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**Clinical mycophenolic acid monitoring in liver transplant recipients**

Chen H *et al.* Clinical mycophenolic acid monitoring in LT recipients

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**Abstract**

In liver transplantation, the efficacy of mycophenolate mofetil (MMF) has been confirmed in clinical trials and studies. However, therapeutic drug monitoring for mycophenolic acid (MPA) has not been fully accepted in liver transplantation as no long-term prospective study of concentration controlled *vs* fixed-dose prescribing of MMF has been done. This review addressed MPA measurement, pharmacokinetic variability and reasons of this variation, exposure relating to acute rejection and MMF-associated side effects in liver transplant recipients. Limited sampling strategies to predict MPA area of concentration-time under the curve have also been described, and the value of clinical use needs to be investigated in future. The published data suggested a fixed-dosage MMF regimen might not be suitable and monitoring of MPA exposure seems helpful in various clinical settings of liver transplant recipients.

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**Key words:** Mycophenolate mofetil; Mycophenolic acid; Pharmacokinetics; therapeutic drug monitoring; Liver transplantation

**Core tip:** We discussed the methods of mycophenolic acid (MPA) monitoring, pharmacokinetic characteristics, clinical exposure relating to acute rejection and mycophenolate mofetil (MMF) associated side effects in liver transplant recipients. We also introduced the methods of limited sampling strategies to predict MPA area of concentration-time under the curve. It demonstrated that a fixed-dosage MMF regimen might not be suitable. In clinical setting, monitoring of MPA exposure seems reasonable and necessary.

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**INTRODUCTION**

Mycophenolate mofetil (MMF, CellCept, Hoffman-La Roche) has almost full bioavailability by oral intake and is a pro-drug that is hydrolyzed to release mycophenolic acid (MPA)[1]. Subsequently MPA is metabolized to a major phenolic glucuronide, mycophenolic acid glucuronide (MPAG), and a minor acyl glucuronide (AcMPAG)[2-4]. MPA, the active compound of MMF, is a selective, reversible and non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH) in process of de novo purine synthesis in T and B lymphocytes[5]. As a result nucleic acid synthesis is arrested and immune reaction of allograft is inhibited.

As a major immunosuppressive agent, MPA has been widely used for the prevention of acute rejection in transplant recipients[6]. A dose of 1-1.5 g (fixed-dose) administered orally or intravenously twice a day is recommended for use in renal, cardiac and liver transplant patients in the product leaflet of Hoffman-La Roche Ltd[7]. However, wide inter-patient variability in MPA exposure has been showed in renal, heart and liver transplant patients on a fixed MMF dose[1,8,9]. It’s confirmed in renal transplantation that MPA concentration controlled regimen comparing with fixed-dose regimen can reduce the risk of treatment failure and acute rejection in recipients 12 months post-transplant with no increase in adverse events[10]. Individualizing MMF dose instead of using a fixed dose might be helpful to optimizing immunosuppression and minimize potential toxic effects. Carrying out therapeutic drug monitoring (TDM) seems reasonable and necessary and routine monitoring for MPA is increasingly performed. However, the experience with TDM for MPA in liver transplantation is much limited comparing to lots of investigations performed in kidney transplant patients. At present, a fixed dose of 1 or 1.5 g twice daily of MMF is the standard protocol in liver transplantation with adjustments only in relation to side effects or to its efficacy[11]. No more MPA monitoring-based guidelines for MMF dosage have been set up[12]. It is necessary to study the MPA pharmacokinetics and to carry out TDM of MMF in liver transplant recipients.

In this review, we will focus on four areas in liver transplant recipients: (1) MPA efficacy and MMF-related side effects; (2) methods for measuring MPA concentration; (3) MPA pharmacokinetics; (4) limited sampling strategy (LSS); and (5) MPA concentration–effect relationship.

**MPA EFFICACY AND MMF-RELATED SIDE EFFECTS IN LIVR TRANSPLANATION**

MMF has been successfully used with a reduced dosage of calcineurin inhibitor (CNI) and steroids to reduce the rate of acute rejection, lessen side effects of CNI after liver transplantation and improve long-term survival rate of allografts and recipients[13-15]. In a randomized double-blind comparative study of MMF and azathioprine in primary liver transplant recipients, the incidence of acute rejection or graft loss was 47.7% in the azathioprine patients and 38.5% in the MMF patients during the first 6 months after transplantation[16]. Recently, [Goralczyk](http://www.ncbi.nlm.nih.gov/pubmed?term=Goralczyk%20AD%5BAuthor%5D&cauthor=true&cauthor_uid=22813081) *et al*[17] reported the results of a systematic review and meta-analysis of randomized controlled trials of CNI sparing with MMF in liver transplantation. The authors obtained the conclusion which *de novo* use of MMF in combination with low-dose tacrolimus (TAC) is not associated with increased risk of acute rejection, graft loss, or death and has an acceptable side effect profile. Ringe *et al*[18] reported that use of TAC plus MMF immunosuppressive regimen without corticosteroids from the beginning after liver transplantation led to a graft survival of 83.9 % at 2 years.

MMF has no nephrotoxity and no effect on the lipid profile or other cardiovascular risk factors as systemic hypertension or diabetes mellitus[19]. MMF has been widely used to improve the renal function commonly associated with CNI[20,21]. Nephroprotective effect and promotion of allograft tolerance after liver transplantation were confirmed by MMF with replaced CNI or reduced or interrupted CNI therapy in three randomized controlled trials[22-24]. Recently, Kriss *et al*[25] reported that serum creatinine and calculated glomerular filtration rate (GFR) improved on MMF monotherapy in 23 cases comparing with 23 recipients remaining on CNI-based therapy. Improvement was pronounced significantly in patients with milder renal dysfunction with decrease in serum creatinine (1.63 ± 0.29 *vs* 1.34 ± 0.26 mg/dL, *P* = 0.02) at last follow-up. In a retrospective analysis of pediatric liver transplantation by Evans *et al*[26], there was a statistically significant increase to a median calculated GFR of 69 (28-114) mL/min/1.73 m2 by 1 month and a further increase to a median calculated GFR of 77 (24 – 105) mL/min/1.73 m2 by 2 mo with MMF monotherapy or low-dose cyclosporine A (CsA) or TAC, after which time calculated GFR was maintained. MMF treatment provided safe and effective immunosuppression and allowed CsA or TAC to be discontinued or reduced, leading to improvement of renal function.

CNI increased cardiovascular risk after liver transplantation. Aberg *et al*[27] analyzed the cardiovascular risk of 77 recipients based on CNI and antibodies at 5 years after liver transplantation. At least one cardiovascular risk factor developed in 92% of patients, and the prevalence of treated hypertension, dyslipidemia, overweight, obesity and diabetes were 71%, 61%, 32%, 13% and 10%, respectively. Antibody therapy associated with a 1.49-fold increase in the risk of hypertension (95%CI: 1.15-1.94) and a 6.43-fold increase in the risk of diabetes. In a randomized prospective study by Junge *et al*[28], TAC with MMF comparing TAC with corticosteroid significantly decreased glucose levels with lower HbA1c and the need for insulin as well as reduced significantly serum cholesterol and the incidence of osteopenia. It was confirmed in some studies that immunosuppressive protocol based on reducing doses of TAC[[22](http://www.ncbi.nlm.nih.gov/pubmed?term=Schlitt%20HJ%5BAuthor%5D&cauthor=true&cauthor_uid=11558484),29] or corticosteroids[[30](http://www.ncbi.nlm.nih.gov/pubmed?term=Gerhardt%20T%5BAuthor%5D&cauthor=true&cauthor_uid=19541578)] with MMF could improve blood pressure with reduction of antihypertensive medication.

In summary, the protocol using MMF with reduced TAC improves renal function, decreases the cardiovascular risk and avoids of steroid-associated adverse effects.

The principal complications of MMF are gastrointertinal effects (nausea, vomiting, abdominal pain and diarrhea) and myelosuppression (leucopenia, anaemia and thrombocytopenia)[19]. In the study by Chen *et al*[31], 66.7 % of the patients had at least one episode of MMF-related side effects of hematologic disorder (36.51%), gastrointestinal reaction (25.40%) and infection (20.63%) during the study evaluation up to the third post-transplantation month. For 34 of patients (53.97%), the symptoms disappeared until MMF was decreased gradually in dosage or stopped. Tredger *et al*[32] reported that a total of 96 adverse events possibly associated with MMF therapy were well documented in the 147 adult patients, mainly including gastrointestinal dysfunction, leucopenia and infection.

In the study by Wiesner *et al*[16], diarrhea occurred in 51.3% of liver transplant recipients receiving MMF (1.5 g, twice daily) and corticosteroids. It seems that CNI therapy with MMF has a higher incidence of diarrhea than monotherapy with MMF in liver transplantation. Diarrhea was observed in 31.4% by using MMF combined with CNIs[[33](http://www.ncbi.nlm.nih.gov/pubmed?term=Pfitzmann%20R%5BAuthor%5D&cauthor=true&cauthor_uid=12865798)]. By mono-therapy of MMF, lower rate of diarrhea (14%-15 %) were showed[34-36]. In stable renal transplant recipients, Maes *et al*[37] reported that gastric emptying of solids was significantly faster on TAC compared with CsA. Cantarovich *et al*[13] reported that the incidence of diarrhea was 18% based on cyclosporine and MMF regimen in liver transplantation while occurrence of diarrhea was 38.63% in patients combined with TAC in the study by Zhang *et al*[38].

**METHODS FOR MEASURING MPA CONCENTRATION**

Methods used for measurement of MPA concentration should be sensitive, accurate, specific, rapid, convenient and economical. Different methods were developed to determine total or unbinding MPA (free MPA, fMPA) and MPA metabolites. These methods can be classified as chromatographic method and immunoassay.

***Chromatographic method***

Chromatographic method has the advantages of good specificity and sensitivity. It is especially useful in monitoring the MPA and its metabolites simultaneously. However, these methods have the common shortcomings including the complex in the sample treating, which is labor extensive and time-consuming. Chromatographic methods are suitable for laboratories with large sample load. According to the variance in the detective method, chromatographic based assays used for MPA monitoring include high-performance liquid chromatography (HPLC) with ultra-violet (UV) or fluorescence detector and LC-MS/MS assay.

***Determination of total MPA***

Although LC-MS/MS is the most sensitive assay, HPLC-UV is sufficient in the monitoring of total MPA. Different UV absorption wave length was selected for MPA monitoring[39-41]. Most of these assays had the lower limit of quantification (LLOQ) of about 0.2 μg/mL. The sample preparing procedure of previous studies include solid phased extraction (SPE)[40], liquid-liquid extraction (LLE), and protein precipitation. There is less interference on the chromatography graphs obtained by SPE or LLE method than protein precipitation. However, sample preparing by SPE method include several steps. It is time-consuming and the SPE columns add the cost of determination. LLE method is also labour-intensive, and large quantity of organic solvents used may be harmful. Although protein precipitation does not provide clean extractions like SPE and LLE, it is simpler, more rapid and more economical compared with SPE and LLE. Shipkova *et al*[42] used acetontrile, sodium tungstate and perchloric acid to precipitate protein. Khoschsorur *et al*[43] used 2 folds of acetontrile as sample precipitation reagent. In the study by Chen *et al*[41], one fold of methanol containing 5 % ZnSO4 was used as precipitate reagent, the procedure is very simple and rapid. The result is reliable.

***Determination of total MPA and its metabolites***

As mentioned in the former part, MPA is metabolized primarily by glucuronidation to form MPAG and AcMPAG. Although MPAG is pharmacologically inactive, it can be hydrolyzed back to MPA and absorbed again during enterohepatic recirculation (EHC). AcMPAG has been observed regularly in the plasma of liver, kidney, and heart transplant recipients undergoing treatment with MMF. Chromatically based methods were established to monitor MPA, MPAG and AcMPAG simultaneously, including HPLC-UV methods[39-41] and LC-MS/MS methods[44,45]. To separate MPA from its metabolites sufficiently, both isocratic[41] and gradient[39,40] mobile phase system was used. The peak areas of MPA, MPAG and AcMPAG under 304nm were significantly lower than 215 nm (8.3, 21.8 and 9.4 fold lower, respectively) or 254 nm (2.0, 5.0, 2.7 fold lower, respectively). Higher sensitivity was attained at 215 and 254 nm compared with 304 nm. However, the chromatography of 304 nm provided a cleaner baseline and more reproducible results in our study[41].

Klepacki *et al*[45] established an UHPLC-MS/MS assay using liquid-handling robotic extraction for the quantification of MPA and its metabolites in human plasma and urine. The LLOQ of MPA and its metabolites were 0.097 μg/mL for MPA and MPAG and 0.156 μg/mL for AcMPAG. The total assay run time was 2.3 minutes. The assay has proven to be robust and reliable during the measurement of samples from several pharmacokinetics trials.

***Determination of total fMPA***

The assays for detection fMPA are more complicated due to its very low level in plasma, therefore establishment of more sensitive method is needed[46-49]. The pivotal sample treatment step is to separate fMPA from protein-bounded MPA. Equilibrium dialysis and ultrafiltration can generate comparable results, most studies selected ultrafiltration due to its practicability, accuracy and reproducibility. In the study by Aresta *et al*[46], plasma samples were ultrafiltrated in combination with SPE. The detection wave length was UV 215 nm. The LLOQ was 26 ng/mL. Shen *et al*[47] used a HPLC-fluorescence method to determine total MPA and fMPA. The LLOQ of fMPA was 5 ng/mL. Chen *et al*[48] also developed a HPLC-fluorecence method to determine fMPA in plasma previously. The authors found that at a solvent pH of 8.5, the LLOQ of fMPA reached 2.5 ng/mL, which was much lower than that of HPLC-UV and comparable with LC-MS/MS. The retention time of MPA was about 3 min when pH of the mobile phase was increased to 8.5. To prevent the endogenous interference, TBA was used as ion-pair reagent[48].

The lower limit of assay sensitivity of LC-MS/MS made it the best choice in measuring fMPA concentration. Patel *et al*[49] established an LC-MS/MS assay, the plasma was subjected to ultrafiltration followed by SPE using C18 cartridges. The assay has a LLOQ of 1 ng/mL and an accuracy of > 95%. The method reported has an adequate degree of robustness and dynamic concentration range for the measurement of fMPA for therapeutic drug monitoring purposes or pharmacokinetics investigations. TDM of MPA in saliva offers a favorable non-invasive approach. Besides, concentration of MPA in saliva can be considered as the fMPA approximately. The LC-MS/MS assays for monitoring MPA in saliva were established for adult and pediatric patients.

***Immunoassay***

Immunoassays including series of methods, the mechanism of these methods is the competent combination of antibody with the MPA in plasma and labeled MPA. The most frequently used assay was commercial enzyme multiplied immunoassay technology (EMIT) assay. The advantage of less labor intensive of EMIT rendered this assay more suitable for conventional clinical TDM. Although several studies revealed a 9%-15 % of systematic positive bias between EMIT and HPLC assay, EMIT has been proven to be an efficient method for monitoring of MPA[50-52]. In the study by Chen *et al*[48] on liver transplant patients, 470 total MPA concentrations were determined by both HPLC method and EMIT method. The authors found the relationship of the two methods was EMIT = 1.074 × HPLC + 0.582 (*r*2 = 0.918, *n* = 470, *P* < 0.05) for total MPA, a good correlation between HPLC and EMIT was obtained with a positive bias of EMIT for total MPA (27.0%). The bias of EMIT is suggested causing by the cross-reactivity of AcMPAG.

Chen *et al*[48] established an EMIT method for the determination of fMPA for the first time. The calibration range of fMPA was 0.0050 – 0.50 μg/mL for EMIT method. Mean recovery of the two methods was 97.1%. The intra-day and inter-day coefficient of variations were 4.51%-15.8%, 5.83%-19.5% for EMIT. The authors determined 297 fMPA concentration by both HPLC method and EMIT method, and found relationship of the two methods was EMIT = 1.068 × HPLC + 0.004 (*r*2 = 0.945, *n* = 297, *P* < 0.05), a good correlation between HPLC and EMIT was obtained with a positive bias of EMIT for total MPA (23.3%). Although the LLOQ of EMIT is higher than HPLC method, more than 95% fMPA samples determined by EMIT are higher than LLOQ. EMIT can also be used in monitoring of fMPA.

Other immunoassays including the cloned enzyme donor immunoassay, enzyme inhibition assay[53], and particle enhanced turbimetric inhibition immunoassay[54]. These methods are either under-development or not widely used.

**CHARACERISTICS OF PHARMACOKINETICS OF MPA**

At present, a fixed dose of 1 or 1.5 g twice daily of MMF is the standard protocol in liver transplantation with adjustments only in relation to side effects or to its efficacy[11]. However, there are wide variations in MPA pharmacokinetics reported with standard MMF dosing in liver transplant recipients. Shaw *et al*[8] in his review presented the exposure of MPA AUC in liver transplantation after 1.0 g dosage, twice daily: the range of MPA AUC 5-160 mg.h/L in 22 liver transplant recipients. This kind of variations has been confirmed in some studies in adult (Table 1) or pediatric liver transplantation[55].

The investigations for MPA pharmacokinetics in liver transplantation are focused on the early period after operation. The characteristics of MPA pharmacokinetics in early phase (about within 6 mo): first, mean MPA AUC will increase with the pattern of time-dependence especially in two or three weeks after liver transplantation. Second, large range of MPA pharmacokinetics intra-patient and/or within-patient is observed. Third, the relationship between MMF dosage and MPA pharmacokinetic parameters is variable. Fourth, the difference of MPA exposure in concomitance with different immunosuppressive drug.

Reasons of variation of MPA exposure may include type of recipient and donor graft, the process of liver transplantation, dosage of MMF, enterohepatic recirculation, bowel, liver, and renal dysfunction and drug interactions.

***Type of recipient and donor graft***

In a control study by Jain *et al*[56], the MPA AUC in living donor liver transplant (LDLT) patients were a 4-fold higher than in deceased donor liver transplant (DDLT) patients per 1 g MMF intravenously. The mean plasma concentration of MPAG was 1.4-2.0 times higher in deceased donor liver transplant patients compared with live donor liver transplant patients. A reduced size living donor graft may have lower metabolizing capacity and reduced glucuronidation activity during regeneration. Importantly, the authors suggested the need to use a lower dosage (approximately 30%) of MMF in live donor liver transplant patients compared with deceased donor liver transplant patients. Jain *et al*[57] showed a low bioavailability by oral MMF (mean, 48.5%, within 1 week). The protocol using intravenous MMF can restore full bioavailability and conserve renal function after liver transplantation[58].

In another control study by Shen *et al*[59], the comparison of the pharmacokinetics of MPA and its metabolites between LDLT patients and DDLT patients was taken after oral administration of MMF (1 g, bid). Although the AUC0-12h of MPA and MPAG are not significant difference between the two groups, MPA AUC6-12h were significantly higher in DDLT group than those in LDLT group (*P* < 0.05). Inversely, higher free MPA AUC0-12h and significant free MPA fraction (*P* < 0.05) in LDLT patients were observed in DDLT patients when compared with DDLT group. AcMPAG AUC0-12h was also significantly higher in DDLT group (*P* < 0.05). The activity of glucuronide-conjugating enzymes was decreased due to reduced liver mass during the hepatic regeneration process. These observations suggested that the ability of clearance of MPA has decreased in LDLT patients during the early period after operation. The authors suggested that DDLT patients had higher EHC contributing to total MPA exposure compared with LDLT patients. As free MPA is the pharmacologically active form, lower oral dose of MMF may be administered for LDLT patients.

***Duration posttransplant***

MPA exposure significantly increases with post-transplantation time. In the investigation by Brunet *et al*[11] in 15 liver transplant recipients on a standard 1 g twice-daily dose, mean MPA AUC was 17.4 mg.h/L at day 6, 26.3 mg.h/L at day 10 and 33.6 mg.h/L at month 3. Low MPA AUC in their data was perhaps caused by the external biliary drainage and abnormal value of serum albumin and bilirubin. In another study by Zhang *et al*[38], dose-normalized AUC0–12h of MPA, MPAG and AcMPAG increased significantly in both groups in the later stage (> 1 mo) comparing the data from the early stage (within 2 wk after liver transplantation). Pisupati *et al*[60] observed that MPA AUC0–12h had doubled with 3-6 wk comparing with that first week after transplantation (50.8 *vs* 118 mg.h/L). However, the MPA AUC will tend to be stable after 3 to 6 months. Bencichou *et al*[61] showed that there is no change of MPA AUC and free MPA AUC between at mean 36 days (24-90 d) and at mean 867 days (124-6586 d).

The lower MPA AUC0-12h in the immediate postoperative period is due to a higher apparent oral clearance (CL/F), which may result from a reduced absorption (F) or an increased clearance (CL). Bencichou *et al*[61] assumed that the increase in CL/F is related to an increase in MPA free fraction, leading to lower total MPA AUC0-12h value during the immediate postoperative period. Free fraction of MPA related well with MPA CL/F and decreased significantly as serum albumin level returned to normal which would be consistent with more rapid hepatic and renal extraction, and subsequent biliary and urinary excretion. Pisupati *et al*[60] showed that total MPA CL/F decreased from 32.9 ± 21.4 L/h during the first week to 9.0 ± 4.4 L/h during 3-6 wk. The same authors also showed that there was no change in the intrinsic CL of MPA within the patients and suggested that the lack of a significant change in the intrinsic clearance indicates that the inherent ability of the liver to metabolize and eliminate MPA did not change significantly over time.

The other causes of low MPA exposure during the early stage may relate to the reduction of EHC and low bioavailability.

***Dosage of MMF***

The relationship between MMF dosage and MPA exposure is variable, usually weak or absent. In adult liver transplant recipients, Hwang *et al*[62] showed that there was a crude interindividual correlation between MMF dosage and MPA concentration (*r*2 = 0.271, *P* < 0.001). When assorted according to the post-transplant period, r2 was 0.153 during the first three months, 0.228 for months 4 – 12, 0.508 for years 1 – 2, 0.293 for years 3 – 5, and 0.247 after 5 years. With minimal TAC, a similar degree of interindividual variation was observed (*r*2 = 0.247, P < 0.001). In pediatric liver recipients, Aw *et al*[63] showed MPA AUC0-7h correlated significantly with MMF dose (*r* = 0.552, *P* = 0.010) and MPA C0h (*r* = 0.844; *P* < 0.001). When assorted according to the post-transplant period, r2 was 0.056 during the first three months, 0.162 for months 4-12, 0.085 for years 1-2, 0.071 for years 3-5, and 0.213 after 5 years.

***Enterohepatic recirculation***

MPA undergoes extensive enterohepatic recirculation (EHC) after hydrolysis of its biliary MPAG conjugate by intestinal bacteria and re-absorption of MPA. Hesselink *et al*[64] estimated that the contribution of EHC to the MPA AUC ranges between 10 % and 61 % in human. However, secondary peak is very rare in the initial period after liver transplantation which occurs in approximately 50 % of patients at 1 mo [65]. In some liver transplant patients, the EHC reestablishes around 4 to 8 hours postdosage of MMF[[66](http://www.ncbi.nlm.nih.gov/pubmed?term=Fatela-Cantillo%20D%5BAuthor%5D&cauthor=true&cauthor_uid=17097975)]. Pisupatic *et al*[60] showed that a secondary peak in MPA was seen between 4 and 6 hours after MMF administration in 4 of 10 patients during 3-6 wk and not seen during 1-2 wk. And MPA AUC 3-fold increased approximately which indicated the possible contribution of EHC. In pediatric liver recipients with CsA and MMF, [Lobritto *et*](http://www.ncbi.nlm.nih.gov/pubmed?term=Lobritto%20SJ%5BAuthor%5D&cauthor=true&cauthor_uid=17969194) *al*[55] observed that a second smaller peak was exhibited by some patients (probably due to EHC) although CsA was used which decreased re-circulated MPA concentrations[67].

***Impact of liver and renal dysfunction***

Impairment of liver function has complex effects on MPA kinetics, although cirrhosis affects neither MPA absorption nor MPA plasma protein binding or pharmacokinetics[[68](http://www.ncbi.nlm.nih.gov/pubmed?term=Parker%20G%5BAuthor%5D&cauthor=true&cauthor_uid=8728347)]. It is believed that free MPA levels are affected by hypoalbuminemia, uremia and hyperbilirubinemia[8,69]. Free MPA levels increase markedly in patients with severe renal insufficiency[[70](http://www.ncbi.nlm.nih.gov/pubmed?term=Kaplan%20B%5BAuthor%5D&cauthor=true&cauthor_uid=9583876)].

Chen *et al*[71] showed that MPA AUC0-12h in patients with abnormal albumin level were significantly lower than that in patients with normal albumin level (*P* = 0.009). MPA AUC0-12h was related significantly with serum albumin level (*r2* = 0.412, *P* = 0.001). But other parameters of hepatic function including total serum bilirubin concentration did not influence the change of MPA AUC0-12h. In 8 liver graft recipients, Jain *et al*[65] reported that MPA AUC correlated with serum bilirubin and MPA C0h with albumin concentration. Higher serum bilirubin level may impair hepatic MPAG production, transport and biliary excretion during cholestasis[68]. The decreased hepatic glucuronidation and EHC with moderate hepatic impairment may result in increased urinary MPAG concentrations[65]. Tredger *et al*[32] showed that recipient with low serum albumin level (< 35 g/L) frequently failed to achieve the therapeutic levels of MPA. In adults and children with lower serum albumin concentration, median levels of MPA C0h were 42 % and 19 % respectively in of those in patients with normal serum albumin levels given corresponding doses (*P* < 0.001). However, Brunet *et al*[11] showed no relationship between liver function and MPA exposure.

Tredger *et al*[32] also reported that elevated serum creatinine levels (> 120 mmol/L) were related to higher MPA C0h per unit MMF dose (median increase 38 % early and 50 % late after transplantation, *P* < 0.04) only in adult patients.

***Concomitant immunosuppressive drugs***

CsA but not TAC decreased MPA AUC and increased MPAG AUC0-24h because CsA inhibits MPAG excretion into bile[67]. CsA inhibition of the biliary excretion of MPAG is mediated by the multidrug resistance-associated protein 2 (Mrp2) transporter which leads to the reduction of MPA AUC[72].

In 21 stable pediatric liver transplant recipients, Brown *et al*[73] observed that MPA C0h was significantly lower during CsA co-therapy comparing TAC co-therapy (2.8 *vs* 5.6 mg /L, *P* = 0.006) while MPAG AUC was correspondingly higher (229 *vs* 94 mg/L/h, *P* = 0.012). Higher MMF dosage was demanded with CsA to achieve equivalent MPA C0h level than with TAC (362 *vs*178 mg, *P* = 0.004). The authors suggested contrasting effects of CsA and TAC on MPA glucuronidation or its excretion and EHC.

[Molina](http://www.ncbi.nlm.nih.gov/pubmed?term=Molina%20Perez%20E%5BAuthor%5D&cauthor=true&cauthor_uid=19376423) *et al*[74] reported no interaction between total dose or BMI-adjusted dose of VGC and concomitant administration of MMF in liver transplant recipients.

**LIMITED SAMPLING STRATEGIES FOR MPA**

Up till now, there have been some studies at the establishment of model equations for estimation of MPA AUC using limited sampling strategy in liver transplant recipients.

***Multiple regression analysis***

The most reliable method for judging the exposure of MPA is to calculate MPA AUC0-12h. But monitoring MPA AUC0-12h requires frequent withdraw of blood. It is impractical to obtain 6 − 10 plasma samples for measuring full MPA AUC within 12 hours’ dose interval in clinical setting. Therefore, abbreviated sampling strategies by limited MPA concentration s have been under investigation.

For LSS study, Ting *et al*[75] have some important suggestion: (1) it is essential to validate the predictive performance of the LSS in other patient populations. The prediction bias and prediction precision of the LSS should be determined; (2) a clinically feasible LSS should use 3 or less blood samples, preferably within a short period of time in order to reduce the inconvenience of TDM; and (3) the application of a specific LSS is ideally limited to the population and drug formulation that is used to develop it.

Some studies tried to test whether drug exposure MPA AUC can be accurately estimated from plasma concentrations at single time points, especially at MPA C0h. However, it is very regretful that the relationship between MPA C0h and MPA AUC0-12h is not strong enough. In two studies by Chen *et al*[71] the *r*2 value of MPA C0h was also lower at monitoring MPA concentration by HPLC (*r*2 = 0.300, number of sample = 72) or EMIT (*r*2 = 0.0677, number of sample = 48)[76] at the early stage after liver transplantation. In the study by Brunet *et al*[11], an acceptable correlation between MPA C0h and MPA AUC0-12h of MPA was found (*r* = 0.742, number of samples = 63). In pediatric liver transplantation, Brown *et al*[73] showed the moderate correlation between MPA C0h and MPA AUC0-12h (*r*2 = 0.65, number of sample = 21). In conclusion, MPA AUC0-12h could not be substituted correctly by MPA C0h as well as the other single time-point MPA concentration.

Stepwise regression analysis was used to establish the abbreviated equations for estimated MPA AUC0-12h. All combined models were obtained by using 1 to 4 time point MPA concentrations. A number of regression equations that predict MPA AUC0-12h are undertaken and take the form of the following function:

Estimated MPA AUC0-12h = I + β1C1h + ··· βnCnh

Where I am intercept, β is partial correlation coefficient and C is MPA concentration. The largest *r*2 value was considered as the best regression. Equations with a high coefficient of determination (*r*2) are then validated using data from another group or bootstrap procedure to evaluate their ability to predict the full MPA AUC. The validation step is critically important to assessing reliability of the LSS. There are three main methods to validating an LSS: two-group (model-building group and validating group), bootstrap and jackknife methods.

Chen *et al*[71] developed LSS for the prediction of MPA AUC using 72 profiles (40 cases) by HPLC (Table 2). These authors found that the relationship between estimated MPA AUC0-12h and measured MPA AUC0-12h based on three or four MPA pharmacokinetic parameters was related significantly in some abbreviated models. The best model for prediction of MPA AUC0-12h was by using 1 h, 2 h, 6 h and 8 h time-point MPA concentrations (*r*2 = 0.921, *P* = 0.0001). Bias and prediction are 1.24 ± 11.19 % and 8.24 ± 7.61 % respectively. 63 of 72 (88 %) estimated MPA AUC0-12h was within 15 % of MPA AUC0-12h. Bland-Altman analysis also revealed the best agreement of this equation compared with the others and a mean error of ± 9.89 mg.h/mL. For validation of the accuracy of these equations, Chen *et al*[77] used another group of liver transplant recipients (30 cases). It was confirmed that the equation with C1h, C2h, C6h and C8h had the best ability to predict measured MPA AUC0-12h (*r*2 = 0.936) with the excellent bias (2.18%), precision (5.11%) and the best prediction variation (2SD = ± 7.88 mg.h/L). However, the equation based on C1h, C2h and C4h was more suitable when concerned with clinical convenience, which had shorter sampling interval and had the excellent coefficient of determination (*r*2 = 0.795), the excellent (3.48 %), the acceptable precision (14.37 %) and the good prediction variation (2SD = ± 13.23 mg.h/L).

Although the standard technique for monitoring MPA concentration is HPLC, the EMIT has the advantage of convenience and rapidness in clinical setting for TDM of MMF. Due to the cross-reactivity of the antibody in the EMIT assay with the MPA AcMPAG, the EMIT target concentrations are higher than those for HPLC. The average overestimation by EMIT of MPA levels is approximately 10%-30 %. As AcMPAG is pharmacologically activity in vitro, it has been speculated that EMIT measurement may better reflect immunosuppression than HPLC techniques that only measure the parent compound. Thus, establishment of the abbreviated model for estimation of full MPA AUC on account of EMIT is necessary and valuable. Chen *et al*[76] established some equations for the prediction of MPA AUC using 48 profiles (40 cases) by EMIT (Table 2). The best equation was by using C1h, C2h, C4h and C8h. Forty of 48 (83.33 %) estimated MPA AUC0-12h was within 15 % of MPA AUC0-12h. The bias and precision are 0.27 ± 1.79% and 8.83 ± 1.24% respectively. The best agreement between estimated MAP AUC0-12h and MPA AUC0-12h was also showed with an average error was 9.02 mg.h/L by Bland-Altman analysis. The authors used the Bootstrap analysis with 200 replicated datasets and confirmed the accuracy and robustness of this equation.

In two above investigations by Chen *et al*[71,76], MPA C6h and/or C8h were necessary in the best equations from 3 or 4 time point MPA concentrations. The accurate equation by LSS should include one time-point MPA sample during the interval 6-12 h post-dosage. It is probable that in liver transplant recipients MPA EHC was importantly contributed to the full MPA AUC.

In the study by Attard *et al*[78], a total of 41 MPA AUC0-8h were determined in 41 pediatric liver transplant recipients (Table 2). The best equation by LSS include MPA C0h, C0.67h and C6h with excellent coefficient of determination (*r* = 0.88). For clinical practice, the equation with C0h, C0.33h and C2h are suitable (*r* = 0.74).

***Bayesian analysis***

Maximum a posteriori (MAP) Bayesian assay is based on the concept that prior information or beliefs can be combined with observe data, that is Bayes' theorem[75,79]. Briefly, the priori population PK parameters, in combined with demographic, pathophysiological and limited concentration-time data from the individual are used to predict the individualized parameters. Besides, the uncertainty of the parameters will also be estimated. As the amount of individual data accumulates, the population data contribute less to the overall prediction, and parameter prediction is individualized eventually. Prediction of parameters is achieved by minimizing the Bayesian Function:



Where Ppop is the population average of parameter P; P^ is the individual expected average of parameter *P*; var(P) is the variance of the estimated parameter *P*; Cobs is the observed concentration value; C^ is the predicted concentration value; and var(C) is the variance of the predicted concentration[80].

***Population pharmacokinetic study of MPA***

A reliable Bayesian forecasting method is based on the reliability of population pharmacokinetic (PPK) models established. PPK parameters for commonly used drugs are available in popular Bayesian software programs (eg, NONMEM, ADAPT II, PKS). PPK studies to date have mostly been undertaken in renal transplant recipients, with limited investigation in patients treated with MPA for autoimmune disease or haematopoietic stem cell transplantation. Most of these studies have involved use of the MMF formulation of MPA.

It is a hard work to develop a PPK model of MPA to fully describe the complex physiological processes that occur in relation to the absorption and EHC of this drug. There are more than 20 PPK models have been developed for MPA, and more complex models for description of MPA pharmacokinetics also include modeling of metabolites and free MPA concentrations. However, most of these studies included less than 100 subjects, which is not sufficient to fully characterize the complex kinetics of this agent in different clinical conditions. Population models applied to MAP Bayesian analysis vary somewhat in structure, and separate covariates have been identified as being significant in different studies.

Sampling time of MPA PPK study varied between various studies, however, most studies using rich-time between two doses of MMF. The data also included various stages post-transplantation, the longest time of sampling included data 10 years post-transplantation[81]. The most frequently used structure model is 2-compartmental model. van Hest *et al*[82] collected data 3-140 d post-transplantation from 140 patients. 6523 samples were obtained, they tested 1-, 2- and 3-compartment model, and found 2-compartment model is most rational and suitable. Similar with other immunosuppressive agents, the absorption of MPA is very complex. Shum *et al*[83] tested different absorption model including first order absorption, time-dependent model, Emax model, Weibull model and dual sequential first order absorption process were tested. Finally, first order absorption with a lag time improved the model significantly. Le Guellec *et al*[84] found a 2-compartment model with zero-order absorption, the absorption duration being estimated from the data, provided the best fitting.

***MAP Bayesian for the assessment of MPA AUC***

After the final PPK model of MPA is obtained, the covariates value and selected concentration-time data from the individual patients are input in the model to obtain individualized AUC. Most of studies used the trapezoidal method to estimate the full MPA AUC value, which is considered as reference value. Evaluations have been conducted of how closely MAP Bayesian estimation of MPA AUC matches.

External and internal validation methods can be used in the MAP Bayesian estimation of MPA AUC. External validation involves the application of the developed method to a new dataset, which require the correct covariates and accurate sampling times recorded. It is more stringent in the study design and can provide the strongest evidence for evaluation. Most of studies evaluated using internal validation datasets through data splitting or using a re-sampling technique. In some studies, data were split into a population model-building group and a validation group to evaluate MAP Bayesian forecasting. Other methods of validation include jackknife or Bootstrap method. Optimal sampling theory is based on the notion that there are specific sampling times, or windows of time, containing more information about pharmacokinetic parameters or drug exposure than other sampling times[85]. All these studies tested all combinations of study sampling times in selecting sampling times for Bayesian forecasting. Few studies used D-optimality (within pre-determined time limits). Predictive performance is usually expressed in terms of the *r*2, mean percentage predicted error (MPPE) and relative root mean-squared error (rRMSE) between reference AUC and estimated AUC.

The study of Barau *et al*[86] is the only study on the Bayesian estimation of MPA AUC in 28 pediatric patients received liver transplant. All patients received MMF therapy combined with TAC or CsA. The PPK model was established by using intensive pharmacokinetic datasets obtained from 16 children. A one compartment model with first order absorption and first order elimination was selected. CL/F was estimated at 12.7 l h-1. Ka was estimated at 1.7 h-1 at age 8.7 years with IIV of 308%. V/F was 64.7 L, and increased about 2.3 times in children during the immediate post transplantation period. The individual MPA AUC0-12h was estimated by MAP Bayesian method using pharmacokinetic parameters obtained with the final model, including covariates, through Adapt II software. The MPA AUC0 12h estimated from concentrations measured 0, 1 and 4 h after administration of MMF was in good correlation with the data obtained from trapezoidal method.

MAP Bayesian estimation is more flexible compared with multiple linear regression methods. Drug exposure can be estimated with any number of blood samples taken at any time. Furthermore, with MAP Bayesian forecasting, the information about an individual patient may helpful in the AUC estimation[87]. However, there are still some problems: firstly, the PPK model established for MAP Bayesian estimation may be not the best one for the limited patients. Secondly, the algorithms used to select the optimal sampling time may not be accurate enough. Thirdly, there is still large bias in the prediction in various studies. Finally, the best sampling times by comparison of predictive performance cannot be regarded as truly optimal, because the possible combinations are limited by the study design. These problems should be solved by further studies before the method can be widely used in the individualized therapy of MPA.

**CONCENTRATION-EFFECT RELATIONSHIP**

It has been clearly shown that MMF is a very powerful immunosuppressive drug used in preventing graft rejection. However, there were also plenty of evidences showing that MMF has serious side effects including hematologic and gastrointestinal disorders[4]. The prospective, randomized and double-blind trial performed by van Gelder *et al*[88] showed that the rate of acute rejection decreased significantly in renal transplantation if MPA AUC was in the target range of 32.2-60.6 mg.h/L. Although the results are conflicting among different transplant setting, MPA concentration monitoring is recommended in kidney transplantation by the therapeutic window of 30 to 60 mg.h/L for MPA AUC and of 1 to 3.5 mg/L for MPA C0h[8]. However, it is still not widely accepted to individualize an oral MPA regimen by routinely monitoring MPA pharmacokinetic parameters in liver transplantation currently.

***MPA exposure and acute rejection***

In 147 adult liver transplants, Tredger *et al*[32] observed that nine of the 10 episodes were associated with plasma MPA concentrations less than 1 mg/L, with the exception occurring at 1.8 mg/L in a patient whose serum albumin was 31 g/L and creatinine 236 mmol/L. The relative risk of rejection (95%CI) increased 4.2-, 2.5-, and 1.6-fold, respectively, at plasma MPA concentrations of less than 0.5, 1.0 and 1.5 mg/L (*P* = 0.003, 0.002 and 0.058, respectively). The authors defined a cutoff of 0.85 mg/L in adult liver recipients by receiver operating characteristic (ROC) curve analysis. Besides, they also observed that MMF doses in the patients with rejection were not different from those in the control cohort. In the study by Chen *et al*[31], only recorded two instances of acute rejection were proven by hepatic biopsy in 63 patients (3.2 %) within 3 months after transplantation. MPA C0h were 0.32, 0.6 mg/L respectively and MPA AUC0-12h were 15.18, 32.49 mg.h/L respectively with 7.3, 2.2 ng/L of TAC C0h, respectively. Recently, Sarvary *et al*[89] found the optimal cutoff of MPA C0h on acute rejection (≥ 1.34 mg/L on CsA and ≥ 1.98 mg/L on TAC) in 56 liver transplant recipients during six months’ follow-up. In other studies, no relationship between MPA pharmacokinetics and acute rejection was established.

***MPA exposure and adverse effects***

In 63 liver transplant recipients, Chen *et al*[31] showed Mean MPA C0h and AUC0-12h in patients with side effects increased significantly than those without side effects (C0h: 2,28 *vs*. 1.31 mg/L, *P* < 0.05; AUC0-12h: 49.68 *vs* 37.16 mg.h/L, *P* < 0.01). In addition, the levels of MPA C0h and MPA Cmax were higher in recipients with leucopenia, diarrhea and infection than in those without these effects, but only during the episode of leucopenia significant difference was achieved (2.23 *vs* 1.81, *P* < 0.01). In 147 adult transplant recipients, Tredger *et al*[32] also showed that episodes of leukopenia were associated both with higher median plasma MPA levels (2.8 *vs* 1.4 mg/L, *P* = 0.004). These authors also observed that MPA levels were higher during episodes of bacterial, fungal and viral infections, although this trend failed to achieve significance (1.8 *vs* 1.4 mg/L, *P* = 0.056) and there were no differences in median MPA levels gastrointestinal side effects compared with the control cohort. Brunet *et al*[11] showed significantly elevated mean MPA concentrations at C0.66h for six form 13 patients with diarrhea compared with symptom free patients (22.9 *vs* 7.4 mg/L, *P* < 0.05) and there was not different significantly for MPA C0h and MPA AUC.

ROC curve analyse is also used to test the ability of MPA pharmacokinetic parameters to discriminate between cases with or without side effects in liver transplantation (Table 3). Chen *et al*[31] showed that the thresholds of MPA C0h and MPA AUC0-12h for side effects were 2 mg/L (sensitivity, 52.4%; specificity, 90.5%, *P* = 0.001) and 40 mg.h/L (sensitivity, 71.4%; specificity, 61.9%, *P* = 0.012), respectively. For single sort of side effect, only leukopenia was discriminated effectively by ROC analysis in MPA C0h with the threshold of 2 mg/L (sensitivity, 56.5 %; specificity, 75 %, *P* = 0.026). The relative risks were 1.79 for MPA C0h and 1.65 for MPA AUC to predict the occurrence of MMF-related side effects while 2.11 for MPA C0h and 1.68 for MPA AUC to predict the occurrence of leukopenia. In the study by Tredger *et al*[32], corresponding increases more than 3-fold in the relative risks for leukopenia, infection and gastrointestinal disturbances were showed at MPA concentration from 3 to 4 mg/L. The thresholds of MPA C0h were 2.85 mg/L in infectious episodes (ROC area = 0.634, *P* = 0.056) and 2.25 mg/L in leukopenia (ROC area = 0.780, *P* = 0.003). Although the relative risk of gastrointestinal disorders with increased MPA C0h, there was no significant association (*P* > 0.5). Importantly, the authors observed that a significant association between with MMF dose and episodes of leukopenia (ROC area = 0.750, *P* = 0.007). It is suggested that individualizing MMF dose instead of using a fixed dose might be helpful to optimizing immunosuppression and minimize potential toxic effects. However, Chen *et al*[31] showed no significant difference in MPA pharmacokinetic parameters between patients with infection and those without.

Among immunosuppressive drugs, MMF is the main course of diarrhea when compared with other agents. The mechanism responsible for MMF-related diarrhea is not yet elucidated. In liver transplantation[31,32], the levels of MPA C0h or AUC0-12h were not significantly higher in patients with diarrhea than those without diarrhea. However, Zhang *et al*[38] found that MPA C6h, C10h, C12h and MPA AUC6-12h were significantly higher in patients with diarrhea (*P* < 0.05). These results suggested that higher EHC might contribute to the occurrence of diarrhea.

It was guessed that diarrhea may be related to MPAG or AcMPAG[[90](http://www.ncbi.nlm.nih.gov/pubmed?term=Bailey%20MJ%5BAuthor%5D&cauthor=true&cauthor_uid=12686489)]. However, in the study by Zhang *et al*[38], there was no significant difference (*P* > 0.05) though MPA Cmax and MPA AUC0-12h of MPAG were higher in recipients with diarrhea. Likewise, C0h, Cmax, and AUC0-12h of AcMPAG were also higher in patients with diarrhea, but also no significant difference was found (*P* > 0.05). Arns *et al*[91] suggested that the capacity of enterocytes to participate in MPA metabolism could potentially result in local generation of AcMPAG and MPAG with consequent direct toxic effects on the gastrointestinal tract. Perhaps concentration of AcMPAG in gastrointestinal tract is more important than plasma concentration of AcMPAG for induction of diarrhea.

Another risk of diarrhea was dependent on dosage of MMF. Diarrhea was controlled by decreasing the dosage or interruption even if these patients had the same starting dosage of MMF as those not suffering from diarrhea[31].

**CONCLUSION**

Until now, TDM for MPA has not been fully accepted in liver transplantation as no long-term prospective study of concentration controlled *vs* fixed-dose prescribing of MMF has been done. However, based on published data, it is confirmed that intra- or inter-individual MPA pharmacokinetic variability exists which relates to greater risk on acute rejection at lower MPA concentration and MMF-associated side effects at higher MPA concentration. In another hand, the standard dose of MMF is rarely necessary in liver transplant recipients who had more MMF-related side effects and less acute rejection. These data suggests that monitoring MPA exposure is helpful in clinical setting.

In liver transplantation, it was showed that MPA C0h has more practical benefits over MPA AUC although the relationship between MPA C0h and MPA AUC is not very strong in some studies. Comparing with the therapeutic window in renal transplantation (MPA C0h: 1-3.5 mg/L), it suggests that acute rejection is more likely at concentrations less than 1 to 2 mg/L (μg/mL) and adverse effects at concentrations 3-4 mg/L or greater in liver transplantation[[13](http://www.ncbi.nlm.nih.gov/pubmed?term=Cantarovich%20M%5BAuthor%5D&cauthor=true&cauthor_uid=21454066)]. However, it needs more clinical validation in future. Although MPA AUC is much accurate which reflects the change of MPA pharmacokinetics and relates closely to side effects[31], no recommended therapeutic ranges of MPA AUC could be used in pediatric or adult liver transplant recipients. On the other hand, monitoring of MPA AUC is not practical in clinical using. It should obtain 6-10 plasma samples for measuring full MPA AUC within 12 hours’ dose interval. Although abbreviated sampling strategy by limited MPA concentrations is practical in clinical setting, the equations including MPA concentrations within 2 h with good correlation were only seen in pediatric transplant recipients[78]. In adult liver transplantation, good coefficients of determination (r2) were seen in equations including one MPA concentration at least during 6-12 h after oral MMF[71,76]. Monitoring MPA C0h has more practical benefits than MPA AUC in liver transplantation.

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**Table 1 Pharmacokinetic data of mycophenolic acid in adult liver transplant recipients**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Year** | **Regimen** | **Time since LT** | **n** | **Method** | **AUC0-12h (mg.h /L)** | **mean tmax (h)** | **mean C0h (mg/L)** | **mean Cmax (mg/L)** |
| Jain *et al*[65] | 2001 | TAC + MMF | day 6 - 30 | 8 | HPLC | 40.0 ± 30.9 (7.3 - 102.3) | 1.8 ± 1.6 |  | 10.6 ± 7.5 |
| Mardigyan *et al*[92] | 2005 | TAC + MMF | > 12 mo | 14 | EMIT | 45 ± 22 | 0.5 | 2.1 ± 1.5 | 12.2 ± 75 |
| Pisupati *et al*[60] | 2005 | TAC + MMF | < week 1 | 10 | HPLC | 50.8 ± 42.1 | 1.8 ± 1.2 |  | 9.1 ± 7.2 |
|  |  |  | week 1 - 2 |  |  | 60.3 ± 38.5 | 1.8 ± 1.4 |  | 11.6 ± 6.7 |
|  |  |  | week 3 - 6 |  |  | 118.0 ± 57.6 | 1.3 ± 0.7 |  | 36.7 ± 15.6 |
| Brunet *et al*[11] | 2006 | TAC + MMF | day 6 | 13 | HPLC-UC | 17.4 (13.2 – 39.7) | 2 | 0.4 | 4.6 |
|  |  |  | day 16 | 13 |  | 26.3 (13.1 – 45.8) | 1.2 | 0.6 | 7.7 |
|  |  |  | month 3 | 14 |  | 33.6 (15.1 – 54.6) | 0.7 | 1.3 | 6.6 |
| Chen *et al*[71] | 2007 | TAC + MMF | day 7 | 38 | HPLC | 44.6 ± 16.50 (17.99 – 96.87) | 1.42 ± 0.77 |  | 8.45 ± 4.77 |
|  |  |  | day 14 | 34 |  | 50.54 ± 18.60 (22.78 – 98.73) | 1.45 ± 0.81 |  | 11.29 ± 5.51 |
| Chen *et al*[76] | 2008 | TAC + MMF | day 7 - 14 | 48 | EMIT | 45.77 ± 18.69 (10.66 - 117.01) | 1.94 ± 1.65 | 2.02 ± 1.57 | 11.76 ± 6.34 |
| Kamar *et al*[93] | 2009 | TAC + MMF | day 7 | 15 | HPLC | 36.8 ± 27 |  |  |  |
|  |  |  | day 14 | 15 |  | 32.6 ± 11 |  |  |  |
|  |  |  | day 30 | 15 |  | 36.7 ± 13 |  |  |  |
| Beckebaum *et al*[94] | 2009 | TAC + MMF | day 60 (14 - 230 days) | 18 | LC-MS/MS | 55.9 (22.9 – 144.8) | 0.5 | 3 | 14.2 |
|  |  | CsA + MMF | day 70 (11 - 87 days) | 12 |  | 52.2 (31.8 – 102.1) | 1 | 2.5 | 15.3 |
| Benichou *et al*[61] | 2010 | TAC + MMF | day 12 (4 - 20 days) | 26 | EMIT | 26.8 (21.8 - 39.7) |  |  |  |
|  |  |  | day 36 (24 - 90 days) | 25 |  | 45.2 (26.0 - 57.0) |  |  |  |

**Table 2 Limited sampling strategy for prediction of full mycophenolic acid area of concentration-time under the curve in liver transplant recipients**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Method** | **Regimen** | **Patient population** | **Numbers of**  **files (cases)** | **Sampling times investigated (h)** | **Suggested times of LSS (h)** | **Predicted AUC =** | ***r*2** | **LSS validation** | **Bias** | Precision |
| Attard *et al*[78] | EMIT or | CsA or TAC | Pediatrics | 41 files | 0,0.33,0.67,1.25,2,4,6,8 | 0, 0.33, 2 | 9.1 + 5.7\*C0 + 1.1\*C0.33 + 2.1\*C2 | 0.74 | No | N/A | N/A |
|  | HPLC-UV | + MMF |  | (41 cases) |  | 0, 0.67, 6 | 5.2 + 7.1\*C0 + 1.1\*C0.66 + 5.4\*C6 | 0.88 | No | N/A | N/A |
| Chen *et al*[71] | HPLC | TAC + MMF | Adults | 72 files | 0.5,1,1.5,2,4,6,8,10,12 | 1, 2, 4 | 10.776 + 0.749\*C1h + 1.604\*C2h + 4.116\*C4h | 0.75 | validation | Yes | Yes |
|  |  |  |  | (40 cases) |  | 1, 2, 6 | 10.229 + 0.925\*C1h + 1.750\*C2h + 4.586\*C6h | 0.855 | group | Yes | Yes |
|  |  |  |  |  |  | 1, 2, 6, 8 | 5.503 + 0.919\*C1h + 1.871\*C2h + 3.176\*C6h + 3.664\*C8h | 0.921 |  | Yes | Yes |
|  |  |  |  |  |  | 1, 2, 4, 6 | 6.658 + 0.921\*C1h + 1.573\*C2h + 2.057\*C4h + 3.543\*C6h | 0.899 |  | Yes | Yes |
| Chen *et al*[76] | EMIT | TAC + MMF | Adults | 48 files | 0.5,1,1.5,2,4,6,8,10,12 | 1.5, 6 | 10.56 + 1.55C1.5h + 6.44C6h | 0.859 | Boodstrap | Yes | Yes |
|  |  |  |  | (48 cases) |  | 2, 4, 8 | 9.37 + 2.18C2h + 2.10C4h + 4.71C8h | 0.901 |  | Yes | Yes |
|  |  |  |  |  |  | 1, 2, 4, 8 | 4.46 + 0.81C1h + 1.78C2h + 2.51C4h + 4.94C8h | 0.95 |  | Yes | Yes |
|  |  |  |  |  |  | 1, 2, 4, 6 | 5.92 + 1.10C1h + 1.01C2h + 1.77C4h + 4.80C6h | 0.927 |  | Yes | Yes |

LSS: Limited sampling strategy; MPA: Mycophenolic acid; MPA AUC: MPA area of concentration-time under the curve.

**Table 3 Receiver operating characteristic analyses of mycophenolic acid exposure and mycophenolate mofetil -related side effects in liver transplant recipients**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Ref.** |  | **Area of ROC Curve** | **95 %CI** | **Cut-Off Value** | ***P* value** |
| Chen *et al*[31] | Side effects1 |  |  |  |  |
|  | MPA C0h | 0.748 | 0.619 - 0.877 | 2 mg/L | 0.001 |
|  | MPA AUC0-12h | 0.695 | 0.559 - 0.831 | 40 mg.h/L | 0.012 |
|  | Leukopenia |  |  |  |  |
|  | MPA C0h | 0.67 | 0.534 - 0.805 | 2 mg/L | 0.026 |
| Tredger *et al*[32] | Leukopenia |  |  |  |  |
|  | MPA C0h | 0.78 | 0.642–0.919 | 2.25 mg/L | 0.003 |
|  | MMF dose | 0.75 | 0.662 – 0.837 |  | 0.007 |
|  | Infection |  |  |  |  |
|  | MPA C0h | 0.634 | 0.499–0.770 | 2.85 mg/L | 0.056 |

1Side effects: include leukopenia, diarrhea and infection. MMF: Mycophenolate mofetil; ROC: Receiver operating characteristic; MPA: Mycophenolic acid.