)
for 2 weeks, weekly Vcr (2.0 mg/m²) for 4 weeks, weekly doxoru-
bicin (HR) or daunorubicin (IR), at a dose of 30 mg/m²/day, three
(HR) or four (IR) times. In addition, E. coli asparaginase (dose:
6,500 IU/m²) was given four times followed by cyclophosphamide
(dose: 1,000 mg/m²), low-dose cytarabine and 6-TG. Maintenance
therapy consisted of weekly oral MTX (starting dose: 20 mg/m²)
and daily oral 6-MP, dose adjusted according to TPMT activ-
ity (starting doses, wild-type patients: 75 mg/m²/day, heterozygous
patients: 50 mg/m²/day, TPMT deficient patients: 5–10 mg/m²/day).
In addition, low-risk patients received alternate pulses at four-week intervals Vcr (2.0 mg/m², one dose)/dexamethasone (6 mg/m²/day
for 5 days) and high-dose MTX (5 g/m²/24 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/m²) and dexamethasone (6 mg/m²/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 dexam-
ethasone (dose: orally 6 mg/m²/day) and Vcr dose: i.v. 2.0 mg/m²,
max. dose 2.5 mg for 5 days. For HR patients who were not treated
with HSCT in CR1, two cycles of the LSA2L2 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
day of diagnosis 100 < 200 × 10⁹/L, and/or CNS leukaemia at diagnosis
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received cranial irradiation (24 or 18 Gy) depending on whether or not CNS leukemia 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 jirovecii (Pj), fungal infections or management of febrile neutropenia. The use of myeloid growth factors was optional after courses with high-dose cytarabine (2 g/m²).

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-leukemic 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 infections TRD) as only event, and the following covariates were included: sex, age (< or ≥ 10 years), WBC (< or ≥ 200 × 10⁹/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 ± 0.4%, 2.4 ± 0.5% and 6.7 ± 0.9% for the SR, IR and HR group, respectively (P < 0.005). 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 ± 0.5% and 3.2 ± 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 ≥ 350 × 10⁹/L at diagnosis. ALL, except one patient, died from intracerebral infiltration of leukemic blast cells with or without intracerebral bleeding. One patient with a large mediastinal tumour (WBC at diagnosis: 23 × 10⁹/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-leukemic 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 30 post-HSCT patients) giving a proportion of death in CR1 of 1.8% (3.3% in the low-risk groups and 3.2% in the high-risk group, P = 0.003). 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-L2 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-
Treatment Related Death in ALL

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 polymicrobial or polybacterial, and in 16 cases no microorganism was found. Chemotherapy induced neutropenia (defined as neutrophils ≤ 0.5 × 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 ≥ 200 × 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 ≥ 200 × 10^9/L at diagnosis, T-cell disease, Down syndrome, and HSCT in CR1 were identified as independent risk factors for TRDs.

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

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>TRD (n = 88)</th>
<th>All patients (n = 2735)</th>
<th>HR (95% CI), simple regression</th>
<th>Adjusted HR (95% CI), multiple regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>51</td>
<td>1,231</td>
<td>1.7 (1.1–2.6)</td>
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</tr>
<tr>
<td>Male</td>
<td>37</td>
<td>1,504</td>
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<td>1.0</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>1–9</td>
<td>71</td>
<td>2,274</td>
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<td>1.0</td>
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<tr>
<td>10–14</td>
<td>17</td>
<td>461</td>
<td>1.2 (0.7–2.1)</td>
<td>0.9 (0.51–1.5)</td>
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<td>WBC (&lt; 10^9/L)</td>
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<td>&lt;200</td>
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<tr>
<td>≥200</td>
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<td>132</td>
<td>6.5 (3.9–10.8)</td>
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<td>Immunophenotype</td>
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<td>T-cell</td>
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<td>Protocol</td>
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<tr>
<td>1992</td>
<td>55</td>
<td>1,645</td>
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<tr>
<td>2000</td>
<td>33</td>
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<td>Down syndrome</td>
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<td>Yes</td>
<td>9</td>
<td>59</td>
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<td>CNS</td>
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<td>68</td>
<td>3.8 (1.7–8.2)</td>
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<td>81</td>
<td>2,667</td>
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<td>HSCT in CR1</td>
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<td>Yes</td>
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<td>109</td>
<td>17.7 (8.1–38.6)</td>
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<tr>
<td>No</td>
<td>78</td>
<td>2,626</td>
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</table>

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

<table>
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<th>Major group</th>
<th>Organism</th>
<th>ALL-1992</th>
<th>ALL-2000</th>
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</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Coagulase negative staphylococcus</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bacillus cereus*</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa†</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Eschericia coli</td>
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<td>3</td>
</tr>
<tr>
<td></td>
<td>Klebsiella</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Listeria*</td>
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<td>1</td>
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<tr>
<td></td>
<td>Stenotrophomonas (Xanthomonas) maltophilia</td>
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<td></td>
<td>Enterobacter</td>
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<tr>
<td></td>
<td>Stomatococcus</td>
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<tr>
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<td>Micrococcus</td>
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<tr>
<td></td>
<td>Unspecified</td>
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<td>2</td>
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<tr>
<td>Virus</td>
<td>Cytomegalovirus**</td>
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<td>2</td>
</tr>
<tr>
<td></td>
<td>Adenovirus</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Influenza B</td>
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<td>1</td>
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<tr>
<td></td>
<td>Respiratory syncytial virus</td>
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<td>1</td>
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<tr>
<td>Fungi</td>
<td>Candida*</td>
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<tr>
<td></td>
<td>Geotrichium capitatum</td>
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<tr>
<td></td>
<td>Pneumocystis jiroveci</td>
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<tr>
<td></td>
<td>Unspecified†</td>
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<td>1</td>
</tr>
<tr>
<td>Polybacterial or microbial**</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Polybacterial or microbial**††</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

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). *Central nervous system infections (n = 2).

**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 closely 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 treat-
ment with reconstituting 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]. Rasbur-
case 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 admin-
istration 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 distur-
bances, for example hyperphosphatemia, these should be corrected 
before start of anti-leukaemic therapy.

The treatment-induced immunosuppression includes neutrope-
ia, 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 dom-
inating lymphocyte subset during maintenance therapy. We have 
shown that infections remain the most common cause of TRD com-
prising 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 
to due 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 neutrope-
ia varies widely between treating centres even within the same 
collaborative group. In a British survey of 21 United Kingdom Chil-
dren’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 Pseu-
domonas 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 neu-
 tropenia, but no data on routine supportive care have been registered 
as part of this study.

There was no uniform approach to PI infection prophylaxis dur-
ing 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 PI 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 induc-
tion 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 
alveolar oncology patients (including 22 clinical trials) van de Weter-
ing et al. [33] concluded that oral prophylactic antibiotics decreased 
Gram-negative bacteraemia and infection related mortality. How-
ever, 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 neurotox-
icity. Vcr is metabolised by CYPS1A enzymes and azoles are potent 
inhibitors of CYPS1A isoenzymes resulting in higher Vcr concentra-
tions [34,35]. Fluconazole is a relatively weaker inhibitor of CYPS1A 
compared to other azoles (e.g. itraconazole) [35] leaving flucona-
azole 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 immunoreg-
ulating 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 vari-
ations 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 infections 
TRD could include: (i) reduced or modified anti-leukaemic therapy 
intensity, (ii) prophylactic antibiotic therapy, (iii) immunological 
reconstitution (e.g. immunoglobulin substitution) and (iv) granu-
locyte colony-stimulating factor therapy.

Subgroups of patients dying from specific organ toxicities 
(bleeding or thrombosis included) constitute a relatively small frac-
tion 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 
common 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 throm-
botic 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 bleed-
ings excluded), three had accompanying severe thrombocytopaenia 
(platelets <20 x 10^9/L). Three of the patients also had an ongoing

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infection, illustrating the additive risk of infection and coagula-
tion 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. Accord-
ingly, studies addressing the prevention of TRDs have become as important as efforts to overcome the resistance to the anti-leukaemic therapy.

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atric 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 reg-
istry. Ann Asberg, Arja Harila-Saari, Henrik Hasle, Stefan Söderhäll (members of NOPHO Event-group) and Olafur Gisli Jonsson provided clinical data through their roles as national coordinators for this study. Statistical analyses were performed by Bendik Lund and Stian Lydersen.

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