Individualized dosing guidelines for PEGasparaginase and factors influencing the clearance: a population pharmacokinetic model

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Supplemental methods

Measurements
Asparaginase activity levels were measured using the L-aspartic β-hydroxamate (AHA) assay. 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 indoxine, which is quantified by photometric detection at 690 nm. The lower limit of quantification (LLQ) was 10 IU/L.

Population PK 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).

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.

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., described the data better.

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

\[ CL_i = \Theta_{pop} \times e^{(\eta_i + k_i)} \]

Were Θ\textsubscript{pop} is the typical population value for CL. \( \eta_i \) and \( k_i \) represent random effects accounting for individual and occasion variation from the typical value. \( \eta_i \) and \( k_i \) are assumed to be symmetrically distributed with a mean of 0 and estimated variance of \( \omega^2 \) and \( \pi^2 \).

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.

\[ CL_i = \Theta_{pop} \times BSA \times e^{(\eta_i + k_i)} \]
To allow for asparaginase activity levels below the limit of quantification (LLQ), several methods were applied. Both the M3 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:

\[
\left( \frac{\text{covariate}}{\text{median}} \right)^{\theta}
\]

Discontinuous data:

\[
\text{covariate} \times e^{\theta}
\]

For example, the effect of the continuous covariate leukocyte count on Cl was explored by incorporating the leukocyte count divided by the median \((2.4 \times 10^9/L)\) to the power of \(\theta\) in the equations of Cl and Vd. The discontinuous covariate ‘infection’ was explored by multiplying the Cl and Vd by \(\theta\) 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.

To develop dosing guidelines, Monte Carlo simulations were performed. Starting doses were calculated targeting trough asparaginase activity levels >100 IU/L, >250 IU/L and >350 IU/L, taking into account the significant covariates. By stepwise increasing the dose in simulations, it was evaluated which loading and maintenance dose provides adequate trough levels in 95% of the patients.

Dosing guidelines were developed targeting at a trough asparaginase activity level of 100-250 IU/L or 250-400 IU/L based on week- or trough levels. For adjustment of the PEGasparaginase dose based on week levels, trough levels were predicted based on individual simulated time profiles of PEGasparaginase activity.
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 improve 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.² 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.² 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:

\[
Cl \ (first \ 13 \ days) = \theta_1 \cdot e^{\eta + \eta_{IOV}} \cdot BSA
\]

\[
Cl \ (after \ 13 \ days) = \theta_1 \cdot e^{\eta + \eta_{IOV}} \cdot BSA \cdot Cl_{ind}
\]

\[
Cl_{ind} = 1 + \theta_2 \cdot (TAD - \text{split point})
\]

\[
Vd = \theta_3 \cdot e^{\theta_4 \cdot \eta} \cdot BSA
\]

Where TAD is time after dose and the Cl\textsubscript{ind} increases with $\theta_2$ per day. In the equation of Vd, $\theta_4$ 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\textsuperscript{2}, increasing with 0.079 L/day/m\textsuperscript{2} after 12.9 days, and a Vd of 0.92 L/m\textsuperscript{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 m². All patients received bi-weekly steady-state doses of PEG-asparaginase with doses ascending from 100 IU/m² to 3000 IU/m² in 100 IU/m² 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.
References

1. Lanvers C, Vieira Pinheiro JP, Hempel G, Wuerthwein G, Boos J. Analytical validation of a microplate reader-based method for the therapeutic drug monitoring of L-asparaginase in human serum. *Anal Biochem* 2002 Oct 1; **309**(1): 117-126.

2. Wurthwein G, Lanvers-Kaminsky C, Hempel G, Gastine S, Moricke A, Schrappe M, *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 Mar 27.

3. Beal SL. Ways to fit a PK model with some data below the quantification limit. *J Pharmacokinet Pharmacodyn* 2001 Oct; **28**(5): 481-504.
**Supplemental Table 1:** Treatment protocol

| Treatment phase | Therapy |
|-----------------|---------|
| **Protocol 1A** |         |
| Prednisone      | 60 mg/m²/day for 29 days followed by 3x3 days tapering |
| Vincristine     | 1.5 mg/m²/dose at day 8, 15, 22 and 29 |
| Daunorubicin    | 30 mg/m²/dose at day 8, 15, 22 and 29 (not in case of Down syndrome) |
| PEGasparaginase | 1,500 IU/m² 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/m² at day 40 |
| Cyclophosphamide | 1,000 mg/m²/dose at day 36 and 64 |
| Cytarabine | 75 mg/m²/day at days 38 – 41, 45 – 48, 52 – 55, 59 – 62 |
| 6-Mercaptopurine | 60 mg/m²/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/m²/day for 56 days |
| Methotrexate | 5,000 mg/m²/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 |
| PEGasparaginase |         |
| **Protocol IV for SR patients** |         |
| Dexamethasone | 10 mg/m²/day for 15 days followed by 3x3 days tapering |
| Vincristine | 1.5 mg/m²/dose at day 1 and 8 |
| PEGasparaginase | Individualized dose at day 1 |
| **Maintenance for SR patients** |         |
| 6-Mercaptopurine* | 50 mg/m²/day for 81 weeks |
| Methotrexate* | 20 mg/m²/week for 81 weeks |
| **Intensification and maintenance for MR patients** |         |
| Dexamethasone | 6 mg/m²/day for 5 days every 3 weeks until week 82 |
| Vincristine | 2 mg/m²/dose every three weeks until week 82 |
| Doxorubicin | 30 mg/m²/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/m²/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/m²/dose every three weeks from week 85 - 136 |
| 6-Mercaptopurine | 50 mg/m²/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/m²/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/m²/day from days 1 - 14 |
| Methotrexate | 5,000 mg/m² 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/m²/dose at day 15, 16 and 17 |
| Etoposide | 350 mg/m²/dose at day 15, 16 and 17 |
| PEGasparaginase | 1,500 IU/m² at day 22 |
| Vincristine | 1.5 mg/m²/dose at day 22 and 29 |

| HR block 2 |  |
|---|---|
| Methotrexate | 5,000 mg/m² 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/m²/dose at day 15, 16, 17, 18 and 19 |
| Mitoxantrone | 5.25 mg/m²/dose at day 15, 16, 17, 18 and 19 |
| PEGasparaginase | 1,500 IU/m² at day 22 |
| Vincristine | 1.5 mg/m²/dose at day 22 and 29 |

| HR block 3 |  |
|---|---|
| Methotrexate | 5,000 mg/m² 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/m²/dose at day 15, 16 and 17 |
| Fludarabine | 22.5 mg/m²/dose at day 15, 16, 17, 18 and 19 |
| Cytarabine | 1,500 mg/m²/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 |

| Protocol II* |  |
|---|---|
| Dexamethasone | 10 mg/m²/day at days 1 – 21 followed by 3x3 days tapering |
| Vincristine | 1.5 mg/m²/dose at day 8, 15, 22 and 29 |
| Doxorubicin | 30 mg/m²/dose at day 8, 15, 22 and 29 |
| PEGasparaginase | 1,500 IU/m² at day 8 |
| Cyclophosphamide | 1,000 mg/m² at day 36 |
| 6-Thioguanine | 60 mg/m²/day at days 36 – 49 |
| Cytarabine | 75 mg/m²/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/m²/day from week 1 – 37 |
| Methotrexate | 20 mg/m²/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, m² (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** |           |                           |
| Allergy                 | 90 (98%)             | 29 (100%)                 |
| Silent inactivation     | 90 (98%)             | 0                         |
| Central neurotoxicity# | 54 (58%)             | 38 (41%)                  |
| Thrombosis#            | 54 (58%)             | 38 (41%)                  |
| Pancreatitis#          | 88 (96%)             | 4 (4%)                    |
| **Number of infections** |                      |                           |
| Unknown, number of patients (%) | 19 (41%) | 12 (41%)                  |
| unknown, number of patients (%) | 38 (41%) | 4 (14%)                   |
| **Number of ICU admissions** | 3 (41%)          | 2                         |
| unknown, number of patients (%) | 38 (41%) | 4 (14%)                   |
| **Leukocytes, * 10⁹/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 (%)\(^n\) 333 (41%) 228 (56%)

PEGasp AB, OD (median, IQR) Measurements missing (%)\(^n\) 0.019 (0.010 – 0.034) 333 (41%) 0.009 (0.006 – 0.017) 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.

\(^n\) 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°C Celsius) and hospital admission or prescription of antibiotics.

### Supplemental table 3. 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                | 125%            |
| 30 – 49 IU/L                | 150%            |
| 10 – 29 IU/L                | 200%            |
Supplemental figure 1. Goodness of fit plots

Supplemental Figure 1. 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.