Acute leukaemia in children : aspects of diagnosis and treatment

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Cyclosporine A exposure is associated with acute GVHD and relapse in children after stem cell transplantation

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Summary:

Cyclosporine A (CsA) is commonly used after haematological stem cell transplantation (HSCT) as GVHD prophylaxis. In solid organ transplantation, area-under-the-blood-concentration-versus-time-curve (AUC) correlates with clinical outcome. However, in HSCT it has not been determined whether the AUC is superior to trough level monitoring to optimize clinical efficacy of CsA therapy. Therefore, the aim of this study was to investigate the relationships between CsA trough levels and/or AUC early after HSCT, with clinical outcome.

Ninety-one children (1.1 to 17.3 years) were treated consecutively with a HSCT for a haematological malignancy. CsA trough levels were obtained and were used to estimate the AUC, retrospectively, with a NONMEM method. Subsequently, these exposure parameters were correlated to occurrence of acute GVHD, relapse risk and overall survival.

Low CsA trough levels were found to correlate with the occurrence of acute GVHD. In addition, a CsA AUC over 3000 mcg*h/L in AML patients was associated with a reduced overall survival, and a higher relapse risk. This was not the case for ALL patients.

Thus, monitoring CsA exposure early after HSCT and adjusting the CsA dose to a predefined target trough level and AUC may provide a tool to influence the GVHD/GVL balance.

Introduction

Cyclosporine A (CsA) is the most extensively used drug to prevent acute Graft versus Host Disease (GVHD) after allogeneic haematopoietic stem cell transplantation (HSCT)(1). Yet, the optimal therapeutic approach for CsA to prevent acute GVHD is not determined, as reflected by treatment differences worldwide(2). The clinical use of CsA is complicated by considerable intra-patient and inter-patient variability in pharmacokinetics in combination with a relatively small therapeutic window(3). For that reason, it is important to monitor the concentration of CsA in each individual, which generally relies on trough level measurements.

However, trough levels correlate poorly to the area-under-the-blood-concentration-versus-time-curve (AUC)(4). Furthermore, in solid organ transplantation, systemic exposure of CsA, as measured by the AUC, has been shown to correlate well with clinical outcome(5;6). In HSCT, however, it is not clear whether the AUC is superior to trough level monitoring to achieve optimal clinical effect of CsA therapy(7). Therefore, the aim of this retrospective study was to relate CsA trough levels and estimated CsA AUCs during the early post HSCT period, with clinical outcome. Specifically, the relationship of CsA exposure with the occurence of acute GVHD and relapse of haematological malignancy was studied.

Patients and methods

Patients

A total of 145 children, aged 1 tot 17 years, that received a HSCT in the paediatric stem cell transplantation unit of the Leiden University Medical Centre between July 1989 and July 2007 met the inclusion criteria for this study. These criteria were an allogeneic HSCT with a matched sibling donor (MSD) or matched (≥ 9/10 alleles) unrelated donor (UD) for a haematological malignancy, body weight between 10 and 60 kilogram, conventional GVHD prophylaxis consisting of CsA in combination with a short course of methotrexate (as described later) and CsA blood level monitoring. CsA trough levels higher than 800 mcg/L (> +2 SD from the geometric mean), without any clinical consequence or intervention, were considered to be caused by erroneous blood sampling and were excluded from the analysis. Excluded from this study were seven patients who received more than one stem cell graft, as were another 13 patients from whom the exact CsA dosing scheme could not be retrieved.
Data on all remaining 125 patients were used for pharmacokinetic modelling (See Population pharmacokinetic analysis for AUC estimation). However, 25 patients did not provide evaluable trough levels during the second week following the HSCT. Furthermore, in 3 ALL and 6 AML patients the CsA was tapered after 30 days and discontinued shortly thereafter, due to inclusion in a prospective study on immunomodulation to prevent leukaemia relapse.

Therefore, the number of evaluable cases became 91.

Sixty-two children were transplanted with a graft from a matched sibling (MSD) donor, 29 received stem cells from an unrelated donor (UD). Patient characteristics are described in Table 1.

Table 1. Patient and HSCT characteristics

	Matched sibling donor (MSD)	**Unrelated donor (UD)**
Number of patients	62	29
Patient age in yrs, range (median)	1.1 – 17.3 (8.5)	1.5 – 16.4 (10.9)
Patient sex (male/female)	42 / 20	19/ 10
Weight in kg, range (median)	10 – 60 (27)	10 – 58 (38)
Height in cm, range (median)	77 – 175 (135)	47 – 174 (147)
Diagnosis		
ALL	29	6
AML	21	10
NHL	2	0
MDS	9	10
aCML	1	3
Acute GVHD		
Absent	62 (94%)	26 (76%)
Grade 1	4 (6%)	2 (7%)
Grade 2	0	1 (3%)
Grade 3	0	3 (10%)
Grade 4	0	1 (3%)
Chronic GVHD		
Absent	64 (97%)	32 (94%)
Limited	0	0
Extensive	2 (3%)	2 (6%)

The stem cell source was bone marrow in all patients, except for peripheral blood stem cells in 6 UD-HSCT and cord blood stem cells in 2 MSD-HSCT. All patients were nursed in strict protective isolation using positive-pressure laminar-air flow cubicles or rooms, and received total gut decontamination with non-absorbable antimicrobials to suppress the potentially

pathogenic intestinal microflora(8); before discharge they were re-contaminated with strictly anaerobic faecal flora plus BiogardeR. All blood products for infusion were leukocyte-depleted and irradiated with a dose of 25 Gy. All included patients engrafted. Median day of engraftment, defined as the first day at which blood granulocyte counts rose to 0.5 × 109/L (for two consecutive determinations) was day 22 days after SCT (95% CI: 14 – 43 days).

GVHD prophylaxis and treatment

GVHD prophylaxis included CsA in combination with a short course of methotrexate. Short-course MTX was given at a dose of 10 mg/m2 IV at days +1, +3, and +6 (and in some patients, +11), and was combined with a 2-h infusion of CsA 2 mg/kg/day IV divided over two doses, starting 1 to 3 days before infusion of the graft. If considered necessary by the treating physician, dose adjustments were made on the basis of trough level measurements. The route of administration of CsA was changed from a twice daily IV to a twice daily oral regimen following haematological engraftment and in case oral medication was well tolerated, usually at ≥3 weeks post SCT. At the time of conversion, the dose of CsA was tripled with regard to the difference in bioavailability of oral CsA. CsA was usually continued for 180 days, tapered thereafter and finally stopped. Acute and chronic GVHD were diagnosed by clinical manifestation and graded according to standard criteria(9). Acute GVHD was treated with methylprednisolone 2 mg/kg/day IV, followed by tapering upon clinical improvement; exceptionally other drugs were used e.g. ATG, anti-CD52 antibodies and thalidomide. Chronic GVHD was further characterized according to Shulman et al(10).

Cyclosporine A concentration measurements

CsA levels were determined once weekly in whole blood (EDTA) by using the routine methodology of the laboratory for Clinical Pharmacy and Toxicology of the LUMC. Before 2001, CsA concentrations were measured by radioimmunoassay based on monoclonal antibody (RIA, Incstar), later by Fluorescence Polarization Immunoassay (FPIA) using AxSYM analyzer according to manufacturer's instructions (Abbott). The geometric mean of the CsA blood concentration, measured before and after change in analytical method was 100

and 120 µg/L, respectively (p=0.18). The lower limit of quantification (LLQ) was 15 µg/L. From the included patients the CsA trough level measurements from the early post SCT period (day +7 through +14) were retrieved. In a few patients more than one trough level measurement was available during the second week following SCT. In these cases, selection of the lowest trough level and estimated AUC was applied, as reflection of low exposure under treatment.

Population pharmacokinetic analysis for AUC estimation

A pharmacokinetic (PK) model which was developed and described previously(11) was updated with the data obtained for this study using Non-Linear-Mixed-Effects-Modelling (NONMEM, version VI release 1.2, Icon Development Solutions, Ellicott City, Maryland, USA)(12). Modelling results were analyzed using the statistical software package S-Plus® for Windows (version 6.2 Professional, Insightful Corp., Seattle, USA). First order conditional estimation (FOCE) with interaction was used throughout the modelling process. A convergence criterion of 3 significant digits in the parameter estimates was used. For model comparisons the obtained minimum value of the objective function (MVOF) defined as minus twice the log-likelihood, was used. The modelling process was guided by statistical and visual checks (i.e. diagnostic 'goodness of fit' plots). A model parameter was retained in the model when including this parameter in the model resulted in a decrease of 6.63 points in the minimum value of the objective function.

The previously decribed model, build on 17 individuals (17 full 12 hour profiles), was updated with a database consisting of 125 individuals (669 trough level measurements). The combined dataset revealed two relationships that had to be described in order to obtain an unbiased model for parameter estimations. First of all, an allometric relationship between patients' bodyweight and CsA clearance as well as central volume of distribution was incorporated in the model by means of PK=TVPK*(BW/meanBW)exp, with PK as the PK parameter, TVPK as the PK parameter for the typical patient and BW as term for bodyweight. Exp refers to the exponent, typically 0.75 for clearance and 1 for volume of distribution(13). Secondly, a time dependent linear relationship for clearance was identified with a decreasing

clearance from the moment of SCT up to 3 weeks (500 hours) post SCT, described in terms of CL=TVCL*[1 - 0.000918*(time post transplantation - 500)], with TVCL used for the typical CsA clearance of the SCT patient. CsA clearance was up to 30% higher during the first three weeks post SCT compared to the stable period thereafter. These adjustments resulted in a model that adequately described the entire dataset. Consequently, the obtained model was used to derive CsA CL estimates for every patient on basis of his/her collected trough levels. These so called individual empirical Bayes estimates for clearance were used to calculate the AUC with the formula AUC=F*Dose / CL, with F defined as bioavailability.

Statistical analysis

Cumulative incidence curves were used in a competing risks setting, with death being treated as a competing event to calculate probabilities of acute GVHD and relapse. Confidence intervals were obtained from standard error estimates of the Kaplan Meier curves. Univariate and multivariate analyses of CsA pharmacokinetic parameters and patient characteristics on outcome (overall survival, relapse risk and transplant-related mortality) were assessed using the Cox proportional hazards model. The starting point of evaluation of outcome was set at 30 days after SCT as a consequence of the exclusion of the subset of patients with early tapering (at 30 days after SCT) of GVHD prophylaxis. The evaluation cut-off of CsA trough levels (100 mcg/L) and CsA AUC (3000 mcg*h/L) was chosen close to the median. Time of SCT, donor type, time to engraftment and acute GVHD were added as covariates to the analysis. All statistical analyses were performed with the SPSS-16.0 statistical package. P-values less than 0.05 were considered as statistically significant. The evaluation cut-off date was 1-1-2008.

Results

Acute Graft versus Host Disease

For children undergoing an allogeneic SCT with a MSD (n=62), the incidence of acute GVHD was 6%, all grade I (n=4), while the incidence of acute GVHD in UD-SCT (n=29) appeared to be 24% (n=7). Five recipients of an unrelated donor graft experienced a GHVD grade II or higher (Table 1). Lower CsA trough levels during the early post SCT period were associated with a higher risk of acute GVHD. Besides the low CsA trough level (hazard decreases per 10 additional µg/L CsA trough blood concentration), receiving a graft from an unrelated donor also was an independent prognostic factor for the development of acute GVHD (Table 2).

Table 2. Independent prognostic factors for development of acute GVHD according to a multivariate analysis of 91 patients.

Factor	HR	95% CI	P
Donor type (UD vs. MSD)	0.17	0.04-0.66	0.01
CsA trough blood concentration (10 µg/L)	0.79	0.56-0.96	0.02

Abbreviation: HR, hazard ratio.

Interestingly, no correlation was observed between CsA AUC and the incidence of acute GVHD. Because of the low occurrence of acute GVHD in our patient population we were not able to perform a sub analysis of patients with acute GVHD grade 0 and I versus grade II or more. However, in our patients the value of the CsA trough levels and AUCs did not correlate with the severity of the acute GVHD.

Relapse and survival

To determine the relationship between CsA trough levels or AUC and clinical outcome in children with an allogeneic SCT for a haematological malignancy, we evaluated overall survival (OS), relapse risk (RR) and transplant-related mortality (TRM). Overall, the 5-year probability for OS was 60% (95% CI: 50-70%), for RR was 38% (95% CI: 28-48%) and for

TRM was 8% (95% CI: 0-15%), respectively. For children with ALL and AML, the five years probability of OS was 48% and 57%, respectively, and the five years probability of relapse was 58% and 39%, respectively (NS).

Table 3 and 4 demonstrate the clinical relevance of CsA trough level and AUC value during the second week following SCT as markers for clinical outcome. Although CsA trough levels were not related tot overall survival or relapse risk (Table 3), CsA AUC did show a relationship with clinical outcome. AUC values below 3000 mcg*h/L were significantly related to higher survival rates and a decreased risk of relapse for patients diagnosed with AML. In contrast, this is not the case for patients with ALL.

Table 3. Prognostic impact of CsA exposure (Trough level>100 mcg/L vs. <100 mcg/L) during the early post SCT period on OS, RR, and TRM in all 91 SCT patients and those transplanted for ALL and AML, separately.

	Overall (n=91)		**ALL (n=35)**		**AML (n=31)**	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
OS	1.28 (0.67-2.46)	0.46	1.14 (0.44-2.94)	0.79	2.04 (0.68-6.13)	0.20
RR	0.62 (0.31-1.22)	0.17	0.78 (0.31-1.98)	0.60	0.61 (0.19-1.92)	0.40
TRM	1.03 (0.23-4.63)	0.97	0.02 (0-3*105)	0.65	0.23 (0.02-2.60)	0.24

Abbreviations: HR, hazard ratio; OS, overall survival; RR, relapse risk; TRM, transplantation-related mortality.

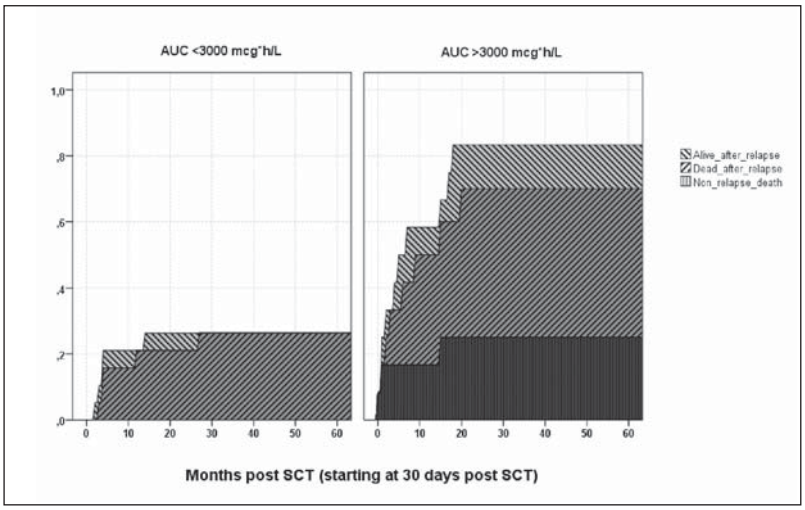
Table 4. Prognostic impact of CsA exposure (AUC>3000 mcg*hr/L vs. AUC <3000 mcg*h/L) during the early post SCT period on OS, RR, and TRM in all 91 SCT patients and those transplanted for ALL and AML, separately.

	Overall (n=91)		**ALL (n=35)**		**AML (n=31)**	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
OS	1.97 (1.02-3.80)	0.04	1.36 (0.29-3.45)	0.52	3.92 (1.27-12.20)	0.02
RR	0.45 (0.24-0.92)	0.03	0.94 (0.38-2.31)	0.89	0.29 (0.09-0.92)	0.04
TRM	0.43 (0.10-1.95)	0.28	0.02 (0-2*105)	0.62	0.01 (0-224)	0.34

Abbreviations: HR, hazard ratio; OS, overall survival; RR, relapse risk; TRM, transplantation-related mortality.

Figure 1 displays a Kaplan-Meier curve, displaying the outcome of patients with AML according to CsA AUC, showing overall survival, disease-free survival, transplant-related mortality and relapse in one graph.

Figure 1. Relationship of CsA-AUC and outcome in children treated for AML. These curves display the outcome of patients with AML, showing overall survival, disease-free survival, transplant-related mortality and relapse in one graph. AUC values below 3000 mcg*h/L are significantly related to higher survival rates and a decreased risk of relapse.



A multivariate Cox regression analysis was performed to investigate the effect on outcome of the following covariates: time of SCT, donor type, time to engraftment and occurrence of acute GVHD. None of these covariates had a significant influence on the outcome parameters in our study population, neither in all patients together, nor in the sub group of AML patients.

Discussion

To date, the optimal therapeutic approach for CsA therapy as GVHD prophylaxis following HSCT remains unclear in terms of dose, as well as monitoring strategy(2). The benefit of adequate GVHD prophylaxis using CsA and MTX has been evaluated mainly using trough levels. Several investigators have recognized CsA exposure in the early post transplantation

period as a risk factor for the development of acute GVHD(14-18). In children after HSCT, a CsA trough lever lower than <85 mcg/L during the first two weeks post transplantation was associated with severe acute GVHD(15). Using trough levels as reflection of CsA exposure has the advantage that it is practical. The usefulness, however, of dose adjustments based on CsA trough levels continues to be controversial. It has been argued that the CsA trough level relates poorly to the AUC(4). In addition, it has been described that the maximal pharmacodynamic response, as measured by inhibition of calcineurin activity, coincides with the peak concentration approximately two h after dose intake(19). This indicates that monitoring the AUC or a concentration 2 hours after CsA dose intake (C2) could be a better measure of drug exposure than trough levels. In fact, a study in solid organ transplantation demonstrates a relationship between C2 and graft rejection(20). In HSCT, however, C2 concentrations did not show any correlation with acute GVHD in adults(21).

To further complicate CsA therapy after HSCT, it has been suggested that a high CsA dose is related to relapse of original disease(22). When taking this into account an optimal therapeutic drug monitoring approach becomes mandatory to control the balance between acute GVHD and relapse of disease. Therefore, the aim of this study was to identify a relationship between a pharmacokinetic parameter and clinical outcome in terms of acute GVHD and relapse of disease. Ultimately this study should indicate whether the use of AUC would potentially add to therapy outcome. Therefore, in this study, CsA trough levels were obtained from 125 children transplanted for a hematological malignancy. These trough levels were used to estimate the AUC with a population pharmacokinetic model that has been described previously(11), but was improved in this study. We choose to evaluate the CsA exposure during the second week following HSCT to omit the acute toxicities of the HSCT and thus allow some time to reach a pharmacological steady state. Furthermore, we felt that the intensity of immunosuppression during this period preceding engraftment of haematopoietic precursor cells could potentially play a role in the development of (subclinical) acute GVHD and GVL effect.

In accordance with reports from others (14-18), we observed a relationship between CsA trough levels and acute GVHD. The AUC, however, did not show any correlation with occurrence of acute GVHD. This is in line with a study from Martin et al. that described

lower CsA trough levels in children who developed grade II-IV acute GVHD than in those developing no GVHD or only grade I, in contrast to the peak blood concentration and AUC that were comparable in both groups(15). Adequate GVHD prophylaxis may perhaps be more related to trough level than to another pharmacological measure of exposure, indicating the need for a sufficiently high blood trough concentration rather than the need for adequate total exposure. Despite these arguments one would still have expected to find a relationship between AUC and acute GVHD. The reason for this absence could be due to the low incidence of acute GVHD in our patient population and/or biases in the AUC estimation. The potential error in the estimations was calculated and found to be approximately 20% in the estimated AUC when assessed on basis of the trough levels in this study. Inclusion of more blood concentration measurements from time points after CsA dose intake might have further improved AUC estimation.

Acute GVHD contributes to both early and late mortality after HSCT, while lower relapse rates have been found in leukaemia patients who develop acute or chronic GVHD. This so-called graft versus leukaemia (GVL) effect, probably mediated by donor alloreactive T cells and associated with the occurrence of acute GVHD, is an important contribution to the control of leukaemia. Its effect is considerably less for cases with acute leukaemia than in CML(23). Furthermore, lymphoid leukaemias seem less sensitive to donor lymphocyte infusions than leukaemia from myeloid origin(24), which is possibly due to their inability to act as antigen-presenting cell, but also their potential to induce tolerance may be involved(25). Also, Locatelli et al., demonstrated that children with acute leukaemia (47 children with ALL and 12 children with AML) receiving CsA at a dose of 1 mg/kg/day had a significantly lower risk of relapse as compared to those who received CsA at a dose of 3 mg/kg/day, probably due to increased graft versus leukaemia effect(22).

Our study shows that low systemic exposure to CsA (~AUC) during the early post transplantation period was significantly associated with lower relapse risk and higher overall survival, independent of development of acute GVHD, in children with AML. This was not the case for the ALL patients in our study, possibly because they are less prone to benefit from the GVL-effect. The results of this study indicate that an AUC not higher than 3000

mcg*h/L, particularly in AML patients, should be used to optimize clinical outcome. We therefore suggest monitoring AUC weekly in the first weeks following HSCT, and propose a target-AUC between 2500 and 3000 mcg*h/L to maximize GVL effect. For adequate GVHD prophylaxis a target trough level of 100 mcg/L would be reasonable. However, the risk of acute GVHD and relapse needs to be weighed against each other for each individual patient in the context of their specific HSCT. Since it is inconvenient for the patient and the hospital staff to obtain sufficient samples to calculate the full AUC0-12h during twice daily dosing, a limited sampling strategy(11) could be used in place. Especially in the paediatric setting, compartmental population pharmacokinetic modeling with Bayesian estimation offers several advantages. The model is flexible, does not depend on exact blood sampling time-points, enables interpretation of sets with missing data and generally can use data from children being given the drug therapeutically. It is therefore useful in clinical practice. Consequently, by using the individual patient covariates and the information obtained from a prior population analysis, one can estimate individual pharmacokinetic parameters(26) or determine the dose that achieves a target concentration in the individual patient(27), allowing further optimization of the intensity of immunosuppression.

The results of this study indicate that a close correlation of AUC with outcome exists and stresses the importance of CsA drug monitoring in SCT recipients to minimize GVHD and maximize GVL effect. The preservation of a GVL-effect (especially for AML) by careful monitoring immunosuppression may further improve the outcome following SCT of children with high risk acute leukaemia.

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