Individualized asparaginase therapy for children with acute lymphoblastic leukemia

Robin Q.H. Kloos

Printing of this thesis was financially supported by:
Erasmus University Rotterdam

ISBN: 978-94-6380-733-3

Printing by: ProefschriftMaken
Cover design: H.H. Kloos

Individualized asparaginase therapy for children with acute lymphoblastic leukemia

Proefschrift

Ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
Op gezag de
rector magnificus

Prof. dr. R.C.M.E. Engels

en volgens het besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
27 oktober 2020

door

Robin Quirine Henriëtte Kloos
geboren te Utrecht

Erasmus University Rotterdam

PROMOTIECOMMISSIE

Promotor:	Prof. dr. R. Pieters
Overige leden:	Prof. dr. R. Mathôt
	Prof. dr. C.A. Uyl-de Groot
	Prof. dr. P. Hoogerbrugge
Co-promotor:	Dr. I.M. van der Sluis

CONTENTS

Part VI

CHAPTER 1

General introduction

INTRODUCTION

Pediatric acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children as it accounts for more than a quarter of all pediatric malignancies.[1] The peak incidence occurs at 3 to 5 years of age.[2] Over the past decades, advances in the treatment of ALL have resulted in current 5-year overall survival rates of approximately 90% in many developed countries.[3-10] In the Netherlands, patients are currently treated according to the Dutch Childhood Oncology Group (DCOG) ALL-11 treatment protocol, which contains several treatment phases: the induction, consolidation, intensification and maintenance phase. These treatment phases consist of a combination of chemotherapeutic agents including vincristine, corticosteroids, methotrexate, cytarabine, cyclophosphamide, mercaptopurine, anthracyclines and asparaginase.
According to DCOG ALL-11, patients are stratified after induction to a standard, medium and high risk group based on treatment response and cytogenetic aberrations of the leukemia. As approximately 70% of the patients is stratified in the medium risk group and these patients are most intensively treated with asparaginase, this thesis mainly focusses on the medium risk treatment protocol.

Asparaginase

Since the 1970's, asparaginase is one of the key components of pediatric ALL therapy. The enzymatic drug catalyzes the hydrolysis of asparagine to aspartic acid and ammonia. Normal cells are able to restore their intracellular asparagine storage from aspartic acid with the enzyme asparagine synthetase. In contrast, leukemic cells depend on extracellular asparagine pools. Hence, extracellular asparagine depletion, accomplished by asparaginase therapy, selectively kills the leukemic cells.[11] This is not necessarily caused by a lack of asparagine synthetase in the leukemic cells: although asparaginase resistance has been associated with asparagine synthetase expression[12, 13], several other studies have failed to reproduce this correlation.[14-16] Thus, other mechanisms may also play a role.[17]

For many years, the general consensus of opinion is that a minimal asparaginase activity level of 100 IU/L is required for complete asparagine depletion in both serum and cerebrospinal fluid (CSF).[18-27] *In vivo*, asparagine measurement is challenging, though, due to *ex vivo* asparaginase activity in blood samples. Due to

this, the asparaginase activity levels currently reported at which asparagine is supposed to be completely depleted vary between 20 – 400 IU/L. In only one out of 11 studies (the study of Angiolillo et al.), asparagine levels rebounded when asparaginase activity levels became <400 IU/L.[28]. All other ten studies, however, report thresholds around 100 IU/L or lower.[19, 20, 29, 30, 22, 24, 25] Table 1 shows the different studies with adequate sample handling (directly put on ice, centrifuged, deproteinized and stored at -20 to -80°C). Thus, an asparaginase activity level of 100 IU/L is currently considered as the minimal asparaginase activity level required.

Table 1. Minimal asparaginase activity level for complete asparagine depletion

Study	Number of patients	Limit of quantification	Minimal asparaginase level
Ahlke et al. 1997[19]	11	0.1 µM	100 IU/L
Riccardi et al. 1982	7 (+ rhesus monkeys)	0.2 µM	100 IU/L
Rizzari et al. 2000[20]	62	0.2 µM	50 IU/L
Albertsen et al. 2001[31]	15	0.2 µM	100 IU/L
Rizzari et al. 2006[22]	20	0.2 µM	30 IU/L
Avramis et al. 2007[23]	5	0.01 µM	100 IU/L
Pieters et al. 2008[25]	32	0.5 µM	20 IU/L
Appel et al. 2008[24]	57	0.2 µM	100 IU/L
Strullu et al. 2010[26]	33	0.1 µM	100 IU/L
Tong et al. 2013[32]	23	0.2 µM	40 IU/L
Angiolillo et al. 2014[28]	165	0.05 µM	400 IU/L

Several formulations of asparaginase, which are derived from bacteria, are available for clinical practice. Asparaginase gained from the *Escherichia coli* bacteria is used in its native form (native *E. coli* asparaginase) but also in a poly-ethylene glycol (PEG) conjugated form, PEGasparaginase. By PEGylating a drug, polyethylene glycol groups are linked to the drug in order to decrease immunogenicity and prolong a drug's half-life.[33, 34] Hence, PEGasparaginase is less immunogenic than native *E. coli* asparaginase and has to be administered less frequently (biweekly versus every three days).[35] Native *E. coli* asparaginase is also available in a recombinant version, produced in *E. coli* cells by recombinant DNA technology, which has equal pharmacokinetic properties as the natural formulation.

A third type of asparaginase is derived from *Erwinia Chrysanthemi* bacteria (*Erwinia* asparaginase) and is, therefore, immunologically distinct from the *E. coli* asparaginase formulations. Unfortunately, *Erwinia* asparaginase therapy has several disadvantages: the drug has a relative short half-life, which results in an

inconvenient dosing schedule as it requires an administration frequency of at least three times a week. Consequently, *Erwinia* asparaginase therapy is relatively expensive when compared to the other formulations. Furthermore, if patients develop an immunological reaction to this formulation, there are no alternatives.

Several studies have shown that intensive and adequate asparaginase treatment improves the event-free survival (EFS) of ALL with approximately 10% (Table 2).[36-39, 3, 4, 10] This conclusion was not only based on studies in which patients were treated either with or without intensified asparaginase therapy, but also on studies that compared identical dose schedules of native *E. coli* asparaginase and *Erwinia* asparaginase, the latter administrated in a less effective dosing schedule due to a lack of pharmacokinetic knowledge in those days.

Table 2. Efficacy of intensified asparaginase treatment

Study	Number of patients	Treatment	Outcome 5-year EFS
Mondelaers et al. 2017[40]	1552	24 vs. 12 doses of native *E. coli* asparaginase	87% vs. 84%
Vilmer et al. 2010[41]	653	Native *E. coli* asparaginase vs *Erwinia* asparaginase, both 25,000 IU/m^2	74% vs. 61% *
Moghrabi et al. 2007[4]	286	Native *E. coli* asparaginase vs. *Erwinia* asparaginase, both 25,000 IU/m^2, weekly	89% vs. 78% *
Pession et al. 2005[3]	355	>20 weeks vs. ≤ 20 weeks of asparaginase	88% vs. 83% *
Silverman et al. 2001[38]	377	>25 weeks vs. ≤ 25 weeks of asparaginase	90% vs. 73% *
Duval et al. 2002[39]	700	Native *E. coli* asparaginase vs. *Erwinia* asparaginase, both 10,000 IU/m^2, weekly	73% vs. 60% *
Rizzari et al. 2001[37]	610	>20 weeks vs. ≤ 20 weeks of asparaginase	75% vs. 72%
Amylon et al. 1999[36]	317	>20 weeks vs. ≤ 20 weeks of asparaginase in patients with T-cell ALL	68% vs. 55% *

EFS: event-free survival; ALL: acute lymphoblastic leukemia; vs.: versus; *: statistically significant difference

Asparaginase toxicity

Although asparaginase is very effective, patients may develop severe adverse events, possibly limiting the efficacy of the therapy. These events include the development of hypersensitivity reactions, pancreatitis, thromboembolic events, central neurotoxicity, hepatotoxicity, hypertriglyceridemia, and bone marrow suppression, which are elaborated below.

Hypersensitivity reactions

Patients may develop a hypersensitivity reaction to asparaginase, neutralizing the drug completely.[42-44] The neutralizing reactions vary from mild allergic reactions to an anaphylactic shock, all accompanied by asparaginase activity levels of zero. In addition, asparaginase may even be neutralized in absence of clinical symptoms, which is called silent inactivation.[45, 19, 26] The rates of these reactions vary in the literature, partly due to the type of asparaginase used. Hypersensitivity reactions have been reported up to 75% in patients treated with native E. coli asparaginase and in 3-30% of the patients treated with PEGasparaginase. [38, 46-50] Hypersensitivity reactions to *Erwinia* asparaginase are reported in 3-37%.[51, 52, 4, 48, 53, 54, 49]
Beside the formulation, the dosing schedule plays an important role: if asparaginase treatment is interrupted for several weeks or months, patients may develop anti-asparaginase antibodies during this period of interruption, causing hypersensitivity reactions to asparaginase directly after this period.[21, 49] Also the route of administration was thought to be a risk factor but a recent review of Beaupin et al. has shown that hypersensitivity reactions occur just as often when asparaginase is administered intravenously as intramuscularly.[55] In the Netherlands, asparaginase is administered intravenously.
Several studies have shown that, although by PEGylation the immunogenicity has been decreased, patients can develop antibodies to the PEG moiety itself, possibly resulting in allergic reactions and/or rapid clearance of the PEGylated drug.[56-60] PEGasparaginase consists of *E. coli* asparaginase, PEG and a succinimidyl succinate linker (SS-linker). Currently, the role of antibodies to the PEG and linker moieties, so other than to the asparaginase itself, in the development of hypersensitivity to PEGasparaginase is unclear.

Pancreatitis

Pancreatitis has been reported in 2-18% of the patients treated with asparaginase.[61-65] These percentages include Common Terminology Criteria for Adverse Events (CTCAE) grade 2 pancreatitis, which, according to version 4.03, is described as enzyme elevation or radiologic findings only. Thus, it can be questioned whether these patients had an actual pancreatitis. Grade 3 and 4 asparaginase-associated pancreatitis occurs in 5-10% of the patients .[61, 63, 65] The pathophysiology of asparaginase-associated pancreatitis is unknown but the systemic asparagine depletion is believed to affect especially organs with a high

protein turnover, as is the pancreas.[66] In addition, genetic predispositions seem to play a role, for example variants in the CPAP2 gene which encodes for the pancreatic zymogen carboxypeptidase.[67] Currently, it has been recommended by the Ponte de Legno Toxicity Working Group to use the following modified Atlanta criteria of which at least two out of the three criteria are required to diagnose asparaginase-associated pancreatitis: 1) abdominal symptoms suggestive of pancreatitis; 2) serum lipase, amylase or both being three times or more than the upper limit of normal; and 3) imaging findings characteristic of acute pancreatitis.[68] As such, a CTCAE grade 2 pancreatitis does not fulfill these criteria. In general, asparaginase will be discontinued in case of pancreatitis although Hijiya et al. recommend to consider continuation if symptoms resolve within 48 hours with no signs of pseudocysts or necrosis on imaging.[69] An international study reported that 23% of the patients who were re-exposed to asparaginase after having experienced pancreatitis developed a second pancreatitis. However, risk factors predicting a second pancreatitis were not found.[68]

Thromboembolic events

Asparaginase treatment is associated with reduced coagulation and fibrinolysis proteins. Mainly the decline in anticoagulant proteins C and S, and antithrombin III levels may lead to thrombotic events, especially in combination with corticosteroids.[70, 71] Previous studies have shown that not necessarily the dosage of asparaginase but the length of exposure is associated with the development of these events.[72, 73] The incidence of thromboembolic events during ALL treatment vary from 1 to 36%, depending upon the patient groups studied (children versus adults), treatment protocols, and study design (symptomatic events only versus screening, and in- and exclusion of thrombotic events related to central venous catheters (CVC)).[71] In children with ALL, the incidence is in the lower range. The meta-analysis of Caruso et al. reports an overall incidence of 5% thrombotic events during pediatric ALL treatment. Almost one third of these events was related to CVC's and more than half of the events occurred in the central nervous system.

Furthermore, most events were during the induction phase, probably due to the intensive treatment and still active disease in this treatment phase.[72] Grace et al. have studied thrombotic events in a treatment protocol similar to the DCOG treatment protocols, with asparaginase during induction followed by 30 weeks of asparaginase during intensification. This study reports an incidence of 5%,

increasing with the patient's age. This incidence, however, also include CVC related thrombotic events (35%).[74] Hijaya et al. recommend to discontinue asparaginase temporarily in case of clinically significant bleeding or thrombosis.[69] However, re-exposure of asparaginase is recommended with low-molecular-weight heparin once clinical symptoms have been resolved, enabling around 75% of the patients to finish their treatment safely. [75, 74, 69]

Central neurotoxicity

Asparaginase-associated central neurotoxicity has been described less extensively in the literature and is often caused by thrombotic events such as sagittal sinus thrombosis or, less frequently, cerebral hemorrhages.[76] Focusing on neurotoxicity not caused by coagulation alterations, the exact relationship between asparaginase treatment and the development of neurotoxicity is unclear.[69] Central neurotoxicity includes symptoms of ataxia, somnolence, depressed level of consciousness, agitation, seizures and posterior reversible encephalopathy syndrome (PRES). Asparaginase-associated central neurotoxicity usually has a good prognosis and symptoms resolve without complications in most cases.[77, 69]

Hepatotoxicity

Hepatotoxicity (hepatic transaminase and bilirubin elevation) caused by asparagine depletion as a result of asparaginase treatment is rarely fatal but may cause a delay in treatment, negatively affecting treatment outcomes.[69] As many drugs concomitantly used with asparaginase can cause hepatotoxicity, it is difficult to study the exact contribution of asparaginase to the occurrence of hepatotoxicity.[46, 35] The incidence of grade 3-4 hepatotoxicity is the highest in adult patients treated with asparaginase (35 to 60% of the patients), and occurs less often in children (4 to 8% of the patients).[78, 69] It has been recommended to postpone an asparaginase dose in case of increased transaminases (>10 times the upper limit of normal) or bilirubin (>3 times the upper limit of normal).

Hypertriglyceridemia

Hypertriglyceridemia is common in pediatric ALL patients treated with asparaginase, especially in combination with corticosteroids, and is described in up to 67% of the patients.[79, 69] Although increased triglyceride concentrations have been associated with the occurrence of pancreatitis and thrombosis, asparaginase induced hypertriglyceridemia is not and has no clinical consequences.[80-82]

Myelosuppression

The precise myelosuppressive effect of asparaginase is unclear: asparaginase either could cause myelosuppression directly or by inducing the myelosuppressive effects of other drugs as methotrexate and 6-mercaptopurine.[69] Merryman et al. have shown that patients have more myelosuppression and require more 6-mercaptopurine and methotrexate dose reductions during concomitant asparaginase therapy than during continuation therapy without asparaginase, showing the myelosuppressive effect of asparaginase. [83]

Previous results

Until April 2012, patients were treated according to the DCOG ALL-10 treatment protocol. According to this protocol, patients were treated with 5,000 IU/m^2 native *E. coli* asparaginase during induction and 2,500 IU/m^2 PEGasparaginase during intensification after an asparaginase-free interval of approximately 12 weeks. Studies of the asparaginase treatment in this protocol have led to several insights on which the aims for this thesis were based.
It was shown that 22% of the patients treated according to ALL-10 developed an allergy to and 8% silent inactivation of PEGasparaginase during intensification, almost exclusively on the second PEGasparaginase dose. These reactions were caused by antibodies to native *E. coli* asparaginase, developed during the asparaginase-free interval after induction, and cross-reacting with PEGasparaginase during intensification. However, these antibody titers also increased in part of the patients without a hypersensitivity reaction.[49] The incidence of hypersensitivity reactions was much lower during the native *E.coli* asparaginase doses administered in induction: 5% had either an allergic reaction to or silent inactivation of the drug. Of the patients without a reaction or silent inactivation, the mean trough

PEGasparaginase activity level was 899 IU/L, which is relatively high compared to the recommended level of 100 IU/L.[49] The asparaginase activity levels measured were positively correlated with triglyceride concentrations and 47% of the patients developed grade 3-4 hypertriglyceridemia during PEGasparaginase treatment. During intensification, grade 3-4 pancreatitis occurred in 5% of the patients; thrombotic events (excluding CVC-related events) in 3%; and central neurotoxicity in 10%.[65]
During induction, the native *E. coli* asparaginase levels ranged between 143 – 182 IU/L , being lower than the PEGasparaginase levels during intensification. Pancreatitis occurred in 1%, and both thrombosis and central neurotoxicity in 2%.[84] Hypertriglyceridemia during induction was not reported.

The DCOG ALL-11 treatment protocol

Based on these results, certain adjustments were made in the subsequent DCOG ALL-11 treatment protocol. First, native *E. coli* asparaginase in induction was replaced by PEGasparaginase so PEGasparaginase was used in both the induction and intensification phase. Thus, medium risk patients are treated with three PEGasparaginase doses during induction and another 14 doses during intensification.
Second, a unique therapeutic drug monitoring (TDM) program was implemented to 1) adjust the PEGasparaginase dosage based on asparaginase activity levels in order to prevent too high trough levels and 2) identify patients with silent inactivation of PEGasparaginase. The first three doses during induction had a fixed dose of 1,500 IU/m^2 PEGasparaginase but after the third dose, the dose was adjusted based on trough PEGasparaginase serum levels. If patients developed a hypersensitivity reaction to PEGasparaginase, the formulation was switched to *Erwinia* asparaginase. Also the *Erwinia* asparaginase treatment was individualized to ensure optimal asparaginase activity levels.

Third, patients were either randomized to a standard discontinuous asparaginase dosing schedule, similar to the previous ALL-10 dosing schedule, or an experimental continuous asparaginase dosing schedule, to study whether this will decrease the occurrence of hypersensitivity reactions.

By continuous administration of the PEGasparaginase doses, patients are treated concomitantly with asparaginase and 6-mercaptopurine, cytarabine and

cyclophosphamide in the first consolidation course, and with asparaginase and high dose methotrexate and 6-mercaptopurine during the second consolidation course. Previous studies have shown a possible effect of asparaginase on methotrexate efficacy and toxicity *in vitro*, depending on the sequence of administration.[85-88] However, the exact effect *in vivo* is unclear. The asparaginase randomization study, thus, allows us to compare the methotrexate efficacy and toxicity with and without concomitant asparaginase treatment.

Aims

The aim of this thesis was to study the feasibility, efficacy and toxicity of individualized asparaginase treatment and optimize the asparaginase treatment of children with ALL.

The specific aims were:

- To study the feasibility, efficacy and toxicity of individualized asparaginase treatment in children with ALL.
- To develop a model to describe the population pharmacokinetics of PEGasparaginase and identify factors explaining variability in order to improve asparaginase therapeutic drug monitoring.
- To study the different types of hypersensitivity reactions to asparaginase.
- To develop a sensitive assay to measure antibodies to the different components of PEGasparaginase (PEG, the SS-linker and asparaginase) and study the different types of antibodies formed.
- To study the influence of asparaginase on methotrexate efficacy and toxicity.
- To compare the costs of an individualized dosing schedule with a fixed dosing schedule.
- To study the cost-efficacy of the *Erwinia* asparaginase.

Outline of the thesis

In chapter 2, the feasibility, efficacy and toxicity of individualized asparaginase treatment in children treated according to the DCOG ALL-11 protocol have been studied. In chapter 3, the population pharmacokinetics of PEGasparaginase are studied using non-linear mixed effects modelling (NONMEM), identifying factors explaining the variability in asparaginase activity that was observed. With this model, dosing guidelines were provided.

In chapter 4, a new, atypical type of hypersensitivity reaction to asparaginase was described. These allergic-like reactions mimic real allergic reactions but are not accompanied by inactivation of the drug. In chapter 5, an assay to measure antibodies against the asparaginase, PEG and the linker was developed. Next, it was studied which types of antibodies were formed in patients with a hypersensitivity reaction to PEGasparaginase.

In chapter 6, the influence of asparaginase on high dose methotrexate efficacy and toxicity was studied.

In chapter 7, the costs of individualized asparaginase treatment were compared with the costs of a fixed dosing schedule. Chapter 8 is a cost-effectiveness analysis of *Erwinia* asparaginase treatment.

In chapter 9, the results of this thesis are discussed.

REFERENCES

1. Kaatsch, P. Epidemiology of childhood cancer. Cancer Treat Rev. 2010;36(4):277-285.
2. Ries, L. A. G., M. A. Smith and J. G. Gurney. Cancer incidence and survival among children and adolescents: United States SEER Program 1975-1995. Bethesda, MD: National Cancer Institute, SEER Program. 1999.
3. Pession, A., M. G. Valsecchi, G. Masera, W. A. Kamps, E. Magyarosy, C. Rizzari, 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.
4. Moghrabi, A., D. E. Levy, B. Asselin, R. Barr, L. Clavell, C. Hurwitz, 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.
5. Conter, V., C. R. Bartram, M. G. Valsecchi, A. Schrauder, R. Panzer-Grumayer, A. Moricke, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood. 2010;115(16):3206-3214.
6. Mitchell, C., S. Richards, C. J. Harrison and T. Eden. Long-term follow-up of the United Kingdom medical research council protocols for childhood acute lymphoblastic leukaemia, 1980-2001. Leukemia. 2010;24(2):406-418.
7. Moricke, A., M. Zimmermann, A. Reiter, G. Henze, A. Schrauder, H. Gadner, et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia. 2010;24(2):265-284.
8. Schmiegelow, K., E. Forestier, M. Hellebostad, M. Heyman, J. Kristinsson, S. Soderhall, et al. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia. 2010;24(2):345-354.
9. Hunger, S. P., X. Lu, M. Devidas, B. M. Camitta, P. S. Gaynon, N. J. Winick, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. J Clin Oncol. 2012;30(14):1663-1669.
10. Pieters, R., H. de Groot-Kruseman, V. Van der Velden, M. Fiocco, H. van den Berg, E. de Bont, et al. Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group. J Clin Oncol. 2016;34(22):2591 - 2601.
11. Muller, H. J. and J. Boos. Use of L-asparaginase in childhood ALL. Crit Rev Oncol Hematol. 1998;28(2):97-113.
12. Aslanian, A. M. and M. S. Kilberg. Multiple adaptive mechanisms affect asparagine synthetase substrate availability in asparaginase-resistant MOLT-4 human leukaemia cells. Biochem J. 2001;358(Pt 1):59-67.
13. Holleman, A., M. H. Cheok, M. L. den Boer, W. Yang, A. J. Veerman, K. M. Kazemier, et al. Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment. N Engl J Med. 2004;351(6):533-542.
14. Ramakers-van Woerden, N. L., R. Pieters, A. H. Loonen, I. Hubeek, E. van Drunen, H. B. Beverloo, et al. TEL/AML1 gene fusion is related to in vitro drug sensitivity for L-asparaginase in childhood acute lymphoblastic leukemia. Blood. 2000;96(3):1094-1099.
15. Stams, W. A., M. L. den Boer, H. B. Beverloo, J. P. Meijerink, R. L. Stigter, E. R. van Wering, et al. Sensitivity to L-asparaginase is not associated with expression levels of asparagine synthetase in t(12;21)+ pediatric ALL. Blood. 2003;101(7):2743-2747.
16. Fine, B. M., G. J. Kaspers, M. Ho, A. H. Loonen and L. M. Boxer. A genome-wide view of the in vitro response to l-asparaginase in acute lymphoblastic leukemia. Cancer Res. 2005;65(1):291-299.
17. Lanvers-Kaminsky, C. Asparaginase pharmacology: challenges still to be faced. Cancer Chemother Pharmacol. 2017.
18. Riccardi, R., J. S. Holcenberg, D. L. Glaubiger, J. H. Wood and D. G. Poplack. L-asparaginase pharmacokinetics and asparagine levels in cerebrospinal fluid of rhesus monkeys and humans. Cancer Res. 1981;41(11 Pt 1):4554-4558.

19. Ahlke, E., U. Nowak-Gottl, P. Schulze-Westhoff, G. Werber, H. Borste, G. Wurthwein, et al. Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during induction therapy in children with acute lymphoblastic leukaemia. Br J Haematol. 1997;96(4):675-681.
20. Rizzari, C., M. Zucchetti, V. Conter, L. Diomede, A. Bruno, L. Gavazzi, et al. L-asparagine depletion and L-asparaginase activity in children with acute lymphoblastic leukemia receiving i.m. or i.v. Erwinia C. or E. coli L-asparaginase as first exposure. Ann Oncol. 2000;11(2):189-193.
21. Albertsen, B. K., H. Schroder, J. Ingerslev, P. Jakobsen, V. I. Avramis, H. J. Muller, et al. Comparison of intramuscular therapy with Erwinia asparaginase and asparaginase Medac: pharmacokinetics, pharmacodynamics, formation of antibodies and influence on the coagulation system. Br J Haematol. 2001;115(4):983-990.
22. Rizzari, C., M. Citterio, M. Zucchetti, V. Conter, R. Chiesa, A. Colombini, et al. A pharmacological study on pegylated asparaginase used in front-line treatment of children with acute lymphoblastic leukemia. Haematologica. 2006;91(1):24-31.
23. Avramis, V. I., S. Martin-Aragon, E. V. Avramis and B. L. Asselin. Pharmacoanalytical assays of Erwinia asparaginase (erwinase) and pharmacokinetic results in high-risk acute lymphoblastic leukemia (HR ALL) patients: simulations of erwinase population PK-PD models. Anticancer Res. 2007;27(4C):2561-2572.
24. Appel, I. M., K. M. Kazemier, J. Boos, C. Lanvers, J. Huijmans, A. J. Veerman, et al. Pharmacokinetic, pharmacodynamic and intracellular effects of PEG-asparaginase in newly diagnosed childhood acute lymphoblastic leukemia: results from a single agent window study. Leukemia. 2008;22(9):1665-1679.
25. Pieters, R., I. Appel, H. J. Kuehnel, I. Tetzlaff-Fohr, U. Pichlmeier, I. van der Vaart, et al. Pharmacokinetics, pharmacodynamics, efficacy, and safety of a new recombinant asparaginase preparation in children with previously untreated acute lymphoblastic leukemia: a randomized phase 2 clinical trial. Blood. 2008;112(13):4832-4838.
26. Strullu, M., N. Corradini, M. Audrain, J. L. Orsonneau, D. Bouige, P. Thomare, et al. Silent hypersensitivity to Escherichia coli asparaginase in children with acute lymphoblastic leukemia. Leuk Lymphoma. 2010;51(8):1464-1472.
27. Tong, W. H., I. M. van der Sluis, C. J. Alleman, R. R. van Litsenburg, G. J. Kaspers, R. Pieters, et al. Cost-analysis of treatment of childhood acute lymphoblastic leukemia with asparaginase preparations: the impact of expensive chemotherapy. Haematologica. 2013;98(5):753-759.
28. Angiolillo, A. L., R. J. Schore, M. Devidas, M. J. Borowitz, A. J. Carroll, J. M. Gastier-Foster, et al. Pharmacokinetic and Pharmacodynamic Properties of Calaspargase Pegol Escherichia coli L-Asparaginase in the Treatment of Patients With Acute Lymphoblastic Leukemia: Results From Children's Oncology Group Study AALL07P4. J Clin Oncol. 2014.
29. Grigoryan, R. S., E. H. Panosyan, N. L. Seibel, P. S. Gaynon, I. A. Avramis and V. I. Avramis. Changes of amino acid serum levels in pediatric patients with higher-risk acute lymphoblastic leukemia (CCG-1961). In Vivo. 2004;18(2):107-112.
30. Tsurusawa, M., M. Chin, A. Iwai, K. Nomura, H. Maeba, T. Taga, et al. L-Asparagine depletion levels and L-asparaginase activity in plasma of children with acute lymphoblastic leukemia under asparaginase treatment. Cancer Chemother Pharmacol. 2004;53(3):204-208.
31. Albertsen, B. K., H. Schroder, P. Jakobsen, H. J. Muller, N. T. Carlsen and K. Schmiegelow. Monitoring of Erwinia asparaginase therapy in childhood ALL in the Nordic countries. Br J Clin Pharmacol. 2001;52(4):433-437.
32. Tong, W. H., R. Pieters, W. C. Hop, C. Lanvers-Kaminsky, J. Boos and I. M. van der Sluis. No evidence of increased asparagine levels in the bone marrow of patients with acute lymphoblastic leukemia during asparaginase therapy. Pediatric blood & cancer. 2013;60(2):258-261.
33. Abuchowski, A., J. R. McCoy, N. C. Palczuk, T. van Es and F. F. Davis. Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. J Biol Chem. 1977;252(11):3582-3586.
34. Abuchowski, A., T. van Es, N. C. Palczuk and F. F. Davis. Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. J Biol Chem. 1977;252(11):3578-3581.

35. Dinndorf, P. A., J. Gootenberg, M. H. Cohen, P. Keegan and R. Pazdur. FDA drug approval summary: pegaspargase (oncaspar) for the first-line treatment of children with acute lymphoblastic leukemia (ALL). Oncologist. 2007;12(8):991-998.
36. Amylon, M. D., J. Shuster, J. Pullen, C. Berard, M. P. Link, M. Wharam, 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.
37. Rizzari, C., M. G. Valsecchi, M. Arico, V. Conter, A. Testi, E. Barisone, 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.
38. Silverman, L. B., R. D. Gelber, V. K. Dalton, B. L. Asselin, R. D. Barr, L. A. Clavell, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97(5):1211-1218.
39. Duval, M., S. Suciu, A. Ferster, X. Rialland, B. Nelken, P. Lutz, 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.
40. Mondelaers, V., S. Suciu, B. De Moerloose, A. Ferster, F. Mazingue, G. Plat, et al. Prolonged versus standard native E. coli asparaginase therapy in childhood acute lymphoblastic leukemia and non-Hodgkin lymphoma: final results of the EORTC-CLG randomized phase III trial 58951. Haematologica. 2017;102(10):1727-1738.
41. Vilmer, E., S. Suciu, A. Ferster, Y. Bertrand, H. Cave, A. Thyss, et al. Long-term results of three randomized trials (58831, 58832, 58881) in childhood acute lymphoblastic leukemia: a CLCG-EORTC report. Children Leukemia Cooperative Group. Leukemia. 2000;14(12):2257-2266.
42. Oettgen, H. F., P. A. Stephenson, M. K. Schwartz, R. D. Leeper, L. Tallai, C. C. Tan, et al. Toxicity of E. coli L-asparaginase in man. Cancer. 1970;25(2):253-278.
43. Peterson, R. G., R. E. Handschumacher and M. S. Mitchell. Immunological responses to L-asparaginase. J Clin Invest. 1971;50(5):1080-1090.
44. Killander, D., A. Dohlwitz, L. Engstedt, S. Franzen, G. Gahrton, B. Gullbring, et al. Hypersensitive reactions and antibody formation during L-asparaginase treatment of children and adults with acute leukemia. Cancer. 1976;37(1):220-228.
45. Asselin, B. L., J. C. Whitin, D. J. Coppola, I. P. Rupp, S. E. Sallan and H. J. Cohen. Comparative pharmacokinetic studies of three asparaginase preparations. J Clin Oncol. 1993;11(9):1780-1786.
46. Avramis, V. I., S. Sencer, A. P. Periclou, H. Sather, B. C. Bostrom, L. J. Cohen, et al. 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(6):1986-1994.
47. Wacker, P., V. J. Land, B. M. Camitta, J. Kurtzberg, J. Pullen, M. B. Harris, et al. 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(9):627-632.
48. Raetz, E. A. and W. L. Salzer. Tolerability and efficacy of L-asparaginase therapy in pediatric patients with acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 2010;32(7):554-563.
49. Tong, W. H., R. Pieters, G. J. Kaspers, D. M. te Loo, M. B. Bierings, C. van den Bos, 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.
50. Liu, W. J., H. Wang, W. D. Wang, M. Y. Zhu, C. C. Liu, J. H. Wang, et al. Use of PEG-asparaginase in newly diagnosed adults with standard-risk acute lymphoblastic leukemia compared with E. coli-asparaginase: a retrospective single-center study. Sci Rep. 2016;6:39463.
51. Billett, A. L., A. Carls, R. D. Gelber and S. E. Sallan. Allergic reactions to Erwinia asparaginase in children with acute lymphoblastic leukemia who had previous allergic reactions to Escherichia coli asparaginase. Cancer. 1992;70(1):201-206.

52. Albertsen, B. K., H. Schroder, P. Jakobsen, V. I. Avramis, H. J. Muller, K. Schmiegelow, et al. Antibody formation during intravenous and intramuscular therapy with Erwinia asparaginase. Med Pediatr Oncol. 2002;38(5):310-316.
53. Vrooman, L. M., J. G. Supko, D. S. Neuberg, B. L. Asselin, U. H. Athale, L. Clavell, et al. Erwinia asparaginase after allergy to E. coli asparaginase in children with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2010;54(2):199-205.
54. Salzer, W. L., B. Asselin, J. G. Supko, M. Devidas, N. A. Kaiser, P. Plourde, et al. Erwinia asparaginase achieves therapeutic activity after pegaspargase allergy: a report from the Children's Oncology Group. Blood. 2013;122(4):507-514.
55. Beaupin, L. K., B. Bostrom, M. J. Barth, I. Franklin, R. Jaeger, P. Kamath, et al. Pegaspargase hypersensitivity reactions: intravenous infusion versus intramuscular injection - a review. Leuk Lymphoma. 2017;58(4):766-772.
56. Ganson, N. J., S. J. Kelly, E. Scarlett, J. S. Sundy and M. S. Hershfield. Control of hyperuricemia in subjects with refractory gout, and induction of antibody against poly(ethylene glycol) (PEG), in a phase I trial of subcutaneous PEGylated urate oxidase. Arthritis Res Ther. 2006;8(1):R12.
57. Koide, H., T. Asai, K. Hatanaka, S. Akai, T. Ishii, E. Kenjo, et al. T cell-independent B cell response is responsible for ABC phenomenon induced by repeated injection of PEGylated liposomes. Int J Pharm. 2010;392(1-2):218-223.
58. Garay, R. P., R. El-Gewely, J. K. Armstrong, G. Garratty and P. Richette. Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents. Expert Opin Drug Deliv. 2012;9(11):1319-1323.
59. Ishida, T. and H. Kiwada. Anti-polyethyleneglycol antibody response to PEGylated substances. Biol Pharm Bull. 2013;36(6):889-891.
60. Verhoef, J. J., J. F. Carpenter, T. J. Anchordoquy and H. Schellekens. Potential induction of anti-PEG antibodies and complement activation toward PEGylated therapeutics. Drug Discov Today. 2014;19(12):1945-1952.
61. Knoderer, H. M., J. Robarge and D. A. Flockhart. Predicting asparaginase-associated pancreatitis. Pediatr Blood Cancer. 2007;49(5):634-639.
62. Kearney, S. L., S. E. Dahlberg, D. E. Levy, S. D. Voss, S. E. Sallan and L. B. Silverman. Clinical course and outcome in children with acute lymphoblastic leukemia and asparaginase-associated pancreatitis. Pediatr Blood Cancer. 2009;53(2):162-167.
63. Samarasinghe, S., S. Dhir, J. Slack, P. Iyer, R. Wade, R. Clack, et al. Incidence and outcome of pancreatitis in children and young adults with acute lymphoblastic leukaemia treated on a contemporary protocol, UKALL 2003. Br J Haematol. 2013;162(5):710-713.
64. Raja, R. A., K. Schmiegelow, B. K. Albertsen, K. Prunsild, B. Zeller, G. Vaitkeviciene, et al. Asparaginase-associated pancreatitis in children with acute lymphoblastic leukaemia in the NOPHO ALL2008 protocol. Br J Haematol. 2014;165(1):126-133.
65. Tong, W. H., R. Pieters, H. A. de Groot-Kruseman, W. C. Hop, J. Boos, W. J. Tissing, et al. Toxicity of very prolonged PEGasparaginase and Erwiniaasparaginase courses in relation to asparaginase activity levels with a special focus on dyslipidemia. Haematologica. 2014.
66. Raja, R. A., K. Schmiegelow and T. L. Frandsen. Asparaginase-associated pancreatitis in children. Br J Haematol. 2012;159(1):18-27.
67. Liu, C., W. Yang, M. Devidas, C. Cheng, D. Pei, C. Smith, et al. Clinical and Genetic Risk Factors for Acute Pancreatitis in Patients With Acute Lymphoblastic Leukemia. J Clin Oncol. 2016;34(18):2133-2140.
68. Wolthers, B. O., T. L. Frandsen, A. Baruchel, A. Attarbaschi, S. Barzilai, A. Colombini, et al. Asparaginase-associated pancreatitis in childhood acute lymphoblastic leukaemia: an observational Ponte di Legno Toxicity Working Group study. Lancet Oncol. 2017;18(9):1238-1248.
69. Hijiya, N. and I. M. van der Sluis. Asparaginase-associated toxicity in children with acute lymphoblastic leukemia. Leuk Lymphoma. 2015:1-31.
70. Athale, U. H. and A. K. Chan. Thrombosis in children with acute lymphoblastic leukemia. Part II. Pathogenesis of thrombosis in children with acute lymphoblastic leukemia: effects of the disease and therapy. Thromb Res. 2003;111(4-5):199-212.
71. Goyal, G. and V. R. Bhatt. L-asparaginase and venous thromboembolism in acute lymphocytic leukemia. Future Oncol. 2015:1-12.

72. Caruso, V., L. Iacoviello, A. Di Castelnuovo, S. Storti, G. Mariani, G. de Gaetano, et al. Thrombotic complications in childhood acute lymphoblastic leukemia: a meta-analysis of 17 prospective studies comprising 1752 pediatric patients. Blood. 2006;108(7):2216-2222.
73. Payne, J. H. and A. J. Vora. Thrombosis and acute lymphoblastic leukaemia. Br J Haematol. 2007;138(4):430-445.
74. Grace, R. F., S. E. Dahlberg, D. Neuberg, S. E. Sallan, J. M. Connors, E. J. Neufeld, et al. The frequency and management of asparaginase-related thrombosis in paediatric and adult patients with acute lymphoblastic leukaemia treated on Dana-Farber Cancer Institute consortium protocols. Br J Haematol. 2011;152(4):452-459.
75. Qureshi, A., C. Mitchell, S. Richards, A. Vora and N. Goulden. Asparaginase-related venous thrombosis in UKALL 2003- re-exposure to asparaginase is feasible and safe. Br J Haematol. 2010;149(3):410-413.
76. Vagace, J. M., M. D. de la Maya, C. Caceres-Marzal, S. Gonzalez de Murillo and G. Gervasini. Central nervous system chemotoxicity during treatment of pediatric acute lymphoblastic leukemia/lymphoma. Crit Rev Oncol Hematol. 2012;84(2):274-286.
77. Hourani, R., M. Abboud, M. Hourani, H. Khalifeh and S. Muwakkit. L-asparaginase-induced posterior reversible encephalopathy syndrome during acute lymphoblastic leukemia treatment in children. Neuropediatrics. 2008;39(1):46-50.
78. Stock, W., D. Douer, D. J. DeAngelo, M. Arellano, A. Advani, L. Damon, et al. Prevention and management of asparaginase/pegasparaginase-associated toxicities in adults and older adolescents: recommendations of an expert panel. Leuk Lymphoma. 2011;52(12):2237-2253.
79. Parsons, S. K., S. X. Skapek, E. J. Neufeld, C. Kuhlman, M. L. Young, M. Donnelly, et al. Asparaginase-associated lipid abnormalities in children with acute lymphoblastic leukemia. Blood. 1997;89(6):1886-1895.
80. Goto, Y., R. Nishimura, A. Nohara, S. Mase, T. Fujiki, H. Irabu, et al. Minimal contribution of severe hypertriglyceridemia in L-asparaginase-associated pancreatitis developed in a child with acute lymphocytic leukemia. Rinsho Ketsueki. 2016;57(8):994-998.
81. Persson, L., A. Harila-Saari, I. Hed Myrberg, M. Heyman, A. Nilsson and S. Ranta. Hypertriglyceridemia during asparaginase treatment in children with acute lymphoblastic leukemia correlates with antithrombin activity in adolescents. Pediatr Blood Cancer. 2017;64(10).
82. Raja, R. A., K. Schmiegelow, D. N. Sorensen and T. L. Frandsen. Asparaginase-associated pancreatitis is not predicted by hypertriglyceridemia or pancreatic enzyme levels in children with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2017;64(1):32-38.
83. Merryman, R., K. E. Stevenson, W. J. Gostic, 2nd, D. Neuberg, J. O'Brien, S. E. Sallan, et al. Asparaginase-associated myelosuppression and effects on dosing of other chemotherapeutic agents in childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2012;59(5):925-927.
84. van der Sluis, I. M., H. de Groot-Kruseman, M. Te Loo, W. J. E. Tissing, C. van den Bos, G. J. L. Kaspers, et al. Efficacy and safety of recombinant E. coli asparaginase in children with previously untreated acute lymphoblastic leukemia: A randomized multicenter study of the Dutch Childhood Oncology Group. Pediatr Blood Cancer. 2018:e27083.
85. Capizzi, R. L., J. R. Bertino, R. T. Skeel, W. A. Creasey, R. Zanes, C. Olayon, et al. L-asparaginase: clinical, biochemical, pharmacological, and immunological studies. Ann Intern Med. 1971;74(6):893-901.
86. Capizzi, R. L., W. P. Summers and J. R. Bertino. L-asparaginase induced alteration of amethopterin (methotrexate) activity in mouse leukemia L5178Y. Ann N Y Acad Sci. 1971;186:302-311.
87. Jolivet, J., D. E. Cole, J. S. Holcenberg and D. G. Poplack. Prevention of methotrexate cytotoxicity by asparaginase inhibition of methotrexate polyglutamate formation. Cancer Res. 1985;45(1):217-220.
88. Sur, P., D. J. Fernandes, T. E. Kute and R. L. Capizzi. L-asparaginase-induced modulation of methotrexate polyglutamylation in murine leukemia L5178Y. Cancer Res. 1987;47(5):1313-1318.

Part I

CHAPTER 2

2

Individualized Asparaginase Dosing in Childhood Acute Lymphoblastic Leukemia

Robin Q.H. Kloos, Rob Pieters, Florine M.V. Jumelet
Hester A. de Groot-Kruseman, Cor van den Bos, Inge M. van der Sluis

Journal of Clinical Oncology, 2020. doi: 10.1200/JCO.19.02292

ABSTRACT

Background In the DCOG ALL-11 protocol, PEGasparaginase and *Erwinia* asparaginase treatment of pediatric acute lymphoblastic leukemia is individualized with therapeutic drug monitoring (TDM). The efficacy of TDM and its effect on asparaginase-associated toxicity is reported.

Methods After induction with 3 fixed intravenous doses of 1,500 IU/m^2 PEGasparaginase, 382 medium risk patients received fourteen individualized doses targeting trough levels of 100–250 IU/L; standard risk patients one individualized dose; high risk patients 2–5 fixed administrations (1,500 IU/m^2). After a neutralizing hypersensitivity reaction, patients started with 20,000 IU/m^2 3x/week *Erwinia* asparaginase. (L-)asparagine was measured monitoring asparaginase efficacy. Several asparaginase-associated toxicities were studied.

Results The final median PEGasparaginase dose could be lowered to a medium of 450 IU/m^2. Overall, 97% of all trough levels of non-allergic patients was >100 IU/L. Asparagine was <0.5 µM in 96% and 67% of the PEGasparaginase and *Erwinia* asparaginase levels >100 IU/L, respectively. Ten percent developed a neutralizing hypersensitivity reaction to PEGasparaginase, of which 40% was silent inactivation. The cumulative incidences of grade 3-4 pancreatitis, central neurotoxicity and thromboses were 12%, 4% and 6%, respectively, and were not associated with asparaginase activity levels. During medium risk intensification, 50% had increased alanine transaminase; 3% hyperbilirubinemia (both correlated with asparaginase activity levels) and 37% hypertriglyceridemia (all grade 3-4). Hypertriglyceridemia occurred less in intensification compared to ALL-10 (37% versus 47%), which is similar to ALL-11 but with higher asparaginase levels during intensification.

Conclusion In conclusion, TDM of asparaginase results in a significant reduction of the PEGasparaginase dose with adequate asparaginase activity levels and sufficient asparagine depletion. Also, with TDM, silent inactivation and allergic-like reactions are identified. However, there is limited effect of reduced asparaginase activity levels on toxicity.

INTRODUCTION

Asparaginase is essential for pediatric acute lymphoblastic leukemia (ALL) treatment. [1-7] The drug starves leukemic cells by converting extracellular asparagine, an essential amino acid for these cells.[8] Asparaginase activity >100 IU/L is considered to be sufficient for complete asparagine depletion. [9-16]
Hypersensitivity reactions to asparaginase occur with or without clinical symptoms of an allergy. The latter is called silent inactivation (SI), neutralizing the drug completely and requiring a switch from *E.coli* asparaginase to *Erwinia* asparaginase.[17] Patients may also develop atypical allergies without inactivation, allergic-like reactions, which do not require a switch in formulation to ensure adequate treatment.[18] Currently, in most countries, polyethylene glycol conjugated *E.coli* asparaginase (PEGasparaginase) is used as a first line formulation and *Erwinia* asparaginase (Erwinase®) as second line.
Also other asparaginase-associated toxicity hampers asparaginase treatment, possibly resulting in worse outcomes.[19] We showed that a fixed PEGasparaginase dose of 2,500 IU/m^2 results in high trough asparaginase activity levels, possibly causing unnecessary toxicity.[20] Furthermore, asparaginase activity levels show large inter- and intra-patient variability.[21, 22] Therefore, therapeutic drug monitoring (TDM) was implemented in the Dutch Childhood Oncology Group (DCOG) ALL-11 treatment protocol. This way, also SI is detected and allergic-like reactions are identified.
Here, the efficacy of the ALL-11 TDM program and the effect on asparaginase-associated toxicity was studied.

METHODS

Patients and therapy

Patients treated according to the ALL-11 protocol in the Netherlands between April 2012 and December 2016 were included. Asparaginase related side effects (hypersensitivity reactions, pancreatitis, central neurotoxicity, thrombosis and infections) were nationally registered by the DCOG. In 50 patients treated in the Erasmus MC Rotterdam or Amsterdam UMC hepatotoxicity, hypertriglyceridemia, the number of transfusions and hyperglycemia were also assessed. Informed consent was signed by patients and parents according to Dutch law. The study (CCMO register: NL50250.078.14) was approved by the institutional review boards according to the declaration of Helsinki.

The ALL-11 protocol is described in Supplemental table 1. The TDM is described in Supplemental figure 1. In induction, patients were treated with three intravenous PEGasparaginase doses (1,500 IU/m^2, biweekly). Thereafter, patients were stratified as standard risk (SR), medium risk (MR) or high risk (HR). After an interval of approximately 12 weeks, SR patients were treated with one individualized dose during protocol IV; MR patients were treated with 14 individualized doses biweekly during intensification. The algorithm of dose reductions is described in Supplemental table 2. HR patients were treated with 2–5 doses of 1,500 IU/m^2 with intervals of approximately 7 weeks.
For the nation-wide TDM program, trough serum asparaginase activity levels were measured for dose adjustments, targeting 100–250 IU/L. Week levels were measured after the first PEGasparaginase dose or the first dose following an asparaginase-free interval for early detection of SI. When asparaginase activity levels were stable within the target range, trough levels were measured every four weeks. In HR patients, week levels were measured after each dose to detect SI. The asparaginase activity level measurements and formulation of dosing advices were performed centrally.
In case of a neutralizing hypersensitivity reaction, patients were switched to intravenous Erwinase®, starting with 20,000 IU/m^2 three times a week for two weeks. After two weeks, the dose and/or dosing schedule was adjusted to ensure Erwinase® activity levels >100 IU/L.

Efficacy

PEGasparaginase doses and trough asparaginase activity levels were analyzed with a target range of 100-250 IU/L. For TDM of Erwinase®, also the adjusted dosing intervals were analyzed. Asparagine and glutamine concentrations were measured in serum samples collected in the Erasmus MC. Beside total asparagine levels, L-asparagine was measured as this type of asparagine is incorporated in proteins and hydrolyzed by L-asparaginase (Supplemental methods).[23]

Toxicity

Asparaginase-associated toxicity (<2 weeks after an asparaginase dose) was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Hypersensitivity reactions (neutralizing allergies, allergic-like reactions or SI), ≥grade 3 central neurotoxicity (ataxia, somnolence, a depressed level of consciousness, agitation, seizures and posterior reversible encephalopathy syndrome (PRES)), ≥grade 3 pancreatitis, fulfilling the criteria described by

Schmiegelow et al.[24], the number of patients with at least one infection (≥grade 2), and ≥grade 3 thrombosis without central line thromboses, were registered by the DCOG. Hepatotoxicity, hypertriglyceridemia, the number of transfusions, and hyperglycemia were analyzed in a subset of 50 patients.
Toxicity was correlated with the asparaginase activity levels and compared to patients treated according to the ALL-10 protocol, which is similar to ALL-11 but with native *E.coli* asparaginase treatment during induction (8x5,000 IU/m^2, trough levels 143-182 IU/L), and PEGasparaginase during intensification (2,500 IU/m^2, mean trough level 899 IU/L) (Table 2).[20, 25]

Measurements and statistical analysis

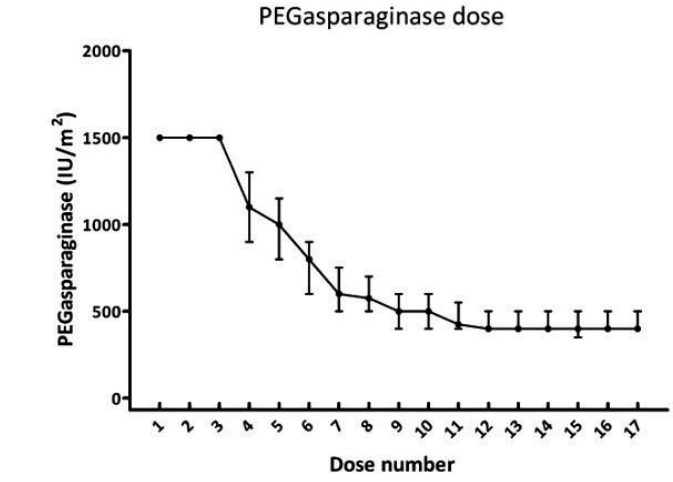
The measurements of asparaginase activity levels, asparagine and glutamine concentrations, and antibodies, as well as the statistical analysis are described in the Supplemental methods.

RESULTS

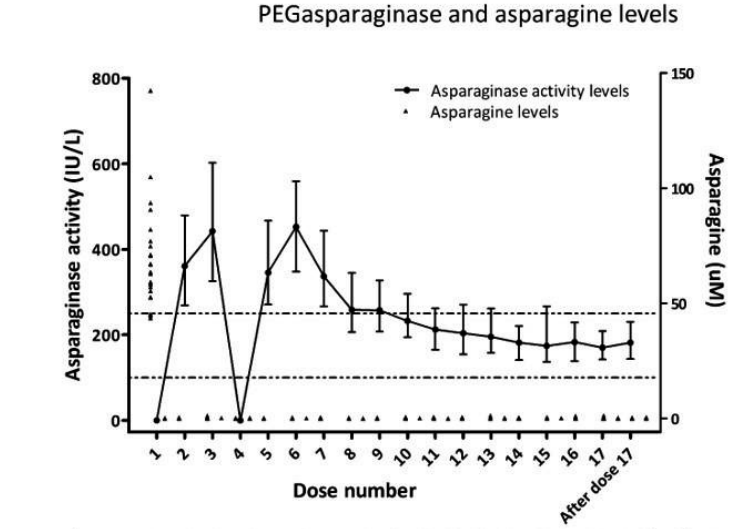
Patient characteristics

Three hundred eighty-two patients were included with a median age of 5.3 years (IQR 3.3–10.3); the median age of the patients in whom the extra laboratory measurements were performed was 4.7, IQR 3.4–7.7 years. After induction, 108 patients were treated as SR, 243 patients as MR, and 18 as patients HR.

Figure 1. Therapeutic drug monitoring of PEGasparaginase



Dose number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Number of patients	323	320	298	283	247	190	183	188	174	170	150	151	130	137	123	115	153



Dose number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	After dose 17
Number of patients	323	319	287	283	247	184	177	187	169	165	144	149	122	129	120	108	148	77

Figure 1 shows the PEGasparaginase dose (median and interquartile range), the trough asparaginase activity levels (median and interquartile range), and corresponding asparagine levels during the different PEGasparaginase doses. The first three doses had a fixed dose of 1500 IU/m^2, after which the doses were individualized.
The asparagine comprised both L- and D-asparagine as L-asparagine was measured in only 50 patients. This figure only includes patients without a neutralizing hypersensitivity reaction.

Efficacy

Figure 1 shows the PEGasparaginase doses and corresponding trough PEGasparaginase activity levels of non-allergic patients. The first three doses were fixed at 1,500 IU/m^2. Thereafter, doses were reduced in all patients targeting trough levels of 100-250 IU/L. Overall, 97% of the trough levels was ≥100 IU/L. After the 10^{th} administration, stable median asparaginase activity levels within range were reached, with a median dose of 450 IU/m^2 (IQR 450 – 500 IU/m^2). There were no PEGasparaginase levels <50 IU/L at that point; 68-87% of the trough levels was within the target range of 100-250 IU/L (Table 1).

Thirty-seven patients started with 20,000 IU/m^2 Erwinase®, three times a week, for two weeks. In 26 patients, the dose and/or dosing frequency was adjusted. During the first two weeks, 89 out of 117 48h levels (76%) and 16 out of 67 72h levels (24%) were >100 IU/L. Thereafter, the Erwinase® dose varied between 15,000–40,000 IU/m^2. Thirteen patients (57%) were treated every-other-day (72% of the 48h levels (120/166 levels) >100 IU/L), and three patients (13%) were treated two times a week (63% 72h levels (10/19) and 44% 96h levels (7/16) >100 IU/L). Of the ten patients who continued treatment 3x/week after the first two weeks, 76% of the 48h levels (99/131) and 34% of the 72h levels (36/105) were >100 IU/L.

Table 1. Asparaginase activity levels of patients without a hypersensitivity reaction

			Trough asparaginase activity level			
		n	**<50 IU/L n (%)**	**50 - 99 IU/L n (%)**	**100 – 250 IU/L n (%)**	**>250 IU/L n (%)**
All patients	After dose 1	319	1 (<0.5%)	7 (2%)	61 (19%)	250 (79%)
	After dose 2	287	4 (1%)	10 (4%)	26 (9%)	247 (86%)
	After dose 3*		-	-	-	-
SR + MR patients	After dose 4	247	6 (2%)	3 (1%)	34 (14%)	204 (83%)
MR patients only	After dose 5	184	2 (1%)	2 (1%)	17 (9%)	163 (89%)
	After dose 6	177	1 (1%)	2 (1%)	37 (21%)	137 (77%)
	After dose 7	187	1 (1%)	1 (1%)	84 (45%)	101 (54%)
	After dose 8	169	0	3 (2%)	77 (46%)	89 (53%)
	After dose 9	165	0	2 (1%)	96 (58%)	67 (41%)
	After dose 10	144	0	5 (4%)	95 (66%)	44 (30%)
	After dose 11	149	0	3 (2%)	101 (68%)	45 (30%)
	After dose 12	122	0	4 (3%)	84 (69%)	34 (28%)
	After dose 13	129	0	7 (5%)	105 (82%)	17 (13%)
	After dose 14	120	1 (1%)	7 (6%)	96 (80%)	16 (13%)
	After dose 15	108	0	6 (6%)	83 (77%)	19 (17%)
	After dose 16	148	0	5 (3%)	129 (87%)	14 (10%)
	After dose 17	77	0	4 (5%)	60 (78%)	13 (17%)

***** There was no asparaginase activity level measured after the third PEGasparaginase dose. Just prior to that dose, a trough level was measured on which the first dose adjustment of the first dose in intensification was based.

Asparagine and glutamine were measured in 754 samples (637 samples PEGasparaginase (asparagine concentrations in Figure 1); 117 samples Erwinase®) in 110 patients. Median baseline asparagine and glutamine levels were 68.52 µM and 557 µM (n=12), respectively. During asparaginase treatment, asparagine varied between 0.03–1.26 µM. Of the samples >100 IU/L, asparagine was <0.2 µM in 45%, and <0.5 µM (<1% of baseline asparagine concentration) in 96% for PEGasparaginase, but in 11% and 67%, respectively, for Erwinase®.
Beside total asparagine levels, L-asparagine was measured. The median L-asparagine concentration in PEGasparaginase samples (n=50, asparaginase activity level 11–752 IU/L) was 0.15 µM (IQR 0.08–0.24 µM), and 0.10 µM (IQR 0.05–0.15 µM) in Erwinase® samples (n=20, asparaginase activity level 13–530 IU/L) (Supplemental figure 3). Overall, 76% of the samples with an asparaginase activity level >100 IU/L had an L-asparagine level <0.2 µM.

The mean glutamine level was 571 µM (SD 159 µM) during PEGasparaginase and 600 µM (SD 192 µM) during Erwinase® treatment, which was not lower than the baseline of 557 µM.

Toxicity

Hypersensitivity reactions

Ten percent (n=40) had a neutralizing hypersensitivity reaction to PEGasparaginase (Table 2): 6% (n=22) had an allergy; 4% (n=18) SI. The incidence was 3% (n=13) during induction and 7% (n=27) during MR intensification. SI and allergies during induction occurred after all doses; during intensification, SI only occurred after the first dose. Of the eight patients with an allergy during MR intensification, five (62%) were during the first and three (38%) during the second dose.
None of the patients with a reaction during induction had PEGasparaginase or native *E.coli* asparaginase antibodies. Of the reactions during the first dose of intensification, 33% were accompanied with PEGasparaginase antibodies and 11% with anti-native *E.coli* asparaginase antibodies. The three patients with an allergy during the second dose of intensification were positive for both antibodies.
Five patients (1%) had an allergic-like reaction (Table 2). PEGasparaginase was completed in 4 patients. One patient was switched to Erwinase® because the reaction was not recognized as an allergic-like reaction.
Of the 37 patients switched to Erwinase®, four patients (10%) developed an allergy (n=2) to or SI (n=2) of Erwinase® (Table 1). Four patients (10%) had an allergic-like reaction; Erwinase® was completed in two of these four.

Pancreatitis

The cumulative incidence of pancreatitis was 12% (n=34, Figure 2).The median age at diagnosis was 8.3 years (IQR 5.0–13.5 years) versus 5.2 years (IQR 3.2–9.8 years) in patients without pancreatitis (p=0.001). The cumulative incidence during induction was 4% (n=14). After induction, this was 0% for SR patients, 8% for MR patients, and 6% for HR patients (one patient). There was no statistically significant correlation between pancreatitis and asparaginase activity levels. PEGasparaginase was successfully reintroduced in three patients.
In ALL-10, pancreatitis occurred in 1% of the patients during induction and in 5% of the patients during intensification (Table 3).[26, 25]

Table 2. Hypersensitivity reactions

Number of patients (%):	Total	Neutralizing hypersensitivity reaction			Neutralizing allergy			Silent inactivation			Allergic-like reaction (without inactivation)		
		During induction	*After induction*	*Total*	*During induction*	*After induction*	*Total*	*During induction*	*After induction*	*Total*	*During induction*	*After induction*	*Total*
Hypersensitivity reactions to PEGasparaginase													
Standard risk	108	2 (2%)	10 (9%)	12 (11%)	2 (2%)	6 (5%)	8 (7%)	0	4 (4%)	4 (4%)	0	2 (2%)	2 (2%)
Medium risk	243	9 (3%)	17 (7%)	26 (10%)	5 (2%)	8 (3%)	13 (5%)	4 (2%)	9 (3%)	13 (5%)	0	3 (1%)	3 (1%)
High risk	18	1 (6%)	0	1 (6%)	1 (6%)	0	1 (6%)	0	0	0	0	0	0
Only induction*	13	1 (8%)	-	1 (8%)	0	0	0	1 (8%)	0	1 (8%)	0	0	0
Total	382	13 (3%)	27 (7%)	40 (10%)	8 (2%)	14 (4%)	22 (6%)	5 (1%)	13 (3%)	18 (4%)	0	5 (1%)	5 (1%)
Stratified only	369	12 (3%)	27 (7%)	39 (10%)	8 (2%)	14 (4%)	22 (6%)	4 (1%)	13 (3%)	17 (4%)			
Hypersensitivity reactions to Erwinase®													
Standard risk	10	1 (10%)	1 (10%)	2 (20%)	0	1 (10%)	1 (10%)	1 (10%)	0	1 (10%)	0	0	0
Medium risk	26	0	1 (4%)	1 (4%)	0	1 (4%)	1 (4%)	0	0	0	0	4 (15%)	4 (15%)
High risk	0	0	0	0	0	0	0	0	0	0	0	0	0
Only induction*	1	1 (100%)	0	1 (100%)	0	0	0	1 (100%)	0	1 (100%)	0	0	0
Total	37	2 (5%)	2 (5%)	4 (10%)	0	2 (5%)	2 (5%)	2 (5%)	0	2 (5%)	0	4 (10%)	4 (10%)
Stratified only	36	1 (3%)	2 (6%)	3 (9%)	0	2 (6%)	2 (6%)	1 (3%)	0	1 (3%)	0	4 (10%)	4 (10%)

*In part of the patients, only induction therapy could be analyzed due to cessation of asparaginase (n=7) or mortality (n=6) during induction.

Table 3. Asparaginase-associated toxicity during DCOG ALL-10 and DCOG ALL-11

	DCOG ALL-10[26, 25]	**DCOG ALL-11**
	Native *E. coli* asparaginase Trough levels: 143 – 182 IU/L	**PEGasparaginase Median trough level: 403 IU/L**
Induction		
Pancreatitis (≥grade 3)	Incidence 1%	Cumulative incidence 4%
Thrombosis (≥grade 3)	Incidence 2%	Cumulative incidence 4%
Central neurotoxicity (≥grade 3)	Incidence 2%	Cumulative incidence 1%
Infections (number of patients with at least one ≥grade 2 infection)	Protocol 1A: 37% Protocol 1B: 41%	Protocol 1A: 35% Protocol 1B: 42%
Hepatotoxicity	Alanine transaminase increased in 36% of the patients Grade 3/4 hyperbilirubinemia in 11% of the patients	Alanine transaminase increased in 26% of the patients Grade 3/4 hyperbilirubinemia in 10% of the patients
Dyslipidemia	Not reported	Grade 3/4 hypertriglyceridemia in 12% of the patients
Intensification	**PEGasparaginase Mean trough level 899 IU/L**	**PEGasparaginase Median trough level: 272 IU/L**
Pancreatitis (≥grade 3)	Incidence 5%	Cumulative incidence 8%
Thrombosis (≥grade 3)	Incidence 3%	Cumulative incidence 2%
Central neurotoxicity (≥grade 3)	Incidence 10%	Cumulative incidence 3%
Infections (number of patients with at least one ≥grade 2 infection)	Week 1-19: 46% Week 20-35: 53%	Week 1-19: 35% Week 20-35: 38%
Hepatotoxicity	Not reported	Alanine transaminase increased in 50% of the patients Grade 3/4 hyperbilirubinemia in 3% of the patients
Dyslipidemia	Grade 3/4 hypertriglyceridemia in 47% of the patients	Grade 3/4 hypertriglyceridemia in 37% of the patients

* The ALL-10 intensification phase included 6 doses of doxorubicin. The ALL-11 intensification phase contained 4 doses or none in case of EVT6/RUNX1 positive leukemia's or Down patients.

Central neurotoxicity

The cumulative incidence of central neurotoxicity was 4% (n=12, Figure 2), the majority having PRES (8/12 patients). Age at diagnosis of the patients with and without neurotoxicity did not differ. During induction, four patients (cumulative incidence 1%) had central neurotoxicity. After induction, this was 0% for SR patients, 3% for MR patients and 6% for HR patients (one patient). There was no statistically significant correlation between central neurotoxicity and asparaginase levels. In all but one patient, asparaginase was completed.
In ALL-10, central neurotoxicity occurred in 2% of the patients during induction and 10% during intensification (Table 3).[26, 25]

Thrombosis

The cumulative incidence of thrombosis was 6% (n=19, Figure 2), of which 84% was a sagittal sinus thrombosis and 16% deep venous thrombosis of an extremity. The median age was 9.2 years (IQR 6.8–11.3 years) versus 5.2 years (IQR 3.2–9.9 years) of patients without thrombosis(p=0.003). The cumulative incidence was 4% (n=14) during induction. After induction, this was 0% in SR and HR patients, and 2% in MR intensification. There was no statistically significant correlation between thrombosis and asparaginase activity levels. Asparaginase, with concomitant LMWH administration, was completed in 18 out of the 19 patients.
In ALL-10, thrombosis occurred in 2% of patients during induction and 3% during intensification (Table 3).[26, 25]

Infections

The number of patients with at least one infection during induction was comparable between ALL-11 and ALL-10: 35% versus 37% in protocol 1A, and 42% versus 41% in protocol 1B. However, less patients had an infection during MR intensification in ALL-11: 35% versus 46% in the first 19 weeks, and 38% versus 53% in week 20-35 (Table 3).[27] ALL-10 intensification, however, included 6 doses of doxorubicin instead of 4 doses (or none in case of EVT6/RUNX1 positive leukemia's or Down patients) in ALL-11 intensification.

Figure 2. Cumulative incidences of pancreatitis, neurotoxicity and thrombosis

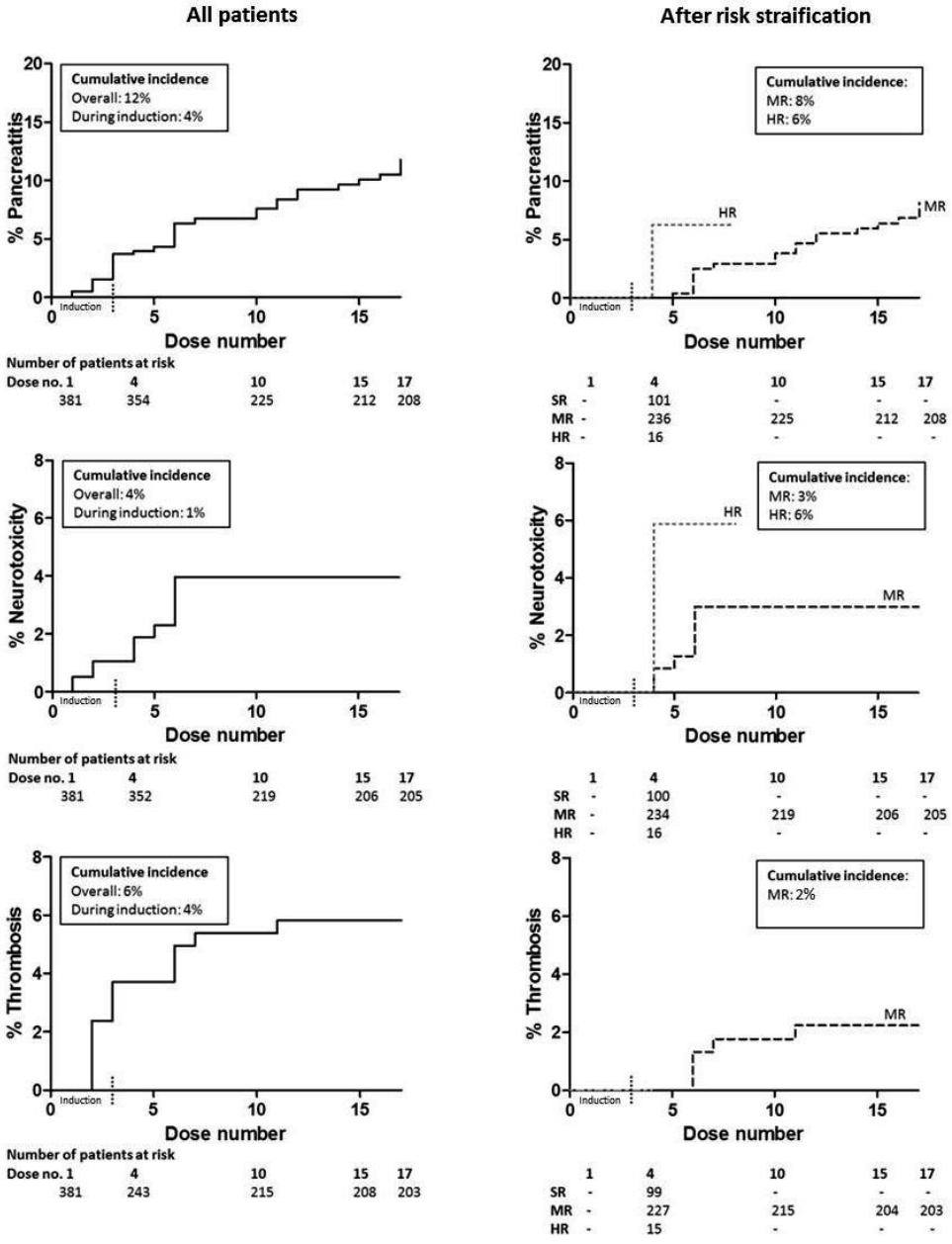


Figure 2 shows the cumulative incidences of asparaginase-associated pancreatitis, central neurotoxicity and thrombosis for all patients (left) and after risk stratification (right) for standard risk (SR), medium risk (MR) and high risk (HR) patients. Of note, only 18 patients were stratified as HR and the cumulative incidence of 6% for pancreatitis and central neurotoxicity reflect only one patient.

The end of induction is indicated with a vertical dotted line. The cumulative incidences after risk stratification did not comprise the toxicity which occurred during induction.

Hepatotoxicity

The hepatotoxicity was reflected by alanine transaminase (ALT) and bilirubin concentrations (Supplemental table 3 and Figure 3). Grade 3/4 increased ALT occurred in 26% of the patients during induction and 50% during intensification; grade 3/4 hyperbilirubinemia occurred in 10% of the patients during induction and 3% during intensification. Longitudinal analysis showed that ALT and bilirubin were higher with higher asparaginase activity levels ($p=0.004$ and $p<0.001$, respectively) (Supplemental figure 2). In ALL-10, ALT was increased in 36% of the patients and grade 3/4 hyperbilirubinemia occurred in 11% of the patients during induction (Table 3).[26, 25]

Hypertriglyceridemia

Grade 3/4 hypertriglyceridemia occurred in 12% of the patients during induction and 37% during intensification (Supplemental table 3), without clinical consequences. Triglycerides were mainly increased prior to the 6th PEGasparaginase dose (Figure 3), directly after administration of dexamethasone. In ALL-10, 47% of the patients had grade 3/4 hypertriglyceridemia during intensification. We found no correlation between triglyceride concentrations and asparaginase activity levels.

Other toxicity

During induction, the median number of erythrocyte and thrombocyte transfusions administrated since the start of asparaginase to two weeks after the third dose was 1 (IQR 1–2 and 0–2, respectively), and 0 (IQR 0–1 and 0–0, respectively) during intensification (Supplemental table 3). Glucose levels were rarely increased throughout the asparaginase treatment (Figure 3), and only one patient had grade 3/4 hyperglycemia (Supplemental table 3).

Figure 3. Laboratory measurements

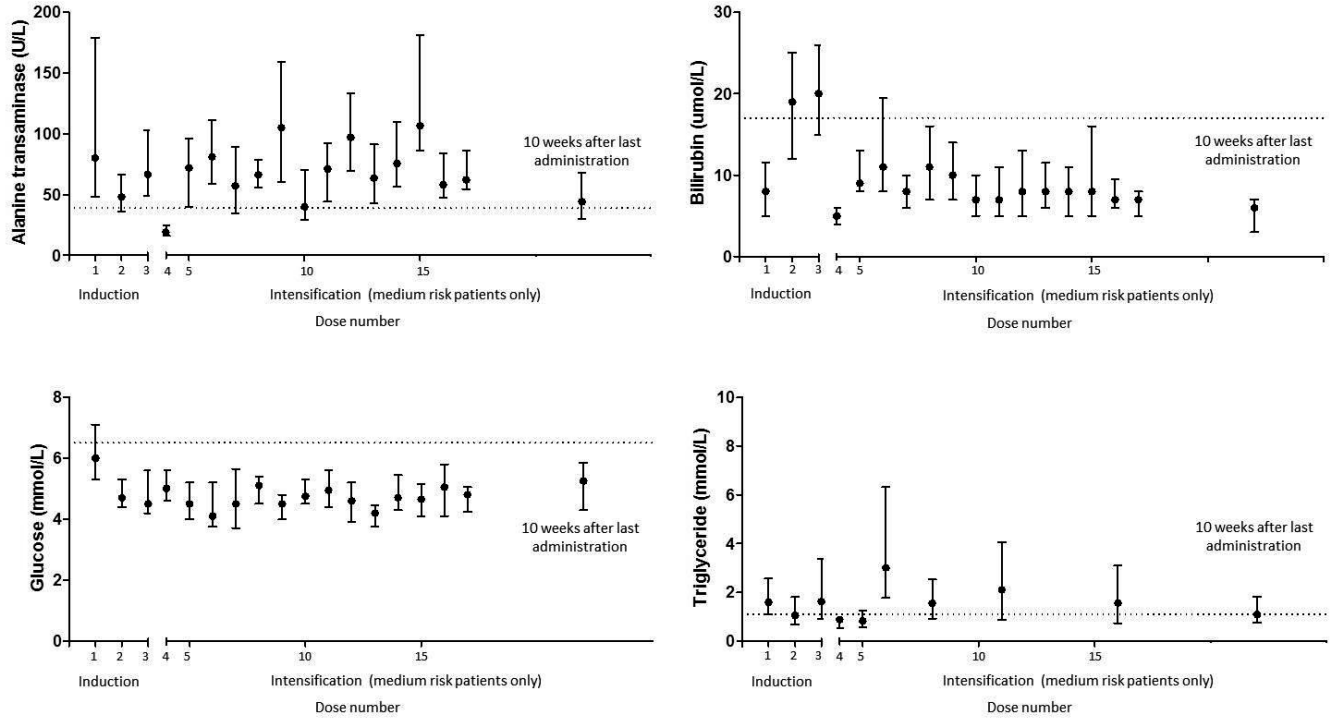


Figure 3 shows the median and interquartile range of the alanine transaminase (ALT), bilirubin, glucose and triglyceride levels throughout the asparaginase treatment. The levels during induction include SR, MR and HR patients (n=50); the other levels are measured during medium risk intensification therapy (n=26). The first measurements were done prior to the first PEGasparaginase dose; the measurement at the 4th dose was done prior to this dose after an asparaginase-free interval of several months. The last measurement was done in week 37, ten weeks after the last asparaginase dose. The horizontal dotted lines indicate the upper limit of normal.

Longitudinal analyses showed a correlation between asparaginase activity levels and ALT and bilirubin levels.

DISCUSSION

The efficacy and toxicity of individualized asparaginase therapy was analyzed. With our nation-wide TDM program, the PEGasparaginase dose was reduced to a median of 450 IU/m^2 with asparaginase activity levels >100 IU/L in 97% of the non-allergic patients. This dose reduction leads to a substantial reduction of the asparaginase-associated costs.[28] The starting dose of 1,500 IU/m^2 proved to be adequate as trough asparaginase activity levels were >100 IU/L in 98% of the patients after the first dose, and in 95% after the second.

Efficacy of the TDM program was evaluated by measurement of asparagine and glutamine. Accurate sample handling is essential to avoid *ex vivo* asparaginase activity.[29, 30] In our samples, asparagine was measurable and glutamine was not decreased, confirming that the samples were adequately processed. Asparagine was <0.5 µM in 96% of the PEGasparaginase samples >100 IU/L but only in 67% of the Erwinase® samples. However, L-asparagine concentrations measured in these samples were very low (73% of the samples <0.2 µM), showing a considerable influence of the irrelevant D-asparagine on total asparagine concentrations. This is the first study reporting L-asparagine concentrations during asparaginase treatment.

Compared to ALL-10, the pattern of occurrence of a hypersensitivity reaction and the formation of antibodies have changed fundamentally in ALL-11. Patients were treated during induction with native *E.coli* asparaginase in ALL-10 and PEGasparaginase in ALL-11, whereas both protocols used PEGasparaginase during intensification. In ALL-10, during induction, 5% had a hypersensitivity reaction to native *E.coli* asparaginase.[25] In ALL-11, this percentage was 3% to PEGasparaginase. During ALL-10 intensification, 30% had a neutralizing hypersensitivity reaction, which is much more than the 7% in ALL-11. In ALL-10, these reactions almost exclusively occurred during the second PEGasparaginase dose, and they were all accompanied with antibodies to *E.coli* asparaginase.[26] In contrast, in ALL-11, the reactions occurred during both the first and second PEGasparaginase dose in intensification, and native *E.coli* asparaginase antibodies were absent in the majority of the patients, as was also the case with the reactions during induction. Only the three patients with a reaction during the second PEGasparaginase dose during intensification had, like ALL-10, antibodies to native *E.coli* asparaginase and PEGasparaginase. Thus, by using PEGasparaginase, patients do not necessarily

seem to develop reactions to the asparaginase moiety, except for the few patients with a reaction during the second dose during intensification.
In ALL-11, PEGasparaginase antibodies were also absent in the far majority of the patients with a neutralizing reaction. More detailed measurements have shown that by coating the plates with PEGasparaginase in a basic carbonate coating buffer (pH 9.5), PEG hydrolyses from PEGasparaginase.[31] Thus, possible anti-PEG antibodies were not measured with the assay used. As anti-PEG antibodies have been described earlier[32], the majority of the hypersensitivity reactions in ALL-11 were probably against the PEG moiety or even against the linker connecting the PEG to asparaginase.[31]

In order to assess whether lowering asparaginase levels by TDM leads to less toxicity, toxicity was correlated with asparaginase activity levels and compared to ALL-10 (Table 2). In ALL-10, the asparaginase activity levels were significantly lower during induction but much higher during intensification compared to ALL-11. During induction, the occurrence of thrombosis and central neurotoxicity was similar between the two protocols and there was no correlation with asparaginase activity levels. Pancreatitis, however, seems to occur more frequently in ALL-11 (4% versus 1% in ALL-10). This might be due to the longer asparaginase exposure during ALL-11 induction (42 days in ALL-11 versus 24 in ALL-10) as there was no correlation between the PEGasparaginase level itself and pancreatitis. The percentage of patients who developed grade 3/4 hyperbilirubinemia did not differ, despite the correlation between asparaginase activity levels and bilirubin levels, which is, though statistically significant, clinically irrelevant.
The toxicity during the intensification phase was comparable as well. Only central neurotoxicity occurred more often (10% versus 3%) and infection occurred more often in ALL-10 versus ALL-11.[27] However, the anthracycline therapy was less intensive in ALL-11, resulting in less myelosuppression, but only during the first 13 weeks of the intensification. Grade 3/4 hypertriglyceridemia occurred less often in ALL-11 (37% versus 47% in ALL-10), although a correlation with asparaginase activity levels was not found. This is in contrast to the correlation found in our earlier studies, which could be explained by less variation in the ALL-11 asparaginase levels as a result of the TDM. Comparing the rough data of ALL-10 triglycerides[26] with ALL-11, the median triglycerides are higher during ALL-10 (4.6 mmol/L) than ALL-11 (2.6 mmol/L), with more outliers in ALL10 (Supplemental figure 4). Thus, the correlation between asparaginase activity levels and triglycerides only holds for higher asparaginase levels.

We conclude that TDM of asparaginase is feasible in a nation-wide program and results in a substantial dose reduction without loss of efficacy of treatment – reflected by asparagine depletion – though survival analyses should be awaited. In addition, TDM leads to detection of SI in 4% and identification of allergic-like reactions in 1% of the patients. However, the effect of the PEGasparaginase dose reduction on asparaginase-associated toxicities is limited.

REFERENCES

1. Amylon, M. D., J. Shuster, J. Pullen, C. Berard, M. P. Link, M. Wharam, 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. Rizzari, C., M. G. Valsecchi, M. Arico, V. Conter, A. Testi, E. Barisone, 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.
3. Silverman, L. B., R. D. Gelber, V. K. Dalton, B. L. Asselin, R. D. Barr, L. A. Clavell, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97(5):1211-1218.
4. Duval, M., S. Suciu, A. Ferster, X. Rialland, B. Nelken, P. Lutz, 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.
5. Pession, A., M. G. Valsecchi, G. Masera, W. A. Kamps, E. Magyarosy, C. Rizzari, 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.
6. Moghrabi, A., D. E. Levy, B. Asselin, R. Barr, L. Clavell, C. Hurwitz, 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.
7. Pieters, R., H. de Groot-Kruseman, V. Van der Velden, M. Fiocco, H. van den Berg, E. de Bont, et al. Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group. J Clin Oncol. 2016.
8. Muller, H. J. and J. Boos. Use of L-asparaginase in childhood ALL. Crit Rev Oncol Hematol. 1998;28(2):97-113.
9. Riccardi, R., J. S. Holcenberg, D. L. Glaubiger, J. H. Wood and D. G. Poplack. L-asparaginase pharmacokinetics and asparagine levels in cerebrospinal fluid of rhesus monkeys and humans. Cancer Res. 1981;41(11 Pt 1):4554-4558.
10. Ahlke, E., U. Nowak-Gottl, P. Schulze-Westhoff, G. Werber, H. Borste, G. Wurthwein, et al. Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during induction therapy in children with acute lymphoblastic leukaemia. Br J Haematol. 1997;96(4):675-681.
11. Rizzari, C., M. Zucchetti, V. Conter, L. Diomede, A. Bruno, L. Gavazzi, et al. L-asparagine depletion and L-asparaginase activity in children with acute lymphoblastic leukemia receiving i.m. or i.v. Erwinia C. or E. coli L-asparaginase as first exposure. Ann Oncol. 2000;11(2):189-193.
12. Albertsen, B. K., H. Schroder, J. Ingerslev, P. Jakobsen, V. I. Avramis, H. J. Muller, et al. Comparison of intramuscular therapy with Erwinia asparaginase and asparaginase Medac: pharmacokinetics, pharmacodynamics, formation of antibodies and influence on the coagulation system. Br J Haematol. 2001;115(4):983-990.
13. Avramis, V. I., S. Sencer, A. P. Periclou, H. Sather, B. C. Bostrom, L. J. Cohen, et al. 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(6):1986-1994.
14. Grigoryan, R. S., E. H. Panosyan, N. L. Seibel, P. S. Gaynon, I. A. Avramis and V. I. Avramis. Changes of amino acid serum levels in pediatric patients with higher-risk acute lymphoblastic leukemia (CCG-1961). In Vivo. 2004;18(2):107-112.
15. Tsurusawa, M., M. Chin, A. Iwai, K. Nomura, H. Maeba, T. Taga, et al. L-Asparagine depletion levels and L-asparaginase activity in plasma of children with acute lymphoblastic leukemia under asparaginase treatment. Cancer Chemother Pharmacol. 2004;53(3):204-208.
16. Pieters, R., I. Appel, H. J. Kuehnel, I. Tetzlaff-Fohr, U. Pichlmeier, I. van der Vaart, et al. Pharmacokinetics, pharmacodynamics, efficacy, and safety of a new recombinant asparaginase

preparation in children with previously untreated acute lymphoblastic leukemia: a randomized phase 2 clinical trial. Blood. 2008;112(13):4832-4838.
17. Pieters, R., S. P. Hunger, J. Boos, C. Rizzari, L. Silverman, A. Baruchel, et al. L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. Cancer. 2011;117(2):238-249.
18. Kloos, R. Q., R. Pieters, G. Escherich and I. M. van der Sluis. Allergic-like reactions to asparaginase: Atypical allergies without asparaginase inactivation. Pediatr Blood Cancer. 2016.
19. Raja, R. A., K. Schmiegelow, B. K. Albertsen, K. Prunsild, B. Zeller, G. Vaitkeviciene, et al. Asparaginase-associated pancreatitis in children with acute lymphoblastic leukaemia in the NOPHO ALL2008 protocol. Br J Haematol. 2014;165(1):126-133.
20. Tong, W. H., R. Pieters, G. J. Kaspers, D. M. te Loo, M. B. Bierings, C. van den Bos, 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.
21. Hempel, G., H. J. Muller, C. Lanvers-Kaminsky, G. Wurthwein, A. Hoppe and J. Boos. A population pharmacokinetic model for pegylated-asparaginase in children. Br J Haematol. 2010;148(1):119-125.
22. Sassen, S. D., R. A. Mathot, R. Pieters, R. Q. Kloos, V. de Haas, G. J. Kaspers, et al. Population pharmacokinetics of intravenous Erwinia asparaginase in pediatric acute lymphoblastic leukemia patients. Haematologica. 2017;102(3):552-561.
23. Visser, W. F., N. M. Verhoeven-Duif, R. Ophoff, S. Bakker, L. W. Klomp, R. Berger, et al. A sensitive and simple ultra-high-performance-liquid chromatography-tandem mass spectrometry based method for the quantification of D-amino acids in body fluids. J Chromatogr A. 2011;1218(40):7130-7136.
24. Schmiegelow, K., A. Attarbaschi, S. Barzilai, G. Escherich, T. L. Frandsen, C. Halsey, et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: a Delphi consensus. Lancet Oncol. 2016;17(6):e231-e239.
25. van der Sluis, I. M., H. de Groot-Kruseman, M. Te Loo, W. J. E. Tissing, C. van den Bos, G. J. L. Kaspers, et al. Efficacy and safety of recombinant E. coli asparaginase in children with previously untreated acute lymphoblastic leukemia: A randomized multicenter study of the Dutch Childhood Oncology Group. Pediatr Blood Cancer. 2018:e27083.
26. Tong, W. H., R. Pieters, H. A. de Groot-Kruseman, W. C. Hop, J. Boos, W. J. Tissing, et al. Toxicity of very prolonged PEGasparaginase and Erwiniaasparaginase courses in relation to asparaginase activity levels with a special focus on dyslipidemia. Haematologica. 2014.
27. Pieters, R., H. de Groot-Kruseman, V. Van der Velden, M. Fiocco, H. van den Berg, E. de Bont, et al. Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group. J Clin Oncol. 2016;34(22):2591 - 2601.
28. Kloos, R. Q. H., C. A. Uyl-de Groot, R. R. L. van Litsenburg, G. J. L. Kaspers, R. Pieters and I. M. van der Sluis. A cost analysis of individualized asparaginase treatment in pediatric acute lymphoblastic leukemia. Pediatr Blood Cancer. 2017.
29. Asselin, B. L., M. Y. Lorenson, J. C. Whitin, D. J. Coppola, A. S. Kende, R. L. Blakley, et al. Measurement of serum L-asparagine in the presence of L-asparaginase requires the presence of an L-asparaginase inhibitor. Cancer Res. 1991;51(24):6568-6573.
30. Lanvers-Kaminsky, C., P. S. Westhoff, M. D'Incalci, M. Zucchetti and J. Boos. Immediate cooling does not prevent the ex vivo hydrolysis of L-asparagine by asparaginase. Ther Drug Monit. 2014;36(4):549-552.
31. Kloos, R., I. van der Sluis, E. Mastrobattista, R. Pieters and J. Verhoef. Acute Lymphoblastic Leukemia Patients Treated with PEGasparaginase Develop Antibodies to PEG and the Succinate Linker. Submitted.
32. Armstrong, J. K., G. Hempel, S. Koling, L. S. Chan, T. Fisher, H. J. Meiselman, et al. Antibody against poly(ethylene glycol) adversely affects PEG-asparaginase therapy in acute lymphoblastic leukemia patients. Cancer. 2007;110(1):103-111.

SUPPLEMENTAL METHODS

Measurements

Asparaginase activity levels were measured using the L-aspartic β-hydroxamate (AHA) assay as described earlier.[1, 2] Asparaginase inactivation was defined as PEGasparaginase activity <100 IU/L after 7±1 days (week level) and/or <10 IU/L after 14±1 days (trough level).

For asparagine and glutamine measurement, whole blood was directly put on ice and 10% sulfosalicylic acid was added. The samples were stored in -80°C until analysis using liquid chromatography tandem-mass spectrometry (limit of quantification: 0.05 µM). Human asparagine measured comprises both L-asparagine and D-asparagine. The latter is also present in humans but not incorporated in proteins, and ranges between 0.017–0.18 µM.[3] Therefore, beside the total asparagine concentration, L-asparagine was measured in 50 patients with varying asparaginase activity levels.

PEGasparaginase and native *E. coli* asparaginase antibodies were measured with Enzyme-Linked Immuno Sorbent Assay (ELISA) as described earlier.[5] The threshold for a positive antibody measurement was calculated using the F-measure.[6]

Statistical analysis

The data was analyzed with the software package SPSS Statistics version 21.0 (IBM Corp, Armonk, New York, USA) and R Sigmaplot Version 3.3.3 (Systat Software Inc, London, UK). Continuous data was presented as mean and standard deviation (SD) or median and interquartile range (IQR). Student t-tests, Mann-Whitney U tests, $\chi 2$-(trend)tests, and Fisher exact tests were used to compare baseline characteristics. To account for the repeated measurement design to estimate the effect of asparaginase activity levels on laboratory measurements, marginal models were estimated. The cumulative incidence of pancreatitis, central neurotoxicity and thrombosis starting from asparaginase treatment were estimated by using Kaplan-Meier's methodology. Due to the presence of repeated measurements, a mixed effect model was estimated to study the association between pancreatitis, central neurotoxicity and thrombosis and asparaginase activity levels.

Supplemental Table 1: DCOG ALL-11 treatment protocol

Treatment phase	Therapy
Protocol 1A	
Prednisone	60 mg/m^2/day for 29 days followed by 3x3 days tapering
Vincristine	1.5 mg/m^2/dose at day 8, 15, 22 and 29
Daunorubicin	30 mg/m^2/dose at day 8, 15, 22 and 29 (not in case of Down syndrome)
PEGasparaginase	1,500 IU/m^2 at day 12, 26
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 15 and 33. Only intrathecal methotrexate at day 1.
Protocol 1B	
PEGasparaginase	1,500 IU/m^2 at day 40
Cyclophosphamide	1,000 mg/m^2/dose at day 36 and 64
Cytarabine	75 mg/m^2/day at days 38 – 41, 45 – 48, 52 – 55, 59 – 62
6-Mercaptopurine	60 mg/m^2/day at days 36 – 63
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 45 and 59
Protocol M for SR and MR patients	
6-Mercaptopurine	25 mg/m^2/day for 56 days
Methotrexate	5,000 mg/m^2/dose at day 8, 22, 36 and 50
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 8, 22, 36 and 50
Protocol IV for SR patients	
Dexamethasone	10 mg/m^2/day for 15 days followed by 3x3 days tapering
Vincristine	1.5 mg/m^2/dose at day 1 and 8
PEGasparaginase	Individualized dose at day 1
Maintenance for SR patients	
6-Mercaptopurine*	50 mg/m^2/day for 81 weeks
Methotrexate*	20 mg/m^2/week for 81 weeks
Intensification and maintenance for MR patients	
Dexamethasone	6 mg/m^2/day for 5 days every 3 weeks until week 82
Vincristine	2 mg/m^2/dose every three weeks until week 82
Doxorubicin	30 mg/m^2/dose at week 1, 4, 7 and 10 (not in case of Down syndrome or TEL/AML1)
PEGasparaginase	Individualized doses biweekly from week 1 – 27**
Methotrexate*	30 mg/m^2/week from week 13 – 84 (or week 2 – 84 in case of Down syndrome or TEL/AML1), not during intrathecal therapy In case of an IKZF1 deletion, 200 mg/m^2/dose every three weeks from week 85 - 136
6-Mercaptopurine*	50 mg/m^2/day from week 1 – 12 in courses of 2 weeks with 1 week interruption (without interruption in case of Down syndrome or TEL/AML1) and from week 13 – 84 daily, without interruption In case of an IKZF1 deletion, 100 mg/m^2/day for 10 days after each methotrexate dose from week 85 - 136
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at week 1, 19, 37, 55 and 73

High risk blocks for HR patients	
HR block 1	
6-Mercaptopurine	25mg/m^2/day from days 1 - 14
Methotrexate	5,000 mg/m^2 at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Cyclophosphamide	1,200 mg/m^2/dose at day 15, 16 and 17
Etoposide	350 mg/m^2/dose at day 15, 16 and 17
PEGasparaginase	1,500 IU/m^2 at day 22
Vincristine	1.5 mg/m^2/dose at day 22 and 29
HR block 2	
Methotrexate	5,000 mg/m^2 at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Cytarabine	1,500 mg/m^2/dose at day 15, 16, 17, 18 and 19
Mitoxantrone	5.25 mg/m^2/dose at day 15, 16, 17, 18 and 19
PEGasparaginase	1,500 IU/m^2 at day 22
Vincristine	1.5 mg/m^2/dose at day 22 and 29
HR block 3	
Methotrexate	5,000 mg/m^2 at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Idarubicin	6 mg/m^2/dose at day 15, 16 and 17
Fludarabine	22.5 mg/m^2/dose at day 15, 16, 17, 18 and 19
Cytarabine	1,500 mg/m^2/dose at day 15, 16, 17, 18 and 19
HR block 4	Equal to HR block 1, but without 6-Mercaptopurine
HR block 5	Equal to HR block 2
HR block 6	Equal to HR block 3, but without Idarubicin
HR Protocol II	
Dexamethasone	10 mg/m^2/day at days 1 – 21 followed by 3x3 days tapering
Vincristine	1.5 mg/m^2/dose at day 8, 15, 22 and 29
Doxorubicin	30 mg/m^2/dose at day 8, 15, 22 and 29
PEGasparaginase	1,500 IU/m^2 at day 8
Cyclophosphamide	1,000 mg/m^2 at day 36
6-Thioguanine	60 mg/m^2/day at days 36 – 49
Cytarabine	75 mg/m^2/day at days 36 – 39 and 43 – 46
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 36 and 43
HR maintenance	
6-Mercaptopurine	50 mg/m^2/day from week 1 – 37
Methotrexate	20 mg/m^2/week from week 1 – 37

* Not if patients are eligible for a stem cell transplantation

Supplemental table 2. Algorithm for dose reductions of PEGasparaginase

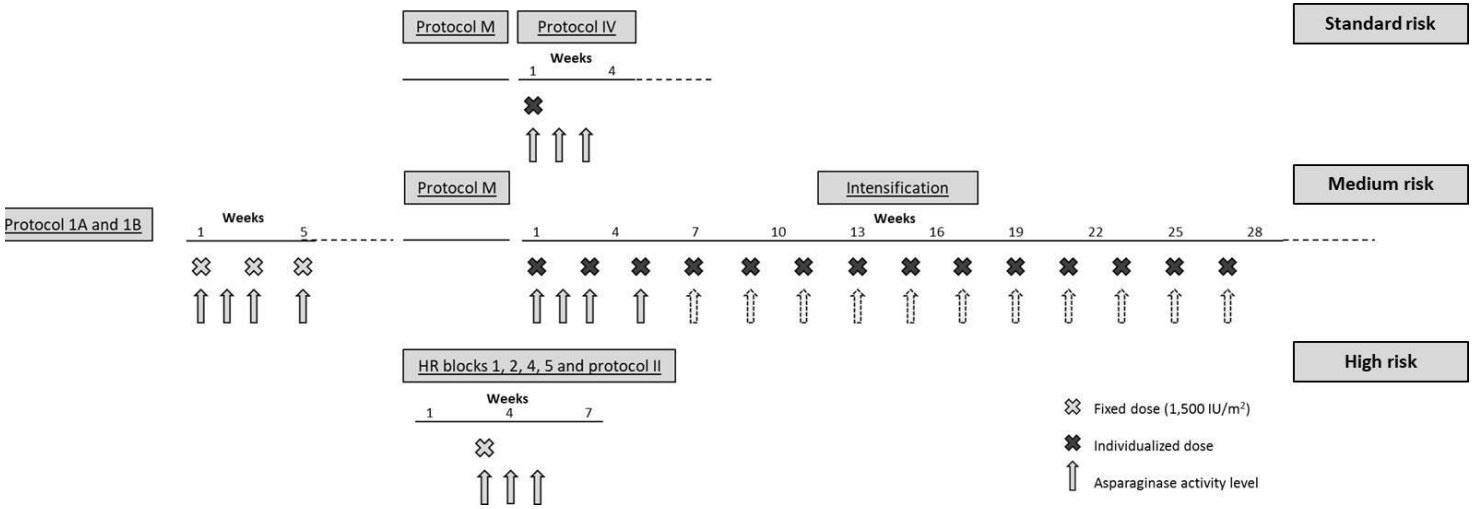
PEGasparaginase trough level	Dose adjustment
>600 IU/L	50%
500 – 599 IU/L	60%
400 – 499 IU/L	70%
300 – 399 IU/L	80%
200 – 299 IU/L	100%
100 – 199 IU/L	100%
50 – 99 IU/L	100%
30 – 49 IU/L	150%
10 – 29 IU/L	200%

Supplemental table 3. Transfusions and laboratory measurements during induction and intensification

Maximal toxicity	Induction N=50		Medium risk intensification N=30	
Number of erythrocyte transfusions (median, IQR)	1 (1 – 2)		0 (0 – 1)	
Number of thrombocyte transfusions (median, IQR)	1 (0 – 2)		0 (0 – 0)	
	Grade 1/2	**Grade 3/4**	**Grade 1/2**	**Grade 3/4**
Increased ALT	36 (72%)	13 (26%)	11 (36%)	15 (50%)
Increased bilirubin	34 (68%)	5 (10%)	15 (50%)	1 (3%)
Increased glucose	21 (42%)	1 (2%)	17 (57%)	0
Hypertriglyceridemia	26 (52%)	6 (12%)	13 (43%)	11 (37%)

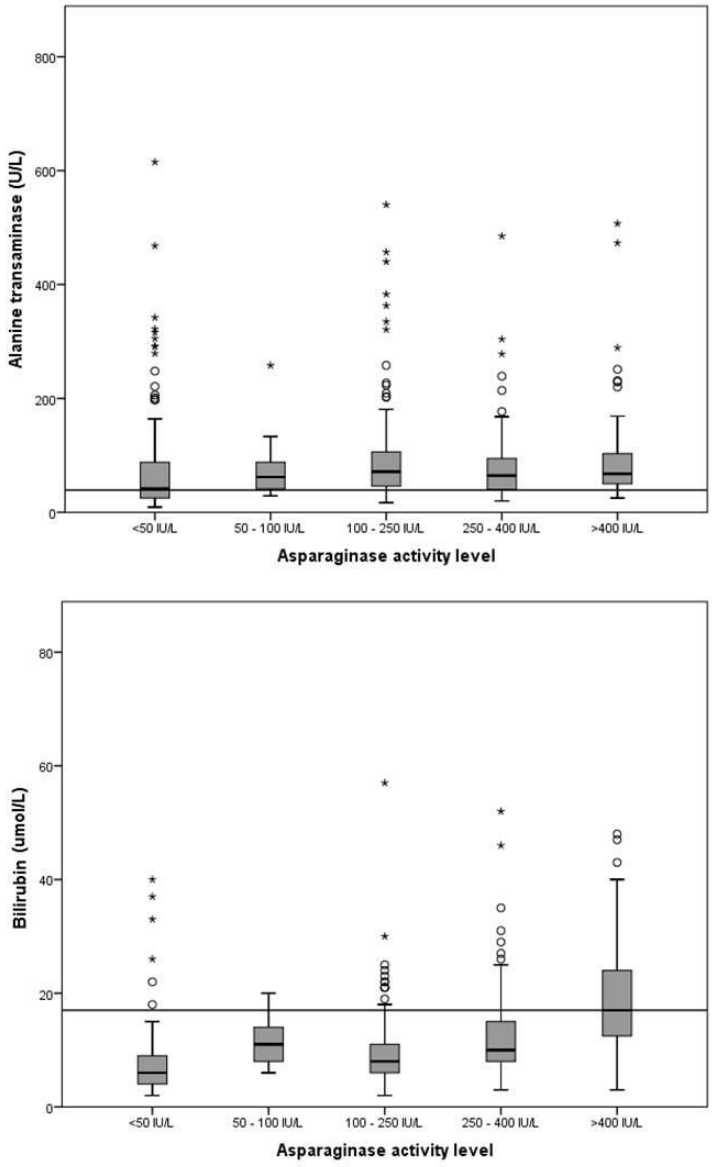
IQR: interquartile range; ALT: alanine transaminase

upplemental figure 1. PEGasparaginase therapeutic drug monitoring



Supplemental figure 1 schematically shows the therapeutic drug monitoring program of the DCOG ALL-11 protocol.
Patients started with three doses of a fixed dose of PEGasparaginase (1,500 IU/m^2) during induction. In standard risk and medium risk patients, the PEGasparaginase doses were individualized after induction. When stable PEGasparaginase levels were reached in medium risk intensification, trough levels were measured only every four weeks (indicated by the dotted arrows). High risk patients were also treated with the fixed dose of induction during the high risk blocks.

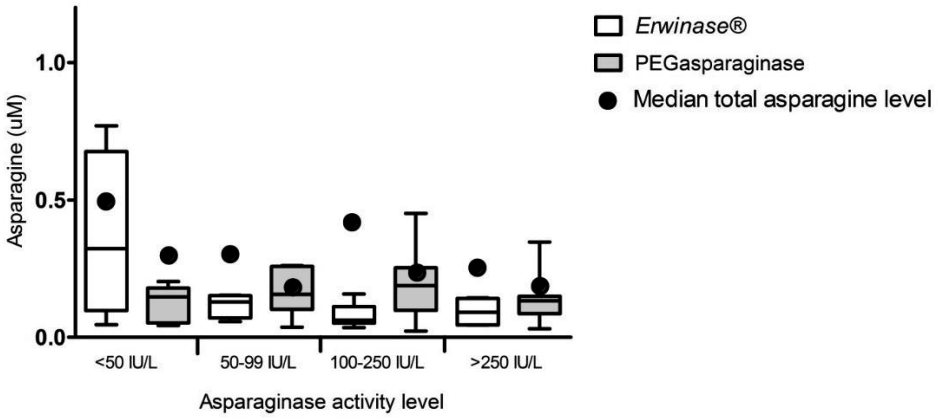
Supplemental figure 2. Correlation asparaginase activity levels and hepatotoxicity



Supplemental figure 2 shows the alanine transaminase (ALT) and bilirubin levels for different asparaginase activity levels. Longitudinal analysis showed that ALT and bilirubin levels were significantly higher with higher asparaginase activity levels ($p=0.004$ and $p<0.001$, respectively).
The triglyceride, amylase and glucose levels were not included in this figure as longitudinal analyses did not show a correlation between these parameters and asparaginase activity levels.

The boxplot includes the 25th, 50th and 75th percentile in the boxes, the outliers (○), extreme outliers (*), and the ranges (indicated by the whiskers).

Supplemental figure 3. Asparagine levels

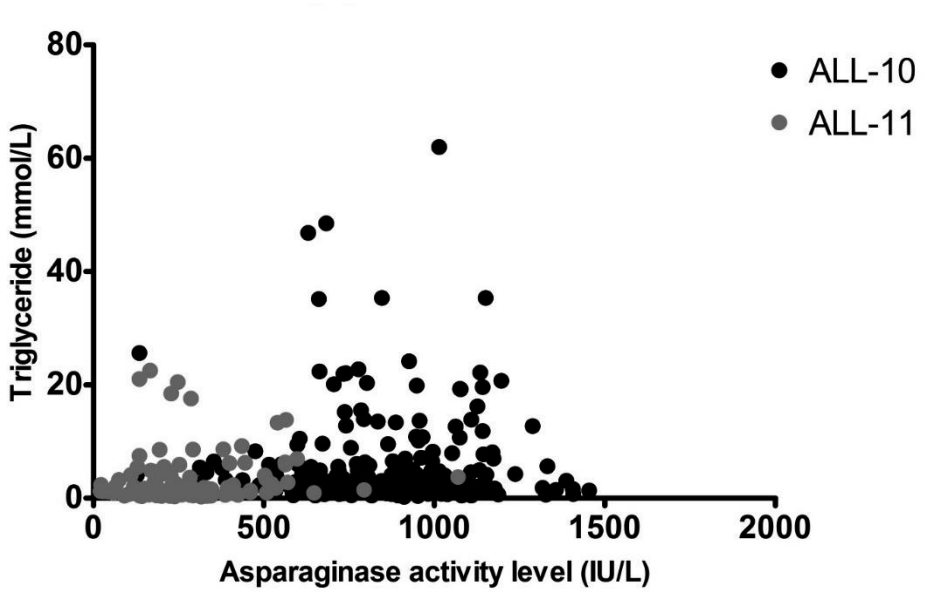


Supplemental figure 3 shows the L-asparagine levels of 50 PEGasparaginase and 20 Erwinase® samples in the box plots. The boxplot includes the 25th, 50th and 75th percentile in the boxes and the ranges (indicated by the whiskers).

For both the PEGasparaginase and Erwinase® samples, there was no association between L-asparagine and asparaginase activity levels (p=0.587 and p=0.267, respectively).

The median total asparagine levels (L- and D-asparagine) are indicated by the black dots.

Supplemental figure 4. Triglycerides in DCOG ALL-10 and ALL-11



Supplemental figure 4 shows the triglyceride levels, plotted against the asparaginase activity levels, for DCOG ALL-10[4] (black dots) and ALL-11 (grey dots).

REFRENCES

1. Lanvers, C., J. P. Vieira Pinheiro, G. Hempel, G. Wuerthwein and J. Boos. 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.
2. Kloos, R. Q., R. Pieters, G. Escherich and I. M. van der Sluis. Allergic-like reactions to asparaginase: Atypical allergies without asparaginase inactivation. Pediatr Blood Cancer. 2016.
3. Visser, W. F., N. M. Verhoeven-Duif, R. Ophoff, S. Bakker, L. W. Klomp, R. Berger, et al. A sensitive and simple ultra-high-performance-liquid chromatography-tandem mass spectrometry based method for the quantification of D-amino acids in body fluids. J Chromatogr A. 2011;1218(40):7130-7136.
4. Tong, W. H., R. Pieters, H. A. de Groot-Kruseman, W. C. Hop, J. Boos, W. J. Tissing, et al. Toxicity of very prolonged PEGasparaginase and Erwiniaasparaginase courses in relation to asparaginase activity levels with a special focus on dyslipidemia. Haematologica. 2014.
5. Tong, W. H., R. Pieters, G. J. Kaspers, D. M. te Loo, M. B. Bierings, C. van den Bos, 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.
6. Van Rijsbergen, C. J. (1979). Information Retrieval. Butterworth.

CHAPTER 3

3

Individualized Dosing Guidelines for PEGasparaginase and Factors Influencing the Clearance: a Population Pharmacokinetic Model

Robin Q.H. Kloos, Ron Mathôt, Rob Pieters, Inge M. van der Sluis

Haematologica, submitted

ABSTRACT

Background Considerable inter- and intra-patient variability exist in serum activity levels of PEGasparaginase, an essential component of pediatric acute lymphoblastic leukemia treatment. Here, a population pharmacokinetic (popPK) model was developed, identifying patient characteristics explaining these variabilities.

Methods Patients (n=92) were treated according to the Dutch Childhood Oncology Group ALL-11 protocol, using therapeutic drug monitoring to individualize the PEGasparaginase doses. Non-linear mixed effects modeling (NONMEM) was used to analyze the popPK evaluating several covariates. The final model was validated using an independent database (n=28) and guidelines for starting doses and dose adjustments were developed.

Results A 1-compartment model with time-dependent clearance (CL) adequately described the popPK. The inter-individual variability in CL and volume of distribution was reduced by normalization of these parameters by body surface area (BSA). CL was 0.084 L/day/m^2 for 12.7 days, increasing with 0.082 L/day/m^2/day thereafter. CL was 38% higher during an infection ($p<0.001$), and 11-19% higher during induction treatment than during intensification and maintenance. Targeting an asparaginase activity level of 100 IU/L, a loading dose of 800 IU/m^2 (induction) and 600 IU/m^2 (intensification) is advised.

Conclusion In conclusion, variability of PEGasparaginase activity levels can be explained by BSA, treatment phase and the occurrence of an infection. With the popPK model developed in this study, PEGasparaginase treatment can be individualized further, taking into account these covariates and the dosing guidelines provided.

INTRODUCTION

Asparaginase plays an important role in the treatment of pediatric acute lymphoblastic leukemia (ALL). The drug converts asparagine in aspartic acid and ammonia, resulting in apoptosis of the leukemic cells, as these cells highly depend on extracellular asparagine pools for protein synthesis.[1-3] An asparaginase activity level of >100 IU/L is considered to be sufficient for complete asparagine depletion.[4-10] Currently, different formulations of asparaginase derived from *Erwinia chrysanthemi* or *E. coli* are available in clinical practice, all with different pharmacokinetic properties.[11, 12] One of these formulations is the polyethylene glycol (PEG) conjugated form of *E. coli* asparaginase, also known as PEGasparaginase, which has several advantages compared to the native *E.coli* asparaginase. First, the risk of developing a neutralizing hypersensitivity reaction to asparaginase is reduced when using PEGasparaginase.[13-15] Second, PEGasparaginase can be administered less frequently than other asparaginase formulations because of its relatively long half-life.[16, 17] Therefore, PEGasparaginase is currently used as a first-line formulation in most developed countries. However, considerable inter- and intra-patient variability of PEGasparaginase levels has been observed and it is, therefore, difficult to determine the right dose for the individual patient.[17, 18] Currently, asparaginase therapy in Dutch pediatric patients with ALL is individualized with therapeutic drug monitoring (TDM). However, more insight in PEGasparaginase population pharmacokinetics (PK) – and especially in characteristics explaining the variability – is needed to optimize individualized dosing. Recently, Hempel et al. have described body surface area (BSA) as an important factor explaining variability in clearance (CL). However, the influence of other patient factors is still unknown.[17] Therefore, the aim of this study was to describe the PK of PEGasparaginase in our cohort, to gain more insight in factors influencing the CL, and to develop a dosing guideline for PEGasparaginase therapy in children.

METHODS

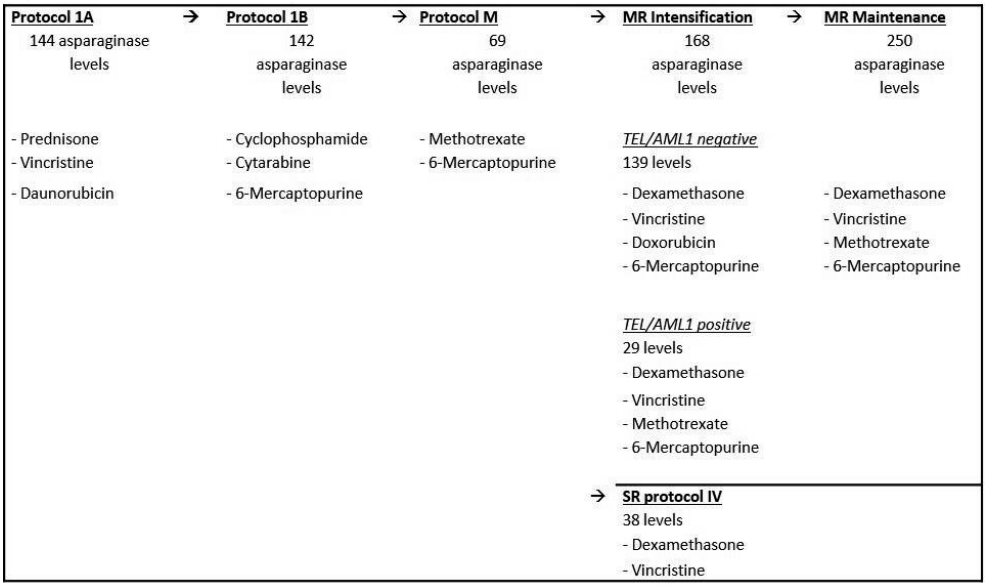
Patients and treatment protocol

Patients (1-18 years old) with newly diagnosed ALL between November 2014 and May 2017, treated according to the Dutch Childhood Oncology Group (DCOG) ALL-

11 treatment protocol in the Sophia Children's Hospital – Erasmus MC, Rotterdam, The Netherlands, were included. In these patients, trough-, top-, week- and other levels were prospectively measured. Data from asparaginase activity levels measured after the last dose as part of our nationwide TDM program from several patients from other Dutch pediatric oncology centers were included as well, providing asparaginase activity levels measured after the last dose. The study was approved by the Institutional Review Board (CCMO register: NL50250.078.14). Informed consent was obtained from patients 12 years or older, the parents or guardians in accordance with the Declaration of Helsinki.

According to the DCOG ALL-11 protocol, patients were stratified in a standard- (SR), medium- (MR) and high risk group (HR) after induction. Supplemental Table 1 describes the complete treatment for each risk group. Figure 1 schematically shows the concomitant chemotherapy per treatment phase.

Figure 1. Concomitant chemotherapy



Regarding the PEGasparaginase therapy, all patients were treated with three doses of PEGasparaginase (1,500 IU/m^2, biweekly) during the induction phase (protocol 1A and 1B). For the SR and MR patients, the subsequent dose(s) were individualized based on trough asparaginase activity levels. SR patients received one individualized dose after an interval of approximately 12 weeks during protocol IV; MR patients received another 14 individualized doses, either directly

after the first three doses or after an interval of approximately 12 weeks during the intensification and maintenance phase as part of a randomized study. HR patients received another 2-5 doses with a fixed dose of 1,500 IU/m^2. When a neutralizing allergy or silent inactivation occurred, patients were switched to *Erwinia* asparaginase. PEGasparaginase was administered intravenously over one hour.

Measurements

Asparaginase activity levels were measured using the L-aspartic β-hydroxamate (AHA) assay as described previously.[19] Briefly, AHA is added to patient serum containing PEGasparaginase and consequently hydrolyzed to L-aspartic acid and hydroxylamine. Hydroxylamine condenses with 8-hydroxyquinoline and oxidizes to indooxine, which is quantified by photometric detection at 690 nm. The lower limit of quantification (LLQ) was 10 IU/L.

Trough asparaginase activity levels and occasional week levels were standardly measured as part of the TDM program. Additional asparaginase activity levels were determined in residual blood, which was left over after other laboratory measurements. Furthermore, at several time points, top levels were measured in blood drawn within 1h after the administration of PEGasparaginase.

Anti-asparaginase antibodies were measured using enzyme-linked immunosorbent assay (ELISA) and expressed as optical density (OD) readings as described earlier.[18]

Population pharmacokinetic analysis

The population pharmacokinetic analysis was performed using NONMEM® Version 7.2 (Icon Development Solutions, Ellicott City, Maryland, USA). Other statistical analyses were performed using IBM SPSS Statistics (IBM Corp, Armonk, New York, USA) version 21.0 for Windows. The graphs to evaluate the models were prepared in R and Sigmaplot Version 3.4.1 (Systat Software Inc, London, UK).

The complete modeling process is described in the Supplemental methods. Briefly, after log transformation of the data, several structural models were evaluated using one- and two compartment models with linear, nonlinear, and time-

dependent elimination. Since body surface area (BSA) is known to highly influence the pharmacokinetics of PEGasparaginase, BSA (calculated with the Mosteller formula[20]) was included in the structural model to scale volume of distribution (Vd) and CL.

Covariate analysis

Several demographic, clinical and therapy-related covariates were evaluated and listed in Supplemental table 2. Beside differences between treatment phase and concomitant chemotherapy, administration of ciprofloxacin, antihistaminic drugs, hydrocortisone, itraconazole, fluconazole, valaciclovir and gabapentin were evaluated. In case of missing data of continuous covariates, the last known value or the median was implemented. Missing discontinuous data was excluded from the analysis.
First, the covariates were explored with univariate analyses after which the significant covariates ($p<0.05$) were evaluated using a stepwise forward inclusion, followed by backward elimination ($p<0.001$) in a multivariate analysis.

Model validation and development of dosing guidelines

The final model was validated using goodness of fit plots and visual predictive checks (VPC) which are described in the Supplemental methods. An independent database, which was obtained by randomly selecting 25% of the total population, was used for external validation of the model.

To develop dosing guidelines for dose adjustments, Monte Carlo simulations were performed. A starting dose for PEGasparaginase was calculated taking into account the significant covariates influencing the PEGasparaginase CL. Doses were calculated targeting trough asparaginase activity levels higher than 100 IU/L, 250 IU/L and 350 IU/L. By stepwise increasing the PEGasparaginase dose in simulations, it was evaluated which loading and maintenance dose provides adequate trough levels in 95% of the simulated patients.

Next, a guideline was developed to adjust the PEGasparaginase dose based on week- or trough levels targeting at a trough asparaginase activity level of 100-250 IU/L or 250-400 IU/L. For adjustment of the PEGasparaginase dose based on levels

measured after one week, trough levels were predicted on the basis of the individual simulated time profiles of PEGasparaginase activity.

RESULTS

Patients and samples

In total, 120 patients were included in the study. Ninety-two patients were included in the index dataset and 28 patients in the validation dataset. Supplemental table 2 describes the patient characteristics of the two datasets. The patients in the validation database were older (median 8.0 years, interquartile range (IQR) 3.3 – 12.5 years) than in the main database (median 4.8 years, IQR 3.3 – 8.2 years), and had a higher weight (median 28.0, IQR 16.6 – 47.9 versus 19.2, 14.9 – 29.3).

Table 1 shows the characteristics and distribution of the samples. In the index database, 816 samples were measured in 92 patients. The majority of the levels were top-, week- or trough levels, and were measured during the intensification and maintenance phase of the MR group. Supplemental figure 1 shows all asparaginase activity levels plotted against the time after dose.

Table 1. Sample characteristics

	Index dataset n = 92	**Validation dataset n = 28**
Total number of samples	816	405
Number of levels per patient, median (IQR) (range)	3 (2 – 12) (1 – 38)	6 (2 – 33) (1 – 39)
Sample time (%)		
0-7 days	371 (45%)	181 (45%)
8-14 days	342 (42%)	187 (46%)
> 14 days	103 (13%)	37 (9%)
Number of levels per treatment phase		
1A	144 (18%)	68 (17%)
1B	142 (17%)	39 (10%)
M	69 (8%)	19 (5%)
MR intensification	168 (21%)	94 (23%)
MR maintenance	250 (30%)	177 (43%)
SR protocol IV	38 (5%)	8 (2%)
HR blocks	5 (1%)	-

Structural model

The development of the structural pharmacokinetic model is described in the Supplemental results. The estimated population PK parameters are given in Table 2. Concentration-time profiles were best described by a one-compartment model. Adding a second compartment did not improve the model. Normalization of the CL by BSA reduced the (unexplained) inter-individual variability (IIV) of this parameter from 29.6% to 24.1%. Intra-patient variability (IOV) was 25.7%. As demonstrated in

Table 2. Pharmacokinetic models

Parameter	Structural model Mean (RSE)	Final model Mean (RSE)	Bootstrap of the final model	
			Estimate	95% CI
OFV	-1140.4	-1203.77		
CL (L/day/m^2)	0.075 (5.2%)	0.084 (4.4%)	0.084	0.078 – 0.090
Vd (L/m^2)	0.92 (4.7%)	0.94 (4.5%)	0.94	0.87 – 1.01
Slope CL_{ind} (L/day/m^2/day)	0.079 (31.5%)	0.082 (20.5%)	0.080	0.052 – 0.115
Split point (days)	12.9 (1.1%)	12.7 (0.2%)	12.7	11.8 – 13.1
Correlation CL and Vd	1.11 (13.4%)	1.26 (10.2%)	1.28	0.98 – 1.51
Covariates				
Treatment phase 1A	-	ref	ref	-
1B	-	1 (fix)	-	-
M	-	0.87 (5.2%)	0.87	0.80 – 0.95
MRG intens.	-	0.89 (5.2%)	0.88	0.82 – 0.98
MRG maint.	-	0.81 (3.9%)	0.81	0.75 – 0.86
SRG protocol IV	-	0.81 (6.5%)	0.81	0.73 – 0.90
Infection	-	1.38 (10.5%)	1.38	1.15 – 1.67
IIV				
CL (%)	24.1 (12.8%)	19.7 (13.2%)	19.5	14.6 – 24.0
Vd	NA	NA	NA	NA
IOV				
CL (%)	25.7 (10.1%)	23.6 (10.3%)	23.1	18.5 – 27.5
Residual variability				
Additional (IU/L)	19.1 (26.2%)	20.2 (18.2%)	20.2	12.7 – 298
Proportional (%)	17.3 (9.2%)	17.0 (9.2%)	16.8	14.2 – 19.5
Shrinkage				
IIV CL	22.0%	24.0%	-	-
IOV	5.0%	15.0%	-	-

Final model:

TAD <12.7 days:

$CL = 0.075 * BSA * 1.38^{INFECTION} * 0.87^{M} * 0.89^{MRG\ INTENS.} * 0.81^{MRG\ MAINT.} * 0.81^{SRG\ PROTOCOL\ IV} * e^{\eta IIV + \eta IOV}$

TAD >12.7 days:

$CL = 0.075 * BSA * (1+0.079 * (TAD - 12.7)) * 1.38^{INFECTION} * 0.87^{M} * 0.89^{MRG\ INTENS.} * 0.81^{MRG\ MAINT.} * 0.81^{SRG\ PROTOCOL\ IV} * e^{\eta IIV + \eta IOV}$

$Vd = 0.92 * e^{1.11 * \eta IIV)}$

Values for INFECTION, M, MG INTENS., MRG MAINT. And SRG PROTOCOL IV: 0 (no) or 1 (yes).

RSE: relative standard error; CI: confidence interval; OFV: objective function value; CL: clearance, Vd: volume of distribution, C_{ind}: induced clearance; MRG intens.: medium risk group intensification; MRG maint.: medium risk group maintenance; IIV: inter-individual variability; IOV: interoccasion variability; NA: not applicable; TAD: time after dose.

Supplemental figure 1, the elimination of PEGasparaginase was not linear. Several models with time dependent CL were evaluated.[21] The structural model – so without the effect of covariates – best described the data with a CL of 0.075 L/day/m^2 for the first 12.9 days after a dose, increasing with 0.079 L/day/m^2 per day thereafter. Thus, during the first 12.9 days, the half-life of PEGasparaginase was 8.5 days and, thereafter, decreased to 4.1 days after one day, and 2.7 days after two days.

Covariate analysis

The univariate analysis resulted in 16 covariates significantly correlated with PEGasparaginase CL (Table 3). In the stepwise forward inclusion procedure, the presence of an infection was first added to the structural model, followed by treatment phase. The CL increased with 38% when a patient had an infection. In comparison with treatment phase 1A, which was used as a reference, CL was lower during protocol M, MR intensification and maintenance, and SR protocol IV. This association was independent of the presence of an infection. The CL in phase 1B was equal to 1A. Only two patients were treated as high risk, so the association between the HR blocks and PEGasparaginase CL could not be estimated reliably and was fixed to one. Adding ICU admission to the model (beside infection and treatment phase), did not improve the fit of the model to the data, nor did the other covariates.

Table 3. Univariate covariate analysis

Covariate	Effect: Θ (95% CI)	Δ OFV	Included after backward elimination?
Anti-native *E. coli* asparaginase antibodies (extinction values)	0.05 (-0.01 – 0.11)	-12.9	No
Anti-PEGasparaginase antibodies (extinction values)	0.04 (-0.01 – 0.10)	-7.3	No
Creatinine	-0.21 (-0.36 – -0.07)	-17.0	No
Leukocytes	-0.09 (-0.13 – 0.05)	-22.9	No
ICU admission	1.61 (1.55 – 1.67)	-31.0	No
Infection	1.45 (1.11 – 1.79)	-52.5	Yes
Treatment phase		-42.3	Yes
1A	ref		
1B	1 (fix)		
M	0.86 (0.78 – 0.94)		
MRG intens.	0.86 (0.77 – 0.95)		
MRG maint.	0.78 (0.71 – 0.85)		
SRG protocol IV	0.78 (0.67 – 0.88)		
Other chemotherapy			
Prednisone	1.11 (0.97 – 1.25)	-5.4	No
Vincristine	0.89 (0.82 – 0.96)	-12.2	No
Daunorubicine	1.11 (0.97 – 1.25)	-5.4	No
6-Mercaptopurine	0.91 (0.84 – 0.99)	-6.8	No
Cyclophosphamide	1.13 (0.98 – 1.22)	-5.3	No
Cytarabine	1.13 (0.93 – 1.33)	-5.3	No
Methotrexate	0.85 (0.80 – 0.91)	-26.2	No
Dexamethasone	0.85 (0.78 – 0.91)	-27.7	No
Doxorubicin	1.01 (0.90 – 1.12)	-0.06	No

* To evaluate any chemotherapy-related effects on clearance on top of the treatment phase, all chemotherapy were first included in the multivariate analysis. Only inclusion of doxorubicin and methotrexate resulted in a significant improvement of the model (OFV -6.0, mean effect (RSE): 1.24 (6%) and OFV -10.3, mean effect (RSE): 0.88 (5%), respectively).
CI: confidence interval; OFV: objective function value; ICU: intensive care unit,
MRG intens.: medium risk intensification; MRG maint.: medium risk maintenance phase.
Covariates significantly influenced the clearance in the univariate analysis when the OFV decreased with >3.84 ($p<0.05$).

Evaluation of the effect of a specific chemotherapeutic agent on CL was difficult because blood concentrations of those agents were not measured and many chemotherapeutics are administrated concomitantly. Therefore, it was assumed that a possible effect of the agent on CL was present during the entire treatment phase. As a result, the treatment phase and the administration of a chemotherapeutic agent was highly correlated.

However, as shown in Figure 1, the intensification phase of MR patients with a TEL/AML1 translocation did not contain doxorubicin but these patients were treated with methotrexate beside dexamethasone, vincristine, 6-mercaptopurine and PEGasparaginase. The other MR patients first received 4x doxorubicin in 12

weeks, followed by methotrexate. Although not significant in the univariate analysis, based on clinical grounds, both doxorubicin and methotrexate were evaluated in the multivariate analysis as the effect of these drugs could be analyzed independently of treatment phase. In the univariate analysis, CL appeared 11% lower during MR intensification and 19% lower during MR maintenance compared to protocol 1A.
Separate addition of the agents to the model both improved the fit of the model (doxorubicin higher CL, methotrexate lower CL). However, in the backward elimination, both drugs turned out not to influence the PEGasparaginase CL significantly and were, therefore, excluded from the final model.

Thus, the final population PK model included, besides BSA, the presence of an infection and treatment phase as covariates significantly associated with the CL of PEGasparaginase. Inclusion of these parameters reduced the IIV of CL from 24.1% to 19.7% (18% reduction), and IOV from 25.7% to 23.6% (8% reduction) (Table 2). In this final model, CL was 0.084 L/day/m^2 for 12.7 days, increasing with 0.082 L/day/m^2/day thereafter.

Goodness of fit and model validation

The parameter estimates of the final model were precise regarding the relative standard errors, and the shrinkage values were acceptable (Table 2). The goodness of fit plots showed an even distribution of the population predictions and individual predictions around the line of unity (Supplemental figure 2). The conditional weighted residuals plotted against the time after dose were also evenly distributed with a mean of zero, and no trend was found. The bootstrap analysis showed that the bootstrap estimates were consistent with the parameter estimates of the final model and the 95% confidence intervals were accurate (Table 2). The visual predictive check of the index dataset is shown in Supplemental figure 3. Both the median estimates and the 95% confidence intervals are within the simulated predicted values. Thus, the final model is accurate in predicting the PEGasparaginase population pharmacokinetics.
As an external validation, the final model derived from the index dataset was used to predict the asparaginase activity levels of the independent validation dataset. The goodness of fit plots and VPC of the validation dataset also show that the final model is adequate to describe the PEGasparaginase pharmacokinetics (Supplemental figure 2 and 3).

Clinical implications

Table 4 shows several dosing regimens for PEGasparaginase (loading dose and maintenance dose) during induction and MRG intensification. Targeting at a trough PEGasparaginase activity level of 100 IU/L, the loading dose recommended during induction is 800 IU/m^2 followed by a biweekly maintenance dose of 600 IU/m^2. For PEGasparaginase treatment during intensification, lower doses should be administered due to decreased CL, being 600 IU/m^2 and 400 IU/m^2, respectively, to target 100 IU/L.

Table 4. Dosing guideline, starting dose

	Target trough asparaginase level	Loading dose	Maintenance dose
Protocol 1A (induction)	100 IU/L	800 IU/m^2	600 IU/m^2
	250 IU/L	1,800 IU/m^2	1,400 IU/m^2
	350 IU/L	2,200 IU/m^2	1,600 IU/m^2
Medium risk intensification	100 IU/L	600 IU/m^2	400 IU/m^2
	250 IU/L	1,500 IU/m^2	1,000 IU/m^2
	350 IU/L	1,800 IU/m^2	1,800 IU/m^2

Based on the half-life during the first 13 days, the steady state is estimated to be reached after 2 doses, after which the dose can be adjusted based on trough or week asparaginase activity levels targeting trough levels between 100 and 250 IU/L or 250 and 400 IU/L (Table 5). If an infection occurs, it is advised to increase the dose with 38%, if clinically possible.

Table 5. Dosing guideline, dose adjustments

Target trough level: 100-250 IU/L			Target trough level: 250-400 IU/L		
Week level	Trough level	Dose adjustment	Week level	Trough level	Dose adjustment
50-100 IU/L	25-50 IU/L	400%	100-200 IU/L	50-100 IU/L	400%
100-250 IU/L	50-75 IU/L	300%	200-300 IU/L	100-150 IU/L	300%
150-200 IU/L	75-100 IU/L	200%	300-250 IU/L	150-200 IU/L	200%
200-450 IU/L	100-250 IU/L	100%	350-450 IU/L	200-250 IU/L	175%
450-550 IU/L	250-300 IU/L	60%	450-700 IU/L	250-400 IU/L	100%
550-750 IU/L	300-400 IU/L	50%	700-900 IU/L	400-500 IU/L	70%
750-1100 IU/L	400-600 IU/L	40%	900-1100 IU/L	500-600 IU/L	60%
1100-1500 IU/L	600-800 IU/L	25%	1100-1250 IU/L	600-700 IU/L	50%
1500-1800 IU/L	800-1000 IU/L	20%	1250-1800 IU/L	700-1000 IU/L	35%

The dose adjustments apply for biweekly administration of PEGasparaginase during steady state. The doses may be adjusted based on week (7 days) or trough (14 days) after administration targeting at trough asparaginase activity levels of 100-250 IU/L or 250-400 IU/L.

DISCUSSION

In this study, the population pharmacokinetics of PEGasparaginase were investigated in order to identify patient and clinical characteristics associated with the CL of PEGasparaginase. Furthermore, the population PK model was used to develop a dosing guideline for the drug. It was shown that the CL is constant during the first 13 days after administration and increases thereafter. In addition, the CL was higher during induction, and during an infection.

During development of the structural model, both first- and zero-order, nor Michaelis Menten elimination described the concentration-time profile of PEGasparaginase adequately. This has also been reported by Hempel et al. and Würthwein et al.[17, 21] In the present study, similar to Würthwein et al., the time dependency of CL proved to be described most adequately with a split model, showing that CL increases substantially after a period of approximately 13 days. This induced CL could be explained by the hydrolysis of the PEG moiety from the PEGasparaginase molecule, resulting in native *E. coli* asparaginase with a linker attached, originally connecting PEG with the asparaginase. Thus, CL will increase to a value more or less comparable with that of native *E. coli* asparaginase, which has a half-life of 1.3 days[16], after several days.

Several associations between covariates and the PEGasparaginase CL were identified. The metabolism of PEGasparaginase appears to depend on treatment phase and is influenced by the presence of an infection. Native *E. coli* asparaginase is being eliminated mainly by the macrophages of the mononuclear phagocyte system (MPS) in the bone marrow, spleen and liver.[22] Although the mechanism of elimination of PEGasparaginase has not been studied, it is likely that this also holds for the PEGylated formulation as other PEGylated drugs are also eliminated by the cells of the MPS.[23]

CL was the highest during protocol 1A containing vincristine, prednisone and daunorubicin. MR intensification, maintenance and SR protocol IV had lower CL and all contain vincristine as well, so an effect of vincristine is unlikely. Especially because the CL during MR maintenance is lower than MR intensification, either doxorubicin and/or methotrexate probably influence the PEGasparaginase CL. The fact that the CL is the highest in induction might be caused by the other anthracycline, daunorubicin, which is administered weekly, whereas doxorubicin, is administered once every 3 weeks in intensification.

Beside the administration of specific chemotherapeutics, other mechanisms may explain the differences in CL between the treatment phases. It has to be taken into account that the physical condition of the patient alters during ALL treatment: during protocol 1A (and also 1B), the tumor load is higher and therapy is more intense compared to protocol M, MR intensification and maintenance, and SR protocol IV, which all had a lower CL. The MPS plays a role in tumor lysis [24, 25], which may explain why the CL is higher during this treatment phase as well, although the asparaginase treatment starts at day 12 of the treatment protocol.

Strikingly, the occurrence of an infection increases the CL with 38%, independent of the treatment phase and BSA, probably because of the activation of the MPS, which is responsible for the CL of asparaginase. This means that the PEGasparaginase activity levels will be 38% lower during an infection and that the PEGasparaginase dose should be increased with this percentage to obtain similar levels. Evidently, this should first be validated in clinical practice, taking into account the clinical situation of the patient.

BSA was included in the structural model, showing that the CL of PEGasparaginase increases with BSA, which is in line with the findings of Hempel et al.[17] Sassen et al., however, have studied the CL of *Erwinia* asparaginase and found the opposite: patients with a lower weight have a higher CL, requiring a higher starting dose of the drug.[26] Shrey et al. have studied the native *E. coli* asparaginase levels in different age groups and also show lower asparaginase activity levels in younger patients, suggesting a higher CL in these patients.[27] Thus, for a PEGylated form of asparaginase, this does not seem to apply, which is also supported by a study of PEGylated interferon alfa-2b in children, which also shows that the CL increases with age.[28]

Based on the population pharmacokinetic model, dosing guidelines were developed taking into account the effect of treatment phase on PEGasparaginase CL. Next, dose adjustments based on both week- and trough levels were suggested targeting 100-250 IU/L or the higher range of 250-400 IU/L. These dosing guidelines, however, should first be validated clinically. In addition, the effect of increasing the dose in case of an infection on PEGasparaginase activity levels should be analyzed.

In conclusion, 18% of the inter- and 8% of the intra-patient variability in CL of PEGasparaginase, normalized for BSA, can be explained by 1) treatment phase showing a higher CL during induction treatment (protocol 1A), and 2) the

occurrence of an infection, which increases the CL. With the population PK model developed in this study, PEGasparaginase treatment may be individualized further, taking into account these covariates and the dosing guidelines provided.

REFERENCES

1. Miller, H. K., J. S. Salser and M. E. Balis. Amino acid levels following L-asparagine amidohydrolase (EC.3.5.1.1) therapy. Cancer Res. 1969;29(1):183-187.
2. Broome, J. D. L-Asparaginase: discovery and development as a tumor-inhibitory agent. Cancer Treat Rep. 1981;65 Suppl 4:111-114.
3. Bussolati, O., S. Belletti, J. Uggeri, R. Gatti, G. Orlandini, V. Dall'Asta, et al. Characterization of apoptotic phenomena induced by treatment with L-asparaginase in NIH3T3 cells. Exp Cell Res. 1995;220(2):283-291.
4. Riccardi, R., J. S. Holcenberg, D. L. Glaubiger, J. H. Wood and D. G. Poplack. L-asparaginase pharmacokinetics and asparagine levels in cerebrospinal fluid of rhesus monkeys and humans. Cancer Res. 1981;41(11 Pt 1):4554-4558.
5. Ahlke, E., U. Nowak-Gottl, P. Schulze-Westhoff, G. Werber, H. Borste, G. Wurthwein, et al. Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during induction therapy in children with acute lymphoblastic leukaemia. Br J Haematol. 1997;96(4):675-681.
6. Rizzari, C., M. Zucchetti, V. Conter, L. Diomede, A. Bruno, L. Gavazzi, et al. L-asparagine depletion and L-asparaginase activity in children with acute lymphoblastic leukemia receiving i.m. or i.v. Erwinia C. or E. coli L-asparaginase as first exposure. Ann Oncol. 2000;11(2):189-193.
7. Albertsen, B. K., H. Schroder, J. Ingerslev, P. Jakobsen, V. I. Avramis, H. J. Muller, et al. Comparison of intramuscular therapy with Erwinia asparaginase and asparaginase Medac: pharmacokinetics, pharmacodynamics, formation of antibodies and influence on the coagulation system. Br J Haematol. 2001;115(4):983-990.
8. Grigoryan, R. S., E. H. Panosyan, N. L. Seibel, P. S. Gaynon, I. A. Avramis and V. I. Avramis. Changes of amino acid serum levels in pediatric patients with higher-risk acute lymphoblastic leukemia (CCG-1961). In Vivo. 2004;18(2):107-112.
9. Tsurusawa, M., M. Chin, A. Iwai, K. Nomura, H. Maeba, T. Taga, et al. L-Asparagine depletion levels and L-asparaginase activity in plasma of children with acute lymphoblastic leukemia under asparaginase treatment. Cancer Chemother Pharmacol. 2004;53(3):204-208.
10. Appel, I. M., K. M. Kazemier, J. Boos, C. Lanvers, J. Huijmans, A. J. Veerman, et al. Pharmacokinetic, pharmacodynamic and intracellular effects of PEG-asparaginase in newly diagnosed childhood acute lymphoblastic leukemia: results from a single agent window study. Leukemia. 2008;22(9):1665-1679.
11. Asselin, B. L., J. C. Whitin, D. J. Coppola, I. P. Rupp, S. E. Sallan and H. J. Cohen. Comparative pharmacokinetic studies of three asparaginase preparations. J Clin Oncol. 1993;11(9):1780-1786.
12. Pieters, R., S. P. Hunger, J. Boos, C. Rizzari, L. Silverman, A. Baruchel, et al. L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. Cancer. 2011;117(2):238-249.
13. Silverman, L. B., R. D. Gelber, V. K. Dalton, B. L. Asselin, R. D. Barr, L. A. Clavell, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97(5):1211-1218.
14. Avramis, V. I., S. Sencer, A. P. Periclou, H. Sather, B. C. Bostrom, L. J. Cohen, et al. 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(6):1986-1994.
15. Hawkins, D. S., J. R. Park, B. G. Thomson, J. L. Felgenhauer, J. S. Holcenberg, E. H. Panosyan, et al. Asparaginase pharmacokinetics after intensive polyethylene glycol-conjugated L-asparaginase therapy for children with relapsed acute lymphoblastic leukemia. Clin Cancer Res. 2004;10(16):5335-5341.
16. Asselin, B. L. The three asparaginases. Comparative pharmacology and optimal use in childhood leukemia. Adv Exp Med Biol. 1999;457:621-629.
17. Hempel, G., H. J. Muller, C. Lanvers-Kaminsky, G. Wurthwein, A. Hoppe and J. Boos. A population pharmacokinetic model for pegylated-asparaginase in children. Br J Haematol. 2010;148(1):119-125.

18. Tong, W. H., R. Pieters, G. J. Kaspers, D. M. te Loo, M. B. Bierings, C. van den Bos, 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.
19. Lanvers, C., J. P. Vieira Pinheiro, G. Hempel, G. Wuerthwein and J. Boos. 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.
20. Mosteller, R. D. Simplified calculation of body-surface area. N Engl J Med. 1987;317(17):1098.
21. Wurthwein, G., C. Lanvers-Kaminsky, G. Hempel, S. Gastine, A. Moricke, M. Schrappe, et al. Population Pharmacokinetics to Model the Time-Varying Clearance of the PEGylated Asparaginase Oncaspar(R) in Children with Acute Lymphoblastic Leukemia. Eur J Drug Metab Pharmacokinet. 2017.
22. van der Meer, L. T., S. Y. Terry, D. S. van Ingen Schenau, K. C. Andree, G. M. Franssen, D. M. Roeleveld, et al. In Vivo Imaging of Antileukemic Drug Asparaginase Reveals a Rapid Macrophage-Mediated Clearance from the Bone Marrow. J Nucl Med. 2017;58(2):214-220.
23. Yang, Q. and S. K. Lai. Anti-PEG immunity: emergence, characteristics, and unaddressed questions. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2015;7(5):655-677.
24. Adams, D. O. (1985). Tumor cell recognition and destruction by activated macrophages: effector and regulatory mechanisms. Dordrecht, Springer.
25. Becker, S. Functions of the human mononuclear phagocyte system. Advanced Drug Delivery Reviews. 1988;2(1-29).
26. Sassen, S. D., R. A. Mathot, R. Pieters, R. Q. Kloos, V. de Haas, G. J. Kaspers, et al. Population pharmacokinetics of intravenous Erwinia asparaginase in pediatric acute lymphoblastic leukemia patients. Haematologica. 2017;102(3):552-561.
27. Schrey, D., S. Borghorst, C. Lanvers-Kaminsky, G. Hempel, J. Gerss, A. Moricke, et al. Therapeutic drug monitoring of asparaginase in the ALL-BFM 2000 protocol between 2000 and 2007. Pediatr Blood Cancer. 2010;54(7):952-958.
28. Xu, C., S. Gupta, G. Krishna, D. Cutler, S. Wirth, C. Galoppo, et al. Population pharmacokinetics of peginterferon alfa-2b in pediatric patients with chronic hepatitis C. Eur J Clin Pharmacol. 2013;69(12):2045-2054.

SUPPLEMENTAL METHODS

Population PK analysis

The activity data were logarithmically transformed and the analysis was performed with the First Order Conditional Estimation method with interaction (FOCE+I).

In the development of the structural model, one- and two compartment models were evaluated. Subsequently, models with first- and zero-order elimination, Michaelis-Menten elimination, and time-dependent elimination were explored. The pharmacokinetics were expressed in terms of clearance (CL) and volume of distribution (Vd).

Time profiles of PEGasparaginase activity versus time were adequately described using a one-compartment model. Addition of a second compartment did not improve the model. Models with first- and zero-order elimination did not describe the data adequately. Models with time-dependent CL, previously described by Würthwein et al.[1] described the data better.

Inter-individual variability and inter-occasion variability, with an occasion defined as administration of a new dose, and correlation between Cl and Vd were assessed in the models. Inter-individual and inter-occasion variability in Cl and Vd was characterized with exponential models. For example, the elimination for the i^{th} patient was estimated using the following equation:

$$CLi = \Theta pop \times e^{(\eta i + ki)}$$

Were Θpop is the typical population value for CL. η_i and k_i represent random effects accounting for individual and occasion variation from the typical value. η_i and k_i are assumed to be symmetrically distributed with a mean of 0 and estimated variance of ω^2 and π^2.

Additional and proportional error models were evaluated to account for the residual error. Furthermore, since body surface area (BSA) is known to be an important covariate for PEGasparaginase pharmacokinetics, this was included in the structural model.

$$CLi = \Theta pop\ x\ BSA \times e^{(\eta i + ki)}$$

To allow for asparaginase activity levels below the limit of quantification (LLQ), several methods were applied. Both the M3 [2] and M2 method, however, resulted

in unstable runs of particularly the time-varying elimination models. Because only 4% of the patients had developed a neutralizing hypersensitivity reaction, this has only little influence on the analysis. Therefore, we have decided to exclude the values <LLQ.

The precision of the parameter estimates, objective function values (OFV's) and goodness of fit plots were used for selection of the models evaluated. A decrease in the OFV of >3.84 points and >10.83 points was considered as a significant improvement of the model with significance of $p<0.05$ and $p<0.001$, respectively.

After obtaining the structural model, several covariates were evaluated as described by the following equations:

Continuous data:

$$\left(\frac{covariate}{median}\right)^{\theta}$$

Discontinuous data:

$$covariate \times e^{\theta}$$

For example, the effect of the continuous covariate leukocyte count on Cl was explored by incorporating the leukocyte count divided by the median ($2.4 * 10^9$/L) to the power of Θ in the equations of Cl and Vd. The discontinuous covariate 'infection' was explored by multiplying the Cl and Vd by Θ in case of an infection.

The covariates were first explored with univariate analysis after which the significant covariates (OFV -3.84) were evaluated with stepwise forward inclusion and backward elimination (OFV -10.83).

A bootstrap analysis with 1000 bootstrap replicates was used to assess the robustness of the model. Visual predictive check (VPC) plots were used for internal validation of the model. An independent validation dataset, obtained by randomly selecting 25% of the total population, was used to validate the final model externally. The VPCs were prediction corrected to correct for the dose adjustments of PEGasparaginase.

SUPPLEMENTAL RESULTS

PK analysis

First, the asparaginase activity levels were log transformed. To account for residual error, an additive and proportional model were evaluated. As a combined model of proportional and additive error was superior, this was further used in development of the model. Linear models and models with time-constant elimination did not adequately describe the data. This analysis, however, showed that a one-compartment model was sufficient and adding body surface area (BSA) as a covariate did significantly improved the model (OFV -22.7). Also inter-individual variability (IIV) on Cl and Vd, inter-occasion variability (IOV) on Cl, and correlation between Cl and Vd significantly improved the model. Next, the models with time-varying clearance as described by Würthwein et al.[1] were tested. These models comprised several exponential elimination equations with initial and induced clearance. However, these models did not adequately describe the data as well. Würthwein et al.[1] have concluded that a split point model best describes the PEGasparaginase pharmacokinetics by exploring transit models. They concluded that the Cl was constant at first but increased after approximately 10 days. Therefore, we next have evaluated a transit model, estimating after how many days the clearance increases.

This model most adequately described the data, estimating the split point at 12.9 days after administration. Hence, the final structural model was as follows:

$$Cl\ (first\ 13\ days) = \Theta_1 * e^{\eta + \eta IOV} * BSA$$

$$Cl\ (after\ 13\ days) = \Theta_1 * e^{\eta + \eta IOV} * BSA * Cl_{ind}$$

$$Cl_{ind} = 1 + \Theta_2 * (TAD - split\ point)$$

$$Vd = \Theta_3 * e^{\Theta_4 * \eta} * BSA$$

Where TAD is time after dose and the Cl_{ind} increases with Θ_2 per day. In the equation of Vd, Θ_4 represents the correlation between Cl and Vd. Table 2 shows the parameter estimates of the final model with a Cl of 0.075 L/day/m^2 , increasing with 0.079 L/day/m^2 after 12.9 days, and a Vd of 0.92 L/m^2. IIV on Vd could not be estimated as this completely correlated with the correlation between Cl and Vd.

After obtaining the structural model, the covariates were evaluated one by one. Univariate analysis resulted in 16 significant covariates influencing the clearance

(Table 3). However, the anti-asparaginase antibodies, creatinine and leukocytes had large relative standard errors and the 95% confidence interval included 0. Infection, treatment phase and intensive care unit (ICU) admission resulted in the largest decrease of OFV and were therefore first evaluated during the multivariate analysis. Multivariate analysis with treatment phase and the presence of an infection significantly improved the model (OFV -21.6) compared to the structural model. Further addition of ICU admission did not improve the model (OFV -2.6) and was, therefore, excluded. Similar results were found for anti-asparaginase antibodies, creatinine and leukocyte levels. As explained in the main article, only methotrexate and doxorubicin significantly improved the model on top of treatment phase and infection (OFV -10.3, mean effect (RSE): 0.88 (5%) and OFV -6.0, mean effect (RSE): 1.24 (6%), respectively). Adding both drugs in the analysis did not improve the model (OFV -0.04) and both drugs were not significant during backward elimination. Finally, treatment phase and infection were included in the final model.

Simulations

Using the final population model, Monte Carlo simulations were performed for 2000 virtual patients with BSA ranging from 0.52 to 2.3 m^2. All patients received bi-weekly steady-state doses of PEG-asparaginase with doses ascending from 100 IU/m^2 to 3000 IU/m^2 in 100 IU/m^2 steps. Trough levels and levels one week after administration were evaluated. Target trough levels of 100 – 250 IU/ml corresponded to levels of 200 – 450 IU/ml at one week after administration. Similarly, target trough levels of 250-400 IU/ml corresponded to levels of 450-750 IU/ml at one week after administration. When simulated levels were outside the target range it was evaluated to what extent the dose had to be increased or decreased to obtain adequate levels.

Supplemental Table 1: DCOG ALL-11 treatment protocol

Treatment phase	Therapy
Protocol 1A	
Prednisone	60 mg/m^2/day for 29 days followed by 3x3 days tapering
Vincristine	1.5 mg/m^2/dose at day 8, 15, 22 and 29
Daunorubicin	30 mg/m^2/dose at day 8, 15, 22 and 29 (not in case of Down syndrome)
PEGasparaginase	1,500 IU/m^2 at day 12, 26
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 15 and 33. Only intrathecal methotrexate at day 1.
Protocol 1B	
PEGasparaginase	1,500 IU/m^2 at day 40
Cyclophosphamide	1,000 mg/m^2/dose at day 36 and 64
Cytarabine	75 mg/m^2/day at days 38 – 41, 45 – 48, 52 – 55, 59 – 62
6-Mercaptopurine	60 mg/m^2/day at days 36 – 63
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 45 and 59
Protocol M for SR and MR patients	
6-Mercaptopurine	25 mg/m^2/day for 56 days
Methotrexate	5,000 mg/m^2/dose at day 8, 22, 36 and 50
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 8, 22, 36 and 50
Protocol IV for SR patients	
Dexamethasone	10 mg/m^2/day for 15 days followed by 3x3 days tapering
Vincristine	1.5 mg/m^2/dose at day 1 and 8
PEGasparaginase	Individualized dose at day 1
Maintenance for SR patients	
6-Mercaptopurine*	50 mg/m^2/day for 81 weeks
Methotrexate*	20 mg/m^2/week for 81 weeks
Intensification and maintenance for MR patients	
Dexamethasone	6 mg/m^2/day for 5 days every 3 weeks until week 82
Vincristine	2 mg/m^2/dose every three weeks until week 82
Doxorubicin	30 mg/m^2/dose at week 1, 4, 7 and 10 (not in case of Down syndrome or TEL/AML1)
PEGasparaginase	Individualized doses biweekly from week 1 – 27**
Methotrexate*	30 mg/m^2/week from week 13 – 84 (or week 2 – 84 in case of Down syndrome or TEL/AML1), not during intrathecal therapy In case of an IKZF1 deletion, 200 mg/m^2/dose every three weeks from week 85 - 136
6-Mercaptopurine*	50 mg/m^2/day from week 1 – 12 in courses of 2 weeks with 1 week interruption (without interruption in case of Down syndrome or TEL/AML1) and from week 13 – 84 daily, without interruption In case of an IKZF1 deletion, 100 mg/m^2/day for 10 days after each methotrexate dose from week 85 - 136
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at week 1, 19, 37, 55 and 73

High risk blocks for HR patients	
HR block 1	
6-Mercaptopurine	25mg/m^2/day from days 1 - 14
Methotrexate	5,000 mg/m^2 at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Cyclophosphamide	1,200 mg/m^2/dose at day 15, 16 and 17
Etoposide	350 mg/m^2/dose at day 15, 16 and 17
PEGasparaginase	1,500 IU/m^2 at day 22
Vincristine	1.5 mg/m^2/dose at day 22 and 29
HR block 2	
Methotrexate	5,000 mg/m^2 at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Cytarabine	1,500 mg/m^2/dose at day 15, 16, 17, 18 and 19
Mitoxantrone	5.25 mg/m^2/dose at day 15, 16, 17, 18 and 19
PEGasparaginase	1,500 IU/m^2 at day 22
Vincristine	1.5 mg/m^2/dose at day 22 and 29
HR block 3	
Methotrexate	5,000 mg/m^2 at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Idarubicin	6 mg/m^2/dose at day 15, 16 and 17
Fludarabine	22.5 mg/m^2/dose at day 15, 16, 17, 18 and 19
Cytarabine	1,500 mg/m^2/dose at day 15, 16, 17, 18 and 19
HR block 4	Equal to HR block 1, but without 6-Mercaptopurine
HR block 5	Equal to HR block 2
HR block 6	Equal to HR block 3, but without Idarubicin
HR Protocol II	
Dexamethasone	10 mg/m^2/day at days 1 – 21 followed by 3x3 days tapering
Vincristine	1.5 mg/m^2/dose at day 8, 15, 22 and 29
Doxorubicin	30 mg/m^2/dose at day 8, 15, 22 and 29
PEGasparaginase	1,500 IU/m^2 at day 8
Cyclophosphamide	1,000 mg/m^2 at day 36
6-Thioguanine	60 mg/m^2/day at days 36 – 49
Cytarabine	75 mg/m^2/day at days 36 – 39 and 43 – 46
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 36 and 43
HR maintenance	
6-Mercaptopurine	50 mg/m^2/day from week 1 – 37
Methotrexate	20 mg/m^2/week from week 1 – 37

* Not if patients are eligible for a stem cell transplantation

Supplemental Table 2. Patient characteristics

	Index dataset n = 92			Validation dataset n = 28		
Sex						
Male	51 (55%)			18 (64%)		
Female	41 (45%)			10 (36%)		
Age, years (median, IQR)	4.8 (3.3 – 8.2)			7.7 (3.3 – 12.5)		
Weight, kg (median, IQR)*	19.2 (14.9 – 29.3)			28.0 (16.5 – 47.9)		
BSA, m^2 (median, IQR)*	0.76 (0.65 – 1.05)			1.04 (0.68 – 1.44)		
Type of ALL						
Pro-B cell	0			0		
Common B-cell	38 (41%)			14 (50%)		
Pre-B cell	11 (12%)			7 (25%)		
Common T-cell	5 (6%)			3 (11%)		
Unknown[%]	38 (41%)			4 (14%)		
Genetics of ALL						
TEL/AML1	19 (21%)			6 (21%)		
t(1;19)	0			1 (4%)		
MLL-rearrangements	0			0		
Hyperdiploid	15 (16%)			5 (18%)		
Other B cell	15 (16%)			9 (32%)		
Other T cell	5 (6%)			3 (11%)		
IKZF1-deletion	5 (6%)			0		
Unknown[%]	38 (41%)			4 (14%)		
Risk group						
Standard risk	13 (14%)			3 (11%)		
Medium risk (%)	76 (83%)			23 (82%		
Continuous	*27*			*4*		
Discontinuous	*49*			*20*		
High risk	2 (2%)			-		
Not stratified	1 (1%)			2 (7%)		
Asparaginase related toxicity	No	Yes	Unknown[%]	No	Yes	Unknown[%]
Allergy	90 (98%)	2 (2%)	0	29 (100%)	0	0
Silent inactivation	90 (98%)	2 (2%)	0	29 (100%)	0	0
Central neurotoxicity[#]	54 (58%)	1 (PRES)	38 (41%)	23 (79%)	1 (4%)	4 (14%)
Thrombosis[#]	54 (58%)	1 (1%)	38 (41%)	23 (79%)	1 (4%)	4 (14%)
Pancreatitis[#]	88 (96%)	4 (4%)	0	22 (75%)	2 (8%)	4 (14%)

Supplemental Table 2 continued

Number of infections[$]	19	12
Unknown, no. of patients (%)[%]	38 (41%)	4 (14%)
Number of ICU admissions	3	2
Unknown, no. of patients (%)[%]	38 (41%)	4 (14%)
Leukocytes, * 10^9/L (median, IQR)	2.4 (1.5 – 4.0)	2.4 (1.6 – 3.4)
Measurements missing (%)[%]	100 (8%)	20 (5%)
AST, U/L (median, IQR)	44 (30 – 65)	46 (33 – 66)
Measurements missing (%)[%]	364 (45%)	155 (38%)
ALT, U/L (median, IQR)	65 (40 – 95)	67 (45 – 97)
Measurements missing (%)[%]	364 (45%)	155 (38%)
Creatinine, µmol/L (median, IQR)	27 (22 – 33)	27 (21 – 38)
Measurements missing (%)[%]	508 (62%)	237 (59%)
Albumin, g/L (median, IQR)	33 (29 – 40)	32 (27 – 39)
Measurements missing (%)[%]	721 (88%)	370 (91%)
Native *E. coli* asp AB, OD (median, IQR)	0.018 (0.010 – 0.030)	0.008 (0.006 – 0.018)
Measurements missing (%)[%]	333 (41%)	228 (56%)
PEGasp AB, OD (median, IQR)	0.019 (0.010 – 0.034)	0.009 (0.006 – 0.017)
Measurements missing (%)[%]	333 (41%)	228 (56%)

IQR: interquartile range; BSA: body surface area; PRES: posterior reversible encephalopathy syndrome; ICU: intensive care unit; AB: antibodies; AST: aspartate transaminase ; ALT: alanine transaminase; OD: optical density; AB: antibodies asp: asparaginase; PEGasp: PEGasparaginase. Laboratory measurements were done during asparaginase activity level measurement.

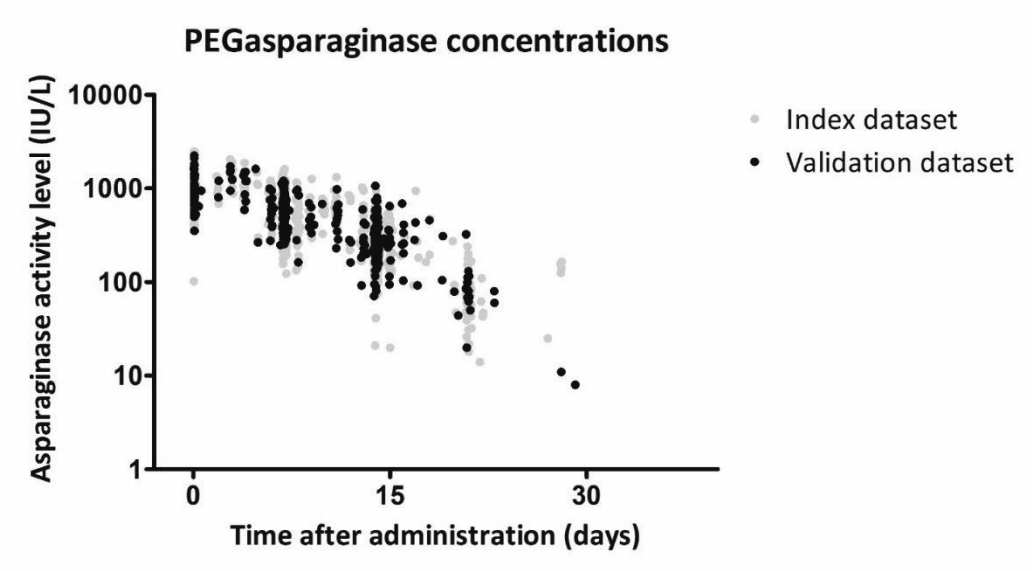
[%] Clinical data of the patients not treated in the Sophia Children's Hospital was missing.

* Weight and BSA measured at start PEGasparaginase therapy.

[#] Only Common Terminology Criteria for Adverse events 4.03 grade 3 and 4.

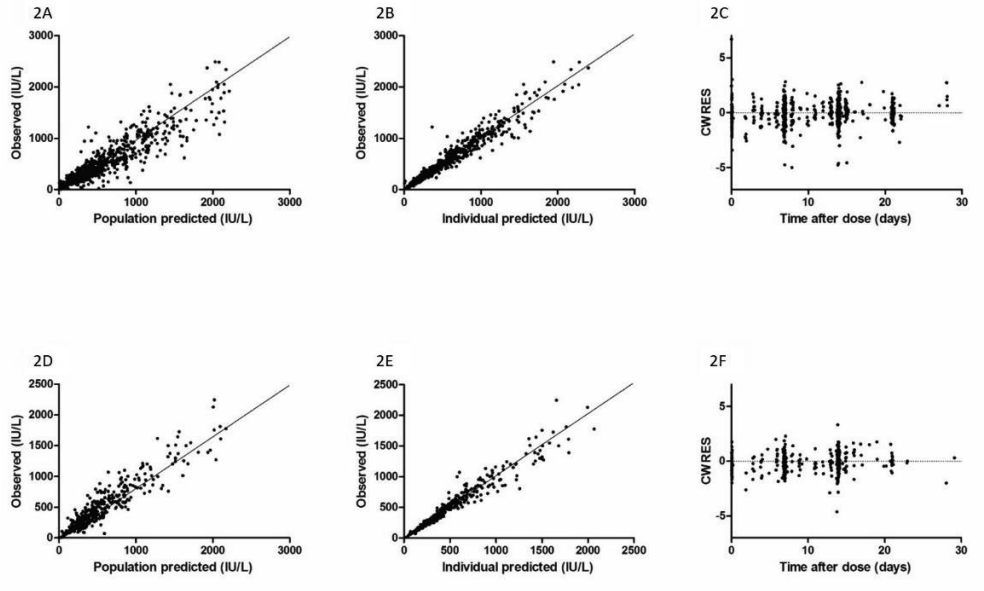
[$] Infections were defined as fever (>38° Celsius) and hospital admission or prescription of antibiotics.

Supplemental figure 1. PEGasparaginase activity levels



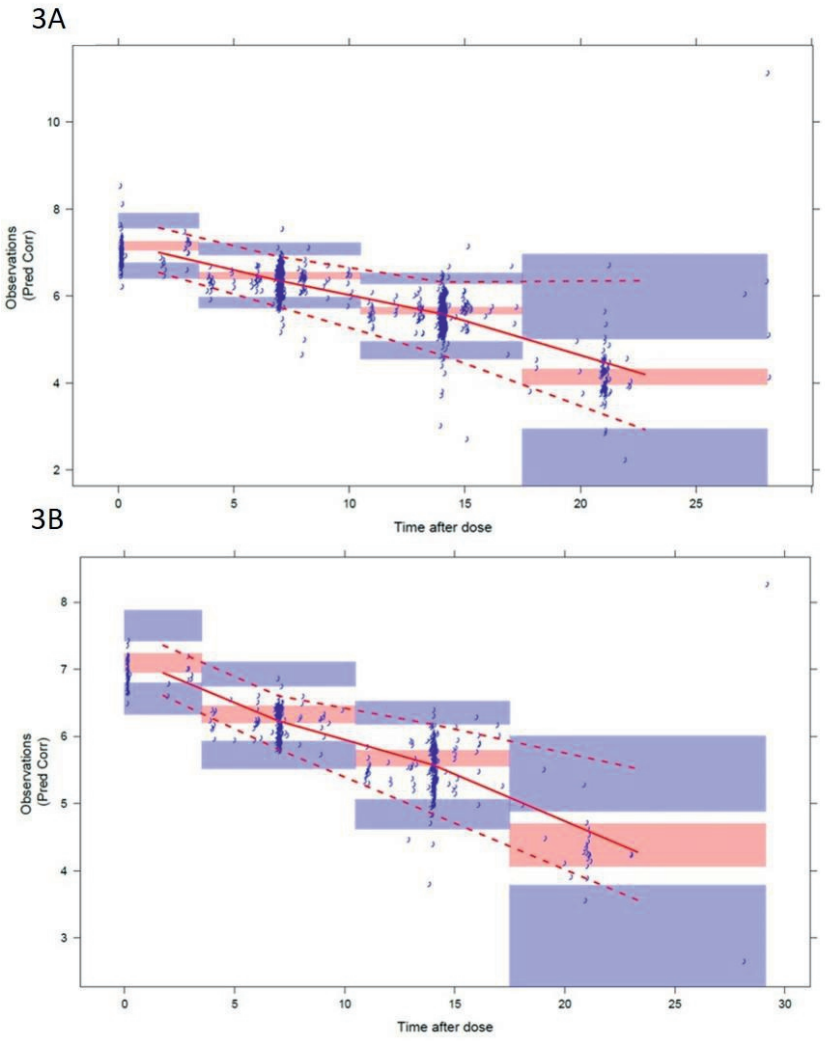
Supplemental Figure 1 shows the PEGasparaginase activity levels versus time after administration for the index database (●, n=92) and the validation database (●, n=28). Note that the asparaginase activity levels nonlinearly decline after 12.7 days. Of note, dose adjustments could (partially) explain the variation in asparaginase activity levels shown in this figure.

Supplemental figure 2. Goodness of fit plots



Supplemental Figure 2A and 2D show the observed asparaginase activity levels plotted against the population predicted values for the main and external database, respectively. In these figures, the dots are evenly distributed around the line of unity.
Figure 2B and 2E show the observed values plotted against the individual predicted values. Also in this figure, the dots are evenly distributed around the line of unity.
Figure 2C and 2F show the conditional weighted residuals (CWRES) plotted against the time after dose. Here, most dots are between -2 and 2, and show no trend.

Supplemental figure 3. Visual predictive check



Supplemental Figure 3A and 3B show the visual predictive checks of the index and validation dataset, respectively. The observations and their corresponding median and 95% confidence intervals are indicated by the points, and the solid and dashed red lines. In both graphs, these lines fall within the 95% confidence intervals of the median and 95% confidence intervals (red and blue shaded areas) as obtained by simulation.

REFERENCES

1. Wurthwein, G., C. Lanvers-Kaminsky, G. Hempel, S. Gastine, A. Moricke, M. Schrappe, et al. Population Pharmacokinetics to Model the Time-Varying Clearance of the PEGylated Asparaginase Oncaspar(R) in Children with Acute Lymphoblastic Leukemia. Eur J Drug Metab Pharmacokinet. 2017.
2. Beal, S. L. Ways to fit a PK model with some data below the quantification limit. J Pharmacokinet Pharmacodyn. 2001;28(5):481-504.

Part II

CHAPTER 4

4

Allergic-like Reactions to Asparaginase: Atypical Allergies Without Asparaginase Inactivation

Robin Q.H. Kloos, Rob Pieters, Gabriele Escherich, Inge M. van der Sluis

Pediatric Blood & Cancer 2016; 63: 1928-34

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.

Methods 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.

Conclusion 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 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 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 the 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 still 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 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 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 IU/m^2 native *E. coli* asparaginase in induction and 15 PEGasparaginase doses (2,500 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/m^2 *Erwinia* asparaginase, administered 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 was hydrolyzed by asparaginase to L-aspartic acid and hydroxylamine. Hydroxylamine (20 µL) was diluted with 8-hydroxiquinoline 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 analyzed with the software package IBM SPSS Statistics (IBM Corp, Armonk, New York, USA) version 21.0 for Windows. The time of occurrence of the reactions after the start of infusion and the presence of anti-asparaginase antibodies were analyzed by non-parametric tests. Symptoms were analyzed 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 1). 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 2).

Table 1. Allergic-like reactions

Asp type	Treatment protocol, phase and dose number	Grade and symptoms	Time after start infusion	Trough level (IU/L)*	Antibodies[$]	Action taken
1 PEGasp	COALL-08-09 Reinduction, 2nd	Grade 1: rash, itchiness	60 min	137~	Unknown	PEGasp continued, finished without other reactions.
2 PEGasp	COALL-08-09 Intensification, 1st	Grade 1: rash, urticaria, fever	24 hours	1,657** 749~	Unknown	PEGasp continued, finished without other reactions.
3 PEGasp	ALL-11 Intensification, 1st	Grade 2: rash, edema, dyspnea	20 min	83**	Negative	Switch to Erw, successfully# finished.
4 PEGasp	ALL-11 Induction, 4th	Grade 2: vomiting, pale	45 min	561** 122	Negative	PEGasp continued and successfully# finished.
5 PEGasp	ALL-11 Intensification, 1st	-	27 min	493**	Negative	Switch to Erw.
Erw	Intensification, 6th	Grade 2: rash, vomiting	12 min	294(T48)	Negative	Erw stopped.
6 Erw	ALL-11 Intensification, 14th	Grade 2: vomiting	53 min	32 (T48)	Negative	Erw stopped.
7 Erw	ALL-11 Intensification, 12th	1. Grade 2: nausea	30 min	157 (T48)	Positive	1. Erw continued with clemastine and reduced
	Intensification, 14th	2. Grade 2: dyspnea	5 min	89 (T72)	Positive	infusion rate. 2. Dose stopped but
	Intensification, 20th	3. Grade 2: nausea, pale	15 min	37 (T72)	Positive	restarted and finished. 3. Dose postponed, Erw successfully# finished.
8 Erw	ALL-11 Intensification, 17th	Grade 2: rash, dyspnea	38 min	146 (T48)	Negative	Erw was continued but not finished because of an allergy.
9 Erw	ALL-11 Intensification, 55th	1. Grade 1: rash	5 min	134 (T48)	Negative	1. Erw continued with HCT and reduced
	Intensification, 58th	2. Grade 1: rash	30 min	139 (T48)	Negative	infusion rate. 2.Dose stopped but
	Intensification, 59th	3. Grade 2: vomiting, edema	10 min	145 (T48)	Negative	restarted and finished. 3. Erw stopped.

Asp, asparaginase; PEGasp, PEGasparaginase; Erw, *Erwinia* asparaginase; min, minutes; T48/72, 48 or 72 hours after previous dose; HCT, hydrocortisone.

* Trough level just before reaction occurred. ** Week level after reaction occurred. ~ Trough level after reaction occurred. # Successfully: with adequate (>100 IU/L) asparaginase activity levels. [$] In case of a reaction to PEGasparaginase, antibodies against PEGasparaginase and native *E. coli* asparaginase.

Table 2. Allergic reactions

Asp type	Treatment protocol, phase and dose number	Grade and symptoms	Time after start infusion	Trough level (IU/L)*	Antibodies[$]	Action taken
10 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash, edema, vomiting	5 min	0	Positive	Switch to Erw, reaction at dose 11, stop Erw.
11 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash, edema, hypotension	10 min	0 0~	Positive	PEGasp continued at first but successfully# switched to Erw after a second reaction.
12 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash, edema	20 min	0 0~	Positive	PEGasp continued at first but successfully# switched to Erw after a second reaction.
13 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash, edema	3 min	0	Positive	Switch to Erw, successfully# finished.
14 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash, edema, vomiting	2 min	0	Positive	Switch to Erw, successfully# finished.
15 PEGasp	ALL10 Intensification, 2nd	Grade 2: Rash, edema, coughing	1 min	0	Positive	Switch to Erw, successfully# finished.
16 PEGasp	ALL10 Intensification, 2nd	Grade 2: Rash, edema, dyspnea	1 min	0	Positive	Switch to Erw, successfully# finished.
17 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash, coughing	1 min	0	Positive	Switch to Erw, successfully# finished.
18 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash	1 min	0	Positive	Switch to Erw, successfully# finished.
19 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash, dyspnea	1 min	0	Positive	Switch to Erw, successfully# finished.
20 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash, coughing	20 min	0	Positive	Switch to Erw, successfully# finished.
21 PEGasp	ALL10 Intensification, 2nd	Grade 2: dyspnea, nauseous	1 min	0	Positive	Switch to Erw, successfully# finished.
22 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash, edema, nauseous	1 min	0	Positive	Switch to Erw, successfully# finished.
23 PEGasp	ALL10 Intensification, 2nd	Grade 2: edema, coughing	5 min	0	Unknown	Switch to Erw, successfully# finished.
24 PEGasp	ALL10 Intensification, 2nd	Grade 2: rash, vomiting	5 min	0	Unknown	Switch to Erw, successfully# finished.

Asp, asparaginase; PEGasp, PEGasparaginase; Erw, *Erwinia* asparaginase; min, minutes.

* Trough level just before reaction occurred. ~ Trough level after the reaction. # Successfully: with adequate asparaginase activity levels (>100 IU/L). [$] Antibodies against PEGasparaginase and native *E. coli* asparaginase

Asparaginase activity levels

The asparaginase activity levels are described in Table 1. Nine levels were obtained just prior to the allergy-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/m^2), 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 2). 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 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 ($p=0.001$).

Clinical symptoms

The clinical characteristics are described in Table 1 for the allergic-like reactions, in Table 2 for the real allergies and summarized in Table 3.

Table 3. Summary of clinical characteristics

	Allergic-like reactions	Allergic reactions	P-value
Symptoms			
Rash/oedema/itchiness/urticaria	9/13 reactions (69%)	14/15 reactions (93%)	0.153
Pulmonary symptoms	3/13 reactions (23%)	6/15 reactions (40%)	0.435
Gastro-intestinal symptoms	6/13 reactions (46%)	5/15 reactions (33%)	0.700
Timing of allergic reaction (median, IQR)	29 (12 – 47)	2 (1 – 5)	<0.001
Anti-asparaginase antibodies present	1/7 patients (14%)	13/13 patients (100%)	0.001

IQR: interquartile range.

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, edema, itchiness or urticaria, b) pulmonary symptoms and c) gastro-intestinal symptoms, 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 minutes, interquartile range (IQR): 12-47 minutes) than real allergic reactions (median 2 minutes, IQR: 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 (IQR: 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.[9, 13, 8] 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.[14, 13, 8] 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, although 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 edema, dyspnea 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. Distinction, however, 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.

REFERENCES

1. Amylon, M. D., J. Shuster, J. Pullen, C. Berard, M. P. Link, M. Wharam, 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. Rizzari, C., M. G. Valsecchi, M. Arico, V. Conter, A. Testi, E. Barisone, 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.
3. Silverman, L. B., R. D. Gelber, V. K. Dalton, B. L. Asselin, R. D. Barr, L. A. Clavell, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97(5):1211-1218.
4. Duval, M., S. Suciu, A. Ferster, X. Rialland, B. Nelken, P. Lutz, 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.
5. Pession, A., M. G. Valsecchi, G. Masera, W. A. Kamps, E. Magyarosy, C. Rizzari, 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.
6. Moghrabi, A., D. E. Levy, B. Asselin, R. Barr, L. Clavell, C. Hurwitz, 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.
7. Sirvent, N., S. Suciu, X. Rialland, F. Millot, Y. Benoit, D. Plantaz, 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, W. H., R. Pieters, G. J. Kaspers, D. M. te Loo, M. B. Bierings, C. van den Bos, 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, E. H., N. L. Seibel, S. Martin-Aragon, P. S. Gaynon, I. A. Avramis, H. Sather, 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., S. P. Hunger, J. Boos, C. Rizzari, L. Silverman, A. Baruchel, et al. L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. Cancer. 2011;117(2):238-249.
11. Vrooman, L. M., K. E. Stevenson, J. G. Supko, J. O'Brien, S. E. Dahlberg, B. L. Asselin, 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., J. P. Vieira Pinheiro, G. Hempel, G. Wuerthwein and J. Boos. 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., J. D. Kawedia, C. Cheng, D. Pei, C. A. Fernandez, X. Cai, et al. Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia. Leukemia. 2012;26(11):2303-2309.
14. Killander, D., A. Dohlwitz, L. Engstedt, S. Franzen, G. Gahrton, B. Gullbring, 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, W. H., R. Pieters, H. A. de Groot-Kruseman, W. C. Hop, J. Boos, W. J. Tissing, et al. Toxicity of very prolonged PEGasparaginase and Erwiniaasparaginase courses in relation to asparaginase activity levels with a special focus on dyslipidemia. Haematologica. 2014.

CHAPTER 5

5

Acute Lymphoblastic Leukemia Patients Treated with PEGasparaginase Develop Antibodies to PEG and the Succinate Linker

Robin Q.H. Kloos, Inge M. van der Sluis, Enrico Mastrobattista
Wim E. Hennink, Rob Pieters, Jan-Jaap F. Verhoef

British Journal of Haematology, 2019. doi: 10.1111/bjh.16254

ABSTRACT

Background Polyethylene glycol (PEG) conjugated asparaginase (PEGasparaginase) is essential for treatment of pediatric acute lymphoblastic leukemia. We developed an assay identifying antibodies against the PEG-moiety, the linker and the drug itself in patients experiencing hypersensitivity reactions to PEGasparaginase.

Methods Eighteen patients treated according to the DCOG ALL-11 protocol, with a neutralizing hypersensitivity reaction to PEGasparaginase to the first PEGasparaginase doses in induction (12 patients) or during intensification after interruption of several months (6 patients) were included. ELISA was used to measure antibodies, coating with the succinimidyl succinate linker conjugated to BSA, PEGfilgrastim and *E. coli* asparaginase, and using hydrolyzed PEGasparaginase and $mPEG_{5.000}$ for competition.

Results Anti-PEG antibodies were detected in all patients (IgG 100%; IgM 67%) of whom 39% had anti-PEG antibodies exclusively. Pre-existing anti-PEG antibodies were also detected in patients who not previously received a PEGylated therapeutic (58% IgG; 21% IgM). Antibodies against the SS-linker were predominantly detected during induction (50% IgG; 42% IgM). Anti-asparaginase antibodies were detected in only 11% during induction but 94% during intensification.

Conclusion Anti-PEG and anti-SS-linker antibodies predominantly play a role in the immunogenic response to PEGasparaginase during induction. Thus, switching to native *E. coli* asparaginase would be an option for adequate asparaginase treatment.

INTRODUCTION

Asparaginase treatment is essential for childhood acute lymphoblastic leukemia (ALL) treatment. The drug depletes extracellular asparagine, an essential amino acid for leukemic cells, selectively killing these cells.[1] Because asparaginase is derived from bacteria, patients can develop antibodies to the non-human epitopes, neutralizing the drug completely. Neutralizing reactions can present with or without symptoms of an allergy, the latter being called silent inactivation.[2-5] These reactions mainly occur after an interruption of asparaginase treatment, during which anti-asparaginase antibody levels increase.[5] Three forms of asparaginase are clinically available which are derived from either *Escherichia coli* or *Erwinia chrysanthemi*. By conjugating native *E. coli* asparaginase with polyethylene glycol (PEG), the drug is less immunogenic.[6-8] Therefore, PEGylated *E.coli* asparaginase (PEGasparaginase) is used for the treatment of pediatric ALL.[9-14] In case of a hypersensitivity reaction, patients have to switch from PEGasparaginase to asparaginase derived from *Erwinia chrysanthemi* bacteria for adequate treatment.[15]

PEGasparaginase consists of *E.coli* derived asparaginase, a 32kDa homotetramer to which 5-10 $mPEG_{5,000}$ chains are conjugated using a succinimidyl succinate linker (SS-linker) on the ε-amino groups of lysine residues of the protein. Although PEGylation reduces the immunogenicity of asparaginase, neutralizing hypersensitivity reactions still occur.[5] Surprisingly, these reactions seem to shift to the first PEGasparaginase doses in induction and not necessarily during the intensification course after an interruption of treatment, as seen in our patient cohort. This leads to the question whether antibodies to the PEG moiety or even the SS-linker may cause these reactions.

Although PEGylation decreases the immunogenicity of biotherapeutics, it has been reported that repeated administration of PEGylated therapeutics can induce anti-PEG antibodies associated with hypersensitivity reactions and rapid clearance.[16, 17] Anti-PEG antibodies have been reported in patients treated with PEG conjugated uricase and PEGinesatide, erythropoietin that is covalently attached to PEG and withdrawn from the market after severe hypersensitivity reactions.[18] Also in ALL-patients treated with PEGasparaginase, the formation of anti-PEG antibodies has been associated with rapid clearance of the drug.[16]

We hypothesize that reactions to PEGasparaginase may be partly triggered by anti-PEG antibodies. Secondly, we hypothesize that the SS-linker can expose a neo-antigen to which antibodies can be formed. The linker contains an ester group

which has limited stability at neutral pH in vitro and is subject to hydrolysis by endogenous esterases in vivo.[19] The exposed succinate group may function as an hapten, enhancing immunogenicity.[20 19]

Because of the lack of proper developed anti-PEG antibody assays, especially lacking proper controls, the aim of this study was to develop a sensitive and specific assay to detect possible antibodies to both PEG and the SS-linker. For this we identified patients who had a neutralizing allergy to or silent inactivation of PEGasparaginase during the first administrations (induction phase) or after an interruption of PEGasparaginase treatment (intensification phase).

METHODS

Patients and treatment protocol

Eighteen children with ALL treated according to the Dutch Childhood Oncology Group (DCOG) ALL-11 protocol between April 2012 and December 2016, who had developed a neutralizing hypersensitivity reaction (silent inactivation or allergy) to PEGasparaginase, were selected for the development of the assay. The DCOG ALL-11 protocol (Dutch Trial Register: NTR3379), including use of patient material, was approved by the Institutional Review Board and informed consent was obtained from patients >12 years old, parents or guardians in accordance with the declaration of Helsinki.

According to the DCOG ALL-11 protocol, all patients started with the induction phase containing prednisolone, vincristine daunorubicin, and PEGasparaginase (1,500 IU/m^2 i.v. administered at day 12, 26 and 40). After induction, patients were stratified in a standard, medium or high risk group. In this study, all patients with a hypersensitivity reaction after induction were treated according to the medium risk group. After induction, two consolidation courses were given leading to an asparaginase-free interval of approximately 12 weeks. Thereafter, medium risk patients were treated with 14 PEGasparaginase doses, individualized based on asparaginase activity levels, in the intensification phase. If a hypersensitivity reaction occurred, patients were switched to *Erwinia* asparaginase.

To study the reactions specifically observed during the first PEGasparaginase doses, 12 patients with a neutralizing hypersensitivity reaction during the induction phase and 6 patients with a reaction during the intensification phase were selected (Table 1). The antibodies were quantified in serum obtained within two weeks after the reaction. Allergies were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Asparaginase activity

Whole blood was taken prior to each PEGasparaginase administration and centrifuged at 800x*g* for 10 minutes at room temperature (RT). Samples were stored at -20°C. PEGasparaginase activity levels were measured after thawing using the L-aspartic β-hydroxamate (AHA) assay as described earlier.[21] Inactivation of PEGasparaginase is defined as PEGasparaginase activity level <100 IU/L at day 7±1 after administration and/or a trough PEGasparaginase activity level (14±1 days after a dose) below the limit of detection (<10 IU/L).

Size-exclusion chromatography of PEGasparaginase

The hydrolysis of $mPEG_{5.000}$ from PEGasparaginase (Oncaspar®, Shire, Amsterdam, the Netherlands) was investigated by size-exclusion chromatography. PEGasparaginase and native *E.coli* asparaginase (Paronal®, Medac, Wedel, Germany) were diluted to 100 IU/mL in 0.1M sodium bicarbonate buffer pH9.5 (Sigma Aldrich Zwijndrecht, the Netherlands) or PBS pH 7.4 (Fisher BioReagents, Landsmeer, the Netherlands) at RT for 24h to investigate the dissociation at different pH. Size-exclusion chromatography was performed on a Waters 2695-Separations Module connected to a Waters 2414-Refractive Index Detector and a Waters 2487 Dual λ-Absorbance Detector (Waters Corporation, Milford, MA) to which a PL-Aquagel-OH mixed 8µm column (Agilent Technologies, Santa Clara, United States) was attached. Samples were incubated in one of the above buffers, and, over a 30 hour time period at RT, injected at 1mL/minute flowrate. UV-absorbance was recorded at 280nm.

Synthesis of the PEG BSA conjugate

BSA (Sigma Aldrich, Zwijndrecht, the Netherlands) was conjugated to succinic anhydride (BSA-SS) (Sigma-Aldrich, Zwijndrecht, the Netherlands) or n-ethyl

maleimide (BSA-MAL) (Sigma-Aldrich, Zwijndrecht, the Netherlands) at a molar ratio of 1:10 in 0.1M sodium carbonate pH8.5, to model the exposed linker upon PEG hydrolysis. The pH was kept constant by adding 0.1M sodium hydroxide to the reaction. Upon conjugation, both solutions were dialyzed against 0.1M sodium carbonate pH8.5 and subsequently against PBS at 4°C. Protein concentration was determined by the Pierce™ BCA Protein Assay (Thermo Fisher Scientific, Breda, the Netherlands). Samples were stored at -20°C.

Anti-drug antibody determination

To investigate the specificity of antibodies against asparaginase, PEG and the SS-linker, an enzyme-linked immunosorbent assay (ELISA) was developed coating medium binding Costar® 96-well ELISA plates (Corning, Amsterdam, the Netherlands) with 0.4 IU/L PEGasparaginase, 1.4 IU/L native *E.coli* asparaginase, or 1 µg/mL of PEGfilgrastim (Neulasta, Amgen), filgrastim (Neupogen, Amgen), BSA or BSA-SS in PBS overnight. PEGfilgrastim, a 19kDa protein to which a 20kDa PEG is coupled by aldehyde chemistry using selective N-terminal amine conjugation, was used to investigate anti-PEG antibodies. Patient sera were screened for anti-filgrastim and anti-BSA antibodies to rule out false positive results. Plates were blocked with 2-5% BSA dissolved in PBS for a minimum of 2 hours at room temperature. Patient sera were incubated for 2 hours in block buffer. Plates were washed 5 times with 0.05% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate CHAPS (Merck Chemicals, Amsterdam, the Netherlands) in PBS. IgG and IgM were detected by rabbit polyclonal anti-IgG or anti-IgM (Abcam, Cambridge, UK).

Patients were positive for antibodies if binding exceeded the cut-off point calculated by absorbance value of sera obtained from patients before their first PEGasparaginase administration who did not experience a hypersensitivity reaction (n=26) as well as sera obtained from healthy donors (n=11) (Mini Donor Dienst, UMCU, the Netherlands). The cut-off point for PEGfilgrastim and PEGasparaginase was determined in the presence of 0.1% $mPEG_{5,000}$ to exclude binding of possible pre-existing anti-PEG antibodies.

Specificity of antibodies was determined by competition with different concentrations of *E.coli* asparaginase, $mPEG_{5.000}$, or hydrolyzed PEGasparaginase. For this, PEGasparaginase was diluted to 375 IU/mL in a 0.1M sodium carbonate buffer pH 9.8 and stored for 20 hours at 37°C. Samples were concentrated by

centrifugation in Vivaspin®20 tubes (GE-Healthcare, the Netherlands) and diluted in PBS. The pH was adjusted to pH7.4 by dropwise addition of 1M HCl.

To exclude any nonspecific competition of $mPEG_{5.000}$, its competitive properties were investigated in a varicella zoster virus (VZV) ELISA, as all patients are expected to be positive for VZV-antibodies. A polyclonal TransChromo bovine anti-PEG IgG antibody (Bristol-Myer Squibb, USA) was provided by Bristol-Myers Squibb to serve as a positive control.

Statistics

SPSS Statistics (IBM Corp, Armonk, New York, USA) version 21.0 and GraphPad Prism (GraphPad Software, Inc, La Jolla, USA) version 5.01 for Windows were used for statistical analyses. Cut-offs are defined as the mean plus one standard deviation. To compare the titers of different antibodies, relative titers were calculated by dividing the patient titer by the corresponding minimal required dilution. Relative titers were plotted with boxplots, with the 25^{th}, 50^{th} and 75^{th} percentiles in the boxes and the ranges indicated by whiskers. Dose response curves for the competition analyses were fitted using nonlinear regression.

RESULTS

Table 1 shows the 18 patients included as described in the Methods section. Of those, nine patients had silent inactivation of PEGasparaginase (6 patients during induction, 3 during intensification); the other patients had an allergy. Asparaginase activity levels measured after this allergic reaction were below the limit of quantification in all patients. Two patients had an allergic reaction against the first dose. Thus, only a small amount of PEGasparaginase was administered.

Antibodies against asparaginase and PEG

Presence of anti-PEGasparaginase antibodies, which could be directed to any epitope in the molecule, was investigated (Figure 1 and Table 1). Anti-PEGasparaginase IgG was detected in 92% (11/12) and 100% of the patients in respectively the induction and intensification phase; anti-PEGasparaginase IgM in 67% (8/12) and 83% (5/6). Antibody titers were higher for IgG than for IgM.

Table 1 Overview reactions and relative antibody titers

Patient number	Type of reaction (grade)	Treatment phase	Dose number	IgG				IgM			
				Dilution PEGasp	Dilution Native *E.coli* asp	Dilution PEG-filgrastim	Dilution BSA-SS	Dilution PEGasp	Dilution Native *E.coli* asp	Dilution PEG-filgrastim	Dilution BSA-SS
1	Allergy (2)	Induction	3rd	16x	*Negative*	16x	2x	2x	*Negative*	1x	2x
2	Allergy (2)	Induction	3rd	128x	*Negative*	512x	1x	4x	*Negative*	4x	1x
3	Allergy (3)	Induction	1st	32x	*Negative*	128x	4x	4x	*Negative*	2x	4x
4	Allergy (2)	Induction	3rd	2x	*Negative*	8x	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
5	Allergy (3)	Induction	3rd	256x	*Negative*	1024x	2x	128x	*Negative*	128x	1x
6	Allergy (2)	Induction	1st	4x	1x	32x	2x	*Negative*	*Negative*	1x	2x
7	SI	Induction	3rd	64x	2x	512x	1x	2x	*Negative*	1x	*Negative*
8	SI	Induction	1st	*Negative*	*Negative*	8x	*Negative*	*Negative*	*Negative*	1x	*Negative*
9	SI	Induction	2nd	8x	*Negative*	8x	*Negative*	1x	*Negative*	*Negative*	*Negative*
10	SI	Induction	2nd	4x	*Negative*	8x	*Negative*	1x	*Negative*	1x	*Negative*
11	SI	Induction	3rd	16x	*Negative*	64x	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
12	SI	Induction	2nd	4x	*Negative*	16x	*Negative*	1x	*Negative*	1x	*Negative*
13	Allergy (3)	Intens.	2nd	256x	8x	512x	*Negative*	2x	*Negative*	2x	*Negative*
14	Allergy (2)	Intens.	2nd	256x	64x	1024x	1x	16x	1x	32x	1x
15	Allergy (2)	Intens.	2nd	64x	4x	512x	*Negative*	1x	*Negative*	2x	*Negative*
16	SI	Intens.	1st	1x	*Negative*	8x	*Negative*	*Negative*	*Negative*	*Negative*	*Negative*
17	SI	Intens.	1st	16x	2x	32x	*Negative*	8x	*Negative*	*Negative*	*Negative*
18	SI	Intens.	1st	32x	8x	64x	*Negative*	64x	4x	*Negative*	*Negative*

The minimal required dilutions to avoid background signal: Anti-PEGasparaginase IgG 1,000x; IgM 2,000x; native *E. coli* asparaginase IgG 1,000x; IgM 2,000x; PEG-filgrastim IgG 75x, IgM 75x; BSA-SS IgG 75x; IgM 75x. As these dilutions were different for the different antibodies, relative titers were calculated to compare the antibody titers. For this, the absolute titers measured were divided by the corresponding minimal required dilutions. For example, a relative titer of 16x means that the titer of that sample was 16 times higher than the negative samples.
PEGasp: PEGasparaginase; asp: asparaginase; BSA-SS: bovine serum albumine - succinimidyl succinate; SI: silent inactivation; Intens.: intensification

Figure 1. Relative titers

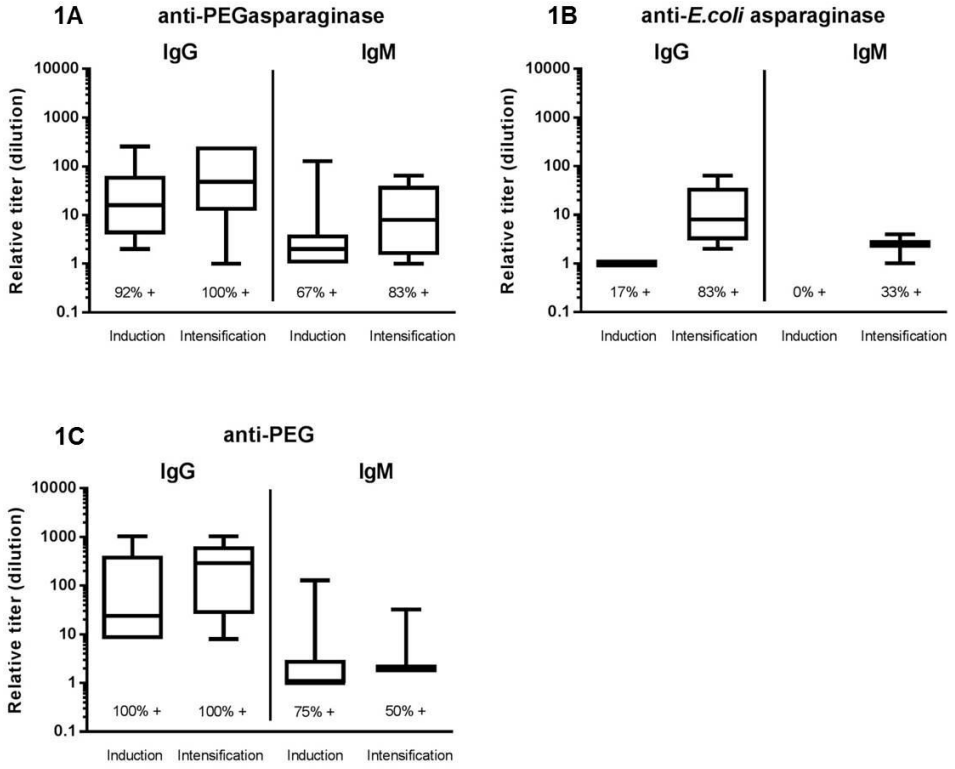


Figure 1 shows the titers relative to the minimal required dilution per epitope for IgG and IgM in patients with a hypersensitivity reaction during the induction and intensification. Each graph also show the percentage of patients positive for the antibodies during induction (n=12) and intensification (n=6). The relative titers were obtained by dividing the titers measured by the corresponding minimal required dilutions.

Figure 1A shows the relative titers of anti-PEGasparaginase antibodies.

Figure 1B shows the relative titers of anti-native *E. coli* asparaginase antibodies.

Figure 1C shows the relative titers of anti-PEG antibodies.

Minimal required dilutions (MRD): Anti-PEGasparaginase IgG 1,000x; IgM 2,000x; native *E. coli* asparaginase IgG 1,000x; IgM 2,000x; PEG-filgrastim IgG 75x, IgM 75x.

Specificity against asparaginase or PEG was investigated by coating plates with native *E.coli* asparaginase or PEGfilgrastim (Table 2). In patients with a hypersensitivity reaction during induction, anti-asparaginase IgG was detected in 17% (2/12) and IgM in none of the patients. In contrast, these antibodies were detected in 83% (5/6) and 33% (2/6) of the patients with a reaction during intensification (Figure 1B and Table 1-2). All patients were positive for anti-PEG IgG antibodies, whereas 75% (9/12) and 50% (3/6) of patients were positive for anti-PEG IgM in the induction and intensification phase, respectively (Figure 1C and Table 1-2).

Table 2. Antibodies against asparaginase, PEG and the SS-linker in patients with a reaction to PEGasparaginase during induction or intensification

Type of antibodies	Induction n=12		Intensification n=6	
	IgG	IgM	IgG	IgM
Anti-PEGasparaginase antibodies	92%	67%	100%	83%
Anti-Asparaginase antibodies	17%	0%	83%	33%
Anti-PEG antibodies	100%	75%	100%	50%
Anti-SS-linker antibodies	50%	42%	17%	17%

All patients were negative for anti-filgrastim IgG and IgM. Anti-PEG titers were around 100x higher for anti-PEG IgG than for anti-PEG IgM. Patients with undetectable PEGasparaginase levels in combination with an allergic reaction had higher anti-PEG IgG titers than patients with silent inactivation. Although the anti-PEG titers were lower in the nine patients with silent inactivation, 6 out of these 9 patients were exclusively positive for anti-PEG antibodies (Table 1). Specificity towards PEG was confirmed by $mPEG_{5,000}$ competition and validated by a polyclonal anti-PEG IgG antibody (Figure 2A).[22] Specificity of $mPEG_{5,000}$ to compete solely with anti-PEG antibodies was confirmed by its non-competitive properties to anti-VZV antibodies (Figure 2D).

Pre-existing anti-PEG IgG antibodies were detected in 58% (14/24) and anti-PEG IgM in 21% (5/24) patients (Figure 2C). These patients did not experience any hypersensitivity reaction to or neutralization of PEGasparaginase during asparaginase treatment.

Figure 2. PEG specificity

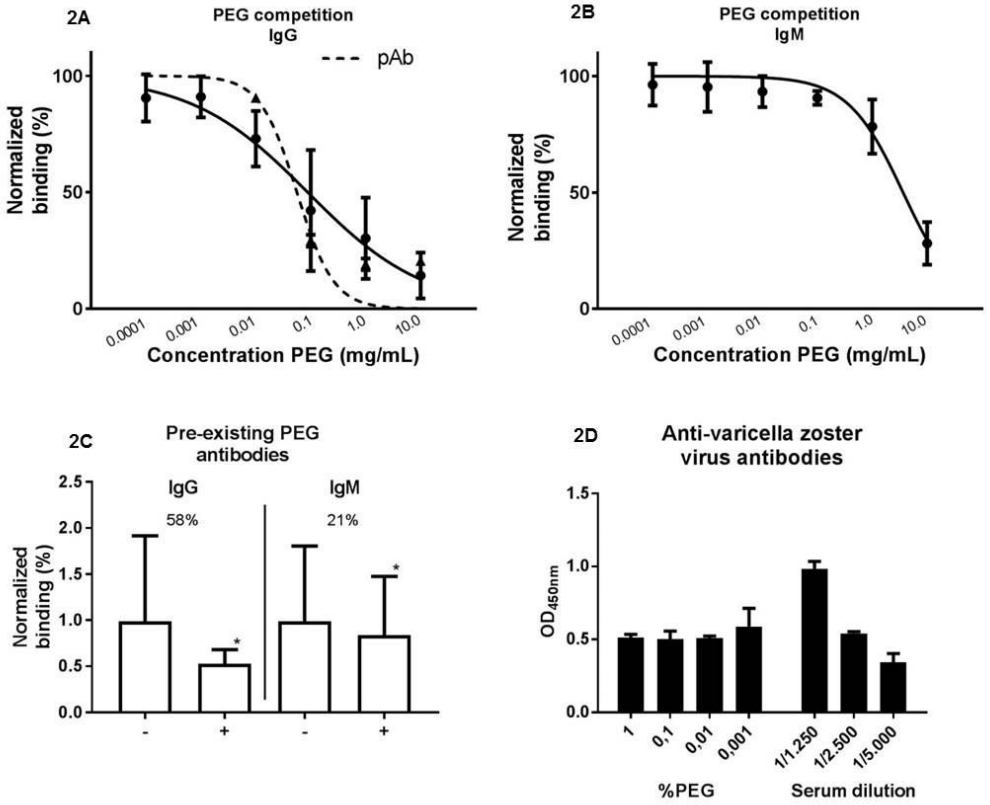


Figure 2 shows the specificity of the anti-PEG antibodies in normalized dose-response curves. Gradually added $mPEG_{5,000}$ concentrations competed with the PEGfilgrastim, decreasing the extinction relative to the extinction without competition (%).

Figure 2A shows the normalized dose-response curve for anti-PEG IgG with PEG-competition (n=5) and the polyclonal anti-PEG IgG (pAb) as a positive control.

Figure 2B shows the percentage of signal remaining after PEG competition for IgM (n=6), which has an approximately 100x lower affinity for $mPEG_{5k}$ than IgG.

Figure 2C shows normalized anti-PEG binding of patient samples obtained prior to the first PEGasparaginase dose, who did not have any symptoms of hypersensitivity or increased clearance of PEGasparaginase during treatment, in (+) or without (-) the presence of 0.1% $mPEG_{5,000}$.

Figure 2D shows binding of healthy donor serum to varicella zoster virus antigen in the presence of different $mPEG_{5,000}$ concentrations. Also dilutions of sera are shown to indicate the doses-response relation in this assay.

Antibodies against the SS-linker

We found that PEG is rapidly hydrolyzed from PEGasparaginase when incubated in sodium bicarbonate pH9.5 (Figure 3A). Specificity of anti-PEGasparaginase antibodies towards the SS-linker was determined by coating plates with BSA-SS. Anti-BSA-SS IgG was detected in 50% (6/12) and 17% (1/6) during the induction and intensification phase; anti-linker IgM in 42% (5/12) and 17% (1/6) (Figure 3B and Table 1-2). No antibodies were found against unmodified BSA or the unrelated BSA-MAL linker (Figure 3C). Competition by hydrolyzed PEGasparaginase inhibited IgG and IgM binding towards BSA-SS in a dose-dependent manner (Figure 3D).

Figure 3. Anti-SS-linker antibodies

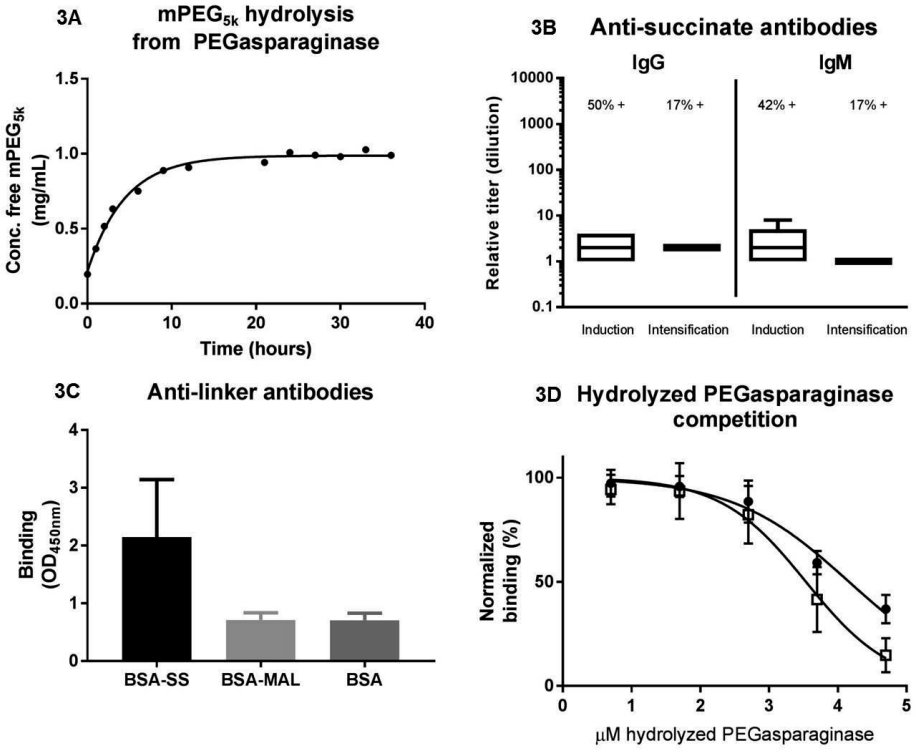


Figure 3 shows the hydrolysis of PEGasparaginase and the detection of anti-succinate linker antibodies.

Figure 3A shows the hydrolysis of $mPEG_{5,000}$ from PEGasparaginase at pH 9.5 and room temperature. After approximately 12 hours, the concentration free $mPEG_{5k}$ stabilizes, showing complete hydrolysis of $mPEG_{5,000}$.

Figure 3B shows the relative titers to the SS-linker conjugated to BSA. The relative titers were obtained by dividing the titers measured by the minimal required dilutions (75x for both IgG and IgM). Percentages indicate fraction of patients positive for anti-succinate antibodies.

In Figure 3C, patients were screened for the n-ethyl maleimide group conjugated to BSA (random linker) and BSA. This figures shows that the antibodies were specific for the succinate group.

Figure 3D shows IgG (●) and IgM (□) normalized dose-response curve of BSA-SS coated plates in presence of different concentrations of hydrolyzed PEGasparaginase.

DISCUSSION

In this study we assessed the specificity of anti-drug antibodies in pediatric ALL patients treated with PEGasparaginase who had a neutralizing hypersensitivity reaction (silent inactivation or allergy) to the drug during the induction or intensification phase.[5, 23] The patients were selected, though randomly, and the conclusions stated below should be confirmed by larger patient samples.

Patients with a hypersensitivity reaction to PEGasparaginase developed IgG and IgM antibodies towards asparaginase, the PEG chain and the linker. The incidence of anti-*E.coli* asparaginase antibodies in our sample was lower during induction (IgG 17% (2/12); IgM 0%) than intensification (IgG: 83% (5/6); IgM: 33% (2/6)), indicating that asparaginase antibodies are mainly developed in an PEGasparaginase-free period and are primarily IgG. Patients with a reaction during induction mainly had anti-PEG (100% IgG and 75% IgM of 12 patients) and anti-succinate linker antibodies (50% IgG and 42% IgM of 12 patients). Thus, after a hypersensitivity reaction during the first PEGasparaginase administrations, patients might benefit from a switch to native *E.coli* asparaginase instead of *Erwinia* asparaginase for adequate treatment.

In contrast to *E.coli* asparaginase antibodies, the patients from our cohort were positive for anti-PEG IgG and IgM antibodies during hypersensitivity reactions both treatment phases. Mainly patients with an allergy had high anti-PEG antibody titers, suggesting that these antibodies may induce clinical symptoms of an allergy. Also, 39% (7/18) with a hypersensitivity reaction had exclusively anti-PEG antibodies, confirming the neutralizing capacity of these antibodies. Overall, IgM titers were low compared to anti-PEG IgG suggesting isotype class switching. Previous articles suggest that anti-PEG antibodies are predominantly induced through a T-cell independent manner.[24] Production of anti-drug antibodies that bypass T-cells are typically IgM or low-affinity IgG.[25] Binding of these antibodies can form immune complexes that are recognized by Fc- or complement-receptors promoting uptake by antigen processing cells such as splenic marginal B-cells.[26] The presence of pre-existing anti-PEG antibodies suggest a mechanism of memory B-cells. Although effective B-cell memory requires involvement of T_h-cells, there is growing evidence that T-cell independent type II antigens can form B-cell memory.[27] T-cell independent type II antigens are associated with polysaccharides, bearing repetitive structures.[28] The repetitive ethylene oxide ($-CH_2-CH_2-O$) units of PEG may be recognized by the same mechanism as these T-cell independent type II antigens.

In line with our observations, Rau et al. reported lower anti-PEG IgM titers than IgG in patients with an allergic reaction to PEGcrisantaspase, a PEGylated form of recombinant *Erwinia* asparaginase using glutaric acid spacer as a linker, administered after a hypersensitivity reaction to PEGasparaginase.[29] Whereas anti-PEG antibodies detected in animals are predominantly reported as anti-PEG IgM, human studies primarily show anti-PEG IgG.[17,30]

In our study, 58% (14/24) of the patients without any reaction had pre-existing anti-PEG IgG, and 21% anti-PEG IgM. Although anti-PEG antibodies were already described in the healthy population[31], this study shows that the antibodies can be developed relatively early in life and may have been formed during previous exposure to PEG containing food or cosmetic products.[32, 33, 31, 34] The fact that also patients without neutralization had anti-PEG antibodies proves that these antibodies do not necessarily possess neutralizing characteristics. Why these antibodies result in a neutralizing hypersensitivity reaction in only part of the patients should be further investigated.

Our study is the first to report that antibodies can be formed against the succinate succinimidyl linker and provides clinical evidence that use of a cleavable linker that remains on the therapeutic protein upon hydrolysis can induce the formation of anti-drug antibodies. Anti-SS-linker antibodies were predominantly found during induction. However, all patients positive for anti-SS-linker antibodies also had anti-PEG antibodies. We could, therefore, not identify if these anti-linker antibodies only bind to the linker or also induce an immunological response. The anti-PEG antibodies may also be directed towards both the linker and the PEG chain. Angiolillo et al. studied the presence of antibodies and occurrence of hypersensitivity reactions in patients treated with PEGasparaginase or Calaspargase pegol, a PEGylated *E.coli* asparaginase with a succinimidyl carbamate linker. Treatment with this type of asparaginase seems to result in a less rapid clearance and slightly less hypersensitivity reactions during induction (3% vs. 7%, although not significant).[35] These findings may imply that PEGylation with the SS-linker results in a more immunogenic therapeutic than by the other linker.

Anti-PEGasparaginase antibodies are frequently measured by various study groups with ELISA using a standard carbonate buffer of pH 9.0–9.5. However, PEG hydrolyses from PEGasparaginase at pH9.5 (Figure 3A). Thus, by using this buffer only anti-*E.coli* asparaginase and anti-SS-linker antibodies can be measured. Therefore, we recommend to coat plates using a buffer of pH7.4 for PEGasparaginase antibody detection.

In conclusion, first, we identified that anti-PEG antibodies play a significant role in the neutralization of PEGasparaginase, mainly during the first doses in induction, since part of the patients are only positive for this type of antibodies. Second, anti-asparaginase antibodies are almost exclusively developed during hypersensitivity reactions after an asparaginase-free interval in our patient cohort. Thus, patients with a reaction during the first doses of PEGasparaginase theoretically could switch to the less expensive native *E.coli* asparaginase for adequate treatment, reserving *Erwinia* asparaginase as an extra alternative. Third, pre-existing anti-PEG antibodies are found in part of the patients with no clinical effect. Therefore, these antibodies not necessarily trigger a hypersensitivity reaction and screening of anti-PEG antibodies is not a good marker to estimate patient outcome. And fourth, the discovery of the SS-linker as a neo-antigen shows that PEGylation through an unstable linker remaining on the therapeutic protein upon hydrolysis can serve as a hapten. Future drug development efforts should therefore focus on stable PEG-linker chemistries.

REFERENCES

1. Muller, H. J. and J. Boos. Use of L-asparaginase in childhood ALL. Crit Rev Oncol Hematol. 1998;28(2):97-113.
2. Oettgen, H. F., P. A. Stephenson, M. K. Schwartz, R. D. Leeper, L. Tallai, C. C. Tan, et al. Toxicity of E. coli L-asparaginase in man. Cancer. 1970;25(2):253-278.
3. Peterson, R. G., R. E. Handschumacher and M. S. Mitchell. Immunological responses to L-asparaginase. J Clin Invest. 1971;50(5):1080-1090.
4. Killander, D., A. Dohlwitz, L. Engstedt, S. Franzen, G. Gahrton, B. Gullbring, et al. Hypersensitive reactions and antibody formation during L-asparaginase treatment of children and adults with acute leukemia. Cancer. 1976;37(1):220-228.
5. Tong, W. H., R. Pieters, G. J. Kaspers, D. M. te Loo, M. B. Bierings, C. van den Bos, 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.
6. Abuchowski, A., J. R. McCoy, N. C. Palczuk, T. van Es and F. F. Davis. Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. J Biol Chem. 1977;252(11):3582-3586.
7. Abuchowski, A., T. van Es, N. C. Palczuk and F. F. Davis. Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. J Biol Chem. 1977;252(11):3578-3581.
8. Knop, K., R. Hoogenboom, D. Fischer and U. S. Schubert. Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. Angew Chem Int Ed Engl. 2010;49(36):6288-6308.
9. Amylon, M. D., J. Shuster, J. Pullen, C. Berard, M. P. Link, M. Wharam, 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.
10. Rizzari, C., M. G. Valsecchi, M. Arico, V. Conter, A. Testi, E. Barisone, 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.
11. Silverman, L. B., R. D. Gelber, V. K. Dalton, B. L. Asselin, R. D. Barr, L. A. Clavell, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97(5):1211-1218.
12. Duval, M., S. Suciu, A. Ferster, X. Rialland, B. Nelken, P. Lutz, 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.
13. Pession, A., M. G. Valsecchi, G. Masera, W. A. Kamps, E. Magyarosy, C. Rizzari, 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.
14. Moghrabi, A., D. E. Levy, B. Asselin, R. Barr, L. Clavell, C. Hurwitz, 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.
15. Pieters, R., S. P. Hunger, J. Boos, C. Rizzari, L. Silverman, A. Baruchel, et al. L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. Cancer. 2011;117(2):238-249.
16. Armstrong, J. K., G. Hempel, S. Koling, L. S. Chan, T. Fisher, H. J. Meiselman, et al. Antibody against poly(ethylene glycol) adversely affects PEG-asparaginase therapy in acute lymphoblastic leukemia patients. Cancer. 2007;110(1):103-111.
17. Verhoef, J. J., J. F. Carpenter, T. J. Anchordoquy and H. Schellekens. Potential induction of anti-PEG antibodies and complement activation toward PEGylated therapeutics. Drug Discov Today. 2014;19(12):1945-1952.

18. Hershfield, M. S., N. J. Ganson, S. J. Kelly, E. L. Scarlett, D. A. Jaggers and J. S. Sundy. Induced and pre-existing anti-polyethylene glycol antibody in a trial of every 3-week dosing of pegloticase for refractory gout, including in organ transplant recipients. Arthritis Res Ther. 2014;16(2):R63.
19. Carter, M. C. and M. E. Meyerhoff. Instability of succinyl ester linkages in O2'-monosuccinyl cyclic AMP-protein conjugates at neutral pH. J Immunol Methods. 1985;81(2):245-257.
20. Turecek, P. L., M. J. Bossard, F. Schoetens and I. A. Ivens. PEGylation of Biopharmaceuticals: A Review of Chemistry and Nonclinical Safety Information of Approved Drugs. J Pharm Sci. 2016;105(2):460-475.
21. Lanvers, C., J. P. Vieira Pinheiro, G. Hempel, G. Wuerthwein and J. Boos. 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.
22. Dong, H., J. R. Mora, C. Brockus, S. D. Chilewski, R. Dodge, C. Merrifield, et al. Development of a Generic Anti-PEG Antibody Assay Using BioScale's Acoustic Membrane MicroParticle Technology. AAPS J. 2015.
23. van der Sluis, I. M., H. de Groot-Kruseman, M. Te Loo, W. J. E. Tissing, C. van den Bos, G. J. L. Kaspers, et al. Efficacy and safety of recombinant E. coli asparaginase in children with previously untreated acute lymphoblastic leukemia: A randomized multicenter study of the Dutch Childhood Oncology Group. Pediatr Blood Cancer. 2018:e27083.
24. Ishida, T., X. Wang, T. Shimizu, K. Nawata and H. Kiwada. PEGylated liposomes elicit an anti-PEG IgM response in a T cell-independent manner. J Control Release. 2007;122(3):349-355.
25. Baker, M. P., H. M. Reynolds, B. Lumicisi and C. J. Bryson. Immunogenicity of protein therapeutics: The key causes, consequences and challenges. Self Nonself. 2010;1(4):314-322.
26. Krishna, M. and S. G. Nadler. Immunogenicity to Biotherapeutics - The Role of Anti-drug Immune Complexes. Front Immunol. 2016;7:21.
27. Good-Jacobson, K. L. and D. M. Tarlinton. Multiple routes to B-cell memory. Int Immunol. 2012;24(7):403-408.
28. Obukhanych, T. V. and M. C. Nussenzweig. T-independent type II immune responses generate memory B cells. J Exp Med. 2006;203(2):305-310.
29. Rau, R. E., Z. Dreyer, M. R. Choi, W. Liang, R. Skowronski, K. P. Allamneni, et al. Outcome of pediatric patients with acute lymphoblastic leukemia/lymphoblastic lymphoma with hypersensitivity to pegaspargase treated with PEGylated Erwinia asparaginase, pegcrisantaspase: A report from the Children's Oncology Group. Pediatr Blood Cancer. 2017.
30. Yang, Q. and S. K. Lai. Anti-PEG immunity: emergence, characteristics, and unaddressed questions. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2015;7(5):655-677.
31. Armstrong, J. K., R. Leger, R. B. Wenby, H. J. Meiselman, G. Garratty and T. C. Fisher. Occurrence of an antibody to poly(ethylene glycol) in normal donors. Blood. 2003;102(11):556a-556a.
32. Richter, A. W. and E. Akerblom. Polyethylene glycol reactive antibodies in man: titer distribution in allergic patients treated with monomethoxy polyethylene glycol modified allergens or placebo, and in healthy blood donors. Int Arch Allergy Appl Immunol. 1984;74(1):36-39.
33. Leger, R. M., P. Arndt, G. Garratty, J. K. Armstrong, H. J. Meiselman and T. C. Fisher. Normal donor sera can contain antibodies to polyethylene glycol (PEG). Transfusion. 2001;41(9):29s-30s.
34. Liu, Y., H. Reidler, J. Pan, D. Milunic, D. Qin, D. Chen, et al. A double antigen bridging immunogenicity ELISA for the detection of antibodies to polyethylene glycol polymers. J Pharmacol Toxicol Methods. 2011;64(3):238-245.
35. Angiolillo, A. L., R. J. Schore, M. Devidas, M. J. Borowitz, A. J. Carroll, J. M. Gastier-Foster, et al. Pharmacokinetic and Pharmacodynamic Properties of Calaspargase Pegol Escherichia coli L-Asparaginase in the Treatment of Patients With Acute Lymphoblastic Leukemia: Results From Children's Oncology Group Study AALL07P4. J Clin Oncol. 2014.

Part III

CHAPTER 6

6

The Effect of Asparaginase Therapy on Methotrexate Toxicity and Efficacy in Children with Acute Lymphoblastic Leukemia

Robin Q.H. Kloos, Rob Pieters, Cor van den Bos
Natasha K.A. van Eijkelenburg, Robert de Jonge, Inge M. van der Sluis

Leukemia and Lymphoma 2019; 23:1-9

ABSTRACT

Background Asparaginase and methotrexate (MTX), both essential for pediatric acute lymphoblastic leukemia therapy, are often used concomitantly. Depending on the sequence, *in vitro*, asparaginase inhibits MTX-polyglutamate (MTXPG) formation, and side effects overlap.

Methods MTX toxicity and efficacy, reflected by intracellular erythrocyte MTXPG's, were compared between children treated with and without asparaginase during high dose MTX (HD-MTX) courses of the DCOG ALL-11 protocol (NL50250.078.14).

Results Seventy-three patients, of whom 23 received asparaginase during the HD-MTX courses, were included. Grade 3-4 leukopenia and neutropenia occurred more often (59% and 86% versus 30% and 62%). The number of infections, grade 3-4 hepatotoxicity, nephrotoxicity and neurotoxicity did not differ. Patients with asparaginase had lower MTXPG levels, although to a lesser extent than *in vitro* studies.

Conclusion Although patients with asparaginase during HD-MTX courses showed more myelosuppression, this had no (serious) clinical consequences. Regarding the MTX efficacy, the schedule-related antagonism seen in *in vitro* seems less important *in vivo*.

INTRODUCTION

Asparaginase and methotrexate (MTX) are both essential for the treatment of pediatric acute lymphoblastic leukemia (ALL). These drugs are often used concomitantly, among other chemotherapeutics, to achieve better survival rates.[1-8] The sequence of administration, however, seems important as several *in vitro* studies have shown an antagonistic effect of native *E.coli* asparaginase on MTX when asparaginase is administered prior to the MTX.[9-12] It has been shown that MTX efficacy, reflected by intracellular MTX polyglutamates (MTXPGs) [13-15], is decreased due to the asparagine depletion caused by asparaginase. The asparagine depletion inhibits folylpolyglutamyl synthetase (FPGS), the enzyme that forms the MTXPGs. [9, 16-18, 12] In contrast, if asparaginase is administered after MTX, there seems to be a synergistic effect *in vitro*.[12] Moreover, *in vivo*, MTX is administered prior to native *E. coli* asparaginase with the Capizzi regimen, increasing the dose guided by toxicity, which permits toleration of higher MTX doses and leads to successful remission rates.[18]
Currently, most treatment protocols use PEGasparaginase, with therapeutic activity of at least two weeks, resulting in continuous asparagine depletion[19]. So treating patients with PEGasparaginase during MTX doses may influence the formation of MTXPGs, independently of the sequence of administration. Treatment protocols using these dosing schedules, however, are successful, suggesting that *in vivo* the effect of asparaginase on MTX efficacy seems less important.[20, 5, 21]

Beside MTX efficacy, concomitant asparaginase and MTX therapy may alter toxicity profiles because the drugs have overlapping side effects, including neurotoxicity, hepatotoxicity and myelosuppression. On the other hand, MTX toxicity can be decreased *in vitro* by drugs that prevent cells from entering the S-phase, which is the case when asparaginase depletes the extracellular asparagine pools.[22]

In the current Dutch Childhood Oncology Group (DCOG) ALL-11 protocol, medium risk patients are being randomized either to a continuous or a discontinuous PEGasparaginase dosing schedule, which contains an asparaginase-free period of several months, to study the effect of the dosing schedule on the occurrence of hypersensitivity reactions. Patients who are treated according to the continuous dosing schedule are concomitantly treated with asparaginase and high dose MTX courses, enabling us to study the possible effects of asparaginase on MTX efficacy and toxicity. The aim of this study is to compare the MTX efficacy, reflected by intracellular erythrocyte MTXPG levels, and toxicity between patients who are

treated with high dose MTX courses with and without concomitant PEGasparaginase treatment.

METHODS

Patients and treatment

Pediatric patients with ALL, diagnosed between November 2014 and June 2017, and treated according to the medium or standard risk group of the DCOG ALL-11 protocol were included in this study. The patients were treated in the Sophia Children's Hospital, Rotterdam, The Netherlands; the Academic Medical Center, Amsterdam, The Netherlands; or the Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands. The study (CCMO register: NL50250.078.14) was approved by the local ethics committee and informed consent was signed by children >12 years old and/or the parents or guardians in accordance with the declaration of Helsinki.

The complete DCOG ALL-11 treatment protocol is described in Table 1. Both standard and medium risk patients treated according to the discontinuous asparaginase dosing group, received three doses of PEGasparaginase (1,500 IU/m^2, IV, biweekly) during induction (course 1A and 1B), followed by an asparaginase-free interval of approximately 12 weeks (remaining course 1B and M). During the following intensification phase, standard risk patients received one more PEGasparaginase dose; medium risk patients another 14 doses. The medium risk patients who were treated according to the continuous dosing schedule, received the PEGasparaginase once every two weeks, also during the consecutive courses 1B and M. The PEGasparaginase doses were individualized after the third dose, based on asparaginase activity levels.[23]

Table 1 DCOG ALL-11 protocol for medium and standard risk patients.

Treatment phase	Therapy
Protocol 1A	
Prednisone	60 mg/m^2/day for 29 days followed by 9 days tapering
Vincristine	1.5 mg/m^2/dose at day 8, 15, 22 and 29
Daunorubicin	30 mg/m^2/dose at day 8, 15, 22 and 29 (not in case of Down syndrome)
PEGasparaginase	1,500 IU/m^2 at day 12, 26
Intrathecal MTX, cytarabine and prednisone	8 – 12 mg MTX, 20-30 mg cytarabine, 8-12 mg prednisone at day 15 and 33. Only intrathecal MTX at day 1.
Protocol 1B	
PEGasparaginase †	1,500 IU/m^2 at day 40
Cyclophosphamide	1,000 mg/m^2/dose at day 36 and 64
Cytarabine	75 mg/m^2/day at days 38 – 41, 45 – 48, 52 – 55, 59 – 62
6-Mercaptopurine	60 mg/m^2/day at days 36 – 63
Intrathecal MTX, cytarabine and prednisone	8 – 12 mg MTX, 20-30 mg cytarabine, 8-12 mg prednisone at day 45 and 59
Protocol M	
6-Mercaptopurine	25 mg/m^2/day for 56 days
MTX	5,000 mg/m^{2}over 24h at day 8, 22, 36 and 50
Intrathecal MTX, cytarabine and prednisone	8 – 12 mg MTX, 20-30 mg cytarabine, 8-12 mg prednisone at day 8, 22, 36 and 50
PEGasparaginase	*Only continuous group:* individualized doses, biweekly

†Medium risk patients in the continuous dosing schedule will continue PEGasparaginase treatment during course 1B and M , administered biweekly. MTX: methotrexate.

The asparaginase-free period for both standard and medium risk patients of the discontinuous dosing schedule started with course 1B containing 6-mercaptopurine, cytarabine and cyclophosphamide, followed by course M with four high dose MTX courses (5,000 mg/m^2/dose IV over 24 hours) and 6-mercaptopurine (25 mg/m^2/day orally) (Table 1). MTX was administered biweekly, except if patients suffered from a (severe) infection, mucositis or hepatotoxicity (AST/ALT >10x upper limit of normal), or when the white blood count was <1.5 * 10^9 or platelets were <50* 10^9. In that case, the course was postponed for at least one week. Folinic acid was administered after the MTX dose until the 48 h plasma MTX level was <0.4 µM or the 72 h plasma MTX level <0.25 µM. The patients with the continuous asparaginase dosing schedule also received PEGasparaginase during course 1B and M. The asparaginase doses were administered biweekly, even if the requirements of MTX administration were not fulfilled and MTX had to be postponed.

This is also true for any delay in protocol 1B, for example if cyclophosphamide has to be postponed. Evidently, patients had to fulfill the requirements for asparaginase administration, which included the absence of hepatotoxicity

(AST/ALT >10x and bilirubin >3x upper limit of normal), jaundice, clinical signs of pancreatitis and cerebral thrombosis.

Toxicity

Methotrexate toxicity was prospectively studied using case report forms which were completed by the physician two weeks after each high dose MTX course. These toxicity forms included central neurotoxicity (ataxia, somnolence, a depressed level of consciousness, agitation, seizures and posterior reversible encephalopathy syndrome), infections, mucositis and diarrhea. In addition, complete blood count, liver enzymes (alanine transaminase (ALT) and aspartate transaminase (AST)), creatinine and albumin concentrations were measured prior to the next high dose MTX courses. Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. In addition, total treatment delay due to toxicity, extra hospital admissions and prolongation of hospital admissions for MTX administration were registered.

MTX polyglutamates

MTX polyglutamates were measured two to three weeks after each MTX course. Blood was drawn to measure intracellular MTXPG concentrations in the erythrocytes as described by Den Boer et al.[24] EDTA whole blood tubes were centrifuged at 2,700 x g for 10 minutes at room temperature. The red cell pellet was harvested and stored at -80°C until analysis. For the analysis, first stable-isotope-labelled internal standards were added, followed by incubation of the sample with 16% perchloric acid for protein precipitation. After centrifugation at 21,350xg for 7 minutes, MTXPG 1-5 concentrations were measured using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). MTXPG 1 is freely transportable out of the cells, so very variable.[25] Therefore, only MTXPG 2-5 were used for the analysis. Beside the intracellular MTXPG levels, 48 h plasma MTX levels were analyzed.

Statistical analysis

SPSS Statistics version 21.0 (IBM Corp, Armonk, New York, USA) and R Sigmaplot Version 3.4.1 (Systat Software Inc, London, UK) were used for the data analysis. Baseline characteristics were stated as mean and standard deviation (SD) for normal distributed data, and median and interquartile range (IQR) for skewed data. Student t-tests, Mann-Whitney U tests, χ^2-(trend)tests, Fisher exact tests and corresponding post-hoc analyses were used to compare the baseline characteristics and maximal toxicity during protocol M. The MTXPG levels were longitudinally analyzed using marginal models to study the levels between patients with and without concomitant asparaginase treatment. The data was log transformed to obtain normally distributed data. We have corrected for the number of days between the MTX dose and sampling, the MTX dose number, age, sex, and whether there was an erythrocyte transfusion administered less than two weeks before the sample. In addition, we have studied whether asparaginase doses were administered directly after the MTX doses or one week after the MTX dose. The 48 h MTX plasma levels were also analyzed with marginal models. The data was log transformed to obtain normally distributed data. In the model, we have corrected for sex, age, the MTX dose number, albumin levels and whether patients had an increased creatinine level. Also in this analysis it was included whether asparaginase doses were administered directly after the MTX doses or one week after the MTX dose.

RESULTS

Baseline characteristics are described in Table 2. In total, 73 patients were included in the study. Twenty-three patients were concomitantly treated with asparaginase during the high dose MTX courses in protocol M. Of the group without asparaginase treatment during the MTX courses, 17/50 (34%) were treated according to the standard risk group; the other 33 patients according to the discontinuous dosing schedule of the medium risk group. Of the group with concomitant asparaginase treatment, one patient had to switch to the high risk group half way during protocol M due to high minimal residual disease.
There were no statistically significant differences in other baseline characteristics between the patients with and without asparaginase treatment during protocol M. Of note, some of the patients were standardly treated with intravenous

immunoglobulins (IVIG) as part of another randomized study. The number of patients standardly treated with IVIG, however, did not differ between the groups.

Table 2 Patient characteristics

	No asparaginase during high dose MTX courses n = 50	Asparaginase during high doses MTX courses n = 23	P-value
Sex (%)			0.621
Male	27 (54%)	14 (61%)	
Female	23 (46%)	9 (39%)	
Age at diagnoses, years, median (IQR)	5.4 (3.0 – 9.2)	4.1 (3.4 – 5.5)	0.319
Type of ALL (%)			0.390
Pre-B cell ALL	12 (24%)	9 (39%)	
Common B-cell ALL	31 (62%)	10 (44%)	
Pro-B cell ALL	1 (2%)	4 (17%)	
T-cell ALL	6 (12%)	0 (0%)	
Risk group (%)			-
Standard risk	17 (34%)	-	
Medium risk,			
Discontinuous group	33 (66%)	-	
Continuous group	-	23 (100%) †	
IVIG therapy during protocol M (%)			0.762
Yes	11 (22%)	4 (17%)	
No	39 (78%)	19 (83%)	
Average trough‡ asparaginase level during protocol M, IU/L, mean (SD)	-	249 (48)	-

† One patient had to switch to high risk therapy due to high minimal residual disease. ‡Trough asparaginase level: 14 ± 2 days after an asparaginase dose. MTX: methotrexate; IQR: interquartile range; ALL: acute lymphoblastic leukemia; IVIG: intravenous immunoglobulins; SD: standard deviation; MRD: minimal residual disease

Toxicity

The maximum toxicity per group and the number of infections and transfusions during protocol M are described in Table 3. Patients with asparaginase treatment during the high dose MTX courses received significantly more erythrocyte and thrombocyte transfusions (median and IQR of 1 (0.75 – 2) and 0 (0 – 0.25), respectively) than patients without concomitant asparaginase treatment (median and IQR of 0 (0 – 0) and 0 (0 – 0), respectively). Patients with asparaginase treatment received a maximum of 5 erythrocyte and 4 thrombocyte transfusions; in the group without asparaginase, the maximum was 1 for both erythrocyte- and thrombocyte transfusions. In addition, the occurrence of grade 3-4 leukopenia and neutropenia was higher in the group of patients with concomitant asparaginase

treatment (leukopenia 59% versus 31%, and neutropenia 86% versus 63%, respectively). However, the number of infections during the high dose MTX courses did not differ between patients with or without concomitant asparaginase treatment.

Table 3 Toxicity during protocol M

	No asparaginase during high dose MTX courses n = 50		Asparaginase during high doses MTX courses n = 22 †		P-value
Number of infections, median (IQR)	0 (0 – 1)		0 (0 – 1)		0.347
Number of transfusions during protocol M, median (IQR)					
Erythrocytes	0 (0 – 0)		1 (0.75 – 2)		<0.001
Thrombocytes	0 (0 – 0)		0 (0 – 0.25)		0.033
	Maximal grade 1-2 n (%)	Maximal grade 3-4 n (%)	Maximal grade 1-2 n (%)	Maximal grade 3-4 n (%)	
Leukopenia	33 (66%) ¶	15 (30%) ¶	9 (41%) ¶	13 (59%) ¶	0.022
Neutropenia	12 (24%)	31 (62%) ¶	3 (14%)	19 (86%) ¶	0.032
Increased ALT/AST	16 (32%) ¶	1 (2%)	20 (91%) ¶	2 (9%)	<0.001
Increased creatinine prior to MTX	4 (8%)	0	1 (5%)	0	0.504
Increased creatinine 48 h after MTX	2 (4%)	0	3 (14%)	0	0.163
Decreased albumine	0[a]	0	14 (100%) §,¶	0	<0.001
Neurotoxicity ‡	5 (10%)	2 (4%)	6 (27%)	0	0.233
Mucositis	18 (36%)	16 (32%)	10 (46%)	5 (23%)	0.580
Diarrhea	10 (20%)	1 (2%)	5 (23%)	1 (5%)	0.572

† In one patient, protocol M was not completed because he had to switch high risk therapy due to high minimal residual disease.
‡ Neurotoxicity included somnolence (grade 1-2), depressed consciousness (grade 1-2), agitation (grade 3-4) and seizures (grade 3-4).
§ Albumine was standardly measured only in part of the patients (n=14 and n=36 for the groups without and with asparaginase, respectively).
¶ Statistically significant ($p<0.05$) after post-hoc analysis.
Grading according to the common terminology criteria for adverse events version 4.03.
MTX: methotrexate; IQR: interquartile range; ALT: alanine transaminase; AST: aspartate transaminase.

Regarding hepatotoxicity, significantly more patients who were treated with asparaginase during the high dose MTX courses had grade 1-2 increased ALT and AST. On the other hand, grade 3-4 increased ALT and AST only occurred in one and two patients of the patients without and with concomitant asparaginase treatment, respectively.
All patients with asparaginase had grade 1-2 hypoalbuminemia, in contrast to patients without asparaginase, who all had normal albumin levels. This, however,

had no clinical consequences.
The number of patients with increased creatinine, neurotoxicity, mucositis and diarrhea did not significantly differ between the groups. Nephrotoxicity 48 hours after one of the high dose MTX courses occurred in 5 patients (3 patients with and 2 patients without asparaginase), all grade 1-2 .

The duration of protocol M, and prolonged and extra hospital admissions during this treatment phase are described in Table 4. Without any delay, protocol M would have a duration of 63 days. The mean duration of protocol M for patients without asparaginase administrations was 68 days (SD 7 days); the mean duration for patients with asparaginase administrations was 80 days (SD 14 days) (p=0.001). The hospital admission duration for MTX administration is usually 2 days, although, among other clinical reasons, admissions may be prolonged if plasma 48 h MTX plasma levels are >0.4 µM. There was no statistically significant difference in the hospital admission duration between the groups. Also, there was no statistically significant difference in the number of extra hospital admissions (median number and IQR of 1 (0 – 1) for both groups).

Table 4 Duration of protocol M

		No asparaginase during high dose MTX courses n = 49†	Asparaginase during high doses MTX courses n = 22†	P-value
Duration of protocol M in days, mean ± SD		68±7	80±14	0.001
Duration of hospital admissions for MTX administration in days, median (IQR)	Dose 1	2 (2 – 4)	2 (2 – 2)	0.087
	Dose 2	2 (2 – 3)	2 (2 – 3)	0.610
	Dose 3	2 (2 – 3)	2 (2 – 2)	0.407
	Dose 4	2 (2 – 4)	2 (2 – 3)	0.099
Extra hospital admissions during protocol M (median, IQR)		0 (0 – 1)	0 (0 – 1)	0.266
T48 MTX plasma level, µM, median (IQR)		0.39 (0.30 – 0.52)	0.39 (0.26 – 0.64)	0.510‡

† In two patients, protocol M was not completed. In one patient due to severe neurotoxicity, protocol M was postponed for several weeks; the other patient had to switch to high risk treatment during protocol M.
‡ The difference in T48 MTX plasma levels between the groups was analyzed using marginal models and corrected for sex, age and albumin levels.
MTX: methotrexate; SD: standard deviation; IQR: interquartile range

MTX polyglutamates

In total, 240 erythrocyte MTXPG samples were obtained. Longitudinally analyzed, MTXPG 2-5 were lower in patients treated with asparaginase during the high dose MTX courses. All MTXPG levels increased in the consecutive high dose MTX courses (Figure 1). The number of days between the MTX dose and sampling, sex and age did not significantly alter the MTXPG levels. Also the timing of the asparaginase dose with respect to the MTX dose did not have a significant influence on the MTXPGs. Figure 1 shows the median MTXPG levels two weeks (12 – 16 days) after the high dose MTX courses, corrected for administration of erythrocyte transfusions. Comparing patients with and without concomitant asparaginase treatment, the median (IQR) MTXPG levels were 4.0 µM (2.1 – 7.6 µM) versus 10.8 µM (6.6 – 19.8 µM) for MTXPG 2 ($p<0.001$), 18.7 µM (9.9 – 27.2 µM) versus 27.5 µM (18.5 – 37.4 µM) for MTXPG 3 ($p=0.004$), 29.2 µM (13.0 – 42.1 µM) versus 37.9 µM (26.6 – 58.3 µM) for MTXPG 4 ($p=0.002$), and 19.4 µM (9.9 – 28.8 µM) versus 32.8 µM (20.3 – 48.1 µM) for MTXPG 5 ($p=0.004$). Overall, the median (IQR) MTXPG 2 – 5 levels were 72.7 µM (37.7 – 110.4 µM) for patients with and 118.6 µM (74.8 – 16.1 µM) for patients without asparaginase treatment during protocol M (<0.001).

Figure 1. Erythrocyte MTXPG concentrations

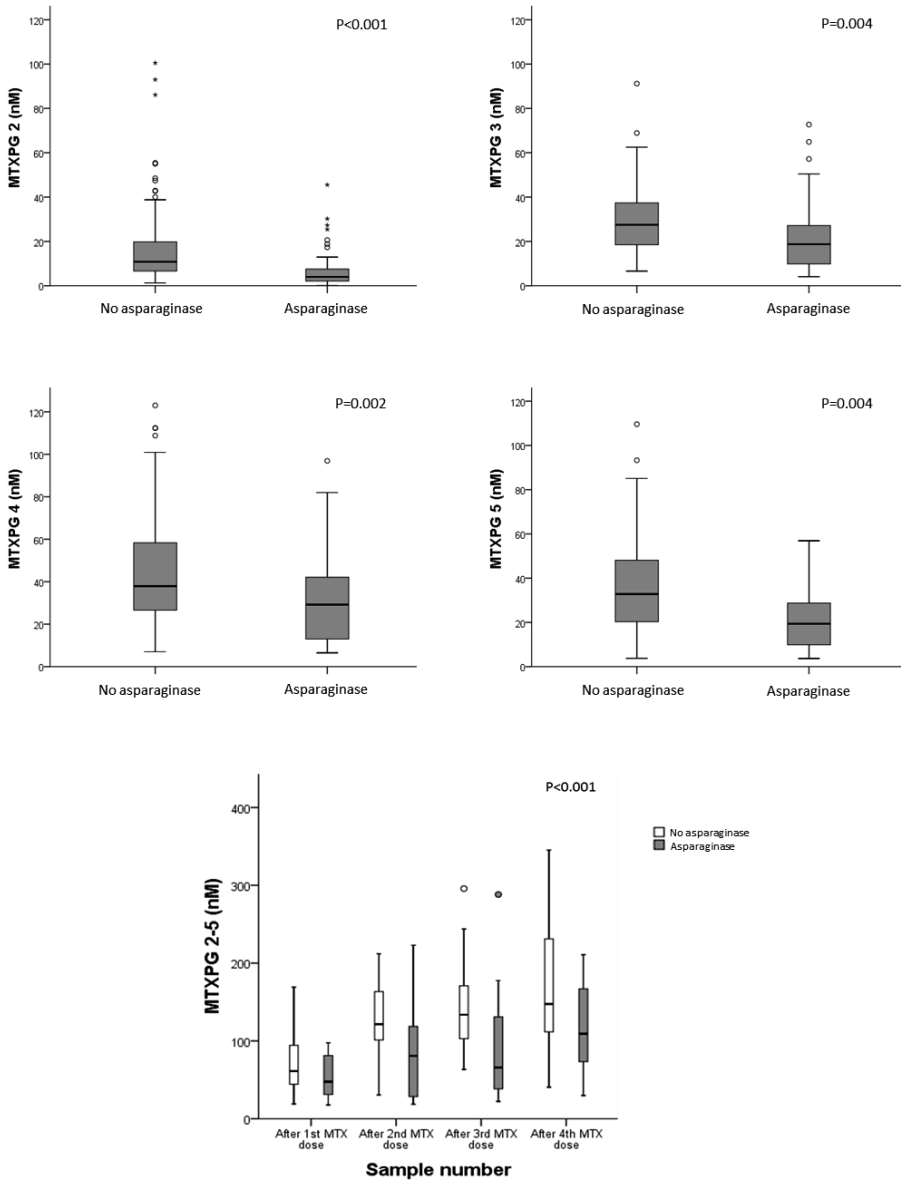


Figure 1 shows the concentrations of erythrocyte MTXPG 2, 3, 4, 5 and 2-5, measured two weeks (12 – 16 days) after the MTX dose, excluding measurements less than two weeks after an erythrocyte transfusion. The p-values have been obtained from the longitudinal analysis. The MTXPG 2-5 levels are shown per MTX dose.
The boxplot includes the 25th, 50th and 75th percentile in the boxes, the outliers (o), extreme outliers (*), and the ranges (indicated by the whiskers).

MTX plasma levels

The 48 h MTX plasma levels are shown in Table 4 and shown in Figure 2. The median 48 h MTX plasma level for patients without concomitant asparaginase treatment was 0.39 µM (IQR 0.30 – 0.52 µM); for patients with concomitant asparaginase treatment this was 0.39 µM (IQR 0.26 – 0.64 µM).

Figure 2. MTX plasma levels

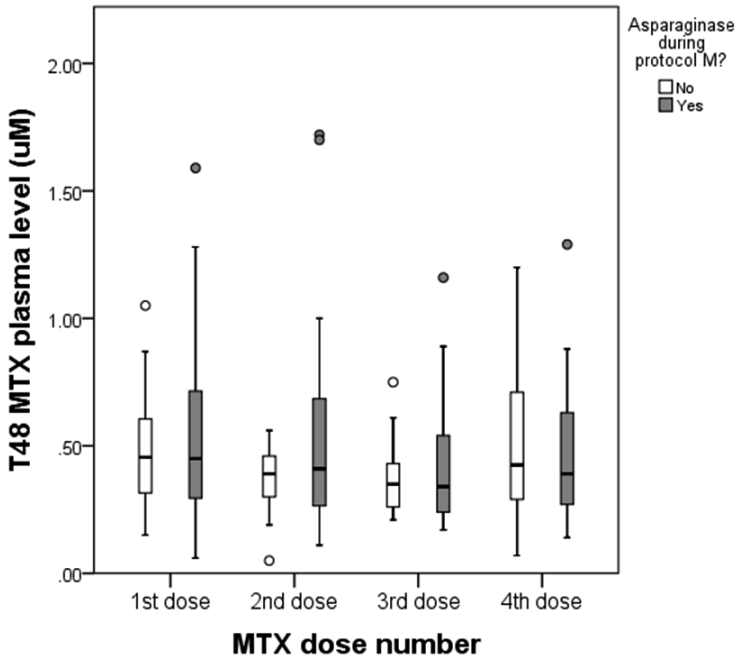


Figure 2 shows the 48 h MTX plasma levels after each high dose MTX for patients with and without concomitant asparaginase treatment. The p-value has been obtained from the longitudinal analysis. The boxplot includes the 25th, 50th and 75th percentile in the boxes, the outliers (○), and the ranges (indicated by the whiskers). The extreme outliers were excluded as described. The T48 MTX plasma levels did not differ between the patients who were treated with and without asparaginase (p=0.510).

In the longitudinal analysis, we have corrected for sex, age and albumin. Creatinine levels were not increased just prior to the MTX doses. During the analysis, outliers violated the normality assumption, even after log-transformation of the data. Therefore, we have performed the analysis also excluding the extreme outliers (n=5). In both models, concomitant asparaginase treatment did not statistically significantly alter the 48 h plasma levels (p=0.624 with outliers and p=0.510 without outliers). Also the albumin levels did not significantly affect the 48 h MTX plasma levels.

DISCUSSION

In this study, the toxicity and efficacy of high dose MTX were analyzed for patients with and without concomitant asparaginase therapy. Patients with asparaginase treatment had more often severe neutropenia and leukopenia, and they received more erythrocyte and thrombocyte transfusions. However, the most important consequence of myelosuppression, namely the occurrence of (severe) infections, did not differ between the groups. As a result of this myelosuppression, the high dose MTX courses had to be postponed more often in patients with asparaginase, resulting in a delay of protocol M. It could be questioned if this delay is clinically relevant. It could even have a positive effect as MTX therapy may be more effective when administered every three weeks instead of two: a possible rescue effect of folinic acid on leukemic cells may be diminished three weeks after the previous dose.[26, 27] In addition to a difference in myelosuppression, all patients in the group with asparaginase had increased ALT and AST, although the far majority had grade 1-2 hepatotoxicity which had no clinical consequences.

Beside differences in myelosuppression and hepatotoxicity, we have found lower albumin levels in patients who were treated with asparaginase. MTX is a week acid and binds to serum albumin. Reiss et al. have shown that hypoalbuminemia is associated with decreased MTX clearance and an increased length of hospitalization.[28] In our patients, however, the 48 h MTX plasma levels and length of hospitalization did not differ in the patients with and without hypoalbuminemia. Unfortunately, in the study of Reiss et al., the severity of hypoalbuminemia was not reported. In our study, patients only had mild (grade 1-2) hypoalbuminemia, possibly explaining the lack of association between albumin levels, and MTX clearance and hospitalization.

The occurrence of nephrotoxicity, neurotoxicity and mucositis did not differ between the groups. The incidence of these side effects was in line with the incidences found by den Hoed et al., who have shown CTCAE grade 3-4 neurotoxicity in 3% and CTCAE grade 3-4 mucositis in 20% of the patients treated with high dose MTX.[27] The occurrence of nephrotoxicity has been reported in 2 – 10% of the patients after high dose MTX courses.[29] These toxicities have been correlated with MTX plasma levels.[29, 27] In our study, asparaginase has no effect on these plasma levels, which may explain that the toxicity did not differ between the groups.

Beside toxicity during the high dose MTX courses, one should consider the toxicity during the intensification phase as well: patients in the continuous asparaginase dosing schedule will receive fewer asparaginase doses during the intensification and maintenance phase. These patients, probably, will tolerate a higher amount of 6-mercaptopurine and MTX, which is administered during these phases when they have completed their asparaginase doses, as reported by Merryman et al.[30] In addition, one could expect fewer infections and less hepatotoxicity during the intensification and maintenance phase in these patients.

Regarding MTX efficacy, we found that erythrocyte MTXPG levels were significantly lower in patients who were treated with asparaginase during the high dose MTX courses. The question is whether this is of clinical relevance. To the best of our knowledge, this is the first study comparing MTXPG levels in patients treated with and without asparaginase during high dose MTX. In earlier studies, Jolivet et al. and Sur et al. have concluded that asparaginase inhibits MTX polyglutamination *in vitro* by inhibition of FPGS due to asparagine depletion.[11, 12] However, this resulted in a decrease of mainly long chain MTXPG levels: MTXPG 4 levels were more than 80% lower, MTXPG 5 was not even measurable, but MTXPG 2 did not alter with asparaginase treatment. In our study, all MTXPG chains were lower, regardless of the chain length and, in contrast to the *in vitro* studies, all long chain MTXPGs were formed. Moreover, the overall decrease in levels is not as large as was found *in vitro* by Jolivet et al. This implies that the effect of asparaginase on MTX efficacy *in vivo* is smaller. However, it also has to be taken into account that erythroblasts, in which the erythrocyte MTX polyglutamination takes place, contain asparagine synthetase[31] and therefore, in contrast to leukemic blasts, do not depend on extracellular asparagine levels. This may influence the effect of asparaginase on MTX polyglutamination. In addition, the type and cytogenetic characteristics of the leukemic cells influence the degree of MTX

polyglutamination. For example, the formation of MTXPGs is increased in hyperdiploid ALL and decreased in T-cell ALL.[32, 33] On the other hand, also the asparaginase sensitivity varies, and leukemic cells which are less sensitive for asparaginase may encounter a smaller effect of asparagine depletion on MTX polyglutamination.[34] When the DCOG ALL-11 has been completed, survival analyses might provide differences between the asparaginase treatment arms, which then may be explained by the difference in MTXPG levels found in this study, although the number of relapses may be too low to draw these conclusions.

Though we found inhibition of MTX polyglutamylation, there was no effect of the timing of asparaginase administration on the MTXPG levels. In our study, asparaginase was either administrated directly or a week after the MTX. Several *in vitro* studies have shown that asparaginase inhibits MTX polyglutamylation and efficacy specifically when asparaginase has been administered prior to the MTX. Vice versa, asparaginase administration after MTX would have a synergistic effect.[9, 17, 11, 12] In line with these findings, several treatment protocols prove to be successful when asparaginase is administered after MTX.[35, 2, 5, 36] These protocols, however, used native *E. coli* asparaginase, which has shorter therapeutic activity than PEGasparaginase (three days versus two weeks). By administering PEGasparaginase biweekly, asparagine is continually depleted, which could explain our finding that the timing of asparaginase administration relative to the MTX doses had no effect on the formation of MTXPGs. However, in our study patients were not treated with asparaginase directly prior to MTX. Therefore, we cannot draw conclusions about a possible effect of very high (top) asparaginase activity levels prior to MTX administration.

We conclude that the schedule-related antagonism seen in the early *in vitro* studies seems less important *in vivo*, especially when patients are treated with PEGasparaginase during the high dose MTX. In addition, the results of this study suggest that MTX toxicity caused by concomitant asparaginase therapy only slightly increases, and would be acceptable.

REFERENCES

1. Bertino, J. R. Karnofsky memorial lecture. Ode to methotrexate. J Clin Oncol. 1993;11(1):5-14.
2. Nachman, J. B., H. N. Sather, M. G. Sensel, M. E. Trigg, J. M. Cherlow, J. N. Lukens, et al. Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. N Engl J Med. 1998;338(23):1663-1671.
3. Amylon, M. D., J. Shuster, J. Pullen, C. Berard, M. P. Link, M. Wharam, 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.
4. Rizzari, C., M. G. Valsecchi, M. Arico, V. Conter, A. Testi, E. Barisone, 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.
5. Silverman, L. B., R. D. Gelber, V. K. Dalton, B. L. Asselin, R. D. Barr, L. A. Clavell, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97(5):1211-1218.
6. Duval, M., S. Suciu, A. Ferster, X. Rialland, B. Nelken, P. Lutz, 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.
7. Pession, A., M. G. Valsecchi, G. Masera, W. A. Kamps, E. Magyarosy, C. Rizzari, 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.
8. Moghrabi, A., D. E. Levy, B. Asselin, R. Barr, L. Clavell, C. Hurwitz, 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.
9. Capizzi, R. L., W. P. Summers and J. R. Bertino. L-asparaginase induced alteration of amethopterin (methotrexate) activity in mouse leukemia L5178Y. Ann N Y Acad Sci. 1971;186:302-311.
10. Capizzi, R. L. Schedule-dependent synergism and antagonism between methotrexate and asparaginase. Biochem Pharmacol. 1974;23(2):151 - 161.
11. Jolivet, J., D. E. Cole, J. S. Holcenberg and D. G. Poplack. Prevention of methotrexate cytotoxicity by asparaginase inhibition of methotrexate polyglutamate formation. Cancer Res. 1985;45(1):217-220.
12. Sur, P., D. J. Fernandes, T. E. Kute and R. L. Capizzi. L-asparaginase-induced modulation of methotrexate polyglutamylation in murine leukemia L5178Y. Cancer Res. 1987;47(5):1313-1318.
13. Chabner, B. A., C. J. Allegra, G. A. Curt, N. J. Clendeninn, J. Baram, S. Koizumi, et al. Polyglutamation of methotrexate. Is methotrexate a prodrug? J Clin Invest. 1985;76(3):907-912.
14. Whitehead, V. M., D. S. Rosenblatt, M. J. Vuchich, J. J. Shuster, A. Witte and D. Beaulieu. Accumulation of methotrexate and methotrexate polyglutamates in lymphoblasts at diagnosis of childhood acute lymphoblastic leukemia: a pilot prognostic factor analysis. Blood. 1990;76(1):44-49.
15. Tsurusawa, M., M. Gosho, T. Mori, T. Mitsui, S. Sunami, R. Kobayashi, et al. Statistical analysis of relation between plasma methotrexate concentration and toxicity in high-dose methotrexate therapy of childhood nonHodgkin lymphoma. Pediatr Blood Cancer. 2014.
16. Vadlamudi, S., B. Krishna, V. V. Reddy and A. Goldin. Schedule-dependent therapeutic synergism for L-asparaginase and methotrexate in leukemic (L5178Y) mice. Cancer Res. 1973;33(9):2014-2019.
17. Chlopkiewicz, B. and J. Koziorowska. Role of amino acid depletion in combined treatment of neoplastic cells with methotrexate and L-asparaginase. Cancer Res. 1975;35(6):1524-1529.
18. Capizzi, R. L. Asparaginase-methotrexate in combination chemotherapy: schedule-dependent differential effects on normal versus neoplastic cells. Cancer Treat Rep. 1981;65 Suppl 4:115-121.

19. Tong, W. H., R. Pieters, G. J. Kaspers, D. M. te Loo, M. B. Bierings, C. van den Bos, 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.
20. Aguayo, A., J. Cortes, D. Thomas, S. Pierce, M. Keating and H. Kantarjian. Combination therapy with methotrexate, vincristine, polyethylene-glycol conjugated-asparaginase, and prednisone in the treatment of patients with refractory or recurrent acute lymphoblastic leukemia. Cancer. 1999;86(7):1203-1209.
21. Pieters, R., H. de Groot-Kruseman, V. Van der Velden, M. Fiocco, H. van den Berg, E. de Bont, et al. Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group. J Clin Oncol. 2016;34(22):2591 - 2601.
22. Chabner, B. A. and D. L. Longo (2006). Cancer chemotherapy & biotherapy, principles and practice.
23. Kloos, R. Q. H., C. A. Uyl-de Groot, R. R. L. van Litsenburg, G. J. L. Kaspers, R. Pieters and I. M. van der Sluis. A cost analysis of individualized asparaginase treatment in pediatric acute lymphoblastic leukemia. Pediatr Blood Cancer. 2017.
24. den Boer, E., R. J. Meesters, B. D. van Zelst, T. M. Luider, J. M. Hazes, S. G. Heil, et al. Measuring methotrexate polyglutamates in red blood cells: a new LC-MS/MS-based method. Anal Bioanal Chem. 2013;405(5):1673-1681.
25. Chabner, B. L. D. L. (2006). Cancer chemotherapy and biotherapy : principles and practice. Philadelphia, Lippincott Williams & Wilkins.
26. Cohen, I. J. Defining the appropriate dosage of folinic acid after high-dose methotrexate for childhood acute lymphatic leukemia that will prevent neurotoxicity without rescuing malignant cells in the central nervous system. J Pediatr Hematol Oncol. 2004;26(3):156-163.
27. den Hoed, M. A., E. Lopez-Lopez, M. L. te Winkel, W. Tissing, J. D. de Rooij, A. Gutierrez-Camino, et al. Genetic and metabolic determinants of methotrexate-induced mucositis in pediatric acute lymphoblastic leukemia. Pharmacogenomics J. 2015;15(3):248-254.
28. Reiss, S. N., L. W. Buie, N. Adel, D. A. Goldman, S. M. Devlin and D. Douer. Hypoalbuminemia is significantly associated with increased clearance time of high dose methotrexate in patients being treated for lymphoma or leukemia. Ann Hematol. 2016;95(12):2009-2015.
29. Widemann, B. C. and P. C. Adamson. Understanding and managing methotrexate nephrotoxicity. Oncologist. 2006;11(6):694-703.
30. Merryman, R., K. E. Stevenson, W. J. Gostic, 2nd, D. Neuberg, J. O'Brien, S. E. Sallan, et al. Asparaginase-associated myelosuppression and effects on dosing of other chemotherapeutic agents in childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2012;59(5):925-927.
31. Chen, L., M. Kostadima, J. H. A. Martens, G. Canu, S. P. Garcia, E. Turro, et al. Transcriptional diversity during lineage commitment of human blood progenitors. Science. 2014;345(6204):1251033.
32. Synold, T. W., M. V. Relling, J. M. Boyett, G. K. Rivera, J. T. Sandlund, H. Mahmoud, et al. Blast cell methotrexate-polyglutamate accumulation in vivo differs by lineage, ploidy, and methotrexate dose in acute lymphoblastic leukemia. J Clin Invest. 1994;94(5):1996-2001.
33. Wojtuszkiewicz, A., G. J. Peters, N. L. van Woerden, B. Dubbelman, G. Escherich, K. Schmiegelow, et al. Methotrexate resistance in relation to treatment outcome in childhood acute lymphoblastic leukemia. J Hematol Oncol. 2015;8(1):61.
34. Chen, S. H. Asparaginase Therapy in Pediatric Acute Lymphoblastic Leukemia: A Focus on the Mode of Drug Resistance. Pediatr Neonatol. 2015;56(5):287-293.
35. Lobel, J. S., R. T. O'Brien, S. McIntosh, G. T. Aspnes and R. L. Capizzi. Methotrexate and asparaginase combination chemotherapy in refractory acute lymphoblastic leukemia of childhood. Cancer. 1979;43(3):1089-1094.
36. Buaboonnam, J., X. Cao, J. L. Pauley, C. H. Pui, R. C. Ribeiro, J. E. Rubnitz, et al. Sequential administration of methotrexate and asparaginase in relapsed or refractory pediatric acute myeloid leukemia. Pediatr Blood Cancer. 2013;60(7):1161-1164.

Part IV

CHAPTER 7

7

A Cost Analysis of Individualized Asparaginase Treatment in Pediatric Acute Lymphoblastic Leukemia

Robin Q.H. Kloos, Carin A. Uyl-de Groot, Raphaële R.L. van Litsenburg
Gertjan J.L. Kaspers, Rob Pieters, Inge M. van der Sluis

Pediatric Blood & Cancer 2017; 64

ABSTRACT

Background Therapeutic drug monitoring (TDM) of asparaginase is necessary to respond to variability in asparaginase activity levels, detect silent inactivation, and distinguish between real allergies and allergic-like reactions with and without asparaginase neutralization, respectively. In this study, the costs of an individualized and fixed asparaginase dosing schedule were compared.

Methods Patients, treated according to the DCOG ALL-11 protocol (individualized PEGasparaginase treatment, starting dose 1,500 IU/m^2) or ALL-10 protocol (native *E.coli* asparaginase followed by 2,500 IU/m^2 PEGasparaginase), were included. To focus on TDM of PEGasparaginase, the costs were also calculated excluding patients treated with *Erwinia* asparaginase, and compared to a hypothetical protocol with a fixed dose of 1,500 IU/m^2 PEGasparaginase. Direct asparaginase-related medical costs, including costs for asparaginase use (calculated with the absolute dose), TDM, laboratory tests, daycare treatment, and outpatient clinic visits, were calculated.

Results Eighty-three ALL-10 patients and 51 ALL-11 patients were included. The asparaginase-related costs were 30.8% lower in ALL-11 than in ALL-10 ($29,048 vs. $41,960). The ALL-11 costs of non-allergic patients were 20.4% lower when using TDM, than the hypothetical protocol with a fixed dose of 1,500 IU/m^2($13,178 vs. $16,551). TDM accounted for 12.4% of the costs. Including asparaginase waste, TDM in ALL-11 will be cost-saving if three doses can be prepared out of one vial, compared to a fixed dose of 1,500 IU/m^2.

Conclusion TDM of asparaginase is cost-saving if calculated with the absolute asparaginase dose and will be if the waste is minimalized by preparing multiple doses out of one vial.

INTRODUCTION

Asparaginase is one of the key components of the treatment of acute lymphoblastic leukemia (ALL) in children and significantly improves the event-free survival (EFS) if patients are treated intensively.[1-6] Unfortunately, hypersensitivity reactions may occur, usually resulting in complete neutralization of the drug.[7, 8] In that case, asparaginase formulations should be switched to maintain optimal treatment. Patients can also develop allergic symptoms without asparaginase neutralization, so called allergic-like reactions.[8] A third type of reaction to asparaginase is silent inactivation, which is neutralization of asparaginase without clinical symptoms. Distinction between these three reactions can only truly be made by continual measurement of asparaginase activity levels.

In addition, high inter- and intra-patient variability of asparaginase activity levels has been observed [9], which requires individualized dosing of the drug. In our previous Dutch Childhood Oncology Group (DCOG) ALL-10 protocol, patients were treated with a fixed dose of 2,500 IU/m^2 PEGasparaginase.[10] These patients had relatively high asparaginase activity levels (mean 899 IU/L), possibly causing unnecessary toxicity since an asparaginase activity level of >100 IU/L is considered sufficient for complete asparagine depletion.[11-18] In addition, 8% of the patients turned out to have silent inactivation.[7] For these reasons, therapeutic drug monitoring (TDM) was implemented in the consecutive DCOG ALL-11 protocol to individualize the asparaginase treatment.

Because asparaginase activity levels are high after the fixed dose of 2,500 IU/m^2, individualized asparaginase treatment will decrease asparaginase use, consequently reducing the costs. On the other hand, the individualization of asparaginase therapy requires the measurement of asparaginase activity levels and the formulation of dosing advices. This will, in turn, increase the costs. PEGasparaginase treatment accounts for a substantial amount of the total ALL therapy costs.[19] Therefore, a change in asparaginase costs will have a significant impact on the total costs.
The aim of this study was to analyze whether TDM of asparaginase is cost saving when compared to the fixed dosing schedule. For this, we have compared the individualized dosing schedule of the DCOG ALL-11 protocol to the fixed dosing schedule of DCOG ALL-10 (2,500 IU/m^2 PEGasparaginase), and to a hypothetical dosing schedule with a fixed dose of 1,500 IU/m^2 PEGasparaginase, the starting dose used in the DCOG ALL-11 protocol.

METHODS

Patients

This retrospective study was performed in in the Sophia Children's Hospital, Rotterdam, the Netherlands and the VU University Medical Center, Amsterdam, the Netherlands. Patients with ALL, diagnosed between May 2009 and March 2015, who were treated according to the medium risk group of the DCOG ALL-10 or ALL-11 protocol, were included. These patients were treated with asparaginase most intensively. The study was approved by the Institutional Review Board in accordance with the Declaration of Helsinki.

Treatment protocols

ALL-10 contained eight doses of native *E.coli* asparaginase (5,000 IU/m^2, every three days) in induction and 15 doses of PEGasparaginase (2,500 IU/m^2, biweekly) in intensification. When an allergy occurred, formulations were switched to *Erwinia* asparaginase (20,000 IU/m^2, three times a week).
In ALL-11, native *E.coli* asparaginase was omitted to prevent hypersensitivity reactions and replaced by three doses of PEGasparaginase (1,500 IU/m^2, biweekly). The intensification phase contained 14 doses, individualized based on trough asparaginase activity levels. Week levels were measured after the first dose in induction and intensification to detect silent inactivation.
In case of asparaginase inactivation, patients were switched to *Erwinia* asparaginase (20,000 IU/m^2, three times a week). The dose and/or dosing schedule was adjusted to ensure adequate levels.

Asparaginase activity analysis

Asparaginase activity levels were measured using the AHA-test as described earlier.[20, 8] Asparaginase inactivation was defined as PEGasparaginase activity <100 IU/L after one week and/or <10 IU/L after two weeks (trough level). The targeted trough asparaginase activity level was 100–250 IU/L.

Costs

Direct medical costs of the asparaginase treatment were calculated from a hospital perspective and were retrieved from medical files. The different cost categories with corresponding costs were described in Table 1 and 2.

Table 1. Cost categories

Cost categories	Costs
PEGasparaginase	$1,387/vial of 3,750 IU
Erwinia asparaginase	$850/vial of 10,000 IU
Native *E. coli* asparaginase	$77/vial of 5,000 IU
Daycare treatment for asparaginase administration	$250/day
Outpatient clinic visits preceding an asparaginase dose or week asparaginase activity level	$152/visit
Laboratory tests*	$7/set of tests
TDM#	$105

TDM: Therapeutic drug monitoring *Tests routinely measured prior to an asparaginase dose, including AST, ALT, bilirubin, amylase and glucose. # The TDM costs include the costs for the AHA-test and the formulation of the dosing advice.

These categories included the costs for asparaginase use ($1,387 per vial of 3,750 IU PEGasparaginase, $850 per vial of 20,000 IU *Erwinia* asparaginase and $77 per vial of 5,000 IU native *E.coli* asparaginase), daycare treatment for asparaginase administration ($250 per day), outpatient clinic visits preceding an asparaginase dose or week level ($152 per visit), specific laboratory tests which were performed prior to an asparaginase dose ($7 for AST, ALT, bilirubin, amylase and glucose measurement), and the costs for TDM ($105 per AHA-test and dosing advice). The latter cost category included the costs for the test itself but also the costs for the preparation of the blood sample, the dose advice and the effort of prescribing the adjusted dose (Table 2). Transport costs were not included because this will considerably differ between countries and pediatric oncology centers.

Table 2. Therapeutic drug monitoring costs

	Costs per hour	Duration (min)	Total costs
Preparation of the blood sample	$30	10	$5
Test*			
Equipment costs	-	-	$9
Chemicals	-	-	$24
Technician costs	$30	41	$22
Administration costs	$30	47	$25
Total costs	-	-	$80
Advise**			
Determine advises dose	$120	5	$10
Prescription of medication	$120	5	$10
Total	-	-	**$105**

*Based on 1200 asparaginase activity samples a year. ** The formulation of the dosing advices is done by pediatric oncologists.

The asparaginase-related costs were calculated by counting the amount of cost categories per patient, multiplied with the unit prices. Dutch tariffs were used for unit prices.[21] All costs were converted to US dollars according to the average currency exchange rate of 2015 (€1=$1,067).

Analysis

The data was analyzed with the software package SPSS Statistics version 21.0 (IBM Corp, Armonk, New York, USA). To calculate the differences between the treatment protocols, we used *t-tests*, χ^2-tests or Mann-Whitney *U*-tests. Two-sided p-values of <0.05 were considered statistically significant.

The following comparisons were made: first, the total asparaginase costs of ALL-11 (individualized dosing, starting with 1,500 IU/m^2 PEGasparaginase) were compared with ALL-10 (fixed dose of 2,500 IU/m^2 PEGasparaginase). Second, the two protocols were compared excluding the patients with a hypersensitivity reaction because the occurrence of hypersensitivity reactions and subsequent use of *Erwinia* asparaginase was strongly influenced by the earlier use of native *E.coli* asparaginase in induction, which has a major impact on the costs.[19] Third, the hypothetical costs of ALL-11 with a fixed dose of 1,500 IU/m^2 and no TDM were calculated using the existing data of ALL-11 and compared with the individualized ALL-11 protocol. Thus, the lower starting dose of ALL-11 compared to ALL-10 was taken into account.

Asparaginase vials must be used directly after opening so waste should be taken into consideration. One vial contains 3,750 IU PEGasparaginase. The number of doses that can be prepared out of a single vial depends on the (mean) absolute dose and the number of patients that are treated with asparaginase on the same day. The total costs per patient were also calculated, taking this into account.

Sensitivity analysis

To evaluate the uncertainty of the data, a one-way sensitivity analysis was performed: the cost categories were varied one by one (mean costs ± 1 SD) to assess their impact on the total costs.

RESULTS

Patient characteristics

The patient characteristics are described in Table 3. In total, 134 patients were included: 83 patients were treated according to the ALL-10 protocol with a fixed dose of 2,500 IU/m^2, and 51 patients were treated according to the individualized dosing schedule of the ALL-11 protocol. The incidence of hypersensitivity reactions was lower in the ALL-11 protocol than in ALL-10 (10% vs. 22%, p=0.077). The patients who were treated according to the ALL-11 protocol were slightly older and had a higher BSA than the ALL-10 patients (both not significantly different).

Table 3. Patient and treatment characteristics

	ALL-10 (n=83)	ALL-11 (n=51)	P-value
Sex (% male, 95% CI)	39% (28 – 49%)	41% (27 – 55%)	0.763
Age (years) (median, range)	5.0 (1.5 – 18.1)	6.4 (1.3 – 17.1)	0.074
BSA (m^2) (median, range)			
Start intensification	0.8 (0.4 – 2.0)	0.9 (0.5 – 2.3)	0.105
Week 19 of intensification	0.8 (0.5 – 2.1)	0.9 (0.6 – 2.2)	0.218
Number of PEGasparaginase doses (median, range) (mean)	15 (0 – 15) (14.5)	17 (1 – 17) (16.4)	<0.001
Number of *Erwinia* asparaginase doses (median, range)	0 (0 – 84)	0 (0 – 99)	0.170
Number of native *E. coli* asparaginase doses (median, range)	8 (3 – 8)	0 (0 – 4)	<0.001
Number of AHA-tests (median, range)	0	16 (2 – 53)	<0.001
Number of out-patient clinic visits (median, range)	23 (2 – 100)	18 (1 – 41)	<0.001
Number of daycare admissions (median, range)	23 (3 – 94)	17 (1 – 105)	<0.001
Number of laboratory tests (median, range)	6 (1 – 23)	16 (6 – 40)	<0.001
Hypersensitivity reactions (%, 95% CI)	22% (13 – 31)	10% (01 – 18)*	0.077
Average absolute PEGasparaginase (IU) dose (median, range)	2,025 (1,225 – 5,188)	726 (366 – 2,400)	<0.001
Average PEGasp dose (IU/m^2) per patient (median, range)	2500	778 (482 – 1500)	<0.001

* Four patients had silent inactivation of PEGasparaginase, one patient had an allergy to PEGasparaginase.

AHA-test: method used to measure asparaginase activity levels.

Asparaginase costs

The total asparaginase-related costs of the ALL-10 protocol and ALL-11 protocol are described in Table 4; the costs of these protocols without patients with a hypersensitivity reaction, and the costs of the hypothetical protocol with a fixed dose of 1,500 IU/m^2 PEGasparaginase and no TDM are described in Table 5.

Table 4. Total asparaginase-related costs ($)

Cost category	ALL-10 Mean ± SD; % of total median (IQR)	ALL-11 Mean ± SD; % of total median (IQR)	Incremental costs Mean	P-value
PEGasparaginase (IU used)	10,604 ± 6,867; 22.4% 10,064 (5,112)	4,363 ± 2,168; 15.0% 3,733 (3,293)	-6,241	<0.001
Erwinia asparaginase (IU used)	18,624 ± 40,552; 44.4% 0 (0)	13,816 ± 43,506; 47.6% 0 (0)	-4,808	0.159**
Native E. coli asparaginase (IU used)	750 ± 284; 1.6% 614 (0)	8 ± 54; 0.03% 0 (0)	-742	<0.001**
Daycare	8,129 ± 5,487; 17.2% 5,771 (0)	5,874 ± 5,493; 20.0% 4,265 (0)	-2255	<0.001*
Outpatient clinic visits	3,803 ± 1,586; 8.0% 3,502 (0)	2,953 ± 1,013; 10.2% 2,741 (152)	-850	<0.001**
Laboratory tests	51 ± 37; 0.1% 43 (50)	118 ± 36; 0.4% 114 (14)	67	<0.001*
TDM	-	1,917 ± 945; 6.6% 1,680 (210)	1,917	<0.001**
Total	41,960 ± 43,205 22,268 (14,529)	29,048 ± 49,787 13,010 (4,283)	-12,912 (30.8%)	<0.001**

No CI because of logarithmic transformation (*) or Mann-Whitney test (**). SD: standard deviation; IQR: interquartile range; TDM: therapeutic drug monitoring.

First, the comparison between the ALL-10 protocol (fixed dose of 2,500 IU/ m^2 PEGasparaginase) and ALL-11 protocol (individualized dosing, starting with 1,500 IU/ m^2 PEGasparaginase) was made (Table 4): the asparaginase-related costs of ALL-11 were 30.8% lower than ALL-10 ($29,048 versus $41,960, p<0.001). These costs were calculated using the absolute asparaginase dose, disregarding asparaginase waste. If the whole vial has to be used for each asparaginase dose, the asparaginase-related costs for ALL-11 would still be less than for ALL-10, although the difference is smaller: $47,624 versus $54,892 for ALL-11 and ALL-10 respectively (p<0.001). One patient of the ALL-11 protocol was initially treated with native *E. coli* asparaginase after silent inactivation of PEGasparaginase but switched to *Erwinia* asparaginase after a few doses. In the ALL-11 protocol, laboratory tests were standardly done before each PEGasparaginase dose, in contrast to the ALL-10 protocol, where they were ordered based on doctor's choice.

Table 5. Total asparaginase-related costs ($) in patients without a hypersensitivity reaction

Cost category	ALL-10 (n=66) Mean ± SD; % of total median (IQR)	ALL-11 (n=46) Mean ± SD; % of total median (IQR)	Incremental costs Mean	P-value
PEGasparaginase (IU used)	13,015 ± 5,744; 56.8% 11,063 (5,042)	4,672 ± 2,043; 35.5% 3,905 (3,204)	-8,343	<0.001
Native E. coli asparaginase (IU used)	747 ± 292; 3.3% 614 (0)	-	-747	<0.001
Daycare	5,662 ± 627; 24.7% 5,771 (0)	4,107 ± 640; 31.2% 4,265 (0)	-1,555	<0.001
Outpatient clinic visits	3,438 ± 399; 15.0% 3,502 (0)	2,658 ± 434; 20.2% 2,741 (38)	-780	<0.001
Laboratory tests	52 ± 37; 0.2% 43 (54)	111 ± 19; 0.8% 114 (16)	59	<0.001
TDM	-	1,630 ± 266; 12.4% 1,680 (131)	1,630	<0.001
Total	22,916 ± 6,373 20,965 (5,503)	13,178 ± 2,660 12,548 (3,345)	-9,738 (42.5%)	<0.001

SD: standard deviation; IQR: interquartile range; TDM: therapeutic drug monitoring.

Secondly, the comparison was made between ALL-10 and ALL-11 without patients who had developed a hypersensitivity reaction (Table 5) because the difference in costs for *Erwinia* asparaginase use contributes to a substantial amount of the incremental costs. The risk of switching to this type of asparaginase was higher in ALL-10 due to native *E.coli* asparaginase use in induction. Excluding hypersensitive patients, the total asparaginase-related costs were 42.5% ($p<0.001$) lower in ALL-11 ($13,178) when compared to ALL-10 ($22,916). The mean costs for the TDM were $1,630 which was 12.4% of the total costs.

Table 6. Total asparaginase-related costs ($), in patients without a hypersensitivity reaction

Cost category	ALL-11 TDM (n=46) Mean ± SD; % of total median (IQR)	ALL-11 Fixed dose (n=46) Mean ± SD; % of total median (IQR)	Incremental costs Mean	P-value
PEGasparaginase (IU used)	4,672 ± 2,043; 35.5% 3,905 (3,204)	9,675 ± 4,218; 58.5% 8,618 (5,678)	-5,003	<0.001
Daycare	4,107 ± 640; 31.2% 4,265 (0)	4,107 ± 640; 24.8% 4,265 (0)	0	-
Outpatient clinic visits	2,658 ± 434; 20.2% 2,741 (38)	2,658 ± 434; 16.1% 2,741 (38)	0	-
Laboratory tests	111 ± 19; 0.8% 114 (16)	111 ± 19; 0.7% 114 (16)	0	-
TDM	1,630 ± 266; 12.4% 1,680 (131)	-	1,630	<0.001
Total	13,178 ± 2,660 12,548 (3,345)	16,551 ± 4,626 15,698 (5,911)	-3,373 (20.4%)	<0.001

SD: standard deviation; IQR: interquartile range; TDM: therapeutic drug monitoring.

Thirdly, the comparison was made between the ALL-11 protocol and the hypothetical ALL-11 protocol with a fixed dose of 1,500 IU/m^2 PEGasparaginase and no TDM (Table 6) because the starting dose of PEGasparaginase was 1,000 IU/m^2 lower in ALL-11 than in ALL-10, which already led to a cost reduction. Furthermore, the fact that three doses of PEGasparaginase were used instead of eight native *E.coli* asparaginase doses in induction resulted in a cost reduction of daycare admissions and outpatient clinic visits. On the other hand, the drug PEGasparaginase is more expensive than native *E.coli* asparaginase (Table 1). The costs of PEGasparaginase use of the ALL-11 protocol ($4,672) were 48.3% lower (p<0.001) than the PEGasparaginase costs of the hypothetical protocol with the fixed dose of 1,500 IU/m^2 ($9,675). Taking into account the costs for the AHA-tests as well, in total, still $3,373 (20.4%) will be saved per patient when using TDM (p<0.001).

In clinical practice, left-over asparaginase after preparation of the dose may have to be discarded if it cannot be used for another patient. However, if patients are treated at the same day, multiple doses can be prepared out of a single vial. Supplemental figure 1 shows the total asparaginase-related costs when vials can be shared between patients, taking into account the PEGasparaginase waste. Depending on the number of patients treated at the same day and the amount of PEGasparaginase administered per dose, multiple doses can be prepared out of

the same vial, resulting in less waste per dose. Supplemental figure 1 shows that, in our cohort, TDM is cost saving when compared to a fixed dose of 1,500 IU/m^2, if three patients are treated with asparaginase on the same day. If only one PEGasparaginase dose can be prepared out of a single vial, TDM would be more expensive than the hypothetical protocol due to the measurement of asparaginase activity levels and the formulation of dosing advices. More information about this analysis is included in the Supplemental results.

Sensitivity analysis

The one-way sensitivity analysis is shown in a tornado diagram (Figure 1). *Erwinia* asparaginase use has the greatest impact on the total costs. Except for the *Erwinia* asparaginase costs, the maximum costs of ALL-11 did not exceed the mean ALL-10 costs when varying the cost categories one by one with 1 SD. The same is true for the minimal costs of ALL-10.

Figure 1. One-way sensitivity analysis of the ALL-10 and ALL-11 costs

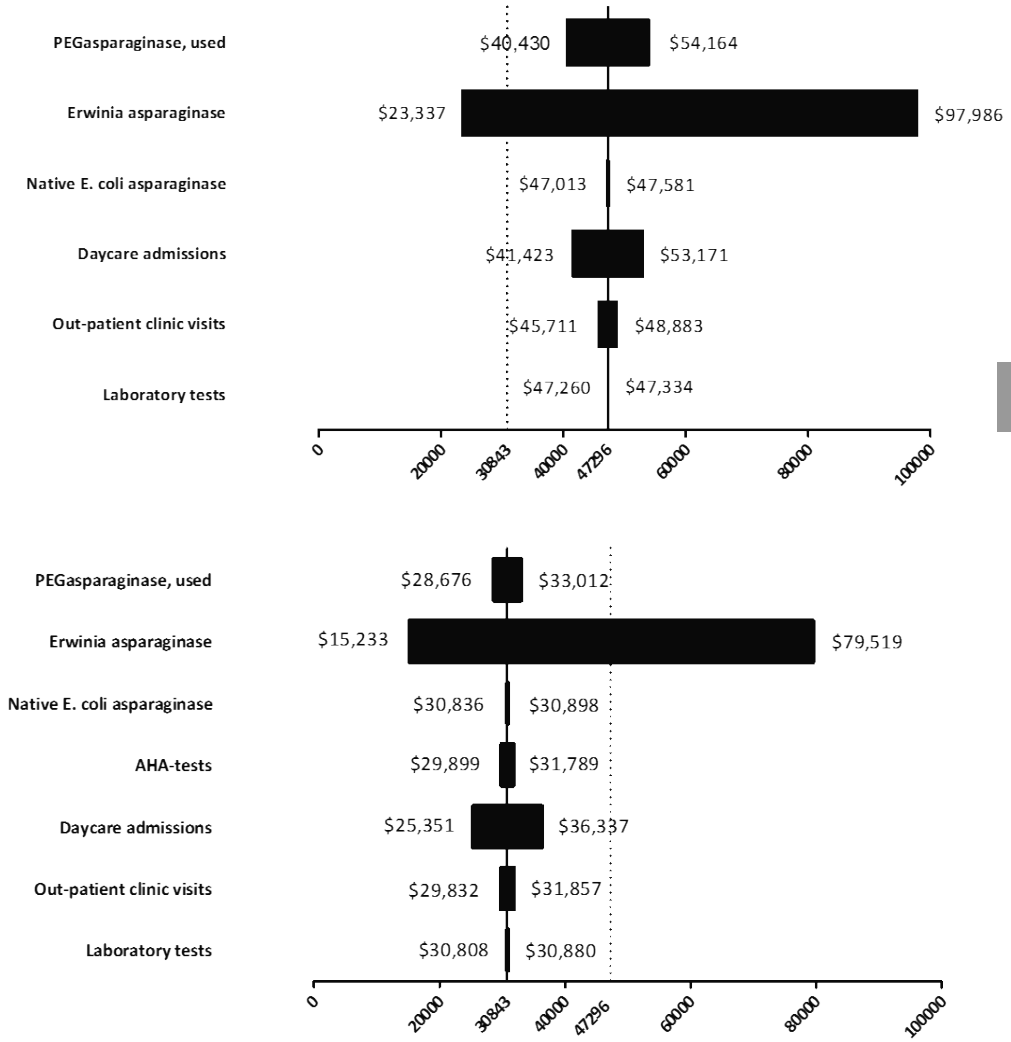


Figure 1a shows the range of mean total asparaginase costs for ALL-10: for each cost category, the mean ± 1 SD of each category was varied to calculate the total asparaginase costs. The minimal costs of *Erwinia* asparaginase were < $0 but were fixed at $0. The dotted line represents the mean total costs of ALL-11 ($29,048).
Figure 1b shows the same for the ALL-11 protocol. Again, the minimal costs of *Erwinia* asparaginase were <$0 and were fixed at $0. The dotted line represents the mean ALL-10 costs ($41,960).

DISCUSSION

In this study, several comparisons were made to study the effect of TDM on the asparaginase-related costs. Although the comparison between real treatment protocols is important, the fairest comparison made was between the ALL-11 protocol and the hypothetical ALL-11 protocol with a fixed dose of 1,500 IU/m^2 and no TDM. This comparison has shown that TDM is costs saving despite the extra costs for the asparaginase activity analysis and dosing advices. However, with the current size of PEGasparaginase vials (3,750 IU), the costs will be higher than a fixed dosing schedule if left-over asparaginase from vials cannot be used for other patients: much asparaginase has to be discarded if only one or two doses can be prepared at the same time, which is the case in many pediatric oncology centers. Hence, centralization of pediatric oncology care would reduce the asparaginase waste and subsequent costs. The size of PEGasparaginase vials is based on the adult asparaginase dose of 2,000 IU/m^2, for whom the average absolute dose is approximately 3,600 IU as the mean adult BSA is 1,79 m^2.[22] Therefore, , smaller vials should become available to reduce the waste for pediatric asparaginase doses and lower doses in adults.

Among the current treatment protocols used to treat children with ALL, asparaginase treatment differs considerably regarding the number of asparaginase doses and asparaginase dosage. Thus, the expenses that could be saved when using TDM will vary between countries. However, as PEGasparaginase is an expensive drug , a dose reduction of approximately 50% will significantly decrease the total treatment costs in most cases. Moreover, the starting dose of ALL-11 (1500 IU/m^2) is relatively low compared to other treatment protocols so theoretically the dose could be reduced even more, which will relatively save even more costs.

Several pharmacoeconomic analyses of pediatric ALL treatment and asparaginase treatment have been performed.[23-25, 19, 26] However, none of these include TDM. Furthermore, analyses about TDM mainly focused on clinical outcomes such as hospitalization or complication rates while our study focused on cost savings due to dose reduction of asparaginase.[27-30]
In this study, we have only performed a cost-analysis, without including the effects of individualized asparaginase doses. In the short term, lower asparaginase activity levels may reduce toxicity such as hepatotoxicity and dyslipidemia. This, however, will have little impact on the total costs but will, of course, be important

for the patient. In addition, also the risk of pancreatitis, thromboembolic events and central neurotoxicity might be reduced, although the incidence of these side effects is relatively low and patient numbers may be too small to observe an effect.
But more importantly, TDM of asparaginase will improve the long-term outcome of children with ALL: first, silent inactivation of asparaginase will be detected, which will result in a better EFS if formulations are switched. [31, 32] This will probably increase the total costs as relatively more patients will be treated with *Erwinia* asparaginase. However, because of a lower relapse rate, the costs may be lower though, as expensive relapse therapy will be given less frequently. In our ALL-11 cohort, only four cases of silent inactivation occurred, which makes it difficult to draw conclusions regarding these costs. Second, allergic-like reactions can only be distinguished from real allergies by TDM.[8] The influence on the costs, however, should be studied more closely because not switching to *Erwinia* asparaginase after an allergic-like reaction to PEGasparaginase will reduce the costs, while continuation of asparaginase after an allergic-like reaction to *Erwinia* asparaginase will increase the direct medical asparaginase-related costs. Regardless of these direct costs, TDM allows patients with an allergic-like reaction to *Erwinia* asparaginase to finish their asparaginase treatment, which will improve their outcome and consequently prevent relapse associated costs.

In our study, the TDM costs accounted for 12% of the total asparaginase-related costs. These costs may be an overestimation because of two reasons: first, the number of asparaginase activity levels measured is relatively high because enough insight in the population pharmacokinetics of PEGasparaginase is lacking. If we would be able to better predict asparaginase activity levels in individual patients, less AHA-tests would be needed, which would reduce the costs. Second, our TDM administration costs are relatively high as we now need 47 minutes per sample for administration. This includes processing the samples before activity measurement and entering all information in the database which is used for the formulation of dosing advices. Especially the latter is time consuming and is partly caused by entering information which is irrelevant for the dosing advices, but necessary for research purposes. Without the administration costs, the actual AHA-test costs $55.

In conclusion, TDM of asparaginase will save expenses when calculated with the amount of IU asparaginase administered. TDM will also be cost saving if the asparaginase waste is minimized by preparing multiple doses out of a single vial or

if smaller vials become available. Although the effects of TDM were not included in the analysis, TDM has several advantages including the possible prevention of toxicity, the detection of silent inactivation, the distinction between real allergies and allergic-like reactions, and consequently the improvement of the EFS. Therefore, we recommend TDM of asparaginase from a clinical and cost perspective.

REFERENCES

1. Amylon, M. D., J. Shuster, J. Pullen, C. Berard, M. P. Link, M. Wharam, 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. Rizzari, C., M. G. Valsecchi, M. Arico, V. Conter, A. Testi, E. Barisone, 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.
3. Silverman, L. B., R. D. Gelber, V. K. Dalton, B. L. Asselin, R. D. Barr, L. A. Clavell, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97(5):1211-1218.
4. Duval, M., S. Suciu, A. Ferster, X. Rialland, B. Nelken, P. Lutz, 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.
5. Pession, A., M. G. Valsecchi, G. Masera, W. A. Kamps, E. Magyarosy, C. Rizzari, 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.
6. Moghrabi, A., D. E. Levy, B. Asselin, R. Barr, L. Clavell, C. Hurwitz, 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.
7. Tong, W. H., R. Pieters, G. J. Kaspers, D. M. te Loo, M. B. Bierings, C. van den Bos, 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.
8. Kloos, R. Q., R. Pieters, G. Escherich and I. M. van der Sluis. Allergic-like reactions to asparaginase: Atypical allergies without asparaginase inactivation. Pediatr Blood Cancer. 2016.
9. Hempel, G., H. J. Muller, C. Lanvers-Kaminsky, G. Wurthwein, A. Hoppe and J. Boos. A population pharmacokinetic model for pegylated-asparaginase in children. Br J Haematol. 2010;148(1):119-125.
10. Pieters, R., H. de Groot-Kruseman, V. Van der Velden, M. Fiocco, H. van den Berg, E. de Bont, et al. Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group. J Clin Oncol. 2016.
11. Riccardi, R., J. S. Holcenberg, D. L. Glaubiger, J. H. Wood and D. G. Poplack. L-asparaginase pharmacokinetics and asparagine levels in cerebrospinal fluid of rhesus monkeys and humans. Cancer Res. 1981;41(11 Pt 1):4554-4558.
12. Ahlke, E., U. Nowak-Gottl, P. Schulze-Westhoff, G. Werber, H. Borste, G. Wurthwein, et al. Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during induction therapy in children with acute lymphoblastic leukaemia. Br J Haematol. 1997;96(4):675-681.
13. Rizzari, C., M. Zucchetti, V. Conter, L. Diomede, A. Bruno, L. Gavazzi, et al. L-asparagine depletion and L-asparaginase activity in children with acute lymphoblastic leukemia receiving i.m. or i.v. Erwinia C. or E. coli L-asparaginase as first exposure. Ann Oncol. 2000;11(2):189-193.
14. Albertsen, B. K., H. Schroder, P. Jakobsen, H. J. Muller, N. T. Carlsen and K. Schmiegelow. Monitoring of Erwinia asparaginase therapy in childhood ALL in the Nordic countries. Br J Clin Pharmacol. 2001;52(4):433-437.
15. Avramis, V. I., S. Sencer, A. P. Periclou, H. Sather, B. C. Bostrom, L. J. Cohen, et al. 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(6):1986-1994.
16. Grigoryan, R. S., E. H. Panosyan, N. L. Seibel, P. S. Gaynon, I. A. Avramis and V. I. Avramis. Changes of amino acid serum levels in pediatric patients with higher-risk acute lymphoblastic leukemia (CCG-1961). In Vivo. 2004;18(2):107-112.

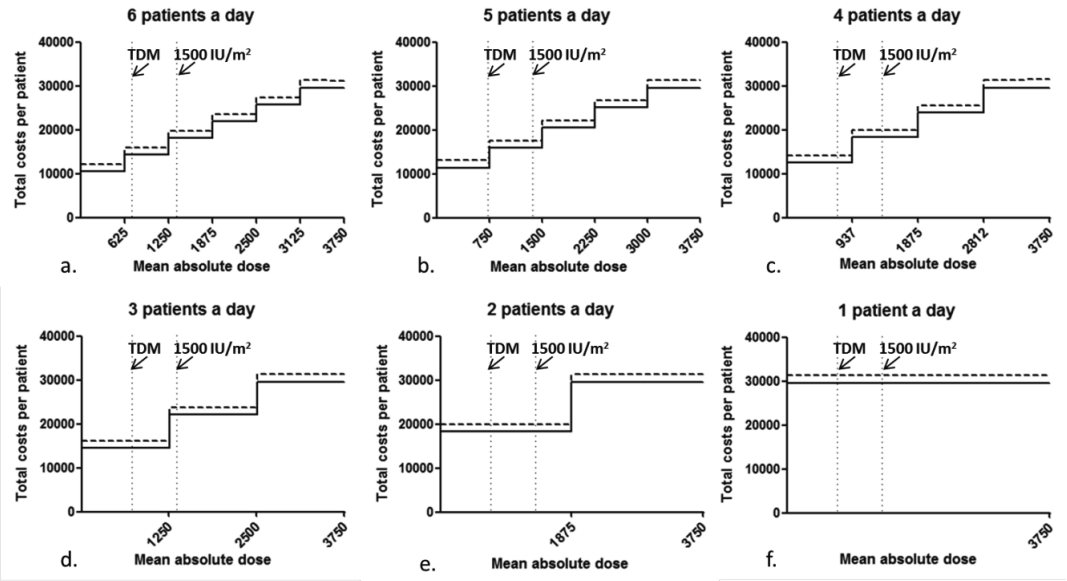
17. Tsurusawa, M., M. Chin, A. Iwai, K. Nomura, H. Maeba, T. Taga, et al. L-Asparagine depletion levels and L-asparaginase activity in plasma of children with acute lymphoblastic leukemia under asparaginase treatment. Cancer Chemother Pharmacol. 2004;53(3):204-208.
18. Pieters, R., I. Appel, H. J. Kuehnel, I. Tetzlaff-Fohr, U. Pichlmeier, I. van der Vaart, et al. Pharmacokinetics, pharmacodynamics, efficacy, and safety of a new recombinant asparaginase preparation in children with previously untreated acute lymphoblastic leukemia: a randomized phase 2 clinical trial. Blood. 2008;112(13):4832-4838.
19. Tong, W. H., I. M. van der Sluis, C. J. Alleman, R. R. van Litsenburg, G. J. Kaspers, R. Pieters, et al. Cost-analysis of treatment of childhood acute lymphoblastic leukemia with asparaginase preparations: the impact of expensive chemotherapy. Haematologica. 2013;98(5):753-759.
20. Lanvers, C., J. P. Vieira Pinheiro, G. Hempel, G. Wuerthwein and J. Boos. 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.
21. Hakkaart, L., S. S. Tan and B. C. A. M. Manual for costing research. Methods and guideline prices for economic evaluations in health care. Rotterdam: Health Cae Insurance Board. 2010.
22. Sacco, J. J., J. Botten, F. Macbeth, A. Bagust and P. Clark. The average body surface area of adult cancer patients in the UK: a multicentre retrospective study. PLoS One. 2010;5(1):e8933.
23. Rahiala, J., P. Riikonen, L. Kekalainen and M. Perkkio. Cost analysis of the treatment of acute childhood lymphocytic leukaemia according to Nordic protocols. Acta Paediatr. 2000;89(4):482-487.
24. Kurre, H. A., A. G. Ettinger, D. L. Veenstra, P. S. Gaynon, J. Franklin, S. F. Sencer, et al. A pharmacoeconomic analysis of pegaspargase versus native Escherichia coli L-asparaginase for the treatment of children with standard-risk, acute lymphoblastic leukemia: the Children's Cancer Group study (CCG-1962). J Pediatr Hematol Oncol. 2002;24(3):175-181.
25. van Litsenburg, R. R., C. A. Uyl-de Groot, H. Raat, G. J. Kaspers and R. J. Gemke. Cost-effectiveness of treatment of childhood acute lymphoblastic leukemia with chemotherapy only: the influence of new medication and diagnostic technology. Pediatr Blood Cancer. 2011;57(6):1005-1010.
26. Kaul, S., E. K. Korgenski, J. Ying, C. F. Ng, R. R. Smits-Seemann, R. E. Nelson, et al. A retrospective analysis of treatment-related hospitalization costs of pediatric, adolescent, and young adult acute lymphoblastic leukemia. Cancer Med. 2016;5(2):221-229.
27. van Lent-Evers, N. A., R. A. Mathot, W. P. Geus, B. A. van Hout and A. A. Vinks. Impact of goal-oriented and model-based clinical pharmacokinetic dosing of aminoglycosides on clinical outcome: a cost-effectiveness analysis. Ther Drug Monit. 1999;21(1):63-73.
28. Ostad Haji, E., K. Mann, A. Dragicevic, M. J. Muller, K. Boland, M. L. Rao, et al. Potential cost-effectiveness of therapeutic drug monitoring for depressed patients treated with citalopram. Ther Drug Monit. 2013;35(3):396-401.
29. Velasco, A., O. Chung, F. Raza, A. Pandey, S. Brinker, D. Arbique, et al. Cost-Effectiveness of Therapeutic Drug Monitoring in Diagnosing Primary Aldosteronism in Patients With Resistant Hypertension. J Clin Hypertens (Greenwich). 2015;17(9):713-719.
30. Deitelzweig, S., M. Evans, E. Hillson, J. Trocio, A. Bruno, W. Tan, et al. Warfarin time in therapeutic range and its impact on healthcare resource utilization and costs among patients with nonvalvular atrial fibrillation. Curr Med Res Opin. 2016;32(1):87-94.
31. Panosyan, E. H., N. L. Seibel, S. Martin-Aragon, P. S. Gaynon, I. A. Avramis, H. Sather, 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.
32. Vrooman, L. M., K. E. Stevenson, J. G. Supko, J. O'Brien, S. E. Dahlberg, B. L. Asselin, 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.

SUPPLEMENTAL RESULTS

Supplemental figure 1 illustrates when TDM will become cost saving, varying the number of doses that can be prepared out of a single vial. The mean absolute dose of ALL-11 with TDM was 726 IU; the mean absolute dose of ALL-11 with a fixed dose of 1,500 IU/m^2 would be 1,350 IU, calculated with the mean overall BSA of ALL-11, which was 0.9 m^2. The figure shows that, in our cohort, TDM is cost saving when compared to a fixed dose of 1,500 IU/m^2, if three patients are treated with asparaginase at the same day: if the doses of these three patients are individualized, they can be prepared out of one vial of 3,750 IU (2,178 IU PEGasparaginase needed in total; mean total costs of the asparaginase treatment: $16,088) but a second vial should be opened if these patients would be treated with a fixed dose of 1,500 IU/m2 (4,080 IU PEGasparaginase needed in total; mean total costs of the asparaginase treatment: $22,041), saving $5,953 per patient, including TDM costs.

Although the costs shown in Supplemental figure 1 are not applicable for other pediatric oncology centers and treatment protocols, the figure can be used to check whether TDM would be cost saving for other centers with certain amounts of patients and absolute doses.

Supplemental figure 1. Mean total costs per patient, taking into account the PEGasparaginase waste.

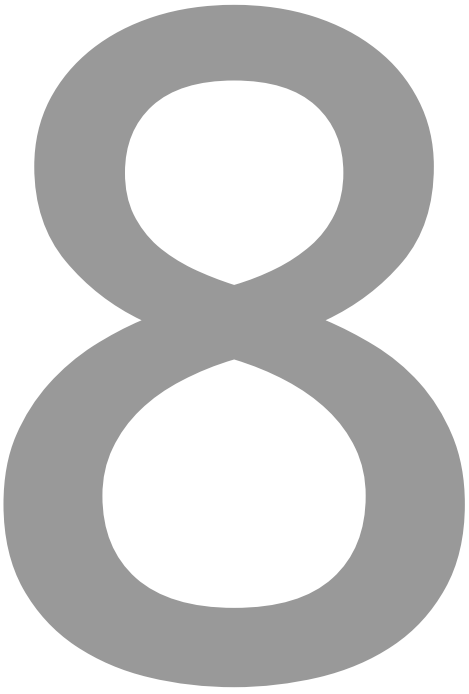


Supplemental figure 1 shows when TDM is cost saving when compared to a fixed dosing schedule, assuming that vials (3,750 IU) should be used completely after opening.

The mean total costs of asparaginase treatment are plotted against the mean absolute asparaginase dose for the situations of six (a), five (b), four (c), three (d), two (e) and one (f) asparaginase administration(s) on the same day: the horizontal dashed lines shows the mean total costs of ALL-11 with TDM; the horizontal solid lines the mean total costs per patient of ALL-11 with a fixed dose of 1500 IU/m^2. The vertical dotted lines represents the mean absolute asparaginase dose of ALL-11 with TDM (726 IU) and with a fixed dose of 1500 IU/m^2 (1,350 IU, calculated with an overall mean BSA of 0.9 m^2 and with the mean number of PEGasparaginase doses of this cohort, 16.4 doses).

The total asparaginase related costs increase when the absolute amount of asparaginase needed exceeds the content of a vial and an extra vial has to be opened for each dose.

CHAPTER 8



A Cost-effectiveness Analysis of Erwinia Asparaginase Therapy in Children with Acute Lymphoblastic Leukemia

Robin Q.H. Kloos, Raphaële R.L. van Litsenburg, Sarah Wolf
Leonoor Wismans, Gertjan J.L. Kaspers, Carin A. Uyl-de Groot
Rob Pieters, Inge M. van der Sluis

Pediatric Blood & Cancer 2018; 66

ABSTRACT

Background *Erwinia* asparaginase is used as second-line formulation after a neutralizing hypersensitivity reaction to the first-line formulation of asparaginase. Here, we have performed a cost-effectiveness analysis of *Erwinia* asparaginase treatment.

Methods Children with acute lymphoblastic leukemia treated according to the Dutch Childhood Oncology ALL-10 or ALL-11 protocol were included and initially treated with PEGasparaginase in the intensification phase. The total treatment costs of this treatment phase, quality of life (QoL), and life years saved (LYS) were studied for two scenarios: 1) patients were switched to *Erwinia* asparaginase treatment after a hypersensitivity reaction; 2) asparaginase would have been permanently stopped.

Results Sixty-eight patients were included. There was no difference in QoL between patients with and without a hypersensitivity reaction. The mean costs of the intensification phase per patient were $40,925 if PEGasparaginase could be continued, $175,632 if patients had to switch to *Erwinia* asparaginase, and $21,190 if asparaginase would have been stopped permanently. An extrapolation of the literature suggests that the 5-year event-free survival would be 10.3% lower without intensive asparaginase treatment, so if asparaginase is stopped after a reaction. Thus, the costs per LYS were $1,892 for scenario 1 and $872 for scenario 2.

Conclusions Switching to *Erwinia* asparaginase increases the costs per LYS by $1,020, which is modest in view of the total costs. Moreover, when asparaginase treatment can be completed by switching to *Erwinia* asparaginase, relapses - and consequential costs - will be avoided. Therefore, from a cost perspective, we recommend a switch to *Erwinia* asparaginase to complete asparaginase treatment.

INTRODUCTION

Asparaginase is a cornerstone of the treatment of acute lymphoblastic leukemia (ALL) in children as adequate, intensive treatment improves the event-free survival (EFS) significantly.[1-7] However, asparaginase treatment may be hampered by the development of hypersensitivity reactions, generally resulting in complete neutralization of the drug. This requires a switch in formulations to maintain adequate asparaginase activity levels.[8-10] In most developed countries, PEGasparaginase is now used as a first-line formulation and *Erwinia* asparaginase as second-line. The latter formulation is administered more frequently than PEGasparaginase (three times a week instead of every other week) due to different half-life's of the two drugs, resulting in a substantial increase in therapy costs.[11, 12] Due to increasing restrictions on healthcare resources, evaluations of costs in relation to benefits become more important, especially for expensive drugs, such as *Erwinia* asparaginase. Therefore, we have performed a cost-effectiveness analysis in which we have compared the costs, quality of life, and life years saved between two scenarios: according to scenario 1, patients were switched to *Erwinia* asparaginase after a hypersensitivity reaction to PEGasparaginase. Scenario 2, which was unethical and therefore hypothetical, described the situation in which the asparaginase treatment was permanently stopped after a hypersensitivity reaction to PEGasparaginase.

METHODS

Patients

The study was performed in the Sophia Children's Hospital, Rotterdam, the Netherlands and the VU University Medical Center, Amsterdam, the Netherlands. Patients were enrolled prospectively between May 2012 and October 2016 and were treated according to the medium risk group of the Dutch Childhood Oncology Group (DCOG) ALL-10 (until April 2012) or the consecutive ALL-11 protocol. The study was approved by the Institutional Review Board. Informed consent was obtained from children >12 years old, parents or children's guardians in accordance with the Declaration of Helsinki.

Treatment Protocols

In this study, the treatment costs of the intensification phase, which contained the majority of the asparaginase doses and hypersensitivity reactions to PEGasparaginase, were calculated. According to ALL-10, patients were treated with native *E. coli* asparaginase (eight intravenous doses of 5,000 IU/m^2) in the induction phase, and 15 doses of PEGasparaginase (2,500 IU/m^2, biweekly, intravenous) in the intensification phase. According to ALL-11, patients were treated with PEGasparaginase in both the induction (three intravenous doses, 1,500 IU/m^2) and the intensification phase (14 intravenous doses, biweekly). In this protocol, therapeutic drug monitoring (TDM) was used to individualize the doses in the intensification phase based on asparaginase activity levels. In case of a hypersensitivity reaction, defined as an allergy to or silent inactivation of PEGasparaginase, patients were switched to *Erwinia* asparaginase (20,000 IU/m^2, three times a week). According to ALL-11, the *Erwinia* asparaginase dose and/or dosing schedule was individualized to ensure adequate asparaginase activity levels.

Table 1 describes an overview of the first 30 weeks of the ALL intensification therapy for ALL-10 and ALL-11.

Table 1. Intensification of the DCOG ALL-10 and ALL-11 treatment protocols

Intensification (30 weeks)	DCOG ALL-10	DCOG ALL-11
Dexamethasone	6 mg/m^2/day orally Day 0-4, every 3 weeks, starting in week 1	6 mg/m^2/day orally Day 0-4, every 3 weeks, starting at week 1
Vincristine	2 mg/m^2/dose intravenously Every 3 weeks, starting in week 1	2 mg/m^2/dose intravenously Every 3 weeks, starting in week 1
Doxorubicin	30 mg/m^2/dose intravenously Weeks 1, 4, 7, 10, 13, 16	30 mg/m^2/dose intravenously Weeks 1, 4, 7, 10 *Not in case of a TEL/AML1 translocation or Down syndrome patients without a IKZF1 deletion*
Methotrexate	30 mg/m^2/ dose intravenously 1x/week, weeks 20 – 30	30 mg/m^2/ dose intravenously 1x/week, weeks 13 – 30
PEGasparaginase	2,500 IU/m^2 intravenously Biweekly, weeks 1 - 29	Dose adjusted based on asparaginase activity levels Biweekly, weeks 1 - 27
6-Mercaptopurine	50 mg/m^2/day orally Courses of 2 weeks starting in weeks 1, 4, 7, 10, 13, 16 Daily from weeks 19 – 30	50 mg/m^2/day orally Courses of 2 weeks starting in weeks 1, 4, 7, 10 Daily from weeks 13 – 30
Intrathecal methotrexate, cytarabine and prednisolone	Methotrexate 8 – 12 mg Cytarabine 20 – 30 mg Prednisolone 8 -12 mg Week 1 and 19	Methotrexate 8 – 12 mg Cytarabine 20 – 30 mg Prednisolone 8 -12 mg Week 1 and 19

Costs Data

The direct medical costs of the intensification phase were retrospectively obtained and calculated from a Dutch hospital perspective.[13] All costs were converted to US dollars according to the average currency exchange rate of 2015 (€1=$1.067). The costs included were costs for (1) PEGasparaginase ($1,387 for one vial of 3,750 IU) and *Erwinia* asparaginase ($850 for one vial of 10,000 IU), rounded to whole vials to take into account the waste; (2) chemotherapy other than asparaginase; (3) supportive care medication; (4) out-patient clinic visits ($175 per visit in an

academic hospital, $85 in a satellite hospital); (5) daycare admissions; (6) inpatient days ($689 per day in an academic hospital, $476 in a satellite hospital); (7) intensive care unit days ($2,163 per day); (8) blood products, (9) laboratory tests; (10) surgical procedure costs (mainly for bone marrow punctures performed under complete anesthesia); and (11) TDM costs ($105, including asparaginase activity level measurements and the formulation of dosing advices[12]). The costs described included costs for staff, materials used, nutrition and overhead. Data were adapted from the medical files of the Erasmus MC Rotterdam and the VU University Medical Center. Dutch tariffs (index year 2015) retrieved from the Dutch Healthcare Authority or the hospitals were used for the unit prices.[14] Costs were discounted by 4% per year to account for the time value of money in accordance with Dutch guidelines.[14]

To calculate the costs of scenario 2 for the patients with a hypersensitivity reaction, the number of outpatient clinic visits was assumed to be equal to the median number of visits of patients without a reaction. In addition, in these patients, the daycare admissions for *Erwinia* asparaginase administration only were excluded. And finally, for ALL-11 patients with a hypersensitivity reaction, only the TDM costs that were part of the PEGasparaginase treatment were included.

Effects Data

To assess the health related quality of life (HRQoL), the Health Utilities Index (HUI) survey version 3.0 [15] was completed by the patient and/or parents in week 1, 3, 4 (in case of a hypersensitivity reaction) and 19 of the intensification phase. The questionnaire included 10 general attributes (vision, hearing, speech, emotion, pain, ambulation, dexterity, cognition, caretaking, and health) each with 5 or 6 levels, describing a patient's health state. The single-attribute utility (SAU) and multi-attribute utility (MAU) scores were calculated, representing the HRQoL for each attribute and overall, respectively. In order to calculate the quality-adjusted-life years (QALYs), the MAU scores were multiplied by the total duration of the treatment phase (30 weeks).

Beside the validated HUI questions, several extra questions about the impact of an allergic reaction and change in dosing schedule were added to the questionnaire. These questions were not validated and, therefore, could not be quantified as part of the HUI analysis.

The number of life years saved was calculated using the EFS described in literature to indicate the difference in EFS between intensified and less intensified asparaginase treatment: a systematic search was performed to find trials studying the effect of intensified asparaginase treatment. Next, a weighted mean difference of the EFS of patients with and without intensified asparaginase treatment was calculated by multiplying the difference in EFS reported with the number of patients included in the study, and dividing this by the total number of patients.

In our study, patients who were switched to *Erwinia* asparaginase after a hypersensitivity reaction to PEGasparaginase were considered to have the same prognosis as patients without an allergy as they were still intensively treated with asparaginase.[16] Because the inclusion of the ALL-10 protocol has been completed, the EFS of this protocol was used for these patients.[7]

All hypersensitivity reactions occurred during the first or second PEGasparaginase dose in intensification. Therefore, not switching would have resulted in a worse prognosis, similar to ALL treatment without asparaginase treatment during intensification. Hence, for the patients with a hypersensitivity reaction in whom, according to scenario 2, the asparaginase therapy would have been permanently stopped, we have subtracted the weighted mean difference in EFS reported in the literature, from the EFS of ALL-10.[7]

Both the QALYs and the number of life years saved were discounted by 1.5% per year to account for the value of time, according to Dutch guidelines.[14] This way, it is taken into account that life years saved in the future are considered as less valuable than life years saved today.

Statistical Analysis

Data was analyzed with SPSS Statistics version 21.0 (IBM Corp, Armonk, New York, USA) and MS Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Multiple imputation was used to impute missing data. *T-tests*, $\chi 2$-tests or Mann-Whitney U-tests were used to calculate the differences between the patients with and without a hypersensitivity reaction, and the two scenarios. The quality of life was longitudinally analyzed using generalized estimating equations. A two-sided p-value of <0.05 was considered statistically different. Data are presented as frequency, median, mean and standard deviation (SD) when appropriate.

Decision Tree Analysis

A decision tree model was developed in order to compare the costs and effects of scenario 1, which included a switch to *Erwinia* asparaginase after a hypersensitivity reaction to PEGasparaginase, to scenario 2, in which the asparaginase therapy was permanently stopped after a hypersensitivity reaction to PEGasparaginase (Figure 1). The mean costs per patient of the intensification phase and the life years saved for patients with and without a hypersensitivity reaction were calculated for both scenarios and multiplied by the probability of developing a hypersensitivity reaction. Next, the costs per life year saved were calculated by dividing the total costs by the number of life years saved.

Sensitivity Analysis

To account for uncertainty in the calculated costs per life year saved, a one-way sensitivity analysis was performed. For this, the costs per life year saved were calculated by varying the probability of developing a hypersensitivity reaction with the 95% confidence interval, and the mean total costs of the intensification phase with one standard deviation for all cost categories. The EFS for patients who would stop asparaginase therapy was varied using the minimal and maximal differences in EFS for intensive and no intensive asparaginase treatment reported.

RESULTS

Patient Characteristics

Table 2 describes the patient characteristics. In total, 68 patients were included in the study. Of these patients, 19 (27.9%) have developed a hypersensitivity reaction to PEGasparaginase. Most patients who developed a hypersensitivity reaction were treated according to the ALL-10 protocol. ALL-11 has a lower risk of hypersensitivity reactions because in the induction phase of this protocol, PEGasparaginase was used instead of native *E.coli* asparaginase. All allergies occurred during the first or second PEGasparaginase dose of the intensification phase. The age, gender and body surface area (BSA) did not statistically differ between the patients with and without a hypersensitivity reaction.

Table 2. Patient characteristics

	Total study group N=68	No hypersensitivity reaction to PEGasp N=49	Hypersensitivity reaction to PEGasp N=19	p-value
Hypersensitivity reaction % (95% CI)	28% (17 – 39%)	-	-	-
Treatment protocol % ALL-10 (95% CI)	57% (46 – 69%)	47% (33 – 61%)	84% (67 – 100%)	0.006
Sex % male (95% CI)	52% (40 – 64%)	49% (35 – 63%)	58% (35 – 81%)	0.594
Age at start intensification years, median (IQR)	7.6 (4.8 – 11.6)	6.9 (4.3 – 11.4)	8.8 (5.4 – 12.9)	0.232
BSA start intensification m^2, median (IQR)	0.92 (0.73 – 1.35)	0.87 (0.69 – 1.30)	1.03 (0.81 – 1.44)	0.194

PEGasp: PEGasparaginase; 95% CI: 95% confidential interval; IQR: interquartile range; BSA: body surface area; SD: standard deviation

Cost Analysis

Table 3 describes the mean costs of the intensification phase for the patients with and without a hypersensitivity reaction, for the different scenarios. The mean total costs per patient were $40,925 without a hypersensitivity reaction to PEGasparaginase, $175,632 when patients were switched to *Erwinia* asparaginase, and $21,190 if the asparaginase therapy was permanently stopped after a reaction. The mean total drug costs for *Erwinia* asparaginase were $126,831, which corresponds with 149 vials of *Erwinia* asparaginase. The costs of the drug asparaginase itself accounted for 44.1%, 74.5% and 19.2% of the total treatment costs of the intensification phase for the three groups respectively. The percentage of costs for asparaginase use for the total study cohort was 63.0% of the total intensification costs. Because TDM was only implemented in the DCOG ALL-11 protocol, the total TDM costs per patient are relatively low in this cohort.

Table 3. Total costs per patient in the intensification phase for the different scenarios

Costs	1. No hypersensitivity reaction to PEGasparaginase Mean ± SD (median) ($)	2. Switch after a hypersensitivity reaction to PEGasparaginase Mean ± SD (median) ($)	3. Stop asparaginase after a hypersensitivity reaction to PEGasparaginase Mean ± SD (median) ($)	P-value 1. vs. 2.	P-value 1. vs. 3.
Out-patient treatment	4,201 ± 723 (4,363)	6,567 ± 1,912 (6,282)	4373 ± 0 (4372)	<0.001	-
Day-care treatment	8,058 ± 1,714 (7,884)	27,541 ± 8,611 (27,389)	2,686 ± 376 (2,905)	<0.001	<0.001
Inpatient care (aca)	2,942 ± 5,033 (0)	2,858 ± 5,094 (687)	2,858 ± 5,094 (687)	0.988	0.988
Intensive care unit admission (aca)	992 ± 5,908 (0)	0 ± 0 (0)	0 ± 0 (0)	0.274	0.274
Out-patient treatment (sat)	20 ± 40 (0)	14 ± 41 (0)	14 ± 41 (0)	0.507	0.507
Inpatient care (sat)	97 ± 307 (0)	75 ± 180 (0)	75 ± 181 (0)	0.923	0.923
PEGasparaginase	18,032 ± 2,382 (19,360)	4,076 ± 4,527 (2,766)	4,076 ± 4,527 (2,766)	<0.001	<0.001
Erwinia asparaginase	0 ± 0 (0)	126,831 ± 51,067 (117,054)	0 ± 0 (0)	<0.001	-
TDM	865 ± 845 (1,466)	600 ± 1,469 (0)	50 ± 123 (0)	0.041	0.001
Blood products	363 ± 547 (215)	283 ± 517 (0)	283 ± 517 (0)	0.288	0.288
Laboratory activities	1,299 ± 1,028 (1,011)	2,077 ± 1,361 (1,512)	2,077 ± 1,361 (1,512)	0.004	0.004
Surgical procedure costs	567 ± 41 (572)	557 ± 66 (572)	557 ± 66 (572)	0.118	0.118
Chemotherapy other than asparaginase	2,077 ± 910 (1,944)	2,203 ± 714 (2,308)	2,203 ± 714 (2,308)	0.448	0.448
Supportive care medication	1,411 ± 1,581 (990)	1,948 ± 2,325 (1,922)	1,948 ± 2,325 (1,922)	0.197	0.197
Total costs	**40,925 ± 10,334 (39,671)**	**175,632 ± 58,765 (174,446)**	**21,190 ± 7,221 (19,687)**	**<0.001**	**<0.001**

SD: standard deviation; aca: academic hospital; sat: satellite hospital; TDM: Therapeutic Drug monitoring

Effects Analysis

Cross-sectional analyses showed that the QALYs of the patients with and without a hypersensitivity did not differ significantly for the questionnaires completed in intensification week 1, 3 and 19. The longitudinal analysis showed that the MAU score overall decreased with 0.12 points per time-point of the questionnaire ($p<0.001$) but the occurrence of a hypersensitivity analysis was not a significant covariate. Thus, the development of a hypersensitivity reaction did not result in a significant change in the HRQoL. Therefore, this analysis was not further included in the decision tree analysis.

Analysis of the extra questions about the burden of the allergic reaction and of switching to *Erwinia* asparaginase are described in table 4 and 5. The question about the burden of (potentially) switching to *Erwinia* asparaginase was answered by both the patients who were switched to *Erwinia* asparaginase and patients without a hypersensitivity reaction (Table 4).

Table 4. The burden of switching to *Erwinia* asparaginase

	Week 1* **Mean ± SD** **median (IQR)**	**Week 3*** **Mean ± SD** **median (IQR)**	**Week 4*** **Mean ± SD** **median (IQR)**	**Week 19*** **Mean ± SD** **median (IQR)**
Allergy	1.43 ± 0.65	1.35 ± 0.61	1.36 ± 0.63	2.00 ± 1.18
	1.00 (1.00 – 2.00)	1.00 (1.00 – 2.00)	1.00 (1.00 – 2.00)	2.00 (1.00 – 3.00)
No allergy	2.11 ± 1.24	2.05 ± 1.24	-	2.09 ± 1.16
	2.00 (1.00 – 3.50)	1.00 (1.00 – 3.00)		2.00 (1.00 – 3.00)

*There was no statistically significant difference between the median scores of patients with and without an allergic reaction. SD: standard deviation; IQR: interquartile range.

Scoring system:

1. Switching to *Erwinia* asparaginase would not be a problem
2. Switching to *Erwinia* asparaginase would partially a problem
3. Switching to *Erwinia* asparaginase would be a growing problem
4. Switching to *Erwinia* asparaginase would be a major problem

There was no statistically significant difference between the scores of both groups at all time-points, including week 19, when all patients with a hypersensitivity reaction had been switched. At these time-points, patients considered switching to *Erwinia* asparaginase as 'no to partially a problem'. In patients who did experience an allergic reaction, the reaction was described as severe, resulting in severe illness and major discomfort during the reaction.

Table 5. Experience of an allergy

	Week 1 Mean ± SD median (IQR)	Week 3 Mean ± SD median (IQR)	Week 4 Mean ± SD median (IQR)	Week 19 Mean ± SD median (IQR)
Severity of the allergic reaction*	1.50 ± 0.67 1.00 (1.00 – 2.00)	2.75 ± 1.76 2.00 (1.00 – 5.00)	4.55 ± 1.21 5.00 (5.00 – 5.00)	4.31 ± 1.49 5.00 (4.50 – 5.00)
Extent of physical illness during the allergic reaction*	1.08 ± 0.29 1.00 (1.00 – 1.00)	2.33 ± 1.83 1.00 (1.00 – 4.75)	4.36 ± 1.21 5.00 (4.00 – 5.00)	4.38 ± 1.50 5.00 (5.00 – 5.00)
Extent of discomfort during the allergic reaction*	1.17 ± 0.58 1.00 (1.00 – 1.00)	2.25 ± 1.66 1.00 (1.00 – 3.75)	4.18 ± 1.40 5.00 (4.00 – 5.00)	4.15 ± 1.52 5.00 (3.50 – 5.00)

*Patients who had inactivation of PEGasparaginase without clinical symptoms of an allergy (silent inactivation) were excluded from this analysis (n=14). SD: standard deviation; IQR: interquartile range.

Scoring system:

Severity of the allergic-reaction:
1. Not applicable
2. No allergic reaction
3. Minor allergic reaction
4. Moderate allergic reaction
5. Severe allergic reaction

Extent of physical illness during the allergic reaction:
1. Not applicable
2. Not ill
3. Minimally ill
4. Moderately ill
5. Severely ill

Extent of discomfort during the allergic reaction:
1. Not applicable
2. No discomfort
3. Minimal discomfort, hampering of activities
4. Moderate discomfort, hampering some activities
5. Major discomfort, hampering most activities

The 5-year EFS of the medium risk group of the DCOG ALL-10 protocol was 88.0% (standard error 2.0%).[7] The EFS of the ALL-11 protocol is not available yet since the protocol is still ongoing. The studies that have reported the effect of asparaginase therapy are described in the supplemental results section. The weighted mean of the differences in 5-year EFS reported is 10.3% (range 3.3 – 17.0%).[1-6] Of note, this percentage is an indication of the actual difference in EFS. Therefore, the 5-year EFS was assumed to be 88.0% for patients without a hypersensitivity reaction to PEGasparaginase and patients who were switched to *Erwinia* asparaginase, and assumed to be 77.7% (88.0% minus 10.3%) when asparaginase would have been permanently stopped. The life expectancy of patients without an event, probably will not differ between the groups. Therefore, if patients had no event within 5 years, their life expectancy was assumed to be equal to the normal population for both groups. However, possible late effects of the ALL treatment could not be taken into account. The mean overall Dutch life expectancy in 2015 was 81.9

years[17]; the mean age of this study population at start of intensification was 8.4 years. Therefore, on average, 73.5 years (81.9 minus 8.4 years) would be saved if the EFS would have been 100%. The EFS was 77.7% if asparaginase would have been permanently stopped after a hypersensitivity reaction so, in this case, the mean number of life years saved would have been 57.1 years (77.7% of 73.5 years; discounted by 1.5% per year to account for the value of time, 38.5 years). If patients were treated intensively with asparaginase, the EFS was 88.0%, so the mean number of life years saved was 64.7 years (88.0% of 73.5 years; discounted, 41.5 years).

Decision Tree Analysis

Figure 1 shows the decision tree of the two scenarios including the costs and life years saved. Taking into account the probability of developing a hypersensitivity reaction to PEGasparaginase, the total costs of scenario 1 were $78,508 versus $35,419 of scenario 2. The discounted number of life years saved were 41.5 for scenario 1 and 40.6 for scenario 2. Thus, the costs per life year saved were $1,892 if patients were switched to *Erwinia* asparaginase after a hypersensitivity reaction and $872 if asparaginase would have been stopped permanently.

Figure 1. Decision tree

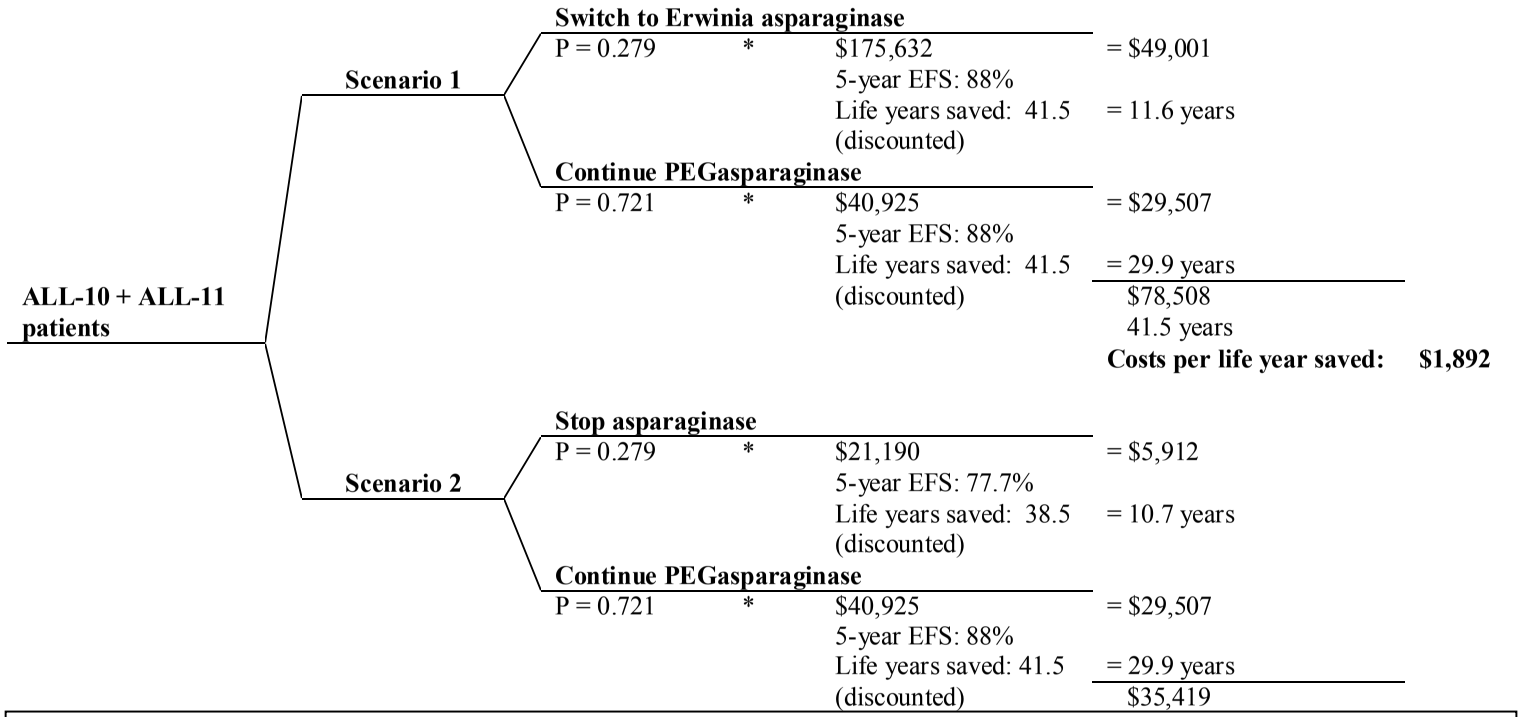


Figure 1 shows the costs and life years saved calculated for each scenario. The HRQoL did not differ between patients with and without a hypersensitivity reaction and was, therefore, not included in this analysis. Patients were switched to Erwinia asparaginase after a hypersensitivity reaction according to scenario 1; asparaginase treatment was permanently stopped after a hypersensitivity reaction according to scenario 2. The Dutch life expectancy in 2015 was 81.9 years, mean age at start intensification was 8.4 years. The life years saved were discounted with 1.5% per year. The mean costs and life year saved were multiplied by the probabilities of each branch and summed up to calculate the totals for each scenario. By dividing the mean total costs by the total number of life years saved, the costs per life year saved were calculated.

Sensitivity Analysis

In Supplemental figure 2, the costs per life year saved are shown, varying the probability of developing a hypersensitivity reaction (95% confidence interval), the total treatment costs (± one standard deviation for all cost categories), and EFS for patients who would have stopped with their asparaginase treatment (variation in EFS differences, reported in the literature). This one-way sensitivity analysis shows that mainly the treatment costs and probability of a hypersensitivity reaction influence the costs per life year saved for each scenario.

DISCUSSION

In this cost-effectiveness analysis of *Erwinia* asparaginase, we have studied the costs of ALL intensification therapy, the HRQoL during asparaginase treatment, and the amount of life years saved for two scenarios. According to these scenarios, patients were either switched to *Erwinia* asparaginase after a hypersensitivity reaction to PEGasparaginase or asparaginase therapy would have been permanently stopped when the reaction occurred. The HRQoL was studied using validated HUI-questionnaires and did not significantly differ between patients with and without a hypersensitivity reaction, although this could possibly be addressed by the relatively small number of patients. Also the extra questions, added to specifically study the burden of switching to *Erwinia* asparaginase, did not show a significant impact. Therefore, the decision tree analysis only included the costs and life years saved.

Switching to *Erwinia* asparaginase would cost $1,020 more per life year saved than permanently stopping asparaginase treatment after a hypersensitivity reaction to PEGasparaginase. Eichler et al. have reviewed cost-effectiveness thresholds reporting different maximal costs per life year saved, for example $93,500 as “rule of thumb” in the US.[18] Although our study has been performed in the Netherlands and the threshold apply to adult patients, with an increase of $1,020 per life year saved for switching to *Erwinia* asparaginase, the costs per life year saved remain far below these costs and would be acceptable. Still, it has to be taken into account that health care costs vary considerably between countries hampering the generalizability of this study.

However, the actual costs per life year saved may vary for different reasons: first,

the actual costs per life year saved for patients who would stop asparaginase may be higher: less asparaginase exposure will not only result in a higher mortality, but also in a higher relapse rate. Ideally, these costs would have been considered in the sensitivity analysis but cost data from relapse patients were not available. Kaul et al. report a threefold increase in costs when patients experience a relapse compared to no relapse, although actual costs of pediatric relapse therapy have not been described.[19] Hence, switching to *Erwinia* asparaginase would save more future costs.

Secondly, our treatment protocol contains relatively many asparaginase doses and, consequently, many *Erwinia* asparaginase doses in case of a hypersensitivity reaction, which increases the total intensification costs tremendously. For treatment protocols with less asparaginase doses, switching to *Erwinia* asparaginase will have less impact on the costs, and the difference in total costs between permanently stopping asparaginase and switching to *Erwinia* asparaginase will be smaller.

Thirdly, the incidence of hypersensitivity reactions influences the costs per life year saved for both scenarios: a lower incidence will result in lower costs per life year saved in scenario 1, due to less *Erwinia* asparaginase use, but also in higher costs per life year saved in scenario 2 as more patients will complete their asparaginase treatment. Most patients in our cohort were treated with native *E.coli* asparaginase in induction (ALL-10), which increases the risk of developing a hypersensitivity reaction. Nowadays, most treatment protocols, including the DCOG ALL-11 protocol, use only the less immunogenic PEGasparaginase, decreasing the number of reactions significantly. Thus, the difference in costs per life year saved between the two scenarios will be even smaller.

Finally, to evaluate the number of life years saved, we have used the EFS of the ALL-10 protocol for the patients who completed their asparaginase treatment. For the patients in scenario 2, in which asparaginase would have been permanently stopped after a hypersensitivity reaction, the EFS was calculated by subtracting the difference in EFS between intensive and no intensive asparaginase reported in the literature, from the EFS of ALL-10. One might question the accuracy of this difference as it is based on former treatment protocols. Ideally, the impact of less asparaginase exposure in our patients should be studied within the treatment protocol used. However, this would be unethical to study so the difference in EFS used is the best available evidence. Besides, the sensitivity analysis showed that varying the EFS only has a minor effect on the costs per life year saved as the costs

barely changed when the difference in EFS between intensive and less intensive asparaginase treatment was varied between 3.3 and 17.0%.

In conclusion, according to this analysis, the costs per life years saved will be higher when patients switch to *Erwinia* asparaginase after a hypersensitivity reaction to PEGasparaginase. However, these costs are only 1% of the costs per life years saved that are considered acceptable.[18] Therefore, we recommend switching to *Erwinia* asparaginase after a hypersensitivity reaction to PEGasparaginase, apart from a clinical perspective, also from a cost perspective.

REFERENCES

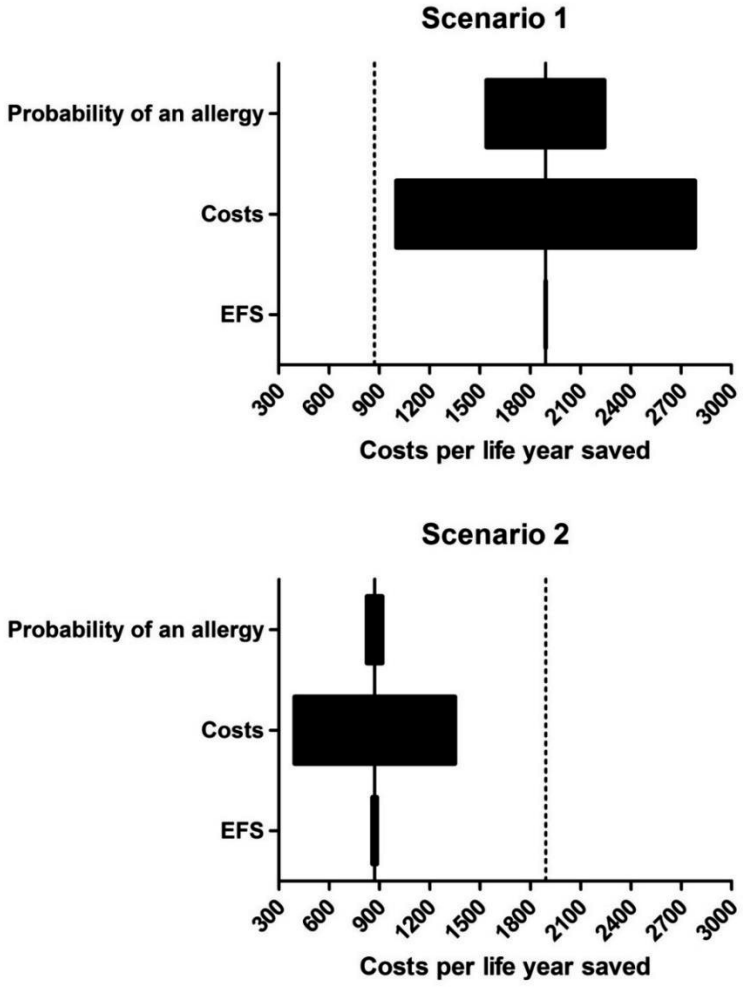
1. Amylon, M. D., J. Shuster, J. Pullen, C. Berard, M. P. Link, M. Wharam, et al. Intensive high-dose asparaginase consolidation improves survival for pediatric patients with T cell acute lymphoblastic leukemia and advanced stage lymphoblastic lymphoma: a Pediatric Oncology Group study. Leukemia. 1999;13(3):335-342.
2. Rizzari, C., M. G. Valsecchi, M. Arico, V. Conter, A. Testi, E. Barisone, 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.
3. Silverman, L. B., R. D. Gelber, V. K. Dalton, B. L. Asselin, R. D. Barr, L. A. Clavell, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97(5):1211-1218.
4. Duval, M., S. Suciu, A. Ferster, X. Rialland, B. Nelken, P. Lutz, 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.
5. Pession, A., M. G. Valsecchi, G. Masera, W. A. Kamps, E. Magyarosy, C. Rizzari, 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.
6. Moghrabi, A., D. E. Levy, B. Asselin, R. Barr, L. Clavell, C. Hurwitz, 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.
7. Pieters, R., H. de Groot-Kruseman, V. Van der Velden, M. Fiocco, H. van den Berg, E. de Bont, et al. Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group. J Clin Oncol. 2016;34(22):2591 - 2601.
8. Hak, L. J., M. V. Relling, C. Cheng, D. Pei, B. Wang, J. T. Sandlund, et al. Asparaginase pharmacodynamics differ by formulation among children with newly diagnosed acute lymphoblastic leukemia. Leukemia. 2004;18(6):1072-1077.
9. Liu, C., J. D. Kawedia, C. Cheng, D. Pei, C. A. Fernandez, X. Cai, et al. Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia. Leukemia. 2012;26(11):2303-2309.
10. Tong, W. H., R. Pieters, G. J. Kaspers, D. M. te Loo, M. B. Bierings, C. van den Bos, 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.
11. Tong, W. H., I. M. van der Sluis, C. J. Alleman, R. R. van Litsenburg, G. J. Kaspers, R. Pieters, et al. Cost-analysis of treatment of childhood acute lymphoblastic leukemia with asparaginase preparations: the impact of expensive chemotherapy. Haematologica. 2013;98(5):753-759.
12. Kloos, R. Q. H., C. A. Uyl-de Groot, R. R. L. van Litsenburg, G. J. L. Kaspers, R. Pieters and I. M. van der Sluis. A cost analysis of individualized asparaginase treatment in pediatric acute lymphoblastic leukemia. Pediatr Blood Cancer. 2017.
13. Husereau, D., M. Drummond, S. Petrou, C. Carswell, D. Moher, D. Greenberg, et al. Consolidated Health Economic Evaluation Reporting Standards (CHEERS)--explanation and elaboration: a report of the ISPOR Health Economic Evaluation Publication Guidelines Good Reporting Practices Task Force. Value Health. 2013;16(2):231-250.
14. Hakkaart, L., S. S. Tan and B. C. A. M. Manual for costing research. Methods and guideline prices for economic evaluations in health care. Rotterdam: Health Cae Insurance Board. 2010.
15. Horsman, J., W. Furlong, D. Feeny and G. Torrance. The Health Utilities Index (HUI): concepts, measurement properties and applications. Health Qual Life Outcomes. 2003;1:54.
16. Pieters, R., S. P. Hunger, J. Boos, C. Rizzari, L. Silverman, A. Baruchel, et al. L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. Cancer. 2011;117(2):238-249.

17. World Health Statistics 2016: Monitoring health for the SDGs Annex B: tables of health statistics by country, WHO region and globally. World Health Organization. 2016.
18. Eichler, H. G., S. X. Kong, W. C. Gerth, P. Mavros and B. Jonsson. Use of cost-effectiveness analysis in health-care resource allocation decision-making: how are cost-effectiveness thresholds expected to emerge? Value Health. 2004;7(5):518-528.
19. Kaul, S., E. K. Korgenski, J. Ying, C. F. Ng, R. R. Smits-Seemann, R. E. Nelson, et al. A retrospective analysis of treatment-related hospitalization costs of pediatric, adolescent, and young adult acute lymphoblastic leukemia. Cancer Med. 2016;5(2):221-229.

SUPPLEMENTAL RESULTS

Several studies have investigated the added value of intensive asparaginase treatment: Silverman et al. have found that the 5-year EFS was 17.0% higher in patients who tolerated >25 weeks of asparaginase than in patients who did not (n=377, EFS 90% vs. 73%).[1] Pession et al. have investigated the effect of 20 weeks of asparaginase therapy in ALL patients and have reported a difference of 5.6% (n=355, 5-year EFS 88.1% vs. 82.5% for patients with and without intensive asparaginase therapy).[2] Amylon et al. studied intensive asparaginase treatment (20 extra weeks vs. no extra asparaginase) in T-cell ALL patients and found an increase in 5-year EFS of 13.4% (n=317, EFS 67.9% vs. 54.5%).[3] The study of Rizzari et al. reported no significant difference in the 5-year EFS in ALL-patients who were treated more (20 weeks of asparaginase) and less intensive with asparaginase (n=610, EFS 75.7% vs. 72.4%).[4] Duval et al. and Moghrabi et al. both compared patients who were treated with E.coli asparaginase vs. Erwinia asparaginase with the same dose.[5, 6] Erwinia asparaginase, however, has a shorter half-life and patients treated with this formulation probably did not had complete asparagine depletion, which may have resulted in the difference in 5-year EFS found: Duval et al. report a difference of 13.6% in favor of patients who were weekly treated with 10,000 IU/m2 native E.coli asparaginase compared to Erwinia asparaginase (n=700); Moghrabi et al. a difference of 11% in patients who were weekly treated with 25.000 IU/m2 of one of these formulations (n=286).[5, 6] All differences in EFS reported were multiplied by the number of patients per study and divided by the total number of patients to obtain a weighted difference is EFS. This resulted in a difference in EFS of 10.3% (3.3% – 17%), which was used as indication of the actual difference in EFS between the two scenario's.

Supplemental figure 1.



Supplemental figure 1 shows the costs per life year saved calculated for both scenarios, varying the event-free survival (EFS), the costs, and the probability of an allergy. The dotted vertical line represents the costs per life year saved for the other scenario ($872 for scenario 2 and $1,892 for scenario 1).
The differences in EFS for intensive versus less intensive asparaginase varied between 3.3 - 17% in literature. Using this variation reported, the costs per life year saved for scenario 2 varied between $858 and $884.
The total costs were varied by calculating the mean costs ± one standard deviation for all cost categories to show the maximum impact of uncertainty in costs. For scenario 1, the costs per life year saved varied between $1,002 and $2,781; and for scenario 2 between $395 and $1,349.
The probability of a hypersensitivity reaction was varied by the 95% confidence interval (17.1% - 38.7%). For scenario 1, the total costs per life year saved varied between $1,541 and $2,242; and for scenario 2 between $858 and $884 (the costs per life year saved increased when the probability of an allergy

REFERENCES

1. Silverman, L. B., R. D. Gelber, V. K. Dalton, B. L. Asselin, R. D. Barr, L. A. Clavell, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97(5):1211-1218.
2. Pession, A., M. G. Valsecchi, G. Masera, W. A. Kamps, E. Magyarosy, C. Rizzari, 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.
3. Amylon, M. D., J. Shuster, J. Pullen, C. Berard, M. P. Link, M. Wharam, 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.
4. Rizzari, C., M. G. Valsecchi, M. Arico, V. Conter, A. Testi, E. Barisone, 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.
5. Duval, M., S. Suciu, A. Ferster, X. Rialland, B. Nelken, P. Lutz, 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.
6. Moghrabi, A., D. E. Levy, B. Asselin, R. Barr, L. Clavell, C. Hurwitz, 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.

Part V

CHAPTER 9

9

General discussion

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

In this thesis, several aspects of asparaginase treatment of children with acute lymphoblastic leukemia (ALL) were studied. First, the efficacy of the asparaginase therapeutic drug monitoring (TDM) program, and its effect on asparaginase-associated toxicity were evaluated. To gain more insight in variability in PEGasparaginase activity levels, a population pharmacokinetic model was developed providing patient- and treatment characteristics explaining this variability. Second, hypersensitivity reactions to asparaginase were further explored: by measuring asparaginase activity levels, allergic-like reactions were recognized. And in order to study hypersensitivity reactions specifically to PEGasparaginase, an assay was developed to measure antibodies not only to asparaginase but also to PEG and the linker. Third, the effect of asparaginase on high dose methotrexate (MTX) efficacy, reflected by MTX polyglutamination, and toxicity was studied. Fourth, the costs of the TDM program were compared to a fixed dosing schedule, and the cost-effectiveness of *Erwinia* asparaginase treatment was analyzed.

Therapeutic drug monitoring

Efficacy

The Dutch Childhood Oncology Group (DCOG) has incorporated a unique national TDM program to individualize the asparaginase treatment of pediatric patients with ALL treated according to the DCOG ALL-11 protocol. As described in chapter 2, based on asparaginase activity levels, the PEGasparaginase dose has been reduced substantially targeting a trough asparaginase activity level of 100 – 250 IU/L. After approximately 10 doses, of which the first three doses had a fixed dose of 1,500 IU/m^2, the median dosage was 450 IU/m^2, which is five times lower than the fixed dose used in DCOG ALL-10 (2,500 IU/m^2).

Although the target level used has been associated with complete asparagine depletion[1], the efficacy of individualized asparaginase treatment had to be verified by measuring asparagine concentrations. To avoid possible *ex vivo* asparaginase activity, a strict protocol was used by putting samples directly on ice and adding sulfosalicylic acid for deproteinization as was previously suggested by others.[2, 3] As asparagine was measurable in all samples and glutamine was not depleted, it was

shown that the samples had been handled properly and *in vivo* asparagine concentrations were adequately reflected, With a very low limit of quantification of 0.05 µM, asparagine was measurable in all samples, though at very low levels, including those with high (>500 IU/L) asparaginase activity levels. For the majority of the PEGasparaginase samples with asparaginase activity levels >100 IU/L, asparagine concentrations were <0.5 µM (96% of the PEGasparaginase samples and 67% of the *Erwinia* asparaginase samples), which is very low compared to baseline asparagine concentrations, confirming efficacious asparaginase treatment in the DCOG ALL-11 patients.

Human asparagine concentrations consist of L-asparagine and D-asparagine, the latter not being incorporated in proteins and, therefore, clinically irrelevant. D-asparagine, as other D-amino acids, are known to be present in humans but their origin is currently unknown.[4] Previous studies focused on total asparagine concentrations and the minimal asparaginase activity for complete asparagine depletion reported varied between 50 – 400 IU/L.[5-8] This could partly be explained by inadequate handling of the samples but also by interference of D-asparagine, which varies between 0.017–0.18 µM.[4] It would be interesting to focus more on L-asparagine concentrations for evaluation of the efficacy of asparaginase treatment. In chapter 2, both total and L-asparagine concentrations were measured in a limited number of samples. It was expected that L-asparagine concentrations would more strongly correlate with asparaginase activity levels as the clinically irrelevant D-asparagine was not included. However, L-asparagine did not differ between samples with various asparaginase activity levels, even though samples with low (<50 IU/L) asparaginase activity levels were included in the analysis. This may be explained by the fact that the low asparaginase levels are already sufficient to deplete asparagine in the serum. The L-asparagine levers were very low (>95% reduction), though, which is probably effective. Still, the role of L-asparagine in the verification of asparaginase treatment efficacy should be further studied, starting with L-asparagine measurement in more samples.

Beside the role of L-asparagine in the reflection of the efficacy of asparaginase treatment, it is essential to study the correlation between asparaginase activity levels – which are relatively easy to measure – and treatment outcome. The number of relapses in DCOG ALL-11, however, is too low to draw conclusions about this correlation because confounders such as cytogenetic aberrations and toxicity resulting in treatment delay, should be taken into consideration in this

analysis. The next international ALL-Together treatment protocol, in which the DCOG will participate, will enable us to analyze the correlation in a larger group of patients.

Silent inactivation

With TDM not only the dose and/or dosing schedule can be individualized: also silent inactivation is detected. Vrooman et al. previously have shown that the event-free survival (EFS) is significantly higher when the asparaginase treatment is individualized, which was a direct result of the detection of silent inactivation and a consequential switch to *Erwinia* asparaginase.[9] Therefore, (early) detection of this type of reaction is crucial.

In the DCOG ALL-11 protocol, silent inactivation occurred in 4% of the patients treated with PEGasparaginase and 5% of the patients treated with *Erwinia* asparaginase. Especially during induction, the occurrence of silent inactivation did not follow a clear pattern and it was seen at all three doses administered according to DCOG ALL-11. During intensification, on the other hand, all silent inactivation reactions occurred at the first dose. Therefore, it is recommended to measure extra week levels for early detection of silent inactivation after all doses during induction, and after the first dose after an interruption of asparaginase treatment.

Beside the detection of silent inactivation, TDM is essential for the identification of allergic-like reactions. This type of reaction will be discussed below.

Erwinia asparaginase

According to DCOG ALL-11, *Erwinia* asparaginase treatment started with 20,000 IU/m^2, three times a week. The dose and/or dosing schedule was adjusted based on the 48h and 72h asparaginase activity levels measured during the first two weeks. As only one fourth of these 72h levels was >100 IU/L, the starting dose or dosing interval seems inadequate for the majority of the patients. We and others reported that 67 – 87% of the patients treated with the same dosing schedule has 72h asparaginase activity levels <100 IU/L in the first two weeks of treatment.[10, 11] In contrast to DCOG ALL-11, the dose was not adjusted in these studies. In DCOG

ALL-10, the *Erwinia* asparaginase activity levels significantly increased after the first two weeks resulting in adequate asparaginase exposure in the majority of the patients (77% of the samples being >100 IU/L).[11] Another study from our group using population pharmacokinetic analysis of *Erwinia* asparaginase, also described a lower clearance – and consequentially higher *Erwinia* asparaginase activity levels – after the first month of therapy.[12] In the DCOG ALL-11 patients, only half of the patients eventually had to switch to an every-other day schedule of *Erwinia* asparaginase despite the low 72h levels during the first two weeks, which indicates a lower clearance after a few months. However, there was much variation in the dose (15,000 – 40,000 IU/m^2) and the higher doses also result in higher 72h levels. It, however, could be questioned whether trough asparaginase activity levels should constantly be >100 IU/L for *Erwinia* asparaginase to be effective. Due to low relapse rates, this is difficult to investigate. Still, adjusting the dose and dosing schedule based on the first two weeks seems to be too early and the dose should be evaluated after four weeks. Evidently, *Erwinia* asparaginase activity levels should be measured during the first weeks to detect possible silent inactivation. Furthermore, to ensure adequate *Erwinia* asparaginase exposure during these first weeks, a starting dose of 25,000 IU/m^2 should be considered.[12]

Toxicity

With TDM, high trough asparaginase activity levels were avoided, possibly resulting in less toxicity. However, the effect of the substantial dose reduction on asparaginase-related toxicity seems limited. Major toxicity, including pancreatitis, thrombosis and central neurotoxicity, were not correlated with the asparaginase activity levels. We did, however, found a significant correlation between hepatotoxicity, reflected by the bilirubin and alanine transaminase levels, and asparaginase activity levels, but this was not clinically relevant. Previously, we have reported a correlation between triglycerides and asparaginase activity levels.[13] In chapter 2, however, it was described that this was not confirmed by the DCOG ALL-11 data, although this could be explained by the lower asparaginase activity levels with less outliers compared with DCOG ALL-10. Vrooman et al., compared individualized treatment with native *E. coli* asparaginase with a fixed dosing schedule, also found no difference in toxicity between both groups was found but the difference in asparaginase activity levels between the individualized and the fixed dosing group were rather small. [9] Still, there seems to be no clinically relevant effect of lower asparaginase activity levels on asparaginase-associated toxicity.

PEGasparaginase clearance

Asparaginase is believed to be cleared by the mononuclear phagocyte system (MPS) as the drug has a molecular weight (170 kDa) which exceeds the limit for renal filtration (approximately 60 kDa).[14, 15] The MPS, or the reticuloendothelial system, is a class of hematopoietic myeloid cells, originating from progenitor cells in the bone marrow, which differentiate to monocytes and dendritic cells and then enter tissues as the liver and spleen to become tissue macrophages.[16] Van der Meer et al. have shown in mice that native *E. coli* asparaginase is cleared by the phagocytic cells in the liver, spleen and bone marrow.[15] As PEGasparaginase has an even higher molecular weight due to the PEGylation and other PEGylated drugs are eliminated by the MPS[17], PEGasparaginase is likely to be eliminated by the MPS as well. In chapter 3, a pharmacokinetic model of PEGasparaginase was developed. This model showed that the clearance of PEGasparaginase is linear during the first 13 days but increases exponentially thereafter. Würthwein et al. showed similar findings.[18] As described in chapter 5, PEG rapidly hydrolyzes from PEGasparaginase in high pH but, also in a more neutral environment, for example inside the human body, the PEG probably detaches, increasing the clearance. The consequential non-linear clearance of PEGasparaginase complicates the TDM and, therefore, dosing guidelines were provided. Currently, trough asparaginase activity levels are targeted between 100 to 250 IU/L. As high asparaginase activity has a limited effect on asparaginase-associated toxicity, we recommend to only aim for a minimal asparaginase level, though a maximum level could be considered from a cost perspective. This minimal level should be determined based on L-asparagine depletion, as it is described above and/or survival if possible.

In chapter 3, it was also reported that the clearance is higher in patients with a higher body surface area, during induction and, more important, also during an infection, which has not been reported earlier. This has serious clinical consequences as the dose should be increased with 40% to obtain therapeutic activity in case of an infection. Evidently, this increase should not comprise the clinical situation or the safety of patients. The higher clearance in case of an infection is probably due to activation of the MPS[19] or eventually also by induction of the hydrolysis of PEG from the PEGasparaginase. In chapter 5, it was shown that the latter is the case with increasing pH but the effect of other environmental alterations on the hydrolysis of PEG remain unknown.

Costs

As TDM resulted in a substantial reduction of the PEGasparaginase dose, the costs of an individualized dosing schedule were compared with a fixed dosing schedule in chapter 7. It was shown that, with an individualized dosing schedule, the costs are only reduced if a vial of PEGasparaginase can be used for multiple administrations and waste can be limited. For the DCOG ALL-11 protocol, the costs of individualized asparaginase treatment were lower when at least three patients could be treated at the same day. By centralization of the pediatric oncology care, this is achievable but in smaller pediatric oncology centers, relatively much waste has to be discarded. The TDM costs itself were very low and this should therefore not be a barrier to use TDM in order to detect silent inactivation of asparaginase.

Hypersensitivity reactions to asparaginase

Allergic-like reactions

By measurement of asparaginase activity levels, it was noted that not all allergic reactions to PEGasparaginase or *Erwinia* asparaginase were accompanied by neutralization of asparaginase. These are called allergic-like reactions. This is in contrast to our previous study, showing neutralization of asparaginase even in case of mild allergic reactions.[11] This study, however, was performed in 89 patients, which is a relatively small group compared to the number of DCOG ALL-11 patients analyzed in chapter 2.

Allergic-like reactions are often not accompanied by antibodies and usually occur relatively late after start infusion. However, this is not always true and asparaginase activity levels should be determined to confirm the type of reaction. We also reported that asparaginase activity levels are already zero just prior to real, neutralizing reactions, which was also confirmed in chapter 2.[11] Thus, the asparaginase activity level measured prior to an allergic-reaction can be used to distinct between allergic-like reactions and neutralizing allergies. However, if asparaginase activity levels are not standardly measured or if an allergic reaction occurs during the first dose, conclusions have to be drawn based on asparaginase activity levels measured directly after the reaction. In that case, the amount of asparaginase administered should be taken into account when interpreting these levels. As was shown in chapter 2, the far majority of patients with an allergic-like reaction during DCOG ALL-11, were able to complete their asparaginase treatment

with the same formulation. If *Erwinia* asparaginase has to be stopped prematurely, there are no alternative formulations available. Therefore, in case of allergic-like reactions to *Erwinia* asparaginase, the identification of these reactions and continuation of therapy probably contributes to a more favorable outcome.

Hypersensitivity reactions to PEGasparaginase

In DCOG ALL-10, patients were first treated with native *E. coli* asparaginase during induction and with PEGasparaginase during intensification. We have shown that one third of the patients in ALL-10 developed a reaction to PEGasparaginase, almost exclusively during the second dose in intensification and all in the presence of native *E. coli* asparaginase antibodies.[11] Hypersensitivity reactions to native *E. coli* asparaginase during induction occurred in 5% of the patients.[20]
In DCOG ALL-11, when PEGasparaginase was administered both in induction and intensification, the pattern of occurrence of hypersensitivity reactions and especially the antibodies formed, changed fundamentally. During intensification, only 7% of the patients had a hypersensitivity reaction, of which 60% was during the first and 40% during the second dose. On the other hand, still 3% developed a reaction during induction, which is more or less comparable with DCOG ALL-10. As described in chapter 5, the patients with a reaction to PEGasparaginase during induction rarely had native *E. coli* asparaginase antibodies but mainly had antibodies against the PEG and the succinimidyl succinate linker (SS-linker). However, native *E. coli* asparaginase antibodies were detected in all but one of the six patients with a hypersensitivity reaction during intensification. By coincidence, the three allergies included in the study were during the second dose. And as described in chapter 2, specifically the patients with a reaction during the second dose of intensification had native *E. coli* asparaginase antibodies, whereas the patients with a reaction during the first dose did not. Thus, only hypersensitivity reactions against the second PEGasparaginase dose during intensification are accompanied with native *E. coli* asparaginase antibodies. Therefore, the question arose whether reactions during the first PEGasparaginase dose after an interruption of treatment were also evoked by PEG and/or SS-linker antibodies. Unfortunately, antibodies against the PEG were not measured in these patients because the assay of PEGasparaginase antibodies turned out to be unsuccessful in measuring PEG antibodies. Still, it could carefully be concluded that, in contrast to in DCOG ALL-10, only the reactions to the second dose in intensification (1% of the medium risk patients) seem to be against the asparaginase moiety itself. The other

reactions (during induction and the first dose in intensification) seem to be against the PEG and/or the linker moieties. This means that most patients with a reaction to PEGasparaginase may benefit from a switch to – the less expensive – native *E. coli* asparaginase instead of *Erwinia* asparaginase, which has a more inconvenient dosing schedule. Evidently, if these patients also develop antibodies to native *E. coli* asparaginase, they should switch to *Erwinia* asparaginase.

Chapter 5 comprised both patients with an allergy to and silent inactivation of PEGasparaginase. Although the number of patients included is limited, the antibody titers of especially PEG antibodies are higher in patients with an allergic reaction to PEGasparaginase. This implies that these antibodies not only neutralize the drug but also evoke symptoms of an allergy.

In general, PEGylated drugs are less immunogenic than non-PEGylated formulations, as is also the case for PEGasparaginase. However, a rapid clearance of these drugs has been observed, despite the PEGylation, probably due to PEG antibodies.[21, 17] Several studies claim the formation of PEG antibodies in patients treated with PEGylated drugs, including studies about PEG-modified bovine adenosine deaminase[22], PEG-interferon therapy in hepatitis C patients[23], and PEGylated phenylalanine ammonia lyase in phenylketonuria patients[24], but the antibodies described turned out not to be specifically against PEG itself.[17] An actual correlation between the presence of PEG antibodies and reduced therapeutic efficacy has been observed only for PEGasparaginase and PEG-uricase.[25, 26] Neutralizing antibodies against PEG have been reported previously in patients treated with PEGasparaginase by Armstrong et al.[27] In patients with gout treated with PEGylated uricase, the presence of PEG antibodies was associated with lower uricase activity levels and loss of efficacy of the drug.[25, 26] Also in our study, described in chapter 5, several patients with neutralization of PEGasparaginase proved to have PEG antibodies in absence of antibodies against the asparaginase and the SS-linker.

PEG antibodies have been described in healthy individuals as well.[28-30] In line with these findings, in chapter 5, it was described that pre-existing PEG antibodies were present prior to the first PEGasparaginase dose in one third of the patients without any reaction. PEG has been widely used in cosmetics (shampoo, soap, lotions etc.) and processed foods, which is believed to be the explanation for the pre-existing PEG antibodies. In addition, macrogol, which is often used as a laxative, consists of

PEG and possibly may play a role in the development of PEG antibodies and hypersensitivity reactions against macrogol are reported.[31] Yang et al. suggest that due to frequent lacerations and skin tears, local inflammatory reactions in close proximity to products containing PEG, may induce the formation of PEG antibodies. Evidence supporting this theory, however, is lacking.

Asparaginase and methotrexate

Methotrexate polyglutamates inhibit dihydrofolate reductase (DHFR), an essential enzyme for the intracellular folate homeostasis and inhibit the thymidylate and purine biosynthesis.[32] MTX is polyglutaminated inside the cell which leads to better retention and more cytotoxicity.[33-37] In the 1970's and 1980's, several *in vitro* studies showed that, if asparaginase has been administrated prior to a MTX dose, the MTX polyglutamination is inhibited in cell culture experiments and mouse models. In contrast, administration of asparaginase after the MTX, in turn, appeared to have a synergistic effect. [33, 34, 36, 37] Specifically the asparagine depletion caused by asparaginase seemed to cause the decrease of polyglutamination, probably by inhibition of folylpolyglutamyl synthetase (FPGS).[36, 37] Whether the antagonistic effect of asparaginase on MTX plays a role *in vivo* is unknown. In chapter 6, we have described the influence of asparaginase on MTX toxicity and therapeutic efficacy, the latter reflected by intracellular MTX polyglutamination. It appeared that concomitant asparaginase and high dose MTX treatment leads to more myelosuppression resulting in treatment delay of on average one week. The occurrence of grade III/IV toxicity was similar with and without asparaginase. Thus, considering toxicity, it is safe to administer asparaginase during high dose MTX treatment. However, the effect of asparaginase on MTX efficacy is less clear. As described in chapter 6, we found that asparaginase does inhibit MTX polyglutamination measured in erythrocytes of children with leukemia *in vivo*, although to a lesser extent than *in vitro*. Moreover, all polyglutamates were formed, including the long chain polyglutamates. The question whether the inhibition of polyglutamination is clinically relevant is difficult to investigate because the incidence of relapse and death are low.

Costs

Erwinia asparaginase

Switching to *Erwinia* asparaginase after a hypersensitivity reaction to native *E. coli* or PEGasparaginase may, especially in less developed countries, be a problem due to the relatively high costs of *Erwinia* asparaginase. In chapter 8, a cost-effectiveness analysis of *Erwinia* asparaginase was performed. Although *Erwinia* asparaginase is an expensive drug, the costs per life year saved only increase with $1,020 when patients are switched to *Erwinia* asparaginase after a reaction to PEGasparaginase. Evidently, these costs apply to patients treated according to DCOG ALL-11 – which has a very extensive asparaginase schedule – and the exact costs per life year saved may differ between countries with other treatment protocols and/or healthcare prices.
Apart from possible differences with other countries, it has to be taken into account that incomplete and less intensive asparaginase treatment could result in more relapses, which results in higher costs.[38-41] Therefore, it pays off to switch to *Erwinia* asparaginase after a reaction and accept the high direct costs of the *Erwinia* asparaginase treatment.

Conclusion and future perspectives

In conclusion, TDM of asparaginase leads to a substantial reduction of the PEGasparaginase dose. However, this has no large impact on the reduction of asparaginase-associated toxicity. However, individualization of asparaginase can lead to a cost reduction and, most importantly, it optimizes the treatment by detection of silent inactivation and identification of allergic-like reactions. Therefore, TDM is recommended to improve treatment outcomes of those patients who develop silent inactivation or allergic-like reactions.

Beside this, it can be concluded that the use of only PEGasparaginase instead of native *E. coli* asparaginase has led to a change in the occurrence of hypersensitivity reactions: first, the percentage of patients developing a reaction has significantly been decreased (35% to 10% in total). Second, the majority of the remaining inactivating reactions seem to be evoked by the PEG or the linker, connecting the PEG to asparaginase. This is important for ALL patients as patients with a reaction to these antigens may be switched to native *E. coli* asparaginase. Future research should focus on the immunogenic characteristics of different linkers, and it could

be questioned whether PEGylation of drugs is the best way to reduce immunogenicity of drugs.

Although this thesis gave more insight in several aspects of the asparaginase treatment in children with ALL, new questions have arose based on these insights. The minimal asparaginase activity level for adequate asparagine depletion should be further investigated. Very important is to determine the correlation between asparaginase activity levels and outcome.
The population pharmacokinetic model, including the effect of infections and treatment phase, should be prospectively evaluated. The efficacy of using the loading and maintenance dose, adjusting the dose based on week or trough levels, and increasing the dose when an infection occurs, should be studied.
Third, the precise role of PEG- and SS-linker antibodies should be investigated. For this, the current assay should be validated before it can be used as a diagnostic tool. Finally, the clinical implications of hypersensitivity reactions accompanied by different types of antibodies should be explored.

REFERENCES

1. Lanvers-Kaminsky, C. Asparaginase pharmacology: challenges still to be faced. Cancer Chemother Pharmacol. 2017.
2. Asselin, B. L., M. Y. Lorenson, J. C. Whitin, D. J. Coppola, A. S. Kende, R. L. Blakley, et al. Measurement of serum L-asparagine in the presence of L-asparaginase requires the presence of an L-asparaginase inhibitor. Cancer Res. 1991;51(24):6568-6573.
3. Lanvers-Kaminsky, C., P. S. Westhoff, M. D'Incalci, M. Zucchetti and J. Boos. Immediate cooling does not prevent the ex vivo hydrolysis of L-asparagine by asparaginase. Ther Drug Monit. 2014;36(4):549-552.
4. Visser, W. F., N. M. Verhoeven-Duif, R. Ophoff, S. Bakker, L. W. Klomp, R. Berger, et al. A sensitive and simple ultra-high-performance-liquid chromatography-tandem mass spectrometry based method for the quantification of D-amino acids in body fluids. J Chromatogr A. 2011;1218(40):7130-7136.
5. Riccardi, R., J. S. Holcenberg, D. L. Glaubiger, J. H. Wood and D. G. Poplack. L-asparaginase pharmacokinetics and asparagine levels in cerebrospinal fluid of rhesus monkeys and humans. Cancer Res. 1981;41(11 Pt 1):4554-4558.
6. Ahlke, E., U. Nowak-Gottl, P. Schulze-Westhoff, G. Werber, H. Borste, G. Wurthwein, et al. Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during induction therapy in children with acute lymphoblastic leukaemia. Br J Haematol. 1997;96(4):675-681.
7. Appel, I. M., K. M. Kazemier, J. Boos, C. Lanvers, J. Huijmans, A. J. Veerman, et al. Pharmacokinetic, pharmacodynamic and intracellular effects of PEG-asparaginase in newly diagnosed childhood acute lymphoblastic leukemia: results from a single agent window study. Leukemia. 2008;22(9):1665-1679.
8. Angiolillo, A. L., R. J. Schore, M. Devidas, M. J. Borowitz, A. J. Carroll, J. M. Gastier-Foster, et al. Pharmacokinetic and Pharmacodynamic Properties of Calaspargase Pegol Escherichia coli L-Asparaginase in the Treatment of Patients With Acute Lymphoblastic Leukemia: Results From Children's Oncology Group Study AALL07P4. J Clin Oncol. 2014.
9. Vrooman, L. M., K. E. Stevenson, J. G. Supko, J. O'Brien, S. E. Dahlberg, B. L. Asselin, 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.
10. Vieira Pinheiro, J. P., E. Ahlke, U. Nowak-Gottl, G. Hempel, H. J. Muller, K. Lumkemann, et al. Pharmacokinetic dose adjustment of Erwinia asparaginase in protocol II of the paediatric ALL/NHL-BFM treatment protocols. Br J Haematol. 1999;104(2):313-320.
11. Tong, W. H., R. Pieters, G. J. Kaspers, D. M. te Loo, M. B. Bierings, C. van den Bos, 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.
12. Sassen, S. D., R. A. Mathot, R. Pieters, R. Q. Kloos, V. de Haas, G. J. Kaspers, et al. Population pharmacokinetics of intravenous Erwinia asparaginase in pediatric acute lymphoblastic leukemia patients. Haematologica. 2017;102(3):552-561.
13. Tong, W. H., R. Pieters, H. A. de Groot-Kruseman, W. C. Hop, J. Boos, W. J. Tissing, et al. Toxicity of very prolonged PEGasparaginase and Erwiniaasparaginase courses in relation to asparaginase activity levels with a special focus on dyslipidemia. Haematologica. 2014.
14. Brueck, M., D. Koerholz, W. Nuernberger, H. Juergens, U. Goebel and V. Wahn. Elimination of l-asparaginase in children treated for acute lymphoblastic leukemia. Dev Pharmacol Ther. 1989;12(4):200-204.
15. van der Meer, L. T., S. Y. Terry, D. S. van Ingen Schenau, K. C. Andree, G. M. Franssen, D. M. Roeleveld, et al. In Vivo Imaging of Antileukemic Drug Asparaginase Reveals a Rapid Macrophage-Mediated Clearance from the Bone Marrow. J Nucl Med. 2017;58(2):214-220.
16. Hume, D. A. The mononuclear phagocyte system. Curr Opin Immunol. 2006;18(1):49-53.
17. Yang, Q. and S. K. Lai. Anti-PEG immunity: emergence, characteristics, and unaddressed questions. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2015;7(5):655-677.

18. Wurthwein, G., C. Lanvers-Kaminsky, G. Hempel, S. Gastine, A. Moricke, M. Schrappe, et al. Population Pharmacokinetics to Model the Time-Varying Clearance of the PEGylated Asparaginase Oncaspar(R) in Children with Acute Lymphoblastic Leukemia. Eur J Drug Metab Pharmacokinet. 2017.
19. Zhang, L. and C. C. Wang. Inflammatory response of macrophages in infection. Hepatobiliary Pancreat Dis Int. 2014;13(2):138-152.
20. van der Sluis, I. M., H. de Groot-Kruseman, M. Te Loo, W. J. E. Tissing, C. van den Bos, G. J. L. Kaspers, et al. Efficacy and safety of recombinant E. coli asparaginase in children with previously untreated acute lymphoblastic leukemia: A randomized multicenter study of the Dutch Childhood Oncology Group. Pediatr Blood Cancer. 2018:e27083.
21. Verhoef, J. J., J. F. Carpenter, T. J. Anchordoquy and H. Schellekens. Potential induction of anti-PEG antibodies and complement activation toward PEGylated therapeutics. Drug Discov Today. 2014;19(12):1945-1952.
22. Chaffee, S., A. Mary, E. R. Stiehm, D. Girault, A. Fischer and M. S. Hershfield. IgG antibody response to polyethylene glycol-modified adenosine deaminase in patients with adenosine deaminase deficiency. J Clin Invest. 1992;89(5):1643-1651.
23. Tillmann, H., N. J. Ganson, K. Patel, A. J. Thompson, M. Abdelmalek, T. Moody, et al. High Prevalence of Pre-Existing Antibodies against Polyethylene Glycol (Peg) in Hepatitis C (Hcv) Patients Which Is Not Associated with Impaired Response to Peg-Interferon. J Hepatol. 2010;52:S129-S129.
24. Longo, N., C. O. Harding, B. K. Burton, D. K. Grange, J. Vockley, M. Wasserstein, et al. Single-dose, subcutaneous recombinant phenylalanine ammonia lyase conjugated with polyethylene glycol in adult patients with phenylketonuria: an open-label, multicentre, phase 1 dose-escalation trial. Lancet. 2014;384(9937):37-44.
25. Ganson, N. J., S. J. Kelly, E. Scarlett, J. S. Sundy and M. S. Hershfield. Control of hyperuricemia in subjects with refractory gout, and induction of antibody against poly(ethylene glycol) (PEG), in a phase I trial of subcutaneous PEGylated urate oxidase. Arthritis Res Ther. 2006;8(1):R12.
26. Hershfield, M. S., N. J. Ganson, S. J. Kelly, E. L. Scarlett, D. A. Jaggers and J. S. Sundy. Induced and pre-existing anti-polyethylene glycol antibody in a trial of every 3-week dosing of pegloticase for refractory gout, including in organ transplant recipients. Arthritis Res Ther. 2014;16(2):R63.
27. Armstrong, J. K., G. Hempel, S. Koling, L. S. Chan, T. Fisher, H. J. Meiselman, et al. Antibody against poly(ethylene glycol) adversely affects PEG-asparaginase therapy in acute lymphoblastic leukemia patients. Cancer. 2007;110(1):103-111.
28. Armstrong, J. K., R. Leger, R. B. Wenby, H. J. Meiselman, G. Garratty and T. C. Fisher. Occurrence of an antibody to poly(ethylene glycol) in normal donors. Blood. 2003;102(11):556a-556a.
29. Garay, R. P., R. El-Gewely, J. K. Armstrong, G. Garratty and P. Richette. Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents. Expert Opin Drug Deliv. 2012;9(11):1319-1323.
30. Chen, B. M., Y. C. Su, C. J. Chang, P. A. Burnouf, K. H. Chuang, C. H. Chen, et al. Measurement of Pre-Existing IgG and IgM Antibodies against Polyethylene Glycol in Healthy Individuals. Anal Chem. 2016;88(21):10661-10666.
31. Wenande, E. and L. H. Garvey. Immediate-type hypersensitivity to polyethylene glycols: a review. Clin Exp Allergy. 2016;46(7):907-922.
32. Chabner, B. A. and D. L. Longo (2006). Cancer chemotherapy & biotherapy, principles and practice.
33. Capizzi, R. L., W. P. Summers and J. R. Bertino. L-asparaginase induced alteration of amethopterin (methotrexate) activity in mouse leukemia L5178Y. Ann N Y Acad Sci. 1971;186:302-311.
34. Chlopkiewicz, B. and J. Koziorowska. Role of amino acid depletion in combined treatment of neoplastic cells with methotrexate and L-asparaginase. Cancer Res. 1975;35(6):1524-1529.
35. Lobel, J. S., R. T. O'Brien, S. McIntosh, G. T. Aspnes and R. L. Capizzi. Methotrexate and asparaginase combination chemotherapy in refractory acute lymphoblastic leukemia of childhood. Cancer. 1979;43(3):1089-1094.
36. Jolivet, J., D. E. Cole, J. S. Holcenberg and D. G. Poplack. Prevention of methotrexate cytotoxicity by asparaginase inhibition of methotrexate polyglutamate formation. Cancer Res. 1985;45(1):217-220.

37. Sur, P., D. J. Fernandes, T. E. Kute and R. L. Capizzi. L-asparaginase-induced modulation of methotrexate polyglutamylation in murine leukemia L5178Y. Cancer Res. 1987;47(5):1313-1318.
38. Amylon, M. D., J. Shuster, J. Pullen, C. Berard, M. P. Link, M. Wharam, 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.
39. Duval, M., S. Suciu, A. Ferster, X. Rialland, B. Nelken, P. Lutz, 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.
40. Pession, A., M. G. Valsecchi, G. Masera, W. A. Kamps, E. Magyarosy, C. Rizzari, 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.
41. Moghrabi, A., D. E. Levy, B. Asselin, R. Barr, L. Clavell, C. Hurwitz, 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.

CHAPTER 10

10

Samenvatting/Summary

SAMENVATTING

In dit proefschrift hebben we onderzocht of het individualiseren van asparaginase tot een effectieve behandeling leidt en wat het effect is op de toxiciteit van asparaginase. Hiervoor hebben we verschillende onderzoeken gedaan.

In hoofdstuk 2 hebben we laten zien dat het individualiseren van de asparaginase behandeling tot een significante reductie van de asparaginase dosis leidt. Dit had echter weinig effect op de toxiciteit. Wel wordt stille inactivatie van asparaginase gedetecteerd door asparaginase spiegels te meten. Patiënten die het middel inactiveerden zonder allergische symptomen konden op deze manier toch adequaat behandeld worden door te switchen naar *Erwinia* asparaginase.
Meer dan 95% van de asparaginase dalspiegels was hoger dan 100 IU/L. De asparagine concentraties, en met name de L-asparagine concentraties, waren bijna onmeetbaar. Daarom kunnen we concluderen dat de geïndividualiseerde asparaginase behandeling effectief is. De correlatie tussen asparaginase en asparagine concentraties, en outcome moet verder onderzocht worden.

In hoofdstuk 3 hebben we een model ontwikkeld dat de populatie farmacokinetiek van PEGasparaginase beschrijft. Het bleek dat de klaring van PEGasparaginase hoger is in het eerste deel van de behandeling – de inductie fase – en bij patiënten met een groter lichaamsoppervlak. Ook wordt het middel sneller uitgescheiden tijdens infecties. Daarom wordt aanbevolen om een hogere dosis PEGasparaginase toe te dienen tijdens de inductie en als een patiënt een infectie heeft.
Verder zagen we dat de klaring van PEGasparaginase lineair is tijdens de eerste 13 dagen maar daarna snel toeneemt, waarschijnlijk als gevolg van hydrolyse van de PEG.

In hoofdstuk 4 worden allergic-like reacties tegen asparaginase beschreven. Deze reacties lijken klinisch sterk op echte, neutraliserende allergieën maar bij dit type reactie wordt de asparaginase niet geneutraliseerd. Allergic-like reacties treden relatief laat op na het starten van de infusie en patiënten hebben meestal geen antistoffen. Toch is het nodig om asparaginase spiegels te meten om daadwerkelijk onderscheid te maken tussen dit type reactie en neutraliserende reacties. Dit is belangrijk omdat patiënten met een allergic-like reactie behandeld kunnen blijven worden met dezelfde soort asparaginase.

Hoofdstuk 5 beschrijft een assay om specifiek antistoffen tegen PEG en de succinimidyl succinaat linker aan te tonen. Uit dit hoofdstuk, en uit hoofdstuk 2, is

gebleken dat een groot deel van de reacties tegen PEGasparaginase niet zo zeer tegen de asparaginase maar tegen de PEG is. Alleen reacties tegen de tweede gift na een pauze in de behandeling lijkt tegen de asparaginase zelf te zijn. Patiënten met een reactie tegen de eerste giften of tegen de eerste gift na een pauze hadden over het algemeen geen antistoffen tegen de asparaginase maar juist tegen de PEG en/of de linker. Deze patiënten zouden daarom kunnen switchen naar native *E. coli* asparaginase. Aan de andere kant bleek ook dat een deel van de patiënten zonder reactie ook PEG antistoffen heeft dus wat de precieze rol van de PEG en linker antistoffen is, moet verder onderzocht worden.

In hoofdstuk 6 is de invloed van asparaginase op de werkzaamheid en het optreden van bijwerkingen van methotrexaat onderzocht. Patiënten die tijdens de hoge dosis methotrexaat kuren gelijktijdig met asparaginase behandeld werden, hadden meer beenmergsuppressie en milde leverfunctiestoornissen maar er was geen verschil in het optreden van ernstige toxiciteit. Ook werd methotrexaat minder gepolyglutamineerd met gelijktijdige asparaginase behandeling, al was dit effect minder dan *in vitro* en werden zowel de korte als de lange ketens gevormd.

In hoofdstuk 7 zijn de kosten van een geïndividualiseerde asparaginase behandeling vergeleken met de kosten van een vast doseerschema. Op basis van een berekening met de absolute dosis, leidt geïndividualiseerde asparaginase behandeling tot een dusdanig lagere dosis, dat dit opweegt tegen de extra kosten van het meten van asparaginase spiegels en formuleren van dosisadviezen. De PEGasparaginase ampullen zijn echter relatief groot en rekening houdend met de restjes, zouden, om kosten te besparen, meerdere patiënten op één dag behandeld moeten worden zodat ampullen gedeeld kunnen worden.

De kosteneffectiviteit analyse in hoofdstuk 8 laat zien dat switchen naar *Erwinia* asparaginase de kosten per gewonnen levensjaar met $1,020 verhoogt, wat relatief weinig is in het perspectief van de kosten van de gehele behandeling. Ook wordt in dit hoofdstuk beschreven dat de kwaliteit van leven niet verandert als patiënten moeten switchen naar dit middel.

In hoofdstuk 9 worden alle resultaten in perspectief geplaatst en wordt een voorstel gedaan waar het vervolgonderzoek zich op kan richten. Dit proefschrift heeft bijgedragen aan nieuwe inzichten betreft de farmacokinetiek, farmacodynamiek, en immunologische en economische aspecten van asparaginase. In de toekomst zal ten eerste de correlatie tussen asparaginase concentraties en outcome beter onderzocht moeten worden. Verder moet het

model van de populatie farmacokinetiek gevalideerd worden. Tenslotte moet de rol van anti-PEG en anti-linker antistoffen verder worden onderzocht.

SUMMARY

The aim of this thesis was to study the feasibility, efficacy and toxicity of individualized asparaginase treatment to optimize the asparaginase treatment of children with acute lymphoblastic leukemia. For this, several studies were performed.

In chapter 2, it was shown that the Dutch therapeutic drug monitoring (TDM) program of asparaginase was feasible and resulted in a significant reduction of the PEGasparaginase dose. However, no effect on the asparaginase-related toxicity was observed. However, with TDM, silent inactivation of asparaginase was detected. This way, we were able to ensure adequate asparaginase treatment by switching those patients to *Erwinia* asparaginase.
As the far majority (>95%) of the trough PEGasparaginase activity levels were >100 IU/L. In addition, the asparagine concentrations, and especially L-asparagine concentrations, were very low. Thus, it was concluded that the TDM program results in effective asparaginase treatment. The correlation between asparaginase and asparagine concentrations, and outcome should, however, be further investigated.

Chapter 3 was a population pharmacokinetic analysis of PEGasparaginase revealing that treatment phase, body surface area and the presence of an infection explain part of the variability in the clearance. Therefore, it was recommended to use a higher PEGasparaginase dose during the induction phase and if a patient develops an infection.
Furthermore, it was confirmed that the PEGasparaginase clearance is constant during the first 13 days but increases thereafter, probably due to hydrolysis of the PEG.

In Chapter 4, allergic-like reactions were described This reaction clinically mimics a real, neutralizing allergy but is not accompanied by inactivation of the drug. Although allergic-like reactions occur relatively late after start of infusion and are usually not accompanied by the presence of antibodies, measurement of asparaginase activity levels is necessary to distinct between these reactions and inactivating allergic reactions. This is important because patients with an allergic-like reaction can continue asparaginase treatment with the same formulation.

In chapter 5, an assay to detect antibodies against PEG and the succinimidyl succinate linker was described. In both this chapter and chapter 2, it was

concluded that a substantial part of the hypersensitivity reactions to PEGasparaginase was to the PEG moiety. Only the reactions to the second dose after an asparaginase-free interval seemed to be against the asparaginase moiety. The other reactions, during induction or against the first dose after an asparaginase-free interval, seem to be evoked by PEG and/or the linker. Therefore, these patients could switch to native *E. coli* asparaginase. As also patients without any reaction proved to have pre-existing PEG antibodies, the exact role of these antibodies has to be further investigated.

In chapter 6, the influence of asparaginase on methotrexate toxicity and efficacy was described. It was shown that with concomitant asparaginase therapy, patients had more myelosuppression and relevant hepatotoxicity during high dose methotrexate courses, but these patients did not have more clinically severe toxicity. In the presence of asparaginase, less methotrexate polyglutamates were formed. However, the effect of asparaginase on methotrexate polyglutamination was less than *in vitro* and both short chain and long chain polyglutamates were formed.

The costs of the TDM program were calculated and compared to the costs of a fixed dosing schedule in chapter 7. Considering the absolute dose, the total costs of asparaginase therapy are lower using TDM than a fixed dosing schedule as less asparaginase has to be used. However, taking into account the waste, to save costs, multiple patients should be treated with asparaginase at the same day to share vials between patients.

In chapter 8, a cost-effectiveness analysis on *Erwinia* asparaginase was performed, showing that switching would increase the costs per life year saved with only $1,020, which is modest considering the total treatment costs. Also, it was shown that the quality of life of patients who had to switch to *Erwinia* asparaginase did not differ from patients who were able to complete the PEGasparaginase treatment.

Finally, in chapter 9, the results were discussed and future perspectives were described. This thesis contributes to the insights into the pharmacokinetic, pharmacodynamic, economic and immunogenic characteristics of asparaginase. However, several new research questions arose. First, the correlation of asparaginase activity levels and outcome should be further studied. Second, the population pharmacokinetic model of PEGasparaginase should be prospectively

validated. And third, more insight in the role of PEG and linker antibodies is needed.

Part VI

AFFILIATIONS CO-AUTHORS

Cor van den Bos	Princess Máxima Center for Pediatric Oncology, Utrecht University, Utrecht, the Netherlands
	Department of Pediatric Oncology and Hematology, Emma Children's Hospital, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands
Natasha KA van Eijkelenburg	Princess Máxima Center for Pediatric Oncology, Utrecht University, Utrecht, the Netherlands
Gabriele Escherich	Clinic of Pediatric Hematology and Oncology, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany
Hester A de Groot-Kruseman	Princess Máxima Center for Pediatric Oncology, Utrecht University, Utrecht, the Netherlands
	Dutch Childhood Oncology Group, Utrecht, the Netherlands
Wim E Hennink	Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands
Robert de Jonge	Department of Clinical Chemistry, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Florine M.V. Jumelet	Department of Pediatric Oncology and Hematology, Sophia Children's Hospital, Erasmus Medical Center, Erasmus University, Rotterdam, the Netherlands
Gertjan Kaspers	Princess Máxima Center for Pediatric Oncology, Utrecht University, Utrecht, the Netherlands Department of Pediatric Oncology and Hematology, VU University Medical Center, Amsterdam, the Netherlands
Raphaele van Litsenburg	Princess Máxima Center for Pediatric Oncology, Utrecht University, Utrecht, the Netherlands Department of Pediatric Oncology and Hematology, VU University Medical Center, Amsterdam, the Netherlands
Ron Mathôt	Department of Hospital Pharmacy, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands
Enrico Matrobattista	Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands
Rob Pieters	Princess Máxima Center for Pediatric Oncology, Utrecht University, Utrecht, the Netherlands
Inge M van der Sluis	Princess Máxima Center for Pediatric Oncology, Utrecht University, Utrecht, the Netherlands

	Department of Pediatric Oncology and Hematology, Sophia Children's Hospital, Erasmus Medical Center, Erasmus University, Rotterdam, the Netherlands
Carin A. Uyl-de Groot	Institute of Health Care Policy & Management / Institute for Medical Technology Assessment, Erasmus University, Rotterdam, the Netherlands
Jan-Jaap F. Verhoef	Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands
Sarah Wolf	Institute of Health Care Policy & Management / Institute for Medical Technology Assessment, Erasmus University, Rotterdam, the Netherlands

LIST OF PUBLICATIONS

THIS THESIS

Kloos RQH, Pieters R, Jumelet FMV, de Groot-Kruseman HA, van den Bos C, van der Sluis IM.
Individualized Asparaginase Dosing in Childhood Acute Lymphoblastic Leukemia.
Journal of Clinical Oncology, 2020. doi: 10.1200/JCO.19.02292

Kloos RQH, Mathôt R, Pieters R, van der Sluis IM.
Individualized dosing guidelines for PEGasparaginase and factors influencing the clearance: a population pharmacokinetic model.
Haematologica, submitted

Kloos RQH, van der Sluis IM, Mastrobattista E, Hennink WE, Pieters R, Verhoef JJ.
Acute lymphoblastic leukemia patients treated with PEGasparaginase develop Antibodies to PEG and the succinate linker.
British Journal of Haematology, 2019. doi: 10.1111/bjh.16254

Kloos RQH, Pieters R, van den Bos C, van Eijkelenburg NKA, de Jonge R, van der Sluis IM.
The effect of asparaginase therapy on methotrexate toxicity and efficacy in children with acute lymphoblastic leukemia.
Leukemia and Lymphoma 2019; 23:1-9

Kloos RQH, van Litsenburg RRL, Wolf S, Wismans L, Kaspers GJL, Uyl-de Groot CA, Pieters R, van der Sluis IM.
A cost-effectiveness analysis of *Erwinia* asparaginase therapy in children with acute lymphoblastic leukemia.
Pediatric Blood and Cancer 2019; 66:e27458. doi: 10.1002/pbc.27458

Kloos RQH, Uyl-de Groot CA, van Litsenburg RRL, Kaspers GJL, Pieters R, van der Sluis IM.
A cost analysis of individualized asparaginase treatment in pediatric acute lymphoblastic leukemia.
Pediatric Blood and Cancer 2017; 64:doi: 10.1002/pbc.26651

Kloos RQH, Pieters R, Escherich G, van der Sluis IM.
Allergic-like reactions to asparaginase: atypical allergies without asparaginase inactivation.
Pediatric Blood and Cancer 2016; 63:1928-34

OTHER MANUSCRIPTS

Sassen SD, Mathôt R, Pieters R, **Kloos RQH**, de Haas V, Kaspers GJL, van der Bos C, Tissing WJ, te Loo M, Bierings MB, Kollen WJ, Zwaan CM, van der Sluis IM.
Population pharmacokinetics of intravenous *Erwinia* asparaginase in pediatric acute lymphoblastic leukemia patients.
Haematologica 2017; 102:552-61

Thus KA, van Halteren AG, de Hond TA, van Dijk MR, **Kloos RQH**, Lankester AC, Bierings MB, Spierings E.
The number of T cell allo-epitopes associates with CD4+ and CD8+ T cell infiltration in pediatric cutaneous GVHD.
Cellular Immunology 2015; 295:112-117

Kloos RQH, Boelens JJ, de Jong J, Versluys B, Bierings MB.
Hemorrhagic cystitis in a cohort of paediatric transplants: incidence, treatment, outcome and risk factors.
Biology of Blood and Marrow Transplantation 2013; 19:1263-1266

Kloos RQH, Bierings MB, Solinge WW, Schutgens R, van Wijk R.
Zeldzame hereditaire enzymstoornissen van de rode bloedcel.
Nederlands Tijdschrift voor Hematologie 2013; 10:54-64

PhD PORTFOLIO

Name PhD candidate:	Robin Q.H. Kloos
Erasmus MC department:	Pediatric Oncology
PhD period:	June 2014 – August 2018
Promotor:	Prof. dr. R. Pieters
Co-promotor:	Dr. I.M. van der Sluis

Meetings, seminars and workshops

Weekly research meetings of pediatric oncology	2014 – 2018	2.0 ECTS
Weekly pharmacy meetings	2014 – 2018	2.0 ECTS
Monthly NONMEM meetings	2014 – 2018	0.5 ECTS
Erasmus PhD day	2014	0.2 ECTS
Sophia Research Day	2014	0.2 ECTS
ASH, San Fransisco	2014	1.0 ECTS
Workshop solliciteren VVAA	2015	0.1 ECTS
Sophia Research Day	2015	0.2 ECTS
Symposium Zorginstituut Nederland	2016	0.2 ECTS
Workshop onderhandelen VENA	2016	0.1 ECTS
SIOP, Dublin	2016	1.0 ECTS
Sophia Research Day	2016	0.2 ECTS
ASH, Atlanta	2017	1.0 ECTS
Sophia Research Day	2018	0.2 ECTS

CLLS meeting, Helsinki	2018	1.0 ECTS

Presentations

Allergic-like reactions to asparaginase: atypical allergies without asparaginase inactivation. Poster presentation, SIOP, Dublin	2016	1.0 ECTS
A cost analysis of individualized asparaginase treatment in pediatric acute lymphoblastic leukemia. Poster presentation, Sophia Research Day	2017	1.0 ECTS
A cost analysis of individualized asparaginase treatment in pediatric acute lymphoblastic leukemia. Poster presentation, Figon Dutch Medicine Days	2017	1.0 ECTS
A Cost-effectiveness Analysis of Erwinia Asparaginase Therapy in Children with Acute Lymphoblastic Leukemia. Poster presentation, CLLS meeting, Helsinki	2018	1.0 ECTS

Awards

Best Poster Award SIOP, Dublin	2016	

Research skills

Endnote course	2014	0.1 ECTS
BROK-course	2014	1.0 ECTS
Scientific integrity course	2014	0.3 ECTS
Biostatistical Methods I: Basis principles	2015	5.7 ECTS

Biostatistical Methods II: Classical Regression Models	2016	4.3 ECTS
Open Clinica course	2016	0.3 ECTS
Biomedical English Writing course	2016	2.0 ECTS
CPO course	2016	0.3 ECTS
Principles of Pediatric Clinical Pharmacology course	2016	1.3 ECTS
Repeated Measurements	2017	1.4 ECTS

Teaching activities

Lectures students minor Pediatric Oncology	2014 – 2017	0.5 ECTS
Supervising master students: S. Wolf, F. Heijboer, S. Fernandez Martin, F. Jumelet	2015 – 2018	4.0 ECTS
Tutor of first year medical students	2017 – 2018	1.0 ECTS

Other skills

Peer review of articles for scientific journals	2014 – 2018	2.0 ECTS
Quality team of Pediatric Oncology and Hematology	2017	0.5 ECTS
Board member Sophia Researches Association	2014 - 2016	

ABOUT THE AUTHOR

Robin Kloos was born on April 23rd 1989 in Utrecht. In 2007, she received her Gymnasium degree at 'Het Nieuwe Lyceum' in Bilthoven after which she moved to Utrecht for her medical training at the Faculty of Medicine of the Utrecht University. Her enthusiasm for pediatric oncology grew in her 4th year when she performed a study on hemorrhagic cystitis in pediatric stem cell transplantation patients, supervised by dr. M.B. Bierings at the department of Pediatric Oncology of the Wilhelmina Children's Hospital in Utrecht. This was followed by an elective internship at this same department and a research internship at the HLA laboratory of the University Medical Center in Utrecht, supervised by dr. E. Spierings. In her final year, she spent 6 months in the Research Laboratory of Pediatric Oncology of the Erasmus MC – Sophia Children's Hospital in Rotterdam to study the role of extracellular vesicles in the interaction between leukemic cells and their bone marrow microenvironment, supervised by dr. R. Stam. Her final year was completed with 3 month internships at the pediatric department of the Diakonessenhuis in Utrecht and the department of Clinical Genetics of the University Medical Center in Utrecht.

In June 2014, Robin started her PhD-project about the asparaginase treatment of children with acute lymphoblastic leukemia at the department of Pediatric Oncology of the Erasmus MC – Sophia Children's Hospital in Rotterdam (promotor prof. dr. R. Pieters, co-promotor dr. I.M. van der Sluis). During this period, she was a member of the Sophia Researchers Association and tutor of first-year medical students.

In September 2018, Robin started as an intern at the pediatric department of the Maasstad Hospital in Rotterdam. She is looking forward to start her pediatric residency and become a pediatric oncologist.

DANKWOORD

Het dankwoord is het meest gelezen deel van een proefschrift. Niet geheel onterecht, dit proefschrift zou namelijk niet tot stand gekomen zijn zonder de hulp van anderen.

Allereerst wil ik graag alle patiënten en hun ouders bedanken voor het meedoen aan de verschillende onderzoeken die we hebben uitgevoerd. Ik vind het bewonderingswaardig hoe iemand in zo'n zware en moeilijke periode zó voor een ander klaar kan staan, in de hoop dat de behandeling voor toekomstige patiënten weer een beetje beter wordt.

Geachte prof. Pieters, beste Rob, ik realiseer me dat het niet gemakkelijk geweest moet zijn om naast het bouwen van het Prinses Máxima Centrum en het behandelen van kinderen met kanker, ook nog promovendi te begeleiden. Ik heb er dan ook diep respect voor hoe ons onderzoek telkens weer beter werd tijdens onze besprekingen en hoe je het overzicht hield. Als ik van tevoren dacht dat ik alles goed op een rijtje had, wist je altijd weer díe vraag te stellen waar ik geen antwoord op had. Dankjewel, ik heb ontzettend veel van je geleerd.

Geachte dr. van der Sluis, beste Inge, de afgelopen 4 jaar was woensdagochtend om 9 uur vaste prik. Maar ook daar buiten had je áltijd tijd, zelfs als je het verschrikkelijk druk had. Je kennis over leukemie en asparaginase is onuitputtelijk en door jouw begeleiding is dit een proefschrift geworden waar ik trots op ben. Dankjewel voor alles, mijn opvolger is een geluksvogel.

Geachte prof. Mathôt, beste Ron, bedankt dat je de tijd hebt genomen me mee te nemen in de wereld van NONMEM. Ik heb veel van je geleerd. Door al die uren modellen draaien, hebben we er een mooi artikel van weten te maken.

Geachte prof. Uyl-de Groot, beste Carin, door jou ben ik enthousiast geworden over het financiële aspect van de gezondheidszorg. We hebben twee mooie kostenanalyses gedaan.

De overige leden van de commissie, prof. Hoogerbrugge, prof. Zwaan, dr. Rijneveld en prof. Tissing, wil ik hartelijk bedanken voor het beoordelen van mijn proefschrift en het plaatsnemen in mijn commissie.

Beste co-auteurs, dank voor de input, moeite en tijd die jullie in onze artikelen hebben gestoken. Beste dr. van Litsenburg, beste Raphaële, bedankt voor je hulp

bij het doorspitten van al die papieren statussen en het doen van de kostenanalyses. Ik voelde me erg welkom. Dear dr. Escherich, dear Gaby, thank you for your help with unraveling the allergic-like reactions. Beste dr. de Groot-Kruseman, beste Hester, ik snap nog steeds niet hoe jij het overzicht over alle Nederlandse oncologie patiënten houdt. Dank voor al je hulp met de SKION-data! Beste Jan-Jaap, vele uren in het lab hebben ervoor gezorgd dat we het PEG-mysterie grotendeels hebben kunnen oplossen. We zijn een goed team. Beste dr. van den Bos, beste Cor, bedankt voor je hulp bij de methotrexaat- en de toxiciteit studie. De inclusies in het AMC liepen als een trein. Beste dr. van Eijkelenburg, Natasha, en prof. dr. de Jonge, Robert, ook jullie wil ik graag bedanken voor jullie hulp bij de methotrexaat studie. Beste prof. dr. Kaspers, beste Gertjan, bedankt voor je hulp bij de kostenstudies. Beste Sara, Leonoor en Florine, als studenten hebben jullie een mooie bijdrage kunnen leveren aan de verschillende studies, bedankt!

Beste Carla, Patty, Fred, Tineke, Henk, en Linda, wat hebben jullie een hoop samples voor me verwerkt en gemeten. De deur van het lab stond altijd open – net als de dropjes-la – en jullie wisten altijd wel ergens een plekje voor me vrij te maken als ik weer eens samples kwam uitzoeken.

Team van research verpleegkundigen, ik wil jullie graag bedanken voor het includeren van de patiënten. Ik vind het erg knap hoe jullie het overzicht weten te bewaren op een afdeling waar zo veel onderzoeken door elkaar lopen. Bedankt!

Lieve Na17 – later Na15 –en andere collega's, ik ga jullie missen! Al die gesprekken over alles en niks, al die kopjes heet water en al die stukken taart en borrels hebben er mede voor gezorgd dat mijn proefschrift nu daadwerkelijk voor jullie neus ligt. Fijne collega's zijn zo belangrijk en ik heb het geluk gehad dat jullie dat waren, dankjulliewel!

Lieve Lisette, Manouk, Esther en Paola van het SOV bestuur, het was fijn om samen deze verantwoordelijkheid op ons te nemen. Wat hebben we gelachen en ik weet zeker dat we elkaar tegen zullen blijven komen!

Lieve collega's van het Maasstadziekenhuis, we zijn een fantastisch team. Heel mooi hoe we altijd voor elkaar klaar staan. Bedankt voor alles!

Lieve vrienden en vriendinnen, voor de één was ik 'nog steeds?!' met mijn onderzoek bezig en de ander was verbaasd dat we nu alweer 4 jaar verder waren. Of het nu een lange adem was of dat het voorbij gevlogen is, zonder jullie was het

hem niet geworden. Alle borrels, etentjes, reizen, skivakanties en zeilweekendjes waren fantastisch. Dank voor jullie gezelligheid, interesse en geduld.

Lieve Jennifer, met jou aan mijn zijde als paranimf komt het allemaal goed. Van keten op de 17e tot fijne gesprekken met lekker eten en een goed glas wijn, mede dankzij jou kijk ik met een fantastisch gevoel terug op de afgelopen jaren. Whisky zal ik nooit leren drinken maar ik heb nog genoeg recepten die ik op jou en Niels wil uitproberen!

Lieve Rob en Marie-Christine, na velen jaren bij jullie over de vloer komen, zijn jullie mijn tweede ouders geworden. Het is fijn hoe ik bij jullie tot rust kan komen. Dankjulliewel voor jullie liefde en steun, ik hou van jullie!

Lieve Eveline, Lodewijk en Janna, bij tweede ouders horen ook een tweede broer en (schoon)zus! Wat hebben we al een hoop leuke dingen meegemaakt en wat gaan we nog een hoop meemaken. Lieve Eef, dank dat je mijn ski's opraapte als ik ze weer eens verloren was in een valpartij. Lieve Lo, het is een geruststelling dat we altijd nog een carrière als Andrea Bocelli en Laura Pausini kunnen beginnen. Lieve Janna, officieel zijn we de koude kant van dit gezin maar zo voelt dat zeker niet. Ik lig altijd weer dubbel om jouw Fryske nuchterheid!

Lieve Pieter, van Fryske nuchterheid naar Limburgse nuchterheid. Tranen in mijn ogen van het lachen als jij weer eens een goed verhaal hebt. En werkelijk alles weet jij op te lappen. En als je even niet weet hoe het moet, dan kijk je toch gewoon een youtube filmpje?

Lieve Pom, wat ben jij een steun en toeverlaat. Ik heb zo veel respect voor je gedrevenheid, integriteit, veerkracht en de manier waarop jij altijd voor iedereen klaar staat. Volgens Roderick spreken we onze eigen taal maar we hebben aan één woord genoeg. Niet meer dan logisch dat ik jou als paranimf wil! Dank dat je mijn grote, kleine zusje bent.

Lieve papa en mama, dit proefschrift draag ik graag aan jullie op. Lieve mama, mijn stress verdwijnt direct als jij een kopje thee (of glaasje wijn) voor me neerzet. Dankjewel dat ik altijd mijn verhaal bij je kwijt kan en dat je altijd trots op me zal zijn, wat er ook gebeurt. Lieve papa, ik zou willen dat ik kan wat jij kunt. Dankjewel dat je mijn grote vriend bent en me altijd stimuleert om het beste uit mezelf te halen. Van jullie heb ik geleerd dat je alles kunt, als je je maar focust. Semper, knvf!

Lieve, lieve Roderick, zonder jou was dit me nooit gelukt. We houden elkaar in balans en je brengt me tot rust. Jij bent mijn thuis, mijn alles. Ik hou de wereld van je, voor altijd en altijd.