MONITORING NEW IMMUNOSUPPRESSIVE AGENTS
Are the Methods Adequate?

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There is an expanding range of immunosuppressive drugs and it is common practice for measurements of these drugs to be made as a guide to therapy or during their initial evaluation. A recent meeting of clinical and laboratory scientists at Chateau Lake Louise produced guidelines on the measurement of cyclosporin, tacrolimus, rapamycin (sirolimus) and mycophenolic acid /1-4/. Amongst the recommendations of three of these consensus panels was the need for external quality assessment of centres performing these analyses. The purposes of external quality assessment include establishing the accuracy and performance of the different methods to measure these drugs. It is often from the data generated by these schemes that assessments can be made of the adequacy of the methods in clinical practice /5,6/. However, all the schemes are comparative with method means and some of the schemes do not supply patient-derived material. None of the schemes is backed by accepted reference methods for the measurement of the drug being tested. Reference methods should be: based on a traceable standard; linked with mass-spectrometric detection; able to identify metabolites of the drug in question; able to deal with difficult sample matrices; able to exclude interference from other drugs or endogenous compounds.

Of the four drugs mentioned above there are commercial assays available for three of them, and a commercial assay is in development for the remaining drug (sirolimus). This last drug is undergoing clinical trials in kidney transplantation, but the other drugs are widely available for a variety of indications. Although cyclosporin can hardly be regarded as a new immunosuppressive agent, there is still substantial interest in its measurement, in part because of the introduction of a new formulation (*Neoral*®), which has substantially different pharmacokinetic properties compared with the original

formulation (*Sandimmun*®) /7/. There are also a number of generic formulations being evaluated.

Other drugs are under active clinical or pre-clinical evaluation, including leflunomide, brequinar and deoxyspurgualin. In addition, there is interest in the development of protein binding assays which might reflect the immunosuppressive efficacy of both the parent drug and its metabolites, as well as assays to assess the pharmacodynamic effect of immunosuppressive drugs and metabolic status. Of particular interest are binding assays for tacrolimus and sirolimus and an assay to measure the activity of inosine monophosphate dehydrogenase.

However, for all practical purposes in this field, the most important analytes for which assays are currently needed are cyclosporin, tacrolimus, mycophenolic acid and sirolimus. Methodological problems associated with the measurement of cyclosporin have been addressed by a number of consensus documents /1,8-12/. Issues which have been particularly important are the choice of sample matrix, calibrator accuracy, assay repeatability, assay sensitivity and the specificity of antibodies used in immunoassays.

Quality assessment schemes are already well established for two of the drugs - cyclosporin and tacrolimus. There have been a number of peer reviewed publications on the cyclosporin schemes /5,6/, but data are only starting to emerge on the practical problems associated with the measurement of tacrolimus /13,14/.

CYCLOSPORIN

Whilst the measurement of cyclosporin was dogged by numerous methodological problems when the drug was first introduced, the influence of consensus guidelines has ensured more uniformity in the choice of sample matrix and the specificity of the analytical technique. There are still some differences between the results produced by three main immunoassays used to measure cyclosporin - Abbott TDx monoclonal, Behring EMIT and INCSTAR CYCLOTrac SP - due, in part, to calibration. In addition, there are some differences in the specificity of the antibodies used in these assays, especially for the measurement of cyclosporin in samples containing relatively high concentrations of cyclosporin metabolites, such as those collected following liver or heart transplantation. The differences are exemplified in Figure 1, showing results for the measurement of

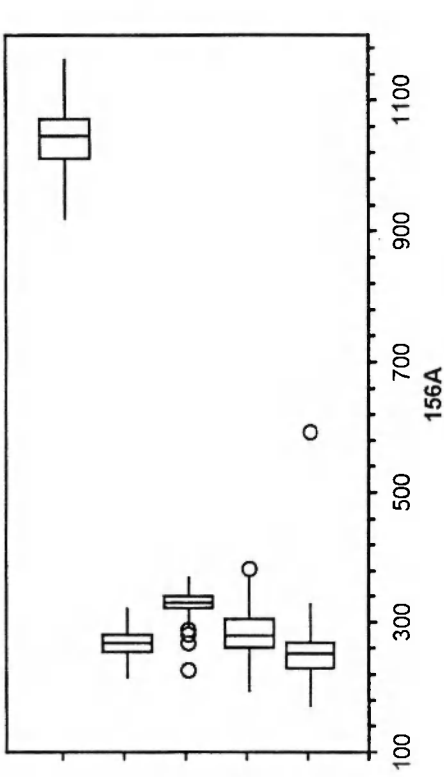

Fig. 1: Typical data for the measurement of cyclosporin in a pooled blood sample from heart transplant patients receiving cyclosporin. The data are shown by analytical method as a box and whisker plot. The default boxplot consists of a box, whiskers and outliers. The line drawn across the box is the median. The lower edge of the box is drawn at the first quartile (Q1, the 25th percentile) and the upper edge at the third quartile (Q3, the 75th percentile). The number of results for each method are shown in parentheses. Data UK Cyclosporin QA Scheme.

cyclosporin in a pooled sample from heart transplant patients. All the immunoassays with a high specificity for the parent compound give median values higher than high-performance liquid chromatography (HPLC). The Abbott TDx assay gives the highest result for this sample, as it uses an antibody with a broader spectrum of cross-reactivity with the metabolites of cyclosporin. The high proportion of cyclosporin metabolites in this sample is reflected by the more than four-fold difference between the result produced using HPLC and that produced by the Abbott TDx polyclonal assay, which is not selective for the parent compound. Whilst the differences between the assays with high specificity for cyclosporin are obvious they tend to make little difference to patient management if a single assay method is used within a centre. However, problems can arise if assays used out-of-hours are different from those used within normal working hours.

There are increasing demands on the dynamic range encompassed by these assays, due to the use of low doses of cyclosporin in combination with other drugs, and the growing interest in the measurement of area under the time-concentration curve (AUC) /15/. The use of low doses of cyclosporin means that the lower limit of quantification should be defined carefully by each laboratory, by including cyclosporin-free samples regularly in assay runs, so that non-compliance can be assessed with confidence. Measurement of AUC often results in cyclosporin concentrations spanning a wide range, especially when *Neoral*® is used /14/. Peak concentrations can be in excess of 2000 µg/l, and assays with a narrow dynamic range, such as the Behring EMIT assay, can result in the need for a large number of sample dilutions to measure accurately the higher concentrations in a profile of samples.

TACROLIMUS

Doses of tacrolimus have tended to decline since the pivotal studies performed in liver transplant patients, and this has caused some problems for the measurement of the consequently lower drug concentrations. Current recommendations on the monitoring of tacrolimus suggest whole blood concentrations consistent with efficacy are of the order of 5-15 µg/l /2/, although it is not uncommon

for patients on long-term maintenance therapy to have good clinical efficacy despite lower concentrations.

Early studies were based on a sensitive ELISA assay produced by the Fujisawa Co. The antibody used in this assay cross-reacted to a limited extent with metabolites of tacrolimus and results for patient samples were higher than those produced by HPLC. The original ELISA is no longer in production but a commercial ELISA based on the same antibody is available from the INCSTAR Corporation /17/. However, most centres are using a semi-automated microparticulate enzyme immunoassay produced by Abbott (IMx) /18/. This assay, again, uses the same antibody as the other immunoassays. Although a rapid assay, suited to routine monitoring of patients, the IMx assay was never optimised to measure within the target range now advocated for tacrolimus.

There are limited data available on comparative assay performance for the measurement of tacrolimus. The European Tacrolimus QA scheme has been operating for almost two years and, at the time of writing, has more than 130 members. There is a comparable scheme in the USA and tacrolimus is now being added to samples circulated by the College of American Pathologists in their cyclosporin proficiency scheme. The difficulties relating to assay sensitivity are illustrated in Figure 2, which shows the results for the measurement of tacrolimus in a blood sample spiked to a nominal concentration of 5 μg/l. Whilst the median values for each method are close to the target value, and very close for the one centre using HPLC with mass spectrometric detection, there is a very wide scatter of results. This is true even for a more sensitive version of the IMx assay (Tacrolimus II), which is replacing the original IMx assay. At higher concentrations, in the range 10-15 μg/l, between-centre precision (CV%) is still of the order 10-15%. Thus, the rather precise target ranges advocated in some studies would be difficult or impossible to achieve using any of the commercially available immunoassays /19,20/.

MYCOPHENOLIC ACID

Mycophenolic acid is formed following the *in vivo* hydrolysis of the pro-drug mycophenolate mofetil (*CellCept*®) /21/. Since the drug is distributed almost entirely within the plasma in whole blood, the recommended sample matrix for monitoring mycophenolic acid is

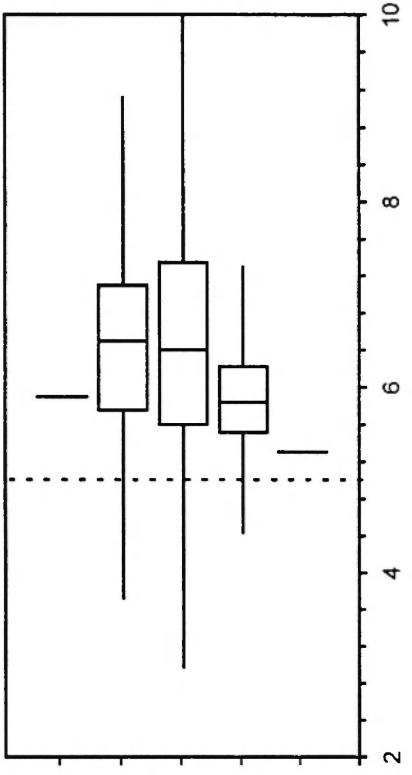

Fig. 2: Between-assay variability for the measurement of tacrolimus in a tacrolimus-free blood sample spiked with tacrolimus to a nominal concentration of 5 µg/l. Data are displayed by analytical method as a boxplot (see Figure 1 for explanation). Data European Tacrolimus QA Scheme.

plasma rather than whole blood /4/. The drug is given in relatively large doses - in the pivotal studies doses of 2 or 3 g per day were used - resulting in much higher concentrations than for the other drugs under consideration /22/. In a pre-dose sample the concentration of mycophenolic acid in plasma is of the order of 3-6 mg/l. Therefore, the problems associated with the measurement of this compound should be less than those encountered when measuring cyclosporin, tacrolimus or sirolimus.

High-performance liquid chromatographic assays have been described for the measurement of mycophenolic acid and its glucuronide metabolite /23/. Recently, Behring Diagnostics introduced an EMIT assay for the measurement of mycophenolic acid. Preliminary work in this laboratory has shown that the EMIT assay is suitable for routine monitoring of mycophenolic acid, being rapid and showing acceptable reproducibility across the range 0.5-15.0 mg/l. Typical data showing the performance for the measurement of three control samples supplied with the kit are illustrated in Figure 3.

There is a growing use of the assay in European centres and an external quality assessment scheme, co-ordinated from this laboratory,

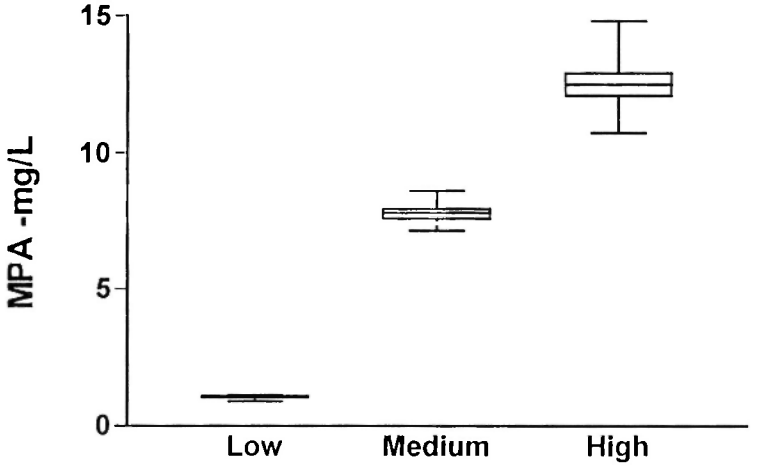

Fig. 3: EMIT assay measurement of mycophenolic acid in three control samples supplied with the Behring EMIT assay. Data are displayed as a boxplot (see Figure 1 for explanation). Data Analytical Unit.

has been introduced. The role of monitoring mycophenolic acid to optimise therapy with mycophenolate mofetil is not yet established. There is growing anecdotal evidence that measurement may be of value but the ideal sample in which to make the measurement is still under consideration. Two methodological aspects to be considered are the dynamic range of the assays and the possibility of interference from the glucuronide metabolite.

The use of AUC measurements has been advocated as a guide to therapy. Peak concentrations can easily exceed 15 mg/l, necessitating sample dilution if the top calibrator is exceeded. Whilst the glucuronide metabolite is stated to interfere only slightly with the measurement of mycophenolic acid using the EMIT assay - less than 0.2% - the metabolite can be present at very high concentrations relative to mycophenolic acid. Comparative data for chromatographic assays and the immunoassay in routine use are awaited.

SIROLIMUS

The clinical investigation of sirolimus is at a relatively early stage /24/. Initially, it was used in combination with cyclosporin, but there is an on-going study using the drug as primary immunosuppression in renal transplant patients.

Sirolimus is extensively distributed into red blood cells and consensus guidelines are for measurements to be made in whole blood /3/. High-performance liquid chromatography, with either ultraviolet or mass spectrometric detection, has been used for the regulatory studies, with these measurements made in central laboratories. Target whole blood concentrations have been in the range 5-30 µg/l, depending on the other drug therapy and the time since transplantation. The HPLC assays have proved sufficiently robust to provide a clinical service, but they are somewhat tedious. A semi–automated immunoassay for sirolimus is in development.

The role of sirolimus monitoring is still under evaluation, but data so far suggest that between-patient pharmacokinetic variability will justify its use /25,26/. Plans are in place to introduce an external quality assessment scheme if the measurement of this compound spreads to more laboratories.

CONCLUSIONS

In conclusion, there is good agreement between clinicians and laboratory scientists on guidelines for the measurement of four of the immunosuppressive drugs available or in evaluation. These guidelines are due to be up-dated towards the end of 1997 at a meeting of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology. External quality assessment schemes have played a part in establishing the comparative performance of the various assays available. The introduction of reference methods for the drugs and their metabolites would improve the quality of the findings these schemes can generate on the comparative performance of the various analytical techniques.

Most of the methodological issues related to the measurement of cyclosporin appear to have been resolved. For tacrolimus the need is for a more sensitive and reproducible assay; preliminary data suggest that the new IMx assay has improved the situation. The EMIT assay for mycophenolic acid could become a valuable adjunct in assessing dose optimisation with mycophenolate mofetil, and the measurement of mycophenolic acid should present few major problems. For sirolimus, methods capable of measuring relatively low concentrations in blood will be required. The current HPLC assays are unlikely to find favour in many laboratories and it remains to be seen whether a robust immunoassay can be developed.

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