original paper

haematologica 2001; 86:311-315
http://www.haematologica.it/2001_03/0315.htm

Pharmacokinetic study of the new cyclosporin-A formulation (Neoral™) in adult allogeneic bone marrow transplant recipients

GIANPIETRO DOTTI, FLAVIO GASPARI, RAFFAELE CARUSO, NORBERTO PERICO, GIUSEPPE REMUZZI, TIZIANO BARBUI, ALESSANDRO RAMBALDI

Divisione di Ematologia, Ospedali Riuniti di Bergamo, and Istituto di Ricerche Farmacologiche Mario Negri Bergamo, Italy

Correspondence: Alessandro Rambaldi, M.D. Divisione di Ematologia, Ospedali Riuniti di Bergamo, largo Barozzi, 1 24128 Bergamo, Italy. Phone: international +39-035-266808 - Fax: international +39-035-266147 - E-mail: rambaldi.ematologia@cyberg.it

Background and Objectives. A major problem encountered during oral cyclosporin-A (CsA) administration to prevent acute graft-versus-host-disease (GVHD) after allogeneic bone marrow transplantation (allo-BMT) is its irregular pharmacokinetics. The aim of this study was to evaluate the pharmacokinetics of Neoral™, a new water-free microemulsion formulation of CsA.

Design and Methods. Eighteen patients aged over 18 were enrolled into the study. When able to eat normally after allo-BMT, patients received CsA orally and after 4 days a 12-hour CsA pharmacokinetic profile was constructed. Three patients received Sandimmune™ 10 mg/kg/day, 5 patients received Neoral™ 7.5 mg/kg/day and 10 patients Neoral™ 5 mg/kg/day. CsA concentration was analyzed on whole blood by high-performance liquid chromatography (HPLC).

Results. Neoral™ showed concentration-time profiles characterized by a smooth and faster rise to the C_{max} value compared to that produced by Sandimmune™. The comparison between pharmacokinetic parameters obtained in patients receiving Neoral™ 5 mg/kg/day or 7.5 mg/kg/day showed a proportional increase of the AUC (4776±1084 vs. 7746±2006 ng/mL h) and C_{max} (1027±203 vs. 1514±231 ng/mL). In all patients to whom 7.5 mg/kg/day of Neoral™ were given, C_{trough} levels were always above the threshold of 200 ng/mL.

Interpretation and Conclusions. Our data suggest that oral administration of Neoral™ 7.5 mg/kg/day early after allo-BMT may represent an appropriate dose resulting in adequate CsA C_{trough} levels without significant renal toxicity.

Key words: allogeneic bone marrow transplantation, cyclosporin-A, immunosuppression, graft-versus-host disease

Cyclosporin-A (CsA) given either as a single agent or in combination with corticosteroids and/or methotrexate (MTX) is widely used to prevent acute graft-versus-host-disease (GVHD) after allogeneic bone marrow transplantation (allo-BMT).[1-3] Although several variables influence the occurrence of acute GVHD,[4] the monitoring of trough CsA concentration (C_{trough}) is usually considered an appropriate parameter to evaluate the efficacy of the immunosuppression and to prevent the toxic effects secondary to overexposure to CsA.[3,5,6]

A major problem encountered during oral CsA administration is its irregular absorption in the gastrointestinal tract due to a bile-dependent process influenced also by liver dysfunction, food intake and irregular gastrointestinal motility.[7] This irregular bioavailability may cause a remarkable inter- and intra-patient variability of pharmacokinetic profiles.[5,8] To minimize this problem, a new water-free microemulsion formulation of CsA (Neoral™) has been introduced into the market.[9] Since Neoral™ is adsorbed in the small bowel and requires the presence of bile to a lesser extent, its bioavailability is significantly enhanced compared to the conventional Sandimmune™ formulation.[9]

In solid organ transplant recipients the conversion from Sandimmune™ to microemulsion formulation Neoral™ maintaining the same dose (1 to 1 conversion) has been associated with increased maximum drug concentration (C_{max}), area under the time-concentration curve (AUC) and C_{trough} level, thus suggesting that a reduction of CsA dose could be adopted for the new microemulsion formulation.[10-13] The narrow therapeutic range of CsA and the increased bioavailability of Neoral™ observed in solid organ transplant recipients suggest that the simple 1 to 1 conversion from Sandimmune™ to the Neoral™ preparation might also expose patients undergoing allo-BMT to higher, potentially toxic blood levels of CsA. We, therefore, decided to evaluate the pharmacokinetic profiles of Neoral™ in allo-BMT patients early after transplantation and besides the measurement of CsA C_{trough}, which is routinely used

in daily clinical practice, we also evaluated the AUC. This latter is, in fact, more informative about the real exposure to CsA and represents the most appropriate parameter to predict graft rejection and CsA-related toxicity after solid organ transplantation.[14,15] Unfortunately, the high number of samples usually required to perform a conventional determination of AUC limits the clinical applicability of this parameter. Previous experience in kidney transplant recipients showed that the reproducible pharmacokinetics observed after Neoral™ allowed an innovative three point sampling strategy to be used to predict the CsA AUC.[16] We, therefore, decided to apply the same approach to allo-BMT patients.

Design and Methods

Patients

Eighteen consecutive non-randomized patients (7 females and 11 males) aged over 18 were enrolled into the study. Seven patients had chronic myelogenous leukemia, 5 patients acute myelogenous leukemia, 4 patients acute lymphoblastic leukemia, 1 patient multiple myeloma and 1 patient myelodysplastic syndrome. The conditioning regimens were as follows: busulphan/cyclophosphamide (10 patients), total body irradiation/cyclophosphamide (3 patients), total body irradiation/melphalan (3 patients), thiotepa/cyclophosphamide (1 patient), and busulphan/melphalan (1 patient). Thirteen patients received peripheral blood progenitor cells (PBPC) from an HLA-identical sibling, 1 patient received bone marrow (BM) from an HLA-identical sibling and 4 patients received BM from an HLA-identical unrelated donor. All patients were regularly followed at the Bone Marrow Transplant Unit at Divisione di Ematologia, Ospedali Riuniti di Bergamo. The study protocol was described in detail to all patients before admission and informed consent to the study was obtained in each instance.

Study schedule

For all patients the prophylaxis for acute GVHD consisted of intravenous CsA (1 mg/kg/day over 24 hours as a continuous infusion) starting the day before the infusion of BM or PBPC.[17] In addition, all patients received intravenous infusion of MTX (15 mg/m^2 on day +1 and 10 mg/m^2 on day +3, +6 and +11). When patients were able to eat normally, usually between day 20 and 30, they were changed to the oral CsA administration and after four days, a 12-hour CsA pharmacokinetic profile was measured after the morning dose of CsA. The pharmacokinetics was based on analysis of blood samples collected from the antecubital vein just before the dose (C_0 or C_{trough}), and 30 minutes as well as 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours (C_{12}) after drug administration. During the same period 3 patients received Sandimmune™ 10 mg/kg/day, 5 patients received Neoral™ 7.5 mg/kg/day and 10 patients received Neoral™ 5 mg/kg/day. The planned dose of CsA was administered in two divided doses every 12 hours. During the pharmacokinetic study, all patients were monitored daily for vital signs and twice weekly for laboratory variables (renal and liver function tests). After discharge patients were followed twice weekly for one month and the CsA dose was modified to maintain the whole blood CsA C_{trough} within 200 and 400 ng/mL. For all patients, in case of acute GVHD occurrence, intravenous CsA was re-established at the dose of 3 mg/kg/day in association with methylprednisolone (2 mg/kg/day).

CsA pharmacokinetic evaluation

Blood samples were analyzed by high-performance liquid chromatography (HPLC) as previously described.[16] The blood concentration-time profile of CsA was recorded for all patients together with C_{trough}, C_{12}, C_{max}, and the time of maximum observed concentration (T_{max}) of blood CsA. The AUC from T_0 to the last sampling point (12 hr) ($AUC_0 \rightarrow 12$) was calculated by the trapezoidal rule. Predicted AUC after Neoral™ administration was estimated using a three-point sampling strategy (sampling points 0, 1, and 3 h), as previously described.[16]

Results and Discussion

When the Neoral™ formulation became available, we treated 3 initial patients with 10 mg/kg/day in two divided doses maintaining the 1 to 1 conversion with the conventional Sandimmune™ formulation according to the manufacturer's instruction. However, in these patients we noticed that CsA C_{trough} levels measured during the early days after the beginning of Neoral™ administration were usually very high, consistently above the value of 500 ng/mL. The last of these patients developed clinical and laboratory evidence of thrombotic thrombocytopenic purpura (TTP) concomitant with a CsA C_{trough} of 1,119 ng/mL and this is in line with reports on a possible role of CsA overexposure.[18,19] From this experience we decided to perform a pharmacokinetic study in order to optimize the dose of Neoral™.

The CsA whole blood 12-h concentration profiles recorded for patients given either Sandimmune™ or Neoral™ are shown in Figure 1. The profiles obtained for patients given Neoral™, regardless of the dose employed (5 mg/kg/day, Panel A or 7.5 mg/kg/day, Panel B) showed a smooth and faster rise to the C_{max} value compared to that given by Sandimmune™ (10 mg/kg/day, Panel C). Although, we studied few cases with Sandimmune™, our results concerning the poor pharmacokinetic profiles of this CsA formulation are in keeping with those reported in the literature.[2,5,7] We, therefore, suggest that a more consistent CsA concentration time profile is obtained after Neoral™ administration in allo-BMT patients as previously reported for solid organ transplant recipients.[10,11,20] The comparison between pharmacokinetic parameters obtained in patients receiving Neoral™ 5 or 7.5 mg/kg/day, showed a 50% increase of AUC (4776±1084 vs. 7746±2006 ng/mL h) and C_{max} (1027±203 vs. 1514±231 ng/mL), and a 80-90% increase of both C_{trough} (186±80 vs. 348±90 ng/mL) and C_{12} (184±103 vs. 325±109 ng/mL) (Table 1). In all patients to whom 7.5 mg/kg/day of Neoral™ were giv-

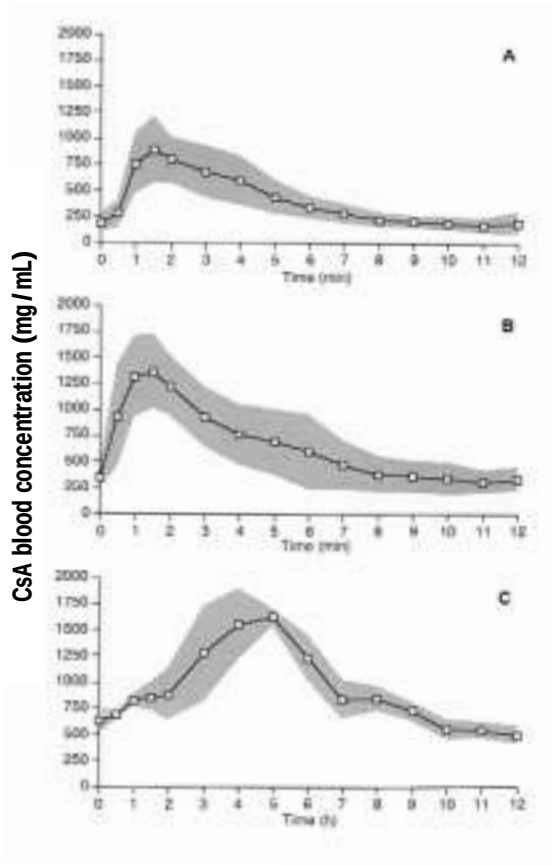

Figure 1. Pharmacokinetic profiles after administration of CsA Neoral™ or Sandimmune™ in allo-BMT recipients. When able to eat normally, the patients were given the CsA orally. Four days later, CsA pharmacokinetic profile was measured after the morning dose of drug. Ten patients received Neoral™ 5 mg/kg/day (Panel A), 5 patients received Neoral™ 7.5 mg/kg/day (Panel B) and 3 patients received Sandimmune™ 10 mg/kg/day (Panel C). The shaded area represents ± 1 SD.

en, C_{trough} as well as C_{12} levels were always above the threshold of 200 ng/mL.

The existence of a tight relationship between CsA concentration and appropriate GVHD prophylaxis has long been recognized.[2-7] Most data reported in the literature have been obtained by the evaluation of serum or plasma CsA C_{trough} using HPLC or radioimmunoassy (RIA) either with polyclonal or monoclonal antibodies.[2-7]. In particular, Yee and co-workers suggested that a serum CsA C_{trough} between 200 and 400 ng/mL measured with a polyclonal-RIA was associated with a lower risk of acute GVHD.[3] Nowadays, however whole blood CsA concentrations evaluated by a specific monoclonal antibody are usually recommended in routine clinical activity[21] and it is difficult to compare CsA concentrations evaluated by different methods and in different matrices. In general, CsA concentration in whole blood is almost double that in serum,[21,22] and C_{trough} levels measured with RIA-methods tend to be higher (ranging from 1.37 to 1.5 times) than those measured by HPLC.[23,24] Putting these data, we arbitrarily suggest that C_{trough} levels of 348±90 ng/mL obtained after Neoral™ 7.5 mg/kg/day in this study can be compared to the therapeutic range of 200-400 ng/mL (measured on serum with a polyclonal-RIA) reported by Yee *et al*.[3] CsA C_{trough} was also evaluated in ten additional patients, followed outside the pharmacokinetic study, to whom an oral dose of Neoral™ 7.4±0.5 mg/kg/day was administered in the early period after transplantation. In these patients we obtained mean C_{trough} levels of 513±244 ng/mL (measured on whole blood with a monoclonal-RIA) 4-6 days after beginning Neoral™. In four of these patients CsA C_{trough} was higher than 500 ng/mL and consequently the dose of CsA was reduced to 5 mg/kg/day and after that the C_{trough} was 338±71 ng/mL. The suggested dose of 7.5 mg/kg/day of Neoral™ as a starting oral dose of CsA in the setting of allo-BMT is in keeping with data recently reported by Parquet *et al.*[25] who suggest that for patients receiving CsA 3 mg/kg by continuous i.v. infusion, the appropriate starting oral dose of Neoral™ is twice the last i.v. dose.

Acute and chronic renal dysfunction are frequently encountered during CsA administration and these are usually dose-dependent.[7] In Table 2, we summarize the serum creatinine levels measured at different time

Table 1. CsA parmacokinetic parameters in allogeneic bone marrow transplant recipients after Sandimmune™ or Neoral™ administration.

No. of patients	*CsA formulation*	*Dose (mg/kg/day)*	*AUC (ngh/mL)*	*C_0 C_{trough} (ng/mL)*	*C_{12} (ng/mL)*	*C_{max} (ng/mL)*	*T_{max} (h)*
3	Sandimmune™	10	11227 ± 507	628 ± 118	412 ± 198	1735 ± 176	4.7 ± 0.6
10	Neoral™	5	4776 ± 1084	186 ± 80	184 ± 103	1027 ± 203	2.1 ± 1
5	Neoral™	7.5	7746 ± 2006	348 ± 90	325 ± 109	1514 ± 231	1.3 ± 0.6

Table 2. Serum creatinine levels in allogeneic bone marrow transplant recipients receiving CsA.

No. of patients	*Csa formulation*	*Dose (mg/kg/day)*	*S-creatinine (mg/dL) day 0*	*S-creatinine (mg/dL) day +4*	*S-creatinine (mg/dL) day +14*	*S-creatinine (mg/dL) day +30*
3	Sandimmune™	10	1.1±0.18	0.8±0.14	1.17±0.21	1.4±0.53
10	Neoral™	5	0.83±0.18	0.92±0.16	0.98±0.17	0.96±0.18
5	Neoral™	7.5	0.82±0.18	0.95±0.06	1±0.16	1.12±0.33
10*	Neoral™	7.4±0.5	1.06±0.23	1.11±0.18	1.11±0.42	1.04±0.38

**Patients evaluated outside the pharmacokinetic study.*

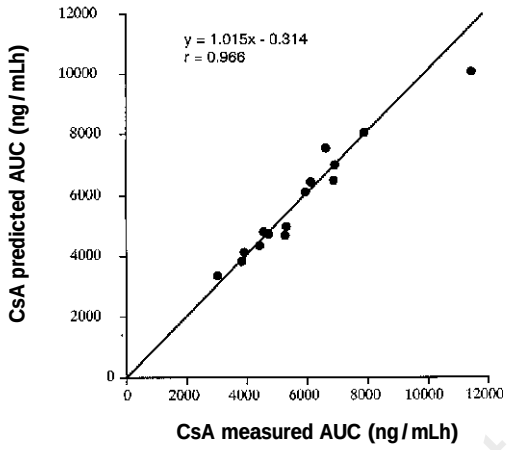

Figure 2. Correlation between measured and predicted AUC after administration of CsA Neoral™ in allo-BMT recipients. For patients receiving Neoral™, the AUC measured by the conventional 12-hour pharmacokinetic profile four days after the shift to oral CsA was correlated to the estimate using the three-point sampling strategy (0, 1, and 3 h), as demonstrated by the following equation: AUC = 5.189 × [0 h] + 1.267 × [1 h] + 4.150 [3 h] + 135.079.[16]

points in patients receiving either Sandimmune™ or Neoral™. In no patients to whom 5 or 7.5 mg/kg/day of Neoral™ were given, did we observe an increase of the serum creatinine level above the threshold of 2 mg/dL within the period of observation.

The evidence that the AUC is a more accurate indicator of total CsA drug exposure in solid organ transplant recipients[14,15] and the consistency of Neoral™ CsA profiles prompted us to investigate whether an innovative three-point sampling strategy early after CsA dosing (at 0, 1, and 3 h),[16] could be used to predict AUC in allo-BMT patients. As shown in Figure 2, the predicted AUC calculated by this strategy was accurate (4879±1242 ng/mL h after 5 mg/kg/day and 7445±1364 ng/mL h after 7.5 mg/kg/day) and not statistically different from the conventionally evaluated areas (4776±1084 ng/mL h after 5 mg/kg/day and 7746±2006 ng/mL h after 7.5 mg/kg/day). Moreover, the calculated error (measured as: AUC measured – AUC predicted/AUC measured ×100) was very modest with a mean value of 0.81±7.55% (range 14.4±14.3%).

Taken together these data suggest that the use of 7.5 mg/kg/day of Neoral™ may represent an appropriate dose during the early oral CsA administration in patients undergoing allo-BMT, resulting in adequate CsA trough levels without significant acute renal toxicity. Whether this significantly prevents the chronic renal toxicity induced by CsA still remains to be demonstrated. Finally, the easy determination we proposed for CsA AUC could be validated within prospective clinical studies designed to evaluate the ability of this pharmacokinetic parameter to predict the risk of acute GVHD and CsA-related toxicity.

Contributions and Acknowledgments

GD and FG contributed equally to this work. GD, FG and AR designed the study and prepared the manuscript. FG and RC carried out HPLC measurements. NP, GR and TB contributed to the discussion and approved the final version to be submitted. We wish to thank all the nursing staff for their excellent support in the clinical management of the Bone Marrow Transplant Unit.

Funding

This work was supported in part by grants from the Associazione Italiana per la Ricerca contro il Cancro (AIRC), from the Consiglio Nazionale per le Ricerche (CNR) Target Project on Biotechnology and from Associazione Paolo Belli, Lotta alla Leucemia.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Prof. Jorge Sierra, who acted as an Associate Editor. The final decision to accept this paper was taken jointly by Prof. Sierra and the Editors. Manuscript received November 17, 2000; accepted February 14, 2001.

Potential implications for clinical practice

The reproducibility of Neoral™ pharmacokinetics and the predictable AUC calculated by the simple three-point method may offer the opportunity to evaluate the significance of the AUC parameter in GVHD prevention in prospective studies.

References

1. Deeg HJ, Storb R, Thomas ED, et al. Cyclosporine as prophylaxis for graft-versus-host disease: a randomized study in patients undergoing marrow transplantation for acute nonlymphoblastic leukemia. Blood 1985; 65:1325-34.
2. McGuire TR, Yee GC, Emerson S, Gmur DJ, Carlin J. Pharmacodynamic studies of cyclosporine in marrow transplant recipients. A comparison of three assay methods. Transplantation 1992; 53:1272-5.
3. Yee GC, Self SG, McGuire TR, Carlin J, Sanders JE, Deeg HJ. Serum cyclosporine concentration and risk of acute graft-versus-host disease after allogeneic marrow transplantation. N Eng J Med 1988; 319:65-70.
4. Sullivan KM. Graft-versus-host disease. In: Thomas ED, Blume KG, Forman SJ, eds. Hematopoietic Cell Transplantation. 2nd ed. Malden, MA; 1999:515-36.
5. Gluckman E, Lokiec F, Devergie A. Pharmacokinetic monitoring of cyclosporine in allogeneic bone marrow transplants. Transplant Proc 1985; 12:500-1.
6. Kennedy MS, Yee GC, McGuire TR, Leonard TM, Crowley JJ, Deeg HJ. Correlation of serum cyclosporine concentration with renal dysfunction in marrow transplant recipients. Transplantation 1985; 40:249-53.
7. Kahan BD. Cyclosporine. N Engl J Med 1989; 321:1725-38.
8. Clardy CW, Schroeder TJ, Myre SA, et al. Clinical variability of cyclosporine pharmacokinetics in adult and pediatric patients after renal, cardiac, hepatic, and bone-marrow transplants. Clin Chem 1988; 34:2012-5.
9. Friman S, Backman L. A new microemulsion formulation of cyclosporin: pharmacokinetic and clinical features. Clin Pharmacokinet 1996; 30:181-93.
10. Mueller EA, Kovarik JM, van Bree JB, Lison AE, Kutz K. Pharmacokinetics and tolerability of a microemulsion formulation of cyclosporine in renal allograft recipients: a concentration-controlled comparison with the commercial formulation. Transplantation 1994; 57:1178-82.
11. Hemming AW, Greig PD, Cattral MS, et al. A microemulsion of cyclosporine without intravenous cyclosporine in liver transplantation. Transplantation 1996; 62:1798-802.
12. Melter M, Rodeck B, Kardorff R, Hoyer PF, Brodehl J. Pharmacokinetics of cyclosporine in pediatric long-term liver transplant recipients converted from Sandimmune to Neoral. Transpl Int 1997; 10:419-25.
13. Senel FM, Yildirim S, Karakayali H, Moray G, Haberal M. Comparison of Neoral and Sandimmune for induction and maintenance immunosuppression after kidney transplantation. Transpl Int 1997; 10:357-61.
14. Grevel J, Welsh MS, Kahan BD. Cyclosporine monitoring in renal transplantation: area under the curve monitoring is superior to trough-level monitoring. Ther Drug Monit 1989; 11:246-8.
15. Keown P, Kahan BD, Johnston A, et al. Optimization of cyclosporine therapy with new therapeutic drug monitoring strategies: report from the International Neoral TDM Advisory Consensus Meeting (Vancouver, November 1997). Transplant Proc 1998; 30:1645-9.
16. Gaspari F, Anedda MF, Signorini O, Remuzzi G, Perico N. Prediction of cyclosporine area under the curve using a three-point sampling strategy after Neoral administration. J Am Soc Nephrol 1997; 8:647-52.
17. Zikos P, Van Lint MT, Frassoni F, et al. Low transplant mortality in allogeneic bone marrow transplantation for acute myeloid leukemia: a randomized study of low-dose cyclosporin versus low-dose cyclosporin and low-dose methotrexate. Blood 1998; 91:3503-8.
18. Pettitt AR, Clark RE. Thrombotic microangiopathy following bone marrow transplantation. Bone Marrow Transplant 1994; 14:495-504.
19. Paquette RL, Tran L, Landaw EM. Thrombotic microangiopathy following allogeneic bone marrow transplantation is associated with intensive graft-versus-host disease prophylaxis. Bone Marrow Transplant 1998; 22:531-7.
20. Fiocchi R, Mamprin F, Gamba A, et al. Pharmacokinetic profile of cyclosporine in long-term heart transplanted patients treated with new oral formulation. Transplant Proc 1994; 26:2994-5.
21. Shaw LM, Yatscoff RW, Bowers LD, et al. Canadian Consensus Meeting on cyclosporine monitoring: report on the consensus panel. Clin Chem 1990; 1990:36:1841-6.
22. Quesniaux VF. Pharmacology of cyclosporine (sandimmune) III. Immunochemistry and monitoring. Pharmacol Rev 1989; 41:249-58.
23. Faulds D, Goa KL, Benfield P. Cyclosporine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. Drugs 1993; 45:953-1040.
24. Keown PA, Glenn J, Denegri J, et al. Therapeutic monitoring of cyclosporine: impact of a change in standards on 125I-monoclonal RIA performance in comparison with liquid chromatography. Clin Chem 1990; 36:804-7.
25. Parquet N, Reigneau O, Humbert H, et al. New oral formulation of cyclosporin A (Neoral) pharmacokinetics in allogeneic bone marrow transplant recipients. Bone Marrow Transplant 2000; 25:965-8.