Impact of Cyclosporin A Pharmacokinetics on the Presence of Side Effects in Pediatric Renal Transplantation

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Abstract. Cyclosporin A (CsA) is a potent immunosuppressant that has many side effects, including hypertrichosis, gingival hyperplasia, and tremor. To evaluate whether there is a relationship between the CsA-pharmacokinetics (PK) and these side effects, their presence and intensity were observed in 46 renal transplanted children/adolescents during two regular visits, and the occurrence of the side effects was correlated with CsA-PK. CsA doses had been unchanged for at least 6 mo. CsA blood concentrations were measured at time 0, 1, 2, and 4 h after the CsA morning dose. An abbreviated area under the curve (AUC) was calculated using C0, C2, and C4. Hypertrichosis positively correlated with C2, C4, Cmax, and AUC. An AUC ≥ 4158 ng/ml per h was the best predictor for the presence of hypertrichosis. Tremor was also positively correlated with C2, Cmax, and AUC. A Cmax ≥ 878 ng/ml was the best predictor for the appearance of tremor. These values of Cmax and AUC are within the therapeutic range of CsA as demonstrated by the studies of calcineurin inhibition by CsA. Gingival hyperplasia was not associated with any of the CsA-PK studied parameters. However, it was associated with the concomitant use of nifedipine. These data show that there is a correlation between the CsA side effects and its pharmacokinetics and that it is possible to decrease the incidence and intensity of such side effects by monitoring the CsA-PK parameters, providing they are under or at the proposed cutoff levels. Nifedipine should also be avoided to reduce the presence of gingival hyperplasia.

Cyclosporin A (CsA) is a potent immunosuppressive drug that has considerably improved graft survival in transplantation. However, its use is also associated with the presence of major adverse effects such as chronic nephrotoxicity, as well as with some minor side effects that include hypertrichosis, gingival hyperplasia, and tremor of the hands. In pediatric recipients and women, these side effects contribute to decrease the health-related quality of life (1), increase nonadherence to the drug, and, sometimes, require the discontinuation of the medication.

At our center, the incidence of these side effects has increased substantially after the introduction of the CsA-microemulsion (Neoral®), probably because we have been monitoring this CsA-microemulsion still using the same trough levels we used with the old formulation. The maintenance of the same CsA trough level that was being used with the old formulation (Sandimmun®) leads to a higher drug exposure, measured by a higher Cmax and an enlarged AUC (2–7). Therefore, it is possible that the current increased incidence of these side effects is related to a higher drug exposure. However, this point still remains to be proven.

To our knowledge, no studies in pediatric recipients have analyzed the correlation between the CsA side effects (hypertrichosis, tremor, and gingival hyperplasia) and the pharmacokinetics (PK) of this drug. Determining such a relation would permit a theoretical basis for CsA dosing to decrease both the incidence and the severity of adverse effects but still maintaining blood concentrations within the therapeutic levels of the drug.

In this study, we examined the presence and intensity of CsA non-nephrotoxic effects and their association with CsA-PK in a population of children and adolescents with stable renal transplants.

Materials and Methods

All renal transplanted children/adolescents with an unchanged dose of CsA for 6 or more months who agreed to participate in the study were enrolled. Observation of the CsA side effects and monitoring of the CsA-PK are routine at our outpatient clinic. Therefore, no specific consent for these procedures was required. However, consent was required to participate in the study. This was given by the parents. The monitoring of CsA-PK was approved by the hospital ethics committee.

Patients were examined during a regular visit for the observation of the following CsA side effects: hypertrichosis, gingival growth, and fine tremor of the hands. These CsA side effects were then classified as absent, mild, moderate, or intense.

The clinical subjective observation of the side effects was performed independently by two examiners. The intensity of the side effects was assessed as absent, mild, moderate, or intense.
effects was subjectively graded according to previous consensus between the investigators, i.e., gingival hyperplasia that had been recently submitted to surgical resection was classified as intense regardless of the current state of the gum. Hypertrichosis was considered to be intense whenever facial hypertrichosis was present, even when, in other areas, it could not be classified accordingly. Tremor of the hands was considered to be at least of moderate intensity if the patient/parent had already realized this side effect before the examination. Because evaluation of these side effects had not been done in a systematic way before this study, only the observation of the side effects done during this study were taken into account.

For the final analysis, the intensity of the side effects was classified as absent only if both investigators concluded it was absent. However, for a mismatching classification between the investigators, the higher intense grade was adopted.

At our center, Neoral® has been used as the only cyclosporine formulation in the last 2 yr. Before this period, all of the children were on Sandimmum®. CsA is given twice a day, usually at 8 a.m. and 8 p.m. Patients were instructed to take their regular CsA evening dose on the previous day. The next morning, they were to arrive at the clinic half an hour before the time of taking the morning dose. An intravenous in-dwelling catheter was inserted in an arm vein and maintained with heparin solution. Blood was harvested at time 0, and 1, 2, and 4 h (C0, C1, C2, and C4, respectively) after taking the regular morning dose. Tubes were immediately sent to the laboratory for CsA total blood concentration. CsA was measured by fluorescence polarization immunoassay (TDX; Abbott Laboratories, Chicago, IL).

In our center, nifedipine is the drug of choice for the treatment of hypertension in patients on CsA. Because nifedipine aggravates gingival hyperplasia (8), we also analyzed the impact of the association of nifedipine and CsA specifically on gum hyperplasia.

All collected data were entered in our computerized patient file for further analysis. For the purpose of this study, C0 was defined as the trough concentration (Cmin), and the highest level among C0, C1, C2, and C4 was defined as the maximal concentration (Cmax). Tmax was defined as the time to the maximal concentration.

The area under the time curve (AUC) of CsA was calculated according to the equation for abbreviated AUC developed at our center for long-term transplanted children, which takes into consideration C0, C1, C2, and C4 only (AUC = 453/[3.71 × C0] + [1.68 × C2] + [4.3 × C4]). This formula gives an abbreviated CsA-AUC that correlates extremely well with the complete AUC (R = 0.94, P < 0.001) (unpublished data). Our formula is very similar to the one proposed by Meir-Kriesche et al. for the abbreviated CsA-AUC in pediatric recipients (9). C1 was measured to obtain the best accurate Cmax. CsA trough levels are often used by transplant centers to monitor CsA. For this reason, CsA trough level (C0) is always reported in this study even when it has no correlation with the side effects.

### Statistical Analyses

Values are always expressed as mean ± SD when the analyzed parameter had a normal distribution. Pearson product moment correlation was used to identify any correlation between side effects and the parameters of the CsA-PK. One-way ANOVA was used to compare one parameter of the CsA-PK with the intensity of the side effect. The Fisher exact test was used to compare the proportion of patients in different groups. A paired t test was used to compare data before and after a reduction of CsA doses.

A logistic regression model was used to study the possibility of a patient to show one side effect (dependent variable) according to the CsA-PK parameter that showed the best statistical significance in the multivariate analysis (independent variable). Initially, we used a model that contained all of the variables, excluding those that interacted with each other. Then we progressively removed the variable with the higher descriptive level until we reached one model that contained only the variable(s) significantly associated with the analyzed dependent variable. The cutoff level for this independent variable was then calculated as the one with the best sensitivity and specificity. A receiver operator characteristic curve was then constructed for that variable. The SAS (Statistical Analysis System) software was used for the statistics analysis.

### Results

#### General Data

Forty-six children/adolescents (23 boys and 23 girls) were enrolled and all of them completed the study. They were transplanted for 4.6 ± 3 yr and had been receiving CsA since then. Mean age at the time of transplantation was 9.6 ± 3.3 yr old (4 to 17 yr old), and at the time of the study 14.3 ± 3.9 yr old (7 to 23 yr old). Thirty-eight (83%) were Caucasian, six (13%) were Afro-Brazilian, and two (4%) were Oriental. The average CsA daily dose was 6 ± 1.9 mg/kg per d (2.1 to 10.7 mg/kg per d). All patients were also receiving prednisone and azathioprine. Renal function was stable and considered adequate for this period of follow-up. Average serum creatinine was 1.6 ± 0.6 mg/dl (0.8 to 3.7 mg/dl), and calculated creatinine clearance was 55 ± 17 ml/min per 1.73 m² (26 to 95 ml/min per 1.73 m²).

#### CsA Pharmacokinetics

Table 1 summarizes the mean CsA-PK parameters of the studied population.

### Side Effects

Hypertrichosis was present in 36 of 46 (78%) of the children, an incidence as high as that of the gingival hyperplasia, which was seen in 40 of 46 (87%) of the patients. Both of these side effects were much more frequent than tremor, which was seen in only 20 of 46 (44%) of the patients (P < 0.001). Mean

### Table 1. Measured parameters of the CsA-PK

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>C0 (ng/ml)</td>
<td>203 ± 75</td>
<td>400</td>
<td>93</td>
</tr>
<tr>
<td>C1 (ng/ml)</td>
<td>830 ± 476</td>
<td>2014</td>
<td>92</td>
</tr>
<tr>
<td>C2 (ng/ml)</td>
<td>881 ± 400</td>
<td>2147</td>
<td>140</td>
</tr>
<tr>
<td>C4 (ng/ml)</td>
<td>495 ± 185</td>
<td>963</td>
<td>96</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>1042 ± 437</td>
<td>2147</td>
<td>140</td>
</tr>
<tr>
<td>Cmax/dose (ng/ml per dose)</td>
<td>371 ± 188</td>
<td>1126</td>
<td>58</td>
</tr>
<tr>
<td>AUC (ng/ml per h)</td>
<td>4783 ± 1540</td>
<td>8801</td>
<td>1465</td>
</tr>
<tr>
<td>AUC/dose (ng/ml per h per mg/kg)</td>
<td>1684 ± 602</td>
<td>3370</td>
<td>591</td>
</tr>
<tr>
<td>Cav (ng/ml)</td>
<td>398 ± 128</td>
<td>733</td>
<td>122</td>
</tr>
<tr>
<td>Tmax (hours)</td>
<td>1.6 ± 0.6</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

CsA, cyclosporin A; PK, pharmacokinetics; C0, trough level; Cmax, maximal concentration; Tmax, time for Cmax; C1, C2, C4, CsA concentration at times 1, 2, and 4, respectively; Cav, average concentration in 12 h; AUC, area under the CsA time curve.
prednisone dose, at the fourth year after transplantation, was 0.08 ± 0.08 mg/kg per d. There was no correlation between the prednisone oral dose and hypertrichosis among groups (absent 0.08 ± 0.07 versus severe 0.08 ± 0.09 mg/kg per d; P = NS).

Also, the intensity of the side effects differed. Hypertrichosis was classified as mild in 11 (31%), moderate in 12 (33%), and severe in 13 (36%) of the children. Tremor of the hands was considered mild in 12 of 20 (60%), whereas in eight of 20 (40%) it was classified as either moderate or severe. Gingival hyperplasia was mild in 10 (25%), moderate in 12 of 40 (30%), and severe in 18 (45%). Although the majority of the patients with hypertrichosis (11/12/13) and gingival hyperplasia (10/12/18) presented these side effects either as moderate or intense, the intensity of tremor (12/6/2) was mostly classified as mild or moderate (P = 0.043).

When tremor was present (44%), hypertrichosis and gingival hyperplasia was also present in a very high percentage of patients (90 and 95%, respectively). Also, when tremor was absent (56%), the other side effects were also less frequent (in 69 and 81%, respectively).

### Relationship between CsA-PK and Side Effects

#### Hypertrichosis
There was a positive correlation between the presence of hypertrichosis and C2, C4, C_max, and AUC. All of these parameters were higher when hypertrichosis was present than when it was absent (Table 2). By ANOVA, the intensity of the hypertrichosis increased as AUC also increased (P < 0.02) (Figure 1A).

Neither C_max nor C1 showed an association with the presence of hypertrichosis. In a model of logistic regression analysis, C4 showed a good prediction for the presence of hypertrichosis (P = 0.04). Values of C4 > 450 ng/ml showed a high chance to develop hypertrichosis. Every increase of 100 ng in C4 levels showed an odds ratio of 2.9 to develop hypertrichosis.

However, in the logistic regression model using all variables, AUC was the best parameter that predicted the appearance of hypertrichosis according to the equation:

\[
\text{Probability} = \frac{\exp(-3.2937 + 0.0011 \text{ AUC})}{1 + \exp(-3.2937 + 0.0011 \text{ AUC})}
\]

Every increase of 1000 ng/ml in the AUC was associated with an odds ratio of 2.9 for the presence of hypertrichosis (P = 0.014). By analyzing the multiple sensitivity and specificity indexes, the cutoff level found was an AUC = 4158 ng/ml per h. This AUC had a sensitivity of 60% and specificity of 78% for the child to present hypertrichosis (Figure 2).

#### Tremor (of the Hands)
C2, C_max, and AUC were higher in children presenting tremor when compared with those who did not. (Table 2).

By ANOVA, C_max showed a positive correlation with the intensity of tremor as the intensity of tremor progressively increased as the C_max also increased (P = 0.009) (Figure 1B). Neither C0, C1, nor C4 showed an association with tremor of the hands.

In a logistic regression model, C_max was the parameter significantly associated with the probability to present tremor (P = 0.0081) according to the equation:

\[
\text{Probability} = \frac{\exp(-2.7037 + 0.0023 \text{ C}_\text{max})}{1 + \exp(-2.7037 + 0.0023 \text{ C}_\text{max})}
\]

Every increment of 500 ng/ml was associated with an odds ratio of 3.2 for tremor. The receiver operator characteristic curve for C_max and tremor is presented in Figure 3. By analyzing the multiple sensitivity and specificity indexes, the best probability of presenting tremor was at a C_max = 878 ng/ml. This C_max showed a sensitivity of 70% and specificity of 65% for the child to present tremor of the hands.

#### Gingival Hyperplasia
The use of CsA was associated with the presence of gingival hyperplasia. However, there was no correlation between any of the CsA-PK parameters and the gingival hyperplasia. None of the analyzed parameters of the CsA-PK was statistically different in children with gingival hyperplasia when compared to those without (C0: 208 ± 76 versus 164 ± 51 ng/ml; C_max [95% confidence interval]: 898 [738 to 1362] versus 876 [824 to 1142] ng/ml; and AUC: 4834 ± 1626 versus 4443 ± 744 ng/ml per h). In a multiple logistic regression analysis, when the parameters C0, C_max, AUC and the use of nifedipine were studied, only the use of nifedipine predicted the occurrence of gingival hyperplasia (P = 0.048). By χ² analysis, there was an association between the concomitant use of nifedipine, associated with CsA, and the presence of gingival hyperplasia.

Twenty-one of 46 (46%) children were receiving nifedipine as an antihypertensive agent. Of these, gingival hyperplasia

### Table 2. Values of the statistically significant parameters of the CsA-PK associated with the adverse effects in the univariate analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hypertrichosis</th>
<th>Tremor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>C0 (ng/ml)</td>
<td>174 ± 62</td>
<td>210 ± 77</td>
</tr>
<tr>
<td>C2 (ng/ml)</td>
<td>628 ± 242</td>
<td>951 ± 410</td>
</tr>
<tr>
<td>C4 (ng/ml)</td>
<td>354 ± 159</td>
<td>535 ± 174</td>
</tr>
<tr>
<td>C_max (ng/ml)</td>
<td>791 ± 355</td>
<td>1112 ± 436</td>
</tr>
<tr>
<td>AUC (ng/ml per h)</td>
<td>3648 ± 1205</td>
<td>5099 ± 1484</td>
</tr>
</tbody>
</table>

*Abbreviations as in Table 1.
occurred in 21 of 21 (100%) of those on nifedipine in contrast to 19 of 25 (76%) of those without \( (P = 0.02) \).

Reduction of CsA-PK and Consequences on Side Effects

In 10 children with the highest AUC values, a reduction of the CsA dose \( (7.5 \pm 2.5 \text{ to } 4.8 \pm 2.3 \text{ mg/kg per d}, \ P = 0.01) \) was performed. After a mean follow-up of 393 \pm 58 \text{ d} \ (310 \text{ to } 469), \) the consequent impact of this reduction in the presence and intensity of the side effects was observed. None of the children/adolescents presented any rejection episode after the CsA dose reduction. On the contrary, serum creatinine decreased from 1.4 \pm 0.6 \text{ to } 1.2 \pm 0.4 \text{ mg/ml} \ (P = 0.0014). \) C0 decreased from 284 \pm 61 \text{ to } 163 \pm 77 \text{ ng/ml} \ (P = 0.002). \) \( C_{\text{max}} \) diminished from 1466 \pm 492 \text{ to } 998 \pm 527 \text{ ng/ml} \ (P = 0.093). \) AUC also decreased from 6897 \pm 1275 \text{ to } 4450 \pm 1723 \text{ ng/ml pe rh}(P = 0.015), \) therefore leading to a reduction of the CsA average concentration \( (C_{\text{av}}) \) from 566 \pm 79 \text{ to } 371 \pm 143 \text{ ng/ml pe h} \ (P = 0.009).

The presence and the intensity of tremor diminished in these 10 children. Before, only three of the 10 did not have tremor of the hands. At last follow-up, seven of 10 did not present this side effect. In two of 10, it was classified as mild and in one as intense (Figure 4).

The intensity of hypertrichosis also decreased. Before the CsA-AUC reduction, seven of these 10 children had hypertrichosis classified as severe, two of 10 as moderate, and one of 10 as mild. At last follow-up, only one of 10 patients had the severe grade, five of 10 were classified as moderate, three of 10 as mild, and one of 10 as absent \( (P = 0.05) \) (Figure 4).

After CsA-PK reduction, gingival hyperplasia did not clearly change. Before, all of the 10 children showed this side effect. Afterward, nine of 10 patients showed this side effect. However, the intensity somehow decreased because none of the children was classified as having a severe grade of this side effect at last follow-up when compared to three of 10 before the dose reduction. Six children were on nifedipine before CsA-PK reduction. In three of them, this drug was withdrawn. In two, the intensity of gingival hyperplasia decreased and one did not change.

Discussion

In this study, performed in a sizeable population of children and adolescents, we identified cutoff levels for CsA-AUC and
90% of the children. This finding is in accordance with the associated with the presence of the other adverse effects in resistant side effect that develops, because its appearance was without presenting the other. Tremor seems to be the most effects in a study designed to recognize them may have contrib- CsA (4.5 yr) in our patients and the active search for the side reported in the literature (1,10–14). The long-term exposure to one of the CsA side effects.

A much lower rate of the analyzed side effects has been reported in the literature (1,10–14). The long-term exposure to CsA (4.5 yr) in our patients and the active search for the side effects in a study designed to recognize them may have contrib- uated to the high incidence of side effects observed. Nevertheless, it emphasizes that the incidence of these disturbances can be much higher than expected, and this has been described in the literature.

Interestingly, the side effects do not necessarily correlate to each other. This means that one can develop one side effect without presenting the other. Tremor seems to be the most resistant side effect that develops, because its appearance was associated with the presence of the other adverse effects in 90% of the children. This finding is in accordance with the recent data from Palomo et al. (15), who described that the brain is resistant to CsA accumulation, thus suggesting that neurotoxicity is harder to develop.

This study demonstrates a clear relationship between the CsA exposure and the presence of hypertrichosis and tremor of the hands in a pediatric population. Our data showed, for the first time, values of CsA AUC and \( C_{\text{max}} \) that are able to minimize the appearance and the severity of CsA adverse effects. Hypertrichosis can be prevented if CsA-AUC is maintained \( \leq 4200 \text{ ng/ml per h} \), and tremor can be prevented if \( C_{\text{max}} \) is reduced to \( \leq 900 \text{ ng/ml} \). Moreover, these levels not only determine the presence of the side effects, but as they increase there is also a progressive increase in the intensity of the side effects (Figure 1, A and B).

It is also interesting that one side effect (hypertrichosis) is more related to total drug exposure (AUC), whereas the other (tremor) is more related to the peak concentration of the drug (\( C_{\text{max}} \)). This fact has already been demonstrated in CsA acute nephrotoxicity, in which after 2 h of achieving \( C_{\text{max}} \), there is a 30 to 50% decrease in the GFR (16). The GFR fall was not related to the total AUC but only to \( C_{\text{max}} \). Also, the brain is more resistant to CsA because of the low CsA concentrations in that tissue and because of its inadequate amount of cyclophilin (15). Consequently, the need of higher CsA concentrations, as expressed by the \( C_{\text{max}} \), to develop neurotoxicity may explain the relationship between this parameter and the occurrence of tremor.

In stable pediatric recipients transplanted for many years, these AUC and \( C_{\text{max}} \) levels seem to be enough to produce the CsA immunosuppressive effects and, at the same time, do not expose patients to a high incidence of side effects.

One may argue whether it is possible to maintain patients on this low AUC without increasing the chances of developing rejection. This is currently being studied at our center in the early phase after transplantation. However, for long-term transplant recipients the most likely answer to this question is yes. No data are available on what level of AUC or any other PK parameter is satisfactory for an adequate immunosuppression. Nevertheless, a lot of evidence shows that patients are currently receiving more CsA than necessary to avoid the number of rejection episodes that CsA is able to reduce. This overuse of CsA has led to frequent episodes of CsA nephrotoxicity and a high incidence of side effects.

In this study, 10 children/adolescents with the highest CsA-AUC had the drug diminished to achieve the target AUC level of 4200 ng/ml per h. After a period of more than 1 yr of follow-up, none of them developed any acute rejection episode and the renal function even improved. This, once again, demonstrates that the above-mentioned CsA levels are enough to maintain adequate immunosuppression for this period of renal transplantation. Furthermore, an AUC of 4200 ng/ml per h corresponds to a \( C_{\text{av}} \) of approximately 350 ng/ml. Kahan et al. showed that this level is enough to prevent rejection episodes, and increasing it to values higher than 400 to 500 ng/ml does not further reduce the incidence of rejection (17).

The major immunosuppressive action of CsA is to inhibit the calcineurin activity. Calcineurin is a calcium-calmodulin-de- Fig 4. Intensity of tremor and hypertrichosis before and after pendent phosphatase necessary to transduce the signal from the cytoplasm to the nucleus for the production of interleukin-2. Some details of the CsA-calcineurin inhibition have been stud- in vitro, calcineurin inhibition by CsA is rapidly achieved, almost never reaches 100%, and is very slowly reversible even in low concentrations (19). In vivo, calcineurin inhibition also rarely reaches 100%, but the inhibition is rapidly reversible in the presence of CsA extracellular binding sites (20).

In a simultaneous evaluation of the CsA-PK and pharma- dynamics in pediatric recipients of renal transplants, Quien et al. demonstrated that calcineurin inhibition positively corre- lated with CsA blood concentrations. They also observed that the maximal inhibition obtained was around 70%, at a blood...

\( C_{\text{max}} \), above which the presence of side effects (tremor and hypertrichosis) is very likely to occur. These findings may have an impact on the daily clinical use of CsA, since a sensitive parameter to monitor CsA dosing to avoid its side effects has been exhaustively searched. Our group of stable patients had a large range of CsA-PK. Also, the incidence of the minor adverse effects in our pediatric population was extremely high, occurring in almost all pa- tients. Both of these facts should have facilitated the finding of a cutoff level in the CsA-PK that correlated with the presence of one of the CsA side effects.

This study demonstrates a clear relationship between the CsA exposure and the presence of hypertrichosis and tremor of the hands in a pediatric population. Our data showed, for the first time, values of CsA AUC and \( C_{\text{max}} \) that are able to minimize the appearance and the severity of CsA adverse effects. Hypertrichosis can be prevented if CsA-AUC is maintained \( \leq 4200 \text{ ng/ml per h} \), and tremor can be prevented if \( C_{\text{max}} \) is reduced to \( \leq 900 \text{ ng/ml} \). Moreover, these levels not only determine the presence of the side effects, but as they increase there is also a progressive increase in the intensity of the side effects (Figure 1, A and B).

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The major immunosuppressive action of CsA is to inhibit the calcineurin activity. Calcineurin is a calcium-calmodulin-de- pendent phosphatase necessary to transduce the signal from the cytoplasm to the nucleus for the production of interleukin-2. Some details of the CsA-calcineurin inhibition have been stud- ed. Pai et al., in bone marrow transplant recipients, showed that at CsA blood levels of 200 ng/ml or greater there was a maximal inhibition of the calcineurin activity and no apparent increase in such inhibition could be seen at higher CsA levels (400 to 600 ng/ml) (18).

Batiuk et al. demonstrated that in vitro, calcineurin inhibition by CsA is rapidly achieved, almost never reaches 100%, and is very slowly reversible even in low concentrations (19). In vivo, calcineurin inhibition also rarely reaches 100%, but the inhibition is rapidly reversible in the presence of CsA extracellular binding sites (20).

In a simultaneous evaluation of the CsA-PK and pharmaco- dynamics in pediatric recipients of renal transplants, Quien et al. demonstrated that calcineurin inhibition positively corre- lated with CsA blood concentrations. They also observed that the maximal inhibition obtained was around 70%, at a blood...
CsA concentration of only 431 ng/ml. Moreover, at a concentration of 148 ng/ml there still was an inhibition of 50% in the calcineurin activity (21).

In another study on the conversion from Sandimmun® to Neoral®, de Mattos et al. showed that in spite of an increased C_{\text{max}} and C_{\text{av}} after conversion to Neoral®, the inhibition of calcineurin activity did not increase proportionally, perhaps demonstrating that the maximal inhibition had already been achieved with the smaller CsA concentration (22).

Taken together, these data indicate that very high CsA blood concentrations are not necessary to obtain the maximal immunosuppressive effect expected from this drug. Therefore, increasing the drug blood level would only considerably increase the incidence and severity of the side effects with only a minor increment in the desired effect. However, this rationale deserves further investigation for the very early phase after transplantation.

The cutoff levels found in this study (AUC of 4200 ng/ml, C_{\text{av}} of 350 ng/ml, and C_{\text{max}} of 900 ng/ml) are consistent with a calcineurin inhibition of 75% in the peak level and a 50% inhibition at either the trough level or the average concentration (C_{\text{av}}) (21).

Another point to be discussed is the need to perform CsA-PK to monitor the use of this drug. This procedure is both time-consuming and expensive. However, the CsA-AUC seems to be the only way to monitor this drug to obtain its best use.

We collected blood at time 0, and 1, 2, and 4 h after the morning dose. The rationale for this reduced sampling strategy was that by collecting blood at these time intervals, we could obtain the abbreviated AUC (using C0, C2, and C4), the C_{\text{max}} (using C1 and C2), and the trough level (using C0). However, it is also possible to measure either C2 or C4 or both to estimate the abbreviated AUC and C_{\text{max}} (23). In this study, C4 also had a good correlation with the presence of hypertrichosis, because C4 is a good parameter to estimate CsA-AUC. C2 can also be used to estimate C_{\text{max}}. However, in this pediatric population, to establish the best C_{\text{max}} there was a need to measure both C1 and C2, although C1 was not necessary for the estimation of CsA-AUC. In long-term stable transplanted children, C_{\text{max}} is closer to C1 in 44% of them and to C2 in 56%. Consequently, none of these parameters could be used alone to estimate C_{\text{max}} in the designed study. By applying multiple linear regression analysis, C_{\text{max}} is better estimated using both C1 and C2 (R = 0.92, P < 0.001, power = 1), where C_{\text{max}} = 17.732 + (0.767 \times C2) + (0.419 \times C1) (unpublished data). However, in a clinical setting, C2 alone can be used to estimate C_{\text{max}}.

Therefore, it is possible to reduce the sampling strategy used in this study to only C2 and C4. According to our data, only C_{\text{max}} and AUC are necessary to obtain the best relationship between the CsA-PK and side effects. By multiple linear regression analysis, it is possible to obtain an equation using C2 to estimate C_{\text{max}}, where C_{\text{max}} = 269 + (0.877 \times C2) (r = 0.8, P < 0.001). AUC can also be estimated using only C4, where AUC = 974 + (7.687 \times C4) (R = 0.92, P < 0.001).

This two-point sampling strategy would reduce even more the time spent by the child in the laboratory in the clinical setting.

Our patients had an extremely high incidence of gingival hyperplasia. This high rate may be related to poor plaque control observed in the majority of our children. We did not find any correlation between the CsA pharmacokinetic parameters and the presence of gingival hyperplasia. It seemed that gingival growth was found in pediatric recipients undergoing CsA treatment regardless of the intensity of this exposure. CsA probably acts to enhance local growth factors. For example, in biopsies of the gum in patients with gingival hyperplasia, CsA was responsible for a 48-fold increase in gene expression of the platelet-derived growth factor B (24). The association between CsA use and gingival hyperplasia is well documented in the literature, and the exchange of CsA either by azathioprine (25) or by tacrolimus (11) reverses the hyperplasia.

On the other hand, our data showed that children with concomitant use of nifedipine had an immense chance of developing gingival hyperplasia. At our center, long-acting nifedipine is the drug of choice to manage systemic arterial hypertension in renal transplanted patients. This drug does not alter the CsA-PK and avoids the decrease in GFR induced by CsA (26). The relationship between the use of nifedipine and gingival hyperplasia in patients on CsA has been shown in the literature (8,27,28).

All of this information leads to the assumption that by decreasing the CsA exposure alone, it will not be possible to avoid or control the gingival hyperplasia. In fact, this was proven in the 10 children who had the CsA dose reduced. The majority of them still presented gingival hyperplasia 1 yr later, although with a slight decrease in the size of the gum. Therefore, it seems that for the best prevention of gingival hyperplasia, one should avoid high CsA levels and the concomitant use of calcium-channel blockers, especially nifedipine, while working to improve hygienic habits and dental plaque control.

The other important fact obtained from this study was the demonstration that one can reverse the presence and intensity of the tremor and hypertrichosis by adjusting the CsA-AUC/C_{\text{max}}. The majority of the patients who had their CsA-PK adjusted to the described target levels either completely reversed the tremor and hypertrichosis or had the intensity of such side effects considerably reduced. Although beyond the scope of this study, the exposure to the high CsA-PK was causing some degree of nephrotoxicity. A significant reduction of the S_{\text{cr}} was observed in all of the 10 children/adolescents after CsA-PK adjustment.

In summary, our data show that it is possible to minimize the CsA side effects, namely hypertrichosis and tremor, by monitoring the CsA-PK and avoiding AUC >4200 ng/ml per h and C_{\text{max}} >900 ng/ml. These levels are within the therapeutic range of CsA for long-term transplanted children, as evaluated by the recent CsA-calcineurin inhibition studies. Gingival hyperplasia is mainly related to the exposure to CsA and not to the intensity of exposure. However, the addition of nifedipine is a major risk factor for this side effect and should be avoided whenever possible.

The possibility of maintaining CsA-AUC and C_{\text{max}} at the suggested levels also for the immediate period after transplantation without causing preventable rejection episodes needs to be investigated.

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References


Access to UpToDate on-line is available for additional clinical information at http://www.lww.com/JASN.