Ciclosporin kinetics in children after stem cell transplantation

A. J. Willemze,¹ S. C. Cremers,² R. C. Schoemaker,³ A. C. Lankester,¹
J. den Hartigh,² J. Burggraaf³ & J. M. Vossen¹

¹Department of Paediatrics, Division of Immunology, Haematology, Oncology and Bone Marrow Transplantation and Autoimmune Diseases and ²Department of Clinical Pharmacy and Toxicology, Leiden University Medical Centre, and ³Centre for Human Drug Research (CHDR), Leiden, the Netherlands

Correspondence

Annemieke J. Willemze MD, Leiden University Medical Centre, Albinusdreef 2, 2300RC Leiden, the Netherlands.
Tel: +31 71 526 4132
Fax: +31 71 524 8198
E-mail: a.j.willemze@lumc.nl

Keywords

Bayesian, children, ciclosporin, pharmacokinetics, stem cell transplantation

Received

22 January 2008

Accepted

18 April 2008

Published OnlineEarly

20 May 2008

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Ciclosporin is an immunosuppressant drug that is commonly used as prophylaxis against graft-versus-host disease (GVHD) after allogeneic haematopoietic stem cell transplantation (SCT) in children.
- Therapeutic drug monitoring is necessary because of its unpredictable absorption and narrow therapeutic window.
- Data on the pharmacokinetics of ciclosporin and the relation between its systemic exposure, as reflected by the AUC (area-under-the-curve), and the biological effect as GVHD prophylaxis are scarce in children after SCT.

WHAT THIS STUDY ADDS

- An adequate estimate of the systemic exposure of ciclosporin, following limiting sampling after intravenous and oral administration, can be obtained in children after SCT by determining the AUC using a two-compartment pharmacokinetic model with lag time in combination with a limited sampling strategy.
- Large interindividual variability in ciclosporin clearance was found, which was independent of body weight; this finding justifies a dosing strategy distinct from that per kg body weight.
- Limited sampling modelling may serve as a tool to study the relationship between systemic exposure (–AUC) and biological effects of ciclosporin, i.e. facilitation of engraftment, prevention of GVHD and avoidance of toxic effects of ciclosporin.

AIMS

To develop a limited sampling strategy to determine ciclosporin systemic exposure [area-under-the-curve(AUC)]. This is meant to be the first step in a future study of the relationship between AUC and the biological effects of ciclosporin.

METHODS

The pharmacokinetics of ciclosporin was investigated prospectively following stem cell transplantation (SCT) in 17 children, aged 1.8–16.1 years. Ciclosporin was given twice daily, intravenously over a short infusion of 2 h duration during the early post-SCT period, or orally later on, when oral medication was well tolerated. Parameter estimation was performed using nonlinear mixed effect modelling as implemented in the NONMEM program. Individual empirical Bayes estimates of clearance and distribution volume were correlated with the demographic variables.

RESULTS

Pharmacokinetics was described adequately with a two-compartment model with lag time (population estimates: $CL = 11.3 \text{ l h}^{-1}$; $V_c = 16.5 \text{ l}$; $V_p = 59.9 \text{ l}$; $t_{1/2} = 0.78 \text{ h}$, $t_{lag} = 0.6 \text{ h}$). The AUCs, determined for the combination of trough level with one time point between 2 and 3 h after dosing, correlated very well with the reference AUC ($r^2 = 0.97$). No correlation was found between clearance and distribution volume, and the demographic patient variables length, body weight, age and glomerular filtration rate.

CONCLUSION

A two-point limited sampling strategy, in combination with a Bayesian fitting procedure using the pharmacokinetic population model described, can adequately determine the AUC of ciclosporin. Since no correlation between clearance and body weight was found, dosing ciclosporin per kg bodyweight is not supported by the results of this study. We suggest starting with a fixed dose, followed by AUC determination and dose adjustment.

Introduction

During the past three decades, allogeneic haematopoietic stem cell transplantation (SCT) has been used for the treatment of an increasing number of diseases, including inherited immune diseases, benign and malignant haematological disorders and metabolic diseases [1, 2]. A major complication of SCT is acute graft-versus-host disease (GVHD). Ciclosporin, a calcineurin inhibitor, is commonly used as prophylaxis against GVHD after SCT [3]. Ciclosporin is a highly lipophilic drug whose pharmacokinetics are characterized by slow, incomplete and variable absorption, large volume of distribution, liver biotransformation via the 3A4 isoenzyme of cytochrome P450 (CYP) and low clearance (CL) [4]. Although it has been reported that children generally have higher ciclosporin clearance than adults, few paediatric studies have been undertaken to confirm this observation [5, 6]. As the drug has a relatively small therapeutic window, it is important to monitor its concentration in each individual patient [7]. Although systemic exposure is poorly reflected by trough levels [6], the current common practice for ciclosporin drug monitoring relies on trough level measurement [5].

Because of the limited size of the paediatric transplant population, studies carried out to characterize the pharmacokinetics of ciclosporin in children after SCT are limited. Likewise, no systematic analyses have yet been reported concerning the concentration–effect/toxicity relationship.

The aim of this study was to investigate prospectively ciclosporin pharmacokinetics in paediatric recipients of a SCT, after intravenous and oral administration of the drug, in order to develop a limited sampling strategy for the

determination of an area-under-the-curve (AUC), reflecting systemic exposure of ciclosporin. With a defined relationship between systemic exposure (AUC) and trough levels, using a statistical pharmacokinetic model, we will be able to study the clinical (beneficial and adverse) effects of ciclosporin in relation to the trough levels in a large group of paediatric SCT recipients. It will be possible to make accurate ciclosporin dose adjustments, and, in the future, possibly better controlled immunomodulatory interventions, such as intentional graft-versus-host reactions [8, 9].

Patients and methods

Patients and ciclosporin administration

Between January 2002 and October 2005, 17 children undergoing stem cell transplantation in the paediatric stem cell transplantation unit of Leiden University Medical Centre (LUMC), the Netherlands, were enrolled in the study (Table 1). Inclusion criteria were: weight >10 kg (i.e. age at least 1 year), GVHD prophylactic treatment with twice-daily ciclosporin (i.e. every 12 h), a central venous access (second lumen of Hickman catheter or port-a-cath) not in use for intravenous (i.v.) ciclosporin infusion, and at least 2 days of ciclosporin treatment before sampling. Exclusion criteria were: severe renal dysfunction (serum creatinine > 2× normal upper limit for age), severe liver dysfunction (serum bilirubin >50 µmol l⁻¹) and/or coagulation disorder, veno-occlusive disease, respiratory insufficiency, and haemodynamic instability. Sandimmune and Neoral (Novartis Pharma, Basel, Switzerland) were used for i.v. and oral

Table 1

Characteristics of patients and ciclosporin administration

UPN	Sex	Disease	Age (years)	Length (cm)	Weight (kg)	Serum creatinine (µmol l ⁻¹)	Days after SCT	Route	Dose (mg 24 h ⁻¹)	Dose (mg kg ⁻¹ day ⁻¹)	GVHD
1	M	AML	15	175	58	55	33	p.o.	350	6.1	No
2	M	CML	12	155	37	25	14	i.v.	120	3.3	No
3	M	JMML	2	89	14	26	2	i.v.	28	2.0	No
4	M	ALL	7	118	29	43	23	i.v.	58	2.0	No
5	F	ALL	7	127	37	48	30	i.v.	74	2.0	No
6	M	ALL	4	101	20	52	2	i.v.	40	2.0	Yes
7	M	ALL	7	106	19	46	24	i.v.	80	4.2	No
8	M	Thal	6	118	20	9	10	i.v.	42	2.1	No
9	M	MDS	14	160	54	32	12	i.v.	108	2.0	No
10	M	ALL	9	141	30	36	21	i.v.	60	2.0	No
11	M	SAA	5	125	25	38	27	p.o.	200	8.2	No
12	M	SAA	16	168	57	76	29	p.o.	350	5.3	No
13	M	Thal	10	128	24	41	29	p.o.	200	8.4	No
14	F	ALCL	15	165	46	77	36	p.o.	300	6.5	No
15	M	AML	15	164	51	46	28	p.o.	300	5.9	Yes
16	M	Thal	1	89	13	26	43	p.o.	90	6.9	Yes
17	M	Thal	5	110	17	27	25	p.o.	200	11.8	No

ALCL, anaplastic large cell lymphoma; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; i.v., intravenous administration; JMML, juvenile myelomonocytic leukaemia; MDS, myelodysplastic syndrome; p.o., oral administration; SAA, severe aplastic anaemia; Thal, β-thalassaemia major.

Figure 1

Examples of measured ciclosporin blood concentration–time curves and the model-predicted profiles; an individual profile in the top graphs, the average profile (plus SD error bars) in the bottom graphs. Vertical lines correspond to markers used for AUC calculation: 0, 1, 2, 3, 4 and 5 correspond to 0, 2.5, 3, 4, 5 and 7 h for i.v. administration (left) and to 0, 1, 2, 3, 4 and 6 h for p.o. administration (right). Solid line: ciclosporin blood concentration–time curve according to the model after fitting the measured concentrations to the population parameters to the measured concentrations. IV, intravenous administration (2-h infusion, —); PO, oral administration (at $t = 0$, ■)

administration, respectively. For reasons of feasibility the study design did not control for variables such as after SCT or dose adjustments of ciclosporin, which were based on trough levels (see below). Parents and the children aged ≥ 12 years gave written informed consent for participation in the study, which was approved by the ethics committee of LUMC. In all cases i.v. ciclosporin was started the day before infusion of the graft at a total dose of $2 \text{ mg kg}^{-1} \text{ day}^{-1}$ administered over two short i.v. infusions of 2 h duration, separated by a 10-h interruption. Following this initial dosing schedule, in accordance with the SCT guideline of the department, an individualized dose regimen was given based on ciclosporin trough levels in order to keep this level between 50 and $200 \mu\text{g l}^{-1}$. Following haematological engraftment and when oral medication was well tolerated, mostly at ≥ 3 weeks after SCT, the route of administration was changed from i.v. to oral, with the dose of ciclosporin being tripled in order to compensate for the difference in bioavailability of oral ciclosporin, twice daily, i.e. every 12 h. Blood sampling was undertaken from an i.v. cannula that was not in use for the administration of ciclosporin. During i.v. and oral ciclosporin administration the blood sampling was done prior to the dosing ($t = 0$ h; trough level) and at

various time points after start of the administration (see Figure 1). If necessary, dose adjustments were made on the basis of trough level results.

Pharmacokinetics and statistical analysis

Parameter estimation was performed using nonlinear mixed effect modelling as implemented in the NONMEM program [10]. This ‘population approach’ allowed the description of the entire dataset using a common structural model (Table 2). Based on these population estimates, individual empirical Bayes estimates were generated by NONMEM, which in turn were used to generate individual concentration–time profiles. First-order conditional estimation with interaction was used throughout, and a proportional residual error model was implemented. Increase in goodness of fit was assessed for tested models with the likelihood ratio test, using the difference in minimum value of the objective function (MVOF); this difference was compared with a χ^2 distribution with degrees of freedom equal to the difference in the number of freely estimated parameters. In order to examine the adequacy of a limited sampling approach, in combination with Bayesian estimation of model parameters using the obtained population infor-

Table 2

Ciclosporin population pharmacokinetics in children after stem cell transplantation

Parameter	Mean (SEM)	Interindividual coefficient of variation (%)
K_a (h^{-1})	0.831 (0.156)	38
V_c (l)	16.5 (4.72)	0% (fixed)
K (h^{-1})	0.625 (0.120)	35
K_{23} (h^{-1})	0.769 (0.185)	32
K_{32} (h^{-1})	0.265 (0.0429)	0
t_{lag} (h)	0.638 (0.0912)	37
F (%)	38.6 (7.87)	28
Residual variability (%)	19.3%	
$t_{1/2a}$ (h)*	0.777 (0.184)	41
V_p (l)*	59.9 (9.00)	19
CL (l h^{-1})*	11.3 (1.74)	36
Q (l h^{-1})*	12.9 (2.81)	52

*Parameters obtained using alternative parameterization. K_a , absorption rate constant; V_c , volume of distribution of the central compartment; K , elimination rate constant from the central compartment; K_{23} and K_{32} , distribution rate constants between the central and peripheral compartments; t_{lag} , lag time; F , oral bioavailability; $t_{1/2a}$, absorption half-life; V_p , volume of distribution of the peripheral compartment; CL, clearance; Q , intercompartmental clearance.

mation, empirical Bayes estimates were generated. These utilized the population estimates from the model in combination with the original dataset, but contained only a subset of data points. The AUCs, calculated using $F \times \text{dose}/CL$, were estimated for the following single and combined time points: auc0, auc1, auc2, auc3, auc01, auc02, auc03, auc012, auc013, auc023, auc0123, auc012345, and were correlated with the reference AUC. Correspondence between auc-indices and sampling time points is provided in Table 3 (e.g. auc03 means an AUC estimated using samples at 0 h and at 3 h after oral administration, or at 4 h after start of i.v. administration, see also Figure 1). Bland–Altman plots were generated comparing the limited sampling AUCs and the reference AUC. Furthermore, predictive performance of the different sampling strategies was investigated. The prediction bias was calculated as the mean prediction error (MPE; average difference between test and reference AUC) and the prediction precision as the mean absolute prediction error (MAPE; average absolute difference between test and reference AUC) as a percentage of the population average AUC. Smaller values for MPE and MAPE indicate less bias and greater precision. NONMEM estimates were obtained using both rate constants describing transfer between compartments (e.g. K_{23}) and using a more physiologically interpretable set of parameters, such as clearance. Individual empirical Bayes estimates of clearance and distribution volume were correlated with the demographic variables length, weight, age and glomerular filtration rate estimates using the Schwartz and Cockcroft–Gault formulas. Shrinkage for empirical Bayes estimates was calculated using PsN scripts [11].

Table 3

Bias, precision and Pearson correlation coefficients of different combinations of blood sampling time-points

Time-points blood sampling*	MPE (%)	MAPE (%)	r^2
0 (auc0)	−2.3	12.4	0.90
1 (auc1)	4.6	18.9	0.72
2 (auc2)	5.1	15.1	0.85
3 (auc3)	2.0	9.4	0.92
0, 1 (auc01)	0.1	7.9	0.94
0, 2 (auc02)	0.5	6.2	0.97
0, 3 (auc03)	−1.0	6.0	0.97
0, 1, 2 (auc012)	1.4	6.2	0.97
0, 1, 3 (auc013)	−0.0	3.8	0.99
0, 2, 3 (auc023)	0.3	3.3	0.99
0, 1, 2, 3 (auc0123)	0.7	3.4	0.99
0, 1, 2, 3, 4, 5 (auc012345)	0.4	1.6	1.00

MPE, mean prediction error; average difference between test and reference AUC (in %); MAPE, mean absolute prediction error; average absolute difference between test and reference AUC (in %); r^2 , squared Pearson correlation coefficient. *See Figure 1 for explanation of time points.

Drug analysis

Ciclosporin levels were determined in whole blood (ethylenediamine tetraacetic acid) using the routine methodology of the Laboratory for Clinical Pharmacy and Toxicology of the LUMC [fluorescence polarization immunoassay on the AxSYM; Abbott, Abbott Park, IL, USA]. The assay has an interassay coefficient of variation of <10% over a concentration range of 50–800 $\mu\text{g l}^{-1}$ and is linear over the therapeutic range with a sensitivity of 25 $\mu\text{g l}^{-1}$.

Results

Ciclosporin pharmacokinetics

Ciclosporin pharmacokinetics was adequately described by a two-compartment model with first-order absorption when given orally or intravenously at a constant rate 2 h infusion, assuming steady-state conditions with a dosing interval of 12 h. Examples of the ciclosporin blood concentration–time curve are shown in Figure 1, as are the average (with SD error bars) observations and average predicted curves using the individual empirical Bayes estimates. Population mean parameters and their interindividual variability are listed in Table 2.

No correlation was found between the empirical Bayes estimates of clearance and distribution volume and the variables length, body weight (BW), age or glomerular filtration rate for the population under study. In particular, no relation between clearance and BW was noted (Figure 2).

Limited sampling strategy

Bias and precision of the different strategies tested are listed in Table 3. Even though AUC estimation using only the trough level and the population model is more than

Figure 2

Relationship between ciclosporin clearance and body weight. No correlation between clearance and body weight was observed; Pearson r , -0.10 ; Spearman r , -0.00 ($n = 17$). The relationship is shown for i.v. administration (closed circles) and oral administration (open circles) combined

acceptable, improvement of bias as well as precision can be obtained when the AUC is calculated on the basis of additional blood concentration. By including time point 2 or 3 (auc02 and auc03, respectively), the r^2 values increase to 0.97 (see Table 3) with relatively low mean predictive error. Adding other or additional time points does not result in a substantial improvement in prediction.

Discussion

Despite the extensive use of ciclosporin for SCT in children, the information available is mostly non-evidence-based and very few studies on ciclosporin pharmacokinetics in children after SCT have been done. Many factors may influence ciclosporin pharmacokinetics, making comparative interpretation from other patient populations e.g. kidney graft recipients, difficult [5]. Secondary to the preparative regimen used in the SCT, patients have important differences in intestinal integrity and hepatic metabolism compared with solid organ transplant recipients, which may affect the absorption and elimination of ciclosporin [12–14]. Furthermore, important pharmacokinetic differences between adults and children have been reported, both in SCT and in solid organ transplantation. In studies of the pharmacokinetics after oral administration of ciclosporin in SCT patients, dosed per kg BW, significantly lower AUCs were reached in children compared with adults [6, 14]. In renal transplant patients a greater apparent steady-state volume of distribution and more rapid apparent blood clearance in young children (2–5 years) were found than in those >10 years old [15]. In another study, faster absorption and elimination was reported,

which was particularly high in stable renal transplant recipients aged 3–7 years [16]. Finally, study design, analytical methodology and range of age of the individuals differ between studies, complicating the comparison of pharmacokinetic data [7]. Therefore, there is still uncertainty as to the optimal schedule for dosage, route of administration and monitoring of ciclosporin in SCT both in adults and children [17].

In solid organ transplantation, ciclosporin exposure, as measured by AUC, has been shown to correlate well with acute rejection and renal toxicity [18], in contrast to the lack of correlation of trough levels with clinical events [19]. In search of a practical monitoring approach C2, the 2-h post dose level, was identified as the best single predictor of systemic exposure (AUC_{0–4}) in the renal transplant setting. In addition, C2 levels were significantly correlated with the predicted probability of acute rejection [20]. Therapeutic drug monitoring of ciclosporin with C2 has now also been clinically validated in kidney, liver and heart transplant recipients [19].

In our study, the correlation between the AUC, determined by pharmacokinetic modelling, using only the ciclosporin trough concentration, and the reference AUC was remarkably good, in contrast to what has been reported by others in the setting of SCT [6] and solid organ transplantation [19, 21]. However, with the inclusion of more time points in the pharmacokinetic modelling, a more accurate estimate of systemic exposure of ciclosporin was obtained (see Table 3). Schultz *et al.* [6] have evaluated ciclosporin pharmacokinetics in seven children after oral administration. Trough levels correlated poorly with the calculated AUC, and the combination of sampling at 1.5 h and 5 h appeared to be the most appropriate two-point predictor, as was identified using forward selection stepwise regression analysis. In contrast, we used a limited sampling strategy in combination with a pharmacokinetic population model to calculate the AUC, which may have led to different results. An additional advantage of the use of a pharmacokinetic model is its flexibility. There is no need to sample at exact time points. As long as the time of sampling is recorded, the estimate of AUC will not be compromised.

No relation between BW and clearance was found, and the interpatient variability of clearance ranged between 5 and 19 L h⁻¹ (see Figure 2). Dose adjustments occurred frequently in the clinical setting, as can also be seen in Table 1. These findings suggest that our current strategy, where children start on a dose regimen based on an empirical dose per kg BW and where subsequent dose adjustments are made to achieve a target trough blood concentration, more or less arbitrarily chosen between 50 and 200 µg l⁻¹, is inadequate. Similarly, other studies regarding ciclosporin pharmacokinetics in children have called into question the validity of dosing per kg BW. In renal transplant recipients <5 years old, higher doses of ciclosporin per kg BW are required to achieve similar target trough concentrations as

in recipients aged 6–17 years [22]. A similar observation has been made in paediatric liver transplant patients [23]. Furthermore, studies on oral administration of ciclosporin after SCT in children have shown higher clearance and lower AUCs in small children compared with grown-up children [24, 25]. Preliminary data obtained in our own SCT population, i.e. comparing recipients ≤ 10 kg with recipients >10 kg BW, and evaluating the frequency of ciclosporin dose increase needed to reach a trough level of $\geq 50 \mu\text{g l}^{-1}$, support the findings seen in paediatric organ transplant recipients. An explanation of this observation might be a change in the intrinsic ability of the liver to metabolize drugs and/or a change in the 'free drug concentration' in blood and plasma, which may alter the clearance of ciclosporin.

For GVHD prophylaxis no optimum AUC has been defined. However, a target AUC of approximately $3.5 \text{ h mg}^{-1} \text{ l}^{-1}$ over 12 h can be determined as adequate by back-extrapolation from the relation between the reference AUC and our clinically used trough levels. This level of systemic exposure is in fact close to the target AUC used 6 weeks after kidney transplantation in several centres in the Netherlands [26]. Administering an i.v. dose of 2 mg kg^{-1} in a population of children with a mean BW of 35 kg would lead to a mean i.v. dose of 70 mg day^{-1} . Assuming a population mean clearance of 11.3 l h^{-1} , such a dose would result in an AUC of $70/11.3 = 6.2 \text{ h mg}^{-1} \text{ l}^{-1}$ over 24 h, corresponding to an estimate of $3.1 \text{ h mg}^{-1} \text{ l}^{-1}$ over 12 h. Given the current practice, it is probably not acceptable to suggest a flat dosing regimen of 70 mg day^{-1} intravenously for all weight classes between 10 and 60 kg. We therefore suggest starting with a dose of 40 mg day^{-1} for children between 10 and 20 kg BW, of 60 mg day^{-1} for 20–35 kg BW and of 80 mg day^{-1} for 35–60 kg BW, and adjusting the daily dose individually according to the results of AUC determinations, using the limited sampling strategy and the pharmacokinetic population model described. With a combination of limited sampling at $t=0$ and a time point between 2 and 3 h later, and a pharmacokinetic modelling using a Bayesian fitting procedure, an accurate assessment of total drug exposure for a specific patient can be obtained. For an accurate estimate of the AUC, the registration of the actual time of sampling is more important than the exact sampling time itself.

The pharmacological suppression of the alloreactive immune response has played an important role in reducing the morbidity and mortality of GVHD [27]. At the same time, it has become clear that the intensity of post-transplant immunosuppression is an important determinant of relapse risk of leukaemic disease [8, 17, 28]. The use of limited sampling in combination with population pharmacokinetics to determine accurately the systemic drug exposure (AUC) may help our understanding of the relationship between ciclosporin dose and adequate immunosuppression.

We thank the medical, nursing and ancillary staff of the IHOBA unit, Department of Paediatrics, LUMC, for their care and management of patients undergoing bone marrow transplantation.

REFERENCES

- Horwitz ME. Stem-cell transplantation for inherited immunodeficiency disorders. *Pediatr Clin North Am* 2000; 47: 1371–87.
- Sanders JE. Bone marrow transplantation for pediatric malignancies. *Pediatr Clin North Am* 1997; 44: 1005–20.
- Holler E. Risk assessment in haematopoietic stem cell transplantation: GvHD prevention and treatment. *Best Pract Res Clin Haematol* 2007; 20: 281–94.
- Fahr A. Cyclosporin clinical pharmacokinetics. *Clin Pharmacokinet* 1993; 24: 472–95.
- del Mar Fernández De Gatta M, Santos-Buelga D, Domínguez-Gil A, García MJ. Immunosuppressive therapy for paediatric transplant patients: pharmacokinetic considerations. *Clin Pharmacokinet* 2002; 41: 115–35.
- Schultz KR, Nevill TJ, Balshaw RF, Toze CL, Corr T, Currie CJ, Strong DK, Keown PA. Effect of gastrointestinal inflammation and age on the pharmacokinetics of oral microemulsion cyclosporin A in the first month after bone marrow transplantation. *Bone Marrow Transplant* 2000; 26: 545–51.
- Cooney GF, Habucky K, Hopppu K. Cyclosporin pharmacokinetics in paediatric transplant recipients. *Clin Pharmacokinet* 1997; 32: 481–95.
- Bader P, Kreyenberg H, Hoelle W, Dueckers G, Handgretinger R, Lang P, Kremens B, Dilloo D, Sykora KW, Schrappe M, Niemeyer C, Von Stackelberg A, Gruhn B, Henze G, Greil J, Niethammer D, Dietz K, Beck JF, Klingebiel T. Increasing mixed chimerism is an important prognostic factor for unfavorable outcome in children with acute lymphoblastic leukemia after allogeneic stem-cell transplantation: possible role for pre-emptive immunotherapy? *J Clin Oncol* 2004; 22: 1696–705.
- Bader P, Klingebiel T, Schaudt A, Theurer-Mainka U, Handgretinger R, Lang P, Niethammer D, Beck JF. Prevention of relapse in pediatric patients with acute leukemias and MDS after allogeneic SCT by early immunotherapy initiated on the basis of increasing mixed chimerism: a single center experience of 12 children. *Leukemia* 1999; 13: 2079–86.
- Beal SL, Sheiner LB, Boeckmann AJ. NONMEM Users Guides, (1989–2006). Ellicott City, MD: Icon Development Solutions, 2006.
- Lindbom L, Pihlgren P, Jonsson EN. PsN-Toolkit – a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. *Comput Methods Programs Biomed* 2005; 79: 241–57.
- McDonald GB, Shulman HM, Sullivan KM, Spencer GD. Intestinal and hepatic complications of human bone marrow transplantation. Part II. *Gastroenterology* 1986; 90: 770–84.

- 13** McDonald GB, Shulman HM, Sullivan KM, Spencer GD. Intestinal and hepatic complications of human bone marrow transplantation. Part I. Gastroenterology 1986; 90: 460–77.
- 14** Schultz KR, Nevill TJ, Toze CL, Corr T, Currie C, Strong DK, Keown PA. The pharmacokinetics of oral cyclosporin A (Neoral) during the first month after bone marrow transplantation. Transplant Proc 1998; 30: 1668–70.
- 15** Mochon M, Cooney G, Lum B, Caputo GC, Dunn S, Goldsmith B, Baluarte HJ, Polinsky MS, Kaiser BA. Pharmacokinetics of cyclosporine after renal transplant in children. J Clin Pharmacol 1996; 36: 580–6.
- 16** Humbert H, Guest G, Said MB, Cabioc MD, Gagnadoux MF, Niaudet P, Broyer M. Steady-state pharmacokinetics of cyclosporine in renal transplant patients: does an influence of age or body weight exist? Transplant Proc 1994; 26: 2791–7.
- 17** Duncan N, Craddock C. Optimizing the use of cyclosporin in allogeneic stem cell transplantation. Bone Marrow Transplant 2006; 38: 169–74.
- 18** Lindholm A, Kahan BD. Influence of cyclosporine pharmacokinetics, trough concentrations, and AUC monitoring on outcome after kidney transplantation. Clin Pharmacol Ther 1993; 54: 205–18.
- 19** Citterio F. Evolution of the therapeutic drug monitoring of cyclosporine. Transplant Proc 2004; 36: 420S–425S.
- 20** International Neoral Renal Transplantation Study Group. Cyclosporine microemulsion (Neoral) absorption profiling and sparse-sample predictors during the first 3 months after renal transplantation. Am J Transplant 2002; 2: 148–56.
- 21** Kahan BD, Grevel J. Optimization of cyclosporine therapy in renal transplantation by a pharmacokinetic strategy. Transplantation 1988; 46: 631–44.
- 22** Harmon WE, Sullivan EK. Cyclosporine dosing and its relationship to outcome in pediatric renal transplantation. Kidney Int Suppl 1993; 43: S50–5.
- 23** Jain AB, Fung JJ, Tzakis AG, Venkataramanan R, Abu-Elmagd K, Alessiani M, Reyes J, Irish W, Warty V, Mehta S, Todo S, Starzl TE. Comparative study of cyclosporine and FK 506 dosage requirements in adult and pediatric orthotopic liver transplant patients. Transplant Proc 1991; 23: 2763–6.
- 24** Choi JS, Lee SH, Chung SJ, Yoo KH, Sung KW, Koo HH. Assessment of converting from intravenous to oral administration of cyclosporin A in pediatric allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant 2006; 38: 29–35.
- 25** Dupuis LL, Taylor T, Saunders EF. Disposition of two oral formulations of cyclosporine in pediatric patients receiving hematopoietic stem cell transplants. Pharmacotherapy 2006; 26: 15–22.
- 26** Scholten EM, Rowshani AT, Cremers S, Bemelman FJ, Eikmans M, van Kan E, Mallat MJ, Florquin S, Surachno J, ten Berge IJ, Bajema IM, de Fijter JW. Untreated rejection in 6-month protocol biopsies is not associated with fibrosis in serial biopsies or with loss of graft function. J Am Soc Nephrol 2006; 17: 2622–32.
- 27** Hogan WJ, Storb R. Use of cyclosporine in hematopoietic cell transplantation. Transplant Proc 2004; 36: 367S–371S.
- 28** Locatelli F, Zecca M, Rondelli R, Bonetti F, Dini G, Prete A, Messina C, Uderzo C, Ripaldi M, Porta F, Giorgiani G, Giraldi E, Pession A. Graft versus host disease prophylaxis with low-dose cyclosporine-A reduces the risk of relapse in children with acute leukemia given HLA-identical sibling bone marrow transplantation: results of a randomized trial. Blood 2000; 95: 1572–9.