Monitoring immunosuppression after liver transplantation : development of individualized Bayesian limited sampling monitoring

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CHAPTER 2

SWITCHING MONITORING OF EMULSIFIED CYCLOSPORINE FROM TROUGH LEVEL TO 2-HOUR LEVEL IN STABLE LIVER TRANSPLANT PATIENTS

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ABSTRACT

Background: After orthotopic liver transplantation (OLT) many patients use emulsified cyclosporine. Recent data showed that blood levels 2 hours after dosing (C-2) better reflect systemic exposure to the drug (area under the blood concentration time curve) than trough levels (C-0) do.

Methods: We investigated difference in dosage, creatinine clearance (CrCl), blood pressure (BP), freedom from rejection, and relation of C-2, C-0, and AUC while switching 31 stable patients more than 6 months after OLT from C-0 to C-2 monitoring. With C-0 between 90 and 150 ng/ml we collected 24-hour urine, while blood samples were taken at t = 0, 1, 2, 3, 4, 6 and 8 hours after dosing to measure cyclosporine, creatinine, liver tests, and blood pressure and calculated AUC and CrCl. Target AUC was calculated based on C-0. Then the dose was adjusted to two subsequent C-2 values of 600 ng/ml ± 15%, the above was repeated, and the differences were assessed.

Results: Cyclosporine dose was reduced in 21/31 patients (68 %) and remained unchanged in 10/31 (32%) after conversion. Mean lowering was 69 mg daily (26.9 %, P < 0.0001). After dose reduction the mean increase of CrCl was 7.93 ml/min (11.6 %, P = 0.016). Only systolic and mean morning BP decreased slightly but significantly. C-2 correlated better with AUC0-12 (r^2=0.75) than C-0 (r^2=0.64). However, 13/21 patients had a second AUC below target AUC and 2 of these 13 patients developed rejection after conversion to C-2 levels.

Conclusion: While C-0 monitoring frequently results in overdosing and more renal dysfunction, C-2 monitoring may lead to episodes of underdosing and rejection. Therefore better ways of monitoring cyclosporine dosing need to be devised.

INTRODUCTION

After orthotopic liver transplantation (OLT) many centers use the microemulsion formulation of cyclosporine (Neoral®) as immunosuppressant[1]. There is a small therapeutic window between too low systemic exposure to the drug, resulting in rejection, and too high systemic exposure, leading to adverse effects such as renal insufficiency and elevated blood pressure. Usually Neoral is given twice daily. Until recently dosage was based on trough-level (C-0) monitoring. Recent data, however, mostly derived from kidney transplantation but also from heart, lung and liver transplantation, show that blood levels 2 hours after dosing (C-2), better than trough levels reflect the systemic exposure over the first 12 hours after dosing (= AUC as gold standard)[2-5]. Based on these and other studies it has been recommended that monitoring based on trough levels should be replaced by monitoring based on C-2 levels both for initial therapy and for maintenance tretment[6,7]. However, only limited data have been published on the results of C-2 monitoring in liver transplantation[8-15]. In the present study we investigated the possible influence of the conversion from C-0 monitoring to C-2 monitoring in stable patients more than 6 months after liver transplantation in the dose, creatinine clearance (CrCl), blood pressure, and freedom from rejection, with the hypothesis that there was no such influence. Furthermore, we calculated the AUC before and after this change in monitoring, and we investigated relationships between blood concentrations at 0 and 2 hours and systemic exposure to the drug.

PATIENTS AND METHODS

The study included 31 stable patients who were at least 6 months post-OLT (21 men, mean age 52, range 31-64 years; 10 women, mean age 39, range 20-58 years). One patient had a biliodigestive (Roux-en-Y) anastomosis, and 30 patients had a duct-to-duct choledochus anastomosis. All patients received Neoral cyclosporine (Neoral) twice daily and were maintained on a stable Neoral dose with two consecutive trough levels (C-0) between 90 and 150 ng/ml before entering the study. Co-medication consisted of mycophenolate mofetil in 9 patients (4 with prednisone), azathioprine in 8 patients (4 with prednisone), and prednisone alone in 8 patients; 6 patients had no immunosuppressive co-medication.
During the day of the AUC, 24-hour urine was collected for measurement of creatinine concentration. Five minutes before the morning dose (approximately 10:00 AM) of Neoral (t = 0), blood samples were taken for liver and kidney function and Neoral concentration.

Further blood samples for Neoral concentration were taken 1, 2, 3, 4, 6 and 8 hours after the morning dose of Neoral. For t = 12 we took the trough level (t = 0), because all our patients were dosed with Neoral twice daily. Blood was taken using an indwelling catheter and was collected in a vacutainer containing EDTA. Whole blood Neoral concentrations were determined by Fluorescence Polarisation Immuno Assay (FPIA, Axsym, Abbott Diagnostics, Abbott Park, IL). In order to avoid an influence (however small) from meals, the patients were instructed to take only a light breakfast with tea and a biscuit on the morning of measuring the AUC, and until the 2-hour sample (C-2) was taken, the patients took no additional food or drinks[16]. Between t = 1 and t = 2 and between t = 6 and t = 8, blood pressure was measured automatically (Dynamap) for one-half hour (morning BP and afternoon BP) with the patient in a reclining chair. Then, according to the recommendations by E. Cole et al.[6], the dose was adjusted to a Neoral level at t = 2 (C-2, peak level) within the target range of 510 and 690 ng/ml (600 ± 15%) using the formula: new dose = old dose x (600/ C-2). Two weeks after the day the first AUC was measured while on C-0 monitoring ("day 1") and the contingent adjustments, the patients came to the clinic for a checkup and a blood sample, which was taken exactly two hours after the morning dose of Neoral (C-2). Further dose adjustments were made using the same formula within weeks. Blood pressure medication was not adjusted during the study. When two subsequent C-2-values were within the target-range, patients were invited for a second day, when the AUC was measured ("day 2") similar to the first "AUC-day" ("day 1"). Again 24-hour urine was collected for the creatinine concentration and blood samples were taken for liver and kidney function tests. The AUC_{0-12h} of all 62 (2 x 31) curves was calculated using the trapezoidal rule[17], and relationships with C-0 and C-2 were investigated. Differences in second and first C-0, C-2 and AUC and their relation, and changes in renal function, liver functions, and blood pressure were assessed. The "target AUC range" was calculated based on the C-0 range of 90-150 ng/ml, using the linear regression line formula describing the relation of C-0 with AUC_{0-12h}.

Statistical Analysis
Statistical analysis was performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL). Results are expressed as mean ± SEM and as median and range (Wilcoxon-test). Potential differences were explored with Paired-Samples T-test, and relationships were investigated using Pearson correlation test and linear regression analysis. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Dose Adjustments

Of the 31 patients 21 (68%) needed a lower dose of Neoral when dosing was based on C-2 monitoring instead of C-0 monitoring. In 10 patients (32%) no change in the dosage of Neoral was necessary and none of the patients required a higher dosage after conversion to C-2 monitoring. In patients in whom the dose was lowered, the dose on day 2 (median 200 mg, range 150-250 mg) was significantly lower than the dose on day 1 (median 250 mg, range 200-350 mg), reduction of 26.9 % of initial dose, $P < 0.0001$, Fig. 1.
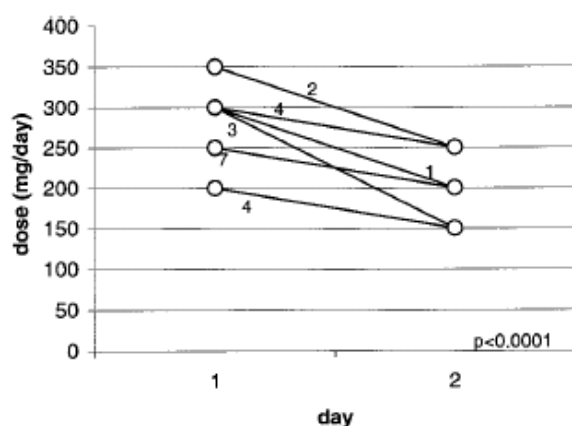

Figure 1. The dose of Neoral on day 1 (269 ± 10 mg) and day 2 (198 ± 9 mg) for patients whose dose was reduced (n = 21). The numbers represent the number of patients per line.
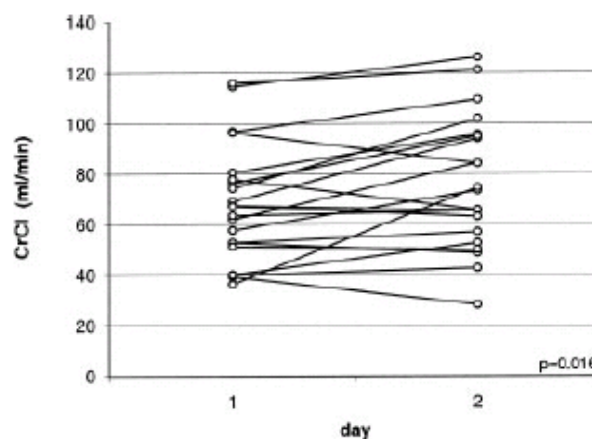
Figure 2. The creatinine clearance (CrCl) on day 1 (68.2 ± 5.1 ml/min) and day 2 (76.1 ± 5.7 ml/min) for patients with dose reduction (n = 21).

Kidney Function and Blood Pressure

Of the 21 patients whose dose was lowered, we calculated the creatinine clearance (CrCl) before (day 1) lowering and after (day 2) lowering of the dose. The mean increase of the CrCl in these patients was 7.93 ± 3.0 ml/min (11.6% of initial CrCl, $P = 0.016$, Fig. 2). The change in systolic blood pressure (morning and afternoon) was − 4.1 ± 1.6 mmHg and +1.52 ± 1.95 mmHg (− 3.1 % and +1.2%, $P = 0.018$ and $P = 0.444$). The change in diastolic blood pressure (morning and afternoon) was − 1.33 ± 0.98 mmHg and +0.048 mmHg ± 1.26 (−1.6 % and 0.00 %, $P = 0.188$ and $P = 0.970$). The differences in the mean arterial pressure (morning and afternoon) were − 2.62 ± 1.09 mmHg and 0.00 ± 1.52 mmHg (− 2.6 % and 0.00 %, $P = 0.026$ and $P = 1.000$) respectively.

Estimation of Systemic Exposure (AUC) while on C-2 Monitoring versus AUC while on C-0 Monitoring

C-2 monitoring correlated better ($r^2 = 0.75$, Fig. 3) than the C-0 monitoring ($r^2 = 0.64$, Fig. 4) with the area under the curve (AUC0-12h). The mean AUC on day 1 was 4588 ± 171 µg.h/L, median 4229 µg.h/L, range 3261–6423 µg.h/L. The mean AUC on day 2 was 3210 ± 117 µg.h/L, median 3195 µg.h/L, range 2380-4096 µg.h/L, $P < 0.0001$ (Fig. 5). Figure 6 shows the difference of C-0 values on the first and the second day ($P < 0.0001$).
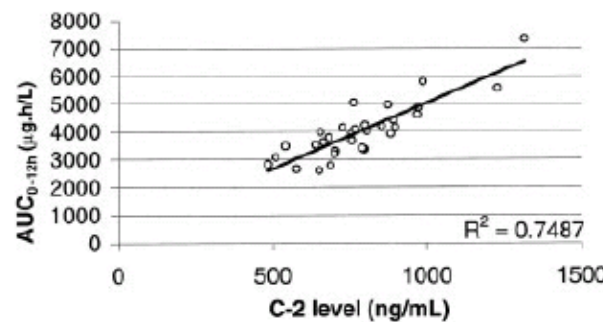

Figure 3. The correlation of the Neoral concentration 2 hours after dosing (C-2) with the area under the curve from 0 to 12 hours after dosing (AUC_{0-12h}) on day 2.
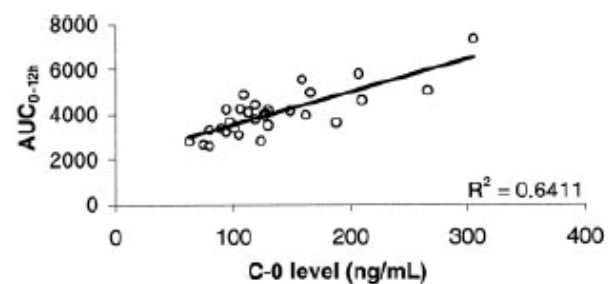

Figure 4. The correlation of the trough-Neoral concentration (C-0) with the area under the curve from 0 to 12 hours after dosing (AUC_{0-12h}) on day 1.
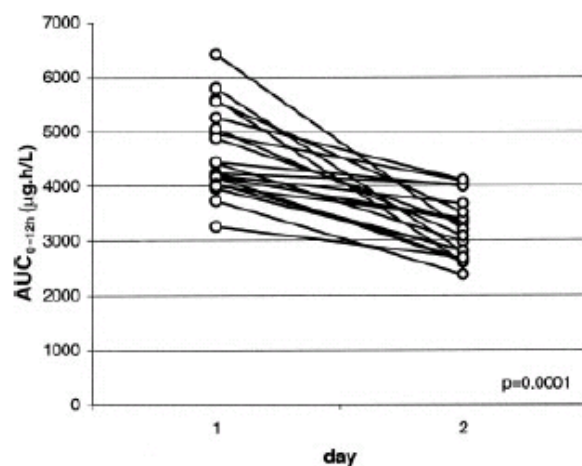

Figure 5. The AUC_{0-12h} of Neoral on day 1 (4588 ± 171 µg.h/L) and day 2 (3210 ± 117 µg.h/L) in patients whose Neoral dose was lowered (n = 21).
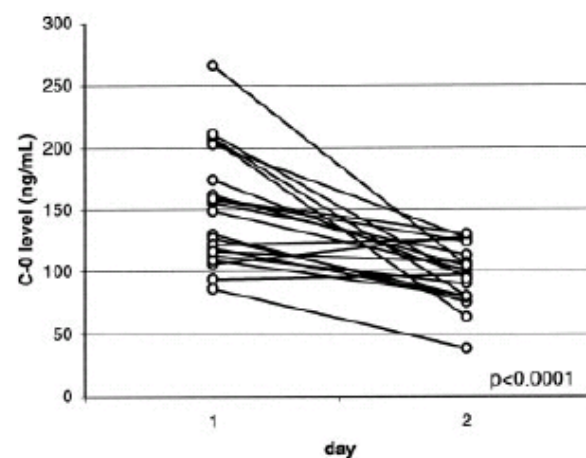

Figure 6. The C-0 of Neoral on day 1 (mean, 151 ± 10 ng/mL) and day 2 (97 ± 5 ng/mL) in patients whose dose was reduced (n = 21).

C-2 Values on Day 1 and Day 2 in Relation to C2 Target Range

As mentioned above, while on C-0 monitoring, C-2 was above the target C-2 in 21/31 patients. In 10/21 patients whose Neoral dose was lowered there were variable C-2 levels; C-2 was outside the target range on day 2 with the same dose after two subsequent C-2 values of 600 ng/mL ± 15%. Mean C-2 value in the 21 patients whose dose was lowered was 666 ± 23 ng/mL (Fig. 7); however, on day 2 just 1/21 of C-2-values was below the target range (C-2 = 485ng/mL) and 9/21 were above the C-2 target range (mean of these 9: 765 ± 20 ng/mL). Also, 7/10 patients with an unchanged Neoral dose had variable C-2 levels with values of C-2 outside the C-2 target range on day 2 (the second "AUC-day").
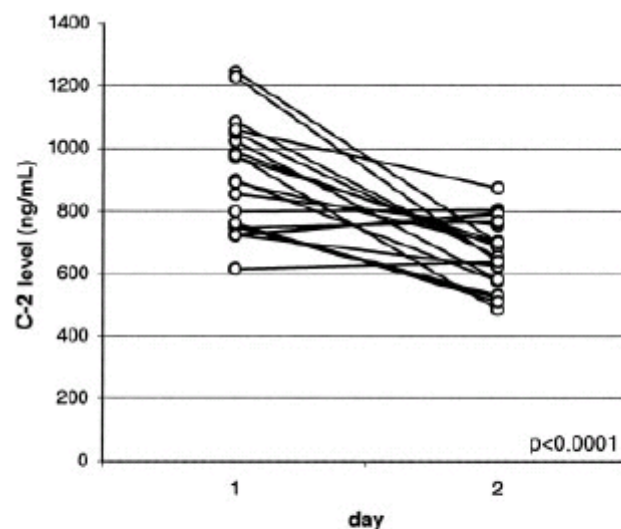
Figure 7. The C-2 of Neoral on day 1 (mean, 898 ± 38 ug/L) and day 2 (666 ± 23 ng/mL) in patients whose dose was reduced (n = 21).

AUC on Day 2 in Relation to Target AUC

We found that 13/21 patients whose Neoral dose was lowered ended below the "target AUC" and were therefore below the lowest exposure on C-0 monitoring. This target AUC is based on the C-trough (C-0) and was calculated with linear regression analysis (Fig. 4). The formula of the line is:

$AUC_{0-12h} = 14.75 \times C\text{-trough} + 2053$ (trendline).

The target range of the trough-levels is 90 - 150 ng/mL; therefore, the AUC target range is 3380 - 4266 µg.h/L. The other 8/21 patients showed a second AUC within the range of the target AUC. As expected, no patient whose Neoral dose was lowered had an AUC on day 2 above the highest AUC on day 1.

C-2 and AUC on Day 2 in Relation to Each Other and in Relation to the Target Ranges

Table 1 shows the C-2 and AUC0-12h of Neoral on day 2 in relation to the target ranges of C-2 and AUC0-12h in the patients in whom the dose was lowered (n = 21). Mean AUC on day 2 was 3543 ± 109 µg.h/L. Of those patients in whom the Neoral dose was lowered and whose second AUC was below the target AUC, 2/13 developed acute cellular rejection with aminotransferases up to 500 U/L, requiring additional corticosteroids and an increase in Neoral dose after the second AUC. These 2 patients were 9 and 10 months after OLT; both had prednisone as co-medication and one also had mycophenolate mofetil (MMF) as co-medication. Of the 31 patients, 4 were within 6 - 12 months after OLT; the low AUCs were not limited to these 4 patients. However, the two patients experiencing rejection were among these 4 patients.

Table 1. The C-2 and AUC$_{0-12h}$ of Neoral on Day 2 in Relation to the Target Ranges of C-2 and AUC$_{0-12h}$ in the Patients in Whom the Dose Was Lowered (n = 21)

	AUC Below Target Range (<3380 µg.h/L)	AUC in Target Range (3380–4266 µg.h/L)	AUC Above Target Range (>4266 µg.h/L)	Total
C-2 below target range (<510 ng/mL)	1	0	0	1
C-2 in target range (510–690 ng/mL)	8	3	0	11
C-2 above target range (>690 ng/mL)	4	5	0	9
Total	13	8	0	21

In order to reach the subsequent C-2 values of 600 ng/mL ± 15 %, we needed 1.57 ± 0.19 (median 1.00; range 1-3) dose adjustments. Patients with the peak level at 1 hour after dosing had an AUC within the target range as often as did patients with the peak level at 2 hours post-dosing. Table 2 shows the C-2 and AUC0-12h of Neoral on day 2 in relation to the target ranges of C-2 and AUC0-12h in the patients whose dose was not changed (n=10).

Table 2. The C-2 and AUC$_{0-12h}$ of Neoral on Day 2 in Relation to the Target Ranges of C-2 and AUC$_{0-12h}$ in the Patients Whose Dose Was Not Changed (n = 10)

	AUC Below Target Range (<3380 µg.h/L)	AUC in Target Range (3380–4266 µg.h/L)	AUC Above Target Range (>4266 µg.h/L)	Total
C-2 below target range (<510 ng/mL)	0	0	0	0
C-2 in target range (510–690 ng/mL)	0	3	0	3
C-2 above target range (>690 ng/mL)	1	3	3	7
Total	1	6	3	10

Differences between Subgroups of Patients

Because only 1 patient with a hepaticojejunostomy was included, no differences between this patient and the other 30 with a duct-to-duct anastomosis could be assessed. No differences in C-2 or AUC of patients with different immunosuppressive co-medications were found, although the number of patients is too small to reliably assess differences between these groups.

Sparse Sampling and AUC0-12h

If AUC is calculated, using the trapezoidal rule, from cyclosporine levels on time points 0, 1, 2, and 3 hours, the correlation with AUC0-12h was $r^2 = 0.96$.

DISCUSSION

During the conversion from C-0 to C-2 cyclosporine monitoring in stable patients more than 6 months after liver transplantation, we saw a significant decrease in cyclosporine dose in two-thirds and an unchanged dose in one-third of the patients. Dose reduction resulted in lower systemic exposure and an improvement of renal function, but only small changes in morning systolic and mean morning blood pressures were observed, with questionable clinical significance. The fact that the kidney function did not improve in all patients may be due to long-term exposure to Neoral, which may have caused a fixed renal insufficiency. Also, further improvement in renal function may require more time. Based on calculating the area under the curve from 0 to 12 hours (cyclosporine blood levels), the correlation of C-2 with AUC was better than the correlation of C-0 with AUC from 0-12 hours. However, in almost one-half of the patients, there was significant intrapatient variability of the C-2 blood levels with the same dose. This made therapeutic drug monitoring with C-2 levels less accurate and may induce many unnecessary subsequent changes in drug dose, which is inconvenient for patients, doctors, and nurses. We found it disturbing that, although two preceding C-2 levels were within the 600 ng/mL ± 15% range, in 13/21 patients whose dose was lowered the second AUC was below the target AUC, while indeed 2 of these 13 patients developed rejection. The fact that these patients were 9 and 10 months post OLT may mean that the dose recommendations of G. Levy and not those of E. Cole should be followed when using C-2 monitoring[6,7]. Further investigations assessing this point may be needed. While on C-2 monitoring, 17/31 patients had a second AUC outside the target AUC. For all patients it may not be necessary to have an AUC within the range of the "target range AUC", but it certainly seems safer if this is the case. Probably the best situation is to have an AUC on day 2 in the lower half of first AUCs, which is

3380 – 3823 µg.h/L. Because 11/13 patients with a second AUC below the target AUC did not develop rejection, some patients may tolerate lower AUCs.

Other studies saw a better correlation of C-2 with AUC when compared to trough-level monitoring in renal and liver graft recipients[3-15]. Most studies in renal transplantation and the limited studies in liver transplantation using C-2 monitoring also showed improved kidney function, and often blood pressure and serum cholesterol also improved. In those studies no rejection occurred despite lower exposure to cyclosporine. However, in the liver transplant studies mentioned AUC was calculated by measuring Neoral blood levels during 4 and 6 hours only, while we used 0-12 hour AUCs. This fact may explain part of the difference between these and our studies. Another explanation may be the lower maintenance levels used in liver transplantation when compared to kidney transplantation: further lowering of the dose may more easily lead to rejection. All samples were taken as recommended[6,7,18] and within 2 minutes from the targeted time (although 10 minutes are allowed); if sampling time would have been more variable (as may be the case in daily practice), an even lower accuracy of C-2 monitoring and inappropriate dose adjustments might occur[19]. In renal transplantation variable cyclosporine levels may contribute to chronic rejection[20]. Although chronic ductopenic rejection has become less common after liver transplantation in the past decade, it forms a continuum with acute cellular rejection; chronic underexposure to cyclosporine can be a cause[21-24]. In renal transplant studies it was shown that absorption profiling over the first 4 hours was superior to trough-level monitoring, with C-2 as the best single-point predictor of AUC[3,25-28]. The clinical superiority of such absorption profiling over C-2 levels has not been examined in those studies. Our data demonstrate that in stable liver transplant patients trough-level monitoring frequently leads to overdosing of cyclosporine, while monitoring by C-2 may cause episodes of underdosing. Therefore, better ways of monitoring cyclosporine dosing in liver transplantation remain to be devised. Because both IL2 blood concentration and 12-hour AUC are related to cyclosporine exposure in the first 4 hours after dosing it seems logical to use a sparse-sampling method over the first hours after dosing. In accordance with others, our data demonstrate that, if AUC is calculated from cyclosporine levels, using the trapezoidal rule, in the first three hours after dosing the correlation with AUC_{0-12h} is 0.96[25, 29]. Thus use of this method may avoid over- and underdosing and unnecessary changes in dose. A disadvantage is the need for fixed time points. The ideal model should be easy to use and flexible, without the rigid time points used in current multiple-sampling methods, and it should be based both on population kinetics and on individual pharmacokinetics[30-34]. We are currently developing such a model.

In conclusion, while C-0 monitoring frequently results in overdosing and more renal dysfunction, C-2 monitoring may lead to episodes of underdosing and rejection. Therefore, better ways of monitoring cyclosporine dosing need to be devised.

REFERENCES

1. Dunn CJ, Wagstaff AJ, Perry CM, Plosker GL, Goa KL. Cyclosporin: an updated review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral®) in organ transplantation. Drugs. 2001;61(13):1957-2016.

2. Levy GA. C2 monitoring strategy for optimising cyclosporin immunosuppression form the Neoral formulation. BioDrugs. 2001;15(5):279-90.

3. Nashan B, Cole E, Levy G, Thervet E. Clinical validation studies of neoral C2 monitoring: a review. Transplantation 2002;73:S3-S9 .

4. Cantarovich M, Elstein E, de Varennes B, Barkun JS. Clinical benefit of Neoral® dose monitoring with cyclosporine 2-hour post-dose levels compared with trough levels in stable heart transplant patients. Transplantation 1999; 68: 1839-42.

5. Glanville AR, Hopkins PM, AboyounCL, Chhajed PN, Plit ML, Malouf MA. Clinical utility of cyclosporin C2 monitoring after lung transplantation. J Heart Lung Transpl 2002; 21: 143.

6. Cole E, Midtvedt K, Johnston A, Pattison J, O'Grady C. Recommendations for the implementation of Neoral C(2) monitoring in clinical practice. Transplantation. 2002 May 15;73(9 Suppl):S19-22.

7. Levy G, Thervet E., Lake J, Uchida K on behalf of the CONCERT group. Patient management by Neoral® C_2 monitoring: an international consensus statement. Transplantation 2002; 73 (9) (Suppl): S12-218.

8. Cantarovich M, Barkun JS, Tchervenkov JI, Besner JG, Aspeslet L, Metrakos P. Comparison of neoral dose monitoring with cyclosporine through levels versus 2-hr postdose levels in stable liver transplant patients. Transplantation. 1998 Dec 27;66(12):1621-7.

9. Grant D, Kneteman N, Tchervenkov J, Roy A, Murphy G, Tan A, Hendricks L, Guilbault N, Levy G. Peak cyclosporine levels (Cmax) correlate with freedom from liver graft rejection: results of a prospective, randomized comparison of neoral and sandimmune for liver transplantation (NOF-8). Transplantation. 1999 Apr 27;67(8):1133-7.

10. Lake JR, on behalf of the Neo-INT-06 Study Group. Benefits of cyclosporine microemulsion (Neoral) C_2 monitoring are sustained at 1 year in de novo liver transplant recipients. Transplant Proc 2001; 33: 3092-3093.

11. Levy GA. Neoral C_2 in liver transplant recipients. Transplant Proc 2001; 33: 3089-3091.

12. Levy G, O´Grady C, Lilly LB, Grant D, Girgrah N, Greig PD. C_2 monitoring in liver transplantation with Neoral immunosuppression: Effect of achieving C_2 target early on efficacy and safety. Am J Transplant 2001; 1 (suppl 1): 310.

13. Dunn S, Falkenstein K, Cooney G. Neoral C_2 monitoring in pediatric liver transplant recipients. Transplant Proc 2001; 33: 3094-3095.

14. Barakat O, Peaston R, Rai R, Talbot D, Manas D. Clinical benefit of monitoring cyclosporine C2 and C4 in long-term liver transplant patients. Transplant Proc 2002; 34(5): 1535-7.

15. Levy G, Burra P, Cavallari A, Duvoux C, Lake J, Mayer AD, Mies S, Pollard SG, Varo E, Villamil F, Johnston A. Improved clinical outcomes for liver transplant recipients using cyclosporine monitoring based on 2-hr post-dose levels (C_2). Transplantation 2002; 73: 953-959.

16. Kahan BD, Dunn J, Fitts C, et al. Reduced inter- and intrasubject variability in cyclosporine pharmacokinetics in renal transplant recipients treated with a microemulsion formulation in conjunction with fasting, low-fat meals, or high-fat meals. Transplantation 1995; 59: 505-511.

17. Rowland M, Tozer TN. Clinical pharmacokinetics, concepts and applications. Lea & Febiger, Philedelphia, 1989:459-463.

18. Kahan BD, Keown P, Levy GA, Johnston A. Therapeutic drug monitoring of immunosuppressant drugs in clinical practice. Clin Ther 2002; 24 (3): 1-21.

19. Saint-Marcoux F, Rousseau A, Le Meur Y, Estenne M, Knoop C, Debord J, Marquet P. Influence of Sampling-Time Error on Cyclosporine Measurements Nominally at 2 Hours after Administration . Clinical Chemistry. 2003;49:813-815.

20. Stoves J, Newstead CG. Variability of cyclosporine exposure and its relevance to chronic allograft nephropathy: a case-control study. Transplantation 2002; 74: 1794-7.

21. Wiesner RH, Ludwig J, Krom RA, Hay JE, van Hoek B. Hepatic allograft rejection: new developments in terminology, diagnosis, prevention, and treatment. Mayo Clin Proc. 1993 Jan;68(1):69-79.

22. van Hoek B, Wiesner RH, Krom RA, Ludwig J, Moore SB. Severe ductopenic rejection following liver transplantation: incidence, time of onset, risk factors, treatment, and outcome. Semin Liver Dis. 1992 Feb;12(1):41-50.

23. Wiesner RH, Ludwig J, van Hoek B, Krom RA. Current concepts in cell-mediated hepatic allograft rejection leading to ductopenia and liver failure. Hepatology. 1991; 14(4 Pt 1):721-9.

24. Wiesner RH, Demetris AJ, Belle SH, et al. Acute hepatic allograft rejection: incidence, risk factors and impact on outcome. Hepatology 1998; 28: 638-645.

25. International Neoral Renal Transplantation Study Group. Randomized, international study of cyclosporine microemulsion absorption profiling in renal transplantation. Am J Transplant 2002; 2(2): 157-66.

26. Kazancioglu R, Goral S, Shockley SL, Feurer ID, Ramanathan V, Helderman JH, VanBuren D. A systematic examination of cyclosporin area under the curve in renal transplant recipients. Transplantation 2002;73:301-302.

27. Canadian Neoral Renal Transplantation Study Group. Absorption profiling of cyclosporine microemulsion (Neoral) during the first 2 weeks after renal transplantation. Transplantation 2001; 72: 1024-32.

28. Johnston A, David OJ, Cooney GF. Pharmacokinetic validation of Neoral absorption profiling. Transplant Proc 2000; 32 (3Suppl.1): S 53-S 56.

29. Sindhi R, LaVia MF, Paulling E, et al. Stimulated response of peripheral lymphocytes may distinguish cyclosporine effect in renal transplant recipients receiving a cyclosporine + rapamycin regimen. Transplantation 2000; 15: 69: 432-436.

30. Camps-Valls G, Porta-Oltra B, Soria-Olivas E, Martin-Guerrero JD, Serrano-Lopez AJ, Perez-Ruixo JJ, Jimenez-Torres NV. Prediction of cyclosporine dosage in patients after kidney transplantation using neural networks. IEEE Trans Biomed Eng 2003; 50: 442-8.

31. Leger F, Debord J, Le Meur Y, Rousseau A, Büchler M, Lachatre G, et al. Maximum a posteriori Bayesian estimation of oral cyclosporine pharmacokinetics in stable renal transplant patients. Clin Pharmacokinet 2002;41:71-80.

32. Monchaud C, Léger F, Rousseau A, David O, Cooney G, Marquet P. Bayesian forecasting of oral cyclosporine in cardiac transplant recipients. Ther Drug Monit 2001;23:468.

33. Debord J, Risco E, Harel M, Le Meur Y, Büchler M, Lachâtre G, et al. Application of a gamma model of absorption to oral cyclosporin. Clin Pharmacokinet 2001;40:375-382.

34. Rousseau A, Monchaud C, Debord J, Vervier I, Estenne M, Thiry P, et al. Bayesian forecasting of oral cyclosporine pharmacokinetics in stable lung transplant recipients with and without cystic fibrosis. Ther Drug Monit 2003;25:28-35.