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American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

Relapse risk following truncation of PEG-asparaginase in childhood acute lymphoblastic leukemia

Tracking no: BLD-2020-006583R2

Sofie Gottschalk Højfeldt (Aarhus University Hospital, Denmark) Kathrine Grell (Department of Pediatrics and Adolescent Medicine, Rigshospitalet, Copenhagen, Denmark) Jonas Abrahamsson (Institute of Clinical Sciences, Queen Silvias Childrens Hospital, Sweden) Bendik Lund (St. Olavs University hospital, Norway) Kim Vettenranta (University of Helsinki, Finland) Olafur Jonsson (Children´s Hospital, Landspitali University Hospital, Iceland) Thomas Frandsen (Rigshospitalet, University Hospital of Copenhagen, Denmark) Benjamin Ole Wolthers (Rigshospitalet, Denmark) Hanne Vibeke Marquart (Copenhagen University Hospital (Rigshospitalet), Denmark) Goda Vaitkeviciene (Vilnius University Hospital Santaros Klinikos and Vilnius University, Lithuania) Kristi Lepik (Tallinn Children's Hospital, Estonia) Mats Heyman (Karolinska University Hospital, Sweden) Kjeld Schmiegelow (Rigshospitalet, University of Copenhagen, Denmark) Birgitte Albertsen (Aarhus University Hospital, Denmark)

Abstract:
Truncation of asparaginase treatment due to asparaginase related toxicities or silent inactivation (SI) is common and may increase relapse risk in acute lymphoblastic leukemia (ALL). We investigated relapse risk following suboptimal asparaginase exposure among 1401 children aged 1–17 years, diagnosed with ALL between July 2008 and February 2016, and treated according to the NOPHO ALL2008 protocol including extended asparaginase exposure (1,000 IU/m^2 intramuscularly weeks 5 to 33). Patients were included with delayed entry at their last administered asparaginase treatment or detection of SI and followed until relapse, death, secondary malignancy, or end of follow-up (median: 5.71 years, interquartile range: 4.02–7.64). In a multiple Cox model comparing patients with (n=358) and without (n=1043) truncated asparaginase treatment due to clinical toxicity, the adjusted relapse-specific hazard ratio (aHR) was 1.33 (95% confidence interval [CI]: 0.86–2.06, *P*=0.20). In a substudy including only patients with information on enzyme activity (n=1115), the 7-year cumulative incidence of relapse for the 301 patients with truncation of asparaginase treatment or SI (157 hypersensitivity, 53 pancreatitis, 14 thrombosis, 31 other, 46 SI) was 11.1% (95% CI: 6.9–15.4) versus 6.7% (95% CI: 4.7–8.6) for the 814 remaining patients. The relapse-specific aHR was 1.69 (95% CI: 1.05–2.74, *P*=0.03). The unadjusted bone-marrow relapse-specific HR was 1.83 (95% CI: 1.07–3.14, *P*=0.03) and 1.86 (95% CI: 0.90– 3.87, *P*=0.095) for any CNS relapse.

These results emphasize the importance of therapeutic drug monitoring and appropriate adjustment of asparaginase therapy when feasible.

Conflict of interest: COI declared - see note

COI notes: Birgitte Klug Albertsen is sponsor for the investigator-initiated NOR-GRASPALL 2016 study (Clinicaltrials.gov: NCT03267030). Kjeld Schmiegelow has received Speaker and/or Advisory Board Honoraria from Jazz Pharmaceuticals and Servier; Speaker fee from Amgen and Medscape; and Educational grant from Servier. None of the other authors have any relevant conflict of interests.

Preprint server: No;

Author contributions and disclosures: Conception, design and collection of data: all authors. Data analysis and interpretation: KG, SGH, BKA, KS. SGH wrote the paper and it was critically reviewed by KG, KS and BKA. All authors approved the final manuscript.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: For original data, please contact biralber@rm.dk

Clinical trial registration information (if any):

Relapse risk following truncation of PEG-asparaginase in childhood acute lymphoblastic leukemia

Sofie Gottschalk Højfeldt, MD[1], Kathrine Grell MSc, PhD[2,3], Jonas Abrahamsson, MD, PhD[4], Bendik Lund, MD, PhD[5], Kim Vettenranta, MD, PhD[6], Ólafur G. Jónsson, MD[7], Thomas L. Frandsen, MD, PhD[3], Benjamin O. Wolthers, MD, PhD[3], Hanne Vibeke Marquart, MD, PhD[8], Goda Vaitkeviciene, MD, PhD[9], Kristi Lepik, MD[10], Mats Heyman, MD, PhD[11], Kjeld Schmiegelow, MD, DrMedSci[3,12], Birgitte Klug Albertsen, MD, PhD[1] on behalf of the Nordic Society of Pediatric Hematology and Oncology, NOPHO group.

[1]Children and Adolescent Health, Aarhus University Hospital, Aarhus, Denmark & Institute of Clinical Medicine, Faculty of Medicine, University of Aarhus, Denmark

[2]Section of Biostatistics, Department of Public Health, University of Copenhagen, Copenhagen, Denmark,

[3]Department of Pediatrics and Adolescent Medicine, University Hospital, Rigshospitalet, Copenhagen, Denmark

[4]Department of Pediatrics, Institution for Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden,

[5]Department of Pediatrics, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway,

[6]Department of Pediatrics, Helsinki University Central Hospital, Helsinki, Finland

[7]Department of Pediatrics, Landspitali University Hospital, Reykjavik, Iceland

[8]Department of Clinical immunology, University Hospital Rigshospitalet, Copenhagen, Denmark

[9] Clinic of Children's Diseases, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

[10] Department of Haematology and Oncology, Tallinn Children´s Hospital, Tallinn, Estonia.

[11]Childhood Cancer Research Unit, Department of Women's and Children's Health Karolinska Institut and Department of Pediatric Oncology, Karolinska University Hospital, Stockholm, Sweden

[12]Institute of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Corresponding author: Birgitte Klug Albertsen, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark, e-mail: biralber@rm.dk, phone: +45 20 22 46 43.

Word count (abstract): 242

Word count (main text): 3801

Number of tables: 4

Number of figures: 3

Number of supplementary files: 1

Reference count: 29

Running Head: Relapse risk following PEG-asparaginase truncation

Key words: Pediatric acute lymphoblastic leukemia, PEG-asparaginase, outcome, toxicities.

Key Points

1) Suboptimal asparaginase exposure leads to an increased risk of relapse in non-high-risk ALL patients.

2) Therapeutic drug monitoring should be used to identify patients with no asparaginase enzyme activity in order to ensure treatment efficacy.

Abstract

Truncation of asparaginase treatment due to asparaginase related toxicities or silent inactivation (SI) is common and may increase relapse risk in acute lymphoblastic leukemia (ALL). We investigated relapse risk following suboptimal asparaginase exposure among 1401 children aged 1–17 years, diagnosed with ALL between July 2008 and February 2016, and treated according to the NOPHO ALL2008 protocol including extended asparaginase exposure (1,000 IU/m^2 intramuscularly weeks 5 to 33). Patients were included with delayed entry at their last administered asparaginase treatment or detection of SI and followed until relapse, death, secondary malignancy, or end of follow-up (median: 5.71 years, interquartile range: 4.02–7.64). In a multiple Cox model comparing patients with (n=358) and without (n=1043) truncated asparaginase treatment due to clinical toxicity, the adjusted relapse-specific hazard ratio (aHR) was 1.33 (95% confidence interval [CI]: 0.86–2.06, *P*=0.20). In a substudy including only patients with information on enzyme activity (n=1115), the 7-year cumulative incidence of relapse for the 301 patients with truncation of asparaginase treatment or SI (157 hypersensitivity, 53 pancreatitis, 14 thrombosis, 31 other, 46 SI) was 11.1% (95% CI: 6.9–15.4) versus 6.7% (95% CI: 4.7–8.6) for the 814 remaining patients. The relapse-specific aHR was 1.69 (95% CI: 1.05–2.74, *P*=0.03). The unadjusted bone-marrow relapse-specific HR was 1.83 (95% CI: 1.07–3.14, *P*=0.03) and 1.86 (95% CI: 0.90– 3.87, *P*=0.095) for any CNS relapse.

These results emphasize the importance of therapeutic drug monitoring and appropriate adjustment of asparaginase therapy when feasible.

Introduction

The importance of asparaginase in the treatment of acute lymphoblastic leukemia (ALL) is well established[1-5]. The overall survival for children with ALL is now above 90% in most contemporary protocols[6], but asparaginase-associated toxicities still constitute a significant problem as they, besides causing acute morbidity and mortality, may cause truncation of the treatment with a subsequent increased risk of relapse[3,7,8]. Balancing the anti-leukemic effect and asparaginase toxicity is challenging, and in spite of multiple studies the optimal asparaginase schedule is still unknown[9,10].

The most frequent toxicities causing asparaginase truncations are hypersensitivity, pancreatitis and thromboembolism[3]. Especially hypersensitivity constitutes a problem due to asparaginase inactivation, not only in patients with clinical hypersensitivity but also among those without clinical symptoms (silent inactivation [SI])[9,11]. Patients with SI have an inferior outcome[7] and thus asparaginase enzyme activity (AEA) levels are used to monitor the treatment with asparaginase in order to identify the patients without enzyme activity and change the asparaginase preparation[12].

In this paper we present results from the NOPHO ALL2008 protocol. The protocol included prospective asparaginase-associated toxicity registration and detailed registration of asparaginase treatment. In addition, samples for AEA measurements were collected before every asparaginase administration and analyzed retrospectively. Firstly we investigated if patients with a truncation of asparaginase therapy had a different risk of relapse compared to patients without truncated treatment, while taking AEA measurements into account. Secondly, we explored if the risk of relapse was different among subgroups based on the asparaginase treatment intensity defined as the total accumulated asparaginase given.

Methods

Patients

The NOPHO ALL2008 protocol opened in July 2008 and the study period ended on February 28th 2016. Children aged 1.0–17.9 years, diagnosed with Philadelphia chromosome negative B-cell precursor or T-cell ALL in Denmark, Estonia, Finland, Iceland, Lithuania, Norway and Sweden and treated according to the protocol were included in this study. The protocol, risk grouping and the stratification criteria have previously been described in detail[13-15]. Definition and grouping of central nervous system (CNS) disease and minimal residual disease (MRD) are described in the supplementary material (S1).

The protocol was approved by the National Medicines Agencies (EudraCT no. 2008-003235-20), relevant ethical committees and registered at clinicaltrials.gov (NCT03987542). Research was conducted in accordance with the Declaration of Helsinki.

Asparaginase in the NOPHO ALL2008 protocol

The standard asparaginase preparation in the NOPHO ALL2008 protocol was PEG-asparaginase (1000 IU/m^2/dose) administered as intramuscular injections. From day 30, at the end of induction therapy, SR and IR patients received five doses of PEG-asparaginase at two-week intervals during consolidation after which patients were invited to participate in a randomization between additional three doses at six-week intervals (experimental arm) or ten doses at two-week intervals (standard arm) during the delayed intensification and the first part of maintenance therapy. The results of the randomization showed no difference in disease-free survival between the two arms and significantly less toxicity in the experimental arm[10]. High-risk patients received seven or nine doses of PEG-asparaginase, given as one dose in each treatment block and two additional doses in the delayed intensification. In case of clinical hypersensitivity, the PEG-asparaginase treatment was truncated and patients switched to Erwinia asparaginase (20.000 IU/m^2/dose): Non-high risk patients were scheduled to receive three Erwinia asparaginase doses per week for two weeks during delayed intensification, replacing only 1 dosage of PEG-asparaginase. High-risk patients were planned to receive three Erwinia asparaginase doses per week in each of the subsequent treatment blocks, corresponding to half of planned PEG-asparaginase treatment. Additional two weeks of Erwinia asparaginase treatment were planned for the delayed intensification. Thus all planned PEG-asparaginase doses were not substituted and these patients were therefore defined as “truncated” even though they received all the planned substitution doses.

The prospective registration of asparaginase-associated toxicities and detailed registration of the asparaginase treatment included: number of PEG-asparaginase dosages given prior to truncation, reason for truncation and number of Erwinia asparaginase doses given.

In assessing the asparaginase treatment intensity, we defined the amount of asparaginase given to the individual patient as the length of asparaginase treatment as follows: i) six Erwinia asparaginase doses counted as one dose of PEG-asparaginase and ii) one dose of PEG-asparaginase corresponded to 18 days of asparaginase treatment (based on previous studies[10,16]) when not given continuously at 14 days intervals.

Asparaginase enzyme activity (AEA)

Samples for AEA measurement were taken before every PEG-asparaginase injection and analyzed retrospectively. The results were not reported to the treating clinician and did therefore not lead to clinical intervention. Samples were stored at -80°C and analyzed retrospectively by either Nessler's reagent or aspartic acid β-hydroxamate assay[16,17]. High-risk patients received PEG-asparaginase at approximately one-month intervals. Since AEA levels were only measured prior to asparaginase administration, the levels in high-risk patients were not informative, due to their dispersed asparaginase therapy. Samples with no measureable AEA were only included in the present study when taken within 14 days since administration. This cutoff relied on several different pharmacokinetic studies[16,18]. Based on the AEA measurements, SR and IR patients were divided into groups prior to any analysis: i) normal enzyme activities; patients with measureable AEA in at least two samples and at least one sample above 100 IU/L, ii) fast metabolizers; patients with AEA in at least two samples but no sample above 100 IU/L, iii) no measureable AEA; patients without enzyme activity in samples taken within 14 days since the latest PEG-asparaginase administration (this included patients with enzyme activity in one sample taken within five days after the first dose of asparaginase or with a single sample with enzyme activity <100 IU/L, but no measureable AEA in any other samples). Patients with hypersensitivity without any samples were also included in group iii, assuming that they did not have any enzyme activity[19].

Remaining patients without samples, patients with samples without any enzyme activity taken at erroneous time points (>14 days since administration), and patients with only one sample were categorized as patients with undetermined enzyme activity. AEA measurements were evaluated at the end of asparaginase treatment, and patients were classified based on all available samples.

Outcome measures and asparaginase exposure groups

Patients were followed from ALL diagnosis with delayed entry at the end of asparaginase treatment until relapse, death in complete remission (DCR1), secondary malignancy (SMN), lost to follow-up or September 18th 2018, whichever came first. The main outcome was the risk of relapse considering DCR1 and SMN as competing events. Secondary, we assessed the risk of any bone marrow relapse and any CNS relapse separately, in each sub-analysis considering also the other type of relapse as competing event.

In the main cohort, we compared patients with truncated asparaginase therapy to the patients who did not have their asparaginase therapy truncated. In a substudy consisting of SR and IR patients with determined AEA, we compared patients with truncated asparaginase therapy or no enzyme activity (truncated$_{orAEA-}$) to the patients who did not have asparaginase therapy truncated and had normal enzyme activity or were fast metabolizers (non-truncated$_{andAEA+}$). Since the group of fast metabolizers was found too small for individual analysis we joined it with the group normal activity, since low AEA levels have been shown to ensure sufficient depletion[20,21]. As sensitivity analyses, we repeated the analyses for the subcohort with exclusion of truncated$_{orAEA-}$ patients who had their asparaginase treatment truncated due to pancreatitis although none were registered with any major treatment modifications in the NOPHO database.

Investigating asparaginase treatment intensity, we categorized the patients in three groups: i) low treatment intensity (asparaginase truncated and less than 10 weeks of asparaginase treatment or no AEA); ii) medium treatment intensity (asparaginase truncated, but at least 10 weeks of asparaginase treatment and normal AEA/fast metabolizers); iii) high treatment intensity (no truncation and normal AEA/fast metabolizers corresponding to non-truncated$_{andAEA+}$). Patients who were truncated due to hypersensitivity and substituted according to protocol were categorized as truncated, since the substitution was inadequate and did not replace each dose of PEG-asparaginase.

Statistics

The follow-up time was estimated using the reverse Kaplan-Meier method. Cumulative incidences of relapse were estimated by the Aalen-Johansen estimator, and Cox proportional-hazards model was used to estimate unadjusted (HR) and adjusted relapse-specific hazard ratios (aHR) with significance evaluated by Wald tests. The multiple models included age group (< or ≥10 years), CNS status at diagnosis (CNS1, CNS2, CNS3, defined in supplementary material S1), MRD day 29 categorized into four groups, and white blood cell (WBC) count at diagnosis (log2-transformed due to skewness; hence, estimates corresponds to a doubling), and for analysis of the main cohort also stratification by risk group. In a post-hoc sub-analysis, multiple Cox models included NCI risk groups defined as NCI-high-risk if age≥10 years or WBC≥50×10^9/L and NCI-SR if age<10 years and WBC<50×10^9/L instead of the above-mentioned variables. Two-sided P-values <0.05 were considered statistically significant. Statistical analyses were performed using R version 3.5.3.

Results

Patient characteristics – main cohort

A total of 1494 patients were included in the NOPHO ALL2008 study cohort and eligible for inclusion in this study. Following exclusion of patients with an event prior to completed asparaginase treatment and patients with missing information about asparaginase truncation, 1401 patients were included in the main cohort (Figure 1). Baseline characteristics of these patients are given in Table 1. There was an equal distribution of males (55%) and females (45%), median age at diagnosis was 4 years (interquartile range [IQR]: 2–8), median WBC was 10.9 $\times 10^9$/L (IQR: 4.5–39.5), and 14% were high-risk patients. The majority of patients (87%) did not have CNS disease at time of diagnosis (CNS1), and 38% had undetectable bone-marrow disease at day 29 (MRD group 4). Of the 1401 patients, 358 (25.6%) had their asparaginase treatment truncated. Median number of asparaginase doses given in the truncated group was 4 (IQR: 3–7) versus 15 (IQR: 8-15) in the non-truncated group. The most frequent reasons for truncation were clinical hypersensitivity (58.1%), pancreatitis (24.6%) and thromboembolism (6.7%) (Table 2).

Cumulative incidences and relapse-specific hazard ratios in the main cohort

With a median follow-up time of 5.7 years (IQR: 4.0–7.6), 13 patients died in DCR1, 11 had developed a SMN, and 97 had developed a relapse with a 7-year cumulative incidence of relapse of 8.5% (95% confidence interval [CI]: 6.8–10.1). The incidence of DCR1 and SMN were similar for the non-truncated and truncated patients: the 7-year cumulative incidence of DCR1 was 4.0% (95% CI: 0–10.2) and 1.3% (95% CI: 0.03–2.7), respectively; and the 7-year cumulative incidence of SMN was 0.7% (95% CI: 0.2–1.3) and 1.2% (95% CI: 0.03–2.4), respectively. The 7-year cumulative incidence of relapse for the non-truncated group was 7.5% (95% CI: 5.6–9.3) and for the truncated group 10.5% (95% CI: 6.7–14.4) with a relapse-specific risk group stratified aHR of 1.33 (95% CI: 0.86–2.06, P=0.20).

Patient characteristics – subcohort

Following exclusion of high-risk patients and patients with undetermined or missing AEA measurements, 1115 patients remained in the subcohort (Figure 1). Overall, the baseline characteristics of the subcohort did not differ from the main cohort (Table 3).

In the subcohort, 255 patients (22.9%) were registered with truncated asparaginase treatment. The most frequent causes of PEG-asparaginase truncation were clinical hypersensitivity (61.6%), pancreatitis (20.8%), and thrombosis (5.5%) (Table 2). Of the 157 patients with clinical hypersensitivity, 130 (82%) had at least one dose of Erwinia asparaginase administered, whereof 57 had following AEA measurements done and 55 of the 57 (96.5%) had sufficient AEA levels >100IU/l. When assessing the AEA measurements in the subcohort, 910 patients (81.6%) had normal enzyme activity levels, 19 (1.7%) were fast metabolizers, and 186 (16.7%) had no enzyme activity. Of the 186, 46 were not registered with a truncation (Table 2); consequently, 301 patients were classified as truncated$_{orAEA-}$, and 814 as non-truncated$_{andAEA+}$. The baseline characteristics of the truncated$_{orAEA-}$ and the non-truncated$_{andAEA+}$ patients are shown in Table 3.

Cumulative incidences and relapse-specific hazard ratios in the subcohort

Median follow-up time was 5.6 years (IQR: 4.0–7.5), and a total of 81 events occurred: two DCR1, eight SMN and 71 relapses with a 7-year cumulative incidence of relapse of 7.9% (95% CI: 6.0–9.7). For the non-truncated$_{andAEA+}$ patients the 7-year cumulative incidence of relapse was 6.7% (95% CI: 4.7–8.6), and for the truncated$_{orAEA-}$ patients it was 11.1% (95% CI: 6.9–15.4) (Figure 2). The relapse-specific HR comparing truncated$_{orAEA-}$ with non-truncated$_{andAEA+}$ was 1.73 (96% CI: 1.07–2.80. *P*=0.03), and the aHR was 1.69 (95% CI: 1.05–2.74, *P*=0.03). Age ≥ 10 years, positive MRD (MRD group 1) and WBC were significantly associated with the relapse-specific aHR (Table 4).

The sensitivity analysis excluding the 53 patients with a truncation due to pancreatitis (six relapses) resulted in a relapse-specific HR of 1.61 (95% CI: 0.96–2.72, *P*=0.07) and aHR of 1.63 (95% CI: 0.97–2.75, *P*=0.07), indicating that the pancreatitis patients alone were not causing the observed relapse association.

The sub-analysis comparing truncated$_{orAEA-}$ with non-truncated$_{andAEA+}$ adjusted for NCI risk groups (344 NCI-high risk, 771 NCI-SR patients) yielded a relapse-specific aHR of 1.77 (95% CI: 1.09–2.85, *P*=0.02) similar to the result adjusted for age, WBC, MRD group, and CNS status.

Investigating the effect of immunophenotype, the 7-year cumulative incidence of relapse was lower for the patients with T-cell ALL: for 65 T-cell and non-truncated$_{andAEA+}$ patients 4.6% (95% CI: 0–9.7), for 749 BCP and non-truncated$_{andAEA+}$ patients 6.8% (95% CI: 4.7–8.9), for 279 BCP and truncated$_{orAEA-}$ patients 12.1% (95% CI: 7.5–16.6). There were no relapses among the 22 patients with T-cell ALL in the truncated$_{orAEA-}$ group; hence, it was not possible to explore whether the

relapse-specific HR for truncated$_{orAEA-}$ versus non-truncated$_{andAEA+}$ differed between patients with BCP and T-cell ALL in a multiple Cox model with interaction.

Type of relapse

Of the 71 relapses in the subcohort, 56 involved the bone marrow, 30 involved the CNS, and 15 involved both. The 7-year cumulative incidence of any bone marrow relapse for the truncated$_{orAEA-}$ patients versus the non-truncated$_{andAEA+}$ patients was 8.8% (95% CI: 5.1–12.6) and 5.4% (95% CI: 3.6–7.2), respectively, and the unadjusted bone marrow relapse-specific HR was 1.83 (95% CI: 1.07–3.14, *P*=0.03). The 7-year cumulative incidence of any CNS relapse for the truncated$_{orAEA-}$ group versus the non-truncated$_{andAEA+}$ group was 5.0% (95% CI: 2.1–7.9) and 2.4% (95% CI: 1.3–3.5), respectively, and the unadjusted CNS relapse-specific HR was 1.86 (95% CI: 0.90– 3.87, *P*=0.01).

Treatment intensity

Based on truncation, AEA and length of asparaginase treatment, we grouped the 301 truncated$_{orAEA-}$ patients into 208 patients with low treatment intensity (truncated$_{lowIntensity}$) and 93 patients with medium treatment intensity (truncated$_{mediumIntensity}$). The 814 non-truncated$_{andAEA+}$ patients were considered to have the highest treatment intensity.

The 7-year cumulative incidence of relapse for the truncated$_{lowIntensity}$ patients was 11.9% (95% CI: 6.4–17.4), for the truncated$_{mediumIntensity}$ patients 9.4% (95% CI: 3.2–15.7), and for the non-truncated$_{andAEA+}$ patients 6.7% (95% CI: 4.7–8.6) (Figure 3). Comparing the truncated$_{mediumIntensity}$ and the truncated$_{lowIntensity}$ to the non-truncated$_{andAEA+}$ patients yielded an unadjusted relapse-specific HR=1.63 (95% CI: 0.77–3.45, *P*=0.206) and HR=1.78 (95% CI: 1.04–3.05, *P*=0.036), respectively. The multiple models showed similar results: aHR=1.49 (95% CI: 0.69–3.18, *P*=0.308) and aHR=1.80 (95% CI: 1.05–3.09, *P*=0.034), respectively.

Defining treatment intensity as number of doses per randomization arm and not considering AEA, we estimated the 7-year cumulative incidence of relapse for the dose-intensity groups with at least three relapses: three, four, and 15 doses in the standard arm with a total of 15 planned doses yielded 10.0%, 14.5%, and 6.2%, respectively, and eight doses in the experimental arm with a total of eight planned doses yielded 9.4%, but all with very wide confidence interval (Supplementary Figure S2).

Discussion

This study investigated the outcome of children with ALL following suboptimal asparaginase exposure in the NOPHO ALL2008 protocol. We found a significantly increased risk of relapse in the truncated$_{orAEA-}$ group compared to the non-truncated$_{andAEA+}$ group. We did several sub-analyses, taking the heterogeneity of the truncated group into account and investigated subgroups receiving different amounts of asparaginase, but overall the increased HR remained the same.

A previous study investigating the event-free survival (EFS) in patients who had their asparaginase therapy truncated due to toxicities found a decreased EFS among those who received <26 weeks of asparaginase treatment compared to those with ≥26 weeks of treatment, without taking AEA measurements into account[3]. In the main cohort of our study we analyzed the relapse-specific HR between truncated and non-truncated without taking enzyme activity levels into account, but as opposed to Silverman et al. we did not find an increased risk of relapse in the truncated group (aHR=1.33, *P*=0.20). Numerous differences between the two studies, including differences in asparaginase formulation and administration, different protocols and substitution regimens could be the cause of the divergent results. Several studies have found decreased EFS in patients with SI of PEG-asparaginase[7,22]. Thus, we analyzed the subcohort including only patients with AEA measurements available. All patients treated suboptimally with asparaginase, i.e. patients who had their asparaginase treatment truncated as well as patients with SI were grouped together in these analyses. Our results showed a significantly increased risk of relapse in the truncated$_{orAEA-}$ group (aHR=1.69, *P*=0.032), which is also in accordance with a recent study from Children's Oncology Group[23]. Our results thus confirm that suboptimal asparaginase treatment leads to an increased risk of relapse. The discrepancy between the results in the main cohort and the subcohort can be due to several factors. The most obvious difference is the lack of AEA measurements as part of the classification in the main cohort, thus patients with SI were not identified. Moreover, it is possible that the truncation of asparaginase treatment in the high-risk group is not as crucial due to a more intensified treatment in these patients.

The optimal cumulative dose of asparaginase in the treatment of ALL is unknown, but has been widely debated. Some studies have shown that more intensified asparaginase treatment (e.g. 20 additional weeks of asparaginase in reinduction) improves EFS[4,5,8,24], while others have found less intensified asparaginase treatment to have comparable outcomes [10,25,26]. The results of the randomization of asparaginase in the NOPHO ALL2008 protocol showed that the disease-free survival did not differ between patients receiving eight doses of PEG-asparaginase (five doses at

two-week intervals and three doses at six-week intervals) compared to patients receiving 15 doses of asparaginase (two-week intervals)[10]. Considering the results of the present study and the NOPHO ALL2008 randomized study, it is evident that non-high risk patients can do well with less intensified asparaginase treatment, but discontinuation due to toxicities or SI potentially still leads to an increased risk of relapse.

Our study did not have the power to investigate how many doses of asparaginase would be needed to avoid the increased risk of relapse in the non-high risk group, but in the sub-analysis we divided patients into three groups based on truncation and weeks of asparaginase treatment. We found increasing cumulative incidence of relapse with a decreasing amount of asparaginase (Figure 3). In the multiple Cox regression analysis, we found a significantly increased risk of relapse in $\text{truncated}_{\text{lowIntensity}}$ group compared to $\text{non-truncated}_{\text{andAEA+}}$ but not when comparing $\text{truncated}_{\text{mediumIntensity}}$ to $\text{non-truncated}_{\text{andAEA+}}$. This could be due to lack of power with only 93 patients in the medium intensity group. It could also indicate that 10 weeks of asparaginase treatment in the non-high risk group was sufficient. However, it is important to keep in mind that we pre-defined the cutoffs for the treatment intensity groups. In addition, due to the randomization, there was an overlap of how many weeks of asparaginase treatment the patients had received in the $\text{truncated}_{\text{mediumIntensity}}$ group and in the $\text{non-truncated}_{\text{andAEA+}}$ group: a patient with a truncation in the standard arm could have received more asparaginase compared to a patient not truncated in the experimental arm, but since the timing of the asparaginase administrations is thought to be of importance, we chose to keep the truncated and non-truncated patients in separate groups.

In the NOPHO ALL2008 protocol Erwinia asparaginase was used in case of hypersensitivity to PEG-asparaginase. Yet, not all doses were replaced as non-high risk patients received only two additional weeks of asparaginase treatment during the delayed intensification following hypersensitivity. Since most of these patients had no AEA during PEG-asparaginase treatment, they received very little effective asparaginase treatment in total. Based on current knowledge, it can seem irrational that the NOPHO ALL2008 protocol did not replace all PEG-asparaginase dosages in case of hypersensitivity; however, it merely reflects that the protocol was planned and was based on the knowledge and literature available at the time, and that new knowledge has emerged meanwhile. In February 2017 therapeutic drug monitoring (TDM) during PEG-Asparaginase treatment became voluntary until closure of the NOPHO ALL2008. Patients identified without enzyme activity were switched to either Erwinia asparaginase or Eryaspase (Clinicaltrials.gov: NCT03267030). In the new ALLTogether protocol TDM is standard of care to allow for the

rational replacement of PEG-Asparaginase in the case of SI and for assessment of allergic-like reactions.

It can seem like a simple recommendation, to substitute all asparaginase doses in case of hypersensitivity. However, in the recent years the world has experienced a shortage of Erwinia asparaginase. To our knowledge, no doses of asparaginase were omitted due to Erwinia asparaginase shortage in the current study, but it is a critical issue that appears to be recurrent. This, combined with the results of our study emphasizes the need for new asparaginase preparations.

We assumed that the increased risk of relapse in the truncated$_{orAEA-}$ group was caused by the lack of asparaginase exposure, but other possible explanations cannot be ruled out. We probed this in a sensitivity analysis excluding the pancreatitis patients since pancreatitis can cause severe illness[28,29] and thereby truncation or postponement of other elements of treatment. As our results did not change significantly, we conclude that the pancreatitis patients alone are not causing the increased aHR. It is however important to stress, that we are studying a single drug in a long and complex treatment and the composition of the ALL treatment differs between the different study groups, thus direct comparisons and recommendations can be difficult. In order to test if the NCI criteria might yield different results, we did a post-hoc analysis adjusting for these risk groups; however, the results did not change markedly. We also made the assumption, that SR and IR patients with hypersensitivity without any AEA measurements had no AEA and included these in the truncated$_{orAEA-}$ group. However, this should not affect the results since all hypersensitivity patients in the NOPHO ALL2008 protocol were truncated due to lack of full asparaginase substitution.

In conclusion we found that patients with truncated asparaginase treatment or no enzyme activity had a significantly increased risk of relapse compared to those with non-truncated therapy and measurable enzyme activity. This is in accordance with previously published studies. Ten weeks of efficient asparaginase treatment seemed to be sufficient in the NOPHO ALL2008 protocol. Our results confirm and emphasize the importance of TDM and substitution of all planned asparaginase doses in order to ensure treatment efficacy that is even more essential now as new protocols are focusing on treatment reduction.

Author contribution

Conception, design and collection of data: all authors. Data analysis and interpretation: KG, SGH, BKA and KS. SGH wrote the paper and it was critically reviewed by KG, KS and BKA. All authors approved the final manuscript.

Conflict of interest: BKA is sponsor for the investigator-initiated NOR-GRASPALL 2016 study (Clinicaltrials.gov: NCT03267030). Kjeld Schmiegelow has received Speaker and/or Advisory Board Honoraria from Jazz Pharmaceuticals and Servier; Speaker fee from Amgen and Medscape; and Educational grant from Servier. None of the other authors have any relevant conflict of interests.

Acknowledgements

The authors sincerely thank laboratory technician, Jane Hagelskjær Knudsen at the Pediatric Research laboratory, Aarhus University Hospital, for excellent work conducting the enzyme analyses**.** Moreover we kindly thank Jacob Bidstrup, MD, for cleaning the Erwinia asparaginase data.

Data sharing

For original data, please email the corresponding author.

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Figure 1. Patient flow diagram of the included patients, subcohorts and subgroups. IR: intermediate risk, SR: standard risk, asp.: asparaginase, AEA: asparaginase enzyme activity

Figure 2. Cumulative incidence of relapse in the subcohort for the two groups based on asparaginase truncation and enzyme activity.Truncated$_{orAEA-}$: 11.1% (95% confidence interval [CI]: 6.9–15.4), non-truncated$_{andAEA+}$: 6.7% (95% CI: 4.7–8.6).
AEA: asparaginase enzyme activity, ALL: acute lymphoblastic leukemia

Figure 3. Cumulative incidence of relapse in the subcohort for the three groups based on intensity of asparaginase treatment. Non-truncated$_{andAEA+}$: 6.7% (95% confidence interval [CI]: 6.0–9.7), truncated$_{mediumIntensity}$: 9.4% (95% CI: 3.2–15.7), truncated$_{lowIntensity}$: 11.9% (95% CI: 6.4–17.4).
AEA: asparaginase enzyme activity, ALL: acute lymphoblastic leukemia

Table 1. Characteristics of the main cohort and the subsets based on asparaginase truncation

Characteristics	Main cohort		Truncated		Non-Truncated	
	No.	%	No.	%	No.	%
No. of patients	1401		358	25.6	1043	74.4
Age at diagnosis, years						
Median [IQR]	4 [2–8]	-	4 [2–8.75]	-	4 [2–8]	-
< 10	1118	79.8	279	77.9	839	80.4
≥ 10	283	20.2	79	28.3	204	19.6
Sex						
Male	765	54.6	193	53.9	572	54.8
Female	636	45.4	165	46.1	471	45.2
NOPHO risk group						
SR	716	51.1	173	48.3	543	52.1
IR	495	35.3	123	34.4	372	35.7
HR	190	13.6	62	17.3	128	12.3
NCI risk group						
SR	884	63.1	219	61.2	665	63.8
HR	517	36.9	139	38.8	378	36.2
CNS status at diagnosis						
CNS 1	1225	87.4	309	86.3	916	87.8
CNS 2	113	8.1	31	8.7	82	7.9
CNS 3	61	4.4	16	4.5	45	4.3
Missing value	2	0.1	2	0.6	-	-
MRD group, day 29						
MRD 1	382	27.3	107	29.9	275	26.4

MRD 2	324	23.1	78	21.8	246	23.6
MRD 3	145	10.3	39	10.9	106	10.2
MRD 4	525	37.5	127	35.5	398	38.2
Missing value	25	1.8	7	2.0	18	1.7
WBC at diagnosis, 10^9/L						
Median [IQR]	10.9 [4.5–39.5]	-	13.0 [4.6–40.4]	-	10.5 [4.5–37.5]	-
Asp. doses given§						
Median [IQR]	15[8–15]	-	4 [3–7]	-	15 [8–15]	-

IQR: interquartile range, SR: standard risk, IR: intermediate risk, HR: high-risk, NCI: national cancer institute, CNS: central nervous system, MRD: minimal residual disease, WBC: white blood cell count, PEG-asp.: PEG-asparaginase.
§PEG-asparaginase doses including recalculated Erwinia asparaginase doses (6 doses of Erwinia asparaginase corresponds to 1 dosage of PEG-asparaginase).

Table 2. Reason for truncation of asparaginase in the main and subcohort

	Main cohort n=1401 n (% of the cohort)	**Subcohort n=1115 n (% of the cohort)**	**No AEA§ n**
Clinical hypersensitivity	208 (14.8%)	157 (14.1%)	139
Pancreatitis	88 (6.3%)	53 (4.8%)	1
Thrombosis	24 (1.7%)	14 (1.3%)	-
Hyperlipidemia	10 (0.7%)	8 (0.7%)	-
Liver toxicity	7 (0.5%)	7 (0.6%)	-
Other*	21 (1.5%)	16 (1.4%)	-
Total number of truncated patients	**358 (25.5%)**	**255 (22.9%)**	**140**
Total number of truncated patients including patients with silent inactivation (truncated$_{orAEA-}$)	**-**	**301**	**186**

* Other: e.g. sepsis, seizure, parental refusal and abdominal pain. § The column "no AEA" only applies for patients in the subcohort. AEA: Asparaginase enzyme activity

Table 3. Characteristics of the subcohort and the subsets based on asparaginase truncation and AEA

Characteristics	Subcohort		Truncated$_{orAEA-}$		Non-Truncated$_{andAEA+}$	
	No.	%	No.	%	No.	%
No. of patients	1115		301	27.0	814	73.0
Age at diagnosis, years						
Median [IQR]	4 [2–7]	-	4 [2–8]	-	4 [2–7]	-
< 10	927	83.1	244	81.1	683	83.9
≥ 10	188	16.9	57	18.9	131	16.1
Sex						
Male	604	54.2	164	54.5	440	54.1
Female	511	45.8	137	45.5	374	45.9
NOPHO risk group						
SR	671	60.2	178	59.1	493	60.6
IR	444	39.8	123	40.9	321	39.4
NCI risk group						
SR	771	69.1	207	68.8	564	69.3
HR	344	30.9	94	31.2	250	30.7
CNS status at diagnosis						
CNS 1	980	87.9	268	89.0	712	87.5
CNS 2	86	7.7	19	6.3	67	8.2
CNS 3	47	4.2	12	4.0	35	4.3
Missing value	2	0.2	2	0.7	-	-
MRD group, day 29						
MRD 1	229	20.5	72	23.9	157	19.3
MRD 2	286	25.7	70	23.3	216	26.5
MRD 3	135	12.1	38	12.6	97	11.9
MRD 4	460	41.3	119	39.5	341	41.9
Missing value	5	0.4	2	0.7	3	0.4
WBC at diagnosis, 10^9/L						
Median [IQR]	9.3 [4.4–31.9]	-	9.4 [4.6–30.7]	-	9.25 [4.3–32.1]	-
Asp. doses given§						

Median [IQR]	15 [8–15]	-	6 [3–11]	15 [8–15]	-

IQR: interquartile range, SR: standard risk, IR: intermediate risk, NCI: national cancer institute, CNS: central nervous system, MRD: minimal residual disease, WBC: white blood cell count, PEG-asp.: PEG-asparaginase, AEA: asparaginase enzyme activity.
[§]PEG-asparaginase doses including recalculated Erwinia asparaginase doses (6 doses of Erwinia asparaginase corresponds to 1 dosage of PEG-asparaginase).

Table 4. Multiple Cox model of time to relapse in the subcohort.

Parameter	Relapse-specific Hazard Ratio	95% CI	*P*
Truncated$_{orAEA-}$ vs. non-truncated$_{andAEA+}$	1.69	1.05 – 2.74	0.03
Age at diagnosis			
≥ 10years vs. <10 years	2.44	1.48 – 4.01	< 0.001
CNS status at diagnosis			
CNS 2 vs. CNS 1	0.69	0.25 – 1.92	0.48
CNS 3 vs. CNS 1	1.16	0.41 – 3.27	0.78
MRD group, day 29			
MRD 3 vs. MRD 4	1.05	0.42 – 2.61	0.92
MRD 2 vs. MRD 4	1.43	0.76 – 2.69	0.27
MRD 1 vs. MRD 4	2.85	1.58 – 5.15	0.001
Doubling in WBC at diagnosis	1.15	1.02 – 1.29	0.02

CI: confidence interval, AEA: asparaginase enzyme activity, CNS: central nervous system, MRD: minimal residual disease, vs.: versus.