

RESEARCH ARTICLE

Allergic-like reactions to asparaginase: Atypical allergies without asparaginase inactivation

Robin Q. H. Kloos¹ | Rob Pieters² | Gabriele Escherich³ | Inge M. van der Sluis¹

¹Pediatric Oncology and Hematology, Erasmus MC—Sophia Children's Hospital, Rotterdam, The Netherlands

²Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

³Clinic of Pediatric Hematology and Oncology, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany

Correspondence

Robin Q. H. Kloos, Department of Paediatric Oncology/Haematology, Erasmus MC—Sophia Children's Hospital, Room Number Na-1603, Weytemaweg 80, 3015 CN Rotterdam, The Netherlands.

Email: r.kloos@erasmusmc.nl

Abstract

Background: Asparaginase is an important component of pediatric acute lymphoblastic leukemia (ALL) therapy. Unfortunately, this treatment is hampered by hypersensitivity reactions. In general, allergies – regardless of severity – cause complete inactivation of the drug. However, we report atypical allergic reactions without inactivation of asparaginase, here called allergic-like reactions.

Procedure: Patients with an allergic-like reaction, who were treated according to the Dutch Childhood Oncology Group ALL-11 or the CoALL 08-09 protocol, were described. The reactions were identified by continual measurement of asparaginase activity levels. Characteristics, including timing of occurrence, symptoms, grade, and the presence of antiasparaginase antibodies, were compared to those of real allergies.

Results: Fourteen allergic-like reactions occurred in nine patients. Five reactions were to PEGasparaginase and nine to *Erwinia* asparaginase. Allergic-like reactions occurred relatively late after the start of infusion compared to real allergies. Antibodies were absent in all but one patient with an allergic-like reaction, while they were detected in all patients with a real allergy. Symptoms and grade did not differ between the groups. Asparaginase was continued with the same formulation in six patients of whom four finished treatment with adequate activity levels.

Conclusions: In conclusion, allergic-like reactions occur relatively late after the start of infusion and without antibodies. Despite these clinical differences, allergic-like reactions can only be distinguished from real allergies by continually measuring asparaginase activity levels. If clinically tolerated, formulations should not be switched in case of allergic-like reactions. Moreover, failure to recognize these reactions may lead to a less favorable prognosis if asparaginase therapy is terminated unnecessarily.

KEYWORDS

acute lymphoblastic leukemia, asparaginase, hypersensitivity, pediatrics

1 | INTRODUCTION

Asparaginase is one of the key components of childhood acute lymphoblastic leukemia (ALL) therapy as intensive dosing schedules improve event-free survival 10–15%.^{1–7} Unfortunately, asparaginase treatment is hampered by hypersensitivity reactions such as clinical allergies and silent inactivation, which neutralize asparaginase completely.⁸ In the case of silent inactivation, asparaginase is inactivated in absence of clinical symptoms. If asparaginase is neutralized due to an allergy or silent inactivation, formulations should be switched to maintain effective asparaginase treatment.^{9–11}

Previously, we have studied trough PEGasparaginase activity levels and allergies to asparaginase in pediatric ALL. The patients were

first treated with several doses of native *Escherichia coli* asparaginase in the induction phase. PEGasparaginase was administered in the intensification phase approximately 12 weeks after the last native *E. coli* asparaginase dose.⁸ Twenty-two percent of the patients developed an allergic reaction. Most importantly, all allergic reactions to PEGasparaginase resulted in complete neutralization of asparaginase. This was regardless of the severity or grade of the reaction and was accompanied by antiasparaginase antibodies. Premedication with clemastine or hydrocortisone reduced symptoms of the allergy but could not prevent neutralization of asparaginase.⁸ Ninety percent of the reactions occurred during the second PEGasparaginase dose. Interestingly, trough asparaginase activity levels already proved to be zero after the first PEGasparaginase dose, meaning

that asparaginase was already neutralized before the allergic reaction occurred.⁸

Beside these neutralizing hypersensitivity reactions, there seem to be atypical allergic reactions to asparaginase, also called allergic-like reactions, not resulting in inactivation of asparaginase. In case of these allergic-like reactions, formulations do not have to be switched to maintain adequate asparaginase therapy. Moreover, therapy may even be withheld unnecessarily when second-line asparaginase is terminated prematurely because of it. Therefore, it is very important to distinguish between real allergies and allergic-like reactions. It is challenging though to interpret activity levels after an allergic reaction when infusion is truncated prematurely and only part of the dose is administered. Fortunately, the trough asparaginase activity level of the preceding dose can be used to evaluate possible neutralization, which can be accomplished by continual therapeutic drug monitoring (TDM). In this article, we describe allergic-like reactions and compare these reactions to real allergies in order to find differences that can be used in clinical practice to distinguish between the two types of reactions.

2 | METHODS

2.1 | Patients and treatment protocols

Patients with allergic symptoms without asparaginase inactivation were described. These patients were treated according to the CoALL 08-09 treatment protocol or the Dutch Childhood Oncology Group (DCOG) ALL-11 protocol in multiple pediatric oncology centers. These protocols are currently open for inclusion and data are not complete yet. Therefore, the frequency of allergies and allergic-like reactions is not available at this time. As a comparison, we used all patients with an allergy and asparaginase inactivation from the Sophia Children's Hospital, Rotterdam, The Netherlands, who were treated according to the DCOG ALL-10 protocol and were partially described earlier.⁸ Use of the data from the enrolled patients was approved by the Institutional Review Board.

The CoALL 08-09 protocol contained three or four PEGasparaginase doses in the intensification phase and one or two in the reinduction phase (2,500 IU/m²) after an asparaginase-free interval of approximately 4 weeks. The doses were administered intravenously over 2 hr: 10% of the dose during the first hour, the remaining during the second. Asparaginase activity levels were measured to detect silent inactivation.

The DCOG ALL-11 protocol included three doses of PEGasparaginase in induction and, after an interval of approximately 12 weeks, 14 doses in the intensification and maintenance phase. After three doses of 1,500 IU/m², TDM was used to individualize the doses based on trough levels.

Patients who were treated according to the DCOG ALL-10 protocol received eight doses of 5,000 IU/m² native *E. coli* asparaginase in induction and 15 PEGasparaginase doses (2,500 IU/m² biweekly) in the intensification and maintenance phases, also after an asparaginase-

free interval of approximately 12 weeks. Trough PEGasparaginase activity levels were measured for research purposes.

In both the DCOG ALL-10 and ALL-11 protocols, asparaginase was administered intravenously over 1 hr. In case of allergy or silent inactivation, patients were switched to 20,000 IU/m² *Erwinia* asparaginase three times a week. TDM was used to adjust the dose schedule for *Erwinia* asparaginase in the DCOG ALL-11 protocol. *Erwinia* asparaginase was administered intravenously over 1 hr. Asparaginase treatment was terminated when patients developed a hypersensitivity reaction to the latter formulation as well.

2.2 | Classification and description of allergic reactions

Allergic reactions were classified as either "real" or allergic-like, based on whether they were accompanied by asparaginase inactivation. An allergic reaction was considered real if trough levels of the preceding dose were already zero before administration of the reaction-inducing dose. In case of allergic-like reactions, asparaginase activity levels were measurable (more than lower limit of quantitation) just prior to or after the reaction-inducing doses.

When symptoms of an allergic reaction occurred, the following characteristics were described: asparaginase activity levels, time of occurrence, symptoms, further treatment, and the presence of antiasparaginase antibodies. Allergic and allergic-like reactions were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

2.3 | Asparaginase activity analysis and antiasparaginase antibody assay

Asparaginase activity levels were measured based on the L-aspartic β -hydroxamate (AHA) assay.¹² AHA is hydrolyzed by asparaginase to L-aspartic acid and hydroxylamine. Hydroxylamine (20 μ l) was diluted with 8-hydroxyquinoline for condensation and oxidation and was quantified by photometric detection at 690 nm. Trough PEGasparaginase activity levels were measured 2 weeks after administration and were considered adequate when >100 IU/l. Trough *Erwinia* asparaginase activity levels were measured 48 or 72 hr after administration and were also considered adequate when >100 IU/l. Antibodies against the different asparaginase formulations were determined by enzyme-linked immunosorbent assays (ELISA) as described earlier.⁸

2.4 | Statistical analysis

The data were analyzed with the software package IBM SPSS Statistics (IBM Corp., Armonk, New York, USA) version 21.0 for Windows. Time of occurrence of the reactions after the start of infusion and presence of antiasparaginase antibodies were analyzed by nonparametric tests. Symptoms were analyzed using the Fisher's exact test. A P-value < 0.05 was considered statistically significant.

TABLE 1 Allergic-like reactions

Patient, treatment protocol	Asp type	Treatment phase (dose ^a)	Grade and symptoms	Time after start infusion	Trough level (IU/l) ^b	AB against nE. coli/PEGasp/Erw	Action taken
1, COALL 08-09	PEGasp	Reind (2)	Grade 1: rash, itchiness	60 min	137 ^c	Unknown	PEGasp continued, finished without other reactions
2, COALL 08-09	PEGasp	Intens (1)	Grade 1: rash, urticaria, fever	24 hr	1,657 (week) 749 ^c	Unknown	PEGasp continued, finished without other reactions
3, ALL-11	PEGasp	Intens (1)	Grade 2: rash, edema, dyspnoea	20 min	83 (week)	Neg./Neg./Neg.	Switch to Erw, successfully ^d finished
4, ALL-11	PEGasp	Induction (4)	Grade 2: vomiting, pale	45 min	561 (week) 122	Neg./Neg./Neg.	PEGasp continued and successfully ^d finished
5, ALL-11	PEGasp Erw	Intens (1) Intens (6)	- Grade 2: rash, vomiting	27 min 12 min	493 (week) 294 (T48)	Neg./Neg./Neg. Neg./Neg./Neg.	Switch to Erw Erw stopped
6, ALL-11	Erw	Intens (14)	Grade 2: vomiting	53 min	32 (T48)	Neg./Pos./Neg.	Erw stopped
7, ALL-11	Erw	Intens (12)	1. Grade 2: nausea 2. Grade 2: edema, dyspnoea 3. Grade 2: nausea, pale	30 min 5 min 15 min	157 (T48) 89 (T72) 37 (T72)	Pos./Pos./Pos. Pos./Pos./Pos. Pos./Pos./Pos.	1. Pretreatment with clemastine and reduced infusion rate for the next doses 2. Dose stopped but restarted and finished 3. Dose postponed, Erw treatment successfully ^d finished
8, ALL-11	Erw	Intens (17)	Grade 2: rash, dyspnoea	38 min	146 (T48)	Pos./Pos./Neg.	Erw was continued but stopped after dose 25 because of an allergic reaction
9, ALL-11	Erw	Intens (55) Intens (58) Intens (59)	1. Grade 1: rash 2. Grade 1: rash 3. Grade 2: vomiting, edema	5 min 30 min 10 min	134 (T48) 139 (T48) 145 (T48)	Pos./Neg./Neg. Neg./Neg./Neg. Neg./Neg./Neg.	1. Erw postponed, HC and reduced infusion rate for the next doses 2. Infusion stopped but restarted and finished 3. Erw stopped

Asp, asparaginase; AB, anti-asparaginase antibodies; nE.coli, native *Escherichia coli* asparaginase; PEGasp, PEGasparaginase; Erw, Erwinia asparaginase; Intens, intensification phase; Reind, Reinduction phase; min, minutes; week, asparaginase activity level measured 1 week after the reaction; T48/72, 48 or 72 hr after previous dose; HC, hydrocortisone; pos., positive; neg., negative.

^aDose number of the treatment phase in which the reaction occurred.

^bTrough level just before the reaction.

^cTrough level after the reaction.

^dSuccessfully: with adequate (> 100 IU/l) asparaginase activity levels.

TABLE 2 Allergic reactions

Patient, treatment protocol	Asp type	Treatment phase (dose ^a)	Grade and symptoms	Time after start infusion	Trough level (IU/l) ^b	AB against nE.coli/PEGasp	Action taken
10, ALL-10	PEGasp	Intens (2)	Grade 2: rash, edema, vomiting	5 min	0	Pos./Pos.	Switch to Erw, reaction at dose 11, stop Erw
11, ALL-10	PEGasp	Intens (2)	Grade 2: rash, edema, hypotension	10 min	0 0 ^c	Pos./Pos.	PEGasp continued at first but successfully ^d switched to Erw after a second reaction
12, ALL-10	PEGasp	Intens (2)	Grade 2: rash, edema	20 min	0 0 ^c	Pos./Pos.	PEGasp continued at first but successfully ^d switched to Erw after a second reaction
13, ALL-10	PEGasp	Intens (2)	Grade 2: rash, edema	3 min	0	Pos./Pos.	Switch to Erw, successfully ^d finished
14, ALL-10	PEGasp	Intens (2)	Grade 2: rash, edema, vomiting	2 min	0	Pos./Pos.	Switch to Erw, successfully ^d finished
15, ALL-10	PEGasp	Intens (2)	Grade 2: rash, edema, coughing	1 min	0	Pos./Pos.	Switch to Erw, successfully ^d finished
16, ALL-10	PEGasp	Intens (2)	Grade 2: rash, edema, dyspnoea	1 min	0	Pos./Pos.	Switch to Erw, successfully ^d finished
17, ALL-10	PEGasp	Intens (2)	Grade 2: rash, coughing	1 min	0	Pos./Pos.	Switch to Erw, successfully ^d finished
18, ALL-10	PEGasp	Intens (2)	Grade 2: rash	1 min	0	Pos./Pos.	Switch to Erw, successfully ^d finished
19, ALL-10	PEGasp	Intens (2)	Grade 2: rash, dyspnoea	1 min	0	Pos./Pos.	Switch to Erw, successfully ^d finished
20, ALL-10	PEGasp	Intens (2)	Grade 2: rash, coughing	20 min	0	Pos./Pos.	Switch to Erw, successfully ^d finished
21, ALL-10	PEGasp	Intens (2)	Grade 2: dyspnoea, nausea	1 min	0	Pos./Pos.	Switch to Erw, successfully ^d finished
22, ALL-10	PEGasp	Intens (2)	Grade 2: rash, edema, nausea	1 min	0	Pos./Pos.	Switch to Erw, successfully ^d finished
23, ALL-10	PEGasp	Intens (2)	Grade 2: edema, coughing	5 min	0	Unknown	Switch to Erw, successfully ^d finished
24, ALL-10	PEGasp	Intens (2)	Grade 2: rash, vomiting	5 min	0	Unknown	Switch to Erw, successfully ^d finished

Asp, asparaginase; AB, antibodies; nE.coli, native *Escherichia coli* asparaginase; PEGasp, PEGasparaginase; Erw, *Erwinia* asparaginase; Intens, intensification phase; min, minutes; pos., positive; neg., negative.

^aDose number of the treatment phase in which the reaction occurred.

^bTrough level just before reaction occurred.

^cTrough level after the reaction.

^dSuccessfully: with adequate asparaginase activity levels (> 100 IU/l).

TABLE 3 Summary of clinical characteristics

	Allergic-like reactions	Allergic reactions	P-value
Symptoms			
Rash/edema/itchiness/urticaria	9/13 Reactions (69%)	14/15 Reactions (93%)	0.153
Pulmonary symptoms	3/13 Reactions (23%)	6/15 Reactions (40%)	0.435
Gastrointestinal symptoms	6/13 Reactions (46%)	5/15 Reactions (33%)	0.700
Timing of allergic reaction (median, 25–95 percentile)	29 (12–47)	2 (1–5)	<0.001
Antiasparaginase antibodies present	1/7 Patients (14%)	13/13 Patients (100%)	0.001

3 | RESULTS

Fourteen allergic-like reactions occurred in nine patients (Table 1). Five reactions were to PEGasparaginase, the remaining nine to *Erwinia* asparaginase. As a comparison, we describe 15 patients with real allergic reactions to asparaginase (Table 2).

3.1 | Asparaginase activity levels

Asparaginase activity levels are described in Table 1. Nine levels were obtained just prior to the reaction-inducing dose, five were measured after the reaction. In patients number one and two, the infusion was not stopped after the allergic-like reaction. Both patients had adequate trough PEGasparaginase activity levels after this dose (137 and 749 IU/l, respectively). Patient number three, who received approximately one-third of the individualized dose (700 IU/m²), had an asparaginase activity level of 83 IU/l 1 week after the reaction to PEGasparaginase.

Most patients had adequate *Erwinia* asparaginase trough levels after a dose interval of 48 hr. Only patient number six had an asparaginase activity level of 32 IU/l just before the reaction-inducing dose. Asparaginase therapy was permanently discontinued after this reaction. Patient number seven had inadequate asparaginase activity levels after each 72-hr dose interval. However, after changing the dose frequency to a 48-hr schedule, asparaginase activity levels were adequate.

All patients with a real allergic reaction already had asparaginase activity levels of zero prior to the reaction (Table 2). Since the majority of the reactions occurred almost immediately after start of an infusion, which was stopped directly, asparaginase activity levels after the reaction would not have been informative and were therefore not measured. However, in patients number 11 and 12, trough activity levels after the reaction were available and were both zero.

3.2 | Antiasparaginase antibodies

Antiasparaginase antibodies were not measured in patients number one and two. The other three allergic-like reactions to PEGasparaginase were not accompanied by antibodies against native *E. coli* asparaginase or PEGasparaginase (Table 1). Antibodies were also absent in all patients with an allergic-like reaction to *Erwinia* asparaginase, except for patient number seven.

In contrast, all 15 patients with a real allergy to PEGasparaginase had detectable antibodies against both PEGasparaginase and native *E. coli* asparaginase, which was significantly more frequent than in patients with an allergic-like reaction ($P = 0.001$).

3.3 | Clinical symptoms

Clinical characteristics are described in Table 1 for the allergic-like reactions, in Table 2 for the real allergies, and summarized in Table 3. The median CTCAE grade was grade two in both allergic and allergic-like reactions. Four of the 14 allergic-like reactions were grade one, the grade of one reaction is unknown, and the remaining 9 were grade two. All real allergic reactions were grade two. The types of reactions, were (i) symptoms of rash, edema, itchiness, or urticaria; (ii) pulmonary symptoms; and (iii) gastrointestinal symptoms, and did not differ between patients with an allergic or allergic-like reaction (Table 3).

Allergic-like reactions occurred significantly later after the start of infusion (median: 29 min, 25–75 percentile: 12–47 min) compared to real allergic reactions (median 2 min, 25–75 percentile: 1–5 min; $P < 0.001$). Patient number two developed an allergic-like reaction 24 hr after administration. It could be questioned if this reaction was associated with the asparaginase infusion. Excluding this case, the allergic-like reactions occurred after a median of 27 min (25–75 percentile: 11–42 min; $P < 0.001$).

3.4 | Further treatment

In three of five patients with an allergic-like reaction to PEGasparaginase (patients number one, two, and four), the drug was successfully continued without new allergic-like reactions. The activity levels were adequate in patient number four, but not measured in the other two patients. In the two other patients (patients number three and five), formulations were switched to *Erwinia* asparaginase directly after the reaction.

In two of five patients with an allergic-like reaction to *Erwinia* asparaginase (patients number five and six), the drug was permanently stopped directly after the reaction. In two patients (patients number eight and nine), the drug was continued initially but was finally terminated after one or more subsequent allergic or allergic-like reactions. Patient number seven successfully completed the *Erwinia* asparaginase doses with adequate levels, although three allergic-like reactions occurred in total.

4 | DISCUSSION

In this article, allergic-like reactions to asparaginase were reported and compared to real allergies. It is important to distinguish between these types of reactions because if allergic-like reactions are incorrectly interpreted as real allergies, asparaginase formulations will be switched or terminated unnecessarily.

Asparaginase activity levels are difficult to interpret when measured after an allergic reaction. A low asparaginase activity level could be caused by either neutralizing antibodies or premature termination of the dose. However, our patients with real allergic reactions already showed complete asparaginase inactivation of the previous dose. Therefore, distinction between allergic-like and real allergic reactions can be made based on the trough level of the preceding dose.

The correlation between hypersensitivity reactions and anti-asparaginase antibodies has been studied frequently. To date, four groups can be distinguished. The first group contains patients with an allergic reaction, accompanied by the presence of anti-asparaginase antibodies.^{8,9,13} Patients in the second group neutralize asparaginase in absence of clinical symptoms, so-called silent inactivation, and also have antibodies against asparaginase.⁸ The third group includes patients without a hypersensitivity reaction to or neutralization of asparaginase, but with anti-asparaginase antibodies, which has been reported in 6–38% of patients treated with asparaginase.^{8,13,14} The fourth group contains patients who have allergic symptoms without development of anti-asparaginase antibodies. This was described by Liu et al. in 4–7% of the patients and by Panosyan et al. in 10%.^{9,13} Unfortunately, both studies did not describe asparaginase activity and these reactions might have been allergic-like. In our cohort, all patients with a real allergic reaction had anti-asparaginase antibodies, whereas antibodies were absent in all but one of the patients with allergic-like reactions ($P = 0.001$). Thus, the occurrence of allergic symptoms in absence of anti-asparaginase antibodies may indicate an allergic-like reaction, without inactivating asparaginase.

Patients with an allergic-like reaction cannot be distinguished from allergic reactions based on clinical symptoms or grade. The only clinical difference between allergic-like reactions and real allergies appeared to be the time of occurrence. In our cohort, allergic-like reactions occurred significantly later after the start of administration. Most real allergic reactions occurred within minutes after start, though two patients developed a real allergic reaction after more than 10 min. Thus, a late timing of the reaction after the start infusion is a strong indication of an allergic-like reaction but distinction cannot be made conclusively.

The mechanism of allergic-like reactions is unclear. Based on a review recently published by Asselin, it can be considered that allergic-like reactions are related to the nonantibody-mediated hypersensitivity reactions that were described. Thus, the allergic-like reactions might be explained by a rapid increase of ammonia levels caused by the administration of asparaginase. Symptoms of this ammonia peak include nausea, vomiting, and rash.¹⁵ Although these symptoms overlap with part of the allergic-like symptoms, half of the patients with an allergic-like reaction had edema, dyspnoea, or urticaria, which cannot be explained by hyperammonemia. On the other hand, Tong et al.

have shown that ammonia levels are higher after *Erwinia* asparaginase therapy than after PEGasparaginase therapy, probably caused by the higher glutaminase activity of *Erwinia* asparaginase.¹⁶ This can explain why allergic-like reactions occur relatively more frequently during *Erwinia* asparaginase treatment. Unfortunately, ammonia levels were not measured in our cohort, but their role in the development and identification of allergic-like reactions should be studied.

In conclusion, we describe allergic-like reactions to PEGasparaginase and *Erwinia* asparaginase, not leading to inactivation of the drug. These reactions occur relatively late after the start of infusion and anti-asparaginase antibodies are absent in the large majority of these patients, but distinction can only be made when asparaginase activity levels are monitored continually, as it is done with TDM. Most importantly, patients are able to complete their asparaginase treatment with the same formulation if clinically tolerated. Although not useful in case of a real allergy, reducing the infusion rate and administering premedication may prevent symptoms in case of an allergic-like reaction.

CONFLICT OF INTEREST

Authors R. Pieters, G. Escherich, and I.M. van der Sluis received research support and consultancy fees from Jazz Pharmaceuticals and Medac.

AUTHORS' CONTRIBUTION

R.Q.H.K. and I.M.S. analyzed the data and wrote the manuscript. R.P. and G.E. interpreted the data and revised the manuscript critically.

ABBREVIATIONS

AHA	L-aspartic β -hydroxamate
ALL	acute lymphoblastic leukemia
CTCAE	Common Terminology Criteria for Adverse Events
DCOG	Dutch Childhood Oncology Group
ELISA	enzyme-linked immunosorbent assays
TDM	therapeutic drug monitoring

REFERENCES

1. Amylon MD, Shuster J, Pullen J, et al. Intensive high-dose asparaginase consolidation improves survival for pediatric patients with T cell acute lymphoblastic leukemia and advanced stage lymphoblastic lymphoma: a Pediatric Oncology Group study. *Leukemia*. 1999;13(3):335–342.
2. Duval M, Suciu S, Ferster A, et al. Comparison of *Escherichia coli*-asparaginase with *Erwinia*-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer-Children's Leukemia Group phase 3 trial. *Blood*. 2002;99(8):2734–2739.
3. Moghrabi A, Levy DE, Asselin B, et al. Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia. *Blood*. 2007;109(3):896–904.
4. Pession A, Valsecchi MG, Masera G, et al. Long-term results of a randomized trial on extended use of high dose L-asparaginase for standard risk childhood acute lymphoblastic leukemia. *J Clin Oncol*. 2005;23(28):7161–7167.

5. Rizzari C, Valsecchi MG, Arico M, et al. Effect of protracted high-dose L-asparaginase given as a second exposure in a Berlin-Frankfurt-Munster-based treatment: results of the randomized 9102 intermediate-risk childhood acute lymphoblastic leukemia study—a report from the Associazione Italiana Ematologia Oncologia Pediatrica. *J Clin Oncol*. 2001;19(5):1297–1303.
6. Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. *Blood*. 2001;97(5):1211–1218.
7. Sirvent N, Suciu S, Rialland X, et al. 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(2):239–247.
8. Tong WH, Pieters R, Kaspers GJ, et al. A prospective study on drug monitoring of PEGasparaginase and Erwinia asparaginase and asparaginase antibodies in pediatric acute lymphoblastic leukemia. *Blood*. 2014;123(13):2026–2033.
9. Panosyan EH, Seibel NL, Martin-Aragon S, et al. 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(4):217–226.
10. Pieters R, Hunger SP, Boos J, et al. L-Asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. *Cancer*. 2011;117(2):238–249.
11. Vrooman LM, Stevenson KE, Supko JG, et al. Postinduction dexamethasone and individualized dosing of *Escherichia coli* L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: results from a randomized study—Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. *J Clin Oncol*. 2013;31(9):1202–1210.
12. Lanvers C, Vieira Pinheiro JP, Hempel G, Wuerthwein G, Boos J. Analytical validation of a microplate reader-based method for the therapeutic drug monitoring of L-asparaginase in human serum. *Anal Biochem*. 2002;309(1):117–126.
13. Liu C, Kawedia JD, Cheng C, et al. Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia. *Leukemia*. 2012;26(11):2303–2309.
14. Killander D, Dohlwitz A, Engstedt L, et al. Hypersensitive reactions and antibody formation during L-asparaginase treatment of children and adults with acute leukemia. *Cancer*. 1976;37(1):220–228.
15. Asselin B. Immunology of infusion reactions in the treatment of patients with acute lymphoblastic leukemia. *Future Oncol*. 2016.
16. Tong WH, Pieters R, de Groot-Kruseman HA, et al. Toxicity of very prolonged PEGasparaginase and Erwinia asparaginase courses in relation to asparaginase activity levels with a special focus on dyslipidemia. *Haematologica*. 2014;99(11):1716–1721.