

Practical approach to therapeutic drug monitoring of mycophenolic acid

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Introduction

The dosing regimens of immunosuppressive drugs applied after organ transplantation aim to balance between the prevention of under-immunosuppression, which may lead to acute rejection, and the prevention of over-immunosuppression, which may cause toxicity, mainly represented by infections. Clearly, accurate and rational dosing of immunosuppressive drugs as well as the monitoring of immunosuppressive therapy is of life-saving importance.

It is therefore no surprise that the dose of all immunosuppressive drugs is somehow individualized. Azathioprine and corticosteroids are mainly dosed based on body weight and the dose of cyclosporine, tacrolimus and sirolimus are based on therapeutic drug monitoring (TDM) strategies.

The exception is mycophenolic acid (MPA), which is usually administered in fixed doses of the prodrugs mycophenolate mofetil (MMF) and enteric coated mycophenolate sodium (EC-MPS). MMF and EC-MPS have been shown to be bioequivalent, but it is noteworthy that the enteric coating of EC-MPS causes a slow release of MPA, resulting in delayed MPA peak concentrations (1 to 8 hours after oral intake) and higher and more variable MPA predose concentrations compared to MMF [1]. The “one-dose-fits-all strategy” for MPA is thought to be one of the major advantages of the drug compared to other immunosuppressive agents, and makes it “easy to use without monitoring” [2]. Given the observations that an area-under-the-

concentration-time curve (AUC_{0-12}) of MPA below 30 mg*h/L is associated with an increased risk for acute rejection, and that the exposure to MPA varies 10-fold between patients with standard doses [3], it is unlikely that optimal MPA exposure, and, therefore, optimal immunosuppression is assured in all patients. Despite the fact that the current practice for MMF has been successful compared to azathioprine [4-6], it is to be expected that some of the adverse events and acute rejection episodes can be prevented in patients with suboptimal MPA exposure. TDM is a suitable approach to identify such patients. This update aims at discussing which renal transplant patients may be monitored at which time-points post transplantation and how the monitoring can be performed.

Which patients are candidates for TDM of MPA?

One of the factors significantly affecting MPA exposure is the concurrent use of ciclosporin A. drug-interaction between MPA and ciclosporin has been shown in clinical studies where patients co-treated with ciclosporine had lower MPA exposure than patients co-treated with sirolimus [7] or tacrolimus [8]. Experimental studies in rats suggested that ciclosporin interfered with the enterohepatic recirculation of MPA, which can account for up to 61% of the total exposure to MPA during a dosing interval, through inhibition of the multi-drug resistance protein 2 (Mrp2) [9-11]. One of the functions of Mrp2 is to excrete the glucuronide metabolite of MPA (MPAG) into bile [12]. Thereafter, MPAG is deglucuronidated in the gut by the intestinal flora to form MPA, which is reabsorbed from the colon, leading to a second peak concentration of MPA. Inhibition of Mrp2 by ciclosporin will thus result in interruption of the enterohepatic recirculation and therefore lower MPA exposure. The clinical consequence of the drug-interaction was shown by a pharmacokinetic meta-analysis: half of the patients who are concurrently treated with MPA and ciclosporine had low MPA exposure (below 30 mg*h/L) in the first week after transplantation [13]. This is of importance because

it has been shown that suboptimal MPA exposure on day 3 after transplantation is associated with a higher incidence of acute rejection [14]. This justifies the recommendation that in those patients who receive MMF or EC-MPS in combination with ciclosporin, the starting dose should be 1500 mg and 1080 mg, respectively, twice daily, instead of the currently recommended 1000 mg and 720 mg twice daily, to reach optimal immunosuppression. This recommendation for the starting dose is not included in the package insert of MMF and EC-MPS. Therefore, it is only recommended in combination with TDM of MPA during the following days and weeks to further guide drug dosing.

Two other factors affecting MPA exposure are plasma albumin level and renal function. The likely mechanism is that acidosis, uremia and accumulation of MPAG, which are all associated with impaired renal function, will decrease MPA binding to plasma albumin [15,16]. Also low plasma albumin levels are likely to decrease MPA protein binding. The resulting increase of MPA free fraction leads to an increase in the amount of MPA available for glucuronidation and hence to a higher total MPA clearance. The result is low MPA exposure in the presence of impaired renal function or low plasma albumin levels, although conflicting results exist [15,17-23]. A complicating factor is that the same change in creatinine clearance or plasma albumin level does not necessarily lead to the same change in MPA exposure in every patient. This indicates that these factors are not suitable to directly serve as a basis for MMF or EC-MPS dose selection. A change in plasma albumin level or creatinine clearance however can provide an indication to measure MPA concentration to check whether exposure has changed and whether dose adjustments are needed to maintain MPA exposure above 30 mg*h/L.

In summary, patients who use MMF or EC-MPS in conjunction with cyclosporine, or patients with poor initial or rapidly changing renal function or plasma albumin levels, are candidates for TDM of MPA in order to achieve or maintain MPA exposure on target.

When to perform TDM of MPA?

Table 1 shows the current consensus about the TDM of MPA [24,25]. In this scheme, MPA exposure is measured four times within the first month after renal transplantation. The first measurement on day 3 aims at reducing between-patient variability and at getting MPA exposure on target. The second and third measurements aim to check whether a dose adjustment on the basis of the first determination of MPA exposure, indeed resulted in the desired MPA exposure. Because MPA exposure will increase in the first weeks after transplantation with 30-50% [26] as a result of recovering renal function and plasma albumin level, a fourth measurement is necessary one month after transplantation. This measurement aims at maintaining MPA target exposure and at preventing overexposure to the drug.

More frequent measurements of MPA exposure are not likely to be necessary given the low within-patient variability in MPA exposure [27]. As a result, there is an increased likelihood that target exposure, once achieved, can be maintained for a long period of time, without the need for frequent measurements of MPA concentrations. Reported values for within-patient variability are $\leq 30\%$ in the maintenance phase after transplantation and 40-50% in the initial period, although the latter values may have been overestimated as it is unclear from these studies whether a correction was made for the increase of MPA AUC_{0-12} over time which occurs in most patient despite a fixed dose [21,28].

Further measurements of MPA exposure may be necessary in the following situations: 1) when changes are planned in the immunosuppressive regimen, e.g. tapering or withdrawal of the calcineurin inhibitor to check whether MPA exposure is high enough to minimize the risk for rejection [29], 2) when rejection or toxicity occurs, to exclude under or overexposure, respectively, and 3) when a change in renal function or plasma albumin level occurs as discussed above.

How to perform TDM of MPA?

MPA plasma concentrations can be measured using high performance liquid chromatography (HPLC) with either ultraviolet or mass-spectrometric (MS) detection or using the enzyme multiplied immunoassay technique (EMIT). These methods are widely available. EMIT measures on average 10 to 30% higher MPA concentrations than HPLC as a result of cross-reactivity with the pharmacologically active acylglucuronide metabolite of MPA [30,31]. Although this metabolite is usually formed in minor quantities, it can accumulate in situations of renal dysfunction leading to a substantially higher overestimation of the MPA concentration. Because EMIT measures both MPA and its pharmacologically active metabolite, it has been suggested that EMIT offers a better reflection of overall immunosuppression [26]. As a result of EMIT measuring higher MPA concentrations than HPLC reference should be given to the method used when pharmacokinetic results are compared between laboratories or studies. Besides, the target concentrations are higher when MPA is determined with EMIT, e.g. MPA AUC₀₋₁₂ of 36.1 mg*h/L by EMIT and a MPA AUC₀₋₁₂ of 33.8 mg*h/L by HPLC, resulted in comparable efficacy [30].

Recently, a new automated enzyme receptor assay has been introduced. This assay uses the biological substrate for MPA, inosine monophosphate dehydrogenase (IMPDH, a key-enzyme in *de novo* synthesis of purine nucleotides, which is necessary for lymphocytes to proliferate), as target enzyme. Total and unbound MPA plasma concentrations measured with the enzyme receptor assay showed a good correlation ($r=0.99$ and $r=0.98$ respectively) with MPA concentrations measured in the same samples by HPLC-UV or HPLC-MS [32]. Coefficients of variation (CV) for precision were <4% [24].

Also a cloned enzyme donor immunoassay (CEDIA) has become available for measurement of total plasma MPA concentrations. Correlation with HPLC-UV was good ($r=0.91$) and CV

values for within- and between run precision were <7%. Like EMIT, cross-reactivity with the acylglucuronide metabolite of MPA has been shown with CEDIA (192%) [33].

There is little clinical experience with the measurement of MPA with the enzyme receptor assay and with CEDIA, so the added value of these assays over EMIT or HPLC remains to be seen.

The most convenient parameter reflecting MPA exposure is the MPA predose concentration (C_0). A disadvantage of this parameter is its poor correlation with AUC_{0-12} (r^2 varies between 0.23 and 0.65) and its less strong correlation with the risk for acute rejection compared to AUC_{0-12} [24,34]. Because full AUC_{0-12} is impractical to obtain during routine clinical practice, alternatives for measuring MPA exposure have been investigated.

One alternative is to estimate the full AUC_{0-12} using limited sampling strategies during the first two or three hours of a dosing interval. Many strategies are published now with acceptable predictive performance ($r^2 > 0.70$). An overview of limited sampling strategies is provided in reference [24]. It is important to realize that a limited sampling strategy developed on the basis of data from renal transplant recipients using oral MMF in combination with cyclosporine collected during the first month after transplantation, can only be used in patients who resemble these characteristics. For patients with another indication for MMF therapy, with another immunosuppressive regimen, who are using intravenous MMF, who are within another period after transplantation or who are using EC-MPS, specifically developed limited sampling strategies should be used. If this is ignored, structural under- or overestimation of MPA exposure may occur. A second important aspect to realize is that the limited sampling strategy to be used should be validated, preferably with data independent from the data that were collected to develop the strategy.

A second alternative to estimate MPA exposure is to use Bayesian algorithms. These algorithms use both data from the patient in whom exposure needs to be estimated and

information from a population pharmacokinetic model. This approach offers several advantages. The first is that the Bayesian approach offers more flexibility than limited sampling strategies for two reasons: 1) Bayesian estimators are less dependent on the number of samples that are drawn from a patient, 2) there is no need to draw samples at predefined time points, as is the case with limited sampling strategies, as long as the time point at which the sample was drawn is known. A second advantage of the Bayesian approach is that the concerned population pharmacokinetic model estimates pharmacokinetic parameters like clearance and volume of distribution including between-patient variability in these parameters. Taking variability into account can improve the accuracy of the estimation of pharmacokinetic parameters, and thus of AUC_{0-12} , whereas ignoring variability can lead to biased estimates [35,36]. A disadvantage of the Bayesian approach is that it is mathematically and statistically complex. This causes the need for specially trained data analysts, and often makes the development of the model time consuming. In addition, the complexity of the method can constitute a barrier for clinicians to accept the results of a Bayesian estimator. They sometimes regard it as a black box, which makes them less eager to use the estimated AUC_{0-12} as a basis for dose adjustment.

A Bayesian algorithm for the estimation of MPA exposure has been made easily accessible through the Internet (<https://pharmaco.chu-limoges.fr/abis.htm>) [37]. The method uses three predefined plasma samples drawn within a wide, flexible, time range: 20 min (± 10 min), 60 min (± 15 min) and 180 min (± 30 min) after MMF administration. Estimated AUC_{0-12} and dosing advice are provided within 48 hours after data entry.

This Bayesian estimator has been used to estimate MPA exposure in a prospective randomized trial (APOMYGRE) where a fixed dose MMF regimen was compared with a MMF dosing regimen based on TDM. The incidence of clinical acute rejection episodes was significantly lower in the TDM group: 12.3% vs 30.7% ($p=0.01$, $n=65$ per group), without an

increase in the incidence of adverse events [38]. Interestingly, a comparable, but much larger (n=901), prospective randomized trial (FDCC) could not show a significant difference between the TDM group and the fixed MMF dose group [39]. An important difference between the two studies was that the latter one used limited sampling strategies to estimate AUC_{0-12} . In addition, it was found that many physicians in the FDCC study were reluctant to increase the MMF dose when low MPA exposure was estimated.

Conclusion

Candidates for TDM of MPA are renal transplant patients who use MPA in conjunction with cyclosporine, or patients with poor initial renal function or low plasma albumin levels as these patients have a higher chance of having an AUC_{0-12} below 30 mg*h/L with standard MMF or EC-MPS doses in the first week post-transplant. Four measurements of MPA exposure during the first month after renal transplantation can be sufficient to reach and maintain optimal MPA exposure above 30 mg*h/L. MPA exposure should be estimated using a limited sampling strategy, or, may be even better, with a Bayesian algorithm. For effective TDM of MPA it seems to be important to actively encourage physicians not be reluctant to apply dose increases when MPA exposure below 30 mg*h/L has been found.

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