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Cure rates of childhood acute lymphoblastic leukemia in Lithuania and the benefit of joining international treatment protocol

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ABSTRACT

Background: Childhood acute lymphoblastic leukemia (ALL) represents the largest group of pediatric malignancies with long-term survival rates of more than 80% achieved in developed countries. Epidemiological data and survival rates of childhood ALL in Lithuania were lacking. Therefore, the aim of this study was to analyze the population-based long-term treatment results of childhood ALL in Lithuania during 1992–2012.

Materials and methods: Data of all 459 children with T-lineage and B-cell precursor ALL treated in Lithuania from 1992 to 2012 were collected and analyzed. Results were compared among four time-periods: 1992–1996 ($N = 132$), 1997–2002 ($N = 136$), 2003–2008 ($N = 109$) and 2009–2012 ($N = 82$).

Results: The incidence of childhood ALL in Lithuania was 3.2–3.6 cases per 100 000 children per year during the study period. Five-year probability of event-free survival increased from 50% ± 4% in 1992–1996 to 71% ± 4% in 2003–2008 ($P < 0.001$). Five-year cumulative incidence of relapses reduced from 27% ± 4.5% in 1992–1996 to 14% ± 3.6% in 2003–2008 ($P = 0.042$). After introduction of high-dose methotrexate of 5 g/m^2, cumulative incidence of CNS-involving relapses reduced from 17% ± 3.9% in 1992–1996 to 1% ± 1.0% in 2003–2008 ($P < 0.001$). Trend for further improvement in survival was seen in 2009–2012 when Lithuania joined international the Nordic Society of Pediatric Hematology and Oncology (NOPHO) ALL-2008 treatment protocol.

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Conclusions: Cure rates of childhood ALL in Lithuania are improving steadily and are now approaching those reported by the largest international study groups. The reasons for such a positive effect are both better financial support for treatment of children with cancer in Lithuania and international collaboration with joining international treatment protocol for childhood ALL.

1. Introduction

Childhood acute lymphoblastic leukemia (ALL) accounts for 20–30% of all pediatric malignancies depending on age [1,2]. Based on risk-adapted antileukemic therapy and improved supportive care, long-term survival rates of 85–90% could be achieved by the best contemporary treatment protocols of childhood ALL which was universally fatal disease in the early 1960s. The Children Oncology Group has recently reported the 88–95% 5-year event-free survival for low risk ALL patients diagnosed in the period 2006–2009 [3]. This development reflects international collaboration of large pediatric oncology groups, inclusion of patients in clinical trials, and systematic reporting of trials results [4–8]. It has been already shown that clinical trials in children with cancer result in significant improvement in their cure rates [9,10]. However, few data on population-based long-term treatment results of childhood ALL with or without international collaboration have been published by pediatric oncology groups from Central or Eastern European countries [11–13]. Treatment results of 208 children with ALL treated in Lithuania from 1986 to 1994 were described in 1995 [14,15].

Due to historical reasons pediatric oncologists in Lithuania did not join international treatment protocols until recently, and because of a population of three million inhabitants conduction of national clinical trials was not possible. Children with cancer in Lithuania were treated according to protocols developed by international study groups that were published or obtained by direct personal contacts to study chairs. Children with ALL from late 1970s to 2008 were treated according to Berlin–Münster–Frankfurt (BFM)-based protocols. However, patients were neither prospectively registered nor enrolled into clinical trials.

In recent decade collaboration developed between Lithuanian pediatric oncologists and Nordic Society of Pediatric Hematology and Oncology (NOPHO). In April 2009, Lithuania joined the international NOPHO ALL-2008 treatment protocol. Here we present the population-based long-term treatment results of childhood ALL in Lithuania in 1992–2012 and discuss the benefits of international collaboration and joining the international treatment protocol.

2. Patients and methods

2.1. Patients

From January 1992 to December 2012, 459 patients were diagnosed with ALL at the Center for Oncology and Hematology, Children's Hospital, Affiliate of Vilnius University Hospital Santariškių Klinikos in which all children with ALL in Lithuania were treated. All children with T-lineage ALL (T-ALL) or B-cell precursor ALL (BCP) aged up to 16 years until January 2003 and subsequently aged up to 18 years (reflecting the age limit for patients in pediatric departments in Lithuania), including six patients with Down syndrome, were included into this population-based study. To evaluate trends in treatment results, the study period was divided into four periods according to the treatment and risk-stratification strategies used: 1992–1996 ($N = 132$), 1997–2002 ($N = 136$), 2003–2008 ($N = 109$) and 2009–2012 ($N = 82$). The 1992–1996 period was excluded from survival analysis for patients with different immunophenotype, since few patients had their immunophenotype determined in 1992–1996 (BCP, $N = 1$ or T-ALL, $N = 3$). For each patient, informed consent to antileukemic therapy was obtained from parents or guardians according to the Declaration of Helsinki. National ethics committee approved the study.

After joining the NOPHO ALL-2008 treatment protocol (EudraCT number: 2008-003235-20) in April 2009, clinical, laboratory and toxicity data of children with ALL started to be registered prospectively into the NOPHO Leukemia Register. For the current study, data were retrieved retrospectively from paper files of the patients diagnosed prior to April 2009 or from the NOPHO ALL-2008 Register for subsequently diagnosed patients. Current status of each patient was checked before including into the study.

Median (75% range) follow-up of 314 patients remaining alive on April 1, 2013, was 10.9 (2.5–19.0) years. Of all 459 patients, 162 patients had a follow-up of ≥10.0 years. Four patients developed a second cancer after a median of 5.9 years from diagnosis of ALL.

2.2. Diagnostic methods

Diagnosis of ALL was established if ≥25% of cells were identified as leukemic blasts in a diagnostic bone marrow. Romanowsky–Giemsa and peroxidase staining methods were used for cytomorphological evaluation of smears until the year 2001. Since 2001 routine immunophenotyping with panels of monoclonal antibodies directed toward lineage-associated antigens according to well established criteria [16] was introduced. G-band karyotyping became routine in 2007. Since 2009 directed analysis by fluorescence in situ hybridization (FISH) and/or reverse transcriptase PCR for translocations t (1;19)(q34;q11)*[BCR-ABL]* or t(1;19)(q23;p13)*[E2A-PBX1]*, and for ic21amp or 11q23/*MLL* aberrations as well as DNA-index by flow cytometry became mandatory as risk stratifying factors in the NOPHO ALL-2008 protocol. Furthermore, since 2009 all

leukemic samples have been also explored for t(12;21)[*ETV6-RUNX1*] translocation. However, the presence of this translocation did not influence treatment stratification except that patients were excluded from dexamethasone induction therapy if white blood cell count (WBC) at diagnosis was at least 100×10^9/L.

In addition, genotyping of thyopurinmethyltransferase by low activity alleles was introduced since joining the NOPHO ALL-2008 protocol as a mandatory test for individual 6-mercaptopurine (6-MP) dose adjustment during consolidation and maintenance therapy [17].

Central nervous system (CNS) disease was defined by presence of leukemic blasts in diagnostic spinal tap and/or peripheral cranial nerve palsy, and/or intracranial leukemic infiltrates detected by imaging methods. Since 2009, CNS1 was defined as no blasts on cytospin, CNS2 as ≥1 and <5 cells per μl with blasts on cytospin, and CNS3 as ≥5 cells per μl with blasts on cytospin of diagnostic spinal tap.

2.3. Response to therapy

In BFM-based era (1992–2008), prednisolone good response or prednisolone poor response was documented when <1000/μL or ≥1000/μL blasts, respectively, were found in peripheral blood after seven days of a prephase with prednisolone and intrathecal (i.th.) methotrexate (MTX). Bone marrow response was evaluated at the end of induction therapy on day 33. In the NOPHO ALL-2008, no prednisolone prephase was given, and bone marrow response was evaluated on day 29.

Complete remission required <5% blasts as detected by cytomorphology by the year 2009, and by molecular methods subsequently. Measurement of minimal residual disease (MRD) by flow cytometry was introduced in routine practice in 2006, while PCR based techniques for immunoglobulin and T-cell receptor (TCR) gene rearrangements were introduced in 2010 following requirements in the NOPHO ALL-2008 protocol for T-ALL MRD measurement [4].

2.4. Risk grouping

In 1992–1996, risk group assignment was based on the presence of hepatosplenomegaly, CNS involvement, response to prednisolone prephase and response to induction therapy (standard risk, SR: no hepatosplenomegaly, no CNS involvement, and no high risk criteria; intermediate risk, IR: hepatosplenomegaly and/or CNS involvement, and no high risk criteria; high risk, HR: prednisolone poor response and/or ≥5% blasts in bone marrow at the end of induction (day 33)).

In 1997–2002, same criteria were used, except SR patients had to be 2.0–11.9 years of age and to have WBC≤20×10^9/L. Since 2001 after immunophenotyping was implemented, patients with T-ALL were excluded from SR.

In 2003–2008, MRD measurement by flow cytometry was implemented for risk stratification: SR if MRD_{d33} negative; IR if MRD_{d33} positive, but $MRD_{d79} < 10^{-3}$; and HR if $MRD_{d79} \geq 10^{-3}$. Other risk criteria remained the same as in previous periods.

In 2009–2012, NOPHO ALL-2008 protocol stratifying criteria were implemented. They had been in detailed described previously [4,18]. Therapeutic risk group assignment was based on WBC< or ≥100×10^9/L, immunophenotype (BCP vs T-ALL), cytogenetics and MRD at days 15, 29 and 79 (SR: WBC < 100×10^9/L, BCP phenotype, day 29 MRD < 10^{-3}, and no IR or HR cytogenetics; IR: (i) BCP with WBC < 100×10^9/L and $MRD_{d29} \geq 10^{-3}$, but <5% or (ii) BCP with WBC ≥ 100×10^9/L or T-ALL and <25% blasts in bone marrow on day 15 and $MRD_{d29} < 10^{-3}$, and no HR cytogenetics; HR: (i) any patient with day 29 ≥5% blasts in bone marrow or (ii) BCP with WBC ≥ 100×10^9/L or T-ALL and day 15 ≥25% blasts in bone marrow and/or $MRD_{d29} \geq 10^{-3}$ or (iii) presence of 11q23/*MLL* rearrangement or hypodiploidy (≤44 chromosomes) or DNA index <0.85, irrespectively of other factors. Patients having t(1;19), dic(9;20) or ic21 amp aberrations were assigned to IR treatment unless HR features were present.

2.5. Treatment

2.5.1. 1992–1996 Period

SR and IR groups. Induction/consolidation: seven days of prephase with prednisolone and i.th. MTX, followed by oral prednisolone 60 mg/m^2/d (21 days, then tapered); weekly vincristine (VCR) 1.5 mg/m^2 (max 2.0 mg) concomitantly with daunorubicin 30 mg/m^2/d i/v (8–29 d.); *E. coli* L-asparaginase 10 000 UI/m^2 i/v, 8 doses (d. 12–33). Cyclophosphamide 1000 mg/m^2 i/v (d. 36, 64); four blocks of four days of cytarabine 75 mg/m^2/d, subcutaneously (d. 38–62), and oral 6-mercaptopurine (6-MP) 60 mg/m^2/d (d. 36–63); i.th. MTX (d. 1, 15, 29, 45, 59). Extra i.th. MTX on d. 8 and 22 for patients with CNS involvement. Extra-myeloid compartment therapy: MTX 1.0 g/m^2/24 h infusion with concomitant i.th. MTX (d. 8, 22, 36, 50); oral 6-MP 25 mg/m^2/d (d. 1–57). Reintensification: same as induction/consolidation except that: (i) daunorubicin was replaced by doxorubicin; (ii) L-asparaginase only three doses given; (iii) cyclophosphamide was given once, on d. 36; (iv) cytarabine two blocks instead of four; (v) oral 6-thioguanine 60 mg/m^2/d instead of 6-MP (d. 36–49), and (vi) i.th. MTX on d. 38 and 45 only. Maintenance with daily oral 6-MP 50 mg/m^2/d, and oral MTX 20 mg/m^2/dose, once per week with doses adjusted according to peripheral blood counts up to two years after diagnosis. IR patients >1.0 year received cranial irradiation 12 Gy before maintenance.

HR patients started block therapy after day 33 of induction. Block HR-1: oral dexamethasone 20 mg/m^2/d (d. 1–5); oral 6-MP 100 mg/m^2/d (d. 1–5); VCR 1.5 mg/m^2/d (max 2.0 mg) (d. 1, 6); MTX 1.0 g/m^2/24 h infusion (d. 1); cytarabine 2.0 g/m^2/dose, ×2 (d. 5); L-asparaginase 25 000 IU/m^2 (d. 6); i.th. triple therapy: cytarabine, prednisolone and MTX (TIT) (d. 1). Block HR-2: oral dexamethasone 20 mg/m^2/d (d. 1–5); oral 6-thioguanine 100 mg/m^2/d (d. 1–5); vindesine 3.0 mg/m^2/d (max 5.0 mg) (d. 1); MTX 1.0 g/m^2/24 h infusion (d. 1); daunorubicine 50 mg/m^2/d (d. 5); ifosfamide 400 mg/m^2/d i/v infusion (d. 1–5); L-asparaginase 25 000 IU/m^2 (d. 6); i.th. TIT (d. 1). Block HR-3: oral dexamethasone 20 mg/m^2/d (d. 1–5); cytarabine 2.0 g/m^2/dose, ×2 (d. 1, 2); vepeside 150 mg/m^2/dose (d. 1, 3, 5); L-asparaginase 25 000 IU/m^2 (d. 6); TIT (d. 1). Blocks were consequently repeated three times making nine HR blocks altogether. Maintenance same as for SR and IR. Cranial irradiation: for ≥1.0 year old patients 12 Gy after the 3rd HR-3 block.

For ≥1.0 year patients with initial CNS involvement cranial irradiation was given dependent on age: 1 – <2.0 y. 18 Gy, and ≥2.0 y. 24 Gy.

2.5.2. 1997–2002 Period

Same treatment as in previous period except that: (i) L-asparaginase dose was reduced to 5000 UI/m^2; (ii) HR blocks were reduced from nine to six blocks; (iii) cranial irradiation for patients with initial CNS involvement was reduced to: <2.0 y. 12 Gy, and ≥2.0 y. 18 Gy.

2.5.3. 2003–2008 Period

Same treatment as in 1997–2002, except that: (i) dose of HD MTX was increased to 5.0 g/m^2/24 h; (ii) in reintensification doxorubicin was replaced by daunorubicin; and (iii) prophylactic cranial irradiation restricted for T-ALL only.

2.5.4. 2009–2012 Period

Since April 2009, all BCP and T-ALL patients were enrolled to prospective NOPHO ALL-2008 protocol which had been described in detailed previously [4,18]. Main differences in therapy compared with previous periods were: (i) cranial radiotherapy was omitted for all patients; (ii) L-asparaginase, i.v. was replaced by pegylated asparaginase (Oncaspar®), i.m.; (iii) treatment with L-asparaginase was delayed until consolidation phase (starting from d. 29); (iv) anthracyclines in induction were reduced to two doses of 40 mg/m^2; (v) individual starting doses of 6-MP dependent on genotype of enzyme thiopurine methyltransferase; (vi) treatment duration was extended up to 2.5 years from diagnosis.

2.6. Hematopoietic stem cell transplantation (HSCT)

Allogeneic hematopoietic stem cell transplantation (HSCT) for children in Lithuania became available in 2002. No strict criteria for HSCT existed before NOPHO-era. In the NOPHO ALL-2008 protocol, recommendations for HSCT were based on treatment response criteria only. HSCT was indicated if: (i) BCP patients with WBC < 100×10^9/L did not reach remission on day 29 (≥5% blasts in BM) or $MRD_{d79} \geq 10^{-3}$; (ii) T-ALL and BCP patients with WBC ≥ 100 had ≥25% blasts on day 15 and ≥5% after block A1, or ≥5% on day 29, or after block B1 MRD ≥ 10^{-3}.

Overall, 20 patients received allogeneic HSCT during the study period in Lithuania in CR1 ($N = 8$) or ≥CR2 ($N = 12$). One patient is alive from the latter group while six patients out of eight who were transplanted in CR1, are alive without disease with a median (range) follow-up of 2.0 (0.2–9.0) years.

2.7. Statistical analysis

The Kruskal–Wallis test was used for comparison of continuous variables, and the chi-square test was used for comparison of categorized variables. Kaplan–Meyer method was used for determination of projected event-free survival (pEFS) and overall survival (pOS). pEFS was calculated from diagnosis to the date of assessment of induction failure, death in first complete remission (DCR1), relapse or the development of a second malignancy (whichever occurred first), or the last known follow-up date for event-free survivors. pOS was calculated from diagnosis to death from any cause. To estimate the cumulative incidences of relapse, induction failure or DCR1, all these events were considered as competing events [19]. Backward stepwise Cox regression analysis was performed to identify independent prognostic factors for differences in outcome. Two-sided P values of <0.05 were regarded as significant. Data were analyzed using statistical package for social sciences (SPSS) software for Windows, version 18.0 (SPSS, Chicago, IL, USA).

3. Results

Characteristics of all 459 study patients are presented in Table 1. Incidence of childhood ALL in Lithuania was 3.2–3.6 cases per 100 000 of children per year during the study period with the dominance of boys (56%) vs. girls (44%). Age (median (75% range): 5.3 (2.3–12.7) years), WBC (median (75% range): 9.2 (2.6–46.4) × 10^9/L for 211 BCP patients, and 90.7 (12.5–382.5) × 10^9/L for 55 T-ALL patients, respectively), and distribution of immunophenotype and cytogenetic aberrations, when the latter two characteristics were available, were in consistence with the findings of other childhood ALL study groups [20–22].

Progressive improvement in pEFS and pOS was observed over time (Table 1, Fig. 1A and B). The 5-year pEFS improved from 50% ± 4% in 1992–1996 to 71% ± 4% in 2003–2008 (pooled $P < 0.001$), and the 5-year pOS improved from 57% ± 4% to 78% ± 4%, respectively (pooled $P < 0.001$). There was a trend for further survival improvement in 2009–2012, however, follow-up time for these patients was relatively short (Table 1, Fig. 1A and B).

Advance was more prominent with a borderline significance for BCP patients with 5-year pEFS increasing from 68% ± 6% in 1997–2002 to 77% ± 4.5% in 2003–2008 ($P = 0.065$), with a trend for further improvement in 2009–2012 (pooled $P = 0.043$) (Fig. 2A). In contrast, survival rates were less prominent for T-ALL (Fig. 2B). However, number of T-ALL patients was small ($N = 17$, 20 and 15 in 1997–2002, 2003–2008 and 2009–2012, respectively). Out of four events for T-ALL patients in the recent period, two deaths appeared for HR patients due to septic complications during block therapy induced myelosuppression ($N = 1$) or cytomegalovirus pneumonia during maintenance ($N = 1$). The rest two events were relapses which are described below.

Five-year cumulative incidence of relapses reduced from 27% ± 4.5% in 1992–1996 to 14% ± 3.6% in 2003–2008 ($P = 0.042$) (Fig. 1A). In 2009–2012, of the 82 patients, four developed early relapses so far (5%) after 0.6–1.4 years from diagnosis. One of them (IR, BCP) developed an isolated bone marrow relapse after parents abandoned the treatment. Another patient (IR, T-ALL) developed an isolated CNS relapse. The remaining two isolated bone marrow relapses occurred for HR ALL patients (T-ALL with hyperleukocytosis and BCP with *MLL*$^-$ rearrangement).

Incidence of CNS disease at diagnosis remained stable during the study period (Table 1), while the 5-year cumulative incidence of CNS involving relapses decreased from 17% ± 3.9% in 1992–1996 to 9% ± 2.9% in 1997–2002 ($P = 0.077$), and after high dose MTX of 5 g/m^2 was introduced in 2003–2008, it decreased further down to 1% ± 1.0% ($P < 0.001$) (Fig. 1B). Importantly, cumulative incidence of CNS involving relapses did not increase in 2009–2012 period (3-year cumulative incidence 2%) after cranial irradiation was omitted for all patients. However, follow-up is short for the recent period.

In contrast, cumulative incidence of induction failure and DCR1 remained high, albeit decreasing, all over the study

Table 1 – Baseline characteristics and treatment results of the patients treated in different time periods.

	1992–1996, N = 132	1997–2002, N = 136	2003–2008, N = 109	2009–2012, N = 82	P
FU, median (75% range), years for alive patients	19.0 (16.6–20.4), N = 71	12.7 (11.3–15.2), N = 89	7.5 (4.7–9.5), N = 84	2.2 (0.5–3.6), N = 70	
Boys, *n* (%)	82 (62)	77 (57)	50 (46)	47 (57)	0.086
Girls, *n* (%)	50 (38)	59 (43)	59 (54)	35 (43)	
WBC, median (75% range), $\times 10^9$/L	9.9 (2.3–56.0)	9.0 (2.8–43.8)	9.2 (2.3–58.4)	18.2 (3.9–101.3)	0.025
Age, median (75% range), years	4.9 (2.1–10.3)	5.3 (2.2–10.9)	6.3 (2.5–13.1)	5.2 (2.3–11.5)	0.14
CNS, *n* (%)					
CNS 1	95 (72)	125 (92)	100 (92)	78 (95)	0.90
CNS 2/3	7 (5)	8 (6)	8 (7)	4 (5)	
NA	30 (23)	3 (2)	2 (2)	0	
Immunophenotype, *n* (%)					
BCP	1 (1)	54 (40)	89 (82)	67 (82)	ND
T-ALL	4 (3)	17 (13)	20 (18)	15 (18)	
NA	127 (96)	65 (48)	0	0	
Cytogenetics, *n* (%)					
Normal karyotype	–	2 (1.5)	11 (10.1)	20 (24.4)	ND
HeH	–	–	19 (17.4)	11 (13.4)	
t(12;21)	–	–	2 (1.8)	19 (23.2)	
t(1;19)	–	–	–	6 (7.3)	
amp(21)	–	–	–	3 (3.7)	
11q23/*MLL*	–	–	–	1 (1.2)	
t(9;22)*[BRL/ABL]*	–	–	3 (2.8)	2 (2.4)	
Hypodiploid	–	–	–	1 (1.2)	
Other	–	–	11 (10.1)	20 (24.4)	
NA	132 (100)	134 (98.5)	63 (57.8)	–	
Primary events, *n* (%)					
IF	18 (14)	8 (6)	6 (6)	2 (2)	0.010
DCR1	20 (15)	13 (10)	11 (10)	7 (9)	0.037
Rel	28 (21)	31 (22)	15 (14)	4 (5)	0.003
CR1	65 (49)	82 (60)	76 (70)	69 (84)	<0.001
SMN	1 (0.8)	2 (1.5)	1 (0.9)	0	
Relapses, *n* (%)					
BM	9 (6.8)	18 (13.2)	13 (11.9)	3 (3.7)	ND
CNS	13 (9.8)	7 (5.1)	0	1 (1.2)	
BM + CNS	3 (2.3)	3 (2.2)	1 (0.9)	0	
Testis	1 (0.8)	2 (1.5)	1 (0.9)	0	
Other	0	1 (0.7)	0	0	
Event-free survival, mean ± SE					
3-year	0.55 ± 0.04	0.70 ± 0.04	0.76 ± 0.04	0.82 ± 0.05	<0.001
5-year	0.50 ± 0.04	0.63 ± 0.04	0.71 ± 0.04	–	
10-year	0.50 ± 0.04	0.61 ± 0.04	0.70 ± 0.04	–	
Overall survival, mean ± SE					
3-year	0.61 ± 0.04	0.73 ± 0.04	0.80 ± 0.04	0.83 ± 0.05	<0.001
5-year	0.57 ± 0.04	0.71 ± 0.04	0.78 ± 0.04	–	
10-year	0.54 ± 0.04	0.66 ± 0.04	0.76 ± 0.04	–	
WBC, mean ± SE, $\times 10^9$/L					
<10, 3-year pEFS	0.57 ± 0.07	0.79 ± 0.05	0.86 ± 0.05	0.89 ± 0.06	0.002
<10, 5-year pEFS	0.52 ± 0.07	0.70 ± 0.05	0.82 ± 0.05	–	
<10, 10-year pEFS	0.50 ± 0.07	0.68 ± 0.06	0.80 ± 0.05	–	
10–99.9, 3-year pEFS	0.58 ± 0.08	0.67 ± 0.07	0.76 ± 0.07	0.84 ± 0.06	0.18
10–99.9, 5-year pEFS	0.56 ± 0.08	0.62 ± 0.07	0.70 ± 0.08	–	
10–99.9, 10-year pEFS	0.56 ± 0.08	0.60 ± 0.07	0.70 ± 0.08	–	
≥100, 3-year pEFS	0.36 ± 0.13	0.27 ± 0.13	0.40 ± 0.13	0.67 ± 0.14	0.19
≥100, 5-year pEFS	0.36 ± 0.13	0.27 ± 0.13	0.33 ± 0.12	–	
≥100, 10-year pEFS	0.36 ± 0.13	0.27 ± 0.13	0.33 ± 0.12	–	

FU, follow-up period; WBC, white blood cell count in peripheral blood at diagnosis; CNS, central nervous system; BM, bone marrow; BCP, B-cell precursor ALL; T-ALL, T-lineage ALL; NA, not available; HeH, high hyperdiploid karyotype (modal chromosome number >50); hypodiploid karyotype, modal chromosome number <45; 11q23/*MLL*, 11q23/*MLL* rearrangement; other, non-stratifying cytogenetic aberrations; IF, induction failure; DCR1, death in first complete remission; Rel, relapse; SMN, second malignancy; EFS, event-free survival. *P* value, determined after comparison of the values among different time-periods; ND, not determined: values were not compared and *P* value was not determined for immunophenotype or cytogenetic aberrations due to a large number of not available results in early time-periods, as well as for relapses due to the different follow-up time for patients in different time-periods.

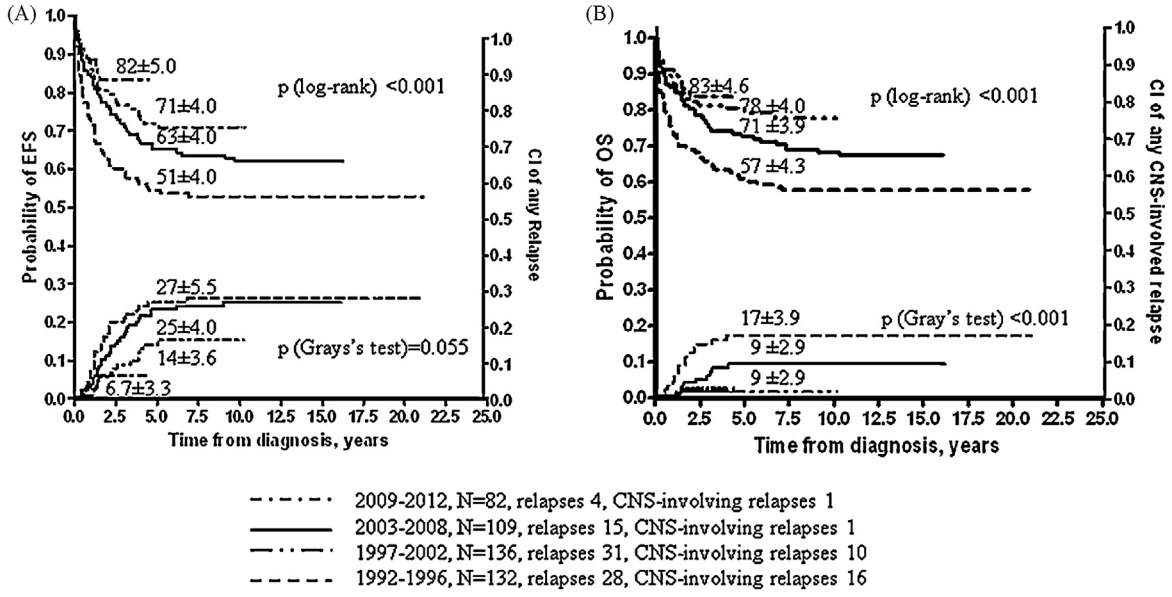

Fig. 1 – Probability of event-free survival and cumulative incidence of any relapse (A) and probability of overall survival and cumulative incidence of any central nervous system relapse (B) in four consecutive time periods.

period (Table 1). Induction failure was considered as death during induction ($N = 30$) or later if remission was not achieved ($N = 1$) or remission status in bone marrow was not evaluated ($N = 3$). Death was induced by infectious complications ($N = 22$), profuse bleeding ($N = 3$), hyperleukocytosis caused complications ($N = 3$), ALL progression ($N = 3$), or the exact cause was difficult to establish in this retrospective study ($N = 3$). Steady improvement in diagnostic work-up which in turn led to better risk classification, and intensification of initial treatment combined with improved supportive care contributed to reduction in cumulative incidence of induction failure from 14% ± 3.0% in 1992–1996 to 2% ± 1.7% in 2009–2012 ($P = 0.008$).

Cumulative incidence of DCR1 has not changed significantly over time ($P = 0.237$). The most common cause of DCR1 (61%) was treatment-related septic complications during myelosuppression ($N = 30$), followed by profuse bleeding ($N = 4$), high dose MTX induced gastroenteritis ($N = 2$), hemorrhagic pancreatitis ($N = 1$), or cerebral venous sinus thrombosis ($N = 1$). The exact reason was difficult to identify with certainty for 11 patients.

Univariate Cox regression analysis revealed WBC at diagnosis, CNS involvement at presentation and two earliest time-periods to have a significant prognostic impact for event (Table 2). However, after including these factors into

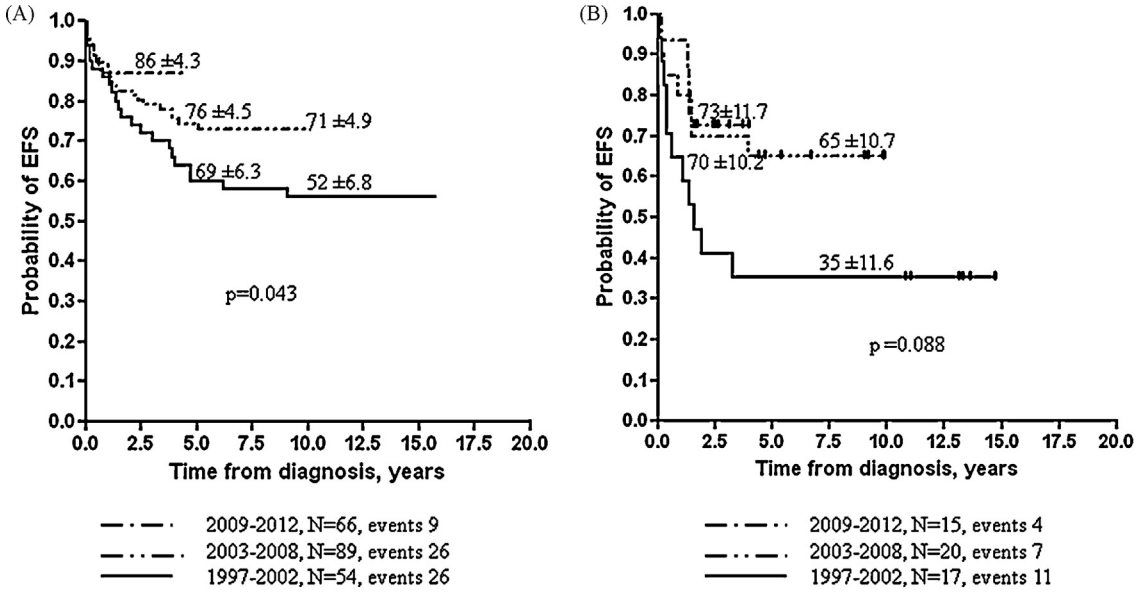

Fig. 2 – Probability of event-free survival for B-cell precursor ALL patients (A) and probability of event-free survival for T-lineage ALL patients (B) in three consecutive time periods.

Table 2 – Results of univariate and multivariate Cox regression analyses to evaluate the risk of different factors for development of an event.

Analysis	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
WBC	1.003 (1.002–1.004)	<0.001*	1.003 (1.002–1.004)	<0.001
Age	1.007 (0.97–1.04)	0.71		
Gender	0.88 (0.65–1.20)	0.41		
CNS involvement	2.41 (1.43–4.05)	0.001*	1.68 (0.96–2.95)	0.07
Time-period				
1992–1996	2.80 (1.54–5.08)	0.001*	2.76 (1.46–5.19)	0.002
1997–2002	1.89 (1.03–3.47)	0.041*	2.22 (1.19–4.11)	0.012
2003–2008	1.42 (0.74–2.70)	0.29	1.53 (0.79–2.93)	0.206
2009–2012	1.00		1.00	

WBC, white blood cell count in peripheral blood at diagnosis; CNS, central nervous system; HR, hazard ratio; CI, confidence interval.
Only factors that were found to be significant risk factors for an event in the univariate analysis were included into the multivariate analysis.

multivariate Cox regression analysis, CNS involvement lost its prognostic significance (Table 2).

4. Discussion

Survival rates of childhood ALL in Lithuania during all time-periods were inferior to those reported by pediatric oncology groups from Western countries [4–6,8,23,24]. However, the gap was decreasing over time from approximately 20% in the earliest period to almost approaching the rates reported by large international groups in the recent period. EFS rate has improved due to a decrease in rates of relapses and induction failure. However, cumulative incidence of induction failure or DCR1 still remained high. These findings have several implications.

First, the Lithuanian health care system used to be characterized by inefficiency, poor health care and a lack of universal access [25]. The restricted access to internationally available research information and international collaboration led Lithuanian physicians to stay behind in the rapidly developing pediatric oncology. Supportive care and nursing practice was also inferior, with duties limited to technical procedures such as delivering prescribed drugs or procedures to the patients. However, with progress in nursing studies, the curriculum is now close to the Western European standards [26].

Second, after gaining independency in 1990 the total health care expenditure per capita in US dollars in Lithuania was only 10% of that in the European Union countries [25]. In 1992–2002 there was a lack of both the supportive care measures such as broad spectrum antibacterial or antifungal drugs, and of antileukemic therapy. Due to health care reforms, health care expenditure per capita increased in 2011 to 1292 $ US, i.e., 35% of that in Western European countries [27]. Furthermore, since the year 2000, approximately 2 million litas (580 thousand euros) was additionally assigned on annual basis by the State for the treatment of children with cancer in Lithuania. This allowed the necessary antileukemic and supportive care drugs to be available for all childhood ALL patients and to perform all required diagnostic procedures in spite of increasing costs [28].

Third, the study indicated a further trend toward survival improval in the 2009–2012 period compared with 2003–2008 period despite the fact that neither financial nor human resources had improved significantly. International collaboration with the NOPHO and finally joining of the NOPHO ALL-2008 treatment protocol could have played a significant role in several ways: (i) internal resources had to be found for implementing new laboratory methods for diagnostic work-up, risk grouping and monitoring of MRD in consistence with protocol requirements. Laboratory tests such as MRD monitoring by PCR method were initially performed in a Nordic lab, and was later implemented in the molecular lab in Lithuania. The Lithuanian flow cytometry lab had to standardize its diagnostic procedures and participate in the NOPHO validation program. These measures in turn led to improved diagnostics and to better risk stratification of children with ALL. (ii) Discussions in the NOPHO ALL-2008 protocol working groups allowed direct comparison of treatment results with results in other centers. (iii) All the features listed above led to a steadily increasing understanding of the biology of childhood ALL which in turn may have improved clinical decisions.

High toxic death rate remains the main challenge in treating children with ALL in Lithuania. It has been for many years close to the limit of unacceptable non-relapse mortality rate proposed for developing countries [29]. Careful monitoring, registration and analysis of toxic events in the NOPHO leukemia register [30] may have already improved the results, however due to short follow-up period this remains to be determined.

5. Conclusions

Cure rates of childhood ALL in Lithuania are steadily improving and are now approaching those reported by the largest international study groups. The reasons for positive effect are both a better financial support for treatment of children with cancer in Lithuania and international collaboration with joining international treatment protocol.

Contribution

G.V. collected and analyzed data; G.V., L.R., and K.S. designed the study and wrote the manuscript; T.Z. revised statistical analysis; R.M. and M.S. were responsible for performing and providing laboratory analyses. All authors revised the manuscript and gave their final approval.

Conflict of interest

The authors state no conflict of interest.

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REFERENCES

[1] Gurney JG, Severson RK, Davis S, Robison LL. Incidence of cancer in children in the United States. Sex-, race-, and 1-year age-specific rates by histologic type. Cancer 1995;15(75):2186–95.
[2] Linabery AM, Ross JA. Trends in childhood cancer incidence in the U.S. (1992–2004). Cancer 2008;15(112):416–32.
[3] Hunger SP, Loh ML, Whitlock JA, Winick NJ, Carroll WL, Devidas M, et al. Children's Oncology Group's 2013 blueprint for research: acute lymphoblastic leukemia. Pediatr Blood Cancer 2013;60:957–63.
[4] Schmiegelow K, Forestier E, Hellebostad M, Heyman M, Kristinsson J, Soderhall S, et al. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia 2010;24:345–54.
[5] Möricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, Gadner H, et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia 2010;24:265–84.
[6] Pui CH, Pei D, Sandlund JT, Ribeiro RC, Rubnitz JE, Raimondi SC, et al. Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. Leukemia 2010;24:371–82.
[7] Conter V, Arico M, Basso G, Biondi A, Barisone E, Messina C, et al. Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) Studies 82, 87, 88, 91 and 95 for childhood acute lymphoblastic leukemia. Leukemia 2010;24:255–64.
[8] Gaynon PS, Angiolillo AL, Carroll WL, Nachman JB, Trigg ME, Sather HN, et al. Children's Oncology Group Long-term results of the children's cancer group studies for childhood acute lymphoblastic leukemia 1983–2002: a Children's Oncology Group Report. Leukemia 2010;24:285–97.
[9] Stiller CA, Kroll ME, Pritchard-Jones K. Population survival from childhood cancer in Britain during 1978–2005 by eras of entry to clinical trials. Ann Oncol 2012;23:2464–9.
[10] Pritchard-Jones K, Stiller C. What can we learn from geographical comparisons of childhood cancer survival? Br J Cancer 2007;96:1493–7.
[11] Stary J, Jabali Y, Trka J, Hrusak O, Gajdos P, Hrstkova H, et al. Long-term results of treatment of childhood acute lymphoblastic leukemia in the Czech Republic. Leukemia 2010;24:425–8.
[12] Karachunskiy A, Herold R, von SA, Miakova N, Timakow A, Mahortih T, et al. Results of the first randomized multicentre trial on childhood acute lymphoblastic leukaemia in Russia. Leukemia 2008;22:1144–53.
[13] Derwich K, Wachowiaki J, Kaczmarek-Kanoldi M, Balcerska A, Balwierz W, Chybicka A, et al. [Treatment results in children with the standard risk acute lymphoblastic leukemia treated with high dose of methotrexate (5.0 g/m^2). 11 years of the Polish Pediatric Leukemia/Lymphoma Study Group experience]. Przegl Lek 2006;63:7–10.
[14] Ragelienė L, Savinas A, Saulienė B, Daugelavičienė V, Kleinotienė G. Vaikų ūminės leukozės gydymo rezultatai gydant pagal BFM schemas Cure rates of children with acute lymphoblastic leukemia treated according to BFM-based chemotherapy Medicina 1995;31:483–90.
[15] Savinas A, Ragelienė L, Saulienė B, Daugelavičienė V, Kleinotienė G, Stankevičienė S. Vaikų ūminės leukozės recidyvai ir ju gydymo rezultatai Survival rates in children with relapsed acute leukemia Medicina 1995;31:491–6.
[16] Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia 1995;9:1783–6.
[17] Schmiegelow K, Forestier E, Kristinsson J, Soderhall S, Vettenranta K, Weinshilboum R, et al. Thiopurine methyltransferase activity is related to the risk of relapse of childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. Leukemia 2009;23:557–64.
[18] Toft N, Birgens H, Abrahamsson J, Bernell P, Griškevičius L, Hallböök H, et al. Risk group assignment differs for children and adults 1–45 years with acute lymphoblastic leukemia treated by the NOPHO ALL-2008 protocol. Eur J Haematol 2013;90:404–12.
[19] Kim HT. Cumulative incidence in competing risks data and competing risks regression analysis. Clin Cancer Res 2007;13(2 Pt 1):559–65.
[20] Vaitkevičienė G, Forestier E, Hellebostad M, Heyman M, Jonsson OG, Lähteenmäki PM, et al. High white blood cell count at diagnosis of childhood acute lymphoblastic leukaemia: biological background and prognostic impact. Results from the NOPHO ALL-92 and ALL-2000 studies. Eur J Haematol 2011;86:38–46.
[21] Forestier E, Schmiegelow K. Nordic Society of Paediatric Haematology and Oncology NOPHO. The incidence peaks of the childhood acute leukemias reflect specific cytogenetic aberrations. J Pediatr Hematol Oncol 2006;28:486–95.
[22] Möricke A, Reiter A, Zimmermann M, Gadner H, Stanulla M, Dordelmann 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;111:4477–89.
[23] Reiter A, Schrappe M, Ludwig WD, Hiddemann W, Sauter S, Henze G, et al. Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. Blood 1994;84:3122–33.
[24] Schrappe M, Reiter A, Ludwig WD, Harbott J, Zimmermann M, Hiddemann W, et al. Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90 German–Austrian–Swiss ALL-BFM Study Group. Blood 2000;95:3310–22.
[25] Bankauskaite V, O'Connor JS. Health policy in the Baltic countries since the beginning of the 1990s. Health Policy 2008;88:155–65.
[26] Toliusiene J, Peicius E. Changes in nursing ethics education in Lithuania. Nurs Ethics 2007;14:753–7.
[27] An online gateway for the latest data and information on the U.S. role in global health; 2013, Available from: http://www.globalhealthfacts.org/.

[28] van Litsenburg RR, Uyl-de Groot CA, Raat H, Kaspers GJ, Gemke RJ. Cost-effectiveness of treatment of childhood acute lymphoblastic leukemia with chemotherapy only: the influence of new medication and diagnostic technology. Pediatr Blood Cancer 2011;57:1005–10.
[29] Hunger SP, Sung L, Howard SC. Treatment strategies and regimens of graduated intensity for childhood acute lymphoblastic leukemia in low-income countries: a proposal. Pediatr Blood Cancer 2009;52:559–65.
[30] Lund B, Asberg A, Heyman M, Kanerva J, Harila-Saari A, Hasle H, et al. Risk factors for treatment related mortality in childhood acute lymphoblastic leukaemia. Pediatr Blood Cancer 2011;56:551–9.