Chapter 3

Cyclosporine A withdrawal during follow-up after pediatric liver transplantation

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Abstract

Background
It is unclear whether Cyclosporine A (CsA) can be withdrawn safely during follow-up after pediatric liver transplantation (LTx). In our transplant program we have been using a strict protocol to withdraw CsA.

Aim
To retrospectively assess the effects of CsA withdrawal after LTx on the incidence of rejection and renal function.

Patients and methods
Between 1986 and 2001, 91 children received CsA for at least 2 years after liver transplantation. Specific criteria for eligibility to withdraw CsA were set.

Results
In 53 of the 91 children CsA was withdrawn. In 35 patients (66%) withdrawal of CsA did not cause rejection. In these patients the renal function improved compared with baseline values (glomerular filtration rate (GFR) 1 year after withdrawal: +16 ml/min/1.73 m², p<0.001; 2 years: +11 ml/min/1.73 m², p<0.05). After CsA withdrawal, 18 patients developed rejection (34%) which could be effectively treated by methylprednisolone and restarting CsA. Failure to withdraw CsA was not associated with increased incidence of graft loss. A body weight below 10 kg at the time of transplantation correlated significantly with successful withdrawal of CsA (<10 kg, 85%; vs. >10 kg, 60%; p<0.05).

Conclusion
CsA can successfully be withdrawn in a major proportion of selected pediatric LTx patients during follow-up. The success rate is the highest in children with a body weight below 10 kg at the time of LTx. Successful withdrawal improves renal function, whereas failure to withdraw is not associated with graft loss or persisting morbidity.

Abbreviations
CsA = cyclosporine A
LTx = liver transplantation
GFR = glomerular filtration rate
Introduction

Soon after its introduction in 1979, cyclosporine A (CsA) became the cornerstone of immunosuppressive therapy after liver transplantation (LTx) due to its strong immunosuppressive effect. However, it has also been associated with side effects, such as nephrotoxicity and hyperlipidemia. The risk of acute rejection decreases in time after transplantation, which may decrease the need for maintenance of immunosuppression during follow-up. Microchimerism, the persistence of low levels of donor cells in the recipient’s blood and tissues, may be responsible for this phenomenon. In selected cases, withdrawal of CsA after LTx was associated with initial improvement of renal function and blood pressure. However, inconclusive data were reported on the incidence of rejection after withdrawal of CsA, ranging from 6 to 50% for cellular rejection and from 12 to 25% for ductopenic rejection. Pediatric patients may be the most important category to minimize side effects after LTx, because of their prolonged life expectancy compared to adult patients. In the Groningen Pediatric Liver Transplant Program, triple therapy consisting of CsA, prednisolone and azathioprine was used as standard immunosuppressive regimen between 1986 and 1998. Simultaneously with the introduction of this regimen, a protocol was implemented for withdrawal of CsA treatment from 2 years after transplantation. The extensive use of the CsA based immunosuppressive regimen for 10 years, with the withdrawal according to protocol after at least 2 years, provides a unique opportunity to retrospectively evaluate the risks and benefits of withdrawal of CsA in pediatric patients after LTx. In the present study we evaluated the consequences of CsA withdrawal on development of rejection (liver histology) and kidney function (based on plasma parameters). We also determined if patient characteristics or surgical parameters concerning the transplantation were associated with a successful withdrawal.
Patients and methods

In the study period between February 1986 and January 2001 a total of 91 patients younger than 18 years underwent a LTx in the University Medical Center Groningen, started with a CsA-based immunosuppressive regimen and had a follow-up of at least 2 years after LTx. Ten patients developed rejection during CsA treatment, and were switched to a tacrolimus-based immunosuppressive regimen. CsA treatment was withdrawn earlier than two years after LTx in 16 patients because of side effects of CsA. These patients were not included in this study. Of the remaining 65 patients, 12 did not meet the criteria for withdrawal and continued with CsA. In 53 patients CsA was withdrawn according to protocol. The medical records of these 53 patients were reviewed and data were collected concerning indications for LTx, age, type of graft, liver and kidney function, and plasma lipid levels in the years following LTx, at the time of CsA withdrawal and 2 years afterwards. The censoring date for the present evaluation (January 1, 2005), allowed for at least 2 years of follow-up after attempted withdrawal of CsA.

Immunosuppressive medication after LTx consisted of cyclophosphamide in a dose of 3 mg/kg during the first 7 days (from 1986 to 1998, deleted from the protocol thereafter), prednisolone (initially 1 mg/kg/day, gradually tapered to 0.25-0.4 mg/kg/2days, alternate day dosing) and azathioprine (2 mg/kg/day). CsA was introduced at the second day after transplantation (trough levels first 4 weeks post-transplantation 200-250 µg/ml, after this period 100 – 150 µg/ml). From 1998 onwards, a tacrolimus-based regimen was used and only patients with an autoimmune hepatitis or sclerosing cholangitis received the CsA-based regimen. Clinically evident and biopsy proven rejections in the first 4 weeks were treated with methylprednisolone intravenously. Rejections after this period were treated with methylprednisolone intravenously or with increasing the oral dose of prednisolone. CsA was withdrawn after the second annual evaluation after transplantation, provided that the graft was functioning well, there were no periods of rejection in the second year and there were no signs of rejection in the biopsy specimen. If criteria were not met, withdrawal of CsA could be reconsidered at later annual evaluations. Withdrawal of CsA was combined with an alteration of the dosages of other immunosuppressive drugs. The withdrawal procedure started with increasing the dosage of prednisolone to 0.25-0.4 mg/kg/day and of azathioprine to 2.5 mg/kg/day. After 4 days the dosage of CsA was decreased by 50% for 1 week, and completely discontinued thereafter. Over a subsequent 6 weeks period, prednisolone dose was tapered to 0.4-0.6 mg/kg/2days, alternate day dosing, azathioprine was continued in the same dose.

Liver biochemical tests and serum creatinine were measured monthly and in case of suspected rejection (elevation of liver enzymes, unexplained fever) a liver biopsy was performed. Liver biopsies according to protocol were performed 1, 2, 3 and 5 years after LTx. Since the maintenance dosage of prednisolone was increased, we reviewed the prednisolone dose 2 years after the CsA withdrawal and in 2004, evaluated the height and most recent bone density (2000–2005) of the lumbar spine by dual-energy X-ray absorptiometry of all the included patients.
Rejection, retransplantation and survival
Outcome parameters were the number of rejections, the number and causes of retransplantations, survival, histology of the liver biopsy specimens, and liver and kidney function. Patient survival was defined as the time between LTx and either the censoring date or patient death.

Kidney function
Kidney function was evaluating by using an estimation of the glomerular filtration rate (GFR) with the Schwartz formula: GFR (ml/min/1.73 m) = [height (cm)/ creatinine (μmol/L] x K, K being a constant reflecting age and muscle mass.\(^{10}\)

Graft function
Liver biopsy specimens taken before and 1 year after withdrawal were analyzed for the presence of histological changes. Rejection was graded according to Banff criteria for grading liver allograft rejection.\(^{11}\) Liver biochemical tests consisted of aspartate aminotransferase and alanine aminotransferase reflecting cellular damage, \(\gamma\)-glutamyl transferase and total bilirubin as indicators for cholestasis, and albumin as indicator of protein synthesis.

Plasma lipid levels
Plasma levels of triglycerides and cholesterol were analyzed from blood samples from the year preceding and 1 year after CsA withdrawal.

Analysis of possibly predictive factors for success/failure of CsA withdrawal
To determine whether predictive factors for a successful CsA withdrawal could be identified, potential contributing factors (such as primary diagnosis, type of graft, age and weight at time of LTx, and the occurrence of early rejection) were analyzed.

Statistical Analysis
Continuous variables are presented as mean with standard deviation and categorical variables as number with percentages. Categorical variables were compared using the Pearson’s chi-square test or the Fisher’s exact test if suspected cell frequency was less than five. The Student’s T-test was used for continuous variables. \(p < 0.05\) was considered statistically significant.

Results
Table 1 shows the demographic details of the 53 included recipients and details of the donors and the LTx procedure. The most frequent indication for LTx was biliary atresia (25 of 53 patients, 47%), followed by a heterogenic group, including various metabolic diseases (9 of 53, 17%) and fulminant hepatic failure (4 of 53, 8%). CsA withdrawal occurred at 2.5±1.1 years after transplantation (mean±SD), and the length of follow-up after (attempted) withdrawal was 8.4±3.5 years.
Incidence of rejection and mortality
Of the 53 patients in which CsA was withdrawn, 35 (66%) did not develop a rejection. Eighteen patients (34%) developed an acute cellular rejection (table 2). Of these 18 patients, 15 (83%) developed a rejection in the first half year after withdrawal. One patient developed a grade 1 rejection 3.5 years after withdrawal of CsA (figure 1). All rejections responded to rejection treatment, consisting of intravenous methylprednisolone and subsequent restarting CsA. Five of these patients who restarted with CsA, switched to tacrolimus at a later stage because of side effects of CsA. In one patient in whom CsA was restarted, it could be withdrawn successfully at 5 years after transplantation. Thus, in 36 patients (68%) CsA was eventually withdrawn successfully.

Table 1. Demographic data of the recipients and variables related to donors and the last transplantation of the 53 patients included in the protocol.

<table>
<thead>
<tr>
<th>Recipients</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25(48%)</td>
</tr>
<tr>
<td>Female</td>
<td>28(52%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>2.7(0.1–17)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>14.6(3.5–56)</td>
</tr>
<tr>
<td>Previous operations</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>19(36%)</td>
</tr>
<tr>
<td>One of more</td>
<td>33(64%)</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Donors</th>
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<tbody>
<tr>
<td>CMV match</td>
<td></td>
</tr>
<tr>
<td>D-/R-</td>
<td>22(42%)</td>
</tr>
<tr>
<td>D+/R-</td>
<td>17(33%)</td>
</tr>
<tr>
<td>D+/R+ D-/R+</td>
<td>13(25%)</td>
</tr>
<tr>
<td>ICU stay (d)</td>
<td>2.2(1–9)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>12(0.5–51)</td>
</tr>
<tr>
<td>D/R age ratio</td>
<td>5.1(0.1–42)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>35(6–85)</td>
</tr>
<tr>
<td>D/R weight ratio</td>
<td>2.6(0.5–10.4)</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Transplantation</th>
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<tbody>
<tr>
<td>First transplantation</td>
<td>42(81%)</td>
</tr>
<tr>
<td>Retransplantation</td>
<td>10(19%)</td>
</tr>
<tr>
<td>Cold ischemia time (h)</td>
<td>10.6(3.5–18.3)</td>
</tr>
<tr>
<td>Anhepatic phase (h)</td>
<td>1.7(0.9–3.65)</td>
</tr>
<tr>
<td>Type of transplantation</td>
<td></td>
</tr>
<tr>
<td>full-size</td>
<td>25(48%)</td>
</tr>
<tr>
<td>partial size</td>
<td>27(52%)</td>
</tr>
</tbody>
</table>

Continuous variables are presented as median (range) and categorical variables as number (percentage).
Abbreviations: CMV: cytomegalovirus; D/R: Donor/Recipient; ICU: Intensive Care Unit.

Incidence of rejection and mortality
Of the 53 patients in which CsA was withdrawn, 35 (66%) did not develop a rejection. Eighteen patients (34%) developed an acute cellular rejection (table 2). Of these 18 patients, 15 (83%) developed a rejection in the first half year after withdrawal. One patient developed a grade 1 rejection 3.5 years after withdrawal of CsA (figure 1). All rejections responded to rejection treatment, consisting of intravenous methylprednisolone and subsequent restarting CsA. Five of these patients who restarted with CsA, switched to tacrolimus at a later stage because of side effects of CsA. In one patient in whom CsA was restarted, it could be withdrawn successfully at 5 years after transplantation. Thus, in 36 patients (68%) CsA was eventually withdrawn successfully.
We reviewed the mortality in the 53 patients where CsA was withdrawn. Of the patients in whom CsA could be successfully withdrawn, three patients died. One patient died from hepatitis C virus infection, complicated by a sepsis. A second patient died from a NK cell lymphoma. The third patient died 10 years after LTx from complications of cirrhosis caused by a stenosis of the biliary system. We could not reasonably relate the causes of death to graft failure caused by the withdrawal of CsA. In the group in which CsA was restarted no patients died.

**Graft function**

In the 53 patients in whom CsA was withdrawn according to protocol, biochemical liver tests before, and 1 and 2 years after withdrawal, did not differ in parameters for cellular damage, cholestasis or protein synthesis (Table 3). Histology of the liver 1 year after successful withdrawal did not show significant changes compared with the biopsy specimens before withdrawal. Chronic or ductopenic rejection was not

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**Table 2.** Characterization of the rejections after CsA withdrawal.

<table>
<thead>
<tr>
<th>Rejection after withdrawal</th>
<th>18/53 (34%)</th>
</tr>
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<tbody>
<tr>
<td>Time after withdrawal (months)</td>
<td>6.8±9.5</td>
</tr>
<tr>
<td>Histological classification (BANFF criteria)</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>4/18 (22%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>12/18 (67%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>2/18 (11%)</td>
</tr>
<tr>
<td>Rejection in patients weight &lt; 10 kg</td>
<td>3/21 (14%) *</td>
</tr>
<tr>
<td>Rejection in patients weight &gt; 10 kg</td>
<td>13/32 (41%) *</td>
</tr>
</tbody>
</table>

* p < 0.05

Continuous variables are presented as median (range) and categorical variables as number (percentage).

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**Figure 1.** The rejection free period after withdrawal of cyclosporine (CsA) in children after liver transplantation (n = 53).

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Cyclosporine A withdrawal after pediatric LTx
seen in any of the liver biopsy specimens 1 year after withdrawal of CsA. Accordingly, no graft loss was reported during the 8.4 ± 3.5 years of follow-up after withdrawal of CsA. One or 2 year after attempted CsA withdrawal, biochemical liver tests did not differ between those in which CsA could be withdrawn successfully and those in which CsA was restarted (not shown).

**Prednisolone dosage**

At two years after CsA withdrawal the dosage of prednisolone was 0.34 ± 0.07 mg/kg/2 days in the CsA-free group and 0.26 ± 0.04 mg/kg/2 days in the group where CsA
was restarted. In 2004 the prednisolone was slowly decreased in most of the patients, but the dosage of prednisolone remained slightly higher in the CsA-free group than in the restart CsA group. The higher dosage of prednisolone in the group, where CsA was successfully withdrawn did not result in a difference in height or bone density between the 2 groups (table 4).

**Renal function**

Renal function was evaluated using estimated GFR before withdrawal, and 1 and 2 years after withdrawal (table 5). The estimated GFR in children, in whom CsA was withdrawn successfully, showed an increase compared with baseline values (1 year: +16 mL/min/1.73 m², p < 0.001; and 2 years: + 11 mL/min/1.73 m², p < 0.05). The estimated GFR in children, in whom CsA had to be restarted, did not alter significantly.

**Plasma lipids**

Evaluation of the levels of triglycerides before and after CsA withdrawal in patients, in whom CsA could be successfully withdrawn, showed a significance decrease (before: 1.6 ± 0.7 mmol/l, after: 1.2 ± 0.6 mmol/l, p < 0.05). Cholesterol levels did not show a significant change after CsA withdrawal.

**Evaluation of prognostic factors related to successful withdrawal**

We analyzed whether specific factors were correlated with a successful withdrawal of CsA. There was no significant correlation between characteristics of the donor or the transplantation procedure, and a successful withdrawal of CsA. Also the primary liver disease (biliary atresia or metabolic liver disease), type of liver graft, age at the time of LTx, or the occurrence of early rejection were not associated with successful or non-successful (failed) withdrawal of CsA. We did find a significant correlation, however, with weight at the time of LTx. In 18 of 21 (85%) children, with a weight of less than 10 kg at the time of LTx CsA, could be successfully withdrawn, compared with 19 of 32 (60%) patients, who had a weight above 10 kg at the time of LTx (p < 0.05), as shown in figure 2.

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**Table 5.** Estimated glomerular filtration rate (GFR) before withdrawal of cyclosporine (CsA) and 1 and 2 years after withdrawal in patients in whom CsA could be withdrawn successfully.

<table>
<thead>
<tr>
<th></th>
<th>Before CsA withdrawal</th>
<th>1 year after CsA withdrawal</th>
<th>2 years after CsA withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR CsA-free group</td>
<td>75 ± 21</td>
<td>91 ± 17*</td>
<td>86 ± 14**</td>
</tr>
<tr>
<td>GFR CsA restart group</td>
<td>81 ± 20</td>
<td>85 ± 18</td>
<td>77 ± 15</td>
</tr>
</tbody>
</table>

Mean ± SD, GFR in mL/min/1.73 m²

* p<0.001, GFR before and one year after withdrawal

** p<0.05, GFR before and two years after withdrawal.

Other differences are not significant.
Discussion

We describe a unique cohort of pediatric patients in whom it was attempted to withdraw CsA during follow-up after LTx, according to a strict protocol. In most of these patients CsA could be withdrawn without the occurrence of rejection or indications of failure or malfunction of the liver graft. Successful withdrawal of CsA was associated with improved kidney function. A weight below 10 kg at the time of LTx was associated with successful withdrawal.

After LTx the risk of acute rejection decreases in time, which allows for a lower level of immunosuppression. In this study we withdrew CsA at least 2 years after LTx in patients, who had not experienced any recent rejection. The withdrawal of CsA led to rejection in a third of the patients. This may be a worrisome sign, but all rejection episodes could be treated successfully with methylprednisolone and restarting CsA.

During follow-up of at least 2 years after the (attempted) withdrawal of CsA, we did not find any graft loss or histological indications of ductopenic rejection either in the group were CsA could be withdrawn with success, or in the group in which CsA had to be restarted.

The immunosuppressive regimens in most transplantation centers include a decrease in steroids in time. Our cohort is unique with respect to CsA withdrawal and steroid increase. The protocol was introduced in 1986 with the introduction of CsA to prevent long-term side effects of CsA. As CsA could be withdrawn in most patients, the protocol was continued until the introduction of a tacrolimus based protocol in 1998. The CsA could be withdrawn at the cost of a higher prednisolone dose, but the difference between the two groups was relatively modest with a dose of only 0.34±0.07 mg/kg/2days, alternate dosing, in the group with successful CsA withdrawal.

We found a significant decrease of approximately 25% in the triglycerides levels af-
Cyclosporine A withdrawal after pediatric LTx

After withdrawal of CsA, but no change in cholesterol levels. Recently, we reported in a subgroup of our patients, that withdrawal of CsA increased bile salt synthesis and concomitantly decreased triglyceride (23%) and cholesterol (18%) levels. Apparently this beneficial effect of CsA withdrawal on plasma lipids is consistent for triglycerides in this much larger cohort studied now.

Nephrotoxicity is one of the most serious side effects of calcineurin inhibitors, such as CsA. Acute nephrotoxicity has been associated with toxic levels of calcineurin inhibitors, and is usually reversible after dose reduction. On the other hand, chronic nephrotoxicity is associated with parenchymal renal lesions: interstitial fibrosis, tubulopathy, and glomerulopathy. There is a general trend to minimize or discontinue calcineurin inhibitors in patients with chronic renal dysfunction after LTx. The available literature, however, does not consistently indicate that renal function persistently improves by this strategy. Withdrawal of CsA in patients after LTx was previously described in a selected group of adults with renal dysfunction. Sandborn et al. showed only a transient, relatively modest improvement of renal function after withdrawal of CsA. Chan et al. and Herrero et al. observed an improved renal function after withdrawal of CsA. Similarly, a prospective randomized study in adult liver transplant patients with moderate renal impairment indicated improved kidney function after CsA withdrawal. In the present study of pediatric patients after LTx, who had only mild or no impairment of the renal function, a significant improvement of the kidney function was observed, in accordance with the data discussed above. It has been shown that there is a further impairment of kidney function in time with the use of calcineurin inhibitors. Withdrawal of CsA could therefore not only improve renal function but could also prevent further damaging of renal function.

All patients that could be successfully weaned from CsA continued with an immunosuppressive regimen of azathioprine and prednisolone. The prednisolone maintenance dose was increased to a dose of 0.4 - 0.6 mg/kg/2 days. Sandborn et al. used a comparable immunosuppressive regimen after withdrawal of CsA, but these authors observed acute rejection in 50% of the patients and a ductopenic rejection in another 25%, leading to the death of two patients. Similarly, Te maintained liver transplantation patients on a regimen of azathioprine and prednisolone, but reported acute rejection in 11% and ductopenic rejection in 22%, yet without any graft loss. In both studies the patients were adults, and concerned relatively small numbers, 12 and 17 respectively. More recently the withdrawal of calcineurin inhibitors with an immunosuppressive regimen based on mycophenolate mofetil has been described in several randomized prospective studies in adult patients with impaired renal function. One of the trials in which a regimen of mycophenolate mofetil monotherapy was used had to be prematurely stopped, however, after three of the five included patients developed a ductopenic rejection needing retransplantation. In the trial by Schlitt in adult patients a lower percentage of rejection was found, particularly in patients, who received an immunosuppressive regimen consisting of mycophenolate mofetil and prednisolone after withdrawal of CsA. The rationale to withdraw CsA in all these patients was related to side effects of the drug, whereas we describe a group of 53 pediatric patients in whom CsA was elect-
tively withdrawn. It seems very well possible that the methodological differences to select our patients and younger age of our patients account for the profoundly better results of our present study. The pediatric immune system may be more amenable to tolerance than that of adults, caused by more circulating naive T cells and a still full functional thymus. This might be especially true for younger children, supported by the fact that we found a significant association between a weight below 10 kg at the time of LTx and successful withdrawal of CsA. Pediatric patients face long-term, possibly even life-long treatment with immunosuppressive medication. CsA has serious side effects on the renal function, cardiovascular system and promotes the development of neoplasms. The present data indicate that CsA can be withdrawn in a selected group of pediatric liver transplant patients from 2 years after LTx with a success rate of approximately 70%. A failed attempt to withdraw CsA does not have apparent negative effects on graft function during prolonged follow-up. In young children with a weight of less than 10 kg at the time of LTx. CsA can be withdrawn successfully in 85%. This suggests that immunosuppressive schemes can be reduced in children, especially young children after LTx. The greatest challenge in the future of immunosuppression, however, remains to predict which particular immunosuppressive regimen would be the best for each individual patient.

References


