A six-hour extrapolated sampling strategy for monitoring mycophenolic acid in renal transplant patients in the Indian subcontinent

Fleming DH, Mathew BS, John GT*, Chandy SJ, Manivannan J*, Jeyaseelan V**

ABSTRACT

Background: Therapeutic drug monitoring for mycophenolic acid (MPA) is increasingly being advocated. The present therapeutic range relates to the 12-hour area under the serum concentration time profile (AUC). However, this is a cumbersome, tedious, cost restricting procedure. Is it possible to reduce this sampling period?

Aim: To compare the AUC from a reduced sampling strategy with the full 12-hour profile for MPA.

Settings and Design: Clinical Pharmacology Unit of a tertiary care hospital in South India. Retrospective, paired data.

Materials and Methods: Thirty-four 12-hour profiles from post-renal transplant patients on Cellcept® were evaluated. Profiles were grouped according to steroid and immunosuppressant co-medication and the time after transplant. MPA was estimated by high performance liquid chromatography with UV detection. From the 12-hour profiles the AUC up to only six hours was calculated by the trapezoidal rule and a correction factor applied. These two AUCs were then compared.

Statistical Analysis: Linear regression, intra-class correlations (ICC) and a two-tailed paired t-test were applied to the data.

Results: Comparing the 12-hour AUC with the paired 6-hour extrapolated AUC, the ICC and linear regression (r²) were very good for all three groups. No statistical difference was found by a two-tailed paired t-test. No bias was seen with a Bland Altman plot or by calculation.

Conclusion: For patients on Cellcept® with prednisolone ± cyclosporine the 6-hour corrected is an accurate measure of the full 12-hour AUC.

KEY WORDS: Mycophenolic acid, therapeutic drug monitoring

Mycophenolic acid, the active immunosuppressant form of the prodrug mycophenolate mofetil (MMF), is widely used for the prophylaxis of acute rejection in renal transplant patients.[1] This tertiary care hospital in India carries out approximately 120 renal transplants annually and in 2002 started prescribing MMF. Previous studies have confirmed the increased risk for acute rejection associated with decreased values for mycophenolic acid (MPA) area under the plasma concentration time curve.[2,3] In addition, studies have reported an increased risk for hematological side-effects associated with increasing MPA AUC values over the dosing interval.[4] Since 1998 it has been agreed that the most reliable indicator of the risk of acute rejection is the full 12-hour AUC (AUC0-12h).[5] Shaw et al[6] discuss the variation in MPA AUC when MPA is administered with different immunosuppressants. For patients receiving cyclosporine and steroids, individualizing the MMF dosage to maintain the MPA AUC0-12h within the 30-60 mg·h/L range is thought to provide a lower risk for acute rejection and hematological side-effects.[7] For a fixed daily dose of MMF there can be more than a tenfold difference between patients in the MPA AUC0-12h,[4] highlighting that therapeutic drug monitoring is required to keep all patients within this therapeutic range. There have been several publications regarding which parameter(s) should be employed in the monitoring of MPA.[8,9] The trough concentration has been shown to have a poor correlation with the AUC0-12h[9] and with the risk of acute rejection as compared to the AUC0-12h.[1] Although the AUC0-12h has been shown to have a proven predictive indication of the clinical outcome, it is a cumbersome, tedious and cost restricting procedure. It also places extra demands on the unit, the patient and their families.

Limited sampling strategies (LSS) have been developed which use only three specimens taken within a range of two to six hours post-dose and to which an appropriate algorithm is applied to predict the AUC0-12h for MPA.[9,10] A number of algorithms have been developed in the West and these vary...
depending on the type of immunosuppressants co-administered. Following the introduction of MPA as treatment, nephrologists referred patients to the Clinical Pharmacology Unit for the routine monitoring of MPA by an AUC_{0-12h} profile. After 10 months it was decided to review the sampling procedure to determine if the sampling time could be truncated without compromising the accuracy. On applying a few of the published LSS to the measured 12-hour AUCs it was found that for each equation, in a number of patients, there was poor agreement between the LSS predicted and the measured AUC. To continue the validation of the various published LSS equations or develop an algorithm applicable to patients of Indian origin would require further time, effort and a large number of 12-hour AUCs. However, in the meantime, for routine drug monitoring, in order to reduce the cost of the test, make it less tedious for the patient and place less demands on the laboratory, a reduced sampling regimen was considered as a viable interim option. On reviewing the 12-hour profiles from our patients it appeared that after six hours the serum concentrations were relatively stable with enterohepatic recirculation adding little to the total AUC. It was decided therefore, as a first step, to evaluate the correlation between a corrected 6-hour sampling regimen against the 12-hour AUC. If there was shown to be good correlation without bias we could immediately halve the time for the test.

**Materials and Methods**

**Clinical methods**

Patients had 12-hour MPA AUC plasma profiles requested as part of therapeutic drug monitoring for the purpose of dosage adjustment of MPA. As a routine, prior to the day of the test patients were seen in the unit and the following were ensured:

- On the day of the test the patient was at steady state with regard to the medication.
- The patient understood clearly the importance of taking the doses at the correct (specified) times on the days and nights prior to the test. On the day of the test compliance was established and if there was any doubt the test was postponed.
- The patient understood that they should not eat after 10 pm on the night prior to the test, but that water could be taken freely and a cup (100 ml) of tea was allowed at 6 am.
- The patient understood the nature of the test including the overall time period.

The profiles for review were chosen in accordance with the following criteria:

**Inclusion criteria (all mandatory):**

- Patient who is post-renal transplant
- Patient between 15 - 85 years of age
- Patient taking Cellcept® (Roche Scientific Co. India Ltd., Basel, Switzerland)

**Exclusion criteria:**

- Patients under 15 and over 85 years of age
- Patients receiving other brands of MMF
- Patients with systemic lupus nephritis

Patient demographics are shown in Table 1. Dosages were given as a twice-daily regimen. Cellcept dosages commonly varied between 1000 mg and 2000 mg daily in divided doses, with one patient prescribed 750 mg and one prescribed 3500 mg per day, after dosage adjustment. All patients were also prescribed prednisolone and/or cyclosporine. Patients reported to the Clinical Pharmacology Unit at 8 am in the morning and a cannula was inserted into a forearm vein. A trough specimen was withdrawn. Cellcept was administered with 100 ml of water and specimens were then withdrawn at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10 and 11 h post dose. As the patients were counseled regarding compliance and were at steady state; the trough concentration is also a 12 hour concentration. Food was allowed two hours post dose. All specimens (approximately five ml each) were collected into EDTA containing vacutainer tubes. Blood specimens were centrifuged and the plasma separated into clean polypropylene tubes. All the specimens were analyzed on the same day as the test and the extraction was done within ten minutes of the blood collection.

**Sample extraction and analysis**

Specimens were assayed by isocratic high performance liquid chromatography (HPLC) with ultraviolet detection. The method was adapted for our column from that published by Svensson et al. to 300 µl of plasma, 40 µl of internal standard (carbamazepine 0.4 mg/ml dissolved in 20% alcohol:80% water) was added and mixed for ten seconds. Four hundred microliters of acetonitrile (ACN) was added and the closed tubes were vortexed at high speed for a further 50 seconds and then centrifuged at 15,800 rpm for eight minutes. The clear supernatant was removed and placed in clean microcentrifuge tubes and 20 µl was injected into the chromatographic system. The mobile phase was 51% ACN and 49% phosphate buffer (20 mM, pH 3.5) at a rate of 1 ml/min. The analytical column was a symmetry (waters) C18, 5 micron, (250 x 4.6 mm). Detection was at 215 nm and the temperature maintained at 30°C. The run time was eight minutes and there were no interferences from concomitant medication. The standard curve for MPA was linear to 100 µg/ml and the minimum detectable concentration was 0.1 µg/ml. Two MPA stock standards are made and used separately for the MPA standard curve and quality controls (QC). The inter-day QC coefficient of variation was 7.9%. The area under the concentration time curve was calculated by the linear trapezoidal rule, shown below. Patient specimens were extracted and analyzed within the same day.

**Equations and statistical analysis:**

1. The trapezoidal equation was used to calculate AUCs from the measured concentration – time data:

   \[
   AUC = \Sigma \left[\frac{(t_{n+1} - t_n)}{2} \times (C_n + C_{n+1})\right]
   \]

**Table 1: Patient demographics (28 patients)**

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.4±12.6</td>
<td>13-63</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weight (Kilogram)</th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>54.8±11.6</td>
<td>34-86</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.7±9.5</td>
<td>13-56</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum creatinine</th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7±0.5</td>
<td>1.0-3.1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum urea</th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.4±18.4</td>
<td>22-94</td>
<td></td>
</tr>
</tbody>
</table>

Fleming, et al.: MPA correlation of 6-hour extrapolated AUC and 12-hour AUC.
Where \( t_0 \) and \( C_0 \) are the time and concentration respectively of \( n \)th specimen.

2. To extrapolate the AUC \( 0-6h \) to AUC \( 0-12h \):
   - The AUC \( 0-6h \) was calculated by the trapezoidal rule and the following corrections applied:
     a. \( \text{AUC}_{0-6h} + 6 \times C_{6} \)
     b. \( \text{AUC}_{0-6h} + (5 \times C_{6}) + C_0 \)
     c. \( \text{AUC}_{0-6h} + (4 \times C_{6}) + (2 \times C_{3}) \)
     d. \( \text{AUC}_{0-6h} + (3 \times C_{6}) + (3 \times C_{3}) \)
     e. \( \text{AUC}_{0-6h} + (2 \times C_{6}) + (4 \times C_0) \)
     f. \( \text{AUC}_{0-6h} + C_6 + (5 \times C_{3}) \)
     g. \( \text{AUC}_{0-6h} + 6 \times C_0 \)
   - The correlation between AUC \( 0-12h \) and each of the above was carried out using linear regression. The equation giving the best correlation and predictive values was then applied to all data.

3. The AUC from enterohepatic re-circulation after six hours will be calculated by the trapezoidal rule. The percentage that this contributes to the full AUC will then be calculated.

4. The correlation between the AUC \( 0-12h \) and AUC corrected was determined using linear regression and intra-class correlation (ICC).

5. A two-tailed paired t-test was also applied to the data with the null hypothesis (\( P < 0.05 \)) that there is no difference between the methods for calculating the AUC.

6. The mean predicted error (MPE) or bias was calculated by the following equation:
   \[
   N \text{ MPE} = \sum \left( \text{pe}_i \right) / N
   \]
   \[N\text{ = number of pairs of estimated and measured.} \]
   \[\text{pe_}i = \text{difference between the estimated and the measured.} \]

7. Bland Altman plots of the mean AUC versus the difference of the AUC \( 0-12h \) and the extrapolated AUC \( 0-6h \) was used to highlight any bias.[12]

**Results**

**AUC corrected**

This was derived from 39 profiles from 31 patients. When the seven corrections for the AUC \( 0-6h \) were correlated with the AUC the two following corrections gave an \( R^2 \) of 0.99:

- c) \( \text{AUC}_{0-6h} + (4 \times C_{6}) + (2 \times C_{3}) \)
- d) \( \text{AUC}_{0-6h} + (3 \times C_{6}) + (3 \times C_{3}) \)

To choose between these two equations the predicted values at each point from the two regressions were compared to the actual value. Equation ‘d’ gave better predicted values reflected in a slightly higher \( R^2 \) value. It was subsequently used for this study.

**Profiles**

1. The MPA 12-hour profiles are now divided into the following four groups:
   - Patients - prednisolone without cyclosporine
     - Less than 1 year post-transplant (A)
     - Greater than 1 year post-transplant (B)
   - Patients - prednisolone plus cyclosporine
     - Less than 1 year post-transplant (C)

Greater than 1 year post-transplant (D)

From the original 39 profiles five were discounted for this further sub-analysis for reasons of, lack of numbers (group A, two profiles), lack of certain transplant details (two profiles) and one specimen could not be withdrawn at the correct time (one profile). Only group B of 9 profiles, C with 14 profiles and D with 11 profiles were considered. The mean plasma concentration time profile plus the standard deviation at each time point for MPA for groups B, C and D are shown in Figure 1. All profiles, except one, had a \( t_{max} \) of two hours or less with 53% (18/34) having a \( t_{max} \) at 0.5h. One patient showed a delayed absorption of 3h but no reason could be identified. Details of the AUCs with means and standard deviations for the three groups are given in Table 2.

2. Enterohepatic re-circulation (EHC): The mean and standard deviation of the AUC from EHC after six hours as a percentage of the total AUC for each of the groups is shown in Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>AUC ( 0-12h )</th>
<th>AUC ( 0-6h ) corrected</th>
<th>EHC as % of AUC ( 0-12h )</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>71.8 (39)</td>
<td>57.0 (41.1)</td>
<td>4.4 (2.9)</td>
</tr>
<tr>
<td>C</td>
<td>23.4 (14.9)</td>
<td>23.8 (15.4)</td>
<td>5.1 (3.7)</td>
</tr>
<tr>
<td>D</td>
<td>42.4 (17.8)</td>
<td>43.1 (19.0)</td>
<td>7.8 (10.9)</td>
</tr>
</tbody>
</table>

Table 2: The means and standard deviations for the area under the curve for 12 hours (AUC \( 0-12h \)) and AUC \( 0-6h \) corrected along with the means and standard deviations of the AUC from enterohepatic recycling after 6 hours expressed as a percentage of the total AUC

<table>
<thead>
<tr>
<th>Statistical parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Statistical parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC (C.I)</td>
<td>0.999</td>
<td>0.971</td>
<td>0.999</td>
<td>Bias (C.I)</td>
<td>-1.17</td>
<td>-2.06</td>
<td>-4.4</td>
</tr>
<tr>
<td></td>
<td>(973–0.998)</td>
<td>(2.06–4.4)</td>
<td>(4.97–2.62)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.999</td>
<td>0.999</td>
<td>0.973</td>
<td>Two-tailed</td>
<td>df 8, ( P = 0.497 )</td>
<td>df 13, ( P = 0.67 )</td>
<td>df 10, ( P = 0.537 )</td>
</tr>
<tr>
<td></td>
<td>0.977</td>
<td>0.977</td>
<td>0.977</td>
<td>paired t-test (C.I)</td>
<td>(0.922–0.994)</td>
<td>(-1.53–3.17)</td>
<td>(-3.50–1.94)</td>
</tr>
</tbody>
</table>

df = degrees of freedom, \( P = P \) value, C. I. = Confidence interval

---

Fleming, et al.: MPA correlation of 6-hour extrapolated AUC and 12-hour AUC
6. Bland Altman Plot: This allows any bias to be visualized. This is shown in Figure 2.
7. Percentage difference: A difference of 10% or less between the AUC\textsubscript{12h} and the AUC\textsubscript{6h} was considered acceptable. From the Bland Altman plot it is observed that the difference in three of the pairs of AUC lay outside the 10%
range in group C and two in group D. In group B no differences in AUC lay outside 10%.

**Discussion**

Johnson et al. reported enterohepatic re-circulation for MPA occurring between four and eight hours post dose. From our profiles 15 of 34 profiles showed some increase in plasma MPA concentration indicative of enterohepatic re-circulation even after eight hours. However, from the full MPA plasma concentration time profiles in patients at steady state the mean contribution of the enterohepatic circulation to the full AUC for groups B, C and D was only 4.4% (3.16 mg.h/L), 5.1% (1.65 mg.h/L) and 7.8% (3.31 mg.h/L) respectively. This indicates that enterohepatic re-circulation after six hours is not a significant contributor to the full AUC irrespective of the concomitant medication and the time after transplant in our patients. The good correlation between the 12-hour AUC and 6-hour corrected AUC in all the three groups further supports this. When comparing the AUCs from the reduced sampling strategy with the full 12-hour AUC the evaluations cannot be classified as independent observations as they are determined on the same patient. For this reason we have applied an ICC. We decided to present the linear regression as well as the ICC in order to allow comparison with other published data. For the AUC 6h corrected against the measured full 12-hour MPA AUC, the ICC for all the groups was greater than 0.97. The linear regressions showed similarly good correlations with r2 values of 0.956 or above for all the groups. Therefore it is valid to make the assumption that concentrations resulting from enterohepatic re-circulation at seven, eight and nine hours are not significantly different from that at six hours and similarly the concentrations at 10 and 11 hours are reflected in the 12h or trough concentration. From the P-values of the t-test, the null hypothesis that there is no difference between AUC 6h and AUC 0-12h can be accepted as correct in all three groups. The equation for bias showed this to be insignificant. This was also seen in the Bland Altman plots. Reviewing the agreement of each of the paired AUC profile values, three profiles in group C and 2 in group D had a difference greater than 10%. However, in none of these would the difference have resulted in an incorrect alteration in the dose. The limitations of this strategy in the long term are obvious; it still involves ten blood specimens over a period of six hours. However, it has allowed the time of the test to be cut by half. To ensure the patients’ comfort we have a cheerfully decorated patient room with comfortable chairs, a bed for any patient who gets tired easily, access to TV and reading material and for the younger patients, drawing materials. Regarding the specimens, the simple extraction, short run time of eight minutes and, if available, an autosampler for the HPLC allows rapid reporting of results. The HPLC consumables are relatively inexpensive and therefore, even with ten specimens per patient, the cost of the test can be kept to a minimum. Based upon this data we suggest that for patients on Cellcept and receiving prednisolone with or without cyclosporine the 6-hour corrected AUC is a valid measure for routine therapeutic drug monitoring. As the next step, we plan to evaluate the current limited sampling strategies to calculate AUC, which are the more practical and accepted approach for both the patient and the laboratory personnel. If no published algorithm is found to be satisfactory then attention will be turned to the development of one.

**Conclusion**

The 6-hour corrected AUC when compared with the full 12-hour AUC showed very good correlation with no statistical difference or bias. From this we conclude that in the absence of a proven limited sampling strategy algorithm, as a viable interim option the AUC 6h corrected is valid for the routine monitoring of mycophenolic acid.

**References**

7. Oellerich M. Symposium on International Association of Pediatric Laboratory Medicine, Egyptian society of Laboratory Medicine and the International Association of therapeutic Drug Monitoring and Clinical Toxicology, Cairo, 2001.

Source of Support: Nil, Conflict of Interest: None declared.