

PEG-Asparaginase Allergy in Children With Acute Lymphoblastic Leukemia in the NOPHO ALL2008 Protocol

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Background. L-Asparaginase is an effective drug in the treatment of childhood acute lymphoblastic leukemia (ALL). The use of L-asparaginase may be limited by serious adverse events of which allergy is the most frequent. The objective of this study was to describe the clinical aspects of PEG-asparaginase allergy in children treated according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL2008 protocol. **Procedure.** Children (1–17 years) enrolled in the NOPHO ALL2008 protocol between July 2008 and August 2011, who developed PEG-asparaginase allergy were identified through the NOPHO ALL2008 toxicity registry. In the NOPHO ALL2008 protocol, patients are randomized to 8 or 15 doses of intramuscular PEG-asparaginase (Oncaspar[®]) 1,000 IU/m²/dose administered at 2 or 6 weeks intervals during a total period of 30 weeks. (Clinical trials.gov no: NCT00819351). **Results.** Of 615 evaluable patients, 79 patients developed clinical PEG-asparaginase

allergy (cumulative risk; 13.2%) and discontinued PEG-asparaginase therapy for that reason. PEG-asparaginase allergy occurred after a median of two doses (75% range 2–4, max 14). In 58% of PEG-asparaginase hypersensitive patients, the clinical allergic reactions appeared within 2 hr after PEG-asparaginase administration and ranged from mild symptoms to systemic anaphylaxis. Nine patients experienced an anaphylactic reaction within 1 hr and 50 min from asparaginase administration; none were fatal. Four of 68 patients (6%) who subsequently received Erwinase therapy also reacted allergic to Erwinase. **Conclusion.** Clinical allergy to PEG-asparaginase occurred early in treatment, was in general moderate in severity, and mostly developed within 2 hr after PEG-asparaginase administration. The risk of subsequent Erwinase allergic reactions was low. *Pediatr Blood Cancer* © 2014 Wiley Periodicals, Inc.

Key words: acute lymphoblastic leukemia; allergy; asparaginase; children

INTRODUCTION

L-Asparaginase is an essential component in the multi-drug treatment used for childhood acute lymphoblastic leukemia (ALL). However, its use may be compromised by serious adverse events, such as clinical allergic reactions, pancreatitis, and thrombosis. Clinical allergy is the most frequent serious adverse event observed, with a reported highly variable frequency of 3–75%, depending on the asparaginase formulation, dosing, and treatment protocol [1–8]. Although the allergic symptoms range from localized erythema to systemic anaphylaxis is the typical clinical presentation of asparaginase hypersensitivity is urticaria [9]. If allergy develops, the substitution of one asparaginase formulation with another is common practice in front-line clinical trials. Currently, three bacterial asparaginase formulations are available: the native- and the pegylated L-asparaginase (PEG-asparaginase) both *Escherichia coli* derived products, and Erwinase, which is derived from *Erwinia chrysantemi*.

Whether clinical asparaginase hypersensitivity has an impact on event-free survival (EFS) is still a matter of debate. Truncated asparaginase therapy, however, has been associated with inferior event-free survival outcomes, in particular CNS relapse [1,10–12]. The impact of asparaginase truncation due to clinical allergy may depend on the timing of the allergic event, successful substitution of the asparaginase drug, and development of asparaginase neutralizing antibodies [1–3,7,13,14].

Clinical asparaginase hypersensitivity, including occurrence and severity of allergy, has mostly been reported in studies using native *E. coli* L-asparaginase as first-line therapy [2,3,7,13,14]. However, due to the advantages of a longer half-life and less immunogenicity of the pegylated asparaginase formulation (PEG-asparaginase), this form of asparaginase is now more frequently used [15].

In the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL2008 protocol, which opened in July

2008, intramuscular PEG-asparaginase 1,000 IU/m²/dose is included as first-line treatment. We here describe the clinical aspects of asparaginase allergy in children (1–17 years) treated according to the NOPHO ALL2008 protocol, including the frequency of allergy, the severity of the allergic reactions, the timing of the allergic event during treatment, the time from L-asparaginase injection to allergic reaction, other allergies, and substitution with Erwinase.

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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Grant sponsor: Danish Childhood Cancer Foundation; Grant sponsor: Danish Cancer Society; Grant sponsor: M.L. Jørgensen and Gunnar Hansen Foundation

Conflict of interest: Nothing to declare.

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Received 15 April 2014; Accepted 22 September 2014

PATIENTS AND METHODS

Treatment, NOPHO ALL2008

The NOPHO ALL2008 protocol opened in July 2008 and is still recruiting. The protocol is open for patients aged one to forty-five years with B-cell precursor or T-cell ALL and treated in the Nordic and Baltic countries (Denmark, Finland, Iceland, Norway, Sweden, Estonia, and Lithuania). Patients are stratified to four risk groups [16], “standard risk” (SR), “intermediate risk” (IR), “high-risk-chemotherapy” (HR-chemo) and a subset of high-risk-chemotherapy are assigned to “high-risk chemotherapy and stem cell transplant” (HR-SCT). For non-transplanted patients, the total duration of therapy is 2.5 years, irrespective of risk group. The entire treatment protocol and risk group assignment has been described in detail in three recent NOPHO publications thus only the PEG-asparaginase therapy is outlined in detail below [16–18]. Figure 1 depicts PEG-asparaginase and concomitant glucocorticosteroid treatment.

Between July 2008 and August 2011, a total of 623 children aged 1–17 years, with Philadelphia chromosome negative B-cell precursor including Down syndrome, T-cell, or bi-lineage ALL were enrolled in the NOPHO ALL2008 protocol. Eight patients with induction failure were excluded as PEG-asparaginase therapy was not given during induction, leaving a total of 615 patients evaluable for this study. Patients were followed at least till the end of L-asparaginase treatment. By January 2012, 82 patients had been identified with clinical PEG-asparaginase allergy through

the NOPHO ALL2008 online toxicity registry described below [17]. Of those 82, three were incorrectly registered.

Patients received PEG-asparaginase (Oncaspar®), 1,000 IU/m² intramuscularly (i.m.) starting post induction on day 30. Patients in the SR and IR treatment groups received the first five doses of PEG-asparaginase at 2-week intervals (protocol week 5–12). From protocol week 13 to 33, patients in the SR and IR treatment groups were randomized to receive PEG-asparaginase at 2 versus 6 weeks intervals. In total patients in the SR and IR treatment groups received 8 or 15 doses of PEG-asparaginase, depending on randomization (Clinical trials.gov no: NCT00819351). Patients who did not take part in the randomized study were assigned to the 2 week interval regime as “standard arm.” Outcome data from the randomization is not yet open for analysis as the study is still recruiting. Patients in the high risk chemotherapy treatment group received 10 or 12 doses of PEG-asparaginase depending on response minimal residual disease level (MRD) after the first intensive HR block (Fig. 1). The first dose was given on protocol day 30 and the subsequent doses distributed with one dose included in each treatment block (7–9 blocks), and two in delayed intensification during treatment week 99 and 101 [16]. Patients with Down syndrome were given an extra dose of PEG-asparaginase at day 8 during induction.

In case of severe allergic reactions, PEG-asparaginase was to be discontinued and replaced by i.m. Erwinase 20,000 IU/m²/dose. According to protocol recommendations, patients in the SR and IR treatment group were to receive i.m. Erwinase 20,000 IU/m²/dose

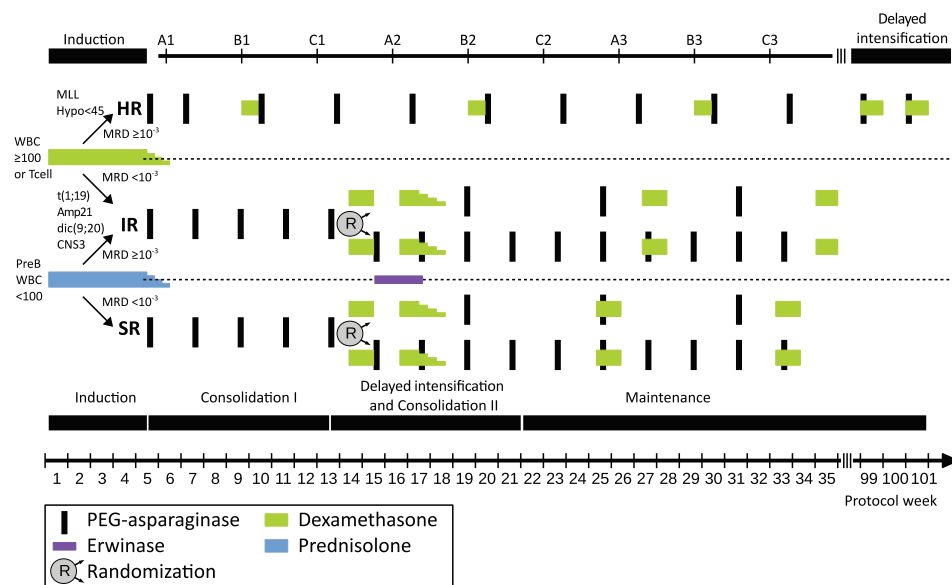


Fig. 1. An outline of PEG-asparaginase and concomitant glucocorticosteroid therapy in the NOPHO ALL2008 protocol. At diagnosis patients with white blood cell (WBC) count $\geq 100 \times 10^9/L$ or T-cell leukemia are assigned dexamethasone (10 mg/m²/day p.o., days 1–21). Doses are tapered over 9 days after day 21). Patients with B-cell precursor leukemia (preB) and WBC $< 100 \times 10^9/L$ are assigned prednisolone (60 mg/m²/day orally, days 1–29. Doses are tapered over 9 days after day 29). Based on cytogenetics (MLL rearrangement, Hypodiploidy < 45 , t(1;19), Amp21, dic(9;20), CNS3 status, and minimal residual disease (MRD), patients are assigned to three treatment risk groups on day 29 (SR, standard risk; IR, intermediate risk; HR, high risk). During delayed intensification IR and SR receive dexamethasone 10 mg/m² orally, days 92–99 and 106–113. In the maintenance phase, SR patients receive dexamethasone (orally 6 mg/m² for 5 days, weeks 25 and 33), where IR patients receive the same dose week 27 and 35. HR patients receive dexamethasone (20 mg/m²/day) days 1–5 in each B block and during delayed intensification orally or i.v. 10 mg/m²/day. A, B, and C symbolize three different blocks of combination chemotherapy in the HR treatment. PEG-asparaginase dose is 1,000 IU/m². The purple horizontal bar between SR and IR indicate 2 weeks of Erwinase treatment (20,000 IU/m²/dose three times a week for 2 weeks) only if PEG-asparaginase is discontinued due to allergy. For more details on the treatment, see Supplementary Tables SI and SII.

three times a week for 2 weeks in the subsequent delayed intensification (Fig. 1). For patients in the HR treatment group, Erwinase were to be administered three times a week for 1 week in the subsequent blocks and delayed intensification phase.

Toxicity Registration and Questionnaires

As part of the NOPHO ALL2008 protocol, an online toxicity registry was developed [17]. Asparaginase allergy is among the 20 pre-defined serious adverse events (SAEs) registered during ALL-treatment. Thus, for all patients on protocol, clinicians registered at three months interval, the occurrence or absence of asparaginase allergy during the preceding three months. The centre response rate to the requested registration is reported to be nearly 99% [17]. Asparaginase associated allergic reactions were graded 1–5 according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.0.

Allergic reactions were defined as follows: grade 1 (transient flushing or rash; drug fever $<38^{\circ}\text{C}$); grade 2 (rash; flushing; urticaria; dyspnea; drug fever $\geq 38^{\circ}\text{C}$); grade 3 (symptomatic bronchospasm, with or without urticaria; parenteral medication(s) indicated; allergy related edema/angioedema; hypotension; grade 4 (anaphylaxis); and grade 5 (death). The registration of asparaginase allergy was mandatory only if allergies was considered severe and/or lead to a change in asparaginase therapy. As part of the present study, treating centers were asked to complete a detailed questionnaire regarding the clinical aspects of asparaginase allergy (i.e., the number of asparaginase injections prior to the allergic reaction, severity of the allergic reaction, time from injection to allergic symptoms, mixing of PEG-asparaginase with lidocaine prior to the intramuscular injection, drugs used to treat the allergic reaction, discontinuation of PEG-asparaginase (Oncaspar[®]), substitution with Erwinase, and presence of other allergies) (Questionnaire form in supplementary online material). Other allergies were defined as Atopy (atopic dermatitis, asthma, allergic rhinitis, and allergic conjunctivitis), drug allergy or food allergy.

Statistics

Statistical analyses were performed using SPSS statistical package for social sciences, version 22.0 (SPSS, Chicago Illinois). Comparisons of continuous variables (i.e., age and white blood cell count at diagnosis) were performed with Mann–Whitney U test for independent samples. Comparisons of categorical variables (i.e., sex, Immunophenotype, and risk groups) were performed with χ^2 -test or Fisher's exact test when any expected value was below five. The cumulative risk of PEG-asparaginase allergy was calculated using the Kaplan–Meier method. Patients that discontinued treatment due to other events (i.e., pancreatitis, relapse, death in complete remission, stem cell transplant, or secondary malignancy) were censored at the time of the event. A Cox proportional hazard model was used for calculations of hazard ratios for PEG-asparaginase allergy. A two-sided P -value of <0.05 was considered statistically significant.

RESULTS

PEG-Asparaginase Allergy

A total of 615 children were included in this study. In the NOPHO ALL2008 toxicity registry 82 patients (1–17 years) were registered with PEG-asparaginase allergy. Three of 82 children were incorrectly registered (they were suspected to have an allergic

reaction to food or other drugs), leaving a total of 79 evaluable patients with clinical PEG-asparaginase allergy. Questionnaires with data on all 79 patients were completed and returned. The cumulative risk of allergy was 13.2%, being 12.3% for SR, 11.6% for IR, and 20% for all HR (Fig. 2). These differences were not statistically significant, log rank, ($P=0.20$). A comparison of allergy-risk in patients in the HR group versus allergy risk in patients in the two lower risk groups (SR- + IR) yielded a borderline statistical significant difference in risk between the HR groups and the lower risk groups, log rank, ($P=0.08$). Details of patients with and without PEG-asparaginase allergy are outlined in Table I. Only age distribution was significantly different in the two groups ($P=0.02$), with a slightly lower median age in the allergic patients. There was no statistical significant difference between the allergic and non-allergic patients with respect to gender ($P=0.72$), immunophenotype ($P=0.15$), risk group ($P=0.17$), or white cell blood count at diagnosis ($P=0.59$).

In a Cox multivariate regression analysis that included sex, age, white blood cell count at diagnosis, and immunophenotype (B-cell precursor and T-Cell), PEG-asparaginase allergy was not statistically significantly associated with any of these variables (Supplementary Table SIII). (Bi-lineage immunophenotype was excluded as this group of patients consisted of only six patients in total). All 79 patients discontinued PEG-asparaginase treatment due to their allergic reaction. However, in two patients registered with allergic reactions (grade 2 and 3) to the second PEG-asparaginase dose, PEG-asparaginase was continued until a new reaction (grade 3 and 4) occurred later in treatment. In another patient, an anaphylactic reaction occurred after the fourth dose of PEG-asparaginase, which was

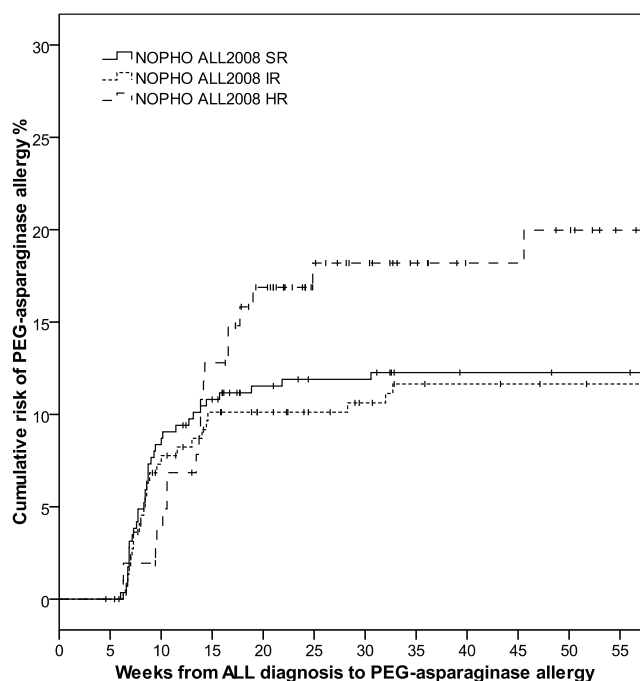


Fig. 2. Kaplan–Meier plot showing the cumulative risk of PEG-asparaginase allergy by treatment risk groups. SR (standard risk), IR (intermediate risk), HR (high-risk chemotherapy + high-risk chemotherapy and hematopoietic stem cell transplant). Acute lymphoblastic leukemia (ALL). Censored patients are marked with vertical bars, (I).

TABLE I. Clinical Characteristics

Patients in total N = 615	Patients with PEG-asp allergy	Patients without PEG-asp allergy	P-Value
Number of patients	79 (12.8%)	536 (87.2%)	
Sex			
Male/Female	40/39 (51%/49%)	285/251 (53%/47%)	0.72
Risk group			
SR/IR/HR	35/25/19 (44%/32%/24%)	254/198/84 (47%/37%/16%)	0.17
Immunophenotype			
BCP/TCL/Bi-lineage	64/13/2 (81%/16%/3%)	461/71/4 (86%/13%/1%)	0.15
Age in years			
Median (range)	3 (1–17)	4 (1–17)	0.02
WBC ($\times 10^9/L$)			
Median (range)	12.3 (0.9–598.0)	10.7 (0.4–1,161.0)	0.59
Trisomy 21	1	16	

SR, standard risk; IR, intermediate risk; HR, high-risk chemotherapy and hematopoietic stem cell transplant; BCP, B-cell precursor; TCL, T-cell; WBC, white blood cell count at diagnosis, PEG-asp, PEG-asparaginase.

mistakenly given intravenously. This reaction occurred within minutes from initiation of i.v. administration. No lidocaine was added to this preparation. Additionally, in 14 of 79 patients, allergy was suspected earlier in treatment, but not registered, as the symptoms were not regarded as PEG-asparaginase allergy at the time they occurred. Symptoms included stomach pain, nausea, vomiting, urticaria, rash, local infiltration at injection site, edema lower lip, and flushing. However, these reactions were either transient, occurred before or late after PEG-asparaginase, or could be explained by other drugs

administered concomitantly. Thus asparaginase was continued until further more evident reactions presented themselves.

PEG-asparaginase allergy developed after a median of 2 doses (75% range 2–4, max 14) and was primarily moderate in severity (grade 2–3 allergy, NCI CTCAE) (Fig. 3). After five doses of PEG-asparaginase, 92% of allergic reacting patients had presented with their allergic reaction. Anaphylactic reactions (grade 4 reactions) were observed only in relation to the 2nd, 3rd, and 4th injection, whereas milder reactions were observed up to the 14th dose of PEG-asparaginase, (Fig. 3).

The allergic symptoms included drug fever, flushing, rash, pruritus, urticaria, edema, bronchospasm, hypotension, and grade 4 anaphylactic reactions. Nine patients (11%) developed an anaphylactic reaction, but none were fatal. All anaphylactic reactions developed within 1 hr and 50 min. Grade 3, 2, and 1 reactions were observed in 36, 29, and 5 patients, respectively. Of 79 patients, only two patients received no supportive treatment as their allergic reactions were mild. Three received corticosteroids only, nine antihistamines only, and depending on symptoms, the remaining 68 patients were treated with combinations of antihistamines, corticosteroids, adrenaline, intravenous fluids, oxygen, and β -2-agonists.

Forty-six patients (58%) developed an allergic reaction within 2 hr after PEG-asparaginase administration whereas 23 patients, (29%) had the reaction between 2 and 6 hr after the intramuscular injection. The latest allergic reaction occurred in the time interval between 24 and 36 hr after the administration of PEG-asparaginase. Figure 4 shows the severity of the reactions over time from injection to development of the allergic reaction.

In the NOPHO ALL2008 protocol L-asparaginase is allowed to be mixed with lidocaine before the intramuscular injection to make the treatment less painful [19]. Of 79 patients reacting with allergy, 37 had received PEG-asparaginase mixed with lidocaine, and 39 had not, in three patients this information was missing. There was no statistically significant difference in severity of the allergic reactions (grade 1–4) between patients who had received asparaginase mixed with lidocaine and those who had not ($P = 0.42$, Fisher's exact test).

Sixteen patients, (20%) were reported to have other allergies. Atopy was predominant among those suffering from other allergies (9/16), but food and drug allergy was also reported and some had more than one other allergy. This information was not available for patients without reactions to PEG-asparaginase.

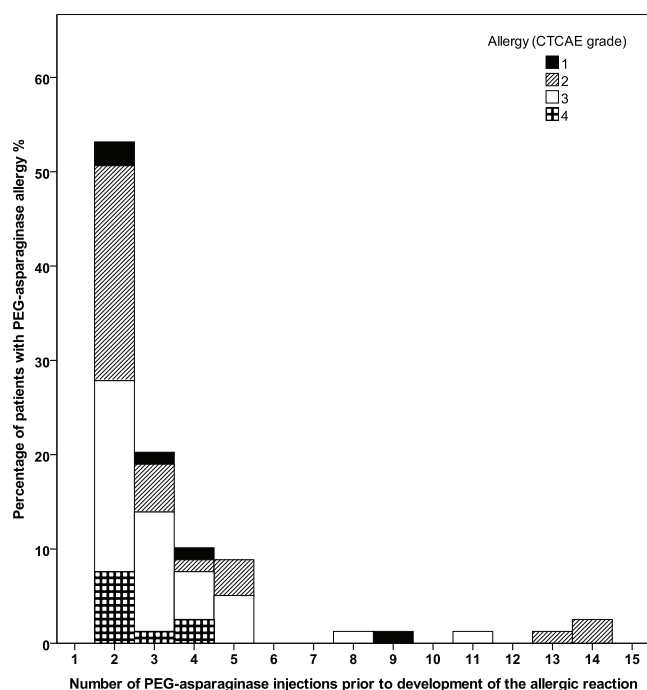


Fig. 3. Number of PEG-asparaginase doses given (including the one that gave the allergic reaction) prior to development of allergy. Patterns denote the severity of allergy CTCAE grade 1–4 (NCI Common Terminology Criteria for serious Adverse Events). Grade 1 being a mild allergic reaction and 4 being an anaphylactic reaction. Patients in total (N = 79).

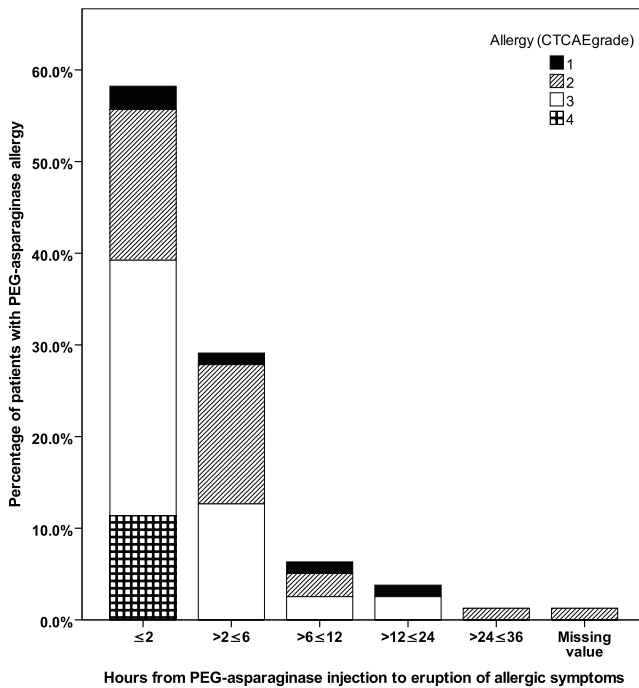


Fig. 4. Time (hours) from PEG-asparaginase administration to development of allergic symptoms. Patterns denote the severity of allergy CTCAE grade 1–4 (NCI Common Terminology Criteria for serious Adverse Events). Grade 1 being a mild allergic reaction and 4 being an anaphylactic reaction. Patients in total (N = 79).

Erwinase Allergy

Among 79 patients with clinical allergy to PEG-asparaginase, 74 were eligible for receiving subsequent Erwinase substitution as the allergic reaction occurred before the beginning of delayed intensification I (scheduled Erwinase for SR and IR).

Of 74 eligible patients 68 patients, (including 30 SR-, 21 IR-, and 16 HR-patients) received Erwinase. Reasons for not giving Erwinase were as follows; one patient had a severe urticaria reaction to PEG-asparaginase; another patient had a previous pulmonary thrombosis during PEG-asparaginase therapy; and one had died before Erwinase was scheduled. In three patients the reason for omitting Erwinase was uncertain. Four of the 68 patients (6%) developed clinical allergy to Erwinase (one SR-, one IR-, and two HR-patients). The allergic reactions to Erwinase all appeared within 2 hr after the injection. Two of four patients (one with a previous anaphylactic reaction to PEG-asparaginase and one with a grade 2 reaction) both had an anaphylactic reaction to Erwinase. The remaining two patients, one with a grade 2 and one with a grade 3 reaction to PEG-asparaginase reacted with similar severity to Erwinase. The number of doses administered prior to reaction towards Erwinase ranged 2–7.

DISCUSSION

Although asparaginase allergy is a well described adverse effect, this is the first detailed report of PEG-asparaginase allergy during prolonged first-line PEG-asparaginase therapy based on a large cohort of pediatric patients. In this cohort, PEG-asparaginase allergy was observed with a cumulative incidence of 13.2%. This is

in line with that reported in other studies using i.m. PEG-asparaginase [1,20]. As the recommendation for registering allergy applied only to severe reactions, there may have been mild reactions, which were not reported. Only few children (6%) developed a subsequent allergic reaction to Erwinase. However, Erwinase was only given during a short period in the majority of patients (SR and IR), and a more prolonged treatment may lead to more allergic reactions.

We report allergy after a median of 2 doses of PEG-asparaginase, whereas other studies have reported asparaginase allergy almost exclusively after several doses [2,3,8,14]. We speculate that the early occurrence of allergy in the NOPHO ALL2008 protocol compared to other settings may reflect the use of first-line PEG-asparaginase instead of native *E. coli* L-asparaginase and also the introduction of PEG-asparaginase post induction, when glucocorticosteroids are being tapered. Most other protocols introduce L-asparaginase in induction, concurrent with full dose of glucocorticosteroids [15]. Pidaparti and Bostrom compared the frequency and severity of allergic reactions to PEG-asparaginase when given intravenously (i.v.) versus intramuscularly (i.m.) [20]. They reported PEG-asparaginase allergy to occur after a median of three doses (both i.v. and i.m.) which is in accordance with our findings. Moreover, they reported no difference in the frequency and severity of clinical allergic reactions between i.v. and i.m. administration indicating that our findings may be transferrable to an i.v. regime.

The severity of the allergic reactions in relation to time after PEG-asparaginase injection has not been reported before. We found all of the anaphylactic reactions (grade 4) to develop within 1 hr and 50 min after intramuscular injection, which would be expected from type 1 allergic IgE mediated reactions. Grade 3 reactions were observed as late as 12–24 hr after PEG-asparaginase administration (Fig. 4). From a clinical point of view this time factor is highly relevant to decide, when it is safe to discharge the child from hospital. Considering the observed time to presentation of anaphylactic reactions it appears reasonable to recommend a minimum of 2 hr in hospital observation.

We found no statistically significant different risk of allergy among the treatment risk groups. However, we suspect that the apparently higher risk seen in patients receiving HR treatment compared with the patients in the SR and IR treatment groups (Fig. 2) may reflect a longer interval between doses (Fig. 1) in the HR treatment. Liu et al. report that a prolonged time interval between doses that is, time off asparaginase exposure, may enhance the risk of clinical allergy [14]. As asparaginase enzyme activity analysis was not included in this study it is not possible to estimate the times off asparaginase exposure. Moreover, the risk groups are different in terms of disease biology. Whether this is of importance for the susceptibility of developing allergy is unknown.

Allergy is a multi-factorial disease with a high prevalence in the general population, and the prevalence differs with age, local environment, country, and over the years [21,22]. In 2012, Mallol et al. reported the prevalence of asthma, rhinoconjunctivitis, and eczema in a large worldwide study (International Study of Asthma and Allergies in Childhood, ISAAC), on children age 6–7 and 13–14 [22]. They found the prevalence of asthma, rhinoconjunctivitis, and eczema to be 11.7%, 8.5%, and 7.9% respectively in 6–7 year old children. Food allergy and drug allergy was not described. We found that 11% of the PEG-asparaginase hypersensitive patients had atopic disease (including asthma,

rhinoconjunctivitis and atopic eczema). Since, we had no data on “other allergies” (atopy, food allergy and drug allergy) in the non-PEG-asparaginase allergic patients it is not possible from this survey to conclude whether presence of “other allergies” is a predictor of development of asparaginase hypersensitivity.

In this survey, half the patients with allergy had received PEG-asparaginase mixed with lidocaine to reduce the treatment related pain; the other half had not. Among clinicians there is a debate whether the use of lidocaine may blur the allergic symptoms or not, given that possible adverse effects of lidocaine (nausea, hypotension, dizziness, and vomiting) may in some ways mimic symptoms of allergy[23]. We cannot rule out this possibility, though the low dose used, makes it less likely. As we had no information on the use of lidocaine in the patients without allergy, it is not possible to evaluate if the use of lidocaine interferes with the frequency of the allergic reactions. However, we found no difference in severity (CTCAE grade) of the allergic reactions in patients who had received lidocaine compared to those without lidocaine.

The importance of PEG-asparaginase allergy falls into three categories: (1) Clinical aspects, that is, occurrence and severity of the actual allergic reaction; (2) Possible development of neutralizing antibodies which may lead to rapid clearance of the drug that is, ineffective treatment; and (3) Possible association with survival outcomes because of the truncation of asparaginase therapy.

In this study, we have only addressed the first issue. It is still too early to evaluate if PEG-asparaginase allergy in the NOPHO ALL2008 protocol is associated with poorer event-free survival or relapse patterns as median follow-up is relatively short. However, truncated L-asparaginase therapy has been associated with inferior event-free survival in other studies and proper substitution with another asparaginase formulation in case of allergy may be highly important [10–12].

The second issue concerns monitoring of enzyme activity and antibodies. Silent (no clinical allergic reaction) anti-asparaginase antibodies of IgG type as well as IgG antibodies in patients with overt clinical allergy have been reported to be associated with fast clearance of all three types of asparaginases (native *E. coli*, Erwinase, and PEG-asparaginase) [14,24–27]. Yet, only one study using PEG-asparaginase as first-line therapy for newly diagnosed ALL has reported on the development of IgG anti-PEG-asparaginase-antibodies and they did not find an association between anti-bodies and low enzyme activity [5].

Direct monitoring of enzyme activity is on the other hand an effective way of evaluating if asparaginase is pharmacologically effective and thus more important than antibody analysis. Moreover, therapeutic drug monitoring of asparaginase enzyme activity levels may support the decision whether or not to switch to another asparaginase preparation when mild clinical allergy is suspected.

Although the registration of allergic reactions was done prospectively, a limitation to the study was the retrospective collection of questionnaire data. Thus, we depended on information from the patient files to be accurate and complete.

In conclusion, we found PEG-asparaginase allergy with a cumulated incidence of 13.2%. In the majority of patients, reactions occurred after only two doses of PEG-asparaginase and were moderate in severity. No anaphylactic reactions developed later than 2 hr after administration of PEG-asparaginase, and thus it may be safe to discharge patients from hospital after 2 hr of observation.

Pediatr Blood Cancer DOI 10.1002/pbc

The early occurrence of allergy emphasizes the need for second line therapy with an alternative asparaginase preparation.

ACKNOWLEDGMENTS

We sincerely thank research nurse Carina Hallberg (Queen Silvias Childrens Hospital, Gothenburg, Sweden) and research nurse Carina Rinaldo (Department of Woman and Child Health, Karolinska institute, Stockholm, Sweden) for their valuable assistance in the collection of data.

REFERENCES

- Silverman LB, Gelber RD, Dalton VK, Asselin BL, Barr RD, Clavell LA, Hurwitz CA, Moghribi A, Samson Y, Schorin MA, Arkin S, Declercq L, Cohen HJ, Sallan SE. Improved outcome for children with acute lymphoblastic leukemia: Results of Dana-Farber Consortium Protocol 91-01. *Blood* 2001;97:1211–1218.
- Wang B, Relling MV, Storm MC, Woo MH, Ribeiro R, Pui CH, Hak LJ. Evaluation of immunologic crossreaction of anti-asparaginase antibodies in acute lymphoblastic leukemia (ALL) and lymphoma patients. *Leukemia* 2003;17:1583–1588.
- Woo MH, Hak LJ, Storm MC, Sandlund JT, Ribeiro RC, Rivera GK, Rubnitz JE, Harrison PL, Wang B, Evans WE, Pui CH, Relling MV. Hypersensitivity or development of antibodies to asparaginase does not impact treatment outcome of childhood acute lymphoblastic leukemia. *J Clin Oncol* 2000;18:1525–1532.
- Evans WE, Tsitsis A, Rivera G, Murphy SB, Dahl GV, Denison M, Crom WR, Barker LF, Mauer AM. Anaphylactoid reactions to *Escherichia coli* and *Erwinia* asparaginase in children with leukemia and lymphoma. *Cancer* 1982;49:1378–1383.
- Avramis VI, Sencer S, Periclou AP, Sather H, Bostrom BC, Cohen LJ, Ettinger AG, Ettinger LJ, Franklin J, Gaynon PS, Hilden JM, Lange B, Majlessipour F, Mathew P, Needle M, Neglia J, Reaman G, Holcenberg JS, Stork L. A randomized comparison of native *Escherichia coli* asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. *Blood* 2002;99:1986–1994.
- Muller HJ, Beier R, Loning L, Blutters-Sawatzki R, Dorffel W, Maass E, Muller-Weihrich S, Scheel-Walter HG, Scherer F, Stahne K, Schrappe M, Horn A, Lumkemann K, Boos J. Pharmacokinetics of native *Escherichia coli* asparaginase (Asparaginase medac) and hypersensitivity reactions in ALL-BFM 95 reinduction treatment. *Br J Haematol* 2001;114:794–799.
- Wacker P, Land VJ, Camitta BM, Kurtzberg J, Pullen J, Harris MB, Shuster JJ. Allergic reactions to *E. coli* L-asparaginase do not affect outcome in childhood B-precursor acute lymphoblastic leukemia: A Children's Oncology Group Study. *J Pediatr Hematol Oncol* 2007;29:627–632.
- Woo MH, Hak LJ, Storm MC, Evans WE, Sandlund JT, Rivera GK, Wang B, Pui CH, Relling MV. Anti-asparaginase antibodies following *E. coli* asparaginase therapy in pediatric acute lymphoblastic leukemia. *Leukemia* 1998;12:1527–1533.
- Narta UK, Kanwar SS, Azmi W. Pharmacological and clinical evaluation of L-asparaginase in the treatment of leukemia. *Crit Rev Oncol Hematol* 2007;61:208–221.
- Sirvent N, Suciu S, Rialland X, Millot F, Benoit Y, Plantaz D, Ferster A, Robert A, Lutz P, Nelken B, Plouvier E, Norton L, Bertrand Y, Otten J. Prognostic significance of the initial cerebro-spinal fluid (CSF) involvement of children with acute lymphoblastic leukaemia (ALL) treated without cranial irradiation: Results of European Organization for Research and Treatment of Cancer (EORTC) Children Leukemia Group study 58881. *Eur J Cancer* 2011;47:239–247.
- Silverman LB, Stevenson KE, O'Brien JE, Asselin BL, Barr RD, Clavell L, Cole PD, Kelly KM, Laverdiere C, Michon B, Schorin MA, Schwartz CL, O'Holleran EW, Neuberg DS, Cohen HJ, Sallan SE. Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985–2000). *Leukemia* 2010;24:320–334.
- Moghribi A, Levy DE, Asselin B, Barr R, Clavell L, Hurwitz C, Samson Y, Schorin M, Dalton VK, Lipshultz SE, Neuberg DS, Gelber RD, Cohen HJ, Sallan SE, Silverman LB. Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia. *Blood* 2007;109:896–904.
- Vrooman LM, Supko JG, Neuberg DS, Asselin BL, Athale UH, Clavell L, Kelly KM, Laverdiere C, Michon B, Schorin M, Cohen HJ, Sallan SE, Silverman LB. *Erwinia* asparaginase after allergy to *E. coli* asparaginase in children with acute lymphoblastic leukemia. *Pediatr Blood Cancer* 2010;54:199–205.
- Liu C, Kawedia JD, Cheng C, Pei D, Fernandez CA, Cai X, Crews KR, Kaste SC, Panetta JC, Bowman WP, Jeha S, Sandlund JT, Evans WE, Pui CH, Relling MV. Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia. *Leukemia* 2012;26:2303–2309.
- Pieters R, Hunger SP, Boos J, Rizzari C, Silverman L, Baruchel A, Goekbuget N, Schrappe M, Pui CH. L-asparaginase treatment in acute lymphoblastic leukemia: A focus on *Erwinia* asparaginase. *Cancer* 2011;117:238–249.
- Toft N, Birgens H, Abrahamsson J, Bernell P, Griskevicius L, Hallbook H, Heyman M, Holm MS, Hulegardh E, Klausen TW, Marquart HV, Jonsson OG, Nielsen OJ, Quist-Paulsen P, Taskinen M, Vaitkeviciene G, Vetterranta K, Asberg A, Schmiegelow K. Risk group assignment differs for children and adults 1–45 yr with acute lymphoblastic leukemia treated by the NOPHO ALL-2008 protocol. *Eur J Haematol* 2013;90:404–412.
- Frandsen TL, Heyman M, Abrahamsson J, Vetterranta K, Sberg A, Vaitkeviciene G, Prunsild K, Toft N, Birgens H, Hallbook H, Quist-Paulsen P, Griskevicius L, Helt L, Hansen BV, Schmiegelow K. Complying with the European Clinical Trials directive while surviving the administrative pressure - An alternative approach to toxicity registration in a cancer trial. *Eur J Cancer* 2014;50:251–259.
- Raja RA, Schmiegelow K, Albertsen BK, Prunsild K, Zeller B, Vaitkeviciene G, Abrahamsson J, Heyman M, Taskinen M, Harila-Saari A, Kanerva J, Frandsen TL. Asparaginase-associated pancreatitis in children with acute lymphoblastic leukaemia in the NOPHO ALL2008 protocol. *Br J Haematol* 2014;165:126–133.
- Albertsen BK, Hasle H, Clausen N, Schroder H, Jakobsen P. Pain intensity and bioavailability of intramuscular asparaginase and a local anesthetic: A double-blinded study. *Pediatr Blood Cancer* 2005;44:255–258.
- Pidaparti M, Bostrom B. Comparison of allergic reactions to pegasparaginase given intravenously versus intramuscularly. *Pediatr Blood Cancer* 2012;59:436–439.

21. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, Williams H. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 2006;368:733–743.
22. Mallol J, Crane J, von ME, Odhiambo J, Keil U, Stewart A. The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three: A global synthesis. *Allergol Immunopathol (Madr)* 2013;41:73–85.
23. Harmatz A. Local anesthetics: uses and toxicities. *Surg Clin North Am* 2009;89:587–598.
24. Albertsen BK, Schroder H, Jakobsen P, Avramis VI, Muller HJ, Schmiegelow K, Carlsen NT. Antibody formation during intravenous and intramuscular therapy with Erwinia asparaginase. *Med Pediatr Oncol* 2002;38:310–316.
25. Willer A, Gerss J, Konig T, Franke D, Kuhnelt HJ, Henze G, von SA, Moricke A, Schrappe M, Boos J, Lanvers-Kaminsky C. Anti-Escherichia coli asparaginase antibody levels determine the activity of second-line treatment with pegylated E coli asparaginase: A retrospective analysis within the ALL-BFM trials. *Blood* 2011;118:5774–5782.
26. Panosyan EH, Seibel NL, Martin-Aragon S, Gaynon PS, Avramis IA, Sather H, Franklin J, Nachman J, Ettinger LJ, La M, Steinherz P, Cohen LJ, Siegel SE, Avramis VI. Asparaginase antibody and asparaginase activity in children with higher-risk acute lymphoblastic leukemia: Children's Cancer Group Study CCG-1961. *J Pediatr Hematol Oncol* 2004;26:217–226.
27. Tong WH, Pieters R, Kaspers GJ, Te Loo DM, Bierings MB, van den Bos C, Kollen WJ, Hop WC, Lanvers-Kaminsky C, Relling MV, Tissing WJ, van der Sluis IM. A prospective study on drug monitoring of PEGasparaginase and Erwinia asparaginase and asparaginase antibodies in pediatric acute lymphoblastic leukemia. *Blood* 2014;123:2026–2033.

PEG-asparaginase allergy in children with acute lymphoblastic leukemia in the NOPHO ALL2008 protocol

Louise Tram Henriksen, Arja Harila-Saari, Ellen Ruud, Jonas Abrahamsson, Kaie Pruunsild, Goda Vaitkeviciene, Ólafur Gisli Jónsson, Kjeld Schmiegelow, Mats Heyman, Henrik Schröder, Birgitte Klug Albertsen.

Supplementary Table I

Table I. Treatment of patients with standard risk and intermediate risk ALL in the NOPHO ALL2008 protocol.

	Non-HR ALL (BCP-ALL WBC <100 × 10 ⁹ /l)	HR-ALL (T-ALL and/or WBC >100 × 10 ⁹ /l)
Induction	Prednisolone 60 mg/m ² /day p.o., days 1–29 Doxorubicin 40 mg/m ² /day i.v., days 1 and 22 Vincristine i.v. 2.0 mg/m ² /days 1, 8, 15, 22 and 29† Methotrexate i.t., days 1, 8, 15 and 29‡	Dexamethasone 10 mg/m ² /day p.o., days 1–21. Doses tapered over 9 d after day 21§ Doxorubicin 40 mg/m ² /day i.v., days 1 and 22 Vincristine i.v. 2.0 mg/m ² , days 1, 8, 15, 22 and 29† Methotrexate i.t. days 1, 8, 15 and 29‡,¶
	Standard risk	Intermediate risk
Consolidation I	HDM 5000 mg/m ² , days 36, 57 and 78 Methotrexate i.t., days 37, 58 and 79‡ Vincristine 2.0 mg/m ² i.v., days 43 and 64† Oral 6-MP 25–75 mg/m ² , days 30–85** PEG-asparaginase 1000 iu/m ² i.m., days 30, 43, 57, 71 and 85	As standard risk apart from CNS3¶
Delayed intensification I and consolidation II	Dexamethasone 10 mg/m ² p.o., days 92–99 and 106–113§ Methotrexate i.t., day 120‡ 6-thioguanine 60 mg/m ² p.o., days 113–127††,‡‡ Vincristine 2.0 mg/m ² i.v., days 92, 99, 106 and 113‡ Ara-C i.v. or i.m. 75 mg/m ² , days 115–118 and 122–125 PEG-asparaginase 1000 iu/m ² i.m., days 99*, 113* and 127	As standard risk apart from: Daunorubicin 30 mg/m ² i.v., days 92, 99, 106 and 113. Cyclophosphamide 1000 mg/m ² , day 127 Methotrexate i.t., days 92 and 127§§
Maintenance I	6-MP 75 mg/m ² p.o., day 134 until 2.5 years from the initial diagnosis‡‡ Methotrexate 20 mg/m ² p.o., once a week from week 20 until 2.5 years after initial diagnosis Dexamethasone p.o. 6 mg/m ² for 5 d, weeks 25, 33, 41, 49 and 57 Vincristine 2.0 mg/m ² , weeks 25, 33, 41, 49 and 57† HDM 5000 mg/m ² , weeks 21, 29, 37, 45 and 53 Methotrexate i.t., weeks 21, 29, 37, 45 and 53‡ PEG-asparaginase 1000 iu/m ² i.m., weeks 21, 23*, 25, 27*, 29*, 31 and 33*	6-MP 75 mg/m ² p.o., day 148 until 2.5 years from the initial diagnosis‡‡ Methotrexate 20 mg/m ² p.o., once a week from week 22 until 2.5 years after initial diagnosis Dexamethasone p.o. 6 mg/m ² for 5 d on weeks 27, 35, 43 and 51 Vincristine 2.0 mg/m ² , weeks 27, 35, 43 and 51 HDM 5000 mg/m ² , weeks 23, 31, 39, 47 and 55 Methotrexate i.t., weeks 23, 31, 39, 47 and 55‡,¶ PEG-asparaginase 1000 iu/m ² i.m., weeks 21, 23*, 25, 27*, 29*, 31 and 33*
Delayed intensification II and consolidation II		Dexamethasone 10 mg/m ² p.o. daily, weeks 60 and 62§ Vincristine 2.0 mg/m ² , weeks 60, 61, 62 and 63† Methotrexate i.t., weeks 60 and 65‡,¶ Cyclophosphamide 1000 mg/m ² , week 63 6-thioguanine 60 mg/m ² , weeks 63 and 64†† Ara-C i.v. or i.m. 75 mg/m ² /dose over 4 d in week 63 and over 4 d in week 64
Maintenance II	Daily 6-MP 75 mg/m ² p.o. is continued until 2.5 years from diagnosis Weekly Methotrexate 20 mg/m ² p.o. is continued until 2.5 years from diagnosis	Daily 6-MP p.o. is continued until 2.5 years from diagnosis Weekly Methotrexate p.o. is continued until 2.5 years from diagnosis Methotrexate i.t. given every 8 weeks until the end of maintenance‡,¶

HR, high risk; ALL, acute lymphoblastic leukaemia; BCP, B-cell precursor; WBC, white blood cell count; p.o., per oral; i.v., intravenous; i.m., intramuscular; i.t., intrathecally; 6-MP, 6-mercaptopurine; HDM, high dose methotrexate; Ara-C, cytosine arabinoside; PEG-asparaginase, pegylated L- asparaginase.

*These doses are not given to patients in the experimental arm of treatment.

†Maximum dose 2.5 mg for patients aged <18 years and 2.0 mg for those >18 years.

‡Age-adjusted doses: 1.0–1.9 years, 8 mg; 2.0–2.9 years, 10 mg; >3.0 years, 12 mg.

§Dexamethasone is tapered over 9 d with 50% for the first 3 d, 25% next 3 d and 12.5% for the final 3 d.

¶If CNS3 involvement found at diagnosis, triple intrathecal therapy (TIT; methotrexate, cytarabine and prednisolone succinate) is given. CNS3 is defined as presence of one of the following: (i) 5 cells/μl cerebrospinal fluid compatible with leukaemic blasts on cytospin, (ii) cranial nerve palsy, (iii) intracranial leukaemic mass on magnetic resonance imaging (MRI) and (iv) retinal involvement confirmed by MRI or biopsy (not mandatory).

**Dosage is reduced to 5 mg/m² for patients with thiopurine methyltransferase (TPMT) deficiency.

††Dosage reduced to 20 mg/m² for patients with TPMT deficiency.

‡‡Dosage reduced to 5 mg/m² for patients with TPMT deficiency. Dosage reduced to 50 mg/m² for patients with TPMT heterozygosity.

§§In case of CNS3, TIT is given.

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PEG-asparaginase allergy in children with acute lymphoblastic leukemia in the NOPHO ALL2008 protocol

Louise Tram Henriksen, Arja Harila-Saari, Ellen Ruud, Jonas Abrahamsson, Kaie Pruunsild, Goda Vaitkeviciene, Ólafur Gisli Jónsson, Kjeld Schmiegelow, Mats Heyman, Henrik Schröder, Birgitte Klug Albertsen.

Supplementary Table II.

Table II. Treatment of patients with high risk ALL in NOPHO ALL2008.

HR treatment	T-ALL or WBC $\geq 100 \times 10^9/l$
Induction	Dexamethasone 10 mg/m ² /day p.o., days 1–21. Doses tapered over 9 d after day 21*. Vincristine i.v. 2.0 mg/m ² /day, days 1, 8, 15, 22 and 29† Doxorubicin 40 mg/m ² /day i.v., days 1 and 22 Methotrexate i.t., days 1, 8, 15 and 29‡,§
High Risk Blocks	Treatment given in the order A1, B1, C1, A2, B2, C2, A3, B3, C3
Block A	Cyclophosphamide 440 mg/m ² /dose i.v., days 1–5 VP16 100 mg/m ² /dose i.v., days 1–5 PEG-asparaginase 1000 iu/m ² /dose i.m., day 6 G-CSF 5 µg/kg i.m., s.c. or i.v., administered 1–5 days after PEG-asparaginase injection. Continued for at least 5 days. Triple intrathecal therapy (methotrexate, cytarabine and prednisolone succinate) is given in Block A2 and A3¶
Block B	Oral 6-MP 100 mg/m ² once daily, days 1–5** Dexamethasone 20 mg/m ² /day given in three daily doses 20 mg/m ² , days 1–5 Vincristine 2.0 mg/m ² , days 1 and 6† HDM 5000 mg/m ² i.v., on day 1 HD-Ara-C 2.0 mg/m ² twice daily, days 5 and 6 Triple intrathecal therapy (methotrexate, cytarabine and prednisolone succinate), day 1¶ PEG-asparaginase 1000 iu/m ² i.m., day 6 G-CSF 5 µg/kg i.m., s.c. or i.v., from day 7. Continued for at least 5 days
Block C	Triple intrathecal therapy (methotrexate, cytarabine and prednisolone succinate), day 1¶ Fludarabine 30 mg/m ² /dose i.v., days 2, 3, 4, 5 and 6 HDAra-C 2.0 g/m ² , i.v. once daily, days 2, 3, 4, 5 and 6 Idarubicin 8 mg/m ² i.v. on days 2 and 4 PEG-asparaginase 1000 iu/m ² /dose i.m. day 6 G-CSF 5 µg/kg i.m., s.c., or i.v. from day 8. Continued for at least 5 days
Maintenance I	6-MP 75 mg/m ² given daily p.o., from week 36 until 2.5 years after diagnosis†† Methotrexate 20 mg/m ² p.o. once a week, from week 36 until 2.5 years after diagnosis HDM 5000 mg/m ² , weeks 42, 66 and 90 Triple intrathecal therapy¶ given alternately, at 6 weeks intervals for the first 60 weeks, with i.t. combination therapy‡‡
Delayed intensification	Dexamethasone given orally or i.v. 10 mg/m ² /day (from day 1 in the block) divided into three doses for 2 weeks separated by 1 week (1 week on, 1 week off, 1 week on)§§ Vincristine i.v. 2.0 mg/m ² , days 1, 8, 15 and 22† Triple intrathecal therapy, day 1¶ Cyclophosphamide 1000 mg/m ² i.v., day 22 Oral 6-Thioguaninen 60 mg/m ² once daily, days 22–36¶¶ Ara-C bolus injection given i.v. (or i.m.) 75 mg/m ² , days 24–27 and 31–34. PEG-Asparaginase 1000 iu/m ² i.m., days 1 and 15
Maintenance II	6-MP 75 mg/m ² p.o. – from week 105 until 2.5 years from initial diagnosis. Same dose as tolerated during previous maintenance treatment. Methotrexate 20 mg/m ² p.o., once a week from week 105 until 2.5 years from initial diagnosis. Same dose as tolerated during previous maintenance treatment.

HR, high risk; ALL, acute lymphoblastic leukaemia; WBC, white blood cell count; p.o., per oral; i.v., intravenous; i.m., intramuscular; i.t., intrathecally; s.c., subcutaneous; 6-MP, 6-mercaptopurine; HDM, high dose methotrexate; (HD)Ara-C, (high dose) cytosine arabinoside; PEG-asparaginase, pegylated L- asparaginase; G-CSF, granulocyte colony-stimulating factor.

*Dexamethasone is tapered over 9 d with 50% for the first 3 d, 25% next 3 d and 12.5% for the final 3 d.

†Maximum dose 2.5 mg for patients aged <18 years and 2.0 mg for those >18 years.

‡Age-adjusted doses: 1.0–1.9 years, 8 mg; 2.0–2.9 years, 10 mg; >3.0 years, 12 mg.

§If CNS3 involvement found at diagnosis, triple intrathecal therapy (methotrexate, cytarabine and prednisolone succinate) is given. CNS3 is defined as presence of one of the following: (i) 5 cells/µl cerebrospinal fluid compatible with leukaemic blasts on cytopspin, (ii) cranial nerve palsy, (iii) intracranial leukaemic mass on magnetic resonance imaging (MRI) and (iv) retinal involvement confirmed by MRI or biopsy (not mandatory).

¶Methotrexate 8 mg (1.0–1.9 years), 10 mg (2.0–2.9 years), 12 mg (3.0+ years). Ara-C 20 (1.0–1.9 years), 26 (2.0–2.9 years), 30 (3.0+ years). Prednisolone succinate 12.5 mg (1.0–2.9 years), 16 mg (3+ years).

**Dosage reduced to 10 mg/m² for patients with thiopurine methyltransferase (TPMT) deficiency. Dosage reduced to 50 mg/m² for patients with TPMT heterozygosity.

††Dosage reduced to 5 mg/m² for patients with TPMT deficiency. Dosage reduced to 50 mg/m² for patients with TPMT heterozygosity.

‡‡Depocyte 25 mg (1.0–2.9 years), 35 mg (3.0–11.9 years), 50 mg (12.0+). Prednisolone succinate 12.5 mg (1.0–2.9 years), 16 mg (3.0–11.9 years), 20 mg (12.0+ years).

§§Tapered during the following 9 d (50% for 3 d, 25% for 3 d, 12.5% for 3 d).

¶¶Dosage reduced to 20 mg/m² for patients with TPMT deficiency.

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Supplementary Table III. Cox regression analysis, PEG-asparaginase allergy

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%CI)	P-value	Hazard ratio (95%CI)	P-value
Sex				
Female	1 (<i>reference</i>)		1 (<i>reference</i>)	
Male	0.96 (0.62 - 1.51)	0.87	0.97 (0.62 - 1.51)	0.88
Age (years)	0.96 (0.91 - 1.02)	0.15	0.95 (0.90 - 1.01)	0.10
Immunophenotype				
B- cell precursor	1 (<i>reference</i>)		1 (<i>reference</i>)	
T- cell	1.28 (0.70 - 2.32)	0.42	1.54 (0.76 - 3.13)	0.23
WBC at diagnosis	1.00 (1.00 - 1.00)	0.91	1.00 (1.00 - 1.00)	0.71

WBC (Highest White Blood Cell count at diagnosis); CI (Confidence intervals)

Questionnaire

Asparaginase associated allergy in children and adolescents treated according to the NOPHO ALL-2008 protocol

This patient was registered in the quarterly SAE report as having had an allergic reaction.

NOPHO ID number:

Country:

Center:

First name:

Last name:

PEG-asparaginase:

1. Was the allergic reaction during PEG-asparaginase treatment?

☐ Yes

☐ No

2. How would you grade the allergic reaction using the Common Terminology Criteria (CTCAE v3.0) Grade 1-5?

Please check one of the following boxes:

1 ☐

2 ☐

3 ☐

4 ☐

5 ☐

ALLERGY/IMMUNOLOGY						
Page 1 of 1						
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Allergic reaction/ hypersensitivity (including drug fever)	Allergic reaction	Transient flushing or rash; drug fever <38°C (<100.4°F)	Rash; flushing; urticaria; dyspnea; drug fever ≥38°C (≥100.4°F)	Symptomatic bronchospasm, with or without urticaria; parenteral medication(s) indicated; allergy-related edema/angioedema; hypotension	Anaphylaxis	Death
REMARK: Urticaria with manifestations of allergic or hypersensitivity reaction is graded as Allergic reaction/hypersensitivity (including drug fever).						
ALSO CONSIDER: Cytokine release syndrome/acute infusion reaction.						

3. Date of the allergic reaction? ____/____/____ (DD / MM / YYYY)

4. What date was the PEG-asparaginase given provoking the allergic reaction? ____/____/____ (DD / MM / YYYY)

5. Was PEG-asparaginase mixed with lidocaine?

☐ Yes

☐ No

6. What was the time between administration of PEG-asparaginase and development of the allergic reaction?

☐ < 2 hours

☐ >2 <= 6 hours

☐ >6 <= 12 hours

☐ >12<=24 hours

☐ >24 <= 36 hours

☐ >36

7. What medication was used to treat the allergic reaction?

8. Number of PEG-asparaginase injections administered prior to the allergic reaction (including the one that provoked the allergic reaction)? _____

9. Did the allergic reaction lead to cessation of PEG-asparaginase administration?

☐ Yes

☐ No If No – why? _____

10. Was PEG-asparaginase discontinued for other reasons than allergy?

☐ Yes

☐ No

If yes – when and why?

___/___/_____ (DD / MM / YYYY)

11. Was PEG-asparaginase dosage modified prior to the allergic reaction?

☐ Yes If yes, how was the dosage modified? _____

☐ No

12. Was allergy suspected (mild reactions) in any of the previous PEG-asparaginase administrations prior to the registration in the database?

☐ Yes

☐ No

If yes, please describe the reaction? _____

13. Does the patient suffer from other allergies / atopic disease?

☐ Yes

☐ No

☐ Not known

If yes – which?

☐ Food allergy

☐ Drug allergy

☐ Atopy (atopic dermatitis, asthma, allergic rhinitis, allergic conjunctivitis)

☐ Other allergy - which? _____

Erwinase:

14. Was Erwinase administered instead of PEG-asparaginase as recommended in the ALL-2008 protocol?

☐ Yes

☐ No If No – why? _____

15. Did the patient develop an allergic reaction during Erwinase treatment?

☐ Yes

☐ No

16. How would you grade the allergic reaction using the Common Terminology Criteria (CTCAE v3.0) Grade 1-5.?

Please check one of the following boxes:

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

ALLERGY/IMMUNOLOGY						
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Allergic reaction/ hypersensitivity (including drug fever)	Allergic reaction	Transient flushing or rash; drug fever <38°C (<100.4°F)	Rash; flushing; urticaria; dyspnea; drug fever ≥38°C (≥100.4°F)	Symptomatic bronchospasm, with or without urticaria; parenteral medication(s) indicated; allergy-related edema/angioedema; hypotension	Anaphylaxis	Death
REMARK: Urticaria with manifestations of allergic or hypersensitivity reaction is graded as Allergic reaction/hypersensitivity (including drug fever).						
ALSO CONSIDER: Cytokine release syndrome/acute infusion reaction.						

17. Date of the allergic reaction? ____/____/____ (DD / MM / YYYY)

18. What date was the Erwinase given provoking the allergic reaction? ____/____/____ (DD / MM / YYYY)

19. Was Erwinase mixed with lidocaine?

☐ Yes

☐ No

20. What was the time between administration of Erwinase and development of the allergic reaction?

☐ < 2 hours

☐ >2 <= 6 hours

☐ >6 <= 12 hours

☐ >12<=24 hours

☐ >24 <= 36 hours

☐ >36

21. What medication was used to treat the allergic reaction?

22. Number of Erwinase injections administered prior to the allergic reaction? _____

23. Did the allergic reaction lead to cessation of Erwinase administration?

☐ Yes

☐ No If No – why? _____

Comments:

Questionnaire completed by _____

Date: _____

Contact person in dept. (e-mail address): _____

Please return the questionnaire to the study coordinator

For any questions regarding the questionnaire please contact the study coordinator