

Cyclosporine Lymphocyte Maximum Level Monitoring in De Novo Kidney Transplant Patients: A Prospective Study

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Objectives: To determine prospectively the temporal variations of cyclosporine-A lymphocyte maximum level, whole blood maximum concentration, and total lymphocyte count in patients with de novo kidney transplantation.

Materials and Methods: Lymphocyte maximum level, whole blood maximum concentration, and total lymphocyte count were prospectively measured in 35 patients at 1, 2, and 3 months after kidney transplantation. Two groups—a biopsy-proven acute rejection group (REJ+) and a rejection-free group (REJ-)—were compared.

Results: Both groups had similar lymphocyte maximum levels, whole blood maximum concentrations, and total lymphocyte counts at the first month after transplantation. REJ+ patients had significantly lower lymphocyte maximum levels at 2 and 3 months (59 ± 34 and 33 ± 9 pg/Lc) and higher total lymphocyte counts ($0.00204 \pm 0.00078 \times 10^9/L$ and $0.00203 \pm 0.00022 \times 10^9/L$) when compared with their REJ- counterparts (87 ± 56 and 63 ± 30 pg/Lc, $P < .05$ and $P < .007$) and ($0.00137 \pm 0.00074 \times 10^9/L$ and $0.0015 \pm 0.0006 \times 10^9/L$, $P < .02$ and $P < .003$) respectively. Whole blood maximum concentrations were significantly higher in patients in the REJ+ group (2050 ± 623 vs 1414 ± 536 ng/mL, $P < .02$) at 2 months. At 3 months, the 2 groups were comparable (1158 ± 340 vs 1365 ± 525 ng/mL, $P = NS$).

Conclusions: These results suggest that acute rejection is associated with a relatively low cyclosporine-A lymphocyte maximum level and high total lymphocyte count in the early posttransplant period.

Cyclosporine-A whole blood maximum concentration failed to correlate with clinical outcome. Cyclosporine-A lymphocyte maximum level seems to offer a more reliable alternative than does whole blood maximum concentration for cyclosporine-A monitoring in patients with kidney transplantation.

Key words: Cyclosporine-A lymphocyte level monitoring, Bioavailability, Bioactivity, Clinical outcome, Immunosuppression

Despite an abundance of literature during the last 2 decades, significant controversy remains regarding the most relevant cyclosporine-A (CsA) therapy monitoring technique [1-5] in solid organ transplantation. To minimize the substantial adverse effects of immunosuppressive drugs on graft loss [6-7] and chronic allograft nephropathy [8], new strategies are needed to optimize the correlation between monitoring methods and clinical outcome and immune responsiveness. Like others [3, 9, 10], we have previously shown a poor relationship between CsA blood levels such as predose trough level (C_0), blood levels 2 hours after dose ingestion (C_2), and maximum blood level after dose ingestion (C_{max}), and histologic diagnosis. Recently, we reported our experiences with CsA lymphocyte levels and monitoring predose lymphocyte level (LT_0L) and maximum lymphocyte level after dose ingestion (LT_mL) as a new alternative for CsA therapy monitoring [9, 10] and tailoring [11] in stable patients and in patients with graft dysfunction.

The aim of this study was to monitor prospectively the temporal variations of CsA C_{max} , LT_mL , and lymphocyte counts in de novo kidney transplant patients while tapering CsA dosages during the first 3 months after transplantation and correlating these parameters with the clinical diagnoses as defined by histologic findings and serum creatinine (Scr) levels.

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Materials and Methods

Thirty-five patients (27 men, 8 women; mean age, 42 years; range, 19-66 years) were included. All patients underwent kidney transplantation for the first time at Rizk Hospital. One received a cadaveric kidney, 7 received living-related grafts, and the remaining 27 were transplanted from living-unrelated donors. All patients received similar induction therapy with an antithymocyte globulin (thymoglobulin) for a total of 12-16 mg/kg body weight to keep the CD4 count ≤ 50 during the first week after transplantation. They were maintained on a CsA-based (initial dosage 7 mg/kg b.i.d.) triple therapy with mycophenolate mofetil (MMF) and prednisone. MMF was begun at 2 g per day (1 g b.i.d.), and the dosage ranged between 1.5-2.5 g/day throughout the study. MMF dosage was adjusted to maintain mycophenolic acid plasma trough levels (MPAC_{min}) between 1.5 and 2.6 $\mu\text{g/mL}$. Prednisone dosage was tapered to 0.1-0.2 mg/kg bodyweight at 3 months posttransplantation. Nine patients with graft dysfunction (defined as no improvement, persistent fluctuation, or a continuous rise in Scr level $\geq 30\%$ from baseline despite CsA tapering and normal graft ultrasound) underwent 9 graft biopsies during the first 3 months posttransplantation: 1 during the first month, 6 during the second month, and 2 during the third month. The remaining 27 patients had stable, normal graft function during the study. Rejection and CsA nephrotoxicity were diagnosed according to Banff criteria [12]. CsA monitoring parameters in whole blood at 1, 1.5, and 2 hours after drug ingestion and their corresponding lymphocyte levels were determined using monoclonal antibodies (Abbott TDX method, Abbott Laboratories, Abbott Park, Ill, USA) for the former and the method of Masri and colleagues [13] for the latter using the MERI solution for CsA extraction from the lymphocytes. C_{max} and LT_{mL}, respectively, were considered as representing the highest CsA whole blood concentration and its corresponding maximum lymphocyte content.

CsA C_{max} and LT_{mL} are expressed as ng/mL and pictogram per lymphocyte (pg/Lc) respectively. CsA LT_{mL} was used for CsA therapy monitoring and dosage tapering. Graft biopsies were performed in patients with persistent graft dysfunction and a drop of LT_{mL} below 50 pg/Lc [10] irrespective of C_{max}. Patients with biopsy-proven acute rejection (n = 4) were assigned to the REJ+ group, and those who did not develop acute rejection (n = 31) were assigned to the REJ- group, which included 26 patients with

normal graft function (NOR) and 5 patients with biopsy-proven CsA nephrotoxicity. All monitoring parameters including complete blood count, total lymphocyte count, Scr level, and urinalysis were determined routinely. In addition, the above-mentioned CsA levels and MPAC_{min} were determined on a monthly basis during the study. These parameters in patients with graft dysfunction and in patients without graft dysfunction also were obtained at the same time after transplantation (during graft biopsy). Results are expressed as means $\pm$ SD and were compared using the Student *t* and chi-square tests or the Fisher exact test when appropriate. Differences were considered significant when values for *P* were less than .05.

Results

Nine patients (25%) developed graft dysfunction during the study. Biopsy-proven acute rejection occurred in 4 patients (11%) during the first month, in 2 within the second month, and in 2 others within the third month after transplantation. The remaining 5 patients (14%) exhibited biopsy-proven acute nephrotoxicity (1 within the first month and 4 others within the second month after transplantation). Results of the CsA and MMF monitoring parameters in both groups (REJ+ and REJ-) are summarized in Table 1. REJ+ patients had significantly lower LT_{mL} than did patients in the REJ- despite similar CsA dosages and slightly higher C_{max}. As expected, the total lymphocyte count was increased in patients in the REJ+ group as compared with those in the REJ- group. Both MMF dosages and MPA C_{min} were comparable in both groups. Moreover, in patients with graft dysfunction

Table 1. Comparison of the different CsA and MMF therapy monitoring parameters and total lymphocyte count in rejecting and nonrejecting patients at the time of graft biopsy. Data in patients with normal graft function were obtained at the same time that graft biopsies were performed.

	CsA dose (mg/kg)	CsA C _{max} (ng/mL)	CsA LT _{mL} (pg/Lc)	Total LC	MMF dose (g)	MPA C _{min} ($\mu\text{g/mL}$)	Serum creatinine ($\mu\text{mol/L}$)
REJ ⁺	4.1 $\pm$ 0.9	1437 $\pm$ 690	41 $\pm$ 5	2.1 $\pm$ 0.9	1.6 $\pm$ 0.4	1.7 $\pm$ 0.5	141 $\pm$ 8.8
REJ ⁻	4.9 $\pm$ 0.8	1265 $\pm$ 536	62 $\pm$ 33	1.5 $\pm$ 0.6	1.7 $\pm$ 0.3	2.1 $\pm$ 1.1	106 $\pm$ 26
<i>P</i>	NS	NS	.001	NS	NS	NS	.0002

C_{max}: maximum CsA blood level after dose ingestion

C_{min}: plasma trough level

CsA: cyclosporine-A

LT_{mL}: maximum lymphocyte level after dose ingestion

MMF: mycophenolate mofetil

MPA: mycophenolic acid

REJ-: rejection-free group

REJ+: biopsy-proven acute rejection group

LC: lymphocyte count

NS: Not significant

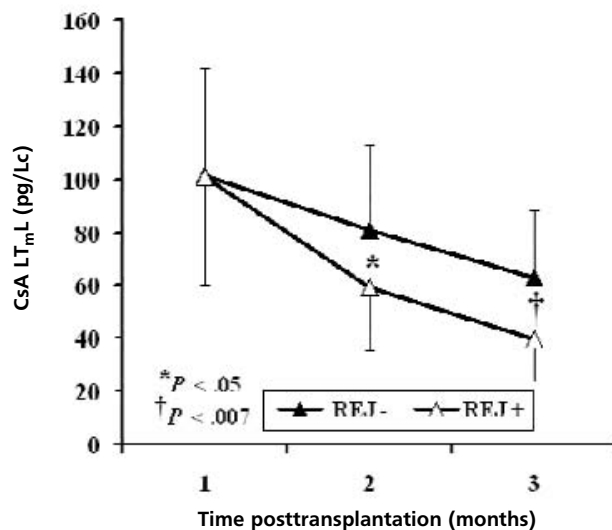


Figure 1. Temporal variations of cyclosporine-A maximum lymphocyte level in rejecting and nonrejecting patients during the first 3 months of the study. Results are expressed as means $\pm$ SD.

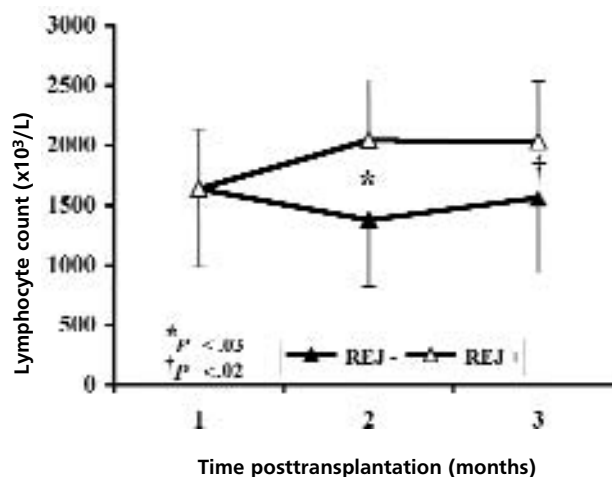


Figure 2. Temporal variations of total lymphocyte count in rejecting and nonrejecting patients during the first 3 months of the study period. Results are expressed as means $\pm$ SD.

(REJ+ and nephrotoxicity), no significant differences were observed in the CsA and MMF monitoring parameters among the 2 groups. Interestingly, patients with CsA nephrotoxicity exhibited similar LT_{mL} (46 ± 32 pg/Lc) despite a noticeably lower C_{max} (1166 ± 817 ng/mL) as compared with those in the REJ+ group (41 ± 5 pg/Lc and 1437 ± 690 ng/mL) respectively. Temporal variations in the CsA monitoring parameters (LT_{mL}, C_{max}) and in the total lymphocyte count over the 3-month study are shown in Figures 1, 2, and 3 respectively. Both groups had similar LT_{mL} (100 ± 43 pg/Lc vs 102 ± 85 pg/Lc), C_{max} (1439 ± 836 ng/mL vs 1471 ± 796 ng/mL), and total lymphocyte count ($0.001637 \pm 0.0011 \times 10^9$ /L vs $0.001638 \pm 0.00114 \times$

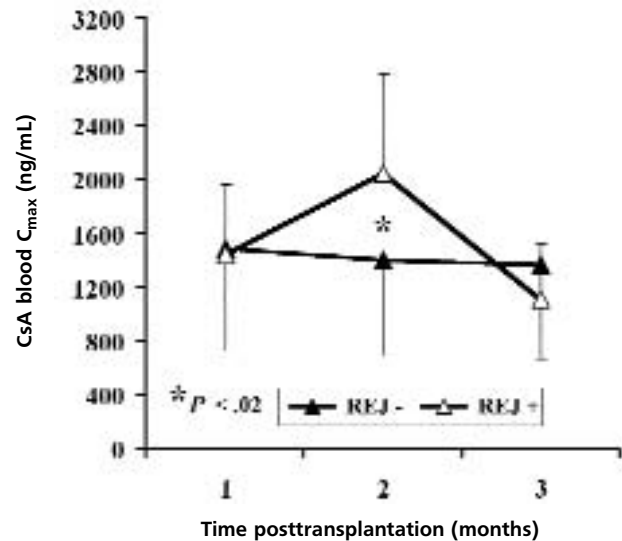


Figure 3. Temporal variations of cyclosporine-A maximum whole blood levels in rejecting and nonrejecting patients during the 3-month study. Results are expressed as means $\pm$ SD.

10^9 /L) at the same CsA dosages (6.5 ± 0.6 mg/kg vs 6.7 ± 1 mg/kg) during the first month after transplantation. REJ+ patients had significantly lower LT_{mL} (59 ± 34 pg/Lc and 39 ± 1.5 pg/Lc) (Figure 1) and higher total lymphocyte count ($0.00204 \pm 0.00078 \times 10^9$ /L and $0.00203 \pm 0.00022 \times 10^9$ /L) (Figure 2) at 2 and 3 months, respectively, when compared with patients in the REJ- group (87 ± 56 pg/Lc and 63 ± 30 pg/Lc, $P < .05$ and $P < .007$) and ($0.00137 \pm 0.00073 \times 10^9$ /L and $0.00156 \pm 0.0006 \times 10^9$ /L, $P < .03$ and $P < .02$). Unexpectedly, C_{max} was significantly higher in patients in the REJ+ group (2050 ± 623 ng/mL vs 1414 ± 536 ng/mL, $P < .02$) at 2 months, and it was comparable to patients in the REJ- group at 3 months (1158 ± 340 ng/mL vs 1365 ± 525 ng/mL, $P = \text{NS}$; Figure 3). During the 3 months after transplantation, MMF dosage and MPA C_{min} temporal variations in both groups were similar (Figures 4A and 4B).

Discussion

Our results demonstrate a strong association between clinical outcome (ie, mainly acute rejection) and CsA lymphocyte content. In fact, during the early post-transplant period, REJ+ patients exhibited significantly lower LT_{mL} than did patients in the REJ- group (Figure 1). This was paralleled by a marked increase in total lymphocyte count in patients in the REJ+ group as compared with those in the REJ- group during the same period (Figure 2). This suggests an inverse correlation between LT_{mL} and the state of immune responsiveness, reflected by the total

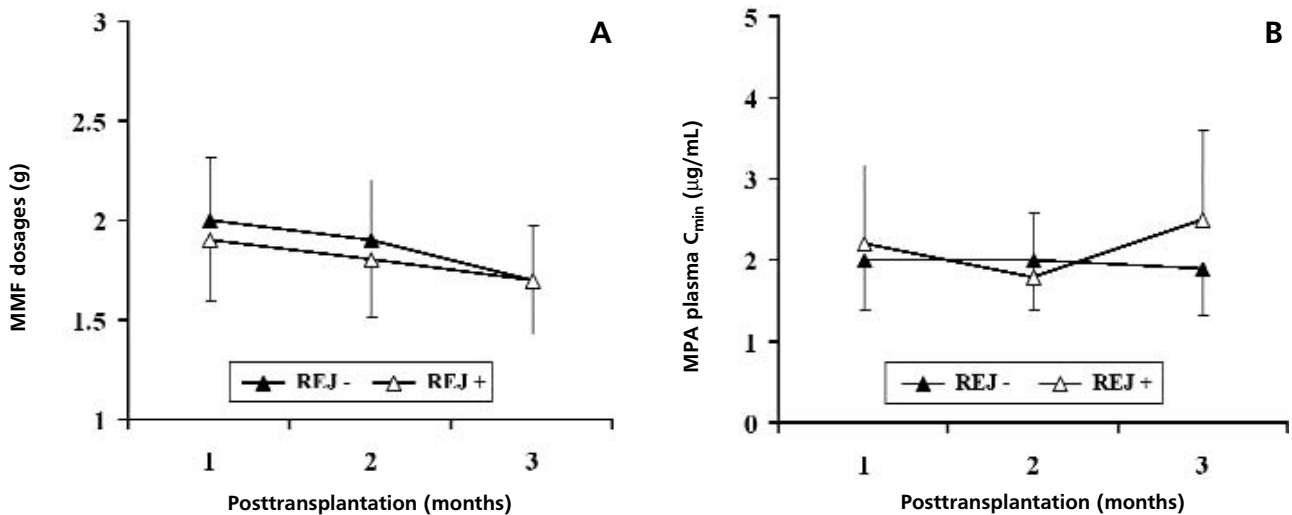


Figure 4. Temporal variations of mycophenolate mofetil monitoring parameters: Mycophenolate mofetil dosage (A) and mycophenolic acid plasma trough level (B) in rejecting and nonrejecting patients during the first 3 months of the study. Results are expressed as means $\pm$ SD.

lymphocyte count. These results seem to confirm our recent findings [9, 10] and are in agreement with recent observations regarding the relationship between CsA lymphocyte content, the degree of calcineurin inhibition [14], and the degree of reduction in lymphocyte proliferation [15].

These differences in CsA LT_mL between the 2 groups could not be attributed to variations in CsA whole blood levels. In fact, and unexpectedly, REJ+ patients exhibited either significantly higher or comparable C_{max} at 2 and 3 months, respectively, when compared with patients in the REJ- group (Figure 3) indicating a lack of association between CsA blood levels and acute rejection. This confirms our and others' recent observations [3, 5, 9, 10]. This could be explained by the lack of correlation between CsA blood levels and total lymphocyte count [9, 10] probably related to the weak correlation between CsA blood levels and the degree of calcineurin inhibition [14, 16]. This is clearly in line with our recent suggestions [10, 17] about the obvious discordance between drug pharmacokinetics and pharmacodynamic effects, and hence, between bioavailability and bioactivity. Other possible mechanisms for the variations in LT_mL between the 2 groups might be a difference in the dose of immunosuppression during either the induction phase or the maintenance phase of therapy after transplantation. This is unlikely to be the case, however, since all patients were subjected to similar induction protocols, similar steroid tapering, and comparable MMF dosage and MPAC_{min} therapeutic

levels as shown in Figures 4A and 4B. This excludes any possible contribution of the above-mentioned parameters to the observed results. Furthermore, all monitoring parameters from the REJ- group also were obtained at the same time after transplantation (ie, when graft biopsies were performed). This is reflected by comparable CsA dosages, C_{max}, MMF dosages, and MPAC_{min}, as shown in Table 1, and indicates that the differences in CsA LT_mL observed between the REJ+ and REJ- groups were mainly related to interpatient variations in CsA lymphocyte content. These variations may be related to the combined effects of both genetics [18, 19, 20] and environmental parameters [10, 21] (eg, hematocrit, the major extra lymphocyte binding site of CsA). However, the comparable mean hematocrit level in the REJ+ and the REJ- groups at 1, 2, and 3 months after transplantation (30%, 37%, 37%, and 30%, 37%, 37%, respectively) excludes this parameter as a possible explanation for the striking differences in LT_mL between the groups.

It is well established that genetic factors and genetic variations for drug metabolizing enzymes [22, 23, 24, 25], drug transporters [21, 26], drug receptors [18, 19], and the baseline activity of a target enzyme and its recovery under immunosuppression [17, 27] account for nearly half of the individual variability in the efficacy and toxicity of drugs. In our study, the difference in LT_mL between the REJ+ patients and REJ- patients might be related to genetic differences in drug transporters and/or receptors that affect the rate of CsA incorporation into lymphocytes [21, 26].

Therefore, a low expression of cyclophilin B (CsA plasma transporter) and/or CsA lymphocyte binding sites, as would be the case in the REJ+ patients (Figure 1, Table 1), could negate the immunosuppressive effect of CsA by means of a lack of calcineurin inhibition. This would increase the risk for acute rejection. Conversely, a higher expression of these parameters in the REJ- patients would ensure adequate immunosuppression and hence, minimize the risk of acute rejection.

Interestingly, our results failed to show an association between CsA nephrotoxicity and either of the CsA monitoring parameters (ie, $C_{\max}$ or LT_mL). Paradoxically, CsA nephrotoxicity patients had noticeably lower $C_{\max}$ than did patients in the REJ+. These findings are in accordance with our recent observations [9, 11] and appear to confirm our previous hypothesis that CsA renal susceptibility also may be genetically controlled [18, 20] and predominantly donor dependant, with variable degrees of recipient influence related to drug absorption, metabolism, and cellular binding [3, 10, 28, 29].

In conclusion, the present study clearly demonstrates in a prospective manner that acute rejection in de novo kidney transplant patients is associated with a relatively lower CsA maximum lymphocyte binding profile and a higher total lymphocyte count in the early posttransplant period. When compared with REJ- patients, REJ+ patients exhibited a low CsA lymphocyte content despite paradoxically higher or similar CsA blood levels ($C_{\max}$). These observations seem to confirm our hypothesis regarding the obvious lack of association between whole blood drug levels (a marker of bioavailability) and target cell drug concentration (an indicator of bioactivity and a strong correlate of the immune response) and the clinical outcome as well, mainly acute rejection. As we have previously demonstrated, all CsA monitoring parameters ($C_{\max}$ and LT_mL) failed to demonstrate a significant relationship with any nephrotoxic effect. In fact, nephrotoxicity patients exhibited lower $C_{\max}$ than did their counterparts in the REJ+ group at the time of graft biopsies despite similar CsA dosages.

Our data suggest that in patients with graft dysfunction and an $LT_mL < 50$ pg/Lc, $C_{\max}$ monitoring may be unreliable and actually could be misleading in predicting the clinical diagnosis. $CsALT_mL \geq 80$ pg/Lc, ≥ 60 pg/Lc, and ≥ 50 pg/Lc during the first, second, and third months after transplantation, respectively, appear to provide adequate protection against acute rejection, irrespective of $C_{\max}$. $CsALT_mL$ seems to offer a new and more reliable alternative to

$C_{\max}$ for CsA therapy monitoring and tapering in kidney transplantation. Larger multicenter and probably multinational studies are required to confirm these interesting preliminary results.

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