A time-to-event model for acute rejections in paediatric renal transplant recipients treated with ciclosporin A

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Ciclosporin A (CsA) is used in immunosuppressive therapy after paediatric kidney transplantation.
- The pharmacokinetic properties of CsA are highly variable and the therapeutic index is narrow, with high CsA exposure resulting in nephrotoxicity.
- Therapeutic drug monitoring has therefore become part of standard care, but how it should be performed remains controversial.

WHAT THIS STUDY ADDS

- We present a time-to-event analysis of a unique data set comprising particularly rich and consistent information on CsA pharmacokinetics and acute rejection events in paediatric kidney transplant recipients.
- None of the potential covariates investigated for the time until first acute rejection (CsA systemic exposure, demographics and transplantation characteristics) was statistically significant, and estimated effects were of low clinical relevance.
- Thus, within the observed CsA exposure range, an increase in exposure was not found to be related to a decrease in acute rejections in the studied population, a finding which may support future dosing decisions.

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AIMS

Ciclosporin A (CsA) dosing in immunosuppression after paediatric kidney transplantation remains challenging, and appropriate target CsA exposures (AUCs) are controversial. This study aimed to develop a time-to-first-acute rejection (AR) model and to explore predictive factors for therapy outcome.

METHODS

Patient records at the Children's Hospital in Helsinki, Finland, were analysed. A parametric survival model in NONMEM was used to describe the time to first AR. The influences of AUC and other covariates were explored using stepwise covariate modelling, bootstrap-stepwise covariate modelling and cross-validated stepwise covariate modelling. The clinical relevance of the effects was assessed with the time at which 90% of the patients were AR free (t_{90}).

RESULTS

Data from 87 patients (0.7–19.8 years old, 54 experiencing an AR) were analysed. The baseline hazard was described with a function changing in steps over time. No statistically significant covariate effects were identified, a finding substantiated by all methods used. Thus, within the observed AUC range (90% interval 1.13–8.40 h mg l⁻¹), a rise in AUC was not found to increase protection from AR. Dialysis time, sex and baseline weight were potential covariates, but the predicted clinical relevance of their effects was low. For the strongest covariate, dialysis time, median t_{90} was 5.8 days (90% confidence interval 5.1–6.8) for long dialysis times (90th percentile) and 7.4 days (6.4–11.7) for short dialysis times (10th percentile).

CONCLUSIONS

A survival model with discrete time-varying hazards described the data. Within the observed range, AUC was not identified as a covariate. This feedback on clinical practice may help to avoid unnecessarily high CsA dosing in children.

Introduction

The introduction of the calcineurin-inhibitor ciclosporin A (CsA) into immunosuppressive therapy was a milestone in renal transplantation, increasing the 1 year survival of kidney grafts from ~50 to ~80–90% [1, 2]. Ciclosporin A also made it possible to avoid long-term high-dose glucocorticoid therapy, which may cause Cushing's syndrome and severe growth impairment in children [3].

Ciclosporin A therapy is complicated by the narrow therapeutic index of the drug; low exposure bears the risk of allograft rejection [4] but, in contrast, high levels of CsA may lead to adverse events, most importantly nephrotoxicity [4]. Thus, both too low and too high CsA exposure pose a severe risk to the kidney transplant recipient. This implies a major impact on the patients' long-term health status and prognosis, because immunosuppression after renal transplantation is a life-long therapy [5]. Adequate dosing is therefore crucial in CsA therapy and, given the large interindividual variability of CsA pharmacokinetics [6], therapeutic drug monitoring has become part of standard care [4, 7].

However, despite ~30 years of experience with CsA therapy, the method for performing the drug monitoring remains controversial [8], particularly with regard to two main aspects: (i) how to determine the systemic exposure in the individual patient; and (ii) how to define a target range for this exposure.

Concerning the exposure, measurement of the complete area under the concentration–time curve (AUC) is the gold standard for monitoring CsA exposure, but, as extensive blood sampling is often challenging in clinical practice, many efforts have been made to replace this method by limited sampling strategies, measurement of an abbreviated AUC or single time point measurements [8]. With regard to the target range, several suggestions have been made, depending on the method of measuring the systemic exposure as well as on the time after transplantation, because highly intensive immunosuppression is usually provided in the early post-transplant phase [9, 10]. Overall, more work on appropriate target ranges for the exposure is deemed necessary [11].

For patients at different risk for transplant rejection, different target ranges may have to be applied. Early acute rejection (AR) events are commonly used as a marker, because they are known to be a major risk factor for chronic rejection, which in turn is an important factor in long-term graft loss in adults as well as in children [12–14]. The identification of risk factors for AR events may therefore contribute to optimization of therapy. Previous studies have identified histocompatibility measures and certain donor information to be amongst such factors [15, 16]. However, owing to differences in the studied populations, observation periods, treatment protocols and methods of analysis, the significance of such factors may not be generalizable to other study populations, making it necessary

to investigate to what extent previous findings can be confirmed in the new population.

New paediatric studies on CsA, which has been part of empirical therapy for many years, will not be conducted without careful deliberation, because ethical considerations forbid the conduct of paediatric studies that are redundant [17]. It is therefore desirable to use any given opportunity to perform retrospective analyses of existing paediatric data to ensure that the available information is analysed exhaustively, before a new intervention is performed.

In the work presented here, we performed a retrospective, systematic analysis by applying a parametric survival model to AR data in paediatric kidney transplant patients by means of nonlinear mixed-effects modelling. The parametric modelling approach has advantages compared with nonparametric and semi-parametric methods (Kaplan–Meier analysis and Cox proportional hazard model), because it allows for the analysis of data sets comprising time-varying covariates, which in addition may influence each other, as well as the simulation time-to-event data based on the final model.

The data analysed were collected at the Helsinki University hospital, where all paediatric kidney transplantations in Finland are performed centrally, implying that the patient population and the study conditions are particularly homogeneous. The data comprise patient demographics, transplantation characteristics, pharmacokinetic (PK) data, including full AUC measurements, and records of AR events. These data permit the investigation of whether CsA exposure or factors known to be relevant in adults or other populations can be connected to AR events in this population. Thereby, subgroups of the patient population who are at particular risk of experiencing acute transplant rejections may be identified, thus contributing to information that may form the basis for clinical decision making.

The aim of this study was to develop a time-to-event model for the time from transplantation until the first AR event and to investigate CsA exposure and further clinical information as potential covariates in order to identify predictors of the outcome of therapy.

Patients and methods

Patients and data acquisition

A retrospective analysis was performed using data collected from paediatric kidney transplant patients at the Children's Hospital in Helsinki, Finland. The study protocol was approved by the Ethics Committee for Pediatrics, Adolescent Medicine, and Psychiatry of the Hospital District of Helsinki and Uusimaa. Pharmacokinetic and pharmacodynamic (PD) data were collected from patient records between 1995 and 2006. During this period, all patient records were eligible for inclusion. The patients received CsA immunosuppression orally in the Neoral® (Novartis

Pharmaceuticals Corporation, East Hanover, NJ, USA) microemulsion formulation and, for all of them, PK profiles were recorded.

After transplantation, the patients, in accordance with the hospital's routine, stayed on the ward for paediatric nephrology for between 3 weeks and 3 months. After they had been discharged, they returned to the hospital every 3–12 months for routine checks and, additionally, with any acute problems. Observation ended either with the patient reaching adulthood and leaving the care of the paediatric department (usually at the age of 18–19 years) or with the end of data extraction in 2006.

The data set comprises the results of PK studies and therapeutic drug monitoring of CsA, information on acute allograft rejection events and further clinical information, such as demographic data and transplantation-related data (see Table 1).

The PK data have been described by a population PK model by Fanta *et al.* [6, 18], and empirical Bayes estimates of CsA bioavailability and clearance obtained with this model were used for the calculation of CsA exposure in the present analysis. The PK information consisted of rich intravenous (i.v.) and oral data from pretransplantation studies. Therapeutic drug monitoring data after renal transplantation included dose interval studies performed 1–3 years

after transplantation, but the bulk of the data were trough concentrations and concentrations after dose, mostly 2 h postdose. Moreover, frequent trough concentration and 2 h postdose monitoring was applied in the first weeks after transplantation. The details of the PK data collection have been described by Fanta *et al.* [6, 18].

Cyclosporin A medication was started preoperatively, and the target trough CsA concentration was $300 \mu\text{g l}^{-1}$ immediately after transplantation. After 6 months, the trough target was reduced to $100 \mu\text{g l}^{-1}$. Concentrations at 2 h postdose of $1500\text{--}1800 \mu\text{g l}^{-1}$ early after transplantation and $800\text{--}1000 \mu\text{g l}^{-1}$ after 6 months were considered additional targets. Additional immunosuppressive medication consisted of basiliximab induction treatment (after 1999), azathioprine or mycophenolate mofetil (after 1999) and methylprednisolone.

Prior to 1999, all patients received triple immunosuppression consisting of methylprednisolone, azathioprine and CsA. Methylprednisolone was started intraoperatively at 100 mg i.v. divided into three doses, and continued postoperatively at 1 mg kg day^{-1} until 3 weeks, when the dose was tapered down to $0.25 \text{ mg kg day}^{-1}$. The dose was further reduced to 0.37 mg kg^{-1} every other day after 3 months. Azathioprine was given intraoperatively at 1.4 mg kg^{-1} i.v. divided into two doses, and continued

Table 1

Summary of patient characteristics and clinical information

Short name	Explanation [categories]	Median (range) or Number of patients in each category
Patient demographics		
Age*	Patient age (years)	7.13 (0.67–19.78)
Baseline age	Patient age at time of transplantation (years)	4.51 (0.67–18.17)
Bodyweight*	Patient bodyweight (kg)	21.6 (8.5–91.5)
Baseline bodyweight	Patient bodyweight at time of transplantation (kg)	19.1 (8.8–68.7)
Sex	Patient sex [male/female]	57/30
Patient condition		
Diagnosis	Diagnosis category: I, congenital nephrotic syndrome; II, posterior urethral valve; III, polycystic kidney disease; IV, nephronophthisis; and V, other [I/II/III/IV/V]	29/10/6/7/35
Dialysis time	Time on dialysis before transplantation (years)	0.96 (0.01–3.86)
Transplantation-specific information		
Basiliximab induction	Patient received basiliximab induction treatment at transplantation [yes/no]	53/34
CMV donor	Transplant donor cytomegalovirus positive [yes/no]	61/26
CMV recipient	Transplant recipient cytomegalovirus positive [yes/no]	30/57
Cold ischaemia time	Renal transplant cold ischaemia time (time of cold storage of the isolated kidney; h)	19.08 (0.48–31.92)
Donor age	Donor age (years)	35.00 (1.00–65.00)
Donor sex	Donor sex [male/female]	53/34
Donor type	Graft from cadaver donor or living related donor [cadaver/living]	71/16
HLA-AB mismatches	Number of HLA-AB mismatches [0/1/2/3]	11/28/42/6
HLA-DR mismatches	Number of HLA-DR mismatches [0/1/2]	28/55/4
Ciclosporin A therapy		
AUC*	Daily CsA exposure (h mg l^{-1})	14.40 (0.70–42.63)
Baseline AUC	Daily CsA exposure at time of transplantation (h mg l^{-1})	3.22 (0.79–19.87)
Daily dose	Daily CsA dose (mg)	185.0 (15.0–1100.0)
Weight-normalized daily dose	Bodyweight-normalized daily CsA dose (mg kg^{-1})	9.57 (0.76–36.92)

Abbreviations are as follows: AUC, area under the concentration-time curve; CMV, cytomegalovirus; CsA, ciclosporin A; HLA-AB, human leukocyte antigen AB; HLA-DR, human leukocyte antigen DR. *Summary of all available measurements, i.e. more than one observation is available for each patient over time.

postoperatively at 2 mg kg⁻¹ day⁻¹, which was reduced to 1 mg kg day⁻¹ after 2 weeks, and increased to 1.4 mg kg day⁻¹ after 3 months when the steroid was reduced.

The immunosuppression protocol was revised in September 1999, when basiliximab induction therapy was introduced. Basiliximab was given in two doses, a bolus of 10 mg in children weighing <30 kg, and 20 mg in those weighing >30 kg, intraoperatively and on 4th day after transplantation. The rest of the immunosuppressive protocol stayed the same. The patients who received their transplants after September 1999 were evaluated 3 months after transplantation, and the immunosuppression was adjusted individually based on graft histology and function. Methylprednisolone dosage was reduced in patients with well-functioning grafts and normal histology. In cases of immunoactivation, methylprednisolone was continued with daily doses. In some patients with immunoactivation (or drug-related adverse effects in few patients), CsA was replaced with tacrolimus, and/or azathioprine was replaced with mycophenolate mofetil.

Acute rejection events were diagnosed based on the results of fine-needle aspiration biopsies [19]. The biopsies were taken routinely on the 5th day after transplantation and at least twice a week until the patient was discharged from hospital. They were also performed whenever a rejection was suspected on clinical grounds, e.g. when fever, a rise in serum creatinine and/or C-reactive protein concentration, tenderness of the graft or a decreased urine output were observed. The treatment of an AR episode was methylprednisolone (3 mg kg day⁻¹) for 5 days or until the blast cell reaction subsided. If no response was seen after 5 days, a renal core biopsy was performed. If the AR was still present, the patient was started on polyclonal antithymocyte globulin or anti-T-cell antibody therapy [20]. The time at which an AR occurred was recorded and, for the patients not experiencing such an event, the time of last observation as defined above was recorded and treated as a censored observation.

Model development

The model was developed in the following two steps: (i) a base model without any explanatory factors apart from time was built; and (ii) potential covariates were explored.

Development of the base model In order to describe the time to the first acute transplant rejection, a parametric survival function according to Equation 1 was used.

$$S(t) = e^{-\int_0^t h(t) dt} \quad (1)$$

The hazard is $h(t)$, and the 'survival' $S(t)$ is a function of the cumulative hazard within the time interval between the

time zero and the time t describing the probability of not experiencing any AR event within this interval. As only one observation was available per individual, random effects on the baseline hazard could not be estimated, i.e. the same baseline hazard was assumed for all subjects.

The base model was developed by exploring different functions for the hazard $h(t)$, starting from a simple time-independent constant hazard and then gradually progressing to more complex functions, including Weibull, Gompertz, log-logistic distributions [21] and hazard functions described as steps over time, i.e. piecewise exponential distributions. Equation 2 gives an example of a step function where the base hazard $h(t)$ changes depending on the time t .

$$h_0(t) = \begin{cases} \theta_1, & \text{if } 0 < t \leq t_1 \\ \theta_2, & \text{if } t_1 < t \leq t_2 \\ \dots & \dots \\ \theta_n, & \text{if } t_{n-1} < t \leq t_n \end{cases} \quad (2)$$

A step-function of this type was used to explore the shape of the hazard function, starting off with 15 steps, i.e. a function of quite narrow steps, and then tentatively removing these one by one; for two similar estimates, the time split between them was removed, and the model was re-run. Whenever this did not significantly increase the objective function value (OFV), the reduced model was kept. The initial time cut-offs for the steps were chosen based on the shape of the raw data survival curve.

Development of the covariate model The potential covariates included the categorical variables cytomegalovirus (CMV) donor, CMV recipient, diagnosis, donor type, donor sex, human leukocyte antigen AB (HLA-AB) mismatches, human leukocyte antigen DR (HLA-DR) mismatches, sex, basiliximab induction and the continuous variables age, AUC, cold ischaemia time, dialysis time, donor age and bodyweight (see Table 1 for explanations). For the time-varying covariates age, bodyweight and AUC, the baseline values, i.e. the values at the time of transplantation, were also tested. The polychotomous covariates diagnosis, HLA-AB mismatches and HLA-DR mismatches were also grouped according to clinical considerations and tested as dichotomous covariates. Diagnosis was grouped into the most common condition (congenital nephrotic syndrome) or any other condition. The HLA-AB mismatches and HLA-DR mismatches were summarized to be zero or any other number of mismatches. The AUC was calculated from the absolute bioavailability f , the daily dose D_{daily} and the total clearance Cl according to Equation 3, thus varying over time. The values for bioavailability and clearance were the individual patient's empirical Bayes estimates obtained based on the observed data and the previously published pharmacokinetic model for this data set by Fanta *et al.* [6, 18].

$$AUC_{daily} = \frac{f * D_{daily}}{CI} \quad (3)$$

The covariates were included into functions (λ_{cov}) that were factors modifying the base hazard h_0 to give the hazard function $h(t)$ (Equation 4).

$$h(t) = h_0(t) * \lambda_{cov1} * \lambda_{cov2} * \dots * \lambda_{covn} \quad (4)$$

Categorical covariates were included as relative factors (Equation 5), where, for the most frequent covariate category, the indicator (*IND*) is set to be 0, otherwise to be 1, so that the covariate parameter λ is estimated for the less frequent category (or, for polychotomous covariates, the n parameters $\lambda_{1...n}$ for n categories). Continuous covariates were included as exponential functions and were centred on the median covariate value (Equation 6). For the AUC, further parameterizations were explored, including different versions of maximum effect models.

$$\lambda_{cov} = 1 + \theta * IND \quad (5)$$

$$\lambda_{cov} = e^{\theta * (cov - cov_{median})} \quad (6)$$

Initially, the covariates were tested in a univariate manner, i.e. each covariate relationship was evaluated on the base hazard separately. Based on the results, covariate relationships were identified for a systematic covariate search by applying stepwise covariate modelling (SCM), i.e. with stepwise forward inclusion followed by backward deletion [22]. In the forward inclusion, the statistical level of significance was set to $P < 0.05$, which corresponds to a reduction of the OFV of at least 3.84, for one degree of freedom. In the backward deletion, the P value was set to $P < 0.01$, corresponding to an increase of the OFV of at least 6.64 to be kept in the model, for one degree of freedom. Categorical and continuous covariates were combined as shown in Equation 4.

In order to determine the appropriate covariate model size with a method specifically designed to provide a good predictive performance, stepwise covariate model building combined with cross-validation (XV-SCM) was performed as described by Katsube *et al.* [23]. In this procedure, the data set was randomly divided into 10 parts of approximately the same size. Of these, nine parts (i.e. 90% of the data) were defined to be the training data, for which a forward SCM with a level of significance of $P < 0.05$ was performed. After each step of this SCM, the resulting model was applied (not estimated) to calculate the OFV (i.e. a partial OFV) for the remaining 10th part, the test data set (containing 10% of the data). This was repeated 10 times, each time with another one of the 10 parts being the test

data set. Thus, for each step of the SCM, i.e. for each number of included relationships, 10 partial OFVs were obtained, which were then added up to the total OFV. The partial OFVs and the total OFV were calculated for each number of included relationships, i.e. for each step in the SCM. The whole procedure, starting with the random division of the data set into 10 new parts, was repeated nine times. A set of overall mean total OFVs was then calculated from the 10 procedures.

As a tool for analysing type I error of covariate inclusion, correlations and interactions between covariate inclusions, selection bias and bias in covariate estimates, bootstrap analyses of the SCM (boot-SCMs) were performed as described by Keizer *et al.* [24]. Two hundred data sets were created by resampling from the original data set with replacement and, for each, SCM was done with a P value of 0.05 for both the forward inclusion and the backward deletion. The P value in the backward step, 0.05, was chosen to assess the inclusion frequency in final models for borderline effects, because applying the conservative value of $P < 0.01$ would exclude them.

Model diagnostics For nested models, decisions about the choice of the best model were made based on the likelihood ratio test principle. The difference in OFV (Δ OFV) between a full and reduced model follows approximately a χ^2 distribution, with the number of degrees of freedom corresponding to the difference in the number of parameters between the two competing models. For a statistical significance level of $P = 0.05$ and one degree of freedom, a Δ OFV of 3.84 is required.

In order to ensure that the models described the data adequately, Kaplan–Meier plots were used. The plots were based on simulations of 100 replicates of the model. In order to enable simulations for time points where no clinical observations had been made, extra data records were added to the data set every 5 days in the time from day 2 to day 30 after transplantation, every 10 days from day 31 to day 90, every 50 days from day 91 to day 365 and every 100 days from day 366 onwards. The covariate information in these additional records was linearly interpolated between the original records.

For non-nested models, strict hypothesis testing was not applied. The OFV was used as a measure of the best fit in combination with simulation-based diagnostics (Kaplan–Meier plots).

For the final model, a nonparametric bootstrap analysis of the data with 1000 resampled data sets was performed, and the uncertainty (95% confidence intervals) of the parameter estimates was obtained from the distribution of the bootstrap estimates (2.5th and 97.5th percentiles).

Decisions about covariate inclusion were made based on the resulting Δ OFV as described above. In order to translate the observed differences in hazard between

different models into a clinically meaningful measure, the time after transplantation at which 90% of the patients were acute-rejection free was calculated. This clinical measure was predicted, taking into account the uncertainty in the estimated covariate effects as well as the distribution of covariates, as follows. For covariate relationships identified in the SCM and boot-SCM as well as for some relationships selected on the basis of clinical importance, bootstrap analyses were performed with 1000 resampled data sets, applying the univariate model. Based on the bootstrap parameter estimates, the time after transplantation at which 90% of the patients were rejection free (t_{90}) was calculated for different covariate values; for the categorical covariates, the comparison was made between the categories, while for the continuous covariates, the calculations were made at the 10th and 90th percentiles of the covariate distributions. Median values and uncertainty (95% confidence intervals) were presented graphically.

Software and estimation method

The data were analysed with nonlinear fixed-effects modelling using NONMEM version 7.2.0 population analysis software [25] with maximal likelihood estimation (\$ESTIMATION METHOD = 0 LIKE). The likelihood function is the conditional probability of the data given the parameters of the model and assuming that the data are independent given the parameters. The likelihood function is the product of the likelihood of each datum. Two types of data occurred, namely uncensored and right censored, denoted unc. and r.c. in the equations below.

For an uncensored datum, with T_i equal to the time of the acute rejection, the individual likelihood is given by Equation 7.

$$L_{i,unc.}(\theta) = \Pr(T = T_i | \theta) \quad (7)$$

For a right censored datum, with T_i equal to the time of the censored observation (last observation time point), the individual likelihood is given by Equation 8.

$$L_{i,r.c.}(\theta) = \Pr(T > T_i | \theta) \quad (8)$$

The overall likelihood function will accordingly be given by Equation 9.

$$L(\theta) = \prod_{T_i \in unc.} L_{i,unc.}(\theta) \prod_{T_i \in r.c.} L_{i,r.c.}(\theta) \quad (9)$$

For execution of estimations and simulations, bootstrap analyses, SCM, boot-SCM and cross-validated SCM, Perl speaks NONMEM (PsN, version 3.5.2) and the PsN-Toolkit were used [26, 27] [<http://psn.sourceforge.net/>,

last accessed on 26 January 2012]. Graphical assessment was done with the R-based model-building aid for population analysis Xpose, version 4.3.2 [28] [<http://xpose.sourceforge.net/>, last accessed on 26 January 2012].

Results

Patients

A total of 89 paediatric renal transplant patients received CsA in the microemulsion formulation between the introduction of this formulation on the ward in June 1995 and the end of data extraction in April 2006. Two of these patients had to be excluded because of incomplete patient records; hence, data from 87 paediatric renal transplant patients were included in the analysis. The study population included all age groups from infants to adolescents, with a median age of 4.5 years at the time of transplantation (Table 1). For five of the patients, it was the second kidney transplantation; for all other patients, it was the first one. The most common diagnosis was congenital nephrotic syndrome of the Finnish type (CNF, NPHS1) and was found in 33% of all patients, followed by posterior urethral valve (10%), nephronophthisis (8%) and polycystic kidney disease (7%). The remaining 40% were numerous smaller subgroups of different conditions and were categorized as 'other diagnoses'. For all patients, PK information was available and had been incorporated into the population PK model by Fanta *et al.* [6].

Acute rejection data

The observed time period differed from patient to patient, with a median value of 3 years and a range from 31 days to 14 years after transplantation.

Of the 87 patients, 54 experienced an AR event during the observation time. The median time until the first AR in these raw data, not taking into account censoring, was 14.5 days (range 0 days to 11 years, interquartile range 8–44 days). For the 33 patients who did not experience any AR, data were analysed as censored events, with the median time for censoring being 1.5 years (range 57 days to 7.2 years). In some of the patients, more than one AR event was observed; 19 patients experienced a second event, seven a third one and one patient a fourth one. Owing to the limited number of these observations, the analysis was restricted to the first event.

Base model

The hazard function giving the best result with regard to OFV and Kaplan–Meier plots was a step-function, defining five time intervals with one constant hazard each. The time cut-offs were determined as described before by tentatively removing the steps of a multistep curve one by one, as long as this did not lead to a significant increase in OFV. The times identified in this manner, where the constant hazards changed, were at 5, 8, 25 and 100 days after

Figure 1

Graphical illustration of the step-function employed for the hazard. The figure shows the shape of the hazard function in a time window of 200 days after transplantation. From day 100 onwards, the hazard stays constant (last data point at 5111 days)

Table 2

Objective function value and number of parameters in different tested survival functions

Survival function*	Objective function value	Number of parameters
Exponential	811.5	1
Gompertz	709.5	2
Weibull	653.7	2
Log-logistic	610.9	2
Step-function exponential	593.2	5

*Exponential, Gompertz, Weibull and log-logistic represent functions used in classical survival analysis [21]. Step-function exponential refers to the final model and corresponds to the step-function of constant hazards shown in Figure 1.

transplantation. The resulting hazard function gives comparably low hazard for the first days after transplantation, followed by a sudden increase in hazard to the maximal value between 5 and 8 days and then a stepwise drop, with the long-term hazard from 100 days from transplantation onwards being close to zero (Figure 1). Table 2 shows the OFVs given by the investigated functions. The Kaplan–Meier plots show good agreement between the observed data and the simulated ‘survival’ (time to rejection; Figure 2). The parameters of the base model were estimated with the precision shown in Table 3.

Covariate model

The results of the univariate testing of the covariate relationships are shown in Table 4. Based on these results, choices were made about which covariates to test in the SCM. Of the AUC measures, baseline AUC provided the best predictive value. Exploration of other models did not result in any improvement. Likewise, baseline bodyweight

Figure 2

Kaplan–Meier plot of the percentage of acute-rejection-free patients vs. time after transplantation in days. Top panel, time period 0–30 days; bottom panel, 0–2920 days (8 years). The shaded area represents 95% confidence intervals for the simulated data. The continuous line represents the real data, with vertical lines marking censored observations. Ten patients were observed for longer than 2920 days. After 3754 days, data were available for only one patient (observed until day 5111)

and baseline age were superior to bodyweight and age. Furthermore, the univariate testing suggested that grouping diagnosis and HLA-DR mismatches into dichotomous covariates would show a stronger effect on the OFV than when incorporated ungrouped. For HLA-AB mismatches, the effect was similarly small for both ways of handling the covariate; for consistency, it was also grouped. This gave 15 covariates to be investigated in the SCM, the

Table 3

Hazard estimates and precision for the final model

Time interval in hazard function	Hazard (day ⁻¹) NONMEM estimate	Bootstrap median	Bootstrap 95% confidence interval
≤5 days	0.00465	0.00465	0.00230–0.01204
>5 ≤ 8 days	0.05780	0.05759	0.03264–0.08923
>8 ≤ 25 days	0.01870	0.01864	0.01049–0.02873
>25 ≤ 100 days	0.00470	0.00461	0.00257–0.00728
>100 days	0.00013	0.00013	0.00004–0.00028

The table shows the estimates of the final NONMEM model and the median and 95% confidence interval for the bootstrap analysis with 1000 samples.

Table 4

Results from the univariate inclusion of covariate

Model	ΔOFV*	P value
Base model	0	–
Donor sex†	0.04	0.8345
CMV recipient†	0.08	0.7734
Donor age†	0.17	0.6765
CMV donor†	0.55	0.4592
HLA-AB mismatches (dichotomous)†	0.59	0.4420
Cold ischaemia time†	0.92	0.3381
HLA-AB mismatches (polychotomous)	3.38	0.3363§
Donor type†	0.98	0.3230
HLA-DR mismatches (polychotomous)	2.34	0.3097‡
Diagnosis (polychotomous)	4.97	0.2900¶
AUC	1.15	0.2827
HLA-DR mismatches (dichotomous)†	1.46	0.2262
Age	1.87	0.1716
Baseline AUC†	2.01	0.1563
Baseline age†	2.21	0.1369
Weight	2.70	0.1006
Sex†	2.99	0.0836
Basiliximab induction†	3.68	0.0550
Diagnosis (dichotomous)†	3.76	0.0524
Baseline bodyweight†	3.85	0.0497
Dialysis time†	4.80	0.0284

Abbreviations are as for Table 1. *ΔOFV is the difference in objective function value between covariate and base model: $OFV_{Covariate} - OFV_{Base}$. †The potential covariate was included in the SCM. ‡Two degrees of freedom. §Three degrees of freedom. ¶Four degrees of freedom. For explanations of the short covariate names, refer to Table 1.

cross-validated SCM and the boot-SCM. These were the categorical variables basiliximab induction, CMV donor, CMV recipient, diagnosis, donor type, donor sex, HLA-AB mismatches, HLA-DR mismatches and sex, and the continuous variables baseline age, baseline AUC, baseline bodyweight, cold ischaemia time, dialysis time and donor age.

In the SCM, three covariates were selected in the forward step, which was performed with a *P* value of 0.05, but were eliminated again in the backward step at a

Figure 3

Results from the cross-validated stepwise covariate model building (XV-SCM). Dashed lines represent single XV-SCM objective function values (OFVs); continuous line shows mean OFV. The data set was randomly divided into 10 equally sized parts. Of these, nine parts were defined to be the training data, for which a forward SCM was performed. After each SCM step, the resulting model was applied to calculate the OFV (i.e. a partial OFV) for the remaining 10th part, the test data set. This was repeated 10 times, each time with another part being the test data set. Thus, for each number of included covariate relations, 10 partial OFVs were obtained, which were then added up to the total OFV. The partial OFVs and the total OFV were calculated for each number of included relationships. The whole procedure was repeated nine times. A set of overall mean total OFVs (continuous line) was then calculated from the 10 procedures (dashed lines). The minimum of the curve points to the optimal number of covariate relationships for the model (in this case, zero)

P value of 0.01 (at a *P* value of 0.05 in the backward step, in contrast, they were kept in the model). These three covariates, in order of selection, were dialysis time, sex and baseline bodyweight.

In the cross-validated SCM (Figure 3), the lowest OFV was found for zero relationships. Had the inclusion of a certain number of covariates improved the model, the cross-validated SCM should have shown the lowest OFV (i.e. a minimum of the curve) for this number of covariates. The absence of such a minimum in Figure 3 suggested that a model without any covariate relationships was preferable.

From the results of the boot-SCM, the inclusion frequencies for the individual covariates were calculated by relating the number of times a covariate was included into a final bootstrap model to the total number of bootstraps (200). The four most commonly included covariate relationships were the dialysis time (inclusion frequency 58%), basiliximab induction (46%), sex (46%) and baseline bodyweight (43%), followed by baseline AUC (27%), diagnosis

Figure 4

Results of repeated stepwise covariate model building in 200 bootstrap data sets. The grey dots represent the parameter estimates for the covariate effects obtained from the first step in each SCM performed on the bootstrap-data sets. The estimates are normalized to the standard deviation of the raw covariate data. Estimates of covariate effects included in the respective final model are shown as black crosses. A high occurrence of black crosses corresponds to a high inclusion frequency. A shift in the distributions of crosses and dots indicates bias in the selection procedure; the size of the bias is quantified by the percentage given on the right-hand side of the plot area. This percentage is calculated by relating the difference between the mean of the included parameter estimates and the mean of all estimates to the mean of all estimates for a covariate: $100 \times (\text{mean}_{\text{incl_par_ests}} - \text{mean}_{\text{all_ests_initial_step}}) / \text{mean}_{\text{all_ests_initial_step}}$. For categorical covariates, the major category is described with the base model, while the parameter is estimated for the less common category, in this case the absence of basiliximab induction, female sex, congenital nephrotic syndrome (nephrot. S.), no HLA-DR mismatches, donor cytomegalovirus (CMV) negative, recipient CMV positive, female donor sex, graft from living donor and no HLA-AB mismatches. ●, not included; +, included

(23%) and HLA-DR mismatches (21%). All of the remaining covariates were included in fewer than 20% of the bootstrap samples. Figure 4 presents information on the parameter estimates in the first (i.e. univariate) SCM step and on the inclusion in the last step for all 15 analysed covariates. The plot suggests that the three covariates chosen in the forward step of the SCM have been selected with biased parameter estimates. It supports that with all sets of estimates, including zero, none of the covariate effects was significant.

Clinical relevance of potential covariate relationships

For the seven covariates with inclusion frequencies above 20% in the boot-SCM, the influence of the covariates was characterized further by translating it into a clinically meaningful measure. Based on the results of individual bootstraps, the time after transplantation at which 90% of the patients were rejection free (t_{90}) was calculated for different covariate values (Figure 5) and, in the following, the results are given as medians (90% confidence interval). For the dialysis time, t_{90} was 5.8 (5.1–6.8) days for patients with a dialysis time of 2.58 years, i.e. at the 90th percentile of values. For patients with a dialysis time of 0.15 years (10th percentile), t_{90} was delayed to 7.4 (6.4–11.7) days. For the patients without any basiliximab induction treatment at transplantation, t_{90} was 6.0 (5.3–6.9) days, while for those who had received the treatment, the time was 1 day later,

at 7.0 (6.1–9.1) days. For male sex, the median time was 6.2 (5.6–7.1) days, while it was about 1 day later, 7.3 (6.2–11.0) days for female patients. Figure 5 shows that similar numbers were found for baseline bodyweight, diagnosis, baseline AUC and HLA-DR mismatches.

Discussion

The time from renal transplantation until the first AR event in 87 paediatric patients was adequately described in a time-to-event model with a step-function of five constant hazards. For none of the 15 potential covariates, including CsA exposure, was a statistically or clinically significant effect found.

Paediatric renal transplantation has been studied previously, including the extensive reports on the North American Pediatric Renal Transplant Cooperative Study, a multicentre study in a heterogeneous population of mixed races treated with various treatment protocols, reporting on data from more than 6000 transplantations [15]. In comparison, the data set presented here included a relatively small number of subjects, but has advantages with respect to the comprehensiveness of the data, the homogeneity of the study population and, in particular, in the focus on CsA therapy. Given that the Children's Hospital in Helsinki is the only hospital in Finland performing paediatric renal transplantations and all patient records, apart from two, were

Figure 5

Plot to illustrate clinical implications of the results: time after transplantation at which 90% of the patients are acute-rejection free (t_{90}). Calculations are based on the parameter estimates resulting from the univariate inclusion of selected covariates using 1000 bootstrap samples. The groups compared against each other are, for categorical data, the different categories and, for continuous data, the 10th and 90th percentile of the covariate values. The plotted areas represent the data between 5th and 95th percentile of the calculated t_{90} . Vertical lines indicate the median values. For categorical data, the plot area is proportional to the percentage (given in parentheses) of each category as observed in the original data set. Abbreviation: nephrot. s., nephrotic syndrome.

available for analysis, there was no bias in the selection of the patients. With all patients treated on the same ward, comparability was very high with regard to treatment protocols, medication, analytical methods and documentation. The patient population was homogeneous with regard to ethnicity, because it consisted of Caucasians only. The analysed patient records reported not only on dosing, but also on PK profiles, with full AUC measurements as well as follow-up data from therapeutic drug monitoring. Furthermore, all included patients received the same formu-

lation, because inclusion was limited to patients receiving CsA in the microemulsion formulation. This formulation was introduced to all patients on the ward in 1995; it demonstrates a higher and less variable bioavailability than the previously used oily suspension [29]. Concerning CsA therapy, the data analysed here therefore offer very rich and consistent information.

Acute rejection events, the pharmacodynamic measure of the study, were diagnosed using fine-needle aspiration biopsy. This method allows for an objective

detection of immunoactivation, but at the same time may lead to the inclusion of cases that clinically might not be defined as an AR.

The analysis showed a very low hazard in the first 5 days after transplantation, followed by an increase on day 6. No information was available that could explain this observation with certainty. It is possible that underlying immunological activation processes were the reason for this lag time.

The investigation of a relationship between CsA exposure and the occurrence of AR events was one major focus of the presented analysis. Daily dosing, absolute and body-weight normalized, was considered, but AUC was preferred as a measure of exposure because it accounts for the variability of bioavailability and elimination. The previously published PK model [6] was developed based on the same data set, i.e. rich PK information, hence it offers a precise estimation of the exposure. The availability of this thorough characterization of PK combined with a long-term follow-up on AR events is a further strength of the presented work, and the results of this analysis may contribute to a re-evaluation of CsA therapy.

With this background, AUC not appearing as a covariate in the model may have clinical implications with regard to CsA therapy in paediatric kidney transplant recipients. Within the observed range of baseline daily CsA AUC values (90% interval 1.13–8.40 h mg l⁻¹), AUC was not found to be correlated to protection from AR in the studied population. Accordingly, in the clinical setting of this study, including target concentration-based CsA dosing, a correlation between AUC and effect could not be observed. This does not, however, not exclude the possibility that a correlation exists. Considering this finding when choosing CsA dosing in the future may help to avoid unnecessarily high exposure to CsA. This is especially important in renal transplant recipients, because the nephrotoxic potential of CsA is regarded with particular concern in this situation. This interpretation, however, has to be put into perspective by taking into account the effects of comedication; all of the patients received several immunosuppressive agents, which made it impossible to identify the effect of CsA without any interference.

Besides CsA exposure, this analysis explored further potential covariates. The analysed demographic information was age, bodyweight and sex. Low paediatric patient age has been found to increase the risk of cadaveric donor graft loss [15]. In a paediatric population, bodyweight is an indirect measure of age, but might in addition be relevant with regard to unequal organ sizes when adult kidneys are transplanted, mainly because of difficulties in maintaining adequate graft perfusion [30], and possibly also with regard to the amount of graft material that confronts the recipient's immune system. Baseline bodyweight being amongst the strongest potential covariate signals found in this analysis seems therefore a plausible finding. For sex, in contrast, there seems to be no rationale to support its

selection as a risk factor. However, with the signal not being statistically significant, the results offer no basis for further interpretation.

The condition of the patients at time of transplantation was characterized by information about the diagnosis and the time for which the patients were on dialysis before transplantation. Owing to a large variety of diagnoses, the largest category was 'other' (mixed diagnoses), which may have made it difficult to detect any clear signal in the analysis. The dialysis time was the strongest signal detected, consistent with previous observations in adult renal transplantation showing decreased overall graft and patient survival with longer dialysis duration [31]. Furthermore, peritoneal dialysis, received by the majority of the patients in this analysis, has been associated with a higher incidence of AR compared with haemodialysis, which has been interpreted as an increased immune competence [32, 33]. Hypothetically, a long time on peritoneal dialysis might imply a longer time of increased immune competence, implying a higher risk of AR. An alternative explanation could be that some patients, due to certain reasons (e.g. the presence of antibodies or rare tissue types), are more likely to wait longer for transplantation (and thus are on dialysis longer) and that these patients, for the same reasons, are more prone to AR. In that case, a longer dialysis time would be connected to an increased occurrence of AR, but would not be the cause.

The analysis included nine transplantation-specific covariates, all of which have been previously reported to be of relevance in renal transplantation patients. In our analysis, however, a potential signal was detected mainly for induction treatment with basiliximab. Basiliximab, a humanized monoclonal antibody targeting the interleukin-2 receptor, is administered at the time of transplantation and, accordingly, prevents AR events in the immediate post-transplantation period. As most of the observed events occurred in the early post-transplantation period (in 50% of the patients during the first 2 weeks and in 25% during the first 8 days, calculated from the raw data without taking into account censoring), induction treatment may have had an influence on the status of the immune system at these times. Cytomegalovirus infection is known to be associated with decreased graft and recipient survival [34]. A cold ischaemia time of longer than 24 h has been described to be a risk factor for graft failure in paediatric recipients of cadaveric renal grafts [15]. Donor age, particularly of donors younger than 5 years of age, was reported to increase the risk of rejection [35]. Moreover, whether the graft originated from a living related donor or a cadaver donor is known to affect rejection and survival in adult as well as paediatric patients, with cadaver donor transplants being a clear risk factor [15, 35]. Likewise, the number of HLA-AB and HLA-DR mismatches, i.e. the degree or lack of histocompatibility between patient and recipient, is known to be of relevance in both children and adults [15, 16]. As risk increases not only with the presence but also

with the number of mismatches, these covariates are polychotomous, making the analysis of the presented data set more difficult owing to low patient numbers in the individual categories. The role of donor sex appears unclear; an analysis of risk factors for rejection in about 900 adults showed a nonsignificant trend for female donor sex to be a risk factor [16].

None of the 15 investigated covariate effects was statistically significant. In the SCM, three covariates were identified in the forward step, but deleted in the backward step. In order to characterize the ambiguous signals further, several procedures were applied in order to corroborate the absence of covariate effects further and evaluate their potential clinical impact. These were boot-SCM, XV-SCM and assessment of clinical relevance of the observed signals. A joint evaluation with these methods is considered particularly beneficial in this situation with a relatively small data set and with signals identified that appear reasonable from a theoretical point of view but are not statistically significant in the standard SCM, where a low significance level ($P < 0.01$) is used to compensate for multiple testing. In contrast to the standard SCM, the XV-SCM is designed to determine an appropriate model size, aiming at high predictive performance [23]. The results of the boot-SCM include frequencies of covariate inclusion and make it possible to identify selection bias in covariate effect sizes [24]. While XV-SCM confirmed that covariate inclusion did not improve the predictive performance of the model, boot-SCM results suggested bias, i.e. they were able to detect a type I error in the analysis. Both XV-SCM and boot-SCM thus support the choice of the base model without any covariates as the final model.

The outcome that none of the investigated covariate effects was significant stands in contrast to previous observations, but conclusions cannot in all cases be transferred between different studies, i.e. between different patient populations. However, this finding might be an indication that there could be other explanatory factors not yet identified. Furthermore, it is also possible that the investigated covariate relationships could not reach statistical significance owing to the limited number of patients. For this reason, the strongest signals found in the covariate analysis were characterized further in order to give the detected changes in hazard clinical meaning. For this, based on the parameter estimates created in bootstrap analyses, the time after transplantation at which 90% of all patients were free of ARs (t_{90}) was calculated for different covariate values. As a result, short dialysis time, female sex and high baseline bodyweight were identified as protective factors. The magnitude of the change in t_{90} , however, was low; for the strongest covariate, dialysis time, the median t_{90} was 5.8 days (90% confidence interval 5.1–6.8) for long (90th percentile) and 7.4 (6.4–11.7) days for short (10th percentile) dialysis times, a shift which is unlikely to be clinically relevant. When considering the extreme values of the con-

fidence intervals, differences of up to 6.6 days are possible, which still represents only a mild effect.

In conclusion, in the presented work, a survival model was developed describing the time between renal transplantation and the first AR event in a homogeneous paediatric population. Demographic and clinical information as well as characteristics of CsA therapy were analysed with different methods for systematic covariate search, but no statistically significant covariate effect was found, and potential signals did not predict a clinically relevant effect. The fact that AUC was not identified as a covariate suggested that, within the observed range, an increase in AUC did not increase protection from AR. This represents relevant feedback for clinical therapy, because it may contribute to the avoidance of unnecessarily high and potentially toxic exposure to CsA in children, thus helping to improve patient safety.

Competing Interests

There are no competing interests to declare.

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