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ORIGINAL PAPER

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# Early prediction of subsequent peg-asparaginase inactivation in acute lymphoblastic leukaemia patients—A NOPHO ALL2008 study

Merete Dam[1,2] | Maddalena Centanni[3] | Daniel Centanni[3] | Lena E. Friberg[3] | Mats O. Karlsson[3] | Line S. Lynggaard[4] | Inga M. Johannsdottir[5] | Kristi Lepik[6] | Goda E. Vaitkeviciene[7] | Laimonas Griskevicius[8] | Tuuli Pöyhönen[9] | Marja Pyörälä[9] | Ólafur G. Jónsson[10] | Petter Quist-Paulsen[11] | Kjeld Schmiegelow[12,13] | Tania Christoforaki[14] | Ulrik Overgaard[15] | Stefan N. Hansen[16] | Birgitte K. Albertsen[1,2]

**Correspondence**
Birgitte K. Albertsen, Department of Paediatrics and Adolescent Medicine, Aarhus University Hospital, Palle Juul-Jensens Blvd. 99, 8200 Aarhus, Denmark.
Email: biralber@rm.dk

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## Summary

Polyethylene glycol (peg)-asparaginase plays a crucial role in acute lymphoblastic leukaemia (ALL) treatment, yet its associated toxicity often leads to treatment discontinuation, elevating relapse risk. Hypersensitivity with inactivation of asparaginase is common and often associated with severe allergic reactions. This study aims to comprehensively analyse asparaginase enzyme activity (AEA) pharmacokinetics, validate a previously developed pharmacokinetic model based on intravenous administration and evaluate its capability to detect changes in clearance before inactivation in patients treated with intramuscular peg-asparaginase. The study, covering 644 patients aged 1–45 with ALL under the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL2008 Protocol (February 2017–December 2022, in Nordic and Baltic countries), included 3003 AEA samples. Sampling occurred 14 days post peg-asparaginase doses, with additional sampling between doses. The incidence of inactivation was 15.2%. Utilizing the pharmacokinetic model, estimates revealed an 87.8% sensitivity and 65.5% specificity for detecting increased peg-asparaginase clearance over time in patients experiencing inactivation. Identification of increased clearance preceding inactivation in the NOPHO ALL2008 dataset highlights the potential of pharmacokinetic sampling to predict inactivation and enable timely intervention before clinical manifestation, with further refinement and inclusion of additional protocols into a unified model offering the promise of improving clinicians' ability to assess individual patient risk.

**KEYWORDS**
ALL, inactivation, peg-asparaginase, pharmacokinetics

For affiliations refer to page 9.

# INTRODUCTION

Asparaginase is a crucial component of the multiagent therapy for acute lymphoblastic leukaemia (ALL).[1,2] While effectively depleting asparagine resulting in apoptosis in the lymphoblasts,[3,4] its use is often challenged by significant toxicity, particularly hypersensitivity reactions.[1,5,6] These toxicities may lead to treatment discontinuation and subsequent relapse.[7,8] Hypersensitivity appears as clinical allergy, silent inactivation (SI) or allergy-like reactions.[9] Clinical allergy and SI involve the inactivation of asparaginase enzyme activity (AEA), whereas allergy-like reactions occur without such inactivation.[5,10]

Therapeutic drug monitoring (TDM) of AEA is important to ensure optimal asparaginase treatment and to identify patients with inactivation of the drug, indicating the potential benefits of switching to another asparaginase preparation.[4] Additionally, TDM makes it possible to distinguish true allergies with inactivation from allergy-like reactions.[10,11]

Polyethylene glycol (peg)-AEA trough concentration ($C_{trough}$) ≥100 iu/L 2 weeks after an administration has been defined as the therapeutic activity target level to ensure complete asparagine depletion.[12,13] Previous studies demonstrated that the number of peg-asparaginase doses could be reduced while maintaining high survival rates and reducing toxicity significantly,[14–16] resulting in less asparaginase treatment in many contemporary protocols compared with previous protocols for patients with standard or low risk disease.[17,18] As a consequence, it is crucial that all doses of peg-asparaginase administered are effective.

Currently, there is a need for predictive tools that can identify patients at risk of inactivation and guide dose adjustments to optimize treatment outcomes. Recently, a pharmacokinetic model was developed on AEA following intravenous (IV) administration of peg-asparaginase.[19] This model confirmed induced clearance in the group of patients who experienced subsequent inactivation in time before the inactivation was present. However, it is important to externally validate the model using a dataset separate from the one on which it was developed. Thus, the primary objectives of this study were to validate the pharmacokinetic peg-asparaginase model based on the ALLTogether pilot protocol data, using an independent dataset, and to evaluate the capability to detect an increase in clearance over time in patients with inactivation undergoing intramuscular (IM) peg-asparaginase therapy. Additionally, the study conducted comprehensive AEA analyses in patients with and without inactivation, all of whom were treated with IM peg-asparaginase according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL2008 protocol during the inclusion period.

# METHODS

See Supporting Information S1 for further details.

## Study population

Patients aged 1–45 years diagnosed with de novo Philadelphia chromosome-negative ALL and treated under the NOPHO ALL2008 protocol from February 2017 to December 2022 with TDM measurements of peg-asparaginase were eligible. The study was registered at www.clinicaltrials.gov (NCT04843514) and complied with the Declaration of Helsinki throughout the study period.

## Patient characteristics and treatment

Baseline patient characteristics, including demographics, diagnosis date, ALL risk groups and toxicities, were extracted from the NOPHO registry. Data on peg-asparaginase administration and hypersensitivity confirmations were collected from participating centres and linked to corresponding AEA measurements.

Patients were stratified into three risk groups: standard risk (SR), intermediate risk (IR) and high risk (HR) based on diagnostic features like white blood cell count, immunophenotype, cytogenetics and minimal residual disease. Peg-asparaginase treatment started on day 30 post-diagnosis, with SR and IR patients receiving eight IM injections of 1000 iu/m$^2$ at specified intervals. HR patients received up to 11 doses based on treatment response, including stem cell transplantation indications (Figure S1).

## TDM and AEA sampling

The NOPHO ALL2008 protocol involved AEA sampling. From July 2008 to February 2017, AEA $C_{trough}$ samples were collected 14 days post-administration and analysed retrospectively. From February 2017, real-time TDM was introduced and unexpected low AEA $C_{trough}$ (<100 iu/L) was reported to clinicians, recommending switching asparaginase preparation. Extended TDM sampling included additional time points (days 1, 4, 7 and 11) post-initial dose. AEA samples post-switch to Erwinase were excluded from analyses. AEA was analysed using aspartic acid β-hydroximate assay at Aarhus University Hospital.

## Inactivation

Inactivation, with or without clinical allergic symptoms (SI), was defined as AEA <100 iu/L at 7 days or AEA ≤5 iu/L (lower limit of quantification, LLOQ) at 14 ± 2 days post-dose. Patients with inactivation switched to Erwinia-derived asparaginase (Erwinase). Hypersensitivity reactions were graded per the Ponte di Legno Working Group, with SI characterized by no allergic symptoms and AEA <LLOQ in samples <16 days post-dose.[9]

## Statistical analyses

Data management and analysis were performed using Stata (Release 17). Comparisons were made between cases with and without inactivation using a linear mixed-effects model. A logistic regression model evaluated the association between initial AEA and subsequent inactivation risk using restricted cubic splines. $p$-values <0.05 were considered statistically significant.

## Pharmacokinetic model

The model applied in this study was originally developed and published in *Leukemia* (2024) using data from the ALLTogether pilot protocol, where peg-asparaginase was administered intravenously. It was designed to characterize clearance dynamics and identify patients at risk of developing inactivation before clinical manifestation. In the development phase, the model demonstrated a sensitivity of 85.4% and a specificity of 71.2% in predicting increased clearance prior to inactivation.[19] Absorption parameters (bioavailability and absorption rate constant) were re-estimated using the NOPHO ALL2008 data. Absorption from the muscle into the systemic circulation was described through a first-order rate constant. The model's capacity to detect increased clearance during treatment was assessed using a mixture model. Patients were classified into two groups: one with stable clearance and another where clearance increased dynamically, modelled using a Hill function to capture the initiation and progression of clearance changes at any time point during the treatment course. This approach enabled accurate classification of patients and supported the model's potential for guiding individualized dosing adjustments. Estimations were made in NONMEM version 7.4.4, with Visual Predictive Checks (VPCs) assessing model prediction accuracy. Sensitivity and specificity of the model classification were calculated for inactivation detection.

# RESULTS

## Patient characteristics

The study included 763 patients, out of which 659 were children (<18 years old) and 106 were adults. The median age at diagnosis was 7.9 years, with 60.0% of patients being male. Patients were categorized into risk groups as follows: SR ($n = 322$, 50.0%), IR ($n = 232$, 36.0%) and HR ($n = 78$, 12.1%). Twelve patients had missing risk group stratification. The baseline characteristics of the patients are outlined in Table 1. Patients with inadequate information about administration dates were excluded, $n = 119$. Furthermore, only samples drawn in the defined time slot were included, $n = 3003$ (Figure 1). The median number of doses per patient was 4 (interquartile range [IQR]: 2–5.5; range: 1–27).

## AEA and inactivation

Of the 116 patients who experienced inactivation, 50 (43.1%) had clinical allergic reactions, while 8 (6.9%) experienced SI. The majority of inactivation events occurred following the second ($N = 50$, 43.1%) or third ($N = 35$, 30.2%)

**TABLE 1** Baseline characteristics of study population.

| | Inactivation | | No inactivation | | Total | |
|---|---|---|---|---|---|---|
| | *N* | % | *N* | % | *N* | % |
| Number of patients | 80 | 12.4 | 564 | 87.6 | 644 | 100 |
| Sex | | | | | | |
| Female | 26 | 32.5 | 230 | 40.8 | 256 | 40.0 |
| Male | 54 | 67.5 | 334 | 59.2 | 388 | 60.0 |
| Age groups, years | 6.8 IQR | | 8.0 IQR | | 7.9 IQR | |
| 1–9 years | 61 | 76.2 | 423 | 74.7 | 484 | 75.2 |
| 10–17 years | 16 | 20.0 | 79 | 14.0 | 95 | 14.7 |
| 18–45 years | 3 | 3.8 | 62 | 11.3 | 65 | 10.1 |
| Median age, years | 6.8 IQR | | | | | |
| Risk groups | | | | | | |
| Standard risk | 34 | 42.5 | 288 | 51.1 | 322 | 50.0 |
| Intermediate risk | 29 | 36.3 | 203 | 36.0 | 232 | 36.0 |
| High risk | 16 | 20.0 | 62 | 11.5 | 78 | 12.1 |
| NA[a] | 1 | 1.2 | 11 | 1.4 | 12 | 1.9 |

Abbreviation: IQR, interquartile range.

[a]Patients with inadequate registration of the risk group in the NOPHO database.

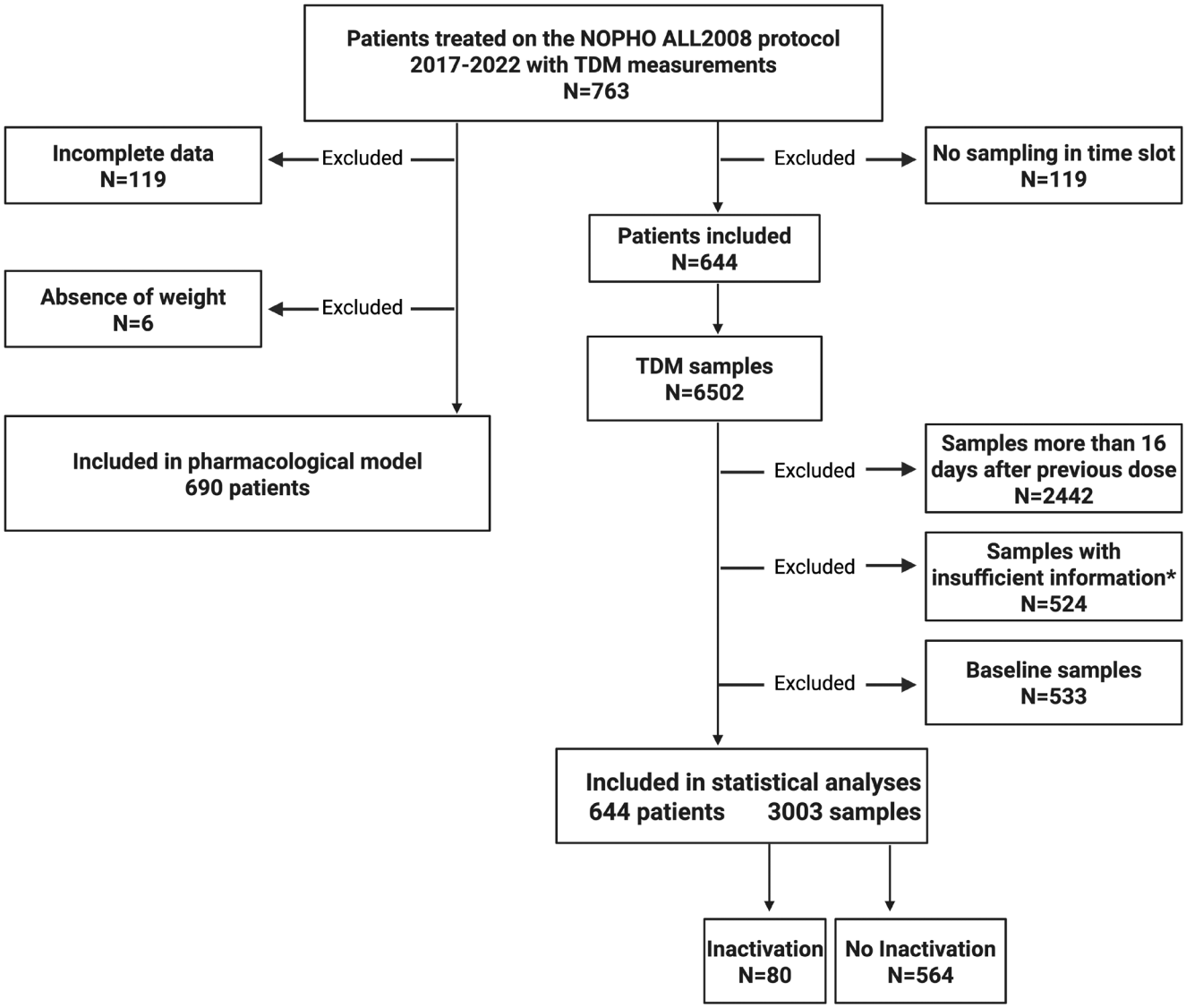


**FIGURE 1** Inclusion of patients and asparaginase enzyme activity samples. *Missing administration date.

peg-asparaginase dose. Inactivation following the fourth ($N = 15$, 12.9%) and fifth ($N = 6$, 5.2%) dose was less frequent, with only a few isolated cases occurring after doses 1, 6, 7 or 8. Among all allergic reactions, 52.5% were classified as severe, while 28.8% were mild. Nine patients (7.6%) initially had a mild allergic reaction followed by a subsequent more severe reaction.

## Statistical analyses

Eighty patients with inactivation underwent sampling for AEA measurements and were included in the statistical analyses (Figures 1 and 2). Among the excluded patients without adequate information on administration dates ($N = 119$), the mean age of them was higher than of the included patients (14.4 vs. 7.8 years), reflecting a greater proportion of insufficient data among adults. No major differences were observed in other basic demographics or risk strata. Mean AEA $C_{trough}$ after the initial dose in the group of patients without inactivation was 174 iu/L (95% confidence interval [CI] 156–183 iu/L). The mean $C_{trough}$ after the second dose was 250 iu/L (95% CI 234–261 iu/L). In patients developing inactivation at any time during treatment, the mean AEA $C_{trough}$ after the initial dose was 79 iu/L (95% CI 28–122 iu/L). Following the second dose, the mean AEA $C_{trough}$ was significantly reduced to 41 iu/L (95% CI 0–85 iu/L), $p < 0.001$ (Table 2). Figure 2 illustrates the AEA after the initial doses in the group with subsequent inactivation and the group with adequate AEA throughout the treatment period (Table S1). The group of patients with inactivation during the treatment period had lower AEA as early as 9–11 days after the initial dose of peg-asparaginase ($p = 0.013$). The AEA from the first 8 days after the initial dose did not display a significant difference ($p = 0.25$) between the two groups. Upon subdividing the inactivation group based on the timing of inactivation (at dose 2, 3 or 4), it was observed that all subgroups exhibited AEA levels

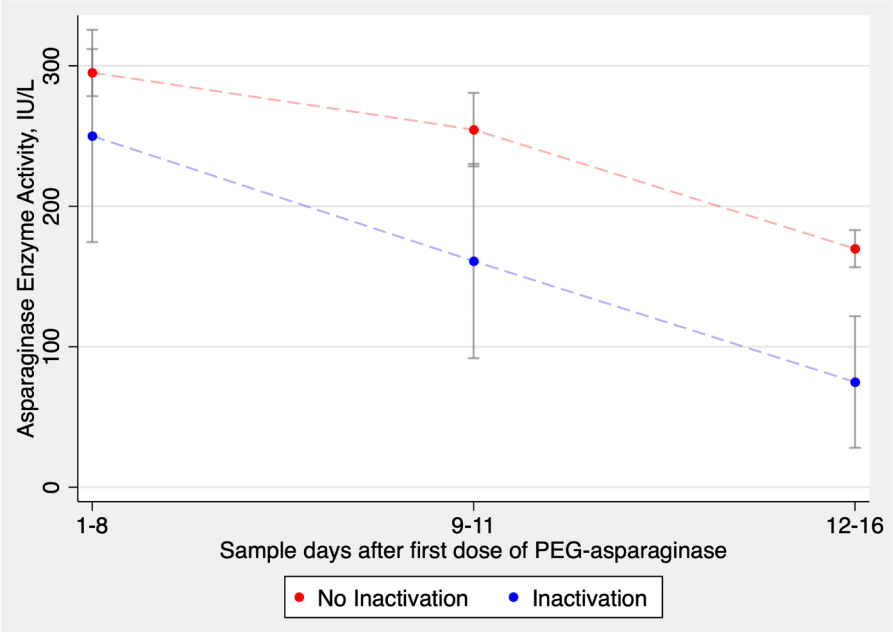


**FIGURE 2** Asparaginase enzyme activity (AEA) measurements and 95% confidence interval after the initial dose of polyethylene glycol-asparaginase separated in the groups: No inactivation (red) and inactivation (blue). AEA measurements at day 1, 4, 7, 11 and 14 (±2) after the initial dose. Details on group sizes are available in Supporting Information S1.

**TABLE 2** Mean asparaginase enzyme activity (AEA) $C_{trough}$ after the initial four doses of peg-asparaginase in the two groups.

| | AEA $C_{trough}$, iu/L | | |
|---|---|---|---|
| | No inactivation | Inactivation | |
| | AEA (samples, *n*) | AEA (samples, *n*) | |
| Dose | Patients, *n* = 564 | Patients, *n* = 80 | *p*-value |
| 1 | 174 iu/L (337) | 79 iu/L (27) | <0.001* |
| 2 | 250 iu/L (250) | 41 iu/L (37) | |
| 3 | 290 iu/L (396) | | |
| 4 | 328 iu/L (328) | | |

*Note*: Inactivation group: 80 patients. No inactivation group: 564 patients. All patients were treated with consecutive IM peg-asparaginase every 14th day during the first five doses.

*Statistically significant differences in AEA after the initial dose of peg-asparaginase in the two groups.

lower than those in the non-inactivation group after the initial dose (Figure 3) (Table S2).

Of the patients who experienced an allergic reaction after the second dose, a total of nine individuals exhibited AEA $C_{trough}$ < LLOQ following their first dose. In eight patients, sampling and data at 14 ± 2 days post their initial dose were missing. Among the patients without $C_{trough}$ measurements, one patient demonstrated AEA <100 iu/L on day 9 after the initial dose, indicating increased clearance. The remaining patients without $C_{trough}$ measurements displayed AEA > 100 iu/L on either day 9 or day 6, which represented the latest AEA measurement corresponding to their initial dose. It remains unknown if these patients would have had AEA $C_{trough}$ < LLOQ if sampling had been done at day 14 (±2).

In the analysis of AEA data utilizing a logistic regression model with AEA $C_{trough}$ values post the initial dose, a noticeable correlation was apparent. Lower AEA was correlated with a heightened risk of subsequent inactivation. For instance, an AEA $C_{trough}$ day 12–16 after the initial dose at 100 iu/L indicated a 7.2% risk (95% CI 3.6–13.7) of experiencing subsequent inactivation. Conversely, with an AEA $C_{trough}$ of 200 iu/L, the risk of subsequent inactivation was 3.5% (95% CI 1.3–8.8) (Figure 4).

## Pharmacokinetic model

AEA samples from 763 patients were initially available (114 [14.9%] with inactivation). Six patients (0.1%) were excluded due to missing weight data, and 68 (9.0%) due to incomplete dose information, yielding a refined dataset of 689 patients (98 [14.2%] with inactivation). Re-estimation of absorption parameters showed a higher bioavailability compared to the previous ALLTogether pilot model analysis, with a bioavailability of 0.844 (RSE 1%) and an absorption rate constant of 0.323 (1/day) relative standard error (RSE 2%). For the current analysis, the positive predictive value (PPV) and negative predictive value (NPV) were 31.4% and 96.7% respectively. Pharmacokinetic analyses showed stable clearance in 56.2% and increased clearance in 43.8% of patients, with 87.6% sensitivity and 67.4% specificity for detecting increased clearance in inactivated patients. VPCs confirmed that the model adequately captured AEA pharmacokinetics and trends, including patients below the LLOQ (5 iu/L; Figure 5). In the

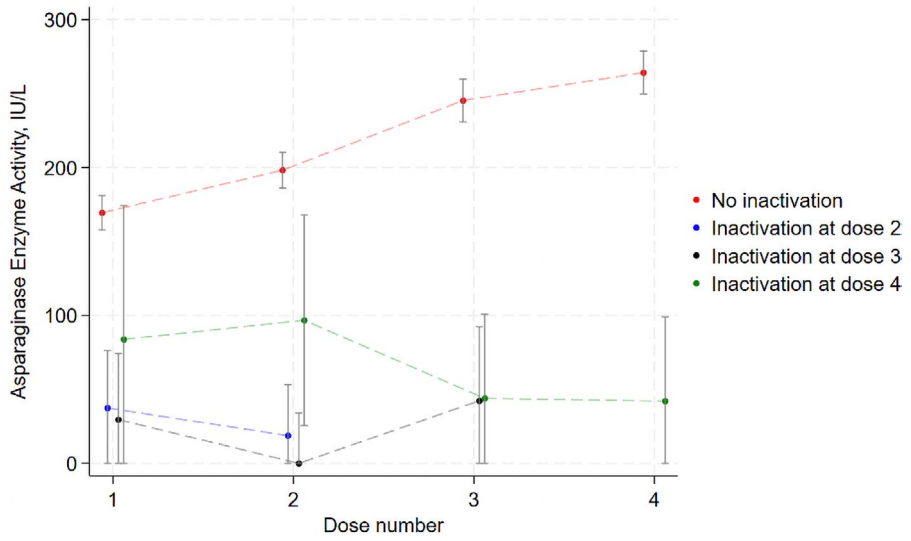


**FIGURE 3** Asparaginase enzyme activity (AEA) measurements and 95% confidence interval after first four doses of polyethylene glycol (peg)-asparaginase separated in the groups: No inactivation (red), inactivation at dose 2 (blue), inactivation at dose 3 (black) and inactivation at dose 4 (green). AEA $C_{trough}$ measurements at day 14 (±2) after the initial four doses of peg-asparaginase. Details on group sizes are available in Supporting Information S1.

group with increased clearance, overprediction was primarily observed in the upper percentile. Mispredictions in the stable clearance group were relatively minor, with no notable trends observed in the VPCs.

## DISCUSSION

This study aimed to explore the pharmacokinetics of IM peg-asparaginase in the NOPHO ALL2008 protocol, focusing on AEA measurements to detect early increases in clearance, potentially forecasting inactivation and allergic reactions. A pharmacokinetic model, previously tested in the ALLTogether pilot protocol, demonstrated promising capacity to capture concentration–time profiles using an external dataset within the NOPHO framework.[19]

In patients aged 1–45 at diagnosis, inactivation (±clinical allergy) occurred in 15.2%, with a lower incidence of 13.8% observed during the early NOPHO ALL2008 years (2008–2016).[20] Early NOPHO ALL2008 phases lacked TDM, likely contributing to the lower observed incidence compared to the ALLTogether pilot study.[19] While allergy-like reactions were likely overestimated as true allergies, their impact is minimal, as such reactions have been reported in 1%–2% of patients.[5,11]

AEA $C_{trough}$ was significantly lower in patients with inactivation (79 iu/L) than in those without (174 iu/L), aligning with the general occurrence of allergic reactions after the second dose of peg-asparaginase. AEA sampling showed significant differences between the first and second doses, supporting that increased clearance may precede inactivation. A regression model confirmed that $C_{trough}$ after the first dose influences the likelihood of inactivation, contrasting with findings from the ALLTogether pilot, where later doses showed poor correlation.[19] Although the mean AEA

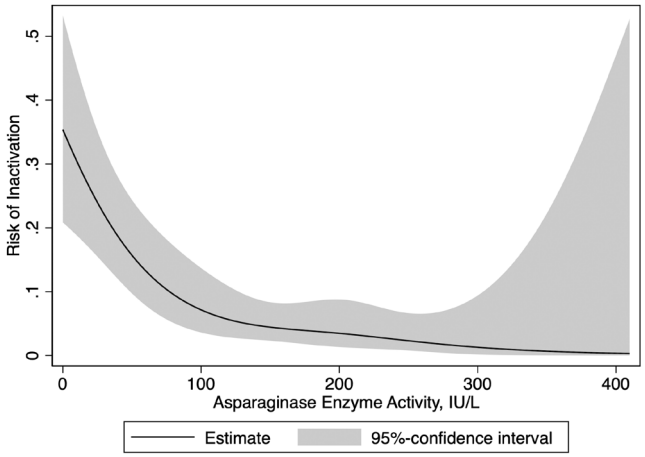


**FIGURE 4** Risk of inactivation of polyethylene glycol-asparaginase. A logistic regression model was applied with asparaginase enzyme activity (AEA) measurements taken between 12 and 16 days (AEA $C_{trough}$) after the initial dose. The figure demonstrates a relationship between AEA $C_{trough}$ day 12–16 after the initial dose at 100 iu/L and a risk of subsequent inactivation at 7.2% risk (95% confidence interval [CI] 3.6–13.7). AEA $C_{trough}$ at 200 iu/L is correlated to a risk of subsequent inactivation at 3.5% (95% CI 1.3–8.8). The large CI observed in patients with AEA >300 iu/L is due to the limited sample size in this category.

levels at day 12–16 differed between patients with and without inactivation, our analysis demonstrated that the high interindividual variability resulted in wide prediction intervals, limiting the clinical usefulness of a single measurement as a stand-alone predictor. This finding underscores the importance of moving beyond isolated time points and instead utilizing longitudinal pharmacokinetic modelling to improve predictive accuracy. Continuous asparaginase treatment in the ALLTogether pilot protocol (Clinicaltrials.gov, NCT03911128) laid the foundation for the investigated

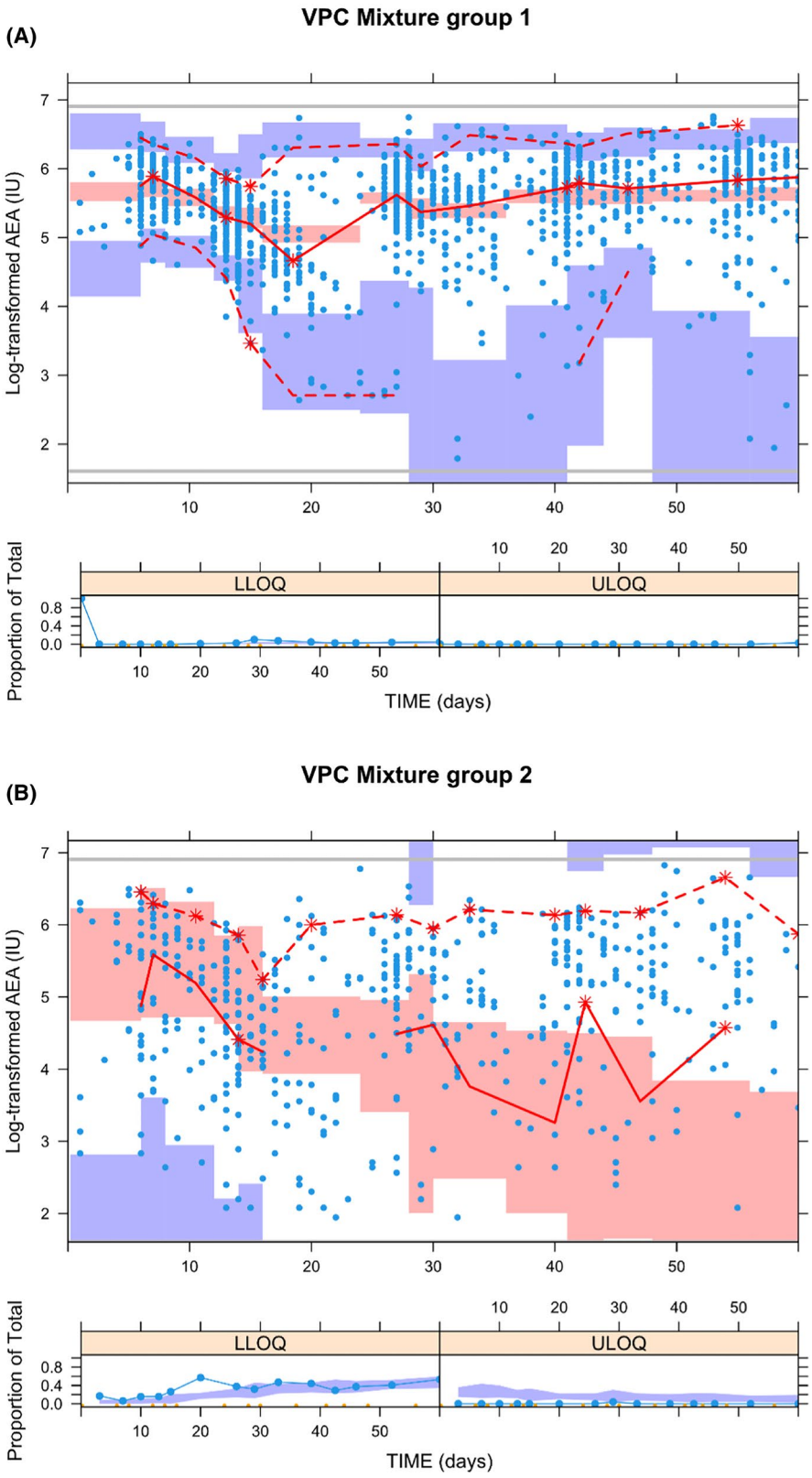


**FIGURE 5** Visual predictive check of pharmacokinetic model. Time is represented in days since the initial dose. (A) Population with no change in clearance over treatment period. (B) Population with increased clearance. Upper figure segments: The dots (•) represent log-transformed individual observed asparaginase enzyme activity (AEA) (iu), the red solid line (—) represents the median observed AEA, whereas the red dashed lines (---) represent the 2.5th and 97.5th percentiles of the observed AEA. The shaded regions indicate the simulated 95% confidence intervals (CIs) for the median, as well as the 2.5th and 97.5th percentiles ($n = 500$). Lower figure segments: The blue dots (•) represent the proportion of patients outside the quantification limits and the shaded regions represent the simulated 95% CIs ($n = 500$). LLOQ, lower limit of quantification, ULOQ, upper limit of quantification.

pharmacokinetic model. In that study, inactivation primarily was found after the fourth dose of peg-asparaginase administered IV. In the current study, most allergic reactions were reported after the second or third dose of IM peg-asparaginase administration. Variables like the administration of various concurrent medications and the initiation timing of asparaginase treatment with respect to the other drugs may potentially affect the timing of inactivation. For instance, modifications in the timing of glucocorticoid treatments have been implemented, which previously have been shown to affect asparaginase through immune modulation, thus warranting assessment in the future.[21,22] Furthermore, introducing an asparaginase-free interval has been shown to bring a higher occurrence of allergic reactions with inactivation primarily being noted on the initial dose following the pause.[5,10,23] Additional protocols, including the ALLTogether1 master protocol, have implemented an asparaginase-free interval leading to discontinued peg-asparaginase. This contrasts with the continuous 14-day administration schedule used in the ALLTogether pilot (Clinicaltrials.gov, NCT03911128) and NOPHO ALL2008 protocol (Clinical trials.gov no: NCT00819351) (first five doses).[16] The generalizability of our model to protocols with discontinuous peg-asparaginase delivery and varying steroid use requires consideration. The ability of this model to capture changes in clearance due to hypersensitivity from the NOPHO ALL2008 pharmacokinetic (PK) data, which consisted solely of data from IM administration, showed slightly lower sensitivity and specificity than the ALLTogether study, where IV administration is more common. Differences in hypersensitivity timing, administration routes, patient demographics and treatment protocols likely contribute to this variation. While some impact on model performance is expected across protocols—especially with the introduction of treatment breaks in the latest Nordic protocol—our external validation suggests these effects are likely minimal.

Before implementing the PK peg-asparaginase model in clinical practice, it is important to define acceptable thresholds for sensitivity, specificity, PPV and NPV. From a clinical perspective, a sensitivity greater than 90% is desirable if the pharmacokinetic model is to remain relevant in the clinical setting. With a sensitivity of 87.8% and an NPV of 96.7%, the model is effective at ruling out hypersensitivity, minimizing the risk of continued inactivated drug administration. However, the low PPV of 31.4% indicates a high false-positive rate, which could lead to unnecessary treatment changes. Given that PPV and NPV are influenced by prevalence, and hypersensitivity rates vary across treatment protocols, differences in model performance should be expected in different clinical settings.

The pharmacokinetic model had 87.8% sensitivity in predicting increased clearance in patients with inactivation, highlighting the potential for early identification and tailored treatment. Clinicians could adjust infusion rates, premedication or consider switching to *Erwinia* asparaginase. Some current protocols employ desensitization with frequent monitoring of AEA, which holds potential for adjusting the peg-asparaginase approach in cases where inactivation is highly likely.[24–28]

The pharmacokinetic model, incorporating re-estimated absorption parameters, adequately captured the observed AEA levels. The revised bioavailability estimate of 0.844 is consistent with the previously reported literature value of 0.82.[29] In contrast, the ALLTogether pilot protocol estimated bioavailability at 0.52, but this estimate was based on a small number of patients receiving peg-asparaginase intramuscularly ($n = 25$). Despite that some overprediction remained for the upper percentile in the group with increased clearance, the model's sensitivity remained robust, which was the primary objective to capture. External prospective validation studies using more diverging datasets are necessary to confirm its reliability and accuracy across different treatment protocols. For instance, evaluating the model's capacity to capture the increased risk of inactivation after an asparaginase-free interval would strengthen its assessment. Specifically testing its ability to detect patterns following treatment discontinuation in more contemporary protocols would enhance the evaluation clinical usefulness.

## Strengths and limitations

The pharmacokinetic model, tested on a robust IM AEA dataset, showed strong potential for predicting inactivation. The NOPHO collaboration provided high-quality data, enabling comprehensive analyses of peg-asparaginase pharmacokinetics, AEA levels and inactivation risk.

The study's inactivation incidence carries some uncertainty, as subtle local IM reactions may be overlooked, and sampling challenges led to the exclusion of numerous samples, particularly those lacking administration dates or taken >16 days post-dose. These exclusions were likely random.

The observed reactions depended on thorough allergy recording (e.g. mild, severe, silent), while the model analyses relied solely on treatment dates and AEA measurements, minimizing bias. Inconsistent real-time TDM in NOPHO ALL2008 contributed to unexplained low AEA levels, whereas contemporary protocols like ALLTogether emphasize real-time TDM for improved asparagine depletion and better detection of inactivation.

Although beyond this study's scope, the model's utility could extend to predicting excessively high AEA levels and informing dose adjustments, further optimizing treatment outcomes.

## CONCLUSION

This study tested a previously developed pharmacokinetic model's[19] capacity to capture increased clearance in patients with inactivation treated with peg-asparaginase, utilizing AEA measurements. The results of the pharmacokinetic model in this study, coupled with sensitivity surpassing 80%,

underscore its potential in addressing the persistent challenge of asparaginase inactivation in ALL treatment. While there are advantages such as personalized treatment and early intervention, considerations regarding generalizability, further model improvement, clinical impact, model implementation and cost-effectiveness should be addressed to ensure the practicality and wider applicability of this approach in routine clinical practice. The future holds the addition of data from the ALLTogether pilot protocol, the NOPHO ALL2008 and the ALLTogether main (ClinicalTrials.gov no: NCT04307576) protocols, which will provide a larger patient cohort and a broader range of parameters across age, sex and other factors—thereby improving prediction of individual patients' risk of peg-asparaginase inactivation at specific time points. Future research should also explore how single-point AEA data can be integrated into multivariate or hybrid models to enhance clinical utility while maintaining strong predictive performance for early inactivation detection.

## AUTHOR CONTRIBUTIONS

BKA conceived and designed the study. MC, LEF, DC and MOK performed the pharmacokinetic model evaluations. MD collected and analysed data in collaboration with SNH. MD, MC, LEF, LSL and BKA wrote the manuscript and all authors gave final approval.

## AFFILIATIONS

[1]Department of Paediatrics and Adolescent Medicine, Aarhus University Hospital, Aarhus, Denmark
[2]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[3]Department of Pharmacy, Uppsala University, Uppsala, Sweden
[4]Department of Hematology, Aarhus University Hospital, Aarhus, Denmark
[5]Department of Paediatric Haematology and Oncology, Oslo University Hospital, Oslo, Norway
[6]Tallinn Children's Hospital, Tallinn, Estonia
[7]Vilnius University Children's Hospital, Vilniaus, Lithuania
[8]Vilnius University Hospital Santaros Klinikos and Vilnius University, Vilniaus, Lithuania
[9]Kuopio University Hospital, Kuopio, Finland
[10]National University Hospital of Iceland, Reykjavik, Iceland
[11]Department of Haematology, St. Olavs University Hospital, Trondheim, Norway
[12]Department of Pediatrics and Adolescent Medicine, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[13]Faculty of Medicine, Institute of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark
[14]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden
[15]Department of Haematology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[16]Department of Public Health, Aarhus University, Aarhus, Denmark

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## CONFLICT OF INTEREST STATEMENT

Jazz Pharmaceuticals and Clinigen have BKA serving on its advisory board, MD and BKA served as consultants for Servier on two occasions. All other authors state that they have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## ETHICS STATEMENT

The study complied with the Declaration of Helsinki throughout the study period.

## CLINICAL TRIAL REGISTRATION

The study was registered at www.clinicaltrials.gov (NCT04843514).

## ORCID

*Merete Dam* https://orcid.org/0000-0002-8857-8629

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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