

## A Review on Therapeutic Drug Monitoring of Immunosuppressant Drugs

<sup>1,2</sup>Niloufar Mohammadpour, <sup>3</sup>Sepideh Elyasi, <sup>3</sup>Naser Vahdati, <sup>\*3,4</sup>Amir Hooshang Mohammadpour,  
<sup>3</sup>Jamal Shamsara

### Abstract

Immunosuppressants require therapeutic drug monitoring because of their narrow therapeutic index and significant inter-individual variability in blood concentrations. This variability can be because of factors like drug-nutrient interactions, drug-disease interactions, renal-insufficiency, inflammation and infection, gender, age, polymorphism and liver mass. Drug monitoring is widely practiced especially for cyclosporine, tacrolimus, sirolimus and mycophenolic acid.

#### Cyclosporine

Therapeutic monitoring of immunosuppressive therapy with cyclosporine is a critical requirement because of intra- and inter-patient variability of drug absorption, narrow therapeutic window and drug induced nephrotoxicity.

#### Mycophenolic acid (MPA)

Some reasons for therapeutic drug monitoring of MPA during post-transplant period include: relationship between MPA pharmacokinetic parameters and clinical outcomes, Inter-patient pharmacokinetic variability for MPA despite fixed MMF doses, alternations of MPA pharmacokinetics during the first months after transplantation, drug- drug interaction and influence of kidney function on MPA pharmacokinetic.

#### Sirolimus

A recent review of the pharmacokinetics of sirolimus suggested a therapeutic range of 5 to 10  $\mu\text{g l}^{-1}$  in whole blood. However, the only consensus guidelines published on the therapeutic monitoring of sirolimus concluded that there was not enough information available about the clinical use of the drug to make recommendations.

#### Tacrolimus

Studies have shown, in kidney and liver transplant patients, significant associations of low tacrolimus concentrations with rejection and of high concentrations with nephrotoxicity. Although the feasibility of a limited sampling scheme to predict AUC has been demonstrated, as yet, trough, or pre-dose, whole blood concentration monitoring is still the method of choice.

**Keywords:** Cyclosporine , Mycophenolic acid, Sirolimus, Tacrolimus, Therapeutic drug monitoring,

1- Department of Microbiology, School of Medicine, Islamic Azad University, Mashhad Branch, Mashhad, Iran

2- Zakariya Research Centre, Islamic Azad University, Mashhad Branch, Mashhad, Iran

3- Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

\*Corresponding author: Tel: +98-511-8823255; Fax: +98-511-8823251; email: mohamadpoorah@mums.ac.ir

4- Pharmaceutical Research Centre, Mashhad University of Medical Sciences, Mashhad, Iran

5- Department of Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

## Introduction

In order to reach the optimum balance between therapeutic efficacy and the occurrence of adverse effects, a physician has to individualize a patient's drug therapy. As all the patients vary in both pharmacokinetics (PK) and pharmacodynamic (PD), obtaining to the goal is always very complicated (1). Thus, the lack of control of drug concentration prevents the patient's clinical response variability when both PK and PD variability are considerable (2-5). In the early 1960s the measurement of the low drug concentrations found in biological fluids during drug treatment was available by using new analytical techniques. Therefore, the process which was known as therapeutic drug monitoring, provided the chance to reduce the pharmacokinetic component of variability by controlling drug therapy using concentrations in the body rather than just by dose (TDM) (1, 6).

A suitable drug should be chosen for therapeutic drug monitoring based on the following criteria:

- 1) The relationship between drug concentration and the effect of the drug should be cleared.

- 2) Narrow therapeutic index; which means the concentrations of separation the drug make therapeutic benefit and those causing adverse effect should be small.

- 3) The difference between-subject pharmacokinetic variability and a poor relationship between dose and drug concentration/response should be noticeable.

- 4) The assessment of the pharmacological response of the drug from the adverse events should be difficult to obtain (7).

The aim of a therapeutic window, especially in combination regimens, should be defined by avoiding under exposure with an increased risk of rejection or over exposure with an increased risk of toxicity. First, as the risk of rejection diminishes over time, the therapeutic window for an immunosuppressive drug may also vary overtime.

Furthermore, as demonstrated for CNI-induced nephrotoxicity, the cumulative risk for toxicity may increase overtime (8, 9).

Most maintenance patients (beyond the first year) can be treated in 2 ways: 1) drug levels derived from a therapeutic window, which can be obtained from the study after transplantation, 2) drug levels derived from an opinion-based therapeutic window.

Second, based on the synergistic of the immunosuppressants for rejection prophylaxis and the potential of their toxicities, the therapeutic window for a given drug in a multi-drug regimen may vary (10).

Additional factors, which can affect the efficacy and toxicity of an immunosuppressive regimen, are donor and recipient characteristics (such as age, number of mismatches, race, delayed graft function, renal and liver function).

Next, the logic of TDM is based on the theory that a proportional increase in dose would result in a proportional increase in exposure.

Although data in maintenance populations may show such linearity, in some cases such as early post-transplant period, the absorption of drugs may change obviously and may not be linear.

Examples are the absorption pattern for CNIS and mycophenolic Acid (MPA), which undergo considerable changes in the early transplant period (11, 12). For example in the first weeks after transplantation, a doubling of MPA dose would result only in approximately 50% in MPA exposure (13, 14).

PD assessment can be achieved by biomarkers which obtained of the investigation of the mechanism of action of immunosuppressive drugs. These biomarkers act specifically for a drug like the target enzyme, for example IMPDH for MPA (19). Calcineurin activity is the other drug target which was studied in transplant patients who received calcineurin inhibitors, i.e., cyclosporine A (CyA) (20, 21).

Cytokines were also studied as PD biomarkers by different research groups.

## Immunosuppressant TDM

Rostaing *et al* investigated the PD influences on cytokines in PBMCs by flow cytometry and found differences in the TH1/TH2 cytokine expression pattern from CyA or tacrolimus treated stable renal allograft recipients compared with healthy volunteers (22, 23). In the other word, cytokine's activated gene expression can be used as a pharmacodynamic readout of immunosuppression (24).

The measurement of the expression and down-regulation of cell surface markers as an estimate of cell cultivation and T-cell function via cytokine expression (25-27) or overall ATP content also can be used (6, 28).

The purpose of this review is to examine the current strategies in use for the therapeutic drug monitoring of immunosuppressant drugs and to discuss some of the factors that impinge on the monitoring of these drugs simply and briefly for clinicians and pharmacists.

### *Cyclosporine A*

In, 1969, the early days of kidney transplantation, there was less than 18% possibility for the 2 years graft survival rate for cadaveric renal transplantation in the USA (29). Five years later, in 1974, Caln (the same author of the previous report) reported that the graft survival for 2 years had developed to over 50% (30). This change in the survival rate was because of better surgical technique and improvement in patient management, but the main drug therapies, azathioprine and prednisolone maintained the same. During the past 16 years, the pharmacological change of the immune system in the field of solid-organ transplantation has undergone remarkable improvements. For example, 1 year kidney allograft survival now approaches 95% for transplanted living related kidneys and 85-90% for cadaveric kidneys (31), whereas before the introduction of CsA. 1 year graft survival was 60%.

Organized investigation on the PK and TDM of immunosuppressive agents began early in the "CsA era" of immunosuppressive therapy in

1683 (6, 32-37). Different absorption and narrow therapeutic index (the drugs which cause irreversible kidney damage when given in extra dose) have lead to the assessment of CsA blood concentrations in order to manage the drug dosage (38).

Two target ranges have been used by most centers. The first one for preliminary therapy (usually up to 6 months posttransplant), and the second for maintenance therapy using lower, target range treatment thereafter. The target ranges differ in the analysis method, transplant type, and transplant center philosophy on approvable immunosuppression intensity. The retrospective review of CsA concentration data and its correlation with clinical events from single-center studies can improve target ranges (39).

In the past, the choice of sample matrix for monitoring (40,41), lack of assay specificity (42) inconsistent assay performance (43) the variable absorption of the drug from the original formulation (sandimmon) (44) and the poor correlation between trough concentration and clinical effects had lead to the reduction of the utility of CsA in therapeutic drug monitoring. During the time, majority of these problems addressed (33); the selected matrix for measurement is blood, not plasma or serum (34, 46), the assay for the parent drug are now more selective (47, 48) most laboratories participate in proficiency testing (49) and for better absorption, the drug has been re-formulated (Neoral1) (50-52). However, trough whole blood concentration remains an imperfect assessment of the total exposure to CsA during a dose interval (53) and a predose blood sample for CsA analysis is used in the was majority of centers.

Lately, it has been approved that capillary blood, gotten by skin puncture is suitable for monitoring CsA (54). This new method can be used specially in pediatric practice.

The relationship between area under the concentration-time curve (AUC) for CsA and

clinical events has been characterized by Kahan and coworkers in the Sandimmun era (55). They concluded that there is just a poor correlation between trough concentrations and the AUC and thereby do not sufficiently reflect CsA exposure, whereas total exposure evaluation (i.e., AUC) would be able to improve correlation with clinical effects of the drug (56, 58).

Despite the acknowledgment of AUC monitoring advantages by many scientists, this method has failed to gain worldwide acceptance because of some difficulties, both for the patient and the clinician (59). The measurement of AUC has been simplified by the arrival of the microemulsion formulation of CsA, Neoral, which has the improved pharmacokinetic characteristics, better absorption and bioavailability (59, 60). Previous studies with Sandimmun have shown that an accurate estimate of CsA AUC can be got by the concentrations of three blood samples, drawn at specific times (61). For Neoral the same level of accuracy can be obtained by two blood samples, collected within the usual 12 hr dose interval, however, it is conflicting (39, 62, 63). Interpatient variability still existed and careful TDM and dose adjustment should be performed (64). The comparison of predose concentration monitoring with little or limited sampling AUC monitoring is now waiting for future studies.

However, it should be noticed that the options for the therapeutic monitoring of CsA are not limited only to predose, trough concentration and AUC monitoring. For some time Cantarovich and coworkers supported the method of using a single timed sample 6h after dosing (65). In a prospective study for controlling CsA therapy in heart transplant patients, comparison between predose and 6h CsA concentration have been done, the use of 6h value showed a 30% lower dose of the drug with the same effectiveness in preventing rejection and the same cardiac and renal function as that seen in those dosed using the predose concentration (66).

These authors have also reported a good correlation between 6h CsA concentrations and efficacy in noninfectious uveitis (67). The usage of CsA blood concentration at 2h postdose is another option for monitoring (C<sub>2</sub>). The reason for this option comes from the observation which was obtained during the clinical development of Neoral when they considered that in liver transplant patients, there was an opposite relationship between the incidence of rejection and the maximum blood CsA concentration (C<sub>max</sub>) (68-70). In a small open-labelled experiment in liver transplant recipients, the usage of C<sub>2</sub> monitoring showed positive results (71, 72). So, the use of C<sub>2</sub> monitoring has been supported by recent studies of the pharmacokinetics and pharmacodynamics of CsA. In a study on nephritic syndrome patients by Naito and his colleagues, it has been shown that C<sub>1</sub> and C<sub>2</sub> are good clinical markers for CsA exposure but not CO (73, 74). Pharmacodynamic studies have shown the correlation between the CsA Concentration 2 hr postdose with maximal calcineurin inhibition (75) and maximal reduction in the number of circulating IL-2<sup>+</sup> CD4<sup>+</sup> peripheral cells (76). Evidence is also beginning to prove that individualizing a patient's absorption phase for CsA which called "absorption profiling" by aiming C<sub>2</sub> concentration results in clinical advantages (77). Different experiments of absorption profiling being conducted in renal, liver and heart transplant patients showed positive results (78, 79). But C<sub>2</sub> can't be used in all populations-A research in Egypt showed that because of the occurrence of schistosomal infection, Egyptians have special characteristics with regard to drug absorption and metabolism, So C<sub>2</sub> can be replaced by C<sub>25</sub> to monitor CsA (80). Pharmacokinetic studies have shown that the first 4 hr postdose, is the most highly variable region of the blood concentration profile CsA absorption phase between patients (81).

## Immunosuppressant TDM

There is also an interaction between cyclosporine and mycophenolate mofetil (MMF), resulted in rise of its concentration and so, reduction of cyclosporine dose may be necessary (82). The correct measurement of cyclosporine has been the subject of many publication (40, 41, 83) and reviews (50, 84).

As general rule, it is identified that without the advantages of prospective concentration-controlled studies done with validated analytical methodology for CsA in multiple centers, the risk/advantage ratio for specific concentrations of the drug in specific patient group is missing. Eight different immunoassay assay systems for the measurement of CsA in whole blood are now produced by five commercial companies. Furthermore, some laboratories are using high performance liquid chromatography (HPLC) to measure the drug. H. P. L.C has been considered as gold standard in CsA measurement because it is especially possible to couple with mass-spectrometry. The disadvantages of HPLC related to poor precision and fault results because of the interference from other sources (85), of the eight immunoassay variants, two are nonspecific and cross-react, markedly, with the metabolites of cyclosporine. The abbott TD×1 drug and metabolite assay uses a polyclonal antibody and produces results that are approximately 3±5 times that of HPLC. where as the DiaSorin CYCLO-Trac NS radio immunoassay uses a monoclonal nonspecific antibody assays to HPLC changes with the metabolite: parent compound ratio in the blood and therefore will vary with transplant type and time after transplant. The results of the non-specific assays have a poor correlation with clinical events (86). The other six immuno assays are concerned as specific for the parent drug but, to a limited amount, cross react with drugs' metabolites and therefore do not give the same results for a given sample. It is noticeable that the differences between the results of the specific assay can partly because of the different cross reactivity of antibodies used. The incorrect

calibration may lead to some of the differences (87). It is interesting to mention that for one of the manufactures the results of their three different assays do not match.

It seems that these differences in measurement correctness do not affect the clinical usefulness of the assays (86), but this lead to increase variability of reported concentrations data in the literature (88) and have an impact on the local target ranges. However, in clinical conditions with high load of CsA metabolite in blood, for example, liver transplant patients immediately post transplant, HPLC is the only method which can precisely measure the parent compound (89).

### *Tacrolimus*

The USA food and drug administration approved tacrolimus (FK-506: Prograf) for prophylaxis of organ rejection in patient receiving allogenic liver transplants. It is generally used in combination with steroids. Tacrolimus is being evaluated in combination with other immunosuppressive agents especially MMF (39) for patients who receive other solid-organ transplants, similar to CsA, too high drug dosage is accompanied with toxicity and too low with rejection. Other similarity is that the whole blood concentration measurement are also used for the monitoring of tacrolimus therapy (90), primary clinical trials which didn't include concentration monitoring lead to patients with neuro-and nephrotoxicity (91). The pharmacokinetics of tacrolimus is highly variable (92). The rationale for therapeutic drug monitoring of tacrolimus is similar to CsA, because it shares many of the pharmacokinetic and pharmacodynamic problems with CsA. An early observational study on correlation between concentration and effect of the blood concentration in kidney transplant patients who didn't experience rejection and those who did (93), however, the other more statistically strict studies on kidney and liver transplant patients, showed significant association of low tacrolimus concentrations with rejection and high

concentration with nephrotoxicity (94). Although the plausibility of a limited sampling scheme to predict AUC has been investigated (95), the method of choice is still trough, or predose, whole blood concentration the timed samples (96) and AUC monitoring has also been investigated, but unlike CsA, they haven't been used in clinical practice yet. This may, partly, because of the high relationship observed between trough concentration and C<sub>max</sub> or AUC (39, 98).

In a prospective study, one hundred twenty renal transplant patients were chosen for an open label clinical trial which concluded five transplant centers. The patients were categorized randomly to one of three target predose tacrolimus blood concentration ranges: Low, middle, or high. Each participating center used quadruple drug therapy, i.e. induction with antilymphocyte globulin and maintenance immunosuppression with tacrolimus, azathioprine, and prednisone. As the result of the 42-day postsurgery study period, the correlation between increasing blood concentration of tacrolimus and (a) the decreasing rate of rejection and (b) the increasing rate of toxicity were both statistically significant (99, 100). In some other studies two sampling time points are chosen as a predictable and precise measure of AUC<sub>0-12</sub> in stable renal transplant patients (101).

In another open label multicenter prospective study investigating the PRO-Trak 11 ELISA method for tacrolimus measurement in liver transplant patients, one hundred and eleven adult liver transplant patients were chosen at six US centers. One of the important results of this research was statistically significant correlation between increasing trough concentrations of tacrolimus and (a) decreasing risk for rejection, according to the lowest blood concentration during the preceding 0 to 7 days, and (b) increasing risk for nephrotoxicity, according to the highest blood concentration during the preceding 7 days (39).

The same cytochrome P<sub>450</sub> 3A enzyme family which is responsible for biotransformation of CsA, sirolimus and prednisone in enterocytes and liver, metabolize tacrolimus. Nevertheless, these metabolites do not accumulate in blood in most transplant patients to the amount observed for CsA, and the metabolite bias observed with immunoassay methods widely used for tacrolimus measurement in whole blood does not seem to be as problematic for tacrolimus as for CsA. Still, more study data in different patient population will be needed (39, 102). Furthermore, tacrolimus metabolism was inhibited by known CYP3A inhibitors such as ketoconazole, cyclosporine A, and nifedipine.

Recent research results on clinical pharmacokinetic show that in transplant patients with CYP3A5 polymorphism the dosage level of tacrolimus must be adjusted (103). As the concentration of tacrolimus found in the blood of stable renal transplant is low, the measurement of the drug became difficult. In-house ELISA, commercial ELISA (Diasorin) and microparticulate enzyme immunoassay, and HPLC. MS (101) methods have been available. The majority of laboratories monitoring tacrolimus use the commercial microparticulate enzyme immunoassay (MEIA. Abbott laboratories) which measures the drug in the range of 3 to 30 µg/L and cross react to just small degree of tacrolimus metabolites(102).

#### ***Mycophenolate mofetil (MMF)***

In 1995, for preventing rejection in renal transplant patients, MMF, the morpholinoethyl ester prodrug from mycophenolic acid (MPA) was approved for clinical use. This drug can be combined with CsA and prednisone and act as a pro-drug for that compound (39, 104-106). When taken orally, because of a rapid conversion to MPA by widely distributed esterases, MMF can't be measured in plasma at any time after oral administration (39, 107). In man, MPA is metabolized to 7-O-MPA glucuronide (MPAG) in liver. This molecule is

## Immunosuppressant TDM

an inactive metabolite that is present in plasma at approximately 20-100 fold higher concentrations than MPA and excreted renally. It was believed that the MPA glucuronide to be the only metabolite of MPA, However, it is known now that there are at least two other metabolites (108). The role of TDM in MMF therapy hasn't been established yet.

Previously no studies have shown that its concentration correlates either to toxicity or acute rejection, but some new studies have shown that there will be a relationship between MPA pharmacokinetic parameters and clinical outcome (109). Some authors believe that as inter individual pharmacokinetic variability is low, the use of TDM in the great number of patients would be limited (110). In contrast, other authors using the same data, believe that the inter individual pharmacokinetic variability is high and that TDM may play a function role in controlling MMF therapy (111, 112).

The latter view was supported by a study of 30 *de novo* heart transplant patients who received tacrolimus and MMF in which the dose of MMF was adjusted to maintain the MPA trough plasma concentration between 2.5 and 4 µg/l (113). These patients were rejection free at 6 months post-transplant and their MMF dose ranged between 0.5 and 6g/day to achieve trough concentration within a target range. The other helping point is the fact that although the bioavailability of MPA is reported high 94% in healthy subjects and renal transplant patients on an exact dose of MMF 2g/day (114). The magnitude of the AUC range was not reduced by the correction of the AUC range was not reduced by the correction of the AUC values for patient weight (115).

To relate the AUC and C<sub>max</sub> of plasma to the incidence of rejection or toxicity for example its leucopenia, one can use logistic regression and the highly statistically significant relationship was found (110-113). The results gathered from the logistic regression and data from other trials (110) suggest that low plasma MPA AUC is an

important risk factor in developing rejection (112, 115, 116). A randomized concentration controlled study of MMF in renal transplant patients results confirmed these data (117). The link between high MPA concentrations and adverse effects has not been recognized. The plasma MPA concentration-time profile for a single dose of oral MMF after an overnight fast shows a rapid increase, then a secondary peak at 6-12 hr. This pattern may be considered as an enterohepatic pathway involving MPAG passage into the gastrointestinal tract via biliary excretion, change to MPA via glucuronidase action in gut flora, and reabsorption of the latter into the general circulation (39).

A retrospective statistical evaluation of MPA dose-interval AUC data correlation with the incidence of acute rejection was performed in patients of a MMF Japanese renal transplant clinical trial (114, 118). The study patients were categorized randomly to one of several doses of MMF, in addition to receiving CsA doses led by blood concentration monitoring and empiric doses of prednisone. A significant correlation ( $P= 0.001$ ) was obtained between risk for rejection (relative to the risk with no MMF) and the natural log of the dose-interval MPA AUC, but not to MMF dose (114).

A prospective multicenter randomized concentration-controlled clinical trial in renal transplant patients, sponsored by Roche global development. The patients (n= 5150) from a total of seven centers in Belgium and the Netherlands were categorized randomly to low, intermediate and high target MPA AUC values. A strategy was developed and agreed to permit continual adjustment of dosing to retain the target AUC values within the 6-month period study: acute rejection incidence and other results were considered. This prospective concentration-clinical response study confirmed the hypothesis of strong statistically significant relationship ( $P= 0.001$ ) between rejection risk and MPA AUC but not MMF dose (118). In another prospective study, they investigated, the

use of a 2 hr abbreviated MPA AUC vs predose MPA plasma concentrations to control the inpatient variance of MPA AUC. The relationship between abbreviated MPA AUC and the full 12 hr AUC was very well, but is much more practical to perform in the clinical setting. Another study which was performed on 21 liver transplant recipients children, showed that AUC 0-7 correlated significantly with MMF dose. As MPA pharmacokinetics varies in pediatric liver transplant recipient, monitoring of MPA plasma level is required (119).

Recent studies have determined that there may be a correlation between drug concentration and its toxicity, for example in a study on kidney transplant patients at a fixed dose of 2g/day, a high C (30 min) is accompanied with increased risk of side effects, supporting the idea that dividing the MMF daily oral dose into more than two divided dose might prevent early MPA toxicity (120). Sometimes, MMF administration may be accompanied with tolerability problems. These problems relates to gastrointestinal adverse effects such as nausea, vomiting, diarrhea, abdominal pain, and gastritis. An enteric-coated formulation of mycophenolate sodium (EC-MPS) has been improved to overcome these disorders. EC-MPS releases MPA in the small intestine instead of the enhanced tolerability relative to MMF. In a study performed by Cattaneo and his colleagues on stable kidney transplant recipients, the pharmacokinetics of MPA released from new EC-MPS is completely variable and unpredictable, comparing with that after MMF dosing. Despite that there are no significant differences in mean MPA exposure. Expressed as dosage-adjusted MPA AUC 0 to 12 and maximum concentration of drug (C<sub>max</sub>) aberrant kinetic curves in individual patients were found, with an extremely high variability in MPA CO. AUC 0 to 12 and t<sub>max</sub>. Moreover, most patients who were given EC-MPS had multiple peaks of MPA in their pharmacokinetic profile that was not seen after long-term MMF

administration. These findings were at variance with those of Arns *et al.* showing similar pharmacokinetic profiles after single EC-MPS of MMF administration to kidney transplant patients. Anyway based on these findings, sodium should be taken into account (121), in patients with diabetes.

Some reports suggested that MPA may act with other drugs as well as immunosuppressants (122). The rate of MPA absorption after oral administration of MMF is delayed MPA/MPA glucuronide and tacrolimus may change the rate and amount of MPA absorption because of its prokinetic properties particularly in patients with diabetic gastroparesis. Jeong *et al.* reported that comparing the tacrolimus-based regimen plus standard dose of MMF with CsA-based regimen in renal transplant recipient with diabetes mellitus, showed that MPA exposure was higher in tacrolimus-based regimen. However, changing CsA to tacrolimus didn't seem to have significant impact on the rate of absorption of MPA, as judged by MPA-T<sub>max</sub> (123, 124).

MMF is commonly administered concomitantly with ganciclovir for managing transplant recipients infected with CMV. A study was conducted by Mohammadpour *et al* to evaluate the probable effects of ganciclovir on MPA was not affected by ganciclovir, but ganciclovir increased MPAG AUC and induced enterohepatic recirculation of MPA (125).

Compared with the other immunosuppressant drugs which are currently used, the plasma concentration of MPA is much higher and this makes HPLC measurement of the drug perform easily. By using this technique the major metabolite MPAG can be resolved and quantified (126).

For accurate and precise measurement of the drug concentration range 0.5-15 mg/ml, use of a commercial homogeneous enzyme immunoassay (Dade Behring) is recommended. (127, 128). Because of the cross-reaction of the above antibody, normally, concentrations of MPA measured by HPLC. (129). A new MPA

## Immunosuppressant TDM

assay based on the enzymatic activity of recombinant IMPDH II (the pharmacological target of MPA) with superb relation with HPLC has been recently produced for the measurement of MPA plasma levels. A study conducted by Marquet *et al* compare this new assay with LC-MS/MS for MPA pharmacokinetic studies in different populations.

MMF was administrated in association with cyclosporine, tacrolimus or sirolimus. The result showed that findings were obviously higher than those obtained with LC-MS/MS in patients on cyclosporine or sirolimus, but not in patients on tacrolimus (130).

### **Sirolimus**

This drug has been recently approved in USA for use with cyclosporine after kidney transplantation, but it can be used in other clinical indication and with tacrolimus (131). The drug was also approved in Europe where the license specifies its use in the prophylaxis of graft rejection in adult kidney transplant recipients, primarily in combination with CsA and with blood concentration monitoring.

Therapeutic drug monitoring of sirolimus is still in its primary stage, but data gathered from several clinical trials which were concentration controlled and used sirolimus as primary

immunosuppressive therapy (132-134). The measurement of the drug is possible by using HPLC with either mass spectrometric or ultraviolet detection. For pivotal phase III studies an investigational immunoassay was used (135). This immunoassay is no longer available. As a result, attention is now focusing on HPLC techniques for routine monitoring of the drug (136, 137). The predose concentrations are generally targeted in the range 4-12 µg/l when sirolimus is used with CsA or tacrolimus.

### **Conclusion**

We are entering an era in which combination therapy will become routine and clinicians will adjust the immunosuppression to the characteristics of the individual patient, changing dose and drugs as time progresses and conditions change. In conclusion, the knowledge of the pharmacokinetic principles of immunosuppressants is critical for the success after transplantation. The use of TDM as an important treatment strategy for improved outcomes, however, the knowledge about the limitations of TDM is equally important for a continued rational development of immunosuppressive drug therapy after transplantation.

### **References**

1. Spector R, Park GD, Johnson GF, Vesell ES. Therapeutic drug monitoring. *Clin Pharmacol Ther* 1988; 43:345-353.
2. Matzke GR, Lucarotti RL, Shapiro HS. Controlled comparison of gentamicin and tobramycin nephrotoxicity. *Am J Nephrol* 1983; 3:11-8.
3. Sanathanan LP, Peck CC, Temple R, Lieberman R, Pledger G. Randomization, PK-controlled dosing and titration: an integrated approach for designing clinical trials. *Drug Inf J* 1991; 25:425-431.
4. Peck CC, Barr WH, Benet LZ, Collins J, Desjardins RE, Furst DE, *et al*. Opportunities for integration of pharmacokinetics, pharmacodynamics, and toxicokinetics in rational drug development. *Clin Pharm Ther* 1992; 51:465-473.
5. Peck CC. Rationale for the effective use of pharmacokinetics and pharmacodynamics in early drug development. In: Yacobi A, Skelly JP, Shah VP, Benet LZ, editors. *Integration of pharmacokinetics, pharmacodynamics and toxicokinetics in rational drug development*. New York: Plenum Press;1993.p.1-5.
6. Budde K, Glander P. Pharmacokinetic principles of immunosuppressive drugs. *Ann Transplant* 2008; 13:5-10.
7. Belitsky P, Levy GA, Johnston A. Neoral absorption profiling: an evolution in effectiveness. *Transplant Proc* 2000; 32:45S-52S.
8. Holt DW, Johnston A. Cyclosporin monitoring: its role in autoimmune indications. *J Autoimmun* 1992; 5:82.
9. Nankivell BJ, Borrows RJ, Fung CL The natural history of chronic allograft nephropathy. *N Engl J Med* 2003; 349:2326-2333.

10. Kahan B. Sirolimus: a comprehensive review. *Expert Opin Pharmacother* 2001; 2:1903–1917.
11. Nashan B, Bock A, Bosmans JL. Use of Neoral C monitoring: a European consensus. *Transpl Int* 2005; 18:768-778.
12. Van Hest RM, van Gelder T, Bouw R. Time-dependent clearance of mycophenolic acid in renal transplant recipients. *Br J Clin Pharmacol* 2007; 63:741–752.
13. Hale MD, Nicholls AJ, Bullingham RE. The pharmacokinetic-pharmacodynamic relationship for mycophenolate mofetil in renal transplantation. *Clin Pharmacol Ther* 1998; 64:672–683.
14. van Hest R, Mathot R,ulto A. Predicting the usefulness of therapeutic drug monitoring of mycophenolic acid: a computer simulation. *Ther Drug Monit* 2005; 27:163–167.
15. Fukudo M. Individualized dosage regimen of immunosuppressive drugs based on pharmacokinetic and pharmacodynamic analysis. *Yakugaku Zasshi* 2007; 127:1081-1089.
16. Oellerich M, Barten MJ, Armstrong VW. Biomarkers: the link between therapeutic drug monitoring and pharmacodynamics. *Ther Drug Monit* 2006; 28:35–38.
17. Dambrin C, Klupp J, Morris RE. Pharmacodynamics of immunosuppressive drugs. *Curr Opin Immunol* 2000; 12:557–562.
18. Yatscoff R, Aspeslet WLJ, Gallant HL. Pharmacodynamic monitoring of immunosuppressive drugs. *Clin Chem* 1998; 44:428-32.
19. Ransom JT. Mechanism of action of mycophenolate mofetil. *Ther Drug Monit* 1995; 17: 681–684.
20. Halloran PF, Helms LM, Kung L, Noujaim J. The temporal profile of calcineurin inhibition by cyclosporine *in vivo*. *Transplantation* 1999; 68:1356–13561.
21. Batiuk TD, Pazderka F, Enns J. Cyclosporine inhibition of calcineurin activity in human leukocytes *in vivo* is rapidly reversible. *J Clin Invest* 1995; 96:1254–1260.
22. Rostaing L, Puyoo O, Tkaczuk J. Differences in Type 1 and Type 2 intracytoplasmic cytokines, detected by flow cytometry, according to immunosuppression (cyclosporine A vs. tacrolimus) in stable renal allograft recipients. *Clin Transplant* 1999; 13:400–409.
23. Rostaing L, Tkaczuk J, Durand M. Kinetics of intracytoplasmic Th1 and Th2 cytokine production assessed by flow cytometry following *in vitro* activation of peripheral blood mononuclear cells. *Cytometry* 1999; 35:318–328.
24. Giese T, Zeier M, Meuer S. Analysis of NFAT-regulated gene expression *in vivo*: a novel perspective for optimal individualized doses of calcineurin inhibitors. *Nephrol Dial Transplant* 2004; 19:55–60.
25. Gummert JF, Barten MJ, Sherwood SW. Pharmacodynamics of immunosuppression by mycophenolic acid: inhibition of both lymphocyte proliferation and activation correlates with pharmacokinetics. *J Pharmacol Exp Ther* 1999; 291:1100–1112.
26. Barten MJ, GummertJF, van Gelder T. Flow cytometric quantitation of calcium- dependent and -independent mitogen-stimulation of T cell functions in whole blood: inhibition by immunosuppressive drugs *in vitro*. *J Immunol Methods* 2001; 253:95–112.
27. Stalder M, Birsan T, Holm B. Quantification of immunosuppression by flow cytometry in stable renal transplant recipients. *Ther Drug Monit* 2003; 25:22–27.
28. Israeli M, Yussim A, Mor E. Preceding the rejection: in search for a comprehensive posttransplant immune monitoring platform. *Transplant Immunol* 2007; 18:7–12.
29. Calne RY. Organ transplantation. The present position and future prospects of organ transplantation. *Trans Med Soc Lond* 1969; 85:56-67.
30. Calne RY. Immunosuppression and clinical organ transplantation. *Transplant Proc* 1974; 6: 51.
31. Terasaki PI, McClelland JD, Yuge J, Cecka JM, Gjertson DW, Takemoto S, *et al*. Advances in kidney transplantation: 1985– 1995. In: Cecka JM, Terasaki PI, eitortrs. *Clinical transplants 1995*. Los Angeles: UCLA Tissue Typing;1996.p.487–501.
32. Shaw LM, Bowers L, Demers L, Freeman D, Moyer T, Sanghvi A. Critical issues in cyclosporine monitoring: report of the task force on cyclosporine monitoring. *Clin Chem* 1987; 33:1269–1288.
33. Kahan BD, Shaw LM, Holt D, Grevel J, Johnston A. Consensus document: Hawk’s Cay meeting on therapeutic drug monitoring of cyclosporine monitoring. *Clin Chem* 1990; 36:1510–1516.
34. Shaw LM, Yatscoff RW, Bowers LD, Freeman DJ, Jeffery JR, Keown PA. Canadian consensus meeting on cyclosporine monitoring: report of the consensus panel. *Clin Chem* 1990; 36:1841–1846.
35. Morris RG, Tett SE, Ray JE. Cyclosporine monitoring in Australia:consensus recommendations. *Ther Drug Monit* 1994; 16:570–576.
36. Holt DW, Johnston A, Roberts NB, Tredger JM, Trull AK. Methodological and clinical aspects of cyclosporine monitoring: report of the Association of Clinical Biochemists task force. *Ann Clin Biochem* 1994;31:420–446.

## Immunosuppressant TDM

37. Oellerich M, Armstrong VW, Kahan B, Shaw L, Holt DW, Yatscoff R. Lake Louise consensus conference on cyclosporin monitoring in organ transplantation: report of the consensus panel. *Ther Drug Monit* 1995;17:642–654.
38. Holt DW, Johnston A, Thomson A, Starzl T. *Immunosuppressive Drugs: Developments in Anti-Rejection Therapy, Pharmacokinetics and monitoring of cyclosporin A*. London: Edward Arnold; 1994.p.3,37-45.
39. Johnston A, Holt DW. Therapeutic drug monitoring of immunosuppressant drugs. *Br J Clin Pharmacol* 1999; 47:339-350.
40. Johnston A, Marsden JT, Holt DW. The influence of haematocrit on blood cyclosporin measurements in vivo. *Br J Clin Pharmacol* 1988; 25:509-513.
41. Johnston A, Marsden JT, Holt DW. Sample pretreatment to minimize interference from whole blood in the radioimmunoassay for cyclosporine. *Transplantation* 1987; 44:332.
42. Holt DW, Marsden JT, Johnston A. Measurement of cyclosporine: methodological problems. *Transplant Proc* 1986; 18:101-110.
43. Johnston A, Marsden JT, Holt DW. The United Kingdom Cyclosporin Quality Assessment Scheme. *Ther Drug Monit* 1986; 8:200-204.
44. Tsang VT, Johnston A, Heritier F, Leaver N, Hodson ME, Yacoub M. Cyclosporin pharmacokinetics in heart-lung transplant recipients with cystic fibrosis. Effects of pancreatic enzymes and ranitidine. *Eur J Clin Pharmacol* 1994; 46:261-265.
45. Holt DW, Marsden JT, Johnston A, Bewick M, Taube DH. Blood cyclosporin concentrations and renal allograft dysfunction. *Br Med J* 1986; 293:1057-1059.
46. Sketris I, Yatscoff R, Keown P. Optimizing the use of cyclosporine in renal transplantation. *Clin Biochem* 1995; 28:195-211.
47. Holt DW, Johnston A. Cyclosporin assay techniques. Accuracy and reproducibility variables impacting on measurements. *Int J Rad Appl Instrum B* 1990; 17: 733-736.
48. Holt DW, Johnston A, den Boer NC, van der Heiden C, Leijnse B, Souverijn JHM. Clinical chemistry plenum publishing corporation; practical applications of therapeutic drug monitoring: The impact of technological developments. *Int J Rad Appl Instrum* 1989; 93-102.
49. Holt DW, Johnston A, Roberts NB, Tredger JM, Trull AK. Methodological and clinical aspects of cyclosporine monitoring: report of the Association of Clinical Biochemists task force. *Ann Clin Biochem* 1994; 31:420-446.
50. Dalrymple-Hay M, Meara M, Reynolds L. Changing stable heart transplant recipients from Sandimmune to Neoral. *Transplant Proc* 1996; 28:2285-2286.
51. Holt DW, Mueller EA, Kovarik JM, van Bree JB, Richard F, Kutz K. Sandimmune neoral pharmacokinetics: impact of the new oral formulation. *Transplant Proc* 1995; 27:1434-1437.
52. Holt DW, Mueller EA, Kovarik JM, van Bree JB, Kutz K. The pharmacokinetics of Sandimmune Neoral: a new oral formulation of cyclosporine. *Transplant Proc* 1994; 26:2935-2939.
53. Holt DW, Johnston A. Monitoring new immunosuppressive agents. Are the methods adequate? *Drug Metabol Drug Interacts* 1997; 14:5-15.
54. Merton G, Jones K, Lee M, Johnston A, Holt DW. Accuracy of cyclosporin measurements made in capillary blood samples obtained by skin puncture. *Ther Drug Monit* 2000; 22:594-598.
55. Lindholm A, Kahan BD. Influence of cyclosporine pharmacokinetics, trough concentrations, and AUC monitoring on outcome after kidney transplantation. *Clin Pharmacol Ther* 1993; 54:205-218.
56. Amante AJ, Kahan BD. Abbreviated area-under-the-curve strategy for monitoring cyclosporine microemulsion therapy in immediate posttransplant period. *Clin Chem* 1996; 42: 1294-1296.
57. El-Agroudy AE. Cyclosporine therapeutic monitoring with Cmax in kidney transplant recipients: does it fit for all populations? *Exp Clin Transplant* 2008; 6:282-286.
58. Kahan BD, Welsh M, Rutzky LP. Challenges in cyclosporine therapy: the role of therapeutic monitoring by area under the curve monitoring. *Ther Drug Monit* 1995; 17:621–624.
59. Holt DW, Johnston A. Cyclosporin monitoring: trough or AUC? *Perspectives* 1996; 2:49-52.
60. Holt DW, Johnston A. Cyclosporin Microemulsion. A guide to usage and monitoring. *Biodrugs* 1997; 7:175-197.
61. Johnston A, Sketris I, Marsden JT. A limited sampling strategy for the measurement of cyclosporine AUC. *Transplant Proc* 1990; 22:1345-1346.
62. Johnston A, Kovarik JM, Mueller EA, Holt DW. Predicting patients' exposure to cyclosporin. *Transplant Int* 1996; 9:110-113.
63. Gaspari F. Abbreviated kinetic profiles in area-under-the-curve monitoring of cyclosporine therapy. Technical note. *Kidney Int* 1998; 54:2146-2150.

64. Fu LW. Cyclosporin pharmacokinetics following administration of capsules and Neoral in paediatric patients with lupus nephritis. *Br J Clin Pharmacol* 1997; 44:125-127.
65. Cantarovich F, Bizollon C, Cantarovich D, Lefrancois N, Dubernard JM, Traeger J. Cyclosporine plasma levels six hours after oral administration. A useful tool for monitoring therapy. *Transplantation* 1988; 45:389-394.
66. Cantarovich M, Besner JG, Fitchett DH, Latter DA. Efficacy and side-effects of cyclosporine dose monitoring with levels 6 h after the morning dose in heart transplant patients. *Clin Transplant* 1997; 11:400-405.
67. Rocha G, Deschenes J, Cantarovich M. Cyclosporine monitoring with levels 6 hours after the morning dose in patients with noninfectious uveitis. *Ophthalmology* 1997; 104:245-251.
68. Grant D, Rochon J, Levy G. Comparison of the long-term tolerability, pharmacodynamics, and safety of Sandimmune and Neoral in liver transplant recipients. Ontario Liver Transplant Study Group. *Transplant Proc* 1996; 28:2232-2233.
69. Grant D, Kneteman N, Tchervenkov J, Roy A, Murphy G, Tan A, *et al*. Peak cyclosporine levels (C<sub>max</sub>) correlate with freedom from liver graft rejection: results of a prospective, randomized comparison of neoral and sandimmune for liver transplantation. *Transplantation* 1999; 67:1133-1137.
70. Levy GA, Grant D. Neoral in liver transplantation. *Transplant Proc* 1996; 28:1019-1021.
71. Levy G. New strategies for therapeutic drug monitoring of Neoral. Two-hour cyclosporine concentration (C<sub>2</sub>) as a monitoring tool for Neoral, Oxford: Blackwell Science;1998.p.19-22.
72. Langers P. Switching monitoring of emulsified cyclosporine from trough level to 2-hour level in stable liver transplant patients. *Liver Transpl* 2004; 10:183-189.
73. Naito M. Monitoring of blood cyclosporine concentration in steroid-resistant nephrotic syndrome. *Int Med* 2008; 47:1567-1572.
74. Takeuchi H. Evidence of different pharmacokinetics between cyclosporine and tacrolimus in renal transplant recipients: why cyclosporine is monitored by C<sub>2</sub> level and tacrolimus by trough level. *Transplant Proc* 2008; 40:2240-2242.
75. Halloran PF, Helms LM, Kung L, Noujaim J. The temporal profile of calcineurin inhibition by cyclosporine in vivo. *Transplantation* 1999; 68:1356-1361.
76. Sindhi R, LaVia MF, Paulling E. Stimulated response of peripheral lymphocytes may distinguish cyclosporine effect in renal transplant recipients receiving a cyclosporine+rapamycin regimen. *Transplantation* 2000; 69:432-436.
77. Belitsky P, Dunn S, Johnston A, Levy G. Impact of absorption profiling on efficacy and safety of cyclosporin therapy in transplant recipients. *Clin Pharmacokinet* 2000; 39:117-125.
78. Cantarovich M, Elstein E, de Varennes B, Barkun JS. Clinical benefit of neoral dose monitoring with cyclosporine 2-hr post-dose levels compared with trough levels in stable heart transplant patients. *Transplantation* 1999; 68:1839-1842.
79. Cantarovich M, Quantz M, Elstein E, Ergina P, Magnan C, de Varennes B. Neoral dose monitoring with cyclosporine 2-hour postdose levels in heart transplant patients receiving anti-thymocyte globulin induction. *Transplant Proc* 2000; 32:446-448.
80. Marquet, P. Performance of the new mycophenolate assay based on IMPDH enzymatic activity for pharmacokinetic investigations and setup of Bayesian estimators in different populations of allograft recipients. *The Drug Monit* 2009; 31:443-450.
81. Johnston A, David OJ, Cooney GF. Pharmacokinetic validation of neoral absorption profiling. *Transplant Proc* 2000; 32:53S-6S.
82. Pourfarziani V, Taher S. Mycophenolate sodium increases cyclosporine blood levels in renal transplant recipients. *Saudi J Kidney Dis Transpl* 2009; 20:991-4.
83. Holt DW, White DJ. How to measure cyclosporin. *Lancet* 1984; 2:228.
84. Holt DW, Johnston A. Cyclosporin A: analytical methodology and factors affecting therapeutic drug monitoring. *Ther Drug Monit* 1995; 17:625-630.
85. Johnston A, Cullen G, Holt DW. Quality assurance for cyclosporin assays in body fluids. *Ann Acad Med Singapore* 1991; 20:3-8.
86. Lindholm A, Dahlqvist R, Groth GG, Sjoqvist F. A prospective study of cyclosporine concentration in relation to its therapeutic effect and toxicity after renal transplantation. *Br J Clin Pharmacol* 1990; 30:443-452.
87. Johnston A, Holt DW. Calibration of the CYCLO-Trac SP cyclosporine radioimmunoassay. *Clin Chem* 1993; 39:2532-2533.
88. McLachlan AJ, Tett SE. Effect of metabolic inhibitors on cyclosporine pharmacokinetics using a population approach. *Ther Drug Monit* 1998; 20:390-395.
89. Schuëtz E, Svinarov D, Shipkova M. Cyclosporin whole blood immunoassays (AxSYM, CEDIA, and Emit): a critical overview of performance characteristics and comparison with HPLC. *Clin Chem* 1998; 44:2158-2164.

## Immunosuppressant TDM

90. Jusko WJ, Thomson AW, Fung J. Consensus document: therapeutic monitoring of tacrolimus (FK-506). *Ther Drug Monit* 1995; 17:606-614.
91. McMaster P, Mirza DF, Ismail T, Vennarecci G, Patapis P, Mayer AD. Therapeutic drug monitoring of tacrolimus in clinical transplantation. *Ther Drug Monit* 1995; 17:602-605.
92. Venkataramanan R, Swaminathan A, Prasad T. Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet* 1995; 29:404-430.
93. Anonymous. Japanese study of FK 506 on kidney transplantation: the benefit of monitoring the whole blood FK 506 concentration. Jap FK 506 Study Group. *Transplant Proc* 1991; 23: 3085-3088.
94. Hedayat S, Kershner RP, Su G. Relationship of whole-blood FK506 concentrations to rejection and toxicity in liver and kidney transplants. *J Biopharm Stat* 1996; 6:411-424.
95. Ku Y-M, Min DI. An abbreviated area-under-the-curve monitoring for tacrolimus patients with liver transplants. *Ther Drug Monit* 1998; 20:219-223.
96. Cantarovich M, Fridell J, Barkun J. Optimal time points for the prediction of the area-under-the-curve in liver transplant patients receiving tacrolimus. *Transplant Proc* 1998; 30:1460-1461.
97. Wong KM, Shek CC, Chau KF, Li CS. Abbreviated tacrolimus area-under-the-curve monitoring for renal transplant recipients. *Am J Kidney Dis* 2000; 35:660-666.
98. Ihara H, Shinkuma D, Ichikawa Y, Nojima M, Nagano S, Ikoma F. Intra- and interindividual variation in the pharmacokinetics of tacrolimus (FK506) in kidney transplant recipients importance of trough level as a practical indicator. *Int J Urol* 1995; 2:151-155.
99. Laskow DA, Vincenti F, Neylan JF, Mendez R, Matas AJ. An open-label, concentration-ranging trial of FK-506 in primary kidney transplantation. *Transplantation* 1996; 62:900-905.
100. Kershner RP, Fitzsimmons WE. Relationship of FK-506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation* 1996; 62:920-926.
101. Taylor PJ, Hogan NS, Lynch SV, Johnson AG, Pond SM. Improved therapeutic drug monitoring of tacrolimus (FK506) by tandem mass spectrometry. *Clin Chem* 1997; 43:2189-2190.
102. Schambeck CM, Bedel A, Keller F. Limit of quantitation (Functional Sensitivity) of the new IMx Tacrolimus II microparticulate immunoassay. *Clin Chem* 1998; 44:2217.
103. Iwasaki K. Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. *Drug Metab Pharmacokinet* 2007; 22:328-335.
104. Weisel M, Carl S. Placebo-controlled study of mycophenolate mofetil combined with cyclosporin, corticosteroids for prevention of acute rejection. European Mycophenolate Mofetil Cooperative Study Group. *Lancet* 1995; 345:1321-1325.
105. Sollinger HW. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. U. S. Renal Transplant Mycophenolate Mofetil Study Group. *Transplantation* 1995; 60:225-232.
106. Sollinger HW. A blinded randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. The Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. *Transplantation* 1996; 61:1029-1037.
107. Bullingham RE, Nicholls A, Hale M. Pharmacokinetics of mycophenolate mofetil (RS61443): a short review. *Transplant Proc* 1996; 28:925-929.
108. Schutz E, Shipkova M, Armstrong VW. Therapeutic drug monitoring of mycophenolic acid: comparison of HPLC and immunoassay reveals new MPA metabolites. *Transplant Proc* 1998; 30:1185-1187.
109. Mohammadpour AH. Estimation of abbreviated mycophenolic acid area under the concentration-time curve during early posttransplant period by limited sampling strategy. *Transplant Proc* 2008; 40:3668-3672.
110. Bullingham RES, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet* 1998; 34:429-455.
111. Hao C. Monitoring mycophenolic acid pharmacokinetic parameters in liver transplant recipients: prediction of occurrence of leukopenia. *Liver Transpl* 2008; 14:1165-1173.
112. Shaw LM, Korecka M, van Breeman R, Nowak I, Brayman KL. Analysis, pharmacokinetics and therapeutic drug monitoring of mycophenolic acid. *Clin Biochem* 1998; 31:323-328.
113. Meiser BM, Pfeiffer M, Jagiello-Kraatz M. Mycophenolate mofetil dose adjustment based on trough levels improves outcome after heart transplantation. *J Heart Lung Transplant* 1998; 17: 85-86.
114. Bullingham RE, Nicholls A, Hale M. Pharmacokinetics of mycophenolate mofetil (RS61443): a short review. *Transplant Proc* 1996; 28:925-929.
115. Nazemian F, Mohammadpour AH, Abtahi B, Naghibi M. influence of renal graft function on mycophenolic acid pharmacokinetic during the period after kidney transplant. *Exp Clin Transplant* 2008; 4:276-281.

116. Mohammadpour AH, Nazemian F, Abtahi B, Naghibi M. Estimation of abbreviated Mycophenolic acid area under the concentration time curve during post transplant period by limited sampling strategy. *Transplant Proc* 2008; 40:3668-3672.
117. van Gelder T, Hilbrands LB, Vanrenterghem Y. A randomized double-blind, multicenter plasma concentration controlled study of the safety and efficacy of oral mycophenolate mofetil for the prevention of acute rejection after kidney transplantation. *Transplantation* 1999; 68:261-266.
118. Takahashi K, Ochiai T, Uchida K, Yasamura T, Ishibashi M, Suzuki S. Pilot study of mycophenolate mofetil (RS-61443) in the prevention of acute rejection following renal transplantation in Japanese patients. *Transplant Proc* 1995; 27:1421-142.
119. Aw MM. Mycophenolic acid pharmacokinetics in pediatric liver transplant recipients. *Liver Transpl* 2003; 9:383-388.
120. Mourad M. Correlation of mycophenolic acid pharmacokinetic parameters with side effects in kidney transplant patients treated with mycophenolate mofetil. *Clin Chem* 2001; 47:88-94.
121. Cattaneo D. Pharmacokinetics of mycophenolate sodium and comparison with the mofetil formulation in stable kidney transplant recipients. *Clin J Am Soc Nephrol* 2007; 2:1147-1155.
122. Nazemian F. Pharmacokinetics of mycophenolic acid during the early period after renal transplant. *Exp Clin Transplant* 2007; 5:658-663.
123. Park JM, Lake KD, Cibrik DM. Impact of changing from cyclosporine to tacrolimus on pharmacokinetics of mycophenolic acid in renal transplant recipients with diabetes. *Ther Drug Monit* 2008; 30:591-596.
124. Nashan B. Pharmacokinetics, efficacy, and safety of mycophenolate mofetil in combination with standard-dose or reduced-dose tacrolimus in liver transplant recipients. *Liver Transpl* 2009; 15:136-147.
125. Mohammadpour AH, Nazemian F, Hassanzadeh khayat M, Bahrami AA, Kazemi M. Effect of ganciclovir on pharmacokinetics of mycophenolic mofetil, in kidney trnsplant patients. *Iran J Basic Med Sci* 2008; 10:233-238.
126. Shipkova M, Niedmann PD, Armstrong VW. Simultaneous determination of mycophenolic acid and its glucuronide in human plasma using a simple high-performance liquid chromatography procedure. *Clin Chem* 1998; 44:1481-1488.
127. Beal JL, Jones CE, Taylor PJ, Tett SE. Evaluation of an immunoassay (EMIT) for mycophenolic acid in plasma from renal transplant recipients compared with a high-performance liquid chromatography assay. *Ther Drug Monit* 1998; 20:685-690.
128. Blanchet B. Comparison of a new enzymatic assay with a high-performance liquid chromatography/ ultraviolet detection method for therapeutic drug monitoring of mycophenolic acid in adult liver transplant recipients. *Liver Transpl* 2008; 14:1745-1751.
129. Saadat-Lajevardi S, Jones K, Lee T. The International Mycophenolic Acid Proficiency Testing Scheme [Abstract]. *Ther Drug Monit* 1999; 21:440-441.
130. Mathew BS. A limited sampling strategy for tacrolimus in renal transplant patients. *Br J Clin Pharmacol* 2008; 66:467-472.
131. McAlister VC, Gao Z, Peltekian K, Domingues J, Mahalati K, MacDonald AS. Sirolimus-tacrolimus combination immunosuppression [letter]. *Lancet* 2000; 355:376-377.
132. Groth CG, Backman L, Morales JM. Sirolimus (rapamycin) -based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group [see comments]. *Transplantation* 1999; 67:1036-1042.
133. Kreis H, Cisterne JM, Land W. Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. *Transplantation* 2000; 69:1252-1260.
134. Kahan BD, Julian BA, Pescovitz MD, Vanrenterghem Y, Neylan J. Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in Caucasian recipients of mismatched primary renal allografts: a phase II trial. Rapamune Study Group. *Transplantation* 1999; 68:1526-1532.
135. Jones K, Saadat-Lajevardi S, Lee TD. An immunoassay for the measurement of sirolimus. *Clin Ther* 2000; 22:49-61.
136. Holt DW, Lee TD, Jones K, Johnston A. Validation of an assay for routine monitoring of sirolimus using HPLC with mass spectrometric detection. *Clin Chem* 2000; 46:1179-1183.
137. Napoli KL. A practical guide to the analysis of sirolimus using high-performance liquid chromatography with ultraviolet detection. *Clin Ther* 2000; 22:B14-B24.