Large scale analysis of routine dose adjustments of mycophenolate mofetil based on global exposure in renal transplant patients.

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Conflict of Interest/Disclosure

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Abstract

Background: We report a feasibility study based on our large-scale experience with mycophenolate mofetil (MMF) dose adjustment based on mycophenolic acid (MPA) inter-dose area under the curve (AUC) in renal transplant patients.

Methods: Between 2005 and 2010, 13930 requests for 7090 different patients (outside any clinical trial) were posted by more than 30 different transplantation centres on a free, secured website for MMF dose recommendations using 3 plasma concentrations and Bayesian estimation.

Results: This retrospective study showed that: (i) according to a consensually recommended 30-60 mg.h/L target, dose-adjustment was needed for about 35% of the patients, 25% being underexposed with the highest proportion observed in the first weeks after transplantation; (ii) when dose adjustment had been previously proposed, the subsequent AUC was significantly more often in the recommended range if the dose was applied than not, at all post-transplantation periods (72-80% vs. 43-54%); (iv) and the interindividual AUC variability in the "respected-dose" group was systematically lower than that in the "not respected-dose" group (depending on the post-transplantation periods, CV% = 31 to 41% versus 49 to 70%, respectively). Further analysis suggested that MPA AUC should best be monitored at least every two weeks during the first month, every 1-3 months between months 1 and 12, while in the stable phase, the odds to be still in the 30-60 range on the following visit was still 75.0% up to one year after the previous dose adjustment.

Conclusion: This study showed that the monitoring of MMF on the basis of AUC measurements is a clinically feasible approach, apparently acceptable by the patients, the nurses and the physicians, owing to its large use in routine clinics.

Introduction:

There is still ongoing debate regarding the added value of therapeutic drug monitoring (TDM) for mycophenolate mofetil (MMF) given as an immunosuppressive drug to prevent acute rejection following solid organ transplantation. As mentioned in the review articles on MMF TDM published over the last ten years (1-11), the general routine use of MMF TDM cannot be formally recommended on the basis of the available evidence. This is mainly due to the conflicting results of randomized studies that examined the clinical benefit of MMF TDM (12, 13).

However, it is not questionable that: (i) MMF exhibits large interindividual pharmacokinetic variability due to numerous factors, such as liver and renal functions, serum albumin levels or associated drugs; (ii) for a daily dose of 2 g, the inter-dose area-under-the curve (AUC) of mycophenolic acid (MPA) can vary 10-fold between patients; and (iii) that MPA $AUC_{0\text{-}12h}$ is better correlated with patients' outcome than any single concentration-time point, as shown at least by retrospective exposure-outcome studies. Moreover a time-dependent increase in dose-standardized MPA exposure as measured by the inter-dose area-under-the-curve (AUC) has been reported (+40% on average over the first 3 months posttransplantation). In this context, consensus conferences converged to the point that an MPA $AUC_{0\text{-}12h}$ between 30 and 60 mg.h/L should be targeted, at least over the first six months post-kidney or heart transplantation and when MMF is used in combination with cyclosporine and steroids. Interestingly, the last consensus report advised to prefer drug dosing based on MPA inter-dose AUC when obtained using limited sampling strategies (LSS) rather than based on single MPA (trough) concentrations (1).

Nevertheless, one of the arguments frequently put forward against MMF monitoring based on AUC is that it would not be feasible, owing to clinical constraints. In this context, based on original pharmacokinetic models built from large populations of transplanted patients enrolled in pharmacokinetic studies promoted and coordinated by the Limoges University Hospital, other academic institutions or by pharmaceutical companies, we previously developed Bayesian estimators to estimate MPA $AUC_{0\text{-}12h}$ using sparse individual data, hence the dose to reach an AUC

target value for each patient (14-16). In April 2005, we launched an expert system for ImmunoSuppressants Bayesian dose Adjustment (ISBA) and made it accessible to all transplantation centres worldwide through a free website (17). The objective of this service is to offer dose adjustment of MMF by providing: individual patient's exposure to the drug (inter-dose area under the curve, i.e., $AUC_{0\text{-}12h}$ for b.i.d. and $AUC_{0\text{-}8h}$ for t.i.d. dosing) estimated using Maximum a Posteriori Bayesian Estimators (MAP-BEs) on the basis of three blood samples collected in the first three hours following drug intake; the modelled concentration-time curve; and one or a range of recommended dose(s) to reach the therapeutic target. MAP-BEs developed and validated in kidney transplant recipients receiving MMF in association with cyclosporine (14-16) or tacrolimus are, among others, available through this website.

To address the concern of MMF dose adjustment feasibility, the objectives of this retrospective analysis of the routine requests for MMF dose adjustment in adult renal transplant patients posted on the ISBA website over the first 5 years of activity, were: (i) to study the MMF doses prescribed; (ii) to describe the distribution of the exposure to MPA after MMF dosing, hence the percentage of patients in whom a dose change was necessary to reach the 'target range' of MPA $AUC_{0\text{-}12h}$; (iii) to evaluate whether the Bayesian estimators used and doses recommended were actually able to increase the probability of the next MPA $AUC_{0\text{-}12h}$ to be in this target range; (iv) to evaluate the extent of intraindividual variability in the AUC; (v) and to propose recommendations about how frequent MMF dose adjustments should be to maintain MPA $AUC_{0\text{-}12h}$ within this range.

Material and methods:

The PK models and Bayesian estimators used by the ISBA expert system for MMF dose adjustment were previously described (14-16). Briefly, concentration data obtained in *de novo* or stable transplant patients can be fitted using: a single-compartment model with a double gamma input allowing good fitting of the double-peak concentration–time curves (most frequently observed in the first months after transplantation); or a 2-compartment model with a single gamma input for the absorption phase (curves with a single peak). Several Bayesian Estimators allowing the determination of individual PK parameters and the calculation of the AUC value were developed, using either the Iterative Two-Stage Bayesian approach (18, 19) or non-linear mixed effects modelling (The program Wings for NONMEM version 405 http://wfn.sourceforge.net/; Globomax, Hanover, USA). Each of them was developed from a specific database characterized by the type of graft, the post-transplantation period, the associated immunosuppressive regimen and the analytical method used for MPA determination. For each of them, either external or internal validation (i.e., in an independent group of patients or using the data-splitting approach, respectively) was performed before any clinical use. These pharmacokinetic tools have been developed from patients given Cellcept® and are not suited for other formulations of mycophenolate mofetil or other forms of mycophenolate. Several of these tools have also been used in TDM-validation or controlled-concentration trials (13,20). The Bayesian estimators used on the website for a dose adjustment of MMF in solid transplantation are all based on the same limited sampling strategy (LSS), i.e. 20min, 60min and 180min after the morning dose. Respecting this LSS is mandatory but, according to the flexibility of any Bayesian estimator and based on the results of validation data of these tools, acceptable ranges around these theoretical sampling times have been defined. Precisely, on the form to fill in on the website, centres are informed that samples must be taken at 20±10min, 60±15min and 180±30min.

All the AUC results and dose adjustments calculated using these tools are validated by a pharmacologist before reporting the results on the ISBA website. This means checking the estimated profile versus the measured concentrations and the current AUC estimate with regards to previous ones for the same patient (if any). In case of unlikely MPA concentrations, bad fitting or discrepancy with previous results, the pharmacologists in charge of the expert system can ask the physician or the clinical chemist for data confirmation or correction, or may model the data again using a close but different Bayesian estimator. For instance, MPA PK "maturation" over post-transplantation time is variable between patients, some reaching a stable clearance (and AUC) more rapidly than others, so that as soon as M3 or M4 in some patients, the Bayesian estimators aimed at the stable period (> 1 year) can be more appropriate than those intended for the M3-M6 period. For the last couple of years, this process has been automated by modeling each profile with 3-5 different models (i.e., for adjacent periods, using one or two peaks), and using the Bayesian Information Criteri (BIC) or Schwarz's criteria to select the model that best explains each individual data with a minimum of PK parameters (in order to prevent overfitting) (21). For each patient, the result corresponding to the lowest AIC value is proposed first hand, and has to be validated by a trained pharmacologist before reporting.

The requests for routine MMF dose adjustment concerning adult renal transplant recipients not enrolled in any kind of concentration-controlled clinical trial and posted on the ISBA website (17) within a 5-year period (i.e., from April 2005 to April 2010) were retrospectively studied. For each request, the following information was obtained: time elapsed since transplantation, MMF daily dose, immunosuppressive co-treatment, date of the previous dose adjustment (if any), calculated AUC value at the different visits (if any), doses proposed to reach AUC values of 30 to 60 mg.h/L.(or $AUC_{0\text{-}8h}$ of 20 to 40 mg.h/L when MMF is given three times daily). In the present study, only the requests where the MPA measurements were performed using either an HPLC technique (UV or MS detection) or the enzyme inhibition assay were considered.

Using this database, we tried to answer the following questions:

1- Depending on the associated CNI and the time elapsed since transplantation, what are the MMF doses prescribed to renal transplant patients, before any dose adjustment?

For this study, only requests corresponding to the first request posted on our website for each patient was considered.

2- What percentage of patients actually needed MMF dose adjustment to reach the 30-60 mg.h/L range?

The percentage of calculated AUCs in the 30-60 mg.h/L range was calculated in subgroups defined by the nature of the associated CNI and the time elapsed since transplantation. Accordingly, the percentages of dose increments or dose decreases proposed were also determined.

3- What is the efficacy of MMF dose adjustment based on Bayesian AUC estimation?

The efficacy of the proposed dose adjustments was evaluated using data from the patients with multiple AUC measurements posted on the website, considering separately those in whom the given dose was still in the dose range proposed at the previous request (i.e., doses to reach AUC values of 30 and 60 mg.h/L, respectively) and those receiving a dose outside this previously proposed dose range. The percentages of patients with current AUC within the therapeutic range were calculated and statistically compared using chi-square tests, taking into account both the post-transplantation period in which each "previous" dose recommendation was made and time space between the previous dose recommendation and the current request.

4- What is the extent of AUC intra-individual variability, and is the dose-exposure relationship linear in the stable period?

We studied the following situations:

- If a patient exhibited an AUC value right in the target range, with no dose correction needed, what were the odds for them to stay in this safe range in the long term? For that, we studied the distribution of the $AUC_{0\text{-}12h}$ values on visit *n+1* when the $AUC_{0\text{-}12h}$ value

on visit *n* was in the 40-50 h.mg/L interval. Patients with the following criteria were considered: multiple AUC measurements beyond the first year after transplantation, no modification of MMF dose and same associated CNI over the follow-up.

- In patients in whom the MMF dose had been doubled between 2 AUC measurements, was the AUC value doubled? For that, we studied the distribution of the individual ratios between AUC on visit n+1 and AUC on visit n. Patients with the following criteria were considered: (i) multiple AUC measurements beyond the first year after transplantation; (ii) AUC value less than 20 mg.h/L on visit *n*; (iii) same associated CNI on visit n+1; and (iv) MMF dose on visit *n+1* double that on visit *n*.

- In patients in whom the MMF dose had been divided by 2 between 2 AUC measurements, was the AUC value decreased by half? For that, we also studied the distribution of the same n+1/n AUC ratio. Patients with the following criteria were considered: (i) multiple AUC measurements beyond the first year after transplantation; (ii) AUC value greater than 90 mg.h/L on visit *n;* (iii) same associated CNI on visit *n+1;* and (iv) MMF dose on visit *n+1* half of that on visit n.

Results:

A total number of 13930 requests, posted on the website from 2005 to 2010, for MMF dose adjustment in renal transplant patients were analysed. This corresponded to a total number of 7090 different patients monitored in 53 different transplantation centres. Among these centres, 37 sent more than 10 requests (of which 33 were French). The repartition of these requests as a function of the CNI combined with MMF and the time elapsed since transplantation is presented in table 1. About a third (4396/13930) of the requests corresponded to the first 3 months post-transplantation. Over this 5-year period, a quite similar number of requests concerned the MMF-CSA and MMF-TAC drug combinations. However, the proportion of patients given MMF-TAC has progressively increased: 26% in 2005, 46% in 2006, 55% in 2007, 57% in 2008, 58% in 2009 and 60% in 2010.

1- Depending on the associated CNI and the time elapsed since transplantation, what are the MMF doses prescribed to renal transplant patients, before any dose adjustment?

Only the first request for each patient was considered, i.e. before any Bayesian dose adjustment performed on the website, resulting in the selection of 7090 AUC values. Approximately 45% (n=3125) of the AUC values were obtained beyond the first year after transplantation, about 40% (n=2842) in the first 3 months and 26% (n=1865) in the first month. Whatever the post-transplantation period, the mean daily dose of MMF given to these patients was higher when associated with CSA than with TAC (overall, 1898 mg/day vs. 1586 mg/day; $p<0.0001$) (figure 1 and table 2). In the first month, the mean daily dose of MMF was 2500mg for patients with CSA versus 1994mg for patients with TAC, and then in both groups it decreased progressively to reach 1600mg and 1200 mg beyond the 1st year post-transplantation.

More precisely, in the first month, 56% of the patients with CSA received 3g/day of MMF and 38% of them 2g/day, whereas these proportions were of 28% and 49% in the following two months. Finally, at the end of the first year, 50% of the patients were given 2g/day. In patients given MMF-

TAC, 73% received 2g/day of MMF in the 1st month versus 17% at the end of the 1st year, when 56% were given 1g/day.

2- What percentage of patients actually needed MMF dose adjustment to reach the 30-60 mg.h/L range?

For this study, 13930 requests were considered. The results of this study are presented in details in table 3. Considering all the post-transplantation periods and associated CNIs, 35% of the patients were outside the 30-60 AUC range. Interestingly, 25% could be considered underexposed, the highest proportion being observed during the first month for patients given MMF-CSA.

In the 1st month, for patients given MMF-CSA, the percentage of AUC values in the 30-60 mg.h/L range was significantly higher when the daily dose was 3g/day than 2g/day (72.3% vs. 47.1%; $p<0.001$).

In the same period, for patients given MMF-TAC, the proportion of AUC values in the recommended range was significantly higher when the daily dose was 2g/day than <2g/day (69.3% vs. 44.6%; $p<0.001$).

From the 3rd month to the end of the 1st year, 54% of the patients receiving ≥3g/day MMF in association with CSA had an AUC value in the recommended range and 40% had an AUC > 60 mg.h/L. In the same period, 74.2% of the AUC values were in the recommended range when patients were given 2g/day MMF.

In patients given MMF-TAC between month 3 and year 1, 66% had an AUC value in the recommended range (but about 25% were below 30 mg.h/L) if they were given less than 1g/day, while 32% of those given 1g/day exhibited an AUC > 60 mg.h/L.

Beyond the 1st year post transplantation, 5.5% (213 out of 3797) of the requests corresponded to patients given MMF-CSA who still received a daily MMF dose of 3g or more, leading to only 4.7%

of AUCs > 60 mg.h/L. Similarly, 16.7% of the requests corresponded to patients given MMF-TAC who still received ≥2g/day MMF, leading to AUC > 60 mg.h/L in 16% of these patients.

3- What is the efficacy of MMF dose adjustment based on Bayesian AUC estimation?

To evaluate the efficiency of Bayesian dose adjustment, we analyzed the data obtained from 3311 renal transplant patients for whom we received at least 2 AUC requests. As far as we could tell from the comparison of the dose proposed in the previous result and the dose reported on the next request form, 65 to 78% of the proposed dose adjustments were actually applied by the clinicians, considering all post-transplantation periods and spaces of time between 2 dose adjustments. In such cases, 72 to 80% of the estimated AUC values were within the 30-60 mg.h/L range. In contrast, when the dose recommendation had not been or was no longer applied, 39 to 57% of the patients were in the target range when the next request was sent. These results are illustrated on figure 2.

The interindividual AUC variability in the "respected-dose" group was systematically lower than that in the "not respected-dose" group (depending on the post-transplantation periods, CV% = 31 to 41% versus 49 to 70%, respectively). As a comparison, the CVs of the AUC values corresponding to a first request on the website ranged from 44 to 64%.

In the 3331 patients, we then studied the effect of time between a dose adjustment proposal and the next AUC measured, at different post-transplantation periods. When considering patients in the first month after transplantation, the percentage of AUCs in the recommended range was significantly higher in the "respected-dose" group than in the "not respected-dose" group when the time elapsed between the 2 visits was less than 2 weeks (79.5 vs 53.7%; $p<0.05$), but not when it was more than 2 weeks (70.4 and 55.5%, respectively). Similarly, between the first and the third months post-transplantation, the percentage of "respected-dose" AUCs in the recommended range tended to be higher when the time elapsed between two dose adjustments was less vs. more than 4 weeks (78.3% vs. 72.6%; ns). When considering dose-adjustments proposed at different post-transplantation

periods between month-3 and year-1, the percentage of subsequent AUCs in the target range was systematically and significantly higher in the "respected-dose" group ($p<0.001$), whatever the time elapsed between the recommendation and the next AUC (although a tendency towards better results was still observed for the shortest periods of time). For dose recommendations made after one year post-transplantation, patients in the "respected-dose" group were also more likely to be in the target than those of the "not respected-dose" group ($p<0.001$), the risk of being outside the therapeutic range remaining stable (i.e. around 25%) when the space of time between dose recommendation and the subsequent AUC was more than 3 months.

4- What is the extent of AUC intra-individual variability, and is the dose-exposure relationship linear in the stable period?

In patients beyond the first year after transplantation with no modification in MMF dose and associated CNI between two visits, we studied the distribution of AUC values on visit *n+1* when the AUC on visit *n* was within a 40-50 interval. When the time elapsed between 2 AUCs measurements was less than 3 months, 77% of the patients were still in a 30-60 range on visit *n+1* (i.e. 77/102). This proportion was not significantly different when increasing the time-intervals between 2 dose adjustments: 81% (56/69) and 71% (i.e. 110/155) for time intervals < 6 months and < 1 year, respectively. Additionally, no difference was observed when stratifying by associated CNI or MMF dose ranges. Globally, when the time elapsed between 2 visits was less than one year, the odds to be still in the 30-60 range on the following visit was 75.0%.

The distribution of AUC values observed on visit *n+1* when the dose was doubled as compared to that of visit *n* is presented in figure 3(a). The AUC_{n+1} / AUC_n ratio was 1.99 ± 0.81 in patients given MMF-TAC (n=52), but it was significantly lower in patients given MMF-CSA (n=60): 1.55 ± 0.74 ($p=0.003$).

The distribution of the AUC values observed on visit *n+1* when the dose was decreased by half is illustrated in figure 3(b). In patients given MMF-TAC, the AUC_{n+1} / AUC_n ratio was 0.69 ± 0.41. This ratio was 0.80 ± 0.41 in patients given MMF-CSA (n=55) (ns).

Discussion:

Although the clinical benefit of MMF monitoring is still debated, partly because the clinical evidence is inconsistent and partly because the best monitoring strategy has still not been agreed upon (1), we report herein large-scale experience with MMF dose adjustment based on MPA inter-dose AUC. The rationale behind this approach is based on the results of pharmacokinetic and comparative, randomized trials and on conclusions of expert consensus conferences, which stated that the inter-dose AUC best reflects the exposure to MPA and that a target range of 30 to 60 mg.h/L for $AUC_{0\text{-}12h}$ (respectively 20 to 40 mg.h/L for $AUC_{0\text{-}8h}$ when MMF is given t.i.d.) probably corresponds to an acceptable, if not the best, efficacy/toxicity compromise for different contexts in transplantation.

Within a 5-year period, transplantation centres have been using the expert system proposed on the ISBA website and have sent a very large number of requests for routine dose adjustment of MMF in different transplantation conditions, which gives an insight into the actual use of MMF monitoring. This has allowed the setting-up of a very large database of routine MPA PK profiles, all estimated using the same PK tools, that we partially analyzed herein. In the present study, only the requests where the MPA measurements were performed using either an HPLC technique (with either ultraviolet or mass-spectrometric detection) or the enzyme inhibition assay were considered. Additionally, an enzyme inhibition assay has emerged. Due to its higher sensitivity and specificity, HPLC-MS is often described as the gold standard technique (22). The enzyme inhibition assay does not seem to be significantly affected by metabolite interference as different studies have reported a good agreement with results from chromatographic assays when measuring MPA concentrations in transplant patients receiving MMF (16, 23, 24). In the report of the last consensus meeting on TDM of MPA (1), a summary of method performance for the measurement of MPA was performed. This study reported that the EMIT assay can lead to an overestimation by approximately 25% due to its cross-reactivity with metabolites of MPA, and exhibits an imprecision of 6 to 8% at a concentration of 1mg/L.

Considering about 7000 kidney transplant patients followed in multiple transplantation centres (most of them being French), the number of requests concerning the MMF-TAC and the MMF-CSA associations was quite similar. However, the proportion of patients with TAC has progressively increased over this period and almost 2/3 of the requests posted in the past months have concerned the MMF-TAC association.

We observed that the mean daily dose of MMF prescribed in the first month after transplantation to patients given MMF-CSA was approximately 2.5g, whereas that of patients given MMF-TAC was close to 2g. These daily doses slightly decrease over post-transplantation time to reach, in the long term, about 1.6g and 1.2g, respectively. The SPC of Cellcept® recommends a dose of 1g twice daily in renal transplant patients. However, greater than 10-fold ranges in MPA-AUC have been observed in different populations of transplanted patients receiving this standard dose (1, 10, 13). MPA pharmacokinetics is affected by the nature of the associated immunosuppressive and evolves with post-transplantation time, MPA-AUC being approximately 30–50% lower in the early post-transplantation period than three months after transplantation, for the same dose (this being sometimes called "AUC pharmacokinetics maturation"). For these reasons, the recommendations made after a roundtable meeting were to start with an MMF dose of 1.5g bid, especially for patients on concurrent CSA (7). In the present study, no more than half of the AUC values were in the 30-60 range for patients given MMF 2g/day in the first weeks after transplantation, whereas about 3/4 reached this range with 3g/day. However, one should not consider 3g/day as a gold standard dose as, for this period, a non negligible percentage of patients needed more than 3g/day or less than 2g.
In a study based on the data of the FDCC trial, van Gelder found a significant relationship between MPA-AUC on day 3 and the incidence of biopsy-proven acute rejection (BPAR) in the first month ($p=0.009$) or in the first year posttransplantation ($p=0.006$) (12). This study also reported that the risk of developing BPAR during the first year was significantly lower in patients with a day-3 AUC > 30 mg.h/L (12). In the APOMYGRE trial (13) where 137 renal allograft recipients were randomized to receive either concentration-controlled doses or a fixed dose of MMF, 7 out of the 10

episodes of BPAR that occurred in the first 3 months post-transplantation were associated with an MPA AUC value <30 mg.h/L, three with a value between 30 and 45 mg.h/L, and none with an AUC >45 mg.h/L. For these reasons, an AUC of 45 mg.h/L may be considered as the best single target in patients on cyclosporine and was recently substituted for the 30-60 h.mg/L range as the dose-adjustment criterion on the ISBA website. When retrospectively considering this single target of 45 mg.h/L in the present study, we calculated that in the first 3 months post-transplantation, 82.7% (1294 out of 1565) of the 'naïve' patients receiving MMF-CSA and 64.9% (1837out of 2831) of those given MMF-TAC were underexposed. This suggests that in CNI withdrawal protocols, patients should be also monitored carefully for MPA exposure to minimize the incidence of acute rejection.

Considering all post-transplant periods and drug combinations, more than 75% of the patients reached the target AUC range at their second visit when they were (still) receiving the dose proposed as a result of a previous dose adjustment on ISBA. When a dose adjustment had been proposed, the subsequent AUC was significantly more often in this recommended range if the dose was (still) applied than not, at all post-transplantation periods (72-80% vs. 43-54%).

When the first dose adjustment was performed in the first two weeks post-transplantation the odds to be (still) in the target range was maximal (i.e., more than 80%) for the following two weeks, but less (even if still better than no dose adjustment) thereafter. This result is in accordance with the systematic increase in MMF exposure in this period and with recommendations that TDM should be performed on days 3, 7 and once between days 10 to 14 post-transplantation (7). For later post-transplantation periods, the percentage of patients in the target was systematically and significantly higher if they received a dose within the previously proposed dose range than not, whatever the time interval between two AUC measurements.

In this study, we proposed to answer to two frequent clinical situations: a patient is in the stable phase (usually beyond the first year post-transplantation) and has either a low or a high AUC value requiring either to double or to halve the dose. In such case, what are the odds to either double or

halve the exposure? We found that when the dose was doubled, the next AUC value was doubled on average in patients given MMF-TAC, however with a large interpatient variability, while it was only multiplied by 1.6 in patients given MMF-CSA. The well-known interaction between CSA and MPA could at least explain these results. On the contrary, when the dose had been divided by 2, the exposure was reduced by 30-40%, without a significant difference between the 2 groups of patients. Thus, from our database, we determined that for patients in the stable post-transplantation period given 2g/day MMF, the mean AUC was 48 ± 14 mg.h/L in the "MMF-TAC" group (n = 497) versus 37 ± 16 mg.h/L in the "MMF-CSA" group (n = 1614). For a daily dose of 1g, these AUC values were 31 ± 15 (n = 1362) and 30 ±14 mg.h/L (n = 865), respectively. This suggests that the interaction with CSA might be negligible for the smallest doses of MMF and more pronounced when MMF doses increase. There are several studies reporting a lower exposure to MPA in patients receiving MMF in combination with CSA than in those receiving MMF and TAC or sirolimus or MMF alone (25-28). Thus, in contrast to TAC, CSA is an inhibitor of OATP1B3 and MPRP2, two transporters involved in the biliary excretion of MPA-phenyl glucuronide, and so decreases the enterohepatic cycling of MPA through inhibition of MPA-phenyl glucuronide excretion into the bile (29). This phenomenon can be schematized as follows: (i) in the presence of CSA, there is a decrease in the number of transporters available for 7-0-MPA-β-glucuronide (MPAG). When the dose of MMF is low, this decrease has very little impact because the number of available receptors is not a limiting factor; (ii) For higher doses of MMF, saturation of the remaining transporters is observed and a higher proportion of MPAG accumulates in the body. Neither the CSA dose nor any trough or C2 concentration was available for this study. However, in a population of 207 kidney recipients in the stable phase, Etienne et al. compared the exposure to MPA between patients receiving CSA dose-adjusted to reach an AUC0-12h of either 4.3 or 2.2 µg.h/L (corresponding to average doses of 2.1 mg/kg/day and 3.1 mg/kg/day, which again shows the non-linearity of the dose-exposure relationship). These authors reported that this decrease in CSA exposure/dose led to a 14% increase in the exposure to MPA.

The main objective of the ISBA website is to help clinicians individualize the dose of immunosuppressive drugs on the basis of their inter-dose AUC. For MMF, results are typically reported with a dose range to reach an AUC in the 30-60 mg.h/L range, together with a personalized comment if the context of the request has been filled in. However, without information on the patient's clinical status, risk of rejection, co-morbidity, side-effects or CNI levels, the proposed dose increases or decreases are suggestions to be appraised globally by the clinician together with the clinical findings. As the final decision always lies with the physician, it might partially explain why approximately one third of the propositions were not followed. However, two thirds were applied and apparently led to approximately 80% patients in the target range, with a decrease in the number of patients with over- or under-exposure. Since February 2008, when filling in the form for dose adjustment, the clinicians are invited to give information about the context of their request. An analysis of 3418 requests posted during 2010 showed that 64% were posted for systematic TDM, 33% for checking a previous dose adjustment, 2% for side-effects, 1% for a suspicion of acute or chronic rejection.

In conclusion, this retrospective analysis of more than 13000 PK profiles obtained from renal transplant patients given MMF, if not able to evaluate the clinical benefit of such a TDM strategy, showed the efficacy of MMF TDM based on AUC measurements in reducing the variability of MPA exposure, early after transplantation as well as in the long term, and in minimizing the frequency of patent under- or over-exposure.

Optimizing MMF TDM does not require a high frequency of AUC measurement to maintain the patients in the target. Actually, if in the early period it was observed that checking the AUC every two to six weeks maximized the chance to maintain the AUC in the target, in the stable phase, when the intra-individual variability is reduced, dose adjustment every 6 months up to one year was not detrimental. In our opinion, MMF TDM should probably also be performed when the associated

immunosuppressant regimen is substantially modified (which was not be studied here apart from the difference between CSA and TAC), or in case of rejection or adverse events. In any case, this study based on a very large number of requests received from many centres show that, despite the inconvenience of drawing blood samples three times within the first 3h post-dose, this approach is clinically feasible, and apparently acceptable by the patients, the nurses and the physicians.

Legends:

Figure 1: Distribution of the daily doses of MMF given to kidney transplant patients at different post-transplantation periods (M: month), sorted by combined calcineurin inhibitor.

Figure 2: Distribution of the MPA AUC values at different post-transplantation periods (M: month) in patients who had benefited from consecutive AUC measurements.

(The line in the box is the median. The lower edge of the box represents the 10th percentile and the upper edge the 90th percentile).

Figure 3:

Distribution of MPA AUC values observed on visit *n+1* when the dose was doubled as compared to that of visit *n (a)* and when the dose was decreased by half as expressed by the AUC_{n+1} / AUC_n ratios. (The line in the box is the median. The lower edge of the box represents the 10th percentile and the upper edge the 90th percentile).

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