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Evaluation of the pharmacokinetics of prednisolone in paediatric patients with acute lymphoblastic leukaemia treated according to Dutch Childhood Oncology Group protocols and its relation to treatment response

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Summary

Glucocorticoids form the backbone of paediatric acute lymphoblastic leukaemia (ALL) treatment. Many studies have been performed on steroid resistance; however, few studies have addressed the relationship between dose, concentration and clinical response. The aim of the present study was to evaluate the pharmacokinetics of prednisolone in the treatment of paediatric ALL and the correlation with clinical parameters. A total of 1028 bound and unbound prednisolone plasma concentrations were available from 124 children (aged 0–18 years) with newly diagnosed ALL enrolled in the Dutch Childhood Oncology Group studies. A population pharmacokinetic model was developed and *post hoc* area under the curve (AUC) was tested against treatment outcome parameters. The pharmacokinetics of unbound prednisolone in plasma was best described with allometric scaling and saturable binding to proteins. Plasma protein binding decreased with age. The AUC of unbound prednisolone was not associated with any of the disease parameters or treatment outcomes. Unbound prednisolone plasma concentrations correlated with age. No effect of exposure on clinical treatment outcome parameters was observed and does not substantiate individualised dosing. Poor responders, high-risk and relapsed patients showed a trend towards lower exposure compared to good responders. However, the group of poor responders was small and requires further research.

Keywords: acute lymphoblastic leukaemia, dexamethasone, NONMEM, paediatrics, pharmacokinetics/pharmacodynamics, prednisolone.

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Introduction

The overall long-term survival of children with acute lymphoblastic leukaemia (ALL) has vastly improved over recent decades.[1–3] Glucocorticosteroids, like prednisolone and dexamethasone, cause apoptosis in malignant lymphoid cells and have significant anti-leukaemic activity, and form the backbone of paediatric ALL treatment.[4] The Berlin–Frankfurt–Münster-based protocols have shown that the day 8 prednisone response is an important prognostic indicator and can be used in risk group stratification.[5,6]

Many studies have been performed on the pharmacodynamic aspects of steroid resistance and sensitivity, both *in vitro* and *in vivo*.[7–10] Differences in prednisolone sensitivity have been found between phenotypes and genetic subtypes.[6,11,12] Patients become more resistant to prednisolone with age (possibly due to higher frequency of T-ALL in older children) and throughout treatment.[12] A poor response to prednisolone is unfavourable and leads to a worse outcome, although this is treatment dependent as is the case for all prognostic factors.[5,6] Pharmacokinetic (PK) studies of glucocorticoids in paediatric ALL treatment are scarce.[13–15]

Dexamethasone is often used in paediatric ALL due to its higher potency and prolonged biological half-life compared to prednisolone. A wide range of equivalent concentrations can be found in literature ranging from fivefold to 16-fold.[16,17] However, a higher incidence of induction-related treatment deaths has been reported in the 10 mg/m^2 dexamethasone *versus* 60 mg/m^2 prednisolone.[18] Prior studies have shown that dexamethasone PKs in paediatric patients with ALL are highly variable with younger patients exhibiting higher clearances compared to older patients. Additionally, a possible effect of asparaginase on dexamethasone PKs was observed.[15,19] It is not known whether this also applies to prednisolone, as studies on the *in vivo* PK exposure to prednisolone in ALL are limited.[13,20]

Prednisolone is highly bound to plasma proteins and shows both linear binding to albumin and non-linear binding to corticosteroid-binding globulin (CBG).[13,21–23] The binding to plasma proteins, and therefore the exposure to the active unbound prednisolone, might be affected by the disease and concomitant chemotherapy. However, no studies have been performed linking unbound prednisolone plasma concentrations to the clinical response in ALL. If a correlation is found between unbound prednisolone and clinical outcome parameters, patients might benefit from individualised dosing.

The aim of the present study was to assess the PKs of unbound prednisolone and its relation to early treatment response in paediatric patients with ALL. The relationship between prednisolone exposure and effect was evaluated using the day 8 prednisone response, the minimal residual disease (MRD) levels and relapse risk in the total population, as well as in well-defined genetic subgroups of sufficient size.[5,6,24,25]

Patients and methods

Patients and treatment

The study was designed as a prospective multicentre Dutch Childhood Oncology Group (DCOG) study, performed in seven paediatric oncology centres within the Netherlands. Patients with ALL aged 0–18 years and treated according to the DCOG ALL-11 (April 2012–July 2020) protocol or Interfant-06 (February 2006–August 2016) protocol were eligible for enrolment. Both protocols were Institutional Review Board approved [European Union Drug Regulating Authorities Clinical Trials Database (EudraCT): 2012-00006725 (ALL-11); Dutch Trial Registry nr. 3379]. Patients with Down syndrome were excluded from this PK study due to potential altered PKs.[26,27] Patients received 60 mg/m^2/day prednisolone either intravenously (i.v.) or orally [*per os* (p.o.)] divided into three single doses per day. During the first week of treatment, patients received prednisolone and one intrathecal methotrexate (MTX) injection at the start of treatment, and patients often switched from i.v. to p.o. prednisolone during the first week. Induction treatment subsequently consisted of p.o. prednisolone with weekly vincristine and daunorubicin, PEG-asparaginase at days 12 and 26, and intrathecal injections (single MTX for prophylaxis, or triple MTX, cytarabine and prednisolone in case of central nervous system involvement), for a total duration of 4 weeks.

Patients were stratified to patients with a prednisone good response (defined as <1000 leukaemic blasts/μl blood on day 8 after 7 days of consecutive prednisolone treatment) and patients with ≥1000 leukaemic blasts/μl who were considered to have a poor response. Risk group classification was done according to the DCOG ALL-11 protocol criteria (Supplement S2).

Sample collection and analysis

Blood samples were collected in the first week of treatment (prior to concomitant chemotherapy), and during week 2–4 of treatment (with concomitant chemotherapy). All samples were collected prior to administration (trough) of prednisolone around the maximum concentration/time of maximum concentration (C_{max}/T_{max}) at 1, 2 and 4 h after administration during steady state (>24 h after the start of prednisolone treatment).[28–30] Blood samples were collected in K2 EDTA tubes and centrifuged at room temperature within 2 h after withdrawal. Supernatant (serum) was collected and stored at −80°C prior to analysis. Samples were analysed with liquid chromatography tandem mass spectrometry [LC-MS/MS; LC: Shimadzu LC-30 Nexera (Nishinokyo-Kuwabaracho, Japan) and MS: AB Sciex 5500 QTrap® (Framingham, MA, USA)] at the Department of Hospital Pharmacy in the Academic University Medical Centre in Amsterdam, the Netherlands. Details are specified in the Supplement S5.

Pharmacokinetic analysis

The concentration time profiles of prednisone and prednisolone were analysed using non-linear effects modelling approach in NONMEM® first-order conditional estimates (FOCE) with interaction (version 7.3, ICON, Development Solutions, Hanover, MD, USA). Pirana software version 2.9.5b (Certara, Princeton, NJ, USA) was used as a modelling environment, and data were further handled in R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). One- and multi-compartment linear models with first-order absorption for p.o. administration were fitted to the unbound prednisolone concentrations. Allometric scaling was implemented *a priori* to normalise the PK parameters over a wide range of body weights, using fixed exponent values of 0.75 for flow-dependent process parameters and 1 for volume-related parameters.[31–33] Parameters were normalised to a weight of 70 kg. The fit of the model was evaluated both numerically by the precision of the estimated PK parameters and the change in the objective function values (OFV) and visually by goodness-of-fit (GoF) plots, and visual predictive checks (VPC). A 3.84-point decrease in OFV for one degree of freedom was considered a significant improvement with a $P < 0.05$. Proportional and constant error models were tested to describe the residual error in plasma concentrations.

Prednisolone exhibits a non-linear (saturable) binding to CBG and a linear binding to albumin.[13,22] Prednisolone plasma protein binding was modelled using the formula reported by Ionita *et al.*[22] (Supplement S1). For missing albumin concentrations, the population median value was used. A schematic overview of the final model is shown in Fig 1.

After finalisation of the structural model, a covariate analysis was performed. Covariates included gender, age, body surface area (BSA), treatment period, albumin, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), bilirubin and urea, treatment block, pharmaceutical formulation (tablet, suspension, i.v.), administration routes (p.o., i.v., tube), creatinine and glomerular filtration rate. Continuous covariates were centred on the median. Missing covariates were replaced by the covariate median. The evaluation of covariates was performed using stepwise regression with iterative forward selection.[34] A decrease of ≥3.84 points in OFV for one degree of freedom was used for forward selection ($P < 0.05$). The robustness of the parameter estimates was evaluated using a non-parametric bootstrap procedure ($n = 1000$). A VPC was performed for internal validation of the model.

Pharmacodynamic analysis

Individual area-under-the-curve (AUC) values were calculated on basis of *post hoc* values for clearance. Correlation was evaluated of exposure and white blood cell (WBC) count, blast count at diagnosis (blood and bone marrow), the prednisone response at day 8, the day 15 bone marrow response and MRD levels on day 15, 33 and 79. For the analysis of prednisone response patients with <1000 blasts/μl at initial diagnosis were excluded from the analysis as their response could not be adequately assessed. Group differences in exposure were evaluated for leukaemia immunophenotype (T-cell or B-cell precursor), and available cytogenetic data [ETS variant transcription factor 6 (*ETV6*)-Runt-related transcription factor 1 (*RUNX1*), transcription factor 3 (*TCF3*)-pre-B-cell leukaemia homeobox 1 (*PBX1*), breakpoint cluster region-Abelson (*BCR-ABL*), hyperdiploidy, histone-lysine *N*-methyltransferase 2A (*KMT2A*)-AF4/FMR2 family member 1 (*AFF1*) and Ikaros family zinc finger protein 1 (*IKZF1*)-del]. Additionally, the AUC of unbound prednisolone was compared between patients who relapsed *versus* patient who did not.

Statistical analysis

Differences between groups were evaluated using Mann–Whiney *U*-test, analysis of variance (ANOVA), Kruskal–Wallis and Fisher's exact test. Relations between variables were evaluated using regression analysis and Spearman's rank correlation. Kaplan–Meier analysis was used to estimate relapse rate stratified by the exposure. A two-sided $P < 0.05$ was considered as statistically significant. Statistical analysis was performed using R (R Foundation for Statistical Computing).

Results

Patients and samples

Blood samples of 132 patients were available. Eight patients were excluded due to incomplete data and 124 patients were used for the PK analysis. A total of 25 prednisolone samples were excluded due to missing information (e.g. time of administration, sampling or dose time), sampling artefacts or concentrations below the lower limit of quantification. A total of 1028 unbound and total prednisolone concentrations were available. The median (range) age of the patients was 6·2 (0·4–17·7) years and the median (range) BSA was 0·86 (0·36–2·2) m^2. The population consisted of 37% girls and 63% boys. Eight patients were classified as prednisone poor responders (PPRs) and 110 as prednisone good responders (PGRs), and six unknowns. A total of 32 of the PGRs had starting leukaemic blasts of <1000/μl and were excluded for the prednisone response analysis. The subset of patients in this study did not differ significantly from the total patients treated according to ALL-11 in the Netherlands with respect to demographics, immunophenotype, risk group stratification, WBC count and prednisone response ($P > 0·05$). An overview of patients and sample characteristics can be found in Table I.

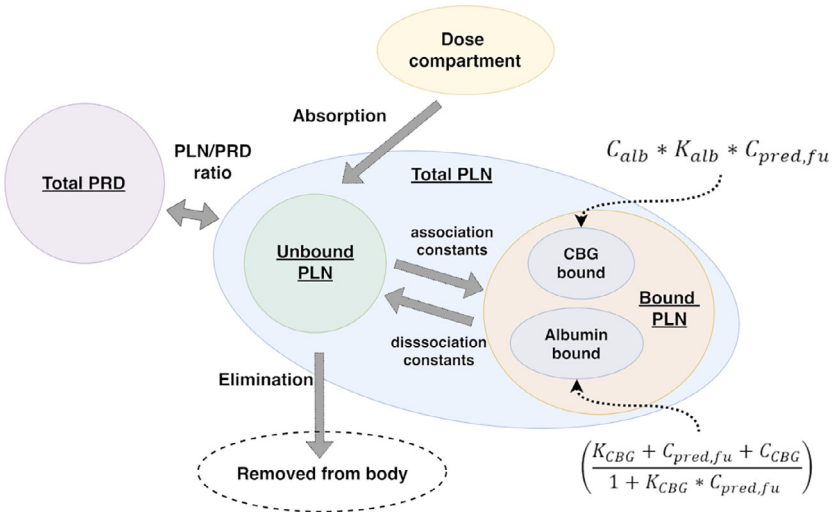

Fig 1. Final model layout. A representation of the final pharmacokinetic model. PLN, prednisolone; PRD, prednisone; CBG, corticosteroid-binding globulin; C_{alb}, albumin concentration; K_{alb}, albumin affinity constant; $C_{pred,fu}$, unbound prednisolone concentration; K_{cbg}, CBG affinity constant; C_{cbg}, CBG concentrations.

Pharmacokinetic analysis

A one-compartment model with first-order absorption best described the unbound prednisolone plasma concentration. Allometric scaling of the PK parameters improved the model significantly ($P < 0{\cdot}001$); the inter-individual variability (IIV) in clearance (CL) and apparent volume of distribution (V) decreased from 54% to 31% and 73% to 33% respectively. Prednisolone is reversibly metabolised into inactive prednisone, which was added to the model. The median (range) percentage of bound prednisolone was 86 (71–99)%. Implementing the ratio of total prednisolone/prednisone concentrations over time significantly improved the model ($P < 0{\cdot}001$). The affinity of the plasma protein CBG for prednisolone (Kcbg) could not be estimated and was fixed to 30 μmol/l, as found in the literature; nor were the CBG levels measured, which were estimated by the PK model.[22] The final structural model was an allometrically scaled one-compartment model with first-order absorption, including plasma protein binding of prednisolone and the ratio of prednisolone/prednisone (Fig 1). Inter-individual variability was described for clearance and distribution, and the residual variability was best described using a proportional error.

The structural model was used for the covariate analysis. In a univariate analysis an association between ALAT, ASAT, bilirubin and treatment phase and both clearance and distribution was observed ($P < 0{\cdot}01$), whereas albumin ($P < 0{\cdot}05$) solely correlated with clearance. The plasma protein binding of prednisolone to CBG was associated with patient age (Fig 2), ASAT and treatment phase ($P < 0{\cdot}01$). After iterative forward inclusion, both ASAT and treatment phase on distribution, and age on CBG concentration remained. ASAT had a positive correlation with distribution; high ASAT was associated with higher distribution ($P < 0{\cdot}001$). The estimated CBG concentration decreased with age ($P < 0{\cdot}001$). Distribution was slightly lower in the treatment phase after week 1 with concomitant chemotherapy ($P < 0{\cdot}001$). The fraction of unbound prednisolone *versus* age over the concentration range is shown in Fig 3. No correlation between clearance of unbound prednisolone (corrected for BSA) and age was observed. An overview of the final parameter estimates can be found in Table II.

In the non-parametric bootstrap procedure 480 of the 500 runs were successful and model estimates were in accordance with the results from the bootstrap replicates, indicating the robustness of the model (Table II). The VPCs for both free and total prednisolone and the GoF plots demonstrate the adequacy of the developed model (Supplement S3). Peak concentrations were slightly under predicted especially for the unbound prednisolone concentrations, probably due to limited samples in the absorption phase. Patients with high peak concentrations were significantly younger.

Pharmacodynamic analysis

Individual *post hoc* estimates of the final model were used to evaluate differences in exposure between and within subgroups of the population. Patients who were PPRs ($n = 8$) seemed to have a slightly lower unbound AUC than patients who were PGRs ($n = 78$); however, the difference was not statistically significant ($P = 0{\cdot}2$), with median AUC values 520 [interquartile range (IQR) 451–577] and 553 (IQR 487–650) ng × h/ml respectively. No differences in AUC were observed between the highest and lowest quartiles of WBC count, blasts at diagnosis (both day 8 blood or 15 bone marrow), and MRD at day 15, 33 and 79, nor in the more resistant subgroups (T-cell phenotype and combined B-cell genetic subtypes *IKZF*-del, *KMT2A-AFF1 and BCR-ABL1*), where we assumed the effect of exposure might be greater due to cellular resistance. Additionally,

Table I. Patients' characteristics at diagnosis.

Characteristic	Total
Patients, *n*	124
Female:male, %	37:63
Median (range)	
Age, years	6·0 (0·4–17·7)
Weight, kg	22 (7–86)
Height, cm	122 (68–188)
BSA, m^2	0·86 (0·36–2·2)
Creatinine, μmol/l	29 (11–92)
ALAT, u/l	45 (5–99)
ASAT, u/l	29 (8–100)
Bilirubin, μmol/l	10 (1–77)
Urea, mmol/l	5·1 (1·6–51)
Albumin, g/l	38 (10–100)
Samples unbound + total, *n*	1 028
Samples per patient, *n*, median (range)	4 (1–10)
Dose prednisolone, mg, median (range)	16·5 (3–45)
B cell, *n*	108
Other, *n*	38
ETV6-RUNX1, *n*	24
IKZF-del, *n*	9
KMT2A-AFF1, *n*	3
TCF3-PBX1, *n*	2
Hyperdiploid, *n*	30
BCR-ABL1, *n*	2
T cell, *n*	16
PPRs:PGRs, *n*	8:110
SR:MR:HR, *n*	30:79:10
Median (range)	
WBC diagnosis, × 10^9/l	12·1 (0·5–366)
WBC day 8, × 10^9/l	1·9 (0·2–73)
MRD day 15, × 10^{-3}	5 (0–2)
MRD day 33, × 10^{-4}	1·5 (0–0·7)

HR, ALL-11 high risk; MR, ALL-11 medium risk; MRD, minimal residual disease; PGRs, prednisone good responders; PPRs, prednisone poor responders; SR, ALL-11 standard risk; WBC, white blood cell count.

the AUC was compared between the different ALL-11 risk groups, standard risk (SR, $n = 30$), medium risk (MR, $n = 79$) or high risk (HR, $n = 10$). Although the AUC seemed to decrease with risk, no significant differences were found; median (IQR) 593 (482–651), 531 (475–651) and 477 (379–652) ng × h/ml respectively (Fig 4). There was no difference between B-cell precursor ($n = 108$) and T-cell ($n = 16$) immunophenotype in the dose-normalised AUC or between the various B-cell precursor genetic subtypes (Fig 5). However, the majority of the patients were *ETV6-RUNX1*, hyperdiploid and B-other, the number of patients in other subtypes was too limited for subgroup analysis.

The Kaplan–Meier shows the probability of relapse-free survival stratified by low (Q1), mid (Q2–Q3) and upper quartile (Q4) AUC of unbound prednisolone. Nine patients relapsed, of which four had an AUC in the lowest quartile, five in the middle and none in the highest quartile (Fig 6). However, the subgroups were very small and therefore only large group effects could be observed. To determine whether the difference in exposure and prednisone response, HR *versus* MR and SR, and relapse-free survival would require over 979, 495 and 364 patients respectively. However, this does not take into account whether this is a clinically relevant difference. The latter is probably not the case due to the large observed variability in exposure in all groups and adjusting the exposure would most likely not result in an improved clinical outcome.

Discussion

Glucocorticoids have an important place in the treatment of paediatric ALL. Patients who are more resistant to prednisolone experience worse outcome.[5,6] The occurrence of resistance to prednisolone in paediatric ALL has been extensively studied. However, the possible association between *in vivo* prednisolone exposure and outcome has not been studied to date. Kawedia *et al.*[14] reported a higher clearance of dexamethasone in younger children, which could result in lower exposure when compared to older children. This might also be the case for prednisolone and might advocate dose modifications in younger patients. However, in the present study no correlation was found between exposure and age. A complicating factor in the treatment with prednisolone may be the concentration-dependent plasma protein binding (from 95% at low concentrations to 60% at high concentrations).[13,21,22] Hence, in the present study, unbound, pharmacologically active, prednisolone was measured in plasma and related to different disease parameters.

The PKs of unbound prednisolone was best described by a one-compartment model with first-order absorption and allometric scaling. This model included the protein binding of prednisolone to the plasma proteins CBG and albumin, and the prednisolone/prednisone ratio. The volume of distribution was smaller in treatment phases with concomitant chemotherapy (>week 1). This might be due to patients receiving hyperhydration in the first week of treatment to prevent tumour lysis syndrome and no asparaginase. In addition, a positive correlation between ASAT and volume of distribution was observed. ASAT was significantly higher in the first week compared to subsequent weeks with chemotherapy (median 32 *versus* 23·5 u/l; $P = 0·02$). The addition of ASAT resulted in a significant improvement of the PK model on top of the treatment phase ($P < 0·01$). ASAT might be used as a marker for liver function; however, it would be expected that high ASAT correlates with a smaller volume of distribution, due to less plasma protein binding. Therefore, it is more likely that the association between volume of distribution and ASAT reflects the collinearity between the latter and the treatment phase, e.g. due to cell decay.

The estimated CBG concentration showed a positive correlation with patient age, with lower CBG concentrations and lower protein binding in older patients. The affinity was set to

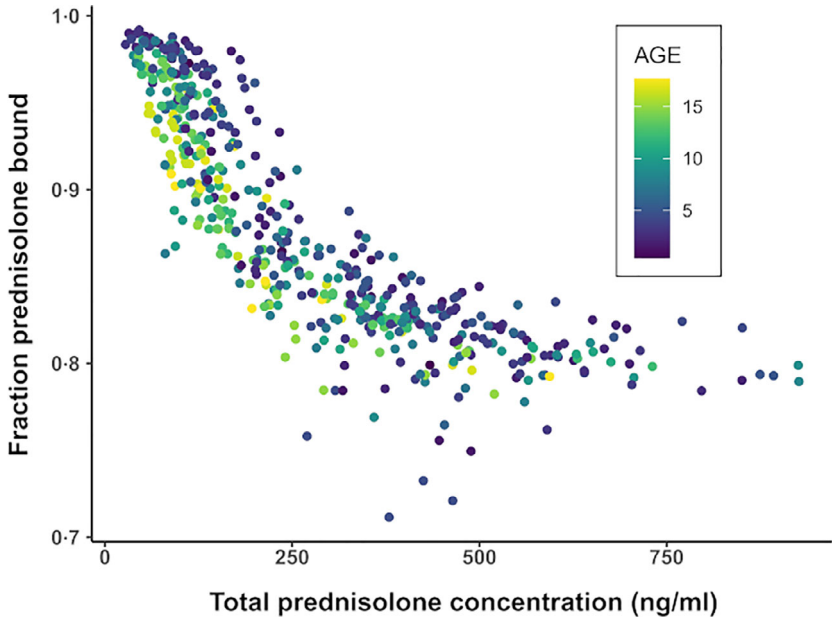

Fig 2. Fraction prednisolone unbound *versus* total concentration by age. The fraction of total prednisolone bound to plasma proteins *versus* the total prednisolone concentration. The colours indicate age, from young to old patients, dark blue to light blue respectively. Younger patients seem to have a higher fraction of prednisolone bound to proteins compared the older patients.

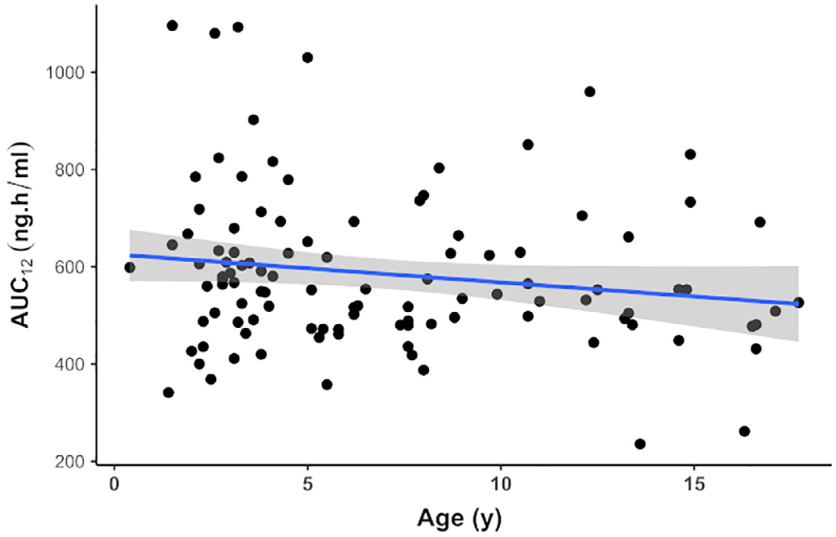

Fig 3. Age *versus* area under the curve (AUC). Dose-corrected unbound $AUC_{[0–12]}$*versus* the age of the patients. No correlation was found between the AUC and patients' age. It shows a large variability in the AUC ($P = 0{\cdot}13$). The line is the regression line.

a fixed value (30 μmol/l as seen in Ionita *et al.*).[22] However, a wide array of affinity constants can be found in the literature.[13,21–23] Due to the fixed affinity of prednisolone and CBG, its role between and within patients could not be evaluated. Prednisone and cortisol bind to CBG as well, although this does not have a large effect on prednisolone.[21,23]

In the present study, the clearance of unbound prednisolone per m^2 did not correlate with age, which is different from results found with dexamethasone clearance, where younger age was associated with higher clearance.[19] Prednisolone has some distinct PK differences compared to dexamethasone. Prednisolone exhibits non-linear binding to plasma proteins whereas dexamethasone does not. The fraction of unbound prednisolone increases when the concentration of total prednisolone increases. Although small differences were observed in protein binding with age and peak concentrations, our present data demonstrated that the AUC of the unbound prednisolone was similar throughout the ages.

The relationship between prednisolone exposure and clinical outcome parameters were studied. No differences in

Table II. Pharmacokinetic parameters and bootstrap.

	NONMEM					Bootstrap		
Parameter	Estimate	RSE, %	95% CI (lower)	95% CI (upper)	Shrink, %	Median	95% CI (lower)	95% CI (upper)
CL/F, l/h/70 kg	100	5	91	109	–	100	91	109
V/F, l/70 kg	589	9	490	688	–	583	499	684
K_a, h	4	2	3·9	4·2	–	4·0	2·0	7·1
C_{cbg}, µmol/l	0·83	6	0·73	0·92	–	0·84	0·74	0·95
K_{alb}, µmol/l	0·002	20	0·001	0·003	–	0·002	0·001	0·003
ASAT–V	0·15	52	0·0	0·29	–	0·16	0·0	0·34
Age–CBG	−0·15	40	−0·27	−0·03	–	−0·15	−0·27	−0·03
Ratio–CL	−0·48	21	−0·68	−0·28	–	−0·49	−0·67	−0·27
Block–V	0·87	5	0·78	0·96	–	0·88	0·78	1·0
IIV CL, %	34	14	26	40	28	34	24	42
IIV V, %	53	15	38	69	21	57	39	78
IIV C_{cbg}, %	29	18	19	39	30	28	15	39
Res err, free	0·63	4	0·58	0·68	5·7	0·63	0·58	0·68
Res err, total	0·36	4	0·33	0·39	8·5	0·36	0·33	0·39

ASAT, aspartate aminotransferase; CBG, corticosteroid-binding globulin; C_{cbg}, CBG concentration; CI, confidence interval; CL, clearance; CL/F, the apparent clearance per bioavailability; IIV, inter-individual variability; K_a, absorption rate constant; K_{alb}, affinity constant for prednisolone to albumin; RSE, relative standard error; Res err, residual error of free and total prednisolone; V, volume of distribution.
V/F, apparent volume of distribution.
Age–CBG, covariate age on prednisolone binding to CBG.
ASAT–V, covariate ASAT on apparent V.
Block–V, covariate treatment block on V.
Ratio–CL, covariate total prednisolone over prednisone ratio on CL.

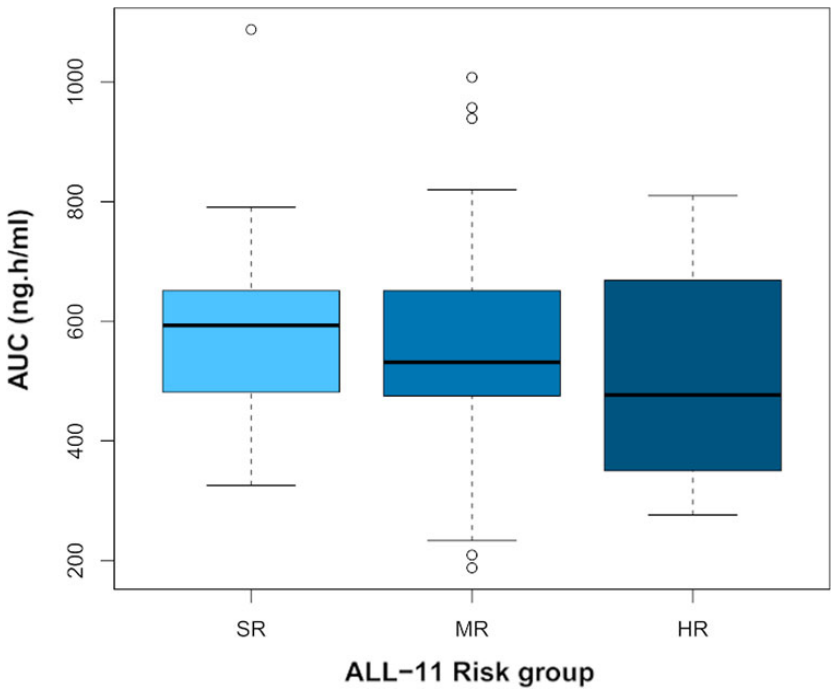

Fig 4. Area under the curve (AUC) of unbound prednisolone *versus* ALL-11 risk groups. In this figure the AUC of unbound prednisolone was compared between patients stratified in different ALL-11 risk groups, standard risk (SR; $n = 30$), medium risk (MR; $n = 79$) and high risk (HR; $n = 10$). The median [interquartile range (IQR)] AUC SR: 593 [482–651], MR: 531 (IQR 475–651) and HR: 477 (IQR 379–652) ng × h/ml (not statistically different; $P = 0·20$).

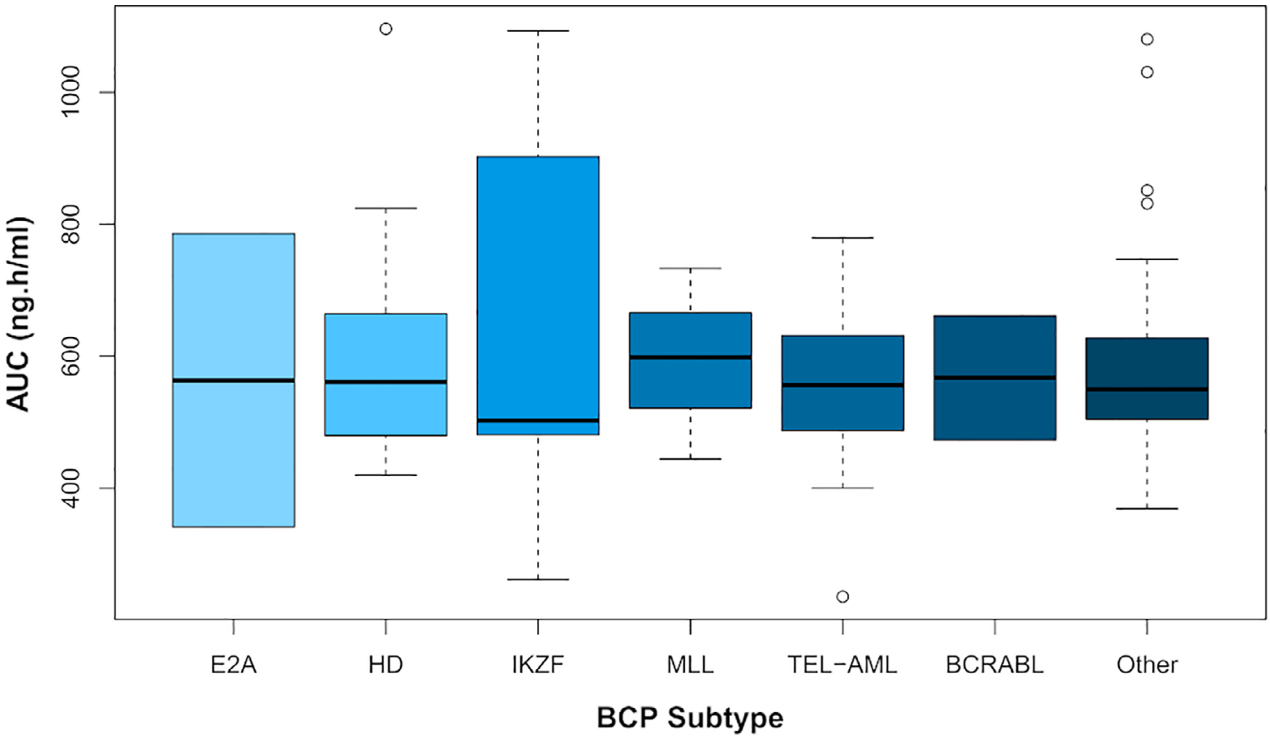

Fig 5. Area under the curve (AUC) *versus* B-cell precursor (BCP) genetic subtype. This figure shows the patients' AUC for different B-cell genotypes. No difference in AUC was found between the groups. Three of the subtypes only had a very limited number of patients *TCF3-PBX1* (n = 2), mixed-lineage leukaemia (MLL): *KMT2A-AFF1* (n = 3), *BCR-ABL1* (n = 2). Hyperdiploid (HD; n = 30), *ETV6-RUNX1* (n = 24), *IKZF*-del (n = 9), B-other (n = 38).

exposure were observed between the PPRs and PGRs. This might suggest that the response is predominantly influenced by the cellular sensitivity to prednisolone and not due to lower exposure to prednisolone. This is also supported by the fact that no difference was found between the AUC quartiles of the more prednisolone resistant phenotype and B-cell genetic subtypes and treatment response. Hence, the present study shows no effect of prednisolone exposure on treatment response after a high dose of 60 mg/m^2/day.

The exposure for patients in the ALL-11 risk groups seemed to slightly decrease with increasing risk. Four (44%) of the nine patients who relapsed had AUC values in the lowest quartile and none in the upper quartile (Q4). The Kaplan–Meier estimates did not show a significant difference between the AUC quartiles and cumulative incidence of relapse (Fig 6). However, the number of patients in the high-risk and more resistant subgroups was small and differences between exposure and outcome within these subgroups could not be well determined.

Conclusion

A PK model was developed to describe the time profile of unbound prednisolone plasma concentration in paediatric patients with ALL. A one-compartment model with allometric scaling and a combined saturable and linear protein binding described the data adequately. Protein binding was slightly higher in younger patients. However, the AUC of unbound prednisolone did not differ with age. In the present study, no differences were observed between exposure and disease outcomes for PGRs, including day 8 prednisone response, blast counts and MRD. The sensitivity to prednisolone is probably the prominent factor regarding prognosis and individualised dosing in this group might not improve outcome. Regarding the PPRs, relapse and high-risk patients, the numbers were small. Future studies might look at whether a combination of increased prednisolone dosing either in combination with sensitisation to prednisolone (e.g. MEK inhibitor) is feasible and beneficial in a hard to treat subset of patients.

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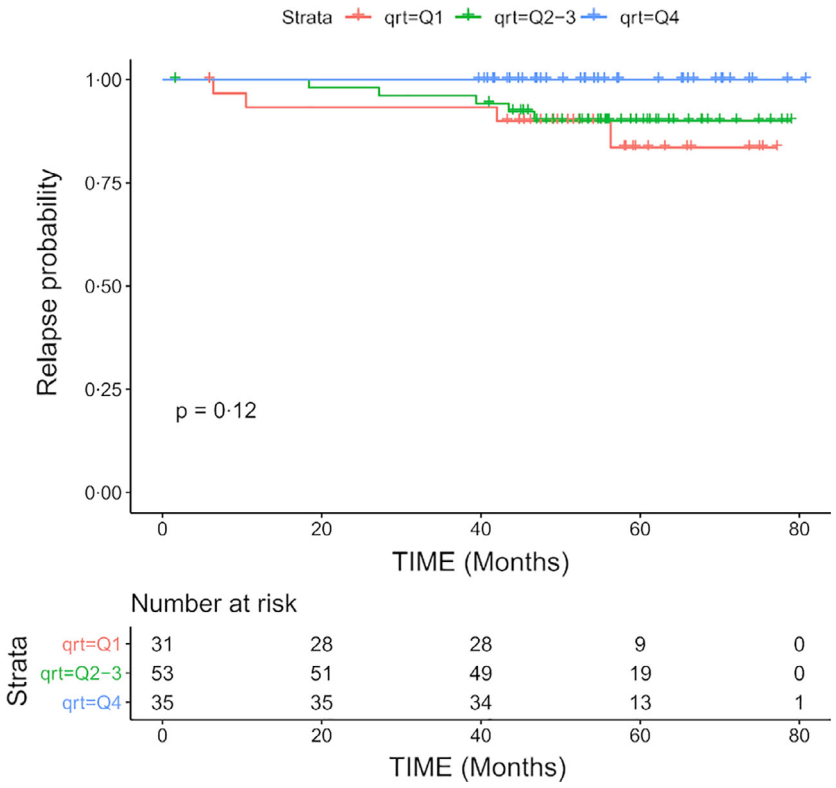

Fig 6. Kaplan–Meier survival and relapse *versus* area under the curve (AUC). Kaplan–Meier of relapse (A) and survival (B) for patients within different AUC quartiles, lower quartile (Q1), mid quartiles (Q2 and Q3) and the upper quartile (Q4) with the highest AUC. Nine patients relapsed of which there were four in Q1 and five in Q2–3. Four patients died of which three were in Q1 and one in Q2–3.

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Conflict of interest

None for the topic under investigation.

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Supporting Information

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

Supplement S1. Equations.

Supplement S2. ALL-11 risk stratification.

Supplement S3. Figures.

Supplement S4. PK model.

Supplement S5. Sample collection and analysis (extended)

Supplement S6. Patient characteristics per AUC quartile stratification.

References

1. Pieters R, Carroll WL. Biology and treatment of acute lymphoblastic leukemia. *Hematol Oncol Clin North Am*. 2010;**24**:1–18.
2. Schrappe M, Nachman J, Hunger S, Schmiegelow K, Conter V, Masera G, et al. Educational symposium on long-term results of large prospective clinical trials for childhood acute lymphoblastic leukemia (1985–2000). *Leukemia*. 2010;**24**:253–4.
3. Silverman LB, Stevenson KE, O'Brien JE, Asselin BL, Barr RD, Clavell L, et al. Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985–2000). *Leukemia*. 2010;**24**:320–34.
4. Inaba H, Pui CH. Glucocorticoid use in acute lymphoblastic leukaemia. *Lancet Oncol*. 2010;**11**:1096–106.
5. Reiter A, Schrappe M, Ludwig WD, Hiddemann W, Sauter S, Henze G, et al. Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. *Blood*. 1994;**84**:3122–33.
6. Dördelmann M, Reiter A, Borkhardt A, Ludwig WD, Götz N, Viehmann S, et al. Prednisone response is the strongest predictor of treatment outcome in infant acute lymphoblastic leukemia. *Blood*. 1999;**94**:1209–17.
7. Haarman EG, Kaspers GJ, Pieters R, Rottier MM, Veerman AJ. Circumvention of glucocorticoid resistance in childhood leukemia. *Leuk Res*. 2008;**32**:1417–23.
8. Haarman EG, Kaspers GJ, Veerman AJ. Glucocorticoid resistance in childhood leukaemia: mechanisms and modulation. *Br J Haematol*. 2003;**120**:919–29.
9. Kaspers GJ, Pieters R, Van Zantwijk CH, VanWering ER, Van Der Does-Van Den Berg A, Veerman AJ. Prednisolone resistance in childhood acute lymphoblastic leukemia: vitro-vivo correlations and cross-resistance to other drugs. *Blood*. 1998;**92**:259–66.

10. Tissing WJ, Meijerink JP, Brinkhof B, Broekhuis MJ, Menezes RX, den Boer ML, et al. Glucocorticoid-induced glucocorticoid-receptor expression and promoter usage is not linked to glucocorticoid resistance in childhood ALL. *Blood*. 2006;**108**:1045–9.
11. Gupta M, Kumar A, Dabadghao S. Resistance of BCR-ABL-positive acute lymphoblastic leukemia to daunorubicin is not mediated by mdr1 gene expression. *Am J Hematol*. 2002;**71**:172–6.
12. Pieters R, den Boer ML, Durian M, Janka G, Schmiegelow K, Kaspers G, et al. Relation between age, immunophenotype and in vitro drug resistance in 395 children with acute lymphoblastic leukemia - implications for treatment of infants. *Leukemia*. 1998;**12**:1344–8.
13. Petersen KB, Jusko WJ, Rasmussen M, Schmiegelow K. Population pharmacokinetics of prednisolone in children with acute lymphoblastic leukemia. *Cancer Chemother Pharmacol*. 2003;**51**:465–73.
14. Kawedia JD, Kaste SC, Pei D, Panetta JC, Cai X, Cheng C, et al. Pharmacokinetic, pharmacodynamic, and pharmacogenetic determinants of osteonecrosis in children with acute lymphoblastic leukemia. *Blood*. 2011;**117**:2340–7.
15. Yang L, Panetta JC, Cai X, Yang W, Pei D, Cheng C, et al. Asparaginase may influence dexamethasone pharmacokinetics in acute lymphoblastic leukemia. *J Clin Oncol*. 2008;**26**:1932–9.
16. Gaynon PS, Lustig RH. The use of glucocorticoids in acute lymphoblastic leukemia of childhood: molecular, cellular, and clinical considerations. *J Pediatr Hematol Oncol*. 1995;**17**:1–12.
17. Kaspers GJL, Veerman AJ, Popp-Snijders C, Lomecky M, Van Zantwijk CH, Swinkels L, et al. Comparison of the antileukemic activity in vitro of dexamethasone and prednisolone in childhood acute lymphoblastic leukemia. *Med Pediatr Oncol*. 1996;**27**:114–121.
18. Möricke A, Zimmermann M, Valsecchi MG, Stanulla M, Biondi A, Mann G, et al. Dexamethasone vs prednisone in induction treatment of pediatric ALL: results of the randomized trial AIEOP-BFM ALL 2000. *Blood*. 2016;**127**:2101–12.
19. Kawedia JD, Liu C, Pei D, Cheng C, Fernandez CA, Howard SC, et al. Dexamethasone exposure and asparaginase antibodies affect relapse risk in acute lymphoblastic leukemia. *Blood*. 2012;**119**:1658–64.
20. Choonara I, Wheeldon J, Rayner P, Blackburn M, Lewis I. Pharmacokinetics of prednisolone in children with acute lymphoblastic leukaemia. *Cancer Chemother Pharmacol*. 1989;**23**:392–4.
21. Rocci ML, D'Ambrosio R, Johnson NF, JusKo WJ. Prednisolone binding to albumin and transcortin in the presence of cortisol. *Biochem Pharmacol*. 1982;**31**:289–92.
22. Ionita IA, Ogasawara K, Gohh RY, Akhlaghi F. Pharmacokinetics of total and unbound prednisone and prednisolone in stable kidney transplant recipients with diabetes mellitus. *Ther Drug Monit*. 2014;**36**:448–55.
23. Boudinot FD, Jusko WJ. Plasma protein binding interaction of prednisone and prednisolone. *J Steroid Biochem*. 1984;**21**:337–9.
24. van Dongen JJ, Seriu T, Panzer-Grümayer ER, Biondi A, Pongers-Willemse MJ, Corral L, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. *Lancet*. 1998;**352**:1731–8.
25. Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grümayer R, Möricke A, 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**:3206–14.
26. Hefti E, Blanco JG. Pharmacokinetics of chemotherapeutic drugs in pediatric patients with down syndrome and leukemia. *J Pediatr Hematol Oncol*. 2016;**38**:283–7.
27. Buitenkamp TD, Mathôt RA, de Haas V, Pieters R, Zwaan CM. Methotrexate-induced side effects are not due to differences in pharmacokinetics in children with down syndrome and acute lymphoblastic leukemia. *Haematologica*. 2010;**95**:1106–13.
28. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Guidance for industry. Population pharmacokinetics. [Internet]. Washington, DC; 1999.
29. Pattar R, Ensom M. Utility of limited sampling strategies for anticancer agents in the clinical arena: a systematic review. *Curr Cancer Ther Rev*. 2009;**5**:45–66.
30. Suarez-Kurtz G, Estrela RD, Salvadori MC. Prednisolone: limited sampling strategies for estimating pharmacokinetic parameters. *Ther Drug Monit*. 2004;**26**:16–22.
31. Holford N, Heo YA, Anderson B. A Pharmacokinetic standard for babies and adults. *J Pharm Sci*. 2013;**102**:2941–52.
32. Savage VM, Deeds EJ, Fontana W. Sizing up allometric scaling theory. *PLoS Comput Biol*. 2008;**4**:e1000171.
33. Wang C, Peeters MY, Allegaert K, Blussé van Oud-Alblas HJ, Krekels EH, Tibboel D, et al. A bodyweight-dependent allometric exponent for scaling clearance across the human life-span. *Pharm Res*. 2012;**29**:1570–81.
34. Hutmacher MM, Kowalski KG. Covariate selection in pharmacometric analyses: a review of methods. *Br J Clin Pharmacol*. 2015;**79**:132–47.

