A prospective study on drug monitoring of PEGasparaginase and *Erwinia* asparaginase and asparaginase antibodies in pediatric acute lymphoblastic leukemia

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Key Points

- Use of native E coli asparaginase in induction leads to high hypersensitivity rates to PEGasparaginase in intensification.
- Switching to Erwinia asparaginase leads to effective asparaginase activity levels in most patients who experienced an allergy to PEGasparaginase.

This study prospectively analyzed the efficacy of very prolonged courses of pegylated Escherichia coli asparaginase (PEGasparaginase) and Erwinia asparaginase in pediatric acute lymphoblastic leukemia (ALL) patients. Patients received 15 PEGasparaginase infusions (2500 IU/m² every 2 weeks) in intensification after receiving native E coli asparaginase in induction. In case of allergy to or silent inactivation of PEG-asparaginase, Erwinia asparaginase (20,000 IU/m² 2-3 times weekly) was given. Eighty-nine patients were enrolled in the PEGasparaginase study. Twenty (22%) of the PEGasparaginase-treated patients developed an allergy; 7 (8%) showed silent inactivation. The PEGasparaginase level was 0 in all allergic patients (grade 1-4). Patients without hypersensitivity to PEGasparaginase had serum mean trough levels of 899 U/L. Fifty-nine patients were included in the Erwinia asparaginase study; 2 (3%) developed an allergy and none silent inactivation. Ninety-six percent had at least 1 trough level ≥100 U/L. The serum asparagine level was not always completely depleted with Erwinia asparaginase in contrast to PEGasparaginase. The presence of asparaginase antibodies was related to allergies and silent inactivation, but with low specificity (64%). Use of native E coli asparaginase in induction leads to high hypersensitivity rates to PEGasparaginase in intensification. Therefore, PEGasparaginase should be used upfront in induction, and we suggest that the dose could be lowered. Switching to Erwinia asparaginase leads to effective asparaginase levels in most patients. Therapeutic drug monitoring has been added to our ALL-11 protocol to individualize asparaginase therapy. (Blood. 2014;123(13):2026-2033)

Introduction

Asparaginase is an enzymatic drug and an essential component of the combination chemotherapy of childhood acute lymphoblastic leukemia (ALL).1 This drug depletes asparagine in the blood, and the malignant lymphoid cells that depend on extracellular asparagine will thus go into apoptosis.2,3 Currently, several asparaginase agents are available on the market. Either these are derived from Escherichia coli in its native form (native E coli asparaginase) or as a pegylated enzyme (PEGAsparaginase). Otherwise, asparaginase is extracted from Erwinia chrysanthemi (Erwinia asparaginase).

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It has been shown that intensified use of asparaginase increases event-free survival (EFS) for children with ALL by 10% to 15%.4-7 Administration of asparaginase can be limited by the occurrence of hypersensitivity reactions to asparaginase, like allergic or anaphylactic reactions.8 Patients with these reactions are switched to another asparaginase product to ensure that they are exposed to asparaginase according to the treatment plan and to ensure an optimal EFS.9 Clinical allergy is associated with inactivation of asparaginase by antibodies.10,11 Formation of asparaginase antibodies (AAAs) can also neutralize
asparaginase without any clinical signs of hypersensitivity, so-called silent inactivation. Panosyan et al and Vrooman et al showed that children with silent inactivation of native E coli asparaginase had a poorer outcome because they were not switched to alternative asparaginase agents, whereas those with clinically overt allergy were switched and had no poorer outcome. In most protocols, asparaginase is given during the induction course, followed by asparaginase-free consolidation courses, and after that asparaginase is again given during the intensification/induction course. The majority of hypersensitivity reactions occur during the intensification phase.

The Dutch Childhood Oncology Group (DCOG) ALL-10 protocol used native E coli asparaginase in induction and the less immunogenic PEGasparaginase in the intensification phase in an attempt to prevent hypersensitivity reactions. In case of either hypersensitivity to PEGasparaginase or silent inactivation, children were switched to Erwinia asparaginase as a second-line agent in intensification. Only a few studies have been performed on silent inactivation using intensive PEGasparaginase or intensive Erwinia asparaginase.

The aim of this prospective drug-monitoring study was to analyze the efficacy of very prolonged use of PEGasparaginase and Erwinia asparaginase by assessing asparaginase activity, aspartate, glutamine, and glutamic acid levels, and AAAs.

Methods

Patients

Children between 1 and 18 years of age with newly diagnosed ALL and stratified as medium-risk patients were included in the prospective PEGasparaginase study from May 2009 until October 2012 in 2 pediatric oncology centers. Patients were assigned to the medium-risk group based on a predelineed good response at day 8, cytomorphic complete remission at day 33, and minimal residual disease positivity at day 33 and/or day 79 (before the start of protocol M), but minimal residual disease level at day 79 , 10^5 and no presence of the t(4;11)(q11;q23) translocation or the corresponding fusion gene MLL/AF4 in the leukemia cells at diagnosis. Children who had an allergy to PEGasparaginase or silent inactivation were switched to Erwinia asparaginase and included in the prospective Erwinia asparaginase study. Because of the expected low number of allergic reactions to PEGasparaginase, the latter study was carried out in all 7 pediatric oncology centers in the same study period.

The Institutional Review Board approved this study before patient enrollment. Informed consent was obtained from parents or guardians and from patients ≥ 12 years of age. This study was in accordance with the Declaration of Helsinki.

DCOG ALL-10 treatment protocol

Patients were stratified into 3 risk groups after induction treatment: standard risk, medium risk, and high risk. The treatment scheme of the ALL-10 protocol and the intensification phase of the medium-risk patients in greater detail are given in supplemental Figure 1 (available in the Blood Web site).

All patients received 8 doses of native E coli asparaginase (5000 IU/m² per dose) every 3 days in induction. If a patient was stratified as medium risk, PEGasparaginase as a first-line agent (2500 IU/m² per dose every other week) was given for a total of 15 doses during the first 30 weeks of intensification. In case of an allergy to or silent inactivation of PEGasparaginase, the drug was replaced by Erwinia asparaginase as a second-line agent (20000 IU/m² per dose) 3 times per week to complete 30 weeks of asparaginase therapy. All asparaginase agents were administered intravenously.

Study design

In the PEGasparaginase study, the PEGasparaginase activity trough levels and AAAs were measured in serum at the start of intensification (week 0); in weeks 2, 4, 6, 8, 10, 14, 16, 24, 26, and 28; and also 1 week after administration in weeks 3, 9, 15, and 25. Serum asparagine, aspartic acid, glutamine, and glutamic acid levels were measured at weeks 0, 2, 4, 14, and 24 during PEGasparaginase therapy.

Children who had an allergy to PEGasparaginase or silent inactivation were included in the Erwinia asparaginase study. For this study, 6 blood samples of Erwinia asparaginase activity levels during the first 2 weeks of therapy were obtained from children who received Erwinia asparaginase (Monday, Wednesday, and Friday). In case of high activity levels (72-hour levels ≥ 100 U/L), the frequency of administration of Erwinia asparaginase was reduced to 2 times per week. Thereafter, every 4 weeks a blood sample was assessed.

Allergy was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Silent inactivation was defined as serum PEGasparaginase activity level ≥ 100 U/L at day 76 ± 1 or ≥ 20 U/L at day 14 ± 1 after administration in a patient without clinical symptoms of allergy. Silent inactivation of Erwinia asparaginase was defined as serum asparaginase activity level > 20 U/L at day 2 after administration of Erwinia asparaginase in patients without clinical allergy.

Laboratory measurements

Serum asparaginase activity and amino acid levels were processed and assessed as described earlier. Antibodies against native E coli asparaginase (Asparaginase medac) (Coli-AAAs), against PEGasparaginase (Onicaspar) (PEG-AAAs), and against Erwinia asparaginase (Erwinase) (Erwinia-AAAs) were measured by enzyme-linked immunosorbent assays (see supplemental Methods). All AAAs were expressed as optical density (OD) readings. Samples were defined as positive for AAAs if the Coli-AAAs OD was 0.13, if the PEG-AAA OD was 0.25, and if the Erwinia-AAOD was 1.96 standard deviations above the negative control processed mean (using Westgard rules).

To evaluate if Coli-AAAs in induction predict an allergy or silent inactivation in intensification, we measured Coli-AAAs after induction in a single center (Rotterdam).

Statistical analysis

The data were analyzed with the software package SPSS, version 20.0.1 (SPSS, Chicago, IL). Repeated measurements of the asparaginase activity levels were evaluated using mixed models analysis of variance (see supplemental Methods). The antibody levels were compared using nonparametric tests. The role of Coli-AAAs at day 79 (at the start of consolidation; protocol M) was analyzed to predict an allergy or silent inactivation at day 140 (start of intensification); for this, sensitivity and specificity were given. Sensitivity and specificity were calculated with the Fisher’s exact test for 2-by-2 contingency tables.

The occurrence of allergy or silent inactivation of PEGasparaginase related to age and gender was investigated with the x² test/Fisher’s exact tests where appropriate. A 2-sided P value < .05 was considered statistically significant. Data are presented as mean ± standard error of the mean (SEM) or specified otherwise.

Results

PEGasparaginase study

Supplemental Table 1 displays the characteristics of 89 patients included in the PEGasparaginase study. Twenty of the 89 (22%) patients had clinical allergic reactions to PEGasparaginase (6 grade 1, 7 grade 2, and 7 grade 3), and 7 of 89 (8%) patients showed silent inactivation. Eighteen of the 20 (90%) allergic reactions occurred on the second PEGasparaginase dose. Age, gender, and ALL immunophenotype did not differ between patients with or without allergy/silent inactivation of PEGasparaginase.
Serum PEGasparaginase activity levels were measured in 592 samples of 89 children (Figure 1A). PEGasparaginase activity levels were not related to age (<10 years or ≥10 years) or gender (both P=0.2). Patients without allergy to and without silent inactivation of PEGasparaginase had a mean trough activity level of 899 U/L (see also supplemental Figure 2). All 20/89 (22%) allergic patients (including grade 1-4 Common Terminology Criteria for Adverse Events) showed PEGasparaginase activity levels of 0. This was not attributable to the fact that the infusion was stopped because 18 patients showed their allergic reactions at the second dose; the serum asparaginase activity level after the first full dose already appeared to be 0 in all 18 cases. Moreover, in 4 cases the second full PEG-asparaginase dose was given with clemastine and hydrocortisone, also resulting in unmeasurable serum activity levels of PEGasparaginase after the second dose. All allergic patients were switched to Erwinia asparaginase. Two of the 7 children with silent inactivation were switched to Erwinia asparaginase. The other 5 patients continued with PEGasparaginase because real-time asparaginase measurements were not available at that moment. Those were excluded from further analysis.

At the start of the intensification phase, the asparagine levels were normal in almost all patients (normal range 40-80 mM). All children without an allergy to PEGasparaginase and without silent inactivation had complete asparagine depletion over time with a lower level of quantification (LLQ) of 0.2 mM (Figure 2A). Children with an allergy to PEGasparaginase or silent inactivation showed no asparagine depletion (Figure 2A). Supplemental Figure 3A shows PEGasparaginase activity levels in relation to the asparagine levels. Figure 2B-D displays aspartic acid, glutamine, and glutamic acid levels. Aspartic acid levels increased after 1 PEGasparaginase infusion in children without allergy and without silent inactivation in line with the asparagine depletion. Thereafter, no changes were seen. No glutamine depletion was seen during PEGasparaginase therapy. Glutamic acid levels increased after the first 2 PEGasparaginase infusions in children without allergy and without silent inactivation; no changes were seen thereafter.

Predictive value of Coli-AAAs in induction for an allergy or silent inactivation in intensification

Coli-AAAs were measured serially in 40 patients; 11/40 (27.5%) had allergy and 4/40 (10%) had silent inactivation, respectively, in intensification. The sensitivity of Coli-AAAs at day 79 to detect allergy or silent inactivation in intensification (day 140) was 87% (95% CI, 60% to 98%), and specificity was 64% (95% CI, 43% to 82%).

At day 79, the Coli-AAA levels of children with allergy or silent inactivation of PEGasparaginase in the subsequent intensification phase were significantly higher than those in children without (P=0.006). The same was found at day 140 (P=0.002). All children allergic to PEGasparaginase, except 1, had Coli-AAAs at the start of intensification (day 140) (Figure 3B). Only 1 patient had PEG-AAAs at day 140 (Figure 3C). The levels of Coli-AAAs and PEG-AAAs increased significantly from day 140 to day 154 after the first PEGasparaginase infusion in allergic patients (P=0.002 and P=0.001, respectively). All allergic children except 1 developed PEG-AAAs after the first PEGasparaginase infusion. All children with silent inactivation had Coli-AAAs before (day 140) and after (day 154) the first PEGasparaginase
infusion. Only 2/7 silent inactivation patients had PEG-AAAs after the first PEG-asparaginase infusion (day 154), and patients with silent inactivation had lower PEG-AAAs at day 154 than patients with clinical allergy ($P \leq 0.001$).

**Erwinia asparaginase study**

Supplemental Table 1 presents the characteristics of the 59 patients with an allergy to or silent inactivation of PEG-asparaginase in this 7-center study; 25 patients had already participated in the PEG-asparaginase study.

Two of the 59 (3%) patients developed an allergy to *Erwinia* asparaginase; one showed a grade 3 allergy at the fourth infusion, and the other a grade 2 allergy at the sixth infusion. These 2 patients did not receive further asparaginase therapy. No patients with silent inactivation of *Erwinia* asparaginase were seen.

*Serum* *Erwinia* asparaginase activity levels were measured in 444 samples of 57 patients without allergy to *Erwinia* asparaginase to complete 30 weeks of exposure (Figure 1B). The 2 children with an allergy to *Erwinia* asparaginase had asparaginase activity levels of 0. This was not attributable to the fact that the infusion was stopped, because the asparaginase level of the previous dose already appeared to be 0 in both patients. Also, in both cases, the next full *Erwinia* asparaginase dose was given with clemastine and hydrocortisone, resulting in unmeasurable serum *Erwinia* asparaginase activity levels.

Table 1 shows the *Erwinia* asparaginase activity levels during intensification therapy. *Erwinia* asparaginase activity levels were not related to age (< 10 years or ≥ 10 years) or gender ($P = 0.7$ and $P = 0.4$, respectively). In the first 2 weeks, all children received 6 doses of *Erwinia* asparaginase. Of the nonallergic *Erwinia*-asparaginase patients in the first 2 weeks, 55/57 (96%) had at least 1 *Erwinia* asparaginase activity level $\geq 100$ U/L, and 57/57 (100%) had $\geq 50$ U/L. In 65% and 85% of all patients, all *Erwinia* asparaginase activity levels in the first 2 weeks were $\geq 100$ U/L and $\geq 50$ U/L, respectively. Median trough levels were 183 U/L at 48 hours and 93 U/L at 72 hours. Nineteen children (33%) were switched to

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**Figure 2. Pharmacodynamics of very intensified PEG-asparaginase courses.** Serum asparagine (A), aspartic acid (B), glutamine (C), and glutamic acid (D) levels (mean ± SEM) during PEG-asparaginase therapy (2500 IU/m$^2$) in children without allergy to PEG-asparaginase and without silent inactivation. Dashed lines show normal values of asparagine (40-80 mM), aspartic acid (4-22 mM), glutamine (457-738 mM), and glutamic acid (18-65 mM). The LLQ of asparagine is 0.2 mM.

**Figure 3. Patients with AAAs during the consolidation phase and in the first two weeks of the intensification.** Serum AAAs against native *E coli* asparaginase (Coli-AAA levels) of patients at day 79 (start of the consolidation phase) and at day 140 (start of the intensification) (A), and Coli-AAA levels and serum AAAs against PEG-asparaginase (PEG-AAA levels) at day 140 (before first PEG-asparaginase dose) and at day 154 (14 days after first PEG-asparaginase dose) (B-C). Dashed lines indicate that samples were defined as positive for AAAs if the Coli-AAA OD was 0.13 and if the PEG-AAA OD was 0.25. Closed circles and blocks show cases with allergy or cases without allergy and without silent inactivation; silent inactivation cases are shown by open circles and blocks. Median Coli-AAA and PEG-AAA levels are indicated by bars. NS, not significant.
twice-weekly infusions because high activity levels (72-hour levels $\geq 100\text{U/L}$) were measured in the first 2 weeks. Children receiving therapy 2 times per week showed a significant decrease of *Erwinia* asparaginase activity levels at day 37 (week 6) as compared with day 11 (week 2) ($P < 0.002$), whereas children continuing *Erwinia* asparaginase 3 times per week showed a significant increase of activity levels ($P < 0.004$) (Figure 1B). The *Erwinia* asparaginase activity levels remained stable thereafter for all patients. Concerning the nonallergic patients during *Erwinia*-asparaginase therapy, 57/57 (100%) had at least 1 *Erwinia* asparaginase activity level $\geq 100\text{U/L}$ after the first 2 weeks. Median trough levels were 177 U/L at 48 hours and 86 U/L at 72 hours. Before the start of *Erwinia* asparaginase, the asparagine levels were normal (normal range 40-80 mM). During repeated asparagine measurements, these levels were strongly depleted but not always fully depleted over time with an LLQ of 0.2 mM (Figure 4A). The 2 children with an allergy to *Erwinia* asparaginase showed no asparagine depletion.

Supplemental Figure 3B shows *Erwinia* asparaginase activity levels in relation to the asparagine levels, and Figure 4B-D displays aspartic acid, glutamine, and glutamic acid levels. No glutamine depletion was seen during therapy; however, after 6 weeks of *Erwinia* asparaginase therapy, the glutamine levels were decreased, but not significantly.

Figure 5 presents the *Erwinia*-AAA and *Erwinia* asparaginase activity levels over time. In total, 38% of patients developed *Erwinia*-AAAs during therapy, all at days 37, 65, and 92. Thereafter, the levels dropped again to baseline values. The presence of *Erwinia*-AAAs did not influence *Erwinia* asparaginase activity levels. No silent inactivation was seen during therapy. Both children (3%) with an allergy to *Erwinia* asparaginase had detectable *Erwinia*-AAAs.

### Discussion

This prospective drug monitoring of asparaginases has resulted in several important findings. First, if patients show no clinical allergy to or silent inactivation of PEGasparaginase, the serum levels are too high using a schedule of 2500 IU/m² every other week. Based on this finding, we suggest that the administered PEGasparaginase dose can be lowered. This dose reduction seems feasible because different

<table>
<thead>
<tr>
<th>Table 1. Serum <em>Erwinia</em> asparaginase trough levels $\geq 20\text{U/L}$, $\geq 50\text{U/L}$, or $\geq 100\text{U/L}$</th>
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<tbody>
<tr>
<td><strong>Erwinase activity median (range)</strong></td>
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<tr>
<td><strong>First 2 weeks of <em>Erwinia</em> asparaginase therapy</strong></td>
</tr>
<tr>
<td>3 times per week</td>
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<tr>
<td>At least 1 sample above 20, 50, or 100 U/L</td>
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<tr>
<td><strong>Week 6 to week 30 of <em>Erwinia</em> asparaginase therapy</strong></td>
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<tr>
<td>3 times per week, 48-h interval</td>
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<tr>
<td>At least 1 sample above 20, 50, or 100 U/L</td>
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<tr>
<td>2 times per week, 72-h interval</td>
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<td>At least 1 sample above 20, 50, or 100 U/L</td>
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*The table is based on 57 patients without allergy to *Erwinia* asparaginase. The 2 patients with clinical allergy to *Erwinia* asparaginase were removed from this group. The table shows the data of the period week 6 to week 30 of *Erwinia* asparaginase therapy of 142 samples of 38 patients who continued infusions 3 times per week after the first 2 weeks of *Erwinia* asparaginase therapy and the data of 71 samples of 19 patients who were switched to infusions 2 times per week after the first 2 weeks of therapy. h, hour. †Number of patients with all samples above 20 U/L, 50 U/L, or 100 U/L.

*For first 2 weeks of *Erwinia* asparaginase therapy, n = 231 samples; for week 6 to week 30 of *Erwinia* asparaginase therapy, n = 213 samples.*
reinduction protocols used 1000 IU/m² PEGasparaginase with adequate trough levels of ≥ 100 U/L in ≥ 80% of the patients.22,23 Appel et al showed earlier that 1000 IU/m² PEGasparaginase in induction resulted in asparaginase levels above 100 U/L for at least 2 weeks with complete asparagine depletion in all patients.24 However, this dose reduction of PEGasparaginase should be used and guided by careful monitoring of asparaginase activity levels. There is a need in the future to evaluate this dose reduction with respect to outcome and toxicity data. Of interest, a dose reduction of PEGasparaginase could lead to cost savings; this should also be studied.

Second, we found a high incidence of inactivation of PEGasparaginase (22% clinical allergy and 8% silent inactivation) in the intensification phase because of antibody development against native E coli asparaginase, which was used in induction. This implies that PEGasparaginase should be used upfront during the induction course instead of native E coli asparaginase because this has been shown to result in less antibody formation.19 A similar inactivation rate of PEGasparaginase (36%) was found 1 week after the PEGasparaginase course by Müller et al using the same asparaginase regimen in induction and a single PEGasparaginase in reinudction.25 However, they reported no allergies in reininduction. If a subsequent second PEGasparaginase course was administered, we believe that the allergy rate of PEGasparaginase would be increased. An important finding is that even very mild allergic reactions (grade 1) also necessitate a switch to Erwinia asparaginase. Continuing with PEGasparaginase combined with pretreatment of clemastine and hydrocortisone is not useful because PEGasparaginase activity levels remain 0 (Figure 1A).

Third, drug monitoring is useful because it detects too high levels in some of the patients and, even more important, silent inactivation in other the patients. Detection of silent inactivation is important to prevent useless continuation of an inactive asparaginase product, which may lead to a worse EFS as shown by Panosyan et al and Vrooman et al.8,12 Nevertheless, drug monitoring is the only way to detect cases of silent inactivation of asparaginase agents.

Fourth, in the case of allergy to or silent inactivation of PEGasparaginase, patients can be treated effectively with Erwinia asparaginase. The majority of the patients that switched to Erwinia asparaginase showed effective asparaginase activity levels during the first 2 weeks of Erwinia asparaginase, namely median trough activity level of 183 U/L (48-hour level) and 93 U/L (72-hour level) and asparaginase activity level ≥ 100 U/L in 100% (48-hour level) and 33% (72-hour level) of the patients. Similar rates were found by Vrooman et al: 83% (48-hour level) and 45% (72-hour level) using a dose of 25 000 IU/m² 3 times per week intravenously.26 These activity levels were lower compared with that of the study by Salzer et al; they found 645 U/L (48-hour level) and 248 U/L (72-hour level) as median trough activity levels and asparaginase activity levels ≥ 100 U/L in 93% (48-hour level) and 88% (72-hour level) of the patients using intramuscular Erwinia asparaginase in a dose of 25 000 IU/m² 3 times per week.27 The route of Erwinia asparaginase administration might explain the higher median asparaginase activity levels that were found by Salzer et al.27 However, previous studies have shown that no differences in mean asparaginase activity levels, asparagine depletion, and AAAs were found after intravenous or intramuscular administration of Erwinia asparaginase.16,28,29

Only 2/59 (3%) developed an allergy to Erwinia asparaginase in our study. Please note that this study does not allow comparison of allergy rates to E coli asparaginase (including the pegylated form) and Erwinia asparaginase because E coli asparaginases were given in induction and intensification with an asparaginase-free interval of 4 months, whereas Erwinia asparaginase was administered continuously without such interval.

Fifth, the mean asparagine levels for both preparations were below the detection level of 0.5 mM as used by others.14,30,32 We observed that asparagine levels were not always completely depleted with our detection level of 0.2 mM21 in Erwinia asparaginase-treated patients in contrast to PEGasparaginase. This may simply reflect the differences in serum drug levels of these 2 compounds. With our very low LLQ of 0.2 mM, 19/57 patients (33%) showed no complete asparagine depletion. If the threshold levels from the literature are used (mean of 0.5 mM), 9 of 57 patients (16%) showed incomplete asparagine depletion.14,30,32 Samples with Erwinia asparaginase activity levels > 100 U/L and asparagine > 0.2 mM were also found in 9 of 57 patients (16%).

Why this difference in asparagine levels during asparaginase therapy exists between Erwinia asparaginase and PEGasparaginase is unclear. Measuring asparaginase activity levels is preferred over

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**Figure 5. Pharmacokinetics of very prolonged Erwinia asparaginase use in relation to AAAs.** Serum Erwinia asparaginase activity (A) and serum AAAs (B) against Erwinia asparaginase (Erwinia AAAs) over time. Serum Erwinia asparaginase activity levels of patients without Erwinia AAAs (C) and with Erwinia AAAs (D) (mean ± SEM). Dashed lines in panels A, C, and D indicate Erwinia asparaginase activity level of 100 U/L. The dashed line in panel B indicates samples defined as positive for Erwinia AAAs if the OD was > 1.96 standard deviations above the negative control processed mean (using Westgard rules).26
measuring asparagine levels to monitor the efficacy of asparaginase therapy because, if not properly handled, asparaginase is very rapidly degraded in vivo. It is remarkable that the glutamine levels of *Erwinia* asparaginase-treated patients were lower as compared with those receiving PEGalasparaginase irrespective of the much lower serum activity levels of *Erwinia* asparaginase. This can be explained by the higher glutaminase activity of *Erwinia* asparaginase. Although glutamine is broken down to glutamic acid by asparaginase, this does not lead to glutamine depletion because this is supplemented from other organ stocks in vivo.

Sixth, our study shows that the presence of AAAs is related to allergy to and silent inactivation of asparaginase as shown by others, but predicting asparaginase allergy or silent inactivation based on antibody formation is hampered by low specificity (64%) of the test. The specificity of the Coli-AAA test in intensification was higher, 73%, but still not perfect. The levels of PEG-AAA were above the cutoff in all allergic patients after the first PEGalasparaginase infusion and negative in children without an allergy. The specificity of the PEG-AAA test is 100% to predict an allergy to or silent inactivation of PEGalasparaginase in intensification. However, the patients with an allergy at the first PEGalasparaginase infusion had no detectable PEG-AAAs (day 0 of intensification). And *Erwinia*-AAAs were found in 38% of the patients but were not associated with inactivation of asparaginase. So, measuring asparagine levels is a more direct and more accurate way of drug monitoring than measuring AAAs. It has been reported that females and toddlers are more prone to develop allergies. However, we found no relation between age, gender, or allergy or antibody formation.

In conclusion, the use of native *E coli* asparaginase in induction leads to a significant rate of allergy to and silent inactivation of PEGalasparaginase in intensification. Switching to *Erwinia* asparaginase in case of allergy to or silent inactivation of PEGalasparaginase leads to effective asparaginase activity levels in most patients, but close drug monitoring remains necessary to ensure adequate drug levels. The relevance of AAAs in clinical practice appears to be limited. It is more useful to monitor the serum asparagine activity levels. In the absence of allergy or silent inactivation, PEGalasparaginase activity levels are too high with a dose schedule of 2500 IU/m² every other week.

This study has therefore resulted in significant changes in the use of asparaginase in the DCOG ALL-11 protocol. PEGalasparaginase is used instead of native *E coli* asparaginase upfront in the induction, and the starting dose of PEGalasparaginase has been lowered to 1500 IU/m². Also, a therapeutic drug monitoring program is now used to individualize the PEGalasparaginase dose and to detect silent inactivation. In case of allergy or silent inactivation, patients are switched to *Erwinia* asparaginase with therapeutic drug monitoring to allow individualized dosing of *Erwinia* asparaginase.

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**Authorship**

Contribution: W.H.T., R.P., and I.M.v.d.S. designed, analyzed, and interpreted the clinical data; C.L.-K. was responsible for the measurements of asparaginase activities and amino acid levels; M.V.R. was responsible for the *Erwinia*-AAAs; G.J.L.K., D.M.W.M.l.L., M.B.B., C.v.d.B., W.J.W.K., and W.J.E.T. were responsible for the inclusion of patients in the other centers; W.H.T. and W.C.J.H. did the statistical analysis; R.P. and I.M.v.d.S. supervised this research; W.H.T., R.P., and I.M.v.d.S. were responsible for the manuscript preparation; and all authors revised the article critically for important intellectual content and approved the final version to be published.

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