Immunosuppressant drugs – the role of therapeutic drug monitoring

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Introduction

Individualizing a patient's drug therapy to obtain the optimum balance between therapeutic efficacy and the occurrence of adverse events is the physician's goal. However, achieving this goal is not always straight forward, being complicated by within and between patient variability in both pharmacokinetics and pharmacodynamics. In the early 1960s new analytical techniques became available allowing the measurement of the low drug concentrations seen in biological fluids during drug treatment. This offered the opportunity to reduce the pharmacokinetic component of variability by controlling drug therapy using concentrations in the body rather than by dose alone. This process became known as therapeutic drug monitoring (TDM) [1].

For a drug to be a suitable candidate for therapeutic drug monitoring it must satisfy the following criteria:-

There should be a clear relationship between drug concentration and effect.

The drug should have a narrow therapeutic index; that is, the difference in the concentrations exerting therapeutic benefit and those causing adverse events should be small.

There should be considerable between-subject pharmacokinetic variability and therefore a poor relationship between dose and drug concentration/response.

The pharmacological response of the drug should be difficult to assess or to distinguish from adverse events.

The immunosuppressive drug cyclosporin satisfies all four of these criteria and yet despite over 19 years of clinical use with therapeutic drug monitoring, there is no firm consensus on the best way to use the drug and monitoring techniques are still evolving [2]. In addition, the number of available agents for use as immunosuppressants has more than trebled in recent years and the range of diseases in which these drugs are used has also widened [3]. The purpose of this review is to examine the current strategies in use for the therapeutic drug monitoring of immunosuppressant drugs [4] and to discuss

some of the factors that impinge on the monitoring of these drugs.

Azathioprine, steroids, antilymphocyte globulin, and OKT3

The combination of azathioprine and prednisolone was responsible for making clinical transplantation viable [5]. With the addition of antilymphocyte globulin [6] (ALG or ATG if human thymocytes instead of human lymphocytes were used to immunize the animal host) they formed the basis of immunosuppression in the early years of transplantation and these drugs are still in widespread use today. Monitoring the blood or plasma concentration of these drugs is not considered worthwhile as they all have relatively wide therapeutic indices. The three agents are generally given in fixed doses and are not subjected to therapeutic drug monitoring.

However, a case can be made for the measurement of the activity of the enzyme thiopurine methyltransferase (TPMT) as an adjunct to azathioprine therapy [7]. Azathioprine is not directly immunosuppressive, the drug must be metabolized first to 6-mercapto-purine, then by TPMT to 6-methyl-mercapto-purine and then on to the pharmacologically active 6-thioguanine nucleotides. The expression of the enzyme TPMT is inherited in an autosomal codominant fashion and consequently varies widely within the population [8] with 11% of the Caucasian population heterozygous and 0.3% homozygous with respect to TPMT deficiency [9]. Potentially fatal complications could be avoided if TPMT activity was monitored in erythrocytes [10]. The therapeutic drug monitoring of azathioprine in cancer chemotherapy is outside the scope of this article but this is reviewed separately in this supplement by Lennard [11].

OKT3 (muromonab-CD3) is a mouse-monoclonal antibody directed against the CD3 complex on T cells [12]. When complexed with its antigen, the antibody prevents the initiation of signal transduction and blocks all T cell function [13]. In a pilot study using OKT3 serum concentrations as a guide to therapy in kidney transplant patients excellent results were reported for the prevention of early graft rejection [14]. Although there is a correlation between OKT3 concentration and T cell killing the relationship is complicated by the patients' antibody

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response to murine-derived protein [15]. In another study using flow cytometry measurements to monitor OKT3 therapy the authors not only measured OKT3 concentration but also anti-OKT3 antibody concentration and the number of CD3⁺ cells (the therapeutic target of OKT3) [16]. Although the authors' conclusions were positive about the use of flow cytometry for monitoring, their overall conclusions were that 'this treatment cannot protect against acute cellular rejection due to the presence of a dimly positive CD3⁺ population'. In those centres using muromonab-CD3, TDM for OKT3 is not in widespread use.

Cyclosporin

In the early days of kidney transplantation Calne gave the 2 year graft survival rate for cadaveric renal transplantation in the USA as less than 18% [17]. Five years later, 1974, the same author reported that graft survival, at 2 years, had risen to over 50% [18]. The change was due to better surgical technique and improved patient management since the main drug therapies, azathioprine and prednisolone, had not changed. Twenty-one years later, in those patients receiving that drug combination, the 2 year graft survival was still only $\approx$ 60% [19]. However, with the discovery [20, 21] and use of cyclosporin for immunosuppression the 2 year graft survival for the majority of patients is now better than 80% [19]. Long-term graft survival has also improved; after the first year from transplant, the estimated graft half-life in patients on cyclosporin alone is 30 years compared with 10.5 years in those patients maintained on azathioprine and steroids [19].

While the introduction of cyclosporin resulted in a marked improvement in graft survival, its use was not without problems. From the initial discovery of cyclosporin [22] to the present day [23], the absorption of the drug has been problematical [24]. Variable absorption and a narrow therapeutic index (the drug causes irreversible kidney damage when given in too high a dose [25]) has resulted in the measurement of cyclosporin blood concentrations so that the dose of the drug can be tailored to the patient in such a way as to maximize therapeutic efficacy while minimizing toxicity [26]. In the past, the utility of cyclosporin therapeutic drug monitoring has been reduced by, choice of sample matrix for monitoring [27, 28], lack of assay specificity [29], inconsistent assay performance [30], the variable absorption of the drug from the original formulation (Sandimmun[®]) [31] and the poor correlation between trough concentration and clinical effects [32]. Over time, the majority of these problems have been addressed [33]; blood, and not plasma or serum, is the chosen matrix for measurement [34, 35], assays are now more selective for the parent compound [36, 37],

most laboratories participate in proficiency testing [38] and the drug has been re-formulated (Neoral[®]) to improve absorption [39–41]. However, trough whole blood concentration remains an imperfect measure of the total exposure to cyclosporin during a dose interval [4]. Recently, it has been shown that capillary blood, obtained by skin puncture, is suitable for monitoring cyclosporin [42]. This may be of particular use in paediatric practice.

In the Sandimmun[®] era substantial efforts were made by Kahan and coworkers to characterize the relationship between area under the concentration–time curve for cyclosporin (AUC) and clinical events. They showed improved correlation between patient outcome and AUC [43]. Although many acknowledge the advantages of AUC monitoring, it has failed to gain widespread acceptance because of the practical difficulties, both for the patient and clinician, in implementing AUC measurements [44]. The advent of the microemulsion formulation of cyclosporin, Neoral[®], with its improved pharmacokinetic characteristics [45], provides an opportunity to simplify the measurement of AUC [44]. Previous studies with Sandimmun[®] have shown that the concentrations of three blood samples, drawn at specific times, can be used to derive an accurate estimate of cyclosporin AUC [46]. For Neoral[®] a similar degree of accuracy in the prediction can be achieved by two optimally timed blood samples [47]. Prospective studies are now underway to compare predose concentration monitoring with sparse, or limited, sampling AUC monitoring.

However, predose, trough concentration and AUC monitoring are not the only options for the therapeutic monitoring of cyclosporin. For some time Cantarovich and coworkers have been advocating the use of a single timed sample 6 h after dosing [48]. In a prospective study of heart transplant patients comparing predose and 6 h cyclosporin concentration to control cyclosporin therapy, the use of the 6 h value resulted in a 30% lower dose of the drug with the same effectiveness in preventing rejection and similar cardiac and renal function as that seen in those dosed using the predose concentration [49]. These authors have also reported a good relationship between 6 h cyclosporin concentrations and efficacy in noninfectious uveitis [50] and other auto-immune diseases [51].

The opportunities presented by new therapeutic drug monitoring strategies to optimize cyclosporin therapy were the subject of a consensus meeting [52]. At that meeting another option that was promoted for monitoring was the use of the cyclosporin blood concentration at 2 h postdose (C₂). The rationale for this comes from the observation that was made during the clinical development of Neoral[®] when it was noted that in liver transplant patients there was an inverse relationship between the incidence of rejection and the maximum blood cyclosporin concentration (C_{max}) [53–56]. The use of C₂

monitoring has been tested in a small open-labelled trial in liver transplant recipients and the results were positive [57].

Recent studies of the pharmacokinetics and pharmacodynamics of cyclosporin have added scientific support to the use of C₂ monitoring. Pharmacokinetic studies have shown for cyclosporin that the absorption phase, the first 4 h postdose, is the most highly variable region of the blood concentration profile between patients [58]. Pharmacodynamic studies have shown that the cyclosporin concentration 2 h postdose not only correlates with the maximal calcineurin inhibition [59] but also coincides with maximal reduction in the number of circulating IL-2⁺ CD4⁺ peripheral T cells [60]. Evidence is beginning to accumulate that individualizing a patient's absorption phase for cyclosporin, so called 'absorption profiling', by targeting C₂ concentration leads to clinical benefits [61]. Trials showing positive benefits of absorption profiling have now been conducted in renal, liver and heart transplant patients [62, 63].

Tacrolimus

Tacrolimus (previously known as FK506), like cyclosporin, has been shown to be an effective immunosuppressive for the prevention of organ rejection after transplantation and, like cyclosporin, too much drug is associated with toxicity and too little with rejection. Again, like cyclosporin, whole blood concentration measurements are used for the monitoring of tacrolimus therapy [64]. Initial clinical trials did not include concentration monitoring and patients experienced neuro- and nephrotoxicity [65].

The pharmacokinetics of tacrolimus are highly variable [66]. Since tacrolimus shares many of the pharmacokinetic and pharmacodynamic problems associated with cyclosporin the rationale for therapeutic drug monitoring is similar. An early observational study correlating concentration and effect failed to show a significant difference between the blood concentration in those kidney transplant patients who did not experience rejection and those who did [67]. However other, more statistically rigorous, studies have shown, in kidney and liver transplant patients, significant associations of low tacrolimus concentrations with rejection and of high concentrations with nephrotoxicity [68]. Although the feasibility of a limited sampling scheme to predict AUC has been demonstrated [69], as yet, trough, or predose, whole blood concentration monitoring is still the method of choice. The use of other timed samples [70] and AUC monitoring has been investigated [71] but, unlike cyclosporin, there is no move towards their use in clinical practice. This may, in part, be due to the high correlation seen between trough concentration and C_{max} or AUC [72].

Mycophenolate mofetil (MMF)

This is the morpholinethylester of mycophenolic acid (MPA) and acts as a pro-drug for that compound [73]. When given orally to man, MMF undergoes rapid and complete absorption. It is hydrolysed, presystemically, to MPA and there is no MMF measurable in plasma [74]. The drug reduces both B and T cell proliferation by inhibition of *de novo* guanine nucleotide production [75]. Since, unlike cyclosporin and tacrolimus, both B and T cells are inhibited it has been suggested that MMF may be effective against both acute and chronic rejection [76]. In man, MPA is metabolized in the liver to 7-O-MPA-glucuronide (MPAG), an inactive metabolite that is present in plasma at approximately 20–100 fold higher concentrations than MPA. The MPA glucuronide was thought to be the only metabolite of MPA, however, it is now known that there are at least two other metabolites. Comparative results for the analysis of clinical samples, using high-performance liquid chromatography and enzyme immunoassay, showed a discrepancy between the assay techniques [77]. Subsequently, it has been shown that this discrepancy was due to the presence of an acyl glucuronide metabolite, which shows *in vitro* pharmacological activity [78]. In addition, a 7-O-glucoside metabolite of MPA has been identified [79].

Although the action of the active moiety, MPA, has been known for over 25 years [80], the amount of published data in peer-reviewed journals, relating concentration to effect, is limited. Large scale multicentre, double-blind, randomised controlled studies in renal transplant patients have shown the effectiveness of MMF in the suppression of acute, biopsy proven, rejection when used in combination with cyclosporin and steroids [81–83]. Logistic regression has been used to relate the AUC and C_{max} of plasma MPA to the incidence of rejection in renal transplant patients and has demonstrated a highly statistically significant relationship [74]. The results of the logistic regression and data from other trials [84] suggest that low plasma MPA AUC is a significant risk factor in developing rejection [85]. These data have been confirmed by the results of a randomised concentration-controlled study of MMF in renal transplant patients [86]. The link between high MPA concentrations and adverse effects has not been characterized.

The role of TDM in MMF therapy has yet to be established. Some authors believe that the interindividual pharmacokinetic variability is low and therefore the utility of TDM in the majority of patients would be limited [84], whereas others, using the same data, believe that the interindividual pharmacokinetic variability is high and that TDM may have a worthwhile function in the control of MMF therapy [87]. Support for the latter view comes from a study of 30 *de novo* heart transplant patients

receiving tacrolimus and MMF in which the dose of MMF was adjusted to maintain the MPA trough plasma concentration between 2.5 and 4 mg l⁻¹ [88]. These patients were rejection free at 6 months post-transplant and their MMF dose ranged between 0.5 and 6 g day⁻¹ to achieve trough concentrations within a target range.

Recent consensus guidelines are circumspect about recommending TDM for the control of MMF therapy since they were written before the clinical potential of AUC and trough concentration monitoring was documented [89]. An update from a recent meeting gives more information [90]. The guidelines suggest that TDM should be used to establish that adequate MPA concentrations are achieved soon after surgery and that it could be useful in cases of adverse reaction to MMF. These guidelines were written from the view point of MMF as secondary immunosuppression to cyclosporin or tacrolimus. However, in a recent study it has been shown that MMF can be used successfully as a primary immunosuppressant [91] and if MMF is to be used as monotherapy the rôle of MPA TDM may assume more importance.

Sirolimus

Recently, this drug has been licensed in the USA for use in combination with cyclosporin following kidney transplantation, but is also being used in other clinical indications and with tacrolimus [92]. The drug was also approved recently in Europe, where the license specifies its use in the prophylaxis of graft rejection in adult kidney transplant recipients, initially in combination with cyclosporin, and with the use of blood concentration monitoring. Therapeutic drug monitoring of sirolimus is still in its infancy but data have accumulated from several clinical trials, some of which were concentration-controlled and used sirolimus as primary immunosuppressive therapy [93–95]. For most Phase II clinical studies the drug was measured using h.p.l.c. with either mass-spectrometric or ultraviolet detection. For the pivotal Phase III studies an investigational immunoassay was used [96]. This immunoassay did not enter commercial production and is no longer available. As a result, attention is now focusing on h.p.l.c. techniques for routine monitoring of the drug [97, 98]. When sirolimus is used in combination with cyclosporin or tacrolimus, predose concentrations are generally targeted in the range 4–12 µg l⁻¹.

Daclizumab and basiliximab

The binding of interleukin 2 (IL-2) to its receptor on antigen activated T cells stimulates clonal proliferation of the T cells that mediate organ allograft rejection [99]. The receptor complex is made up of at least three subunits

(α , β and γ) and, of these, only the α subunit is thought to be specific to IL-2 [100]. This presents a potential therapeutic target for specific immunosuppressive therapy and it has been exploited by a series of monoclonal antibodies raised against the IL-2 receptor's α subunit (IL-2R α). Two such antibodies are currently available. One is a molecularly engineered human IgG1 incorporating the antigen-binding regions of a murine monoclonal antibody (daclizumab [101]) against IL-2R α . The other is a murine-human chimeric antibody (basiliximab [102]) to IL-2R α . Both have been used successfully for the prevention of acute rejection following renal transplantation [103, 104]. The therapeutic index of these antibodies is wide, neither antibody being associated with major adverse effects and, since the half life of both antibodies is long, daclizumab ($t_{1/2} \approx 6$ days [105]) is given as five, and basiliximab ($t_{1/2} > 20$ days [106]) as two, weight related intravenous doses in the first 8 weeks following transplantation. Thus, there appears little to be gained from therapeutic drug monitoring for these agents [107, 108].

The 'therapeutic' range

In a survey of the therapeutic ranges used by 21 transplant centres to guide cyclosporin maintenance therapy in kidney transplant patients there was a six fold range in the concentrations considered effective and a three fold range in those considered toxic [109]. There was also considerable variation in the width of the therapeutic 'window'. Although some of this variation must come from the different combinations of immunosuppressant drugs used and differences in assay methodology [36] and, perhaps, specificity [110], much of the variation is due to the empirical way that these ranges have been derived.

Early attempts to set target ranges to achieve efficacy, while avoiding toxicity, were based on simple clinical observations in patients who had undergone kidney-transplantation [32]. More systematic approaches have been made by the retrospective statistical analysis of large volumes of patient data gathered over a period of years [111]. However, the ranges arrived at are biased by subjective judgements; a more objective approach has been taken by Perna *et al.* [112]. These authors used logistic regression to determine the most probable concentrations for the occurrence of rejection and toxicity in renal transplant patients. This approach has also been used Nicholls [85] to determine the therapeutic range for mycophenolic acid and is applicable to the other immunosuppressant agents and their combinations.

Morris [113] has called the whole concept of the therapeutic range into question. This author suggests that the concept should be abandoned as it puts too much emphasis on achieving the desired numbers rather than treating the patient. He favours a single concentration

approach in which clinicians aim, initially, to dose a patient to achieve a set target concentration. The target is then individualized for that patient based on the number of rejection episodes, occurrence of toxicity, concomitant medication, etc. This has the advantage that pharmacokinetic variability is controlled by the target concentration and pharmacodynamic variability is dealt with by tailoring the target to the patient.

Some of the problems alluded to by Morris could be addressed if the boundaries of the therapeutic ranges were not seen as yes-no cut-off points. To do this the performance characteristics of the concentrations need to be seen in terms of a diagnostic test for determining the probability of drug-effectiveness or toxicity [114]. This, again, requires better information to be available to the clinician so that the blood or plasma concentrations can be interpreted within a Bayesian statistical framework and informed decisions can be made [115].

Assay methodology

Cyclosporin

The correct measurement of cyclosporin has been the subject of many publications [27, 28, 116] and reviews [38, 110]. Currently five commercial companies are producing eight different immunoassay assay systems for the measurement of cyclosporin in whole blood, Table 1. In addition, a number of laboratories are using high performance liquid chromatography (h.p.l.c.) to measure the drug. Using h.p.l.c. it is possible to separate the parent compound from metabolites and for this reason this technique has long been considered the 'gold standard'

in cyclosporin measurement, particularly when coupled with mass-spectrometry. However, although HPLC is specific for cyclosporin, the technique can suffer from poor precision and can give spurious results due to interference from other sources [117].

Of the eight immunoassay variants, two are nonspecific and cross-react, markedly, with the metabolites of cyclosporin, Figure 1. The Abbott TDx[®] Drug and

Figure 1 Measurement of cyclosporin in an aliquot of pooled blood samples from heart transplant patients receiving the drug. The results are shown as Box and Whisker plots (the line is drawn across the box at the median. The left of the box is at the first quartile (Q1), and the right is at the third quartile (Q3) value. The whiskers are the lines that extend from the left and right of the box to the adjacent values within $\pm 1.5 \times (Q3 - Q1)$, values outside the whiskers are plotted as circles. The mean result is shown as a solid circle) for h.p.l.c. (11 centres), TDx nonspecific (19 centres) and r.i.a. nonspecific. The dotted line is at the median value, $190 \mu\text{g l}^{-1}$, for h.p.l.c. (data from the Cyclosporin International Proficiency Testing Scheme [131]).

Table 1 Available assay for the measurement of cyclosporin and abbreviations and manufacturers.

Abbreviation	Assay
H.p.l.c.	High performance liquid chromatography.
TDx	Fluorescence polarization immunoassay with monoclonal specific antibody for the Abbott TDx [®] analyser.
TDxns	Fluorescence polarization immunoassay drug and metabolite with polyclonal nonspecific antibody for the Abbott TDx [®] analyser.
AxSYM	Fluorescence polarization immunoassay with monoclonal specific antibody for the Abbott AxSYM [®] analyser.
R.i.a.	Radioimmunoassay with monoclonal specific antibody, DiaSorin Cyclo-Trac-SP.
R.i.a.ns	Radioimmunoassay with monoclonal nonspecific antibody, DiaSorin Cyclo-Trac-SP.
EMIT	Homogeneous enzyme immunoassay, methanol extraction, Dade Behring EMIT.
CEDIAr	Homogeneous enzyme immunoassay, CEDIA Roche Diagnostics (no results shown, See Schütz <i>et al.</i> [121]).
CEDIA ⁺	Homogeneous enzyme immunoassay, CEDIA CsA Plus, Microgenics AG (Reconfigured and re-launched assay, no results shown, See Schütz <i>et al.</i> [164]).

Metabolite assay uses a polyclonal antibody and produces results that are approximately 3–5 times that of h.p.l.c. whereas the DiaSorin CYCLO-Trac NS radioimmunoassay uses a monoclonal nonspecific antibody and gives results about 5–7 times higher than h.p.l.c. The ratio of the nonspecific assays to h.p.l.c. changes with the metabolite: parent compound ratio in the blood and therefore will vary with transplant type and time after transplant. The results of the nonspecific assays have a poor correlation with clinical events [118].

The other six immunoassays are regarded as 'specific' for the parent drug but do, to a limited extent, cross-react with some of the metabolites of the drug and therefore do not necessarily give the same result for a given sample, Figure 2. Although the differences between the results of the 'specific' assays are due, in part, to the different cross-reactivities of the antibodies used, some of the differences result from incorrect calibration [119]. This can be clearly seen with the measurement of spiked samples (Figure 3). (It is interesting to note that for one of the manufacturers the results of their three different assays do not agree). These differences in measurement accuracy do not seem to affect the clinical usefulness of the assays [118] but do add to variability of reported concentrations in the literature [120] and have an impact on the local target ranges [109]. However, in clinical conditions where the cyclosporin

metabolite load in blood is high, for example, liver transplant patients immediately post-transplant, h.p.l.c. is the only method that can accurately measure the parent compound [121].

Tacrolimus

The concentrations of tacrolimus seen in the blood of stable renal transplant patients is low, $<30 \mu\text{g l}^{-1}$, and this makes the measurement of the drug difficult. In-house ELISA, commercial ELISA (DiaSorin) and micro-particulate enzyme immunoassays, and h.p.l.c.-MS [122] methods have been available. The majority of laboratories monitoring tacrolimus are using the commercial micro-particulate enzyme immunoassay (MEIA, Abbott Laboratories) which measures the drug within the range 3 [123] to $30 \mu\text{g l}^{-1}$ and cross-reacts to a small degree with the metabolites of tacrolimus.

Sirolimus

Therapeutic drug monitoring of sirolimus is still under investigation [124]. Although an immunoassay was available during the Phase III development of sirolimus [96], it has been withdrawn. The assays that are in current use are based on h.p.l.c. using either ultra-violet [98, 125] or mass spectroscopy to detect the drug [97].

Figure 2 Measurement specificity of cyclosporin by method. Twenty-nine aliquots of pooled blood samples from patients receiving cyclosporin were circulated to over 400 laboratories during a 25 month period (June 1998–June 2000 – the Cyclosporin International Proficiency Testing Scheme [131]). The results show the percentage difference of the method mean from the measured h.p.l.c. value for each of the 29 samples as Box and Whisker plots (the line is drawn across the box at the median. The left of the box is at the first quartile (Q1), and the right is at the third quartile (Q3) value. The whiskers are the lines that extend from the left and right of the box to the adjacent values within $\pm 1.5 \times (Q3 - Q1)$, values outside the whiskers are plotted as circles. The mean result is shown as a solid circle).

Figure 3 Trueness of cyclosporin measurement by method. During a 25 month period, 45 aliquots of cyclosporin free blood to which known amounts of the drug had been added were circulated to over 400 laboratories (June 1998–June 2000 – the Cyclosporin International Proficiency Testing Scheme [131]). The results show the percentage difference of the method mean from the expected value for each of the 45 samples as Box and Whisker plots (the line is drawn across the box at the median. The left of the box is at the first quartile (Q1), and the right is at the third quartile (Q3) value. The whiskers are the lines that extend from the left and right of the box to the adjacent values within $\pm 1.5 \times (Q3 - Q1)$, values outside the whiskers are plotted as circles. The mean result is shown as a solid circle).

Receptor assays

Cyclosporin, tacrolimus and sirolimus bind to a group of widely occurring proteins, the immunophilins. The two major immunophilins are cyclophilin, which binds cyclosporin, and FK-binding protein 12, which binds tacrolimus and sirolimus. Receptor assays have theoretical advantages over current immunoassays in that binding is associated with pharmacological activity so that only active drug or active metabolite concentration is measured [126]. However, this contention remains to be proved and these assays are not in routine clinical use.

Mycophenolic acid

Compared with the other immunosuppressant drugs in current use, the plasma concentration of MPA is much higher (of the order $1\text{--}5\text{ mg l}^{-1}$) and this makes h.p.l.c. measurement of the drug straightforward. Using this technique the major metabolite MPAG can be resolved and quantified [127]. A commercial homogeneous enzyme immunoassay (Dade Behring) is available which is capable of accurate and precise measurement of the drug in the concentration range $0.5\text{--}15\text{ mg l}^{-1}$ [128, 129]. On average, concentrations of MPA measured by this immunoassay are about 20% higher than those produced by h.p.l.c. [130], due to the cross-reactivity of the antibody noted above.

Proficiency testing

The difficulties in measuring blood cyclosporin concentration lead to the development of a proficiency-testing scheme for laboratories providing monitoring services for the drug [30]. The International Cyclosporin Proficiency Testing Scheme has been in existence for over 17 years and has over 400 participating centres world-wide [131]. The Scheme circulates three blood samples *per* month to each participating centre and the measurements made on these samples enable laboratories to benchmark their analytical performance against currently available best practice [132]. Initially, the manufacturer of cyclosporin, Sandoz AG (now Novartis AG), to promote measurement accuracy of the drug, funded the Scheme. However, the individual centres, the drug manufacturer and TDM reagent suppliers, now fund it.

In a similar fashion Fujisawa GmbH, the makers of tacrolimus [133], have funded a scheme for that drug and Dade Behring, manufacturer of a kit for the measurement of MPA, have funded a mycophenolate proficiency scheme [134]. These schemes have 260 and 70 members, respectively. A sirolimus proficiency testing scheme, funded by Wyeth-Ayerst Research, was initiated in 1998 to document the proficiency of laboratories offering

support for clinical studies in which the drug was monitored by immunoassay [135]. The scheme is continuing as a service to laboratories measuring the drug by h.p.l.c. The rôle and rationale for proficiency testing schemes in immunosuppressive drug monitoring has been reviewed recently [136]. Full details of the proficiency testing schemes for cyclosporin, tacrolimus, sirolimus and mycophenolate are available on the World Wide Web [137].

Pharmacodynamic monitoring

Blood or plasma drug concentration measurements are only a surrogate for effect. Their use would be unnecessary were it possible to measure the immunosuppressive action of these drugs directly. However, defining the pharmacodynamic target for monitoring is not an easy task. For example, in a recent study investigating immunological monitoring of azathioprine the authors examined multiple subsets of peripheral blood lymphocytes, natural killer activity, the serum concentrations of IgG, IgM, interferon β (IFN β), tumour necrosis factor α (TNF α), interleukin 2 (IL-2), soluble interleukin 2 receptors (sIL-2R), interleukin 6 (IL-6) and the soluble adhesion molecule sICAM-1 [138]. There is a danger that one surrogate will be replaced by another.

However, pharmacodynamic monitoring has been used successfully to control oral prednisolone dose [139]. Based on a previous observation that blood eosinophil counts (BEC) rises beyond $60 \times 10^6/\text{l}$ prior to heart allograft rejection [140], these authors carried out a randomised trial to compare their standard protocol for steroid dose adjustment with a protocol guided by BEC during the first 3 months after heart transplantation. Eighty patients were randomised to either have their EOS reported ($n=40$) and used for steroid dose adjustment or not reported. In the reporting group, patients had their steroid dosage increased if BEC exceeded $60 \times 10^6/\text{l}$. During the first six postoperative weeks, this group of patients had an 83% lower risk of treated rejection ($P<0.05$) and lower median intravenous dose of methyl-prednisolone ($P<0.05$) than the patients in whom EOS was not reported. Overall, the reporting group had less than half the risk of rejection of any grade ($P<0.001$) and significantly more rejection-free biopsies than those treated by the standard protocol ($P<0.01$). There was no increase the incidence of serious steroid-related side-effects.

Cyclosporin and tacrolimus inhibit T-cell proliferation, which is thought to result from their inhibition of calcineurin [141]. This is a serine-threonine phosphatase that plays an essential rôle in intracellular, calcium-dependent, signal transduction [142]. Inhibition of calcineurin in activated T cells reduces the translocation of the cytoplasmic subunit of the nuclear factor to the nuclear subunit

and, hence, impairs the transcription of genes for many of the cytokines essential for the rejection response [143]. For this reason calcineurin has been the primary focus of pharmacodynamic monitoring for cyclosporin. In a study of 62 renal transplant patients the measured calcineurin activity in leucocytes was half that of controls [144]. The trough cyclosporin concentration was inversely related to the calcineurin activity. Since tacrolimus acts on the same target, calcineurin would also be applicable for monitoring that drug. However, the measurement of calcineurin activity is technically challenging and a much simpler procedure would be required before it could be used in the routine setting. In any case, further studies would be needed to confirm that calcineurin activity correlates better with patient outcome than simple blood concentrations.

Mycophenolic acid exerts its immunosuppressive action by inhibition of inosine monophosphate dehydrogenase (IMPDH) and, thereby, blocking *de novo* purine biosynthesis in lymphocytes [145]. An assay has been developed for the measurement of IMPDH activity in whole blood to measure drug effect rather than concentration [146]. As yet there are few published data to support the use of this assay, but initial studies suggest a correlation between IMPDH activity and clinical events [147].

Sirolimus is thought to exert its immunosuppressive activity by blocking the phosphorylation and activation of the P70 S6 kinase that is involved in cell signalling, this prevents, or reduces, lymphocyte proliferation [148]. The measurement of P70 S6 activity is therefore a prime target for pharmacodynamic measurements. An assay has been developed for its measurement in whole blood and studies are ongoing to determine its utility in clinical practice [149].

Antithymocyte globulin (ATG) has been monitored in renal transplant patients using the effect of ATG on subsets of T lymphocytes [150]. In this study, the author identified the CD3⁺ lymphocytes as a pharmacodynamic marker of ATG response. The dose of ATG administered to patients was titrated to maintain the patients' absolute CD3⁺ lymphocyte count at ≈ 50 cells μl^{-1} of blood. Forty-four patients who were treated in this way for steroid resistant rejection had significantly less serious viral infections than 10 patients who were treated on a fixed dose ATG regimen, but their 1 years graft survival was not compromised. Although not a formal pharmacoeconomic study, the author also noted that there was a net saving of £1600 (\$2500) *per patient*.

Benefits of monitoring

Evidence-based medicine is sadly lacking in the area of drug monitoring [151]. Although the perception of therapeutic drug monitoring is that it is beneficial, and aids patient management, there is little hard evidence to

Table 2 Incidence of toxicity and rejection by whole blood tacrolimus concentration [156].

	Tacrolimus ($\mu\text{g l}^{-1}$)		
	< 5	5–15	> 15
Rejection %	34%	17%	5%
Toxicity %	0%	34%	54%

support that view. Outside the field of immunosuppressive drugs there are numerous articles, detailing predominantly retrospective studies, which suggest that TDM has a useful and cost effective rôle in monitoring therapy [152, 153]. However, as the authors of a recent review of clinical pharmacokinetics point out, there is little evidence to support the effect of TDM on true patient outcomes [152].

For the immunosuppressive drugs some positive evidence for TDM is given by the results of prospective concentration-controlled clinical trials [154]. A concentration-controlled trial is one in which patients are dosed to achieve preassigned target concentrations and therefore the results can be assessed in terms of drug concentration and response rather than dose and response. This type of trial has several benefits over the randomized dose-controlled trials [155] and can provide the basis for future TDM decisions.

In a study of tacrolimus, patients were randomly allocated to be targeted at low, medium or high drug blood concentrations [156]. Although there was no difference in the incidence of rejection or toxicity in the three groups in the first 42 days post transplant, logistic regression demonstrated a clear relationship between concentration and effect, Table 2. In a study of similar design using mycophenolate mofetil, patients were randomly allocated to a high, medium or low mycophenolic acid AUC [157]. Again there was a clear concentration related effect of the drug with rejection in the low, medium and high groups of 26, 9 and 6%, respectively.

Conclusion

At the present time the majority of drug regimes in use for transplantation are based on cyclosporin and excellent results can be obtained in solid organ transplantation using this drug. This is also true in the treatment of autoimmune diseases [3]. However, cyclosporin's dominance is being challenged as the number of immunosuppressive agents increases and clinical experience is gained with other drugs. We are entering an era in which combination therapy will be the norm and clinicians will tailor the immunosuppression to the characteristics of the individual patient, changing dose and drugs as time progresses and conditions change [158]. In addition,

generic [159] formulations of cyclosporin [160] will be available in the near future and these together with the new drugs under clinical development, such as FTY720 [161] and everolimus (Certicam®) [162], and the possibilities of xenotransplantation [163] present future challenges which will add to the complexity of TDM.

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