

## Cyclosporine Trough Levels in Renal Graft Recipients

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**Blood Trough Levels (TL) of cyclosporine (CyA) [the drug panimun bioral® cyclosporine oral solution USP - modified-Panacea Biotec Ltd], were monitored in 103 renal transplant patients who were receiving CyA orally. Two hundred and sixty-two blood concentrations of CyA were determined using a validated HPLC assay over a period of two years. Mean dose from week 1 until 2 years ranged from  $7.65 \pm 0.9$  to  $2.73 \pm 0.8$  mg/kg. Mean blood CyA levels ranged from  $197.4 \pm 87.5$  to  $205.9 \pm 113.5$ . The TL concentration changes versus dose reductions were not markedly different after 2-4 weeks and remained within therapeutic range. Stabilised concentrations were achieved after first month. We conclude that the blood TL of CyA were in the nominal therapeutic range suitable for renal transplant patients.**

CyA forms the essential component of the post-transplant (PTx) immunosuppressive drug therapy. High molecular weight, lipophilic nature and window specific absorption decrease the bioavailability of CyA<sup>1,2</sup>. This makes clinical dose adjustments difficult, often resulting in increased incidence of over or under dosing and subjecting more patients to either rejection or nephrotoxicity. Microemulsion formulations of CyA have resulted in enhanced absorption of CyA in the body<sup>3,4</sup>. Linear pharmacokinetics from formulated CyA has been reported<sup>5</sup>.

Adequate CyA dose would reduce incidence of nephrotoxicity without affecting its bioavailability. If the dose is tapered cautiously and critically after transplantation, viable therapeutic concentrations may be achieved. Area under the curve (AUC) and TL<sup>6</sup> are still the most popular methods to monitor CyA levels in the body. While TL is more applicable due to better patient compliance and ethical reasons. Several authors have reported specific TL ranges to achieve adequate immunosuppression<sup>7-12</sup> at different doses.

The aim of our current study was to report the results of TL monitoring strategy in our patients and view these levels at various PTx periods in relation to the dosage.

### METHODS

**Subjects** : One hundred and three inpatients / outpatients (83 males and 20 females) receiving CyA as part of triple drug regimen after kidney transplantation were examined. They were followed up for determinations of their blood CyA levels in PTx period.

**Study design** : Patients were prescribed CyA in twice daily dosing schedule and blood level screening was performed according to post transplant time distribution. Other category of drugs which many of these patients were also receiving included, antibiotics, antianginals, antiulcer, antidiabetics and antituberculosis. Levels were analysed irrespective of these drug interactions.

**Collection of blood samples:** Venous blood samples were collected from the cubital vein in glass tubes containing EDTA. These were kept frozen at  $-20^{\circ}\text{C}$  until assayed in under 1 week.

**Analysis** : All reported blood CyA determinations were analysed by a validated reverse phase HPLC<sup>13</sup>. Commercially available BIO-RAD validation kit was used for analysis.

### RESULTS

PTx classified data from two hundred and sixty-two determinations indicates that  $91.4 \pm 3.9\%$  determinations of CyA TL(s) in whole blood were above 100 ng/ml while only 2% determinations were below 50 ng/ml (Table 1). Only  $14.8 \pm 8.8\%$  determinations exceeded concentrations above 400 ng/ml. Average concentration (Cav) at various PTx periods is shown in Table 2. Average blood CyA concentrations in all PTx periods were confined in the nominal therapeutic TL range of 134.8 - 321.7 ng/ml. The highest average TL(s) [ $321.7 \pm 168.4$ ] were seen in the period between 1 - 3 months at an average mean dose of  $5.8 \pm 1.27$  mg/kg. Percent coefficient of variation (%CV) of average TL(s) in PTx concentration data was high ranging between 40.1 and 58%. (Table 3).

**Table 1 — Whole Blood Cyclosporine Determinations in Different Concentration Ranges in Classified PTx Periods (n = 262). [% observations in parenthesis]**

Concentration range (ng/ml)	1 week after	2-4 weeks after	1-3 months after	3-9 months after	9-24 months after
0-49	0 (0)	1 (2.0)	0(0)	0 (0)	0 (0)
50 -99	4(7.8)	4(8.3)	2(4.3)	4 (5.4)	6(13.9)
100-199	25(49)	15(31.2)	11(23.9)	27(36.5)	18(41.8)
200 - 299	17(33.3)	13(27.0)	13(28.2)	15(20.2)	12(27.9)
300 - 399	3(5.9)	5(10.4)	9(19.5)	14(18.9)	4 (9.3)
> 400	2(3.9)	10(20.8)	11(23.9)	14(18.9)	3 (6.9)
Total (n)	<b>51</b>	<b>48</b>	<b>46</b>	<b>74</b>	<b>43</b>

**Table 2 — Mean ± (SD) Whole Blood Cyclosporine (CyA) Concentrations (Cav) Dose, Dose range and Cyclosporin Level Generated /mg of CyA Dose across the Various PTx Periods in 262 CyA Blood Concentration Determinations in Renal Transplant Patients.**

Time post transplant	(Cav) (ng/ml)	Dose (mg/kg)	CyA level (ng/ml)/dose (mg/kg)
Week 1	197.4 ± 87.5	7.65 ± 0.9	25.96 ± 11.7
2-4 week	270.8 ± 156.9	6.86 ± 1.8	42.48 ± 35.6
1-3 months	321.7 ± 168.4	5.8 ± 1.27	55.0 ± 29.1
3-9 months	269.3 ± 156.3	4.48 ± 1.25	59.1 ± 29.5
9-24 months	205.9 ± 113.5	2.73 ± 0.8	59.4 ± 38.5

The dose of CyA was tailored from an average of 7.65 to 2.73 mg/kg in PTx periods upto 2 years (Table 2). Dose normalised levels (CyA level/ CyA dose) from each determination are also shown in Table 2.

## DISCUSSION

CyA has become well established as a potent immunosuppressive agent in the renal transplantation. However the therapy is complicated due to variable therapeutic effect of the drug. The need to maintain immunosuppression and avoid toxicity resulted in requirement of monitoring CyA levels within a limited range.

The universal use of TL monitoring to maintain CyA levels within the range of a hypothetical therapeutic window and the difficulty of interpreting some unexpected results emphasises the need to improve our understanding of the relationship between the dose and TL(s). Although the TL(s) of CyA are ultimately dependent on its dose but test results may not directly or immediately correlate with dose changes<sup>14</sup>. There is a delay before the levels reflect changes in dose.

**Table 3 — Coefficient of Variation (%CV) of Variabilities in CyA Concentrations, Dose and CyA Level/ mg of CyA Dose from 262 CyA Blood Concentration Determinations in Kidney Transplant Patients**

Time post-transplant	% CV (Cav)	% CV (Dose)
Week 1	44.9	11.7
Week 2- 4	57.5	26.2
1-3 months	52.3	21.8
3-9 months	58.0	27.9
9-24 months	55.1	26.6

It has been reported that despite the variability, majority of the dose changes are identifiable by TL determinations. Frequent changes in the dose also lead to therapeutic problems and the ideal situation may be an increase in interval between dose changes and frequency of TL testing following each

change. However, changing the CyA dosage may be difficult to resist when patients encounter problems with toxicity or rejection.

In our study a retrospective analysis was done and a relationship was observed between the change in dose of CyA and TL(s). Two years TL determinations were above 100 ng/ml in almost 92% of the patients and only 15% determinations exceeded 400 ng/ml. Till date doubt still persists regarding the TL(s) of CyA to prevent the rejection and nephrotoxicity. The mean average dose versus mean average concentration ranged between 7.65 mg/kg to 2.73 mg/kg and 197 mg/ml to 205 ng/ml respectively. The %CV of average dose and concentration stabilised after 2-4 weeks despite the dosage reduction.

Considerable variation in TL(s) can exist even in the absence of dose changes. The other influencing factors are testing errors, taking of samples at other than TL time, patient miscompliance and large bound compartment which makes the maintenance of drug levels within a limited range difficult. The rates of absorption, metabolism and elimination of CyA are also important factors to consider. Our efforts to understand dose adjustment effect on changes on TL(s) indicate that, despite the variabilities, the levels achieved were within the documented therapeutic window in most of the patients. Our results indicate that dose stabilised concentrations were effectively achieved after the first month and mean dose decreased by about 1 mg in given post transplant periods.

Blood levels in patients receiving CyA in progressive PTx periods were in nominal range over a follow up period of upto 2 years and dosage adjustments were therapeutically valid using TL determinations.

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## **REFERENCES**

1. Drewe J, Beglingen C and Kissel T, The absorption site of cyclosporin in the human gastrointestinal tract. *Br J Clin Pharmacol.* 1992 Jan;33(1): 39-43.
2. Raymond JP et al. On the dose dependency of cyclosporinA absorption and disposition in healthy volunteers. *J Pharmacokinetics Biopharm* 1988; 16: 331-353.
3. Mueller EA et al. Program and abstracts of the 13th annual meeting of the American society of transplant physicians. May 16 - 18; Chicago III. Abstract P2-3 1994.
4. Clinical data summary. Sandimmun Neoral, November 22, 1993. Sandoz Pharma Ltd, Basle, Switzerland.
5. Mueller EA, Kovarik JM, van Bree JB, Telzioff W, Grevel J and Kutz K. Improved dose linearity of cyclosporin pharmacokinetics from a microemulsion formulation. *Pharmaceut Res.* 1994 Feb; 11(2): 301-4.
6. Johnston A, Keown PA and Holl DW. Simple bioequivalence Criteria: Are they relevant to critical dose drugs? Experience gained from cyclosporin, *Ther Dr Monit.* 1997;19: 375-381.
7. Lindholm A and Kahan BD. Influence cyclosporin pharmacokinetics, trough concentrations and AUC monitoring on outcome after kidney transplantation. *Clin Pharmacol Ther* 1993 Aug; 54 (2): 205-15.
8. Kivisto KT. The review of assay methods for cyclosporin. Clinical implications. *Clin Pharmacokinetics* 1992 Sep; 23 (3): 173-90.
9. Kahan BD, Shaw LM, Holt D, Grevel J and Johnslon A. Consensus document: Hawk's Cay meeting on therapeutics drug monitoring of cyclosporin. *Clin Chem.* 1990 Aug; 36(8 pt 1): 1510-6.
10. Shaw LM. Advances in Cyclosporin pharmacology, measurement and therapeutic monitoring. *Clin Chem.* 1989 Jul; 35(7): 1299-1308.
11. Perna A, Gotti E, de Bernardis E et al. A logistic regression model provides novel guidelines to maximize the anti-acute rejection properties of cyclosporin with a minimum of toxicity. *J Am Soc Nephrol.* 1996; 7: 786-91.
12. Perico N and Remuzzi G. Prevention of transplant rejection: Current treatment guidelines and future developments. *Drugs* 1997 Oct; 54(4):533-70.
13. Morris RG, Tell SE and Ray JE. Cyclosporin A monitoring in Australia: Consensus recommendations, *Ther Dr Monit.* 1994 Dec; 16(6):570-6.
14. Takasugi M, Takasugi JK and Barba L. Monitoring changes in cyclosporine dosage by trough level testing. *Clin Transplantation.* 1995;9:98-105.