

Title: Allergic-like reactions to asparaginase: atypical allergies without asparaginase inactivation

Authors:

Robin Q.H. Kloos, MD, Pediatric Oncology/Hematology, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands.

Rob Pieters, MD, PhD, Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands.

Gabriele Escherich, MD, PhD, University Medical Centre Hamburg-Eppendorf, Clinic of Pediatric Hematology and Oncology, Hamburg, Germany.

Inge M. van der Sluis, MD, PhD, Pediatric Oncology/Hematology, Erasmus MC – Sophia Children's Hospital, Rotterdam, The Netherlands.

Corresponding author:

Robin Q.H. Kloos, MD

Erasmus MC – Sophia Children's Hospital

Department of Paediatric Oncology/Haematology, room number Na-1607

Weytemaweg 80, 3015 CN, Rotterdam, The Netherlands

E-mail: r.kloos@erasmusmc.nl

Abstract

Background:

Asparaginase is an important component of pediatric acute lymphoblastic leukemia therapy. Unfortunately, this treatment is hampered by hypersensitivity reactions. In general, allergies cause complete inactivation of the drug, regardless of the severity. 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 continuous measurement of asparaginase activity levels. Characteristics, including timing of occurrence, symptoms, grade and the presence of anti-asparaginase 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 and without antibodies. Despite these clinical differences, allergic-like reactions can only be distinguished from real allergies by continuously 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 second line asparaginase therapy is terminated unnecessarily.

Introduction

Asparaginase is one of the key components of childhood acute lymphoblastic leukemia (ALL) therapy as intensive dosing-schedules improve event free survival with 10-15%. [1-7]

Unfortunately, asparaginase treatment is hampered by hypersensitivity reactions like clinical allergies and silent inactivation, which neutralize asparaginase completely.[8] In 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 *E. coli* asparaginase in 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 anti-asparaginase 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 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 continuous 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.

Methods

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 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 ($2,500 \text{ IU/m}^2$) after an asparaginase-free interval of approximately 4 weeks. The doses were administered intravenously over two hours: ten percent 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 $1,500 \text{ IU/m}^2$, 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 $5,000 \text{ IU/m}^2$ native *E. coli* asparaginase in induction and 15 PEGasparaginase doses ($2,500 \text{ IU/m}^2$ biweekly) in the intensification and maintenance phase, 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 protocol, asparaginase was administered intravenously over one hour. 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 one hour. Asparaginase treatment was terminated when patients developed a hypersensitivity reaction to the latter formulation as well.

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 (> 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 anti-asparaginase antibodies. Allergic and allergic-like reactions were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Asparaginase activity analysis and anti-asparaginase 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 μL) was diluted with 8-hydroxyquinoline for condensation and oxidation and was quantified by photometric detection at 690 nm. Trough PEGasparaginase activity levels were measured two weeks after administration and were considered adequate when >100 IU/L. Trough *Erwinia* asparaginase activity levels were measured 48 or 72 hours 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]

Statistical analysis

The data was analysed 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 anti-asparaginase antibodies were analysed by non-parametric tests. Symptoms were analysed using the Fisher's exact test. A p-value <0.05 was considered statistically significant.

Results

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

Asparaginase activity levels

Asparaginase activity levels are described in table I. Nine levels were obtained just prior to the allergy-inducing dose, five were measured after the reaction. In patients number one and two, 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 one week after the reaction to PEGasparaginase.

Most patients had adequate *Erwinia* asparaginase trough levels after a dose interval of 48 hours. 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-hour dose interval. However, after increasing the dose frequency to a 48-hour 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 II). Since the majority of the reactions occurred almost immediately after start of 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.

Anti-asparaginase antibodies

Anti-asparaginase 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 I). 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 ($p=0.001$).

Clinical symptoms

Clinical characteristics are described in table I for the allergic-like reactions, in table II for the real allergies and summarized in table III. The median CTCAE grade was grade two in both allergic and allergic-like reactions. Four out of the 14 allergic-like reactions were grade one, the grade of one reaction is unknown and the remaining nine were grade two. All real allergic reactions were grade two. The type of reactions, i.e. a) symptoms of rash, oedema, itchiness or urticaria, b) pulmonary symptoms and c) gastro-intestinal symptoms, did not differ between patients with an allergic or allergic-like reaction (table III).

Allergic-like reactions occurred significantly later after the start of infusion (median: 29 minutes, 25-75 percentile: 12-47 minutes) compared to real allergic reactions (median 2 minutes, 25-75 percentile: 1-5 minutes) ($p<0.001$). Patient number two developed an allergic-like reaction 24 hours 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 minutes (25-75 percentile: 11-42 minutes) ($p<0.001$).

Further treatment

In three out 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 out 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.

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 show 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 frequently studied. 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 reactions. In our cohort, all patients with a real allergic reaction had anti-asparaginase antibodies whereas antibodies were absent in all but one of the allergic-like patients ($p=0.001$). 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 allergy 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 minutes. 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 discussed that allergic-like reactions are related to the non-antibody 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 oedema, 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 frequent 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 far majority of these patients, but distinction can only be made when asparaginase activity levels are monitored continuously, as it is done with therapeutic drug monitoring. 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.

Acknowledgements

Author contributions: R.Q.H.K. and I.M.S. analysed the data and wrote the manuscript. R.P. and G.E. interpreted the data and revised the manuscript critically.

Conflict of Interest Statement

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

References

1. Amylon MD, Shuster J, Pullen J, Berard C, Link MP, Wharam M, Katz J, Yu A, Laver J, Ravindranath Y, Kurtzberg J, Desai S, Camitta B, Murphy SB. 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, Suci S, Ferster A, Rialland X, Nelken B, Lutz P, Benoit Y, Robert A, Manel AM, Vilmer E, Otten J, Philippe N. 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, 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(3):896-904.
4. Pession A, Valsecchi MG, Masera G, Kamps WA, Magyarosy E, Rizzari C, van Wering ER, Lo Nigro L, van der Does A, Locatelli F, Basso G, Arico M. 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, Conter V, Testi A, Barisone E, Casale F, Lo Nigro L, Rondelli R, Basso G, Santoro N, Masera G, Associazione Italiano Ematologia Oncologia P. 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, Asselin BL, Barr RD, Clavell LA, Hurwitz CA, Moghrabi A, Samson Y, Schorin MA, Arkin S, Declerck L, Cohen HJ, Sallan SE. 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, Suci 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(2):239-247.
8. 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(13):2026-2033.
9. 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, Children's Cancer Group Study CCG. 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, 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(2):238-249.

11. Vrooman LM, Stevenson KE, Supko JG, O'Brien J, Dahlberg SE, Asselin BL, Athale UH, Clavell LA, Kelly KM, Kutok JL, Laverdiere C, Lipshultz SE, Michon B, Schorin M, Relling MV, Cohen HJ, Neuberg DS, Sallan SE, Silverman LB. 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, 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(11):2303-2309.
14. Killander D, Dohlwitz A, Engstedt L, Franzen S, Gahrton G, Gullbring B, Holm G, Holmgren A, Hoglund S, Killander A, Lockner D, Mellstedt H, Moe PJ, Palmblad J, Reizenstein P, Skarberg KO, Swedberg B, Uden AM, Wadman B, Wide L, Ahstrom L. 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, Hop WC, Boos J, Tissing WJ, Van der Sluis IM. Toxicity of very prolonged PEGasparaginase and Erwiniaasparaginase courses in relation to asparaginase activity levels with a special focus on dyslipidemia. *Haematologica* 2014.