Therapeutic Monitoring of Calcineurin Inhibitors for the Nephrologist

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The calcineurin inhibitors (CNI) cyclosporine and tacrolimus remain the backbone of immunosuppression for most kidney transplant recipients. Despite many years of experience, protocols that optimize efficacy with minimal toxicity remain a subject of debate. Nevertheless, studies of the pharmacokinetic properties of the CNI, particularly cyclosporine, have led to improved dosing strategies. The purpose of this article is to review the current understanding of CNI pharmacokinetics and its relevance to proper dosing and monitoring of these medications. This article also reviews the trials that have helped to define the optimal dosages and discusses the effect of adjunctive immunosuppressive agents on CNI pharmacokinetics and dosing.

Pharmacokinetics of Calcineurin Inhibitors

Both calcineurin inhibitors (CNI) cyclosporine and tacrolimus act through an interaction with a cytoplasmic protein, which subsequently binds to and inhibits calcineurin. In the case of cyclosporine, the target is cyclophilin, whereas tacrolimus binds to tacrolimus-binding protein. After a dose of CNI, there is an initial absorption phase, during which blood concentrations reach a peak level (Cmax). Typically, Cmax occurs during the first 2 to 3 h after the dose and corresponds to the time of maximal calcineurin inhibition (1,2). Drug levels then fall as a result of metabolism (also known as the elimination phase) until they are at the lowest, or trough, level (C0) immediately before the next dose. Metabolism is performed chiefly by the cytochrome P450 3A enzyme system in the liver. Both CNI also are metabolized by the intestinal cytochrome P450 3A4 and by P-glycoprotein countertransport in the intestinal mucosa (3,4). The total drug exposure throughout the period from one dose until the next is the area under the concentration-time curve (AUC; Figure 1) (3). Determination of AUC can be made by formal pharmacokinetic testing, which requires blood samples to be drawn at multiple time points throughout the dosing interval. For both CNI, most of the inter- and intrapatient variability occurs in the absorption phase rather than in the elimination phase.

The original corn oil–based preparation of cyclosporine (Sandimmune, Novartis Pharma Canada Inc., Dorval, Canada) was developed to reduce this variability. Neoral was found to have increased and more consistent absorption of cyclosporine, leading to less intrapatient variability than Sandimmune (12), although there remains significant variability in absorption (Figure 2) (13). Randomized, controlled trials confirmed that Neoral was safe in stable (4) and de novo (14,15) renal transplant patients.

Tacrolimus behaves similarly to cyclosporine, with rapid absorption and peak levels being achieved within the first 3 h after a dose. It also shows marked intra- and interpatient variability in absorption (16). Its absorption is not bile dependent but does depend on gastrointestinal transit time and is affected by the presence or absence of food, as well as the lipid content of food (17). In addition, age, gender, race, body mass index, duration of time on tacrolimus, serum albumin, hematocrit, and presence of hepatitis B or C infection or other liver disease all have been shown to influence daily dosage requirements (18,19).

Recently, an extended-release, once-daily formulation of tacrolimus was developed. Modified-release tacrolimus was shown to have an equivalent pharmacokinetic profile in stable patients who were converted from standard tacrolimus in a 1:1 manner (20). Target trough levels for modified-release tacrolimus seem to be the same as for standard tacrolimus in both de novo and maintenance patients (21).

Cyclosporine Monitoring Strategies

Therapeutic drug monitoring is necessary for drugs with a narrow therapeutic index (i.e., the exposure for efficacy is close
Before the introduction of drug monitoring, cyclosporine usage was associated with less rejection but also dosage-related nephrotoxicity and acute renal failure after renal and cardiac transplantation (22,23). In the Sandimmune era, it was demonstrated that empiric cyclosporine dosage reduction was associated with rejection and that blood levels correlated with the degree of immune reactivity (1,24). Furthermore, patients with lower cyclosporine levels were at an increased risk for rejection and graft loss (25). Although patients who had an episode of acute rejection had a lower cyclosporine $C_{\text{max}}$ and $C_0$ levels correlated poorly with the risk for rejection in individual patients (26). Despite this, $C_0$ monitoring of cyclosporine became the standard, because it was simpler than measuring $\text{AUC}$ or determining $C_{\text{max}}$ for each patient.

When Neoral was introduced into clinical use, a series of trials examined its pharmacokinetics in detail. Compared with Sandimmune, patients who received Neoral had similar $C_0$ levels but higher $C_{\text{max}}$ and $\text{AUC}$ (4,14,15,27). In addition, the rate of acute rejection was lower with Neoral in some studies (14). Although some studies showed more early nephrotoxicity with Neoral (28), long-term renal function was equivalent.

These studies also demonstrated that cyclosporine exposure during the first 4 h after a dose (AUC$_{0\to4}$) correlated well with exposure during the entire 12-h dosing interval (AUC$_{0\to12}$). This is consistent with the fact that most of the variability in cyclosporine exposure takes place during the absorption phase. In comparison with AUC$_{0\to4}$, $C_0$ levels correlated poorly ($r^2 = 0.53$) with AUC$_{0\to12}$. Although determining AUC$_{0\to4}$ required four or five blood samples to be drawn, it was also found that the combination of $C_0$ and the 2-h postdose cyclosporine level ($C_2$) provided excellent correlation ($r^2 = 0.945$) with AUC$_{0\to12}$.

A retrospective study subsequently compared AUC$_{0\to4}$ with clinical events in de novo renal transplant recipients. In a group of patients who received cyclosporine, steroids, and a variety of adjunctive agents (azathioprine, mycophenolate mofetil [MMF], and sirolimus) but not antibody therapy, AUC$_{0\to4}$ was lower in patients who had an episode of acute rejection. In addition, patients with the highest AUC$_{0\to4}$ had the highest incidence of nephrotoxicity. Although the groups were small, there were no differences in the relationship among AUC$_{0\to4}$, acute rejection, and nephrotoxicity that was treated with different immunosuppressives. In this study, the optimal AUC$_{0\to4}$, defined by freedom from both acute rejection and nephrotoxicity, was 4400 to 5500 µg/h per L (29). This strategy was subsequently validated prospectively in de novo renal transplant patients (30). These studies also highlighted the importance of achieving adequate cyclosporine exposure early after transplantation. In the prospective study, only one of 11 rejection episodes occurred in a patient who achieved an AUC$_{0\to4} > 4400$ µg/h per L by day 5 after transplantation.

Another randomized, prospective study in patients who received cyclosporine, basiliximab, and prednisone compared a limited sampling strategy to $C_0$ monitoring during the first 3 mo after transplantation (31). In this study, two- or three-point algorithms were used to predict AUC$_{0\to12}$. This study confirmed that adequate early cyclosporine exposure was highly correlated with freedom from acute rejection. Despite this, oc-
currence of the primary end point of acute rejection, graft loss, or death was equal in both groups by the study’s end, as was serum creatinine.

Although these limited sampling strategies were less cumbersome than performing a 12-h pharmacokinetic profile, they still required between two and five blood level measurements to be drawn, which was a deterrent to their implementation. Initial research in long-term heart and liver transplant patients determined that the \( C_2 \) level was the best single-point measurement that correlated with \( \text{AUC}_{0-t} \) (32,33). Further analysis in renal transplant patients confirmed that the \( C_2 \) level was the best correlate of \( \text{AUC}_{0-t} \) in predicting acute rejection (34). Other studies of renal transplant patients during the early posttransplantation period have confirmed that \( \text{AUC}_{0-t} \) is more predictive of rejection than \( \text{AUC}_{0-12} \) and that \( C_2 \) is the best single-point correlate of \( \text{AUC}_{0-t} \) with a correlation \( r^2 \) of 0.83 to 0.85 (10,35).

On the basis of these studies, the CONCERT group published a consensus statement on Neoral monitoring in transplant recipients (36). It concluded that \( C_2 \) monitoring was the optimal method for monitoring Neoral, with the blood drawn within 15 min before or after the 2-h time point. The CONCERT group reiterated that \( C_2 \) monitoring poorly predicts clinical events. It also emphasized the importance of achieving adequate \( C_2 \) levels early after transplantation and that \( C_2 \) monitoring was not associated with impaired renal function, despite leading to the use of higher cyclosporine dosages in the early posttransplantation period. They also noted that some patients may be low absorbers (low \( C_{\text{max}} \)) or slow absorbers (delayed time to \( C_{\text{max}} \)), characteristics that may not be detected or distinguished with a single-point measurement but would be by a limited sampling strategy. In addition, some results from liver, heart, and lung transplant recipients suggested that \( C_2 \) monitoring may reduce nephrotoxicity (33,37,38). Finally, the authors noted that in pharmacoeconomic studies, \( C_2 \) monitoring is at least cost-neutral compared with \( C_0 \) monitoring and may result in cost savings, a finding that has since been confirmed (39,40). However, despite this suggestive evidence, there has never been a randomized, controlled trial of \( C_0 \) versus \( C_2 \) in renal transplantation demonstrating a clinical benefit of \( C_2 \) monitoring.

It is important to note that all of these studies were carried out using the Neoral formulation. Generic formulations of cyclosporine microemulsion are now available, but they may not have identical pharmacokinetics to Neoral or to each other. Although some studies have shown similar pharmacokinetics in transplant patients (41,42), others have not (43), whereas at least one trial showed an increased rate of acute rejection (44). If the cyclosporine formulation that a patient is using is changed, then more frequent monitoring after the switch is made is advisable (45). Furthermore, the optimal monitoring strategy could be different.

Several assays are available to measure cyclosporine. HPLC is less commonly used because of technical difficulties. Fluorescence polarization immunoassay, specific enzyme multiplied immunoassay technique, and cloned enzyme donor immunoassay all are suitable techniques, with whole-blood sampling recommended (3). Because the half-life of cyclosporine is approximately 8 h, the full effect of a dosage adjustment on the cyclosporine level will be seen only after approximately 2 d (4 to 5 half-lives).

**Target Cyclosporine Levels in the First Year after Transplantation**

Adequate cyclosporine exposure early after transplantation decreases the risk for rejection. In one study, a \( C_2 \) level >1700 ng/ml by day 3 after transplantation was associated with a 92% negative predictive value for acute rejection in the first 6 mo. Achieving this level required a mean cyclosporine dosage of 11.7 ± 2.0 mg/kg per d, with a range of 6.8 to 21.5 mg/kg per d. Achieving a \( C_2 \) level >1700 ng/ml by day 5 or 7 after transplantation did not have as strong a predictive value. This relationship did not hold for patients with delayed graft function. However, for patients with immediate graft function, rapid increases in cyclosporine dosage to reach this target level should be made (34).

Although the target \( C_2 \) level of >1700 ng/ml was derived from patients who received cyclosporine, an adjunctive agent, and steroids, this has not been seen in patients who received antibody therapy. In a retrospective analysis of patients who received basiliximab, cyclosporine, MMF, and steroids, a \( C_2 \) level of 1700 ng/ml on day 3 after transplantation did not discriminate between patients who went on to have acute rejection from those that did not (46). In a trial of patients who received basiliximab, cyclosporine, and prednisone without an adjunctive agent, a \( C_2 \) level of >1500 ng/ml by day 3 after transplantation was associated with the lowest risk for rejection (31). However, rather than a threshold value, the risk for rejection seems to be inversely correlated with \( C_2 \) levels during the first year after transplant for patients who receive induction therapy. In a retrospective analysis of a randomized, controlled trial that compared basiliximab with anti-thymocyte globulin followed by cyclosporine, MMF, and steroids, the risk for rejection was 40% at \( C_2 \) levels of 400 ng/ml but declined to 15% when the mean \( C_2 \) was >1500 ng/ml (47). Thymoglobulin allows \( C_2 \) levels to be targeted even lower. A randomized, controlled trial of Thymoglobulin induction, cyclosporine, MMF, and steroids compared \( C_2 \) monitoring with target levels of 1000 to 1200 ng/ml with \( C_0 \) monitoring with a target of 250 to 350 ng/ml during the first 3 mo after transplantation. Both regimens resulted in similar rates of acute rejection, graft loss, or death, but the \( C_2 \) group required lower cyclosporine dosages after the first month (40).

These trials concentrated on the first 3 mo after transplantation. An international randomized, controlled trial compared two \( C_2 \) ranges in patients between 3 and 12 mo after transplantation. All patients received cyclosporine and steroids. Most patients received MMF, with the remainder (11%) receiving azathioprine. Target \( C_2 \) levels for all patients were 1700 ng/ml for the first month, 1500 ng/ml for month 2, and 1300 ng/ml for month 3. After 3 mo, patients were randomly assigned to a higher or lower \( C_2 \) group. Target \( C_2 \) levels were 1100 ng/ml for months 4 through 6 and 900 ng/ml for months 7 through 12 in the higher \( C_2 \) group, whereas patients in the lower \( C_2 \) group had target levels of 900 ng/ml for months 4 through 6 and 700...
ng/ml for months 6 through 12. During the first 3 mo after transplantation, the rate of biopsy-proven acute rejection was 11.7% (48). There was no difference in acute rejection episodes between the two groups between months 3 and 12. At 12 mo, there was no significant difference in GFR, the study’s primary end point. There also were no differences in BP, antihypertensive agent use, or serum total cholesterol, although more patients in the higher C2 group were on lipid-lowering treatment. When patients were reclassified by their achieved C2 levels, there was no difference in GFR at 12 mo, but there was a trend toward lower BP and serum cholesterol in patients with the lowest C2 levels (49).

In summary, these trials have shown that C2 monitoring is safe and effective during the first year after transplantation, and target levels have now been defined (Table 1). However, although C2 is more accurate than C0 monitoring, there is no evidence from randomized, controlled trials that C2 monitoring leads to a reduction in acute rejection, graft loss, or death. For patients who receive antibody therapy, the need to achieve high C2 levels early after transplantation, although the evidence here is not clear-cut (50).

### Target Cyclosporine Levels after the First Year after Transplantation

Long-term graft function and survival often are compromised by chronic allograft nephropathy (CAN), which in some cases seems to be related to CNI toxicity. In addition, higher dosages of CNI increase the incidence of malignancy, hypertension, and hyperlipidemia (51,52). As in de novo renal transplant patients, C0 monitoring correlates poorly with AUC. In a group of long-term patients who were maintained with C0 levels of 206 ± 75 ng/ml, C2 levels ranged from 140 to 2440 ng/ml. Patients with progressively rising serum creatinine values had lower C2 levels (mean 492 ± 327 versus 1054 ± 579 ng/ml) and AUC0 to 12 (mean 3798 ± 1145 versus 6462 ± 1886 μg/h per L), and most had evidence of CAN on biopsy (53). This suggests that underexposure to cyclosporine in long-term transplant recipients is a risk factor for CAN and that C2 monitoring might identify these patients.

C2 monitoring can also identify patients who are receiving excessive cyclosporine dosing. One study showed that patients with a C2 level between 700 and 800 ng/ml had lower serum creatinine values than patients with C2 levels <450 or >950 ng/ml (54). However, this was a cross-sectional study and could not determine whether patients were being kept at lower or higher levels because of renal dysfunction or previous episodes of acute rejection.

In a prospective study (55), 175 patients were converted to C2 monitoring, >90% of whom were >1 yr after transplantation. The target C2 level was set at 800 ng/ml, on the basis of previously published recommendations (56). Approximately half of the patients had a C2 level of >10% above the target C2 after 1 yr after transplantation. C2-guided dosing allowed the mean cyclosporine dosage to fall from 3.5 ± 1.4 to 2.8 ± 1 mg/kg. This reduction in cyclosporine dosage did not result in any episodes of acute rejection. There were improvements in BP and lipid profile, but these did not reach statistical significance.

Among the group with a C2 level >10% above the target level, serum creatinine decreased in half of the patients after cyclosporine dosage reduction, from 153 ± 55 to 132 ± 49 μmol/L.

In another study, patients who were maintained on cyclosporine and steroids were converted from C0 to C2 monitoring and followed for 3 yr. Target levels were 800 to 1000 ng/ml. C2 monitoring showed that half of the patients were above the target range and allowed the mean daily dosage to be reduced by approximately 20%. At 3 yr, few (7.3%) patients had developed CAN. Serum creatinine remained stable through the study period and was accompanied by decreased use of anti-hypertensive agents and mean total cholesterol levels (57).

When histology has been used as an end point to compare cyclosporine and tacrolimus, some trials in de novo recipients have shown more fibrotic changes in patients who received cyclosporine (58–61), but these used C0 monitoring. It is unknown whether C2 monitoring from the time of transplantation will reduce the histologic changes of CAN.

Conversion of stable renal transplant recipients to C2 monitoring is safe and does not lead to an increased risk for acute rejection. It is associated with improvements in BP and lipids and may also improve renal function in patients who are receiving excessive cyclosporine exposure. This improvement in metabolic parameters might decrease the risk for cardiovascular events in this high-risk population. Although reducing cyclosporine overexposure may prevent the development of CAN, no randomized, controlled trials have demonstrated that

### Table 1. Suggested target ranges for renal transplant patients who receive cyclosporine

<table>
<thead>
<tr>
<th>Time</th>
<th>Without Induction Therapy</th>
<th>With IL-2 Receptor Antibody Therapy</th>
<th>Induction with Thymoglobulin</th>
<th>With mTOR Inhibitor</th>
</tr>
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<tbody>
<tr>
<td>0 to 3 mo</td>
<td>C0 &gt;1700 ng/ml by day 5 (54); 1600 to 2000 ng/ml month 1, C2 1400 to 1600 ng/ml month 2, C2 1200 to 1400 ng/ml month 3 (48)</td>
<td>C2 &gt;1500 ng/ml for first 2 mo, C2 1200 to 1400 ng/ml/month 3 (46)</td>
<td>C2 &gt;75 to 125 ng/ml months 1 through 2; C2 50 to 100 ng/ml months 3 through 6 (95,96); reduce C2 target by 50 to 75%</td>
<td>C2 75 to 125 ng/ml months 1 through 2; C2 50 to 100 ng/ml months 3 through 6 (95,96); reduce C2 target by 50 to 75%</td>
</tr>
<tr>
<td>&gt;3 to 12 mo</td>
<td>C2 800 to 1000 ng/ml/months 4 through 6, C2 600 to 800 ng/ml/months 7 through 12 (49)</td>
<td>C2 600 to 1000 ng/ml (46)</td>
<td>C2 50 to 100 ng/ml (95,96); reduce C2 target by 50 to 75%</td>
<td>C2 50 to 100 ng/ml (95,96); reduce C2 target by 50 to 75%</td>
</tr>
<tr>
<td>&gt;12 mo</td>
<td>C2 approximately 800 ng/ml (54–56)</td>
<td>C2 approximately 800 ng/ml (54–56)</td>
<td>C2 approximately 800 ng/ml (54–56)</td>
<td>C2 approximately 800 ng/ml (54–56)</td>
</tr>
</tbody>
</table>

*C0* trough level; *C2*, 2-h postdose cyclosporine level; *mTOR*, mammalian target of rapamycin.
C₂ monitoring reduces CAN, graft loss, or death compared with C₀ monitoring.

**Therapeutic Drug Monitoring of Tacrolimus**

Trough-level monitoring of tacrolimus has been standard practice since its introduction. Similar to cyclosporine, achieving early adequate tacrolimus exposure significantly reduces the risk for acute rejection. In a retrospective analysis of a randomized, controlled trial, the tacrolimus AUC by day 2 after transplantation was found to be a strong predictor of the risk for acute rejection. Patients with a tacrolimus AUC >200 ng/h per ml had a markedly lower risk for acute rejection (17 versus 41%), regardless of whether they received MMF. Tacrolimus C₄ max did not correlate with the risk for rejection. The threshold value of 200 ng/h per ml correlated to a tacrolimus C₀ of 10 ng/ml (62).

Several small trials have assessed the ability of tacrolimus trough levels to predict the tacrolimus AUC. Two trials showed that the tacrolimus C₀ correlated poorly with AUC (r² = 0.11 and 0.362) and that C₄ was the best single-point correlate of AUC (r² = 0.79 and 0.81). Both studies suggested that a two- or three-point limited sampling strategy, both of which incorporated C₄, would predict AUC better than C₀ levels (63,64). Other studies identified C₀ or C₃ as the best correlates of AUC (65,66). However, some studies have shown excellent correlations (r²) in the range of 0.79 to 0.86 between tacrolimus C₀ levels and AUC (65,67,68). No prospective trials have compared outcomes with an AUC, C₀, C₂, C₃, or C₄-guided dosing strategy with those with C₀ monitoring in patients who were treated with tacrolimus. Although C₄ may be the best single-time point for monitoring tacrolimus, the correlation of C₂ with AUC for tacrolimus (r² = 0.87) is similar to that of cyclosporine (61). From a practical point of view, a comparison in the clinic setting of C₀ versus C₂ monitoring of tacrolimus might be interesting. Although such a strategy may not further reduce the already low rate of acute rejection that is seen in patients who are treated with the combination of tacrolimus and MMF, it may decrease the incidence of CNI toxicity.

Like cyclosporine, tacrolimus monitoring should be done with whole-blood samples (16). Its half-life is 12 to 18 h, which suggests that a period of approximately 2.5 d should elapse to assess the effect of a dosage adjustment on the tacrolimus level. Both microparticle enzyme immunoassay and ELISA have excellent correlation with the reference methods of liquid chromatography and mass spectrometry (69).

**Target Tacrolimus Levels**

Target tacrolimus levels in renal transplant patients have been defined by clinical trials (Table 2). These trials usually compared tacrolimus with cyclosporine (both Sandimmune and Neoral) with trough-level monitoring. The trials also varied according to the type of adjunctive therapy, induction therapy, and follow-up.

Data from a phase II clinical trial in renal transplant patients were used to examine the relationship among tacrolimus level, acute rejection, and toxicity. This trial randomly assigned patients to three groups, with tacrolimus trough concentrations between 5 and 14, 15 and 25, and 26 and 40 ng/ml. There were no statistically significant differences among the three groups in terms of acute rejection, but there were more tacrolimus-related adverse events in the two higher dosage groups. In a logistic regression analysis, the risk for acute rejection decreased with increasing tacrolimus levels but at the expense of increased adverse events and nephrotoxicity (70).

The initial phase III clinical trials used tacrolimus C₀ levels as high as 10 to 20 ng/ml during the first 3 mo after transplantation, followed by levels of 5 to 10 ng/ml (71–75). However, significant toxicity was seen with C₀ levels of >15 ng/ml. Subsequent trials often used C₀ ranges between 10 and 15 ng/ml in the early posttransplantation period and 5 to 10 ng/ml after 3 mo, although there is significant variation around these ranges (58,76,77). Patients who receive IL-2 receptor blockade require tacrolimus levels of 10 to 15 ng/ml for only the first 6 wk after transplantation, followed by levels of 5 to 10 ng/ml thereafter (78). More recently, lower levels of tacrolimus (3 to 7 ng/ml) in the early posttransplantation period have been

| Table 2. Suggested target ranges for renal transplant patients who receive tacrolimus |

<table>
<thead>
<tr>
<th>Time</th>
<th>Without Induction Therapy</th>
<th>With IL-2 Receptor Antibody Therapy</th>
<th>Induction with Thymoglobulin</th>
<th>With mTOR Inhibitor</th>
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<tr>
<td>0 to 3 mo</td>
<td>C₀ 10 to 15 ng/ml (71–75)</td>
<td>C₀ 10 to 15 ng/ml first 6 wk, C₀ 5 to 10 ng/ml after week 6 (78); C₀ 3 to 7 ng/ml throughout may be adequate (79)</td>
<td>C₀ 5 to 10 ng/ml (76,77)</td>
<td>C₀ 3 to 7 ng/ml (97)</td>
</tr>
<tr>
<td>&gt;3 to 12 mo</td>
<td>C₀ 5 to 15 ng/ml (71–75)</td>
<td>C₀ 10 to 15 ng/ml first 6 wk, C₀ 5 to 10 ng/ml after week 6 (78); C₀ 3 to 7 ng/ml throughout may be adequate (79)</td>
<td>C₀ 5 to 10 ng/ml (76,77)</td>
<td>C₀ 3 to 7 ng/ml (97)</td>
</tr>
<tr>
<td>&gt;12 mo</td>
<td>C₀ 5 to 10 ng/ml (71–75)</td>
<td>C₀ 10 to 15 ng/ml first 6 wk, C₀ 5 to 10 ng/ml after week 6 (78); C₀ 3 to 7 ng/ml throughout may be adequate (79)</td>
<td>C₀ 5 to 10 ng/ml (76,77)</td>
<td>C₀ 3 to 7 ng/ml (97)</td>
</tr>
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</table>
assessed in a randomized, controlled trial against low- and standard-dosage cyclosporine (both monitored with \( C_0 \) levels) and sirolimus in a quadruple regimen that included daclizumab, MMF, and steroids. Tacrolimus was associated with the lowest risk for acute rejection as well as the highest GFR at 12 mo compared with the other groups (79). Thymoglobulin induction allows for reduction of tacrolimus \( C_2 \) levels to 5 to 10 ng/ml from the time of transplantation (80).

Comparison of Efficacy of Cyclosporine and Tacrolimus

Many trials have compared cyclosporine and tacrolimus in renal transplant recipients (75,81). A recent meta-analysis found fewer acute rejection episodes and graft losses with tacrolimus (82). However, the immunosuppressive protocols in these trials were highly heterogeneous and used a variety of target levels and therapeutic drug-monitoring strategies for cyclosporine and tacrolimus. These trials also used cyclosporine \( C_0 \) monitoring and therefore may have underdosed cyclosporine. A recent retrospective study showed a more rapid decline in GFR in patients who were treated with cyclosporine with \( C_2 \) monitoring compared with tacrolimus, although there was no difference in mean arterial pressure, total cholesterol, or new-onset diabetes (83). In a recent randomized, controlled trial that compared cyclosporine with \( C_2 \) monitoring and tacrolimus, there was no difference in the primary end point of acute rejection, graft loss, or death. However, GFR was slightly but significantly lower with cyclosporine. BP was similar in both groups, but patients who were treated with cyclosporine had higher LDL and HDL cholesterol, whereas there was a higher incidence of new-onset diabetes or impaired fasting glucose in the tacrolimus group (84). In comparison, a study in liver transplant recipients that compared cyclosporine \( C_2 \) with tacrolimus found no difference in renal function or acute rejection (85).

CNI in Combination with Mammalian Target of Rapamycin Inhibitors

The original trials that evaluated sirolimus used full-dosage cyclosporine monitored by \( C_0 \) levels (86,87). Although the acute rejection rate was reduced compared with patients who received cyclosporine and azathioprine, serum creatinine levels were higher. Similar findings were seen in trials that combined full-dosage cyclosporine with everolimus (88,89), and tacrolimus with sirolimus (90–92). There is a small increase in CNI exposure with the addition of mammalian target of rapamycin (mTOR) inhibitors. It therefore is believed that there also must be a substantial increase in tissue CNI exposure with the addition of an mTOR inhibitor, but the mechanism has not yet been elucidated. In addition, cyclosporine and sirolimus should be taken separately, because co-administration increases sirolimus AUC and nephrotoxicity. Whether there is a similar need to separate tacrolimus and sirolimus is unclear (93). Everolimus does not seem to be affected by co-administration with cyclosporine and has been given simultaneously (94).

Patients who are on a combination of CNI and mTOR inhibitor require reduction of the CNI to avoid nephrotoxicity. No randomized, controlled trials have established target \( C_2 \) levels for cyclosporine in combination with sirolimus or everolimus, but CNI dosage reductions of 50 to 75% (or even more) may be necessary to avoid nephrotoxicity (Tables 1 and 2) (95–97). Registry analysis has demonstrated that the combination of a CNI with sirolimus is associated with decreased graft survival compared with a CNI combined with MMF (98,99), but this may be due to nephrotoxicity from the combination of full-dosage CNI and sirolimus. Whether the combination of a low-dosage CNI with an mTOR inhibitor will give equivalent long-term results to a CNI combined with MMF is unknown.

CNI Levels in Steroid-Withdrawal Regimens

Interest has increased in protocols in which corticosteroids are stopped early after transplantation (100–102). Trials with cyclosporine have used trough-level monitoring, either following the same levels as per center practice (103) or choosing levels similar to usual practice (104,105). Trials with tacrolimus have used levels similar to protocols that contain steroids (Table 2), both with (81,106–108) and without (105) induction therapy. These regimens may lead to fewer metabolic complications after transplantation. However, reduction of CNI dosages to avoid nephrotoxicity may be more difficult in the absence of the immunosuppressive effects of steroids.

Limitations of Therapeutic Drug Monitoring

For therapeutic drug monitoring to be useful in clinical practice, it requires consistency in terms of drug administration and sampling. For example, meals may decrease the \( C_{\text{max}} \) and AUC of CNI (3,17). Although this may lead to higher dosage requirements for patients who take their medication with meals, as long as they are consistent, this should not affect drug levels. However, patients who take their medications with meals sometimes and fasting at other times may have more variability in measured levels, which could lead to under- or overdosing.

In addition, blood samples must be drawn at the correct time. For cyclosporine \( C_2 \) monitoring, blood should be drawn within 15 min of the 2-h postdose time point (36). For cyclosporine or tacrolimus trough-level monitoring, blood should be drawn 12 h after the last dose (i.e., immediately before the next dose). Although \( C_2 \) monitoring probably does not require as narrow a therapeutic window as \( C_2 \) monitoring, levels that are drawn at other time points, such as 10 or 15 h after the last dose, may lead to unnecessary dosage adjustments, again leading to under- or overdosing.

Finally, therapeutic drug monitoring is a method of monitoring a medication by its pharmacokinetics. However, the pharmacodynamic effects may not always correlate with pharmacokinetics. Previous studies have attempted to use calcineurin inhibition, IL-2 production, or cytokine mRNA production as a marker of the degree of calcineurin inhibition (1,106,107). A recent study measured expression of nuclear factor of activated T cells–regulated genes and found a close relationship between the degree of gene suppression and the incidence of infections and malignancies (108). However, no pharmacodynamic method has been validated yet in clinical practice.
Conclusion

Both cyclosporine and tacrolimus have a narrow therapeutic window, meaning that monitoring is required. Optimal monitoring can be achieved only with an understanding of the pharmacokinetics of these medications. Underdosing is associated with an increased risk for rejection, whereas overdosing is associated with toxicity and an increased risk for CAN. C₂ monitoring allows more accurate dosing of cyclosporine and better predicts which patients are at risk for acute rejection, and target C₂ levels early and late after transplantation have been defined. Even patients several years after transplantation may benefit from conversion to C₂ monitoring, because this may allow cyclosporine dosage reduction, possibly leading to improvements in renal function and adverse drug effects. Conversely, no randomized, controlled trials have proved conclusively that C₂ monitoring is associated with improved outcomes compared with C₀, and many centers have achieved excellent results using C₀ monitoring combined with the other available immunosuppressants. For this reason, adoption of C₂ monitoring has not been universal. Although tacrolimus C₀ levels correlate better with AUC than cyclosporine C₀ levels, there is new evidence that tacrolimus C₂ and C₄ levels are better surrogates of AUC than C₀. Further studies will be needed to determine whether these newly proposed time points will improve outcomes in patients who are treated with tacrolimus. The goal of such studies would be to reduce tacrolimus-related toxicity while maintaining the low rate of rejection that is seen with the current monitoring strategy.

Disclosures

M.C. has received honoraria from Astellas, Hoffman LaRoche, and Novartis. E.C. has received honoraria and been a member of the Speaker’s Bureau for both Novartis and Astellas.

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