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Therapeutic Drug Monitoring of Micophenolate Mofetil in Cardiac Transplant Patients by Limited Sampling Strategy: An Update

Massimo Baraldo, Sandro Sponga and Ugolino Livi

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Abstract

In the last few years, much progress in avoiding acute and chronic rejection in transplanted patients has been made by introducing new and more effective drugs with different formulations and combinations, and fewer side effects. Standardized protocols have been proposed for different organs, but individualized therapy based on immunosuppressive therapy blood monitoring is necessary because of pharmacological interaction, new generic drug introductions, and different absorptions and biodistributions. In specific mycophenolate mofetil dosing through mycophenolic acid (MPA), therapeutic drug monitoring has demonstrated minimal risk of organ transplant rejection. Even if the MPA area under the 12 h concentration–time curve is more accurate than MPA levels, it appears to be resource consuming and clinically impractical because of the need for numerous blood samples. Limited sampling strategy (LLS) has been proposed to overcome this problem. In heart-transplanted patients, MPA LSS is useful in guiding clinical management and dosing. The purpose of this chapter is to describe the state of the art of MPA LSS employment in heart transplantation and to perform an update of the scientific literature.

Keywords: heart transplantation, immunosuppressive therapy, mycophenolate mofetil, therapeutic drug monitoring, limited sampling strategy

1. Introduction

After heart transplantation, it is usual to administer the triple-drug therapy of induction and maintenance with calcineurin inhibitor (CNI), tacrolimus (TAC) or cyclosporine (CsA),

mycophenolate mofetil (MMF)/enteric-coated mycophenolate sodium (EC-MPS), prodrugs of mycophenolic acid (MPA) or everolimus (EVE)/sirolimus (SIR) and corticosteroids (CSs). TAC should be the preferred CNI and MMF/EC-MPS the preferred cell cycle inhibitor [1]. Baraldo et al., in their review, concluded that CS withdrawal is safe in 50–80% of patients, with late withdrawal being better than early withdrawal. In addition, CS avoidance should be advisable and mandatory in pediatric patients, elderly patients and patients with insulin-dependent diabetes mellitus, metabolic disorders, osteoporosis and infections [2]. While for CNI or mTOR, therapeutic drug monitoring (TDM) is a clinical practice, MPA TDM is already controversial and is not widely used. One of the reasons is that in laboratories it was not a widely distributed platform for the analysis of plasma MPA concentrations, compared to other immunosuppressive drugs such as CsA, TAC, SIR and EVE. Moreover, this drug was promoted as not necessary for TDM and so generally MMF was administered as a fixed dose regimen. However, it was demonstrated that a >10-fold range in MPA dose-normalized area under the curve (AUC) between patients may be observed in heart, renal and liver transplantation, so MPA TDM may be useful [3]. Finally, since it has been demonstrated that the correct use of MPA TDM would require several blood samples to define the AUC_{0-12}, this approach appeared to be laborious, costly and clinically impractical [4]. To overcome the practical problems linked to blood samples to obtain an AUC_{0-12}, limited sampling strategies (LSSs) have been proposed. In a recent Consensus Meeting, LSSs were preferred in solid organ transplantation compared with drug dosing that is based on MPA through concentrations, and the individualization of MMF dosing may minimize the risk of organ transplant rejection [5]. So the $MPA\text{-}AUC_{0-12}$ obtained using the LSS may be useful to guide clinical management and dosing. With the increasing use of MPA in solid organ transplantation, the greater possibility of analyzing at lower costs and the greater diffusion of laboratories that are able to perform the analysis have been recently revised for kidney transplantation [6]. The purpose of this chapter is to describe the state of the art of studies to calculate MPA LSSs in heart transplant recipients and to perform an update of the scientific literature.

2. Mycophenolate mofetil

MMF (Cell Cept®, Roche Pharmaceuticals, Basel, Switzerland) is the morpholino ester pro-drug of MPA and was approved by the Food and Drug Administration in May 1995. MMF became a routine and extensively used part of immunosuppressive regimens, in combination with other immunosuppressant medications, after kidney transplantation, but it is also used after heart, lung, heart/lung and liver transplantation [7]. Attention was focused on the gastrointestinal side effects associated with its use, and an alternative formulation of MPA was explored in an effort to reduce the burden of gastrointestinal toxicity. An enteric-coated formulation of MPA (EC-MPS, Myfortic®, Novartis Pharma AG, Basel, Switzerland) was developed [8]. Equimolar doses of EC-MPS and MMF were shown to produce equivalent MPA exposure and to result in inhibition of the activity of the target enzyme inosine-5-monophosphate dehydrogenase (IMPDH) to a similar degree [9]. The patents have expired for MMF and EC-MPS. Because MPA is not considered a narrow therapeutic index drug, the wider bio-equivalence criteria are applied for the registration of generic MMF formulations [10].

2.1. Pharmacodynamics and pharmacokinetics

MMF is rapidly metabolized to its active constituent MPA, which acts as a specific inhibitor of the proliferation of T- and B-lymphocytes by reversibly inhibiting IMPDH, the key enzyme of the de novo purine synthesis in activated lymphocytes. The inhibition of T- and B-cell proliferation results in diminished cytotoxic T-cell responses and antibody formation against the allograft [11].

MPA bioavailability is >90%. It is mainly metabolized by uridine 5'-diphospho--glucurono-syltransferase [mycophenolic acid glucuronide (MPAG)] in the liver, intestine and kidney in 7-O-glucuronide (inactive) and acyl-glucuronide (active). MPA and MPAG are bound to the protein 97–99 and 82%, respectively, MPA metabolites are eliminated by the kidney and MPA and MPAG are subject to enterohepatic recirculation. The mean 'apparent' half-life and plasma clearance of MPA are 17.9 h and 11.6 L/h, respectively, after oral administration [12].

The clinical pharmacokinetics (PK) of MPA are characterized by a high between-subject and within-subject variability. It also was noted in all types of solid organ transplantation that dose-normalized MPA exposure in the first 3 months after transplantation was increased. The increase in MPA exposure can range from 30–80%. The coadministration of immunosuppressive or other drugs may influence MPA exposure. The MPA-AUC_{0-12} and its glucuronide metabolite were higher in patients with renal impairment than in patients with normal renal function following single dose administration. The MMF PK after a single dose is not altered in patients with cirrhosis. The main side effects of MPA are gastrointestinal disturbances, hematological disorders (e.g. anemia and leucopenia) and infections [13–15].

Moreover, it has been demonstrated that genetic polymorphisms may influence MMF absorption, distribution, metabolism and pharmacological action and may contribute to this interindividual variation in MMF response [16].

3. Mycophenolate mofetil after heart transplantations

Early preclinical studies of MMF demonstrated that MMF significantly prolonged cardiac transplants in rats and that the combination of MMF with CsA was more effective than either agent alone [17]. Furthermore, some trials suggest that MMF, when substituted for azathioprine in standard triple-drug therapy regimens, is well tolerated and might be more efficacious than azathioprine [18–20]. This could be explained by the hypothesis that MMF may provide more synergy with concomitantly administered cyclosporine and/or CSs than azathioprine, therefore demonstrating benefits to both renal [18–22] and cardiac transplant populations. Also, MMF has novel properties that may contribute to the prevention of cardiac allograft rejection and provide benefits in reducing the progression of vascular allograft vasculopathy (CAV).

3.1. Drug administration

Some findings have attempted to correlate MMF pharmacokinetic parameters with outcomes. MMF is a prodrug, so it is rapidly hydrolysed after ingestion to MPA. It must be administered on an empty stomach. TDM in patients receiving MMF has not been extensively investigated,

although preclinical studies demonstrated a correlation between MPA levels and histologic severity of graft rejection. In addition, because appreciable within-patient fluctuations may occur, the dose should not be changed based on a single predose measurement. Another important point is the balance between the different immunosuppressive agents. For example, in previous studies, the risk of rejection was similar between groups with either a higher CsA level and a lower MMF dose or a lower CsA level and a higher MMF dose [23].

3.2. Trials supporting MMF

In 1993, Ensley et al. [18] published one of the first clinical reports describing the use of MMF in cardiac transplantation. This was the first study that found MMF effective to significantly reduce the mean biopsy score with less myelosuppression compared to azathioprine. Some years later, Kobashigawa et al. [24] published the first large multicentre trial in 1998. At the time the trial was initiated, immunosuppressive regimens for heart transplantation relied on a combination of CsA, steroids and azathioprine. The use of MMF demonstrated better survival rates at 1 and 5 years. After these promising results, an analysis of data from the Joint ISHLT/United Network for Organ Sharing Thoracic Registry was conducted in 2001 [25] where the improved long-term survival benefit of MMF therapy was confirmed, suggesting that the positive findings are broadly applicable within the cardiac transplant population. In addition to the randomized, multicentre trials [26–28], other trials and studies evaluated MMF in cardiac transplant recipients in combination with either CsA [29–32] or TAC [30] and in CNI-sparing regimens [33–35]. For example, other studies evaluating the combination of MMF with TAC were published, aimed at determining whether trough-level-adjusted MMF was more effective in combination with TAC or CsA. These results showed that the incidence of acute rejections was lower in patients receiving TAC versus the CsA group, although there was no difference in patient survival. Results from the most recent multicentre, randomized trial involving MMF in cardiac transplant recipients were presented at the ISHLT annual meeting in 2005 [36]. Hence, these authors concluded that in cardiac transplant patients, TAC/MMF appears to offer advantages over TAC/SRL or CsA/MMF when considering any treated rejection and side-effect profiles.

3.3. Advantages and side effects of MMF therapy

Therapy with MMF has peculiar advantages. In patients with chronic renal dysfunction, the reduction in CNI exposure, either through dose reduction or complete withdrawal, has been studied as a means of minimizing further deterioration of renal function. For this purpose, MMF-based CNI-sparing strategies were evaluated in three trials with promising results [34, 35, 37]. A second aspect is related to the anti-inflammatory properties of MMF that may provide long-term benefits in reducing the risk of CAV in cardiac transplant recipients. Furthermore, Weis et al. [37] reported that in cardiac transplant patients the combination of TAC/MMF appeared to be superior to TAC/azathioprine in preserving early coronary vasomotor function, endothelial nitric oxide synthase expression and inducible nitric oxide synthase suppression, as well as cardiac interleukin-6 release. Since these factors, in addition to the risks posed by rejection, are believed to be predictors of CAV, MMF may have a beneficial impact on the subsequent development of CAV.

Being a selective inhibitor of inosine monophosphate dehydrogenase, it is common to have some side effects. The most commonly reported side effects of MMF include leukopenia, anemia, infections, systemic cytomegalovirus disease, hypercholesterolemia and gastrointestinal complications such as diarrhea, nausea and dyspepsia. On the other hand, malignant neoplasms, especially of the skin, are frequent in patients treated with MMF [38, 39].

4. Therapeutic drug monitoring of MPA

Several studies have suggested that therapeutic drug monitoring of MPA concentrations in patients with renal, heart or lung transplants may improve clinical outcomes and allow effective dose individualization of MMF potentially minimizing toxicity [3, 40–43]. In heart transplant recipients, several studies have shown that MPA levels correlate to the risk of rejection [44, 45]. AUC_{0-12} seems to be a better parameter to optimize MPA treatment than the predose measurement (C_0). Unfortunately, measuring AUC_{0-12} requires the collection and analysis of multiple blood samples, which is costly and time-consuming for patients and clinical staff. So, AUC_{0-12} measurement could be simplified by using a technique, initially developed for anticancer drugs, called LSS [46, 47]. It was shown that an equation using three blood samples measured at specific times could approximate or estimate the real AUC_{0-12}. An AUC_{0-12} threshold of 50 mg × h/L was proposed (sensitivity = 77%, specificity = 25%) beyond which the risk of rejection was significantly increased (low vs. high: HR = 3.48 [1.21–10.0], p = 0.0204) [48].

4.1. Analysis of MPA

Quantification of MPA may be performed by high-performance liquid chromatography (HPLC) with ultraviolet detection, liquid chromatography-mass spectrometry (LC–MS/MS) or a commercially available platform assay. In general, laboratories that are providing a routine TDM service will tend to use the platform immunoassay. Those with a large number of sample loads and those with research interests are likely to use the chromatographic technique. HPLC with MS detection is often described as the gold standard technique [49, 50]. One of the problems that can create a bias and alter the calculation of the nomograms are the analytical methods used. It has now been demonstrated that MPA plasma concentrations measured by the immunoassay technique are higher than those determined by HPLC by 25–36%. This overestimation is most likely attributable to the cross-reactivity of the pharmacologically active acyl-glucoronide (Ac MPAG).

4.2. Limited sampling strategy

LSS is a technique aimed at estimating the AUC_{0-12} using a small number of samples, usually three or fewer. Modeling the relationship between the pharmacokinetic parameter and the drug concentration at various times allows this reduction in the number of samples required. The model can then be used to choose the best sampling times to determine the parameter accurately and precisely. The development of such a method requires full pharmacokinetic profiles drawn with sufficient points to measure AUC_{0-12} accurately. Most authors use the trapezoidal method, but there is also linear trapezoidal and linear-logarithmic trapezoidal.

The differences observed between methods are small and there is no clinical significance. To develop an LSS, the first step is arbitrarily splitting the patient data into two groups: a *training group* and a *testing* or *validation group*. The training group is used to determine the relationship between AUC_{0-12} and the timed blood concentration data using a linear regression. The AUC_{0-12} is considered to be the dependent variable; the independent variables are the blood concentrations at each time point. An equation is defined giving the AUC_{0-12} as a function of one or several concentrations:

$$AUC_{0-12} = Constant + (M_1 \times C_1) + (M_2 \times C_2) + (M_3 \times C_3) + (M_x \times C_x)$$

where AUC_{0-12} is the predicted AUC_{0-12}, constant is the intercept on the y-axis, C_1, C_2, C_3, C_x are the blood concentrations measured at time 1, 2, 3, x and M_1, M_2, M_3, M_x are the associated coefficients. The equations are then validated using the testing group. Validation is a compulsory step that must be carried out on a different group to the training group because testing an equation on the group of patients used to generate the equation itself would be self-fulfilling and therefore would produce biased results. Using a fresh data set allows the equations to be tested under real conditions, thus helping in the decision about which equations should be used and which should not. The performance of the equations can be assessed by comparing the *predicted* AUC_{0-12} with the *measured* AUC_{0-12} measuring the mean prediction error or bias (*me*) and the root mean squared prediction error or precision (*rmse*) with their confidence intervals (CIs). The smaller these parameters, the better the prediction [51]. A simpler assessment of the performance of the equations can be achieved by estimating the percentage prediction error (%pe) on the AUC_{0-12}, defined as ([predicted value − measured value]/measured value) times 100. A more clinically orientated method consists of evaluating the proportion of AUC_{0-12} estimated within a percentage prediction error range. Another method consists of expressing the results using the absolute prediction error for a certain percentile of predictions. Some authors 'validated' their equations by calculating the correlation coefficient (*r*) or coefficient of determination (*r*) between the predicted AUC_{0-12} and the measured AUC_{0-12}. This method should not be used because it gives biased results [52].

5. Limited sampling strategy in heart transplants: an upgrade

A search of MEDLINE was done for papers on heart transplantation, MPA and LSS. The following search terms were used: mycophenolic acid, mycophenolate mofetil, heart transplant, solid organ transplant and limited sampling strategy. We utilized this filter: human, adult and English. We considered only papers with these inclusion criteria: age > 18 years, heart transplantation, cotreatment with CyA or TAC and CSs, heart-training group, heart-testing group and plasma MPA concentrations analysed by HPLC. We excluded papers with: age < 18 years, kidney, lung, liver, pancreas transplantations and plasma MPA concentrations analysed by enzyme-multiplied immunoassay technique (EMIT). We found only five studies published, presented in **Table 1**, where we reported studies with the same analytical assay (HPLC) and the nomogram with a coefficient of determination $r^2 > 0.80$.

No. of patients	Model equations $AUC_{0–12} =$	r^2	Method of analysis	Time of AUC; other drugs	Method used	References
9	5.57 + 0.90 * C1.25 + 2.02 * C2 + 4.59 * C6	0.93	HPLC	5 months; CsA, steroids	Training set	[53]
	3.8 + 1.03 * C1.25 + 1.82 * C2 + 1.57 * C4 + 3.48 * C6	0.95				
29	5.57 + 0.90 * C1.25 + 2.02 * C2 + 4.59 * C6	0.87			Validation set	[54]
	3.8 + 1.03 * C1.25 + 1.82 * C2 + 1.57 * C4 + 3.48* C6	0.81				
11	0.10 + 11.15 * C0 + 0.42 * C1 + 2.80 * C2	0.96	HPLC	9 months; CsA, steroids	Training set	[55]
	1.28 + 1.91 * C1 + 0.26 * C2 + 5.91 * C4	0.95				
	−0.51 + 11.47 * C0 + 3.24 * C2	0.94				
	−0.23 + 12.70 * C0 + 3.36 * C2–0.80 * C4	0.94				
28	1.25 * C1 + 5.29 * C4 + 2.90 * C8 + 3.61 * C10	0.95	HPLC	>6 months; TAC	Training set	[56]
	1.33 * C1 + 3.99 * C4 + 3.23 * C6 + 3.81 * C8	0.94				
	1.53 * C1 + 5.51 * C4 + 4.62 * C8	0.91				
	3.93 * C1 + 3.99 * C4 + 3.23 * C6 + 3.81 * C8	0.90				
	3.37 * C0 + 0.97 * C0.5 + 1.20 * C1 + 2.70 * C2	0.87				
	1.11 * C0.5 + 1.16 * C1 + 3.72 * C2	0.84				
20	9.69 + 0.63 * $C_{0.5}$ + 0.61 * C_1 + 2.20 * C_2	0.84	HPLC	1–12 months; CsA, steroids	Training set Validation set	[57]
24	7.93 + 3.89 * C_0 + 0.87 * C_1 + 1.02 * C_2 + 3.72 * C_4	0.89				
	10.2 + 0.64 * C_1 + 0.62 * $C_{1.5}$ + 3.03 * C_4 + 4.23 * C_6	0.85				

AUC = area under the curve; r^2 = coefficient of determination; CsA = cyclosporine; HPLC = high-performance liquid chromatography; TAC = tacrolimus.

Table 1. Limited sampling strategy suggested for MPA-AUC monitoring in combination with cyclosporine A or tacrolimus in heart transplantation.

In 2005, Baraldo et al. wrote the first paper on the use of LSSs to estimate the AUC in heart transplant patients. This was one of two papers that utilized correctly training group and validation group. The authors studied a population with these characteristics: adult >18 years, Caucasian ethnicity, heart transplanted, first 3 months from transplantation, cotreated with

CsA and steroids and good kidney and liver functions. The analysis of MPA plasma concentrations was by HPLC. Multiple stepwise regression analysis was used to define the time points of MPA levels to explain the MPA-AUC_{0-12}. Agreement between abbreviated AUC_{0-12} and full AUC_{0-12} was tested by means of a Bland and Altman analysis. Stepwise linear regression showed that the minimal model with the best estimation of MPA-AUC_{0-12} was obtained at time values of 1.25, 2 and 6 h. The corresponding estimated model was AUC_{0-12} = 5.568 + 0.902 * C(1.25) + 2.022 * C(2) + 4.594 * C(6) (r^2 = 0.926). Bland and Altman analysis revealed good agreement between predicted AUC_{0-12} and full AUC_{0-12}. A further interesting model equation obtained by four samples was AUC_{0-12} = 3.800 + 1.015 * C(1.25) + 1.819 * C(2) + 1.566 * C(4) + 3.479 * C(6) (r^2 = 0.948) [53]. To obtain the validation, these two algorithms proposed were tested in a validation group (29 heart transplant recipients) with the same characteristics of the testing group. The two LSS algorithms used predicted the corresponding MPA-AUC_{0-12} with a mean bias of −4.85 and −3.6% and mean precision of 15.9 and 14%, respectively. Baraldo et al. in conclusion revealed that the MPA-AUC_{0-12} obtained using the LSS may be useful to guide clinical management and dosing, but in heart transplant recipients who share the same characteristics [54].

The study of Wada et al. published in 2007 studied 22 Japanese heart transplant patients approximately 9 months after transplantation and divided them into two groups: 11 who were given MMF + CsA and 11 who were given MMF + TAC. They calculated the entire MPA-AUC_{0-12} and developed an LSS. They suggested a model consisting of three time points and another with two time points that predicted the entire MPA-AUC_{0-12}. We have utilized these two algorithms in heart transplant patients treated with MMF-CsA. The results obtained from this study, however, should be taken with caution because of the limited number of patients evaluated and the ethnic difference, which could influence MPA pharmacokinetics. The patients studied were given the same regimen therapy, the analytical method used was HPLC and the same pharmacokinetic and statistical approaches were used [55].

In 2008, Kaczmarek et al. studied 28 heart transplant patients treated with MMF and TAC. For each patient, the entire MPA-AUC_{0-12} was studied using an LSS. The best estimation of MPA-AUC_{0-12} was obtained with four sampling points: AUC = 1.25 * C_1 + 5.29 * C_4 + 2.90 * C_8 + 3.61 * C_{10} (r^2 = 0.95). The three sampling point equation within the first 2 h was preferred for ambulatory patients: AUC = 1.1 * $C_{0.5}$ + 1.16 * C_1 + 3.72 * C_2 (r^2 = 0.84). The population studied was long-term adult heart transplant recipients (2.5 ± 3 years) with chronic maintenance of immunosuppressive therapy consisting of MMF and TAC [56].

The most recent publication that defined algorithms for the TDM of MPA in heart transplant patients was by Pawinski et al. [57]. The authors studied 20 patients in a first step to obtain a *sampling strategy* and 24 patients in a second step to validate the algorithms. The regression equation for AUC estimation that gave the best fit was: AUC = 9.69 + 0.63 * $C_{0.5}$ + 0.61 * C_1 + 2.20 * C_2 (r^2 = 0.841; *me* = 3.2%; CI 95% (−42.2%; 40.3%)). This global approach appears correct. However, there are some issues that must be discussed: (1) from a statistical point of view, the CI of the *me* is quite wide; (2) the authors calculated the algorithms within the range of 6–8 weeks to 1 year after heart transplant and validated the algorithms in patients more than 1 year after heart transplant, periods that might be characterized by different pathophysiological conditions and concomitant therapy; and (3) the algorithms that include the C_6 blood sample presented the same r^2 = 0.841 and should be considered [58].

Moreover, the study by Dosch et al. presented single-centre preliminary analysis data and is one of the largest published investigations of MPA-AUC$_{0-12}$ in heart transplant recipients to date. The authors, however, did not calculate the entire AUC$_{0-12}$, and used algorithms taken from the literature with algorithms calculated in renal-transplanted patients. Furthermore, MPA plasma concentrations were measured by means of Emit Mycophenolic Acid Assay, which gives slightly higher concentration results compared to HPLC [59].

Ting et al. evaluated 25 heart transplant patients and estimated the MPA-AUC$_{0-12}$. They used an LSS previously developed for lung transplant recipients as well as an LSS used for heart transplant patients published from a different author. The authors concluded that the previously developed LSS used for lung transplant recipients performed well when applied to the heart transplant population for the prediction of MPA-AUC, while the application of the LSS obtained from the literature yielded fewer optimal results. Their conclusion was that: (1) LSS appears to be centre specific, (2) LSS should always be validated before implementation and (3) LSS should be limited to the population and drug therapy that were used to develop it [60].

6. Conclusions

This update has highlighted that research on MPA TDM by LSS in heart-transplanted patients was exhausted in 2009. Over the last 10 years, prevalent MPA TDM by LSS studies have been developed in kidney transplants and revised by van Gelder [6]. From the last Consensus Report, MPA TDM based on LSSs is preferred in solid organ transplantation compared to drug dosing that is based on single MPA trough concentrations. LSS is associated with early postoperative efficacy. The data suggest that specific patient populations might benefit from LSSs to reduce immunological risk in patients who are undergoing minimization or withdrawal of immunosuppressive therapy and patients who are experiencing altered renal, hepatic or bowel function [5]. Even though there is scientific support of its importance, the analysis of MPA plasma concentrations, LLS or Bayesian methodologies is not currently applied on a routine basis after heart transplant, and studies from heart transplant patients remain limited [53–57].

The most recent publication that defined algorithms for the TDM of MPA in heart transplant patients was by Pawinski et al. [57]. The authors calculated the algorithms within the range of 6–8 weeks to 1 year after heart transplant and validated the algorithms in patients more than 1 year after heart transplant. The two periods cannot be compared because the former is more or less rich in clinical problems and drugs, while the latter is usually characterized by a clinical stationarity and fewer medications taken.

Kaczmarek et al. studied 28 heart transplant patients treated with MMF and TAC [56]; the population studied was long-term adult heart transplant recipients (2.5 ± 3 years). These algorithms cannot be compared with MMF-CyA. An algorithm calculated from a TAC + MPA association cannot be used for a CsA + MPA association.

The study of Wada et al. considered patients with the same regimen therapy, analytical method (HPLC) and pharmacokinetic and statistical approaches [55]. In this study the

approach appears well designed; however, ethnic differences could influence MPA pharma-cokinetics, create a bias and generate nomograms that cannot be used for other ethnic groups. It appears that with the same dosage, MPA systemic exposure is higher in Asian renal transplant patients than in Caucasians and American-Africans [61].

Papers by Baraldo et al., ideation groups 2005 and validation groups 2009 are the only studies performed in the first 6 months after transplantation, which are the months where the greatest variability is observed and are the most critical months for the graft [53, 54]. In the Consensus of 2010, the group of experts cited the Kaczmarek, Baraldo and Ting papers, predicting them as reference works for the algorithms in heart transplants. One of the limitations of this study was the limited number of patients in the ideation group [5].

When using the LSS to estimate the MPA-AUC, it is important that the study populations (ideation group and validation group), the drugs and the analytical methods used have characteristics that are always the same and repeatable. Bayesian methodologies have multiple advantages, are more adaptable to different types of patients and are less sensitive to inaccuracies in sampling time [4, 62]. As a result the application of a nomogram from an LSS to estimate AUC_{0-12} is simpler to use but requires greater precision, while Bayesian methodologies are more difficult to use and a specialized technician is required.

Thanks to research done on kidney transplants, today there are automated LC–MS/MS platforms on the market that can perform MPA plasma analysis more accurately, in less time and the costs of the analyses have been significantly reduced. Therefore, there is currently a greater possibility of performing the MPA TDM and a personalization of the therapy.

More accurate MPA TDM may reduce the leukopenia that often leads to discontinuation of MMF therapy and increased risk of rejection. Therefore, in heart transplantations it may be concluded that: (1) the guidelines recommend a C_0 of 1–3.5 mg/L and MPA-AUC_{0-12} values of 30–60 mg · h/L; (2) a population is used similar to that for the calculation of the nomogram (type of transplant, post-transplant period, used therapy, ethnicity, etc.); and (3) analysis of the MPA is performed with a method similar to that used to calculate the nomogram of the LSS.

In conclusion, these results are interesting because LSS MPA-AUC_{0-12} in heart transplant patients remains a sector of clinical pharmacology seldom studied and completes our previous findings with a validation group showing valuable bias and precision values. Future studies are needed to determine whether these algorithms can be clinically applied in a larger cohort of heart transplant patients receiving CsA or TAC associated with MPA therapy.

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Conflict of interest

The authors have no conflicts of interest.

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