# Comparison of Cyclosporine Concentrations 2 Hours Post-Dose Determined Using 3 Different Methods and Trough Level in Pediatric Renal Transplantation

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ABSTRACT

Immunosuppression has been one of the great challenges in pediatric recipients of kidney allografts. Cyclosporine (CsA) has evolved during the past 25 years of transplantation. It requires frequent blood level monitoring because of its narrow therapeutic window and interpatient and intrapatient variability. Neoral (Novartis) is no exception. Ideally, monitoring of blood levels should also include determination of the area under the time-concentration curve (AUC) to better target the therapeutic window, thus avoiding underdosing or overdosing, especially in pediatric patients. A single blood concentration measurement 2 hours after Neoral administration (C2) has been shown to be a more for accurate predictor of drug exposure than trough levels (C0). Therefore, its use may lead to reduction in the incidence and severity of cellular rejection and of CsA toxicity. Some studies have shown that the metabolites/CsA ratio is substantially lower using C2 than C0, however, the between-assay differences for C2 monitoring have not been considered.

The purpose of this study was to evaluate CsA C0 and C2 levels, determined using monoclonal fluorescence polarization immunoassay (FPIA)/TDx and enzyme multiplied immunoassay (EMIT). CsA levels were determined using a radioimmunoassay (RIA) in 30 pediatric transplant recipients with stable renal function within 42.7 mean months follow-up. Mean age was 13.4 years; 15 children were girls; 23 patients were recipients of cadaveric kidneys. The mean CsA microemulsion dose was 5.7 mg/kg/d. The 3 methods showed a high correlation between C0 and C2 ($r \geq 0.97$). A linear regression slope was significantly higher for C0 than C2 ($P < .001$). The CsA concentrations both at C0 and C2 were significantly higher with FPIA than with RIA ($P < .009$) but no differences were found for EMITT ($P = .2$). The mean C0 level for FPIA was 22% and 26% higher than RIA and EMIT, respectively. The mean C2, for FPIA was 7% and 12% higher than RIA and EMIT, respectively. In conclusion, CsA levels determined using RIA or EMIT are better than using FPIA/Tx; also, C2 CsA levels are more accurate than C0 in pediatric transplantation patients.

CYCLOSPORINE (CsA) since its introduction 30 years ago has been an important immunosuppressive drug in transplantation. Carefully designed studies have led to an understanding of its pharmacokinetics and pharmacodynamics, leading to a rapid improvement in patient and graft survivals.[1]

The recognition that pharmacokinetic profiling reveals important drug exposure characteristics was highlighted in crossover studies comparing Sandimmune with Neoral.[2] These studies particularly showed that CsA trough levels did not correlate with drug exposure, and that Neoral still had significant intrapatient and interpatient

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variability, although less than Sandimmune. Afterward, pediatric research on CsA pharmacokinetics showed that C0 levels correlate with CsA exposure in younger pediatric patients.[3]

In renal transplant recipients, the period of greatest variability in drug exposure is the first 4 hours following CsA dosing area under the curve [AUC] (0–4); the interpatient variability being insignificant outside this period. Correlations between drug exposure and clinical outcome demonstrated that patients achieving adequate drug exposure during this interval have low rejection rates and less nephrotoxicity. However, even a 4-hour AUC is not practical in clinical practice. It has recently been shown in adults that a 0–4-hour AUC, which is representative of the absorption period profile, is as accurate as the 0–12-hour AUC.[4–6] Recently, a clinical trial of kidney allograft recipients revealed that those patients who reached target C2 levels experienced superior results.[7]

In pediatric patients, C2 levels also correlate well with drug exposure.[8,9] The pharmacodynamic effect of Neoral C2 monitoring has been validated using calcineurin inhibition and interleukin (IL)-2 expression. Pediatric patients are particularly complicated when treated with CsA, because they have a great variability both in absorption and in drug clearance. Harmon and Matas have identified a relationship between CyA dosing and outcomes. NAPRTCS has indicated that the CyA doses were negatively correlated with risk of rejection (relative risk [RR] = RR 0.9; $P = .02$). The main immunologic assays used to determine CsA levels are as follows: a fluorescence polarization immunoassay FPIA TDx (Abbott Laboratories, Chicago, Ill, United States), and an enzyme multiplied immunoassay EMIT (Dade, Behring, Frankfurt, Germany).

The reference methods for CsA determinations are high-performance liquid chromatography (HPLC) or radioimmunoassay (RIA) owing to their specific analysis of the parent drug without cross-reaction with metabolites.

The aim of the present study was to evaluate 2 automated methods for determination of CyA levels (FPIA and EMIT) at both C0 and C2 to compare their values with the RIA (used as the reference technique).

## MATERIALS AND METHODS

### Patient Samples

CsA levels were determined on 90 whole blood samples from 30 Chilean pediatric recipients of a renal transplant in a public hospital. All children were stable on the same immunosuppressive protocol, at least 6 months posttransplantation. All patients had stable and functioning renal grafts for at least 6 months. Patients who met all study criteria provided written informed consent as approved by the Institutional Investigation and Ethical Committee. Blood samples for trough (C0) CsA levels were obtained the morning prior to the next CsA dose. Afterward, the Neoral dose was administered during nurse supervision. The sample for C2 level determination was obtained 2 hours later. The study period for each patient lasted 2 months for each patient.

Whole blood was collected in coated tubes with EDTA. The initial determinations were performed with FPIA/TDx for C0 and C2 blood samples during regular CsA monitoring. Analyses of the CsA level using EMIT and RIA assay were then performed.

### Control Specimens

Commercially available Lymphocheck whole blood CsA low, medium, and high controls (Lymphocheck Bio-Rad Laboratories, Hercules, Calif, United States) were used to evaluate the within-run precision of the assays. The between-run coefficients of variation of the different methods were determined using 3 different Lymphocheck controls.

### CsA Metabolites

CsA metabolites were provided by Dade Behring of the highest purity available (>95%, as stated by the supplier).

### RIA Method

*EMIT immunoassay.* The reagents for this monoclonal antibody-based EMIT 2000 Cyclosporine Specific Assay were obtained from Dade Behring. The CsA assay was performed on a Hitachi 911 analyzer according to the manufacturer's instructions.

Prior to the assay, 300 $\mu$L pretreatment reagent was added to 100 $\mu$L whole blood; the sample mixture was then spun for at least 10 seconds and centrifuged for at least 10 minutes in a microcentrifuge at 9500*g*. The supernates were then decanted directly into Hitachi sample cups and the samples were assayed immediately. The assay was calibrated using a calibration curve with 5 calibrators. The assay is designed to determine CsA levels up to the highest standard (500 $\mu$g/L). During an 8-week period, 7 calibrations of the system were required.

*Monoclonal FPIA/TDx methods.* The reagents were obtained from Abbott Laboratories. CsA determinations were performed using the Abbott TDx system. Then, 50 $\mu$L solubilization reagent and 300 $\mu$L precipitating reagent were added to 150 $\mu$L whole blood, prior to this mixture being spun. After centrifugation at 9500*g* for 3 minutes, the supernate was decanted and transferred immediately to a TDx analyzer for determining CsA concentration. The assay was performed using a nonlinear calibration curve composed of 6 calibrators designed to determine CsA levels up to the highest standard of 1500 $\mu$g/L. The FPIA/TDx was recalibrated weekly.

### Statistical Methods

We compared CsA levels, determined using RIA and the 2 other methods. Parametric tests were used when the data showed a Gaussian distribution. Paired (T) tests were applied for the differences between mean C0 and C2 CsA concentrations. Correlation analysis was performed to evaluate 2 continuous variables simulta-

**Table 1. CsA Concentrations in C0 and C2 Samples Using RIA, EMIT, and mFPIA Immunoassays From 30 Pediatric Kidney Transplants**

| | RIA | | EMIT | | FPIA | |
|---|---|---|---|---|---|---|
| Method | Mean | SD | Mean | SD | Mean | SD |
| C0 (ng/mL) | 134 | 44 | 127 | 49 | 173 | 54 |
| C2 (ng/mL) | 786 | 187 | 745 | 206 | 845 | 189 |

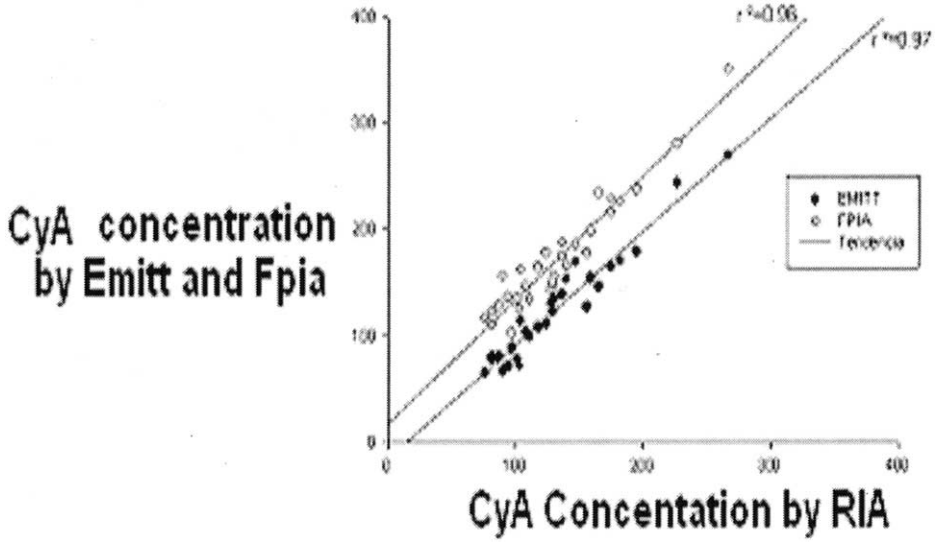


**Fig 1.** Cyclosporinemia concentration (ng/mL) at C0 using RIA vs FPIA and EMIT method.

neously, through the quotient of a Pearson correlation. Analysis of variance (ANOVA) was used for comparison of different methods. $P < .05$ was considered significant. The statistical analysis of the information was performed using Excel 5.0, Statistics for Windows 4.5, and STATA 7.0 packages. The clinically acceptable values for the differences and the standard errors of the estimates were calculated according to previously established criteria, considering the therapeutic range for CsA in our laboratory for C0 to be 100–200 ng/mL and for C2 to be 600–1200 ng/mL in the late renal posttransplantation period.

## RESULTS

Data on CsA levels were from 30 pediatric kidney transplant recipients, including 15 girls; 76% were from cadaveric donors. The overall mean age was 13.4 years (SD, 4.3). Mean follow-up was 42.7 months (SD, 33.9), and then mean creatinine level in the last 6 months was 1.1 mg/dL (SD, 0.63). Mean C0 CsA concentration in the last 6 months was 174.8 ng/mL (DS, 45.3) and Neoral doses in the same period were 5.7 mg/K/d.

Table 1 shows that CsA mean trough level (± SD) for all values was similar for EMIT (127 ± 49) and RIA (134 ± 44) and significantly higher for the FPIA/Tx (173 ± 54) ($P < .02$; Table 1).

Figures 1 and 2 show the correlation of 90 CsA levels from blood samples. Two methods were compared with the RIA reference methods. Correlation coefficients ranged from 0.97 and 0.98 (for EMIT and FPIA/TDx, respectively) for C0 CsA levels; they ranged from 0.91 and 0.94 (EMIT and FPIA/Tx, respectively) for C2 levels. No differences were observed between isolated measure-

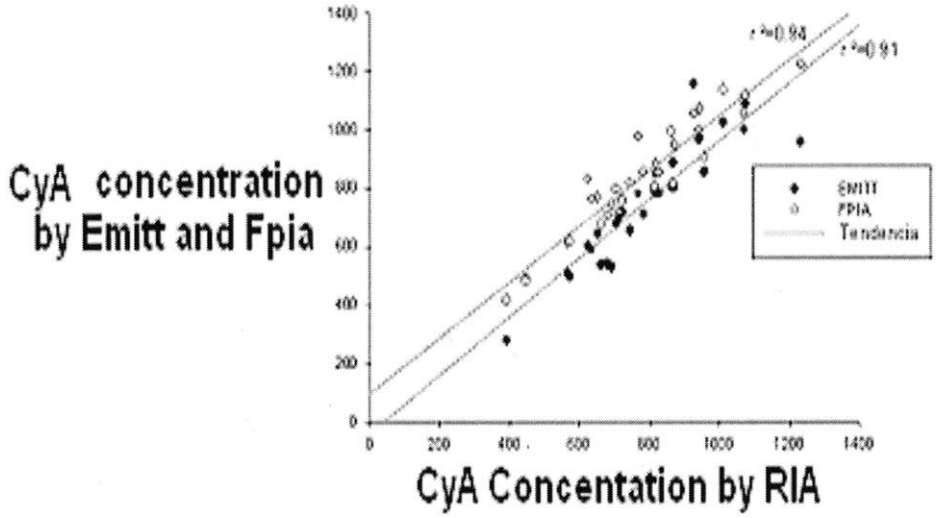


**Fig 2.** Cyclosporinemia concentration (ng/mL) at C2 using RIA vs FPIA and EMIT method.

ments of the 2 methods of CyA level compared with RIA in either C0 or C2 blood samples. However, when lineal multiple regression for repeated measurements method was used, we observed that FPIA levels were significantly greater than those determined using RIA for both C0 and C2 levels ($P = .001$), whereas no significant differences were detected between RIA and EMIT values ($P = .202$; Figs 3 and 4).

## DISCUSSION

Calcineurin inhibitors, especially CsA, have been the cornerstone of immunosuppression in solid organ transplantation. Regular monitoring of CyA blood levels is mandatory to reach the therapeutic window, especially in kidney transplantation, because of its nephrotoxicity. CsA microemulsion (Neoral) minimizes absorption variability of CyA, because it is independent of biliary flow. C0 does not adequately represent the AUC level, especially in children. In fact, there are reports showing an increasing number of kidney rejections among children maintained with acceptable trough levels.

CyA therapeutic levels are focused more for nephrotoxicity than for immunosuppression and consequently are not adequate to represent immunosuppressive levels. Looking for the best point correlating with the AUC, which would represent best the exposure to CyA, would prevent the large number of blood samples, with its associated pain, lost time, and expense involved in performing an AUC, all of which are particularly important in children. C2 represents the best correlation with a short AUC C0–C4 being the closest point to Cmax (peak level postingestion). It shows a good predictive value with kidney rejection after the first 6 months posttransplantation.

In this report, both methods (FPIA and EMIT) correlated with the RIA reference method. The mean value ± SD was similar for the EMIT and RIA, while being significantly higher for the FPIA/TDx. However both immunoassays had higher CsA concentrations than the RIA method. The FPIA methods are 26% above EMIT and 22% above RIA for CsA C0 levels. For C2 CyA levels, FPIA was 12% above EMIT and 7% above RIA. These observations in the pediatric population clearly show that FPIA/TDx values are higher than the other 2

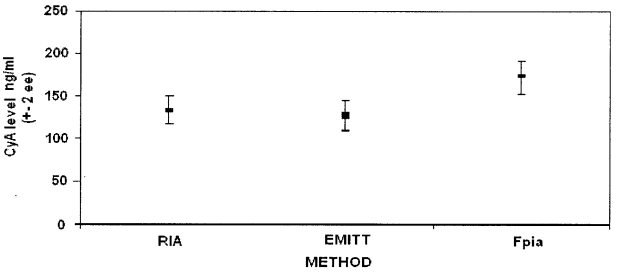


**Fig 3.** CyA levels for C0 blood samples by immunoassays methods.

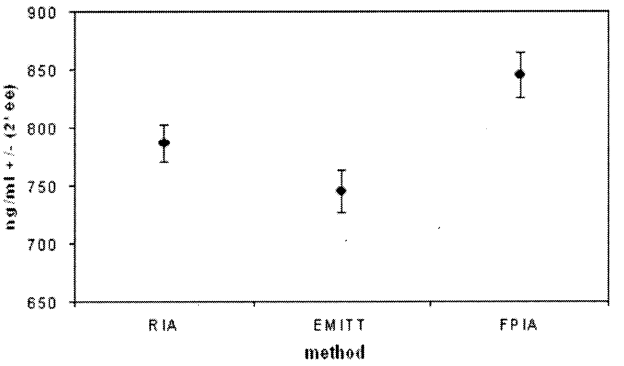


**Fig 4.** C2 cyclosporinemia level using RIA, EMIT and FPIA.

methods, especially when measuring C0 CyA levels. These findings might be due to the relatively high cross-reactivity of this assay with CsA metabolites, some of which may be nephrotoxic, thus potentially leading to chronic allograft nephropathy. Evaluation of 4 automated methods for determination of whole blood has been previously published.[10]

Correlation coefficients for C0 CsA levels ranged from 0.96 (EMIT) to 0.97 for the FPIA/TDx, and for C2 levels 0.91 (EMIT) and 0.94 for the FPIA/TDx.

When a lineal multiple regression method for repeated measured was applied, it showed significant differences between FPIA/TDx and for both C0 and C2 CsA levels blood levels. FPIA/TDx values were higher than RIA ($P = .001$), whereas no differences appeared for RIA and EMIT ($P = .202$).

## REFERENCES

1. Kahan BD: Overview: individualization of cyclosporine therapy using pharmacokinetic and pharmacodynamic parameters. Transplantation 40:457, 1985

2. Kahan BD, Welsh M, et al: Variable oral absorption of cyclosporine. A biopharmaceutical risk factor for chronic allograft rejection. Transplantation 62:599, 1996

3. Johnston A, David VJ, et al: Pharmacokinetic validation profiling. Transplant Proc 32:53S, 2000

4. Oellrrich M, Armstrong VW, Kahan B, et al: Lake Louise consensus meeting on cyclosporine monitoring in organ transplantation: report of the consensus panel. Ther Drug Monit 17:642, 1995

5. Cantarovich M, Besner JG: Two-hour cyclosporine level determination is the appropriate tool to monitor Neoral therapy. Clin Transplant 12:243, 1998

6. Halloran PF, Helms LM, et al: The temporal profile of calcineurin inhibition by ciclosporin in vivo Transplantation 68: 1356, 1999

7. Bohler T, Budde K: Pharmacodynamic monitoring of lymphocyte proliferation and TGF-beta expression of CyA through levels (C0) and 2 hours after intake (C2) of CyA in human renal allograft recipients. Transplant Proc 33:3148, 2001

8. Kovarik JM, Mueller EA, van Bree JB, et al: Cyclosporine pharmacokinetics and variability from a microemulsion formulation. A multicenter investigation in kidney transplant patients. Transplantation 58:658, 1994

9. Filler G, Ehrich JHH: Which cyclosporin formulation? Lancet 348:1176, 1996

10. Am J Clin Pathol 112:358, 1999