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Blood cyclosporin concentrations and the short-term risk of lung rejection following heart-lung transplantation

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1 The relationship between blood cyclosporin concentration ($CyAC_b$) and a patient's risk of organ rejection following heart-lung (HL) transplantation was investigated.

2 Longitudinal data were collected for 90 days post-operation for 31 HL transplant recipients. Following exploratory analysis, a multiple logistic regression model with a binary outcome variable representing presence or absence of lung rejection (as defined on biopsy findings and/or intention to treat) in the next 5 days was fitted to the data.

3 A significant interaction between time post-transplant and $CyAC_b$ was found. During weeks 1–3, the relative risk (RR) of rejection per unit increase in log_e (5-day mean $CyAC_b$) was reduced: RR = 0.29, 95% confidence interval (CI) = (0.12, 0.72). After 3 post-operative weeks, this trend was reversed: RR = 1.61, 95% CI = (0.96, 2.70). Increases in cyclosporin dose ($CyAD$) and in coefficient of variation (CV) for both $CyAD$ and $CyAC_b$ over the previous 10 days significantly increased the risk of rejection: RR per unit increase in log_e (5-day mean $CyAD$) = 2.72, 95% CI = (1.18, 6.25); RR per increase of 10% (i.e. from, say, 20% to 30%) in the CV for $CyAD$ = 1.20, 95% CI = (1.07, 1.36); RR if the CV for $CyAC_b > 40\%$ = 1.51, 95% CI = (1.01, 2.27). Administration of high dose steroids in the previous 5 days was found to protect against further rejection: RR if steroid treatment was given = 0.23, 95% CI = (0.13, 0.38). The model fit was significantly improved by the inclusion of patient-specific effects ($F_{26,1347} = 5.27$, $P < 0.0001$) and an interaction between patient and time post-transplant ($F_{26,1321} = 4.71$, $P < 0.0001$).

4 Plots comparing $CyAC_b$ below various threshold values during rejection and rejection-free periods provided little support for the concept of a therapeutic threshold for $CyAC_b$.

5 We conclude that a negative relationship between $CyAC_b$ and lung rejection exists during the first 3 post-operative weeks. After this, the $CyAC_b$ maintained in these HL patients may be too high to provide any further increase in protective effect. Between-patient differences were also significantly associated with risk of rejection, as was underlying pharmacokinetic instability (reflected by a high CV for $CyAC_b$). The positive $CyAD$-effect relationship was probably due to a tendency for clinicians to increase $CyAD$ in patients whom they suspected were about to reject.

Keywords blood cyclosporin concentration heart-lung transplantation rejection logistic regression

Introduction

Cyclosporin (CyA) is a powerful immunosuppressive drug isolated from the fungus *Tolypocladium inflatum*. It was successfully introduced as an anti-rejection agent in human kidney transplant recipients (Calne *et al.*, 1978) in the late 1970s, and has since become the mainstay of anti-rejection therapy for organ trans-

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plantation throughout the world. In particular, the use of CyA has permitted a substantial improvement in the success of HL transplantation, which was previously impracticable due to poor survival of recipients (Reitz *et al.*, 1980).

Despite the impact of CyA on the success of organ transplantation, infection still remains a major cause of morbidity and mortality in patients receiving the drug (Tolkoff-Rubin & Rubin, 1986; Wreghitt *et al.*, 1987), and nephrotoxicity has also been amply documented as a common and serious side effect (Greenberg *et al.*, 1990). Post-operative management of transplant recipients therefore represents a conflict between preventing organ rejection and minimising the risk of infection or nephrotoxicity. This dilemma is further accentuated by the large inter- and intra-patient variability in the pharmaco-kinetics and -dynamics of CyA, which lead to an unpredictable response to therapy. In order to improve further the success rate of organ transplantation and long-term survival of patients, it is crucial to develop some means of *objectively* predicting the clinical consequences of CyA dosage adjustments in individual patients. To this end, we have undertaken a preliminary investigation of how CyAC_b influences the occurrence of acute organ rejection in 31 HL transplant patients, in an attempt to define a quantitative model of the relationships involved.

Methods

Subjects

Thirty-one consecutive HL transplant patients (19 male; mean age = 36.6 years (s.d. = 9.8)) who received their allografts at Papworth Hospital between October 1988 and March 1990 were studied. Of these, 12 were cystic fibrosis sufferers and 6 had Eisenmenger's syndrome. Other conditions included primary pulmonary hypertension (4) and fibrosing lung disease (2). Each patient was studied for the first 3 post-operative months, since appropriate adjustment to the patient's immunosuppression is most critical during this early period.

Immunosuppressive regimen

All patients were maintained on triple therapy, i.e. CyA (median starting dose = 2.7 mg kg^{-1} day^{-1}, inter-quartile range = 2.0–4.9, increasing to a median maintenance dose of 10.3 mg kg^{-1} day, inter-quartile range = 7.5–17.0 by day 7); azathioprine (1–2 mg kg^{-1} day^{-1} provided the white blood cell count $> 5 \times 10^9 l^{-1}$); and oral prednisone (0.2 mg kg^{-1} day^{-1} commenced on day 14 to allow time for the tracheal anastomosis to heal (White, 1988)). Antithymocyte globulin (ATG) was also given for the first 3 post-operative days and 1375 mg i.v methylprednisolone was administered on day 1. Cy*AD* was given twice daily, except to patients with cystic fibrosis. These patients usually require relatively high doses to achieve CyAC_b comparable with other HL transplant recipients (Tan *et al.*, 1990), and so received Cy*AD* three times per day. Rejection episodes were treated by augmenting the immunosuppression with a short course of high dose i.v. methylprednisolone (1 g day^{-1}). The oral prednisone dose was then increased, and thereafter reduced by 5 mg daily back to the maintenance dose.

Variables

Data were collected on Cy*AD*, CyAC_b and i.v. methylprednisolone treatment for each patient. Cy*AD* information was available daily, whilst the median frequency per patient for measurement of CyAC_b was once every 2.0 days (range = 1.3–3.9) during the in-patient hospital stay. After discharge, the median interval between successive CyAC_b observations increased to 5.6 days (range = 1.7–12.8), giving an overall median measurement interval per patient of 3.3 days (range = 1.9–6.0) for the entire 3 month follow-up period. All CyA data were recorded specifically for this study to ensure accurate measurements and sampling times. Also, all blood samples were taken at least 2 days after any change in Cy*AD* to ensure stable dosing conditions.

Cyclosporin assay

Morning trough CyAC_b (collected approximately 8 or 12 h post-dose according to dosing regimen) were measured in whole blood by selective radioimmunoassay (Cyclo-Trac® SP Kit, Incstar, Stillwater, MN 55082). This procedure uses a monoclonal antibody which selectively recognises the parent drug but not its metabolites. Intra-assay CV were < 10% across the calibration range for this assay.

Definition of rejection

Rejection is diagnosed by histological findings from transbronchial biopsy (Wallwork, 1989). At Papworth, high dose steroid treatment is also administered on the basis of certain clinical and physiological criteria (such as a severe drop in lung function tests) without biopsy confirmation of the rejection episode. Consequently we have analysed all treated episodes of rejection for the purposes of this study, of which 66% were also documented on biopsy.

Exploratory analysis

The data were summarised descriptively by plotting Cy*AD* and CyAC_b over time for each patient, and indicating the days on which rejection occurred. The total number of rejection episodes per patient were also plotted against his or her median CyAC_b before and after 3 post-operative weeks to investigate the trend between overall level of immunosuppression and rejection intensity.

The correlation coefficient between the natural logarithm of the daily Cy*AD* and CyAC_b was determined for each patient. These coefficients were than transformed (Fisher transformation) and pooled to obtain a population estimate of the correlation between Cy*AD* and CyAC_b (see Trull *et al.* (1990) for details of the statistical methods).

Statistical modelling

A quantitative model of the relationship between CyAC_b and the risk of rejection was developed via multiple logistic regression analysis using the GLIM (Generalized Linear Interactive Modelling) function of the statistical package S-PLUS (S-PLUS© Statistical Sciences, Inc., Oxford, 1990). The dependent variable used represented the presence or absence of a rejection episode in the next 5 days. CyAC_b was summarised by taking the natural logarithm of the mean concentration over the previous 5 days. This averaging was performed to minimise the effects of missing data, and to account for a possible time lag in the CyAC_b-effect relationship similar to the lags found by Trull *et al.* (1990) and Yee *et al.* (1988) for other outcome variables and transplant groups. CyAD was averaged over the 5-day period between the previous 3 and 7 days (since the lag between dose and effect is likely to be greater than between concentration and effect) and the natural logarithm taken. Other explanatory variables included in the model were a factor for week post-transplant, a factor indicating whether rejection had occurred within the previous 5 days, and a variable representing rejection intensity (i.e. average number of rejection episodes per week). The CVs in both CyAD and CyAC_b over the previous 10 days were also included as potential risk factors. However, due to the irregular recording of CyAC_b measurements once patients had been discharged from hospital, 17% of the data had to be excluded because the CV's for CyAC_b were based on < three observations in the previous 10 days. A further 12% of the daily records were also excluded from the model because they contained missing values for one or more of the other variables. Consequently, four patients were eliminated from this part of the analysis because they died within 10 days of their operation (none from rejection) and so did not provide sufficient data. To avoid any serious bias being introduced by these exclusions, a separate parameter corresponding to each of the remaining 27 patients was added to the model. This adjusts for the underlying variability between patients so that the effects of all other variables in the model can be interpreted as additional influences on rejection after accounting for the patient-specific differences. Finally, unlike standard regression analyses, the present model is based on longitudinal data. Hence, a robust estimate of the parameter variances proposed by Liang & Zeger (1986) was used in place of the usual GLIM error estimates, in order to account for correlations among the repeated observations for each subject.

Graphical comparison of rejection and rejection-free periods

A graphical analysis was performed to examine whether the pattern of CyAC_b differed in the period immediately before rejection compared with rejection-free periods. For each patient, rejection-free periods were defined as the central week of every 3-week block during which no rejection occurred. This resulted in between 1 and 10 rejection-free weeks per patient. However, two patients had to be excluded because there were no CyAC_b measurements available during their rejection-free weeks. Rejection periods were taken to be the week preceding each rejection episode, of which there were between 0 and 8 per patient. For each period, the mean CyAC_b and the mean deficit between observed CyAC_b and various threshold levels ranging from 100 $\mu g\ l^{-1}$ to 700 $\mu g\ l^{-1}$ were calculated, and plotted against time post-transplant. (The mean deficit was determined by using the trapezoidal rule to calculate the area under the concentration-time curve below the appropriate threshold, and then dividing by the total number of days (i.e. 7). If all observations were greater than the threshold value, a zero deficit was recorded.)

Results

Exploratory findings

The median CyAC_b per patient before and after 3 post-operative weeks were 10.2 mg kg^{-1} day^{-1} and 12.0 mg kg^{-1} day^{-1} respectively (inter-quartile range = 6.1–17.0 and 8.1–17.0, respectively). The median CyAC_b per patient was 347.5 $\mu g\ l^{-1}$ during the first 3 weeks (inter-quartile range = 221.6–439.8). This was significantly lower than during later weeks (median CyAC_b between 4 weeks and 3 months post-transplant = 445.5 $\mu g\ l^{-1}$ (inter-quartile range = 366.8–766.5); 95.0% CI for the difference in medians = (79.5, 273.5)). Figure 1 shows a scatter plot of total number of rejection episodes against median CyAC_b for each patient during these two periods.

The within-patient population correlation coefficient between CyAD and CyAC_b was 0.44, 95% CI = (0.25, 0.59), and exploratory plots of CyAD and CyAC_b over time for two of the patients studied are shown in Figure 2.

Regression model

The initial logistic regression model included the following explanatory variables: patient effects, log_e (5-

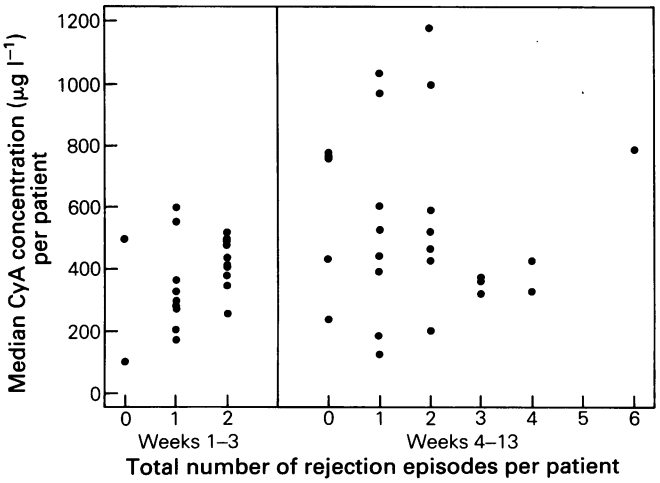

Figure 1 Scatter plot of total number of rejection episodes per patient and median CyAC_b over the first 3 months.

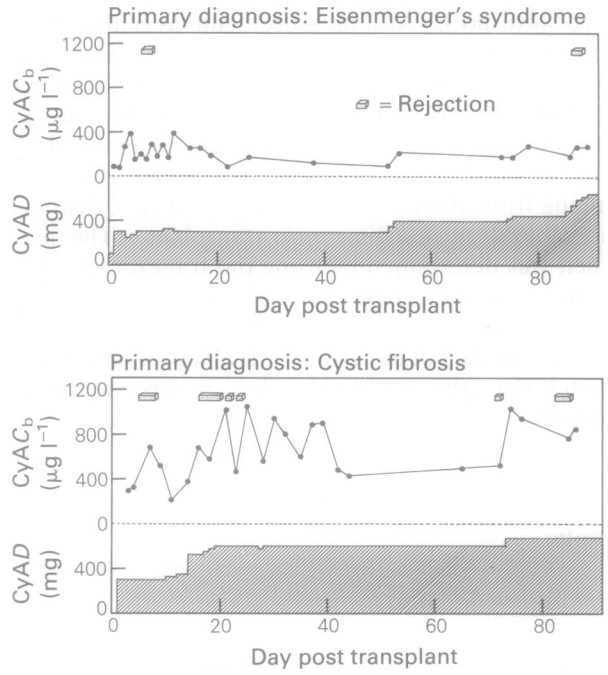

Figure 2 Relationship between CyA*D*, CyAC_b and rejection over time (selected patients).

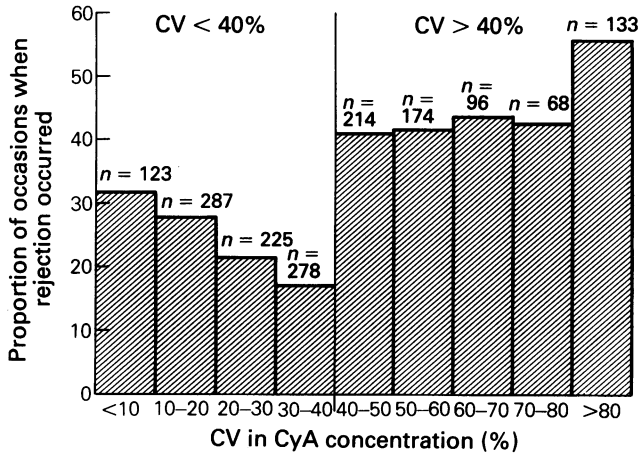

Figure 3 Proportion of days when a rejection episode occurred, plotted according to the corresponding CV in CyAC_b over the previous 10 days.

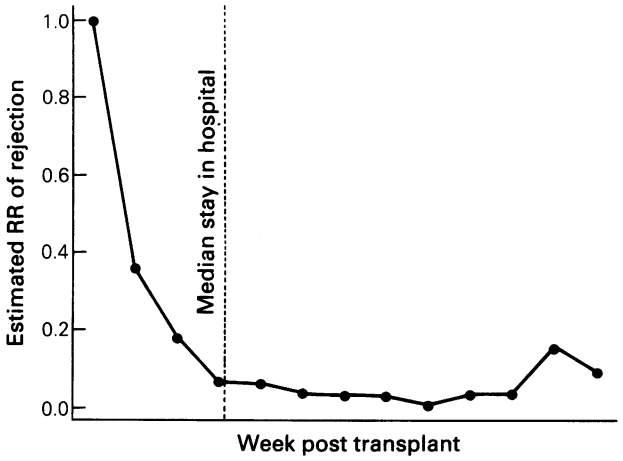

Figure 4 Estimated RR of rejection per week post-transplant (baseline is Week 1).

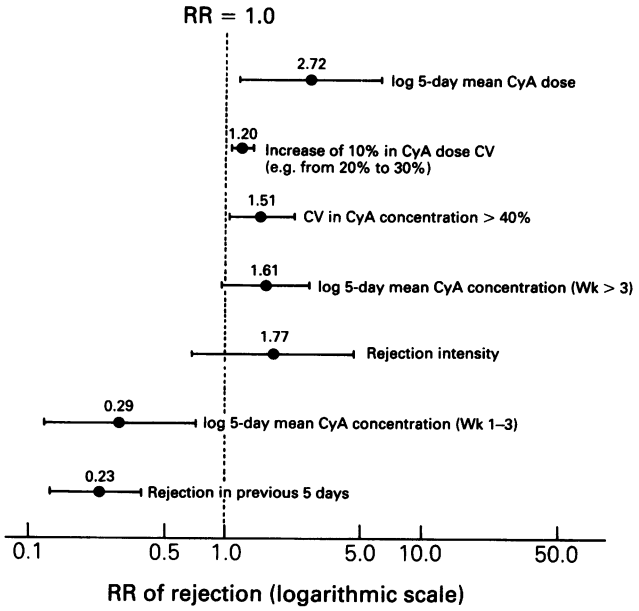

Figure 5 Estimated RR (and 95% CI) of rejection per unit increase (unless otherwise stated) in each explanatory variable in the regression model.

day mean CyAC_b), log_e (5-day mean CyA*D*), week post-transplant, rejection intensity, previous rejection and 10-day CV in CyAC_b. However, on the basis of the histogram in Figure 3, which shows the proportion of 10-day periods preceding a rejection episode categorised by the CV in CyAC_b during that time, we replaced the continuous variable for CV in CyAC_b in the above regression model by a 2-level factor which represented whether the CV in CyAC_b was above or below 40% during the previous 10 days.

The results of the latter regression analysis found that variability in CyAC_b was a significant risk factor for subsequent rejection (RR if the CV in CyAC_b > 40% = 2.2, 95% CI = (1.5, 3.2)), but that no such relationship existed for CyAC_b itself (RR per unit increase in log_e (5-day mean CyAC_b) = 1.2, 95% CI = (0.7, 2.2)). However, the CyAC_b rejection relationship may have been confounded by the sharp decline in risk of rejection seen over the first 3 post-operative weeks (see Figure 4[1]). Therefore, we reduced the original term for week post-transplant to a 2-level effect representing the epochs before and after 3 post-operative weeks, and refitted the logistic regression model to include various interactions between these two epochs and other risk factors. We also included the term for 10-day CV in CyA*D* in this regression model to see if the variability in CyAC_b was simply a reflection of changes in dose.[2]

Figure 5 presents the point estimates and 95% CI for

[1]The apparent subsequent rise in risk seen at week 12 in Figure 4 is probably an artefact due to sparse data at the end of the follow-up period when only the sickest patients were still in hospital and being regularly monitored.

[2]The CV for CyA*D* was left as a continuous variable, rather than being factored like the CV for CyAC_b, since a corresponding histogram to Figure 3 implied a linear relationship between rejection and CV for CyA*D*.

the RR of rejection associated with each of the explanatory variables in the final regression model (apart from individual patient effects). However, these patient effects accounted for 12.8% of the total variation in the data, which corresponds to a significant improvement in model fit ($F_{26,1357} = 5.27$, $P < 0.0001$). The overall fit of the model was further improved by the inclusion of an interaction between epoch and each of the patient effects ($F_{26,1321} = 4.71$, $P < 0.0001$).

Graphical analysis

No graphical distinction was observed between CyAC_b during the week prior to rejection episodes and during rejection-free periods for either mean concentration or mean deficit below a threshold (see Figures 6 and 7 respectively). Plots of mean deficit below other thresholds not illustrated in Figure 7 also showed a similar lack of trend.

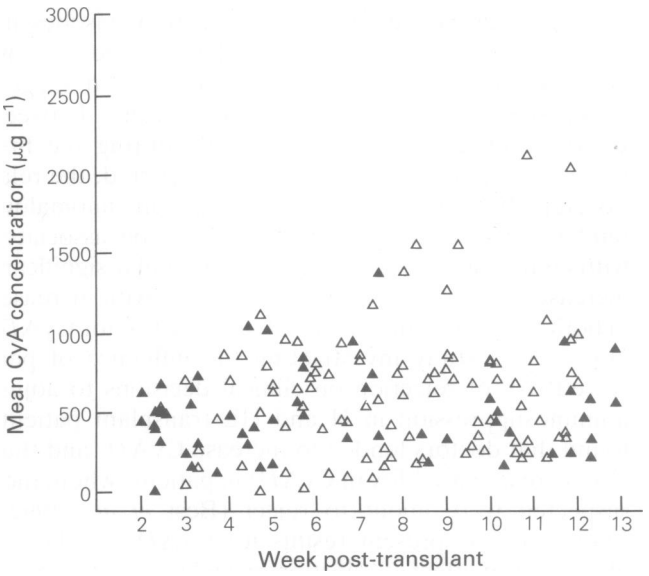

Figure 6 Mean CyAC_b in the week preceding rejection (▲) and during rejection-free periods (△).

Discussion

Although CyA has been used as the major immunosuppressant in HL transplantation for nearly a decade, there remains a paucity of published data concerning the pharmacokinetics or pharmacodynamics of CyA in this particular transplant group. Much of the literature which does exist either ignores or inadequately considers the influence of CyAC_b (for example Carrier *et al.*, 1990; Laufer *et al.*, 1989), or refers to therapeutic ranges for CyAC_b established for non-selective assay methods which detect a combination of parent drug and metabolites. Following the introduction of the selective radioimmunoassay (SRIA), most centres have arbitrarily adjusted the therapeutic range for CyAC_b. The present study therefore represents a preliminary attempt to quantify the relationship between SRIA-measured CyAC_b and the short-term risk of lung rejection. Whilst limitations in design due to missing data necessitate careful interpretation of the findings, the present results provide a useful basis for future larger-scale investigations of this relationship.

Exploratory analysis of the data showed no obvious pattern to the relationship between CyAC_b and rejection. In particular, none of the plots shown in Figures 1,

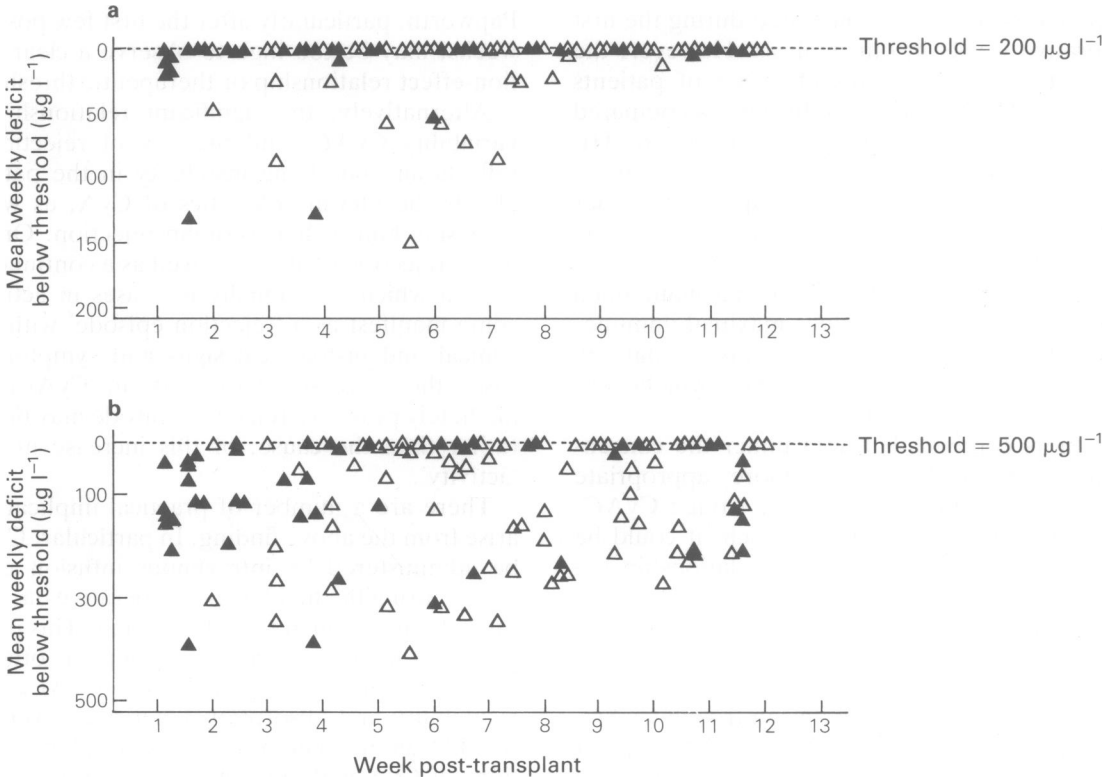

Figure 7 Mean deficit between observed CyAC_b and thresholds of (a) 200 μg l^{-1} and (b) 500 μg l^{-1} in the week preceding rejection (▲) and during rejection-free periods (△).

6 or 7 provide clear evidence that rejection episodes are preceded by lower CyAC_b than rejection-free periods. However, the logistic regression analysis did suggest a negative concentration-effect relationship between CyAC_b and lung rejection, but only during the first three post-operative weeks. After this period, the relationship becomes weaker, showing an anomalous tendency for an increase in CyAC_b to be associated with an increased risk of rejection. A similar significant increase in the risk of rejection was seen with increases in both log_e (5-day mean CyA*D*) and the CV for CyA*D*. However, a study investigating the influence of perceived risk of rejection on clinical decisions to adjust immunosuppression in H and HL transplant patients found that doctors tended to increase CyA*D* (and thus also increase variablity in CyA*D*) in patients whom they suspected were about to reject (Best *et al.*, 1992). Therefore, the present results for CyA*D* may be explained as an effect of rejection brought about by doctor intervention, rather than as a cause. Given the moderate correlation observed between CyA*D* and CyAC_b, this intervention with CyA*D* should also lead to an increase in CyAC_b prior to a suspected rejection episode. The regression model implies that whilst such an increase does reduce the risk of rejection during the initial post-operative period, by the fourth post-operative week, the protective effect of a rise in CyAC_b is entirely lost.

One explanation for the latter result may be attributed to the level of immunosuppression at which these patients are maintained. Sigmoidal log concentration-effect curves are clearly observed for CyA in *in vitro* studies for a range of immunological responses (see, for example, Johansson & Moller, 1990). Therefore, a continuous negative relationship between CyAC_b and the risk of rejection may be a reasonable assumption and is clearly demonstrated during the first 3 post-operative weeks in our analysis. However, the CyAC_b maintained in the present group of patients were significantly higher after the third week compared with the early post-operative period. Furthermore, HL transplant recipients at Papworth are already maintained on relatively high CyAC_b compared to other transplant groups (Trull *et al.*, 1990). Consequently, by the fourth week after transplantation, CyAC_b in many of these HL patients could well be being maintained above the level required to produce maximal immunosuppressive effect. In this case, it is unlikely that any further protective effect of high CyAC_b will be observed in this group of patients.

Some authors contend that area under the concentration-time curve (AUC) is a more appropriate measure for monitoring CyA than are trough CyAC_b values (Kahan & Grevel, 1988). As such, it could be argued that our failure to observe a relationship between CyAC_b and rejection after the third post-operative weeks is partly due to trough CyAC_b *per se* being inadequate for regular monitoring of a patient's level of immunosuppression. However, Trull *et al.* (1990) found a very clear relationship between trough CyAC_b and kidney function in a group of H and HL transplant patients, implying that trough CyAC_b can be used to demonstrate positive CyA concentration-effect relationships.

The logistic regression model also implies that increased variability in both CyA*D* and CyAC_b may be precursors for lung rejection. Reasons for the former effect may relate to doctor intervention as discussed above. However, the effect for CV in CyAC_b is not a simple reflection of these dose changes since both variation in CyAC_b and variation in CyA*D* are identified as significant effects in the same model. This implies that they represent independent short-term risk factors for rejection.

A possible explanation for the influence of variability in CyAC_b on the short-term risk of rejection could relate to the existence of a therapeutic concentration threshold below which rejection is more likley. Given two patients with similar mean CyAC_b, the patient with the higher CV is more likely to be exposed to CyAC_b below this threshold, therefore making him or her more susceptible to rejection. This hypothesis was tested informally tested by plotting the mean deficit between observed CyAC_b and various threshold levels during periods of rejection and no rejection (Figure 7). However, these plots showed virtually no difference between the size of deficits during the different periods, thus providing little support for the above explanation.

In contrast to this latter finding, some authors have observed a significant threshold effect for CyA, although with different outcome measures and transplant groups to the present study. For example, Shaw *et al.* (1990) showed that CyAC_b below 200 $\mu g\ l^{-1}$ were significantly ($P < 0.001$) more likely to be associated with a diagnosis of rejection than of nephrotoxicity in kidney transplant recipients. As illustrated in Figure 7a, there were very few (< 15%) occasions when any of the present HL transplant patients experienced CyAC_b below 200 $\mu g\ l^{-1}$. Such findings again suggest that the CyAC_b maintained in HL transplant recipients at Papworth, particularly after the first few post-operative weeks, may be too high to observe a clear concentration-effect relationship or therapeutic threshold.

Alternatively, the significant relationship between variability CyAC_b and the risk of rejection perhaps reflects an underlying instability in the patient which affects the pharmacokinetics of CyA, as well as predisposing him or her to organ rejection. On the other hand, rejection could be viewed as a continuous phenomenon which occasionally increases in activity to become manifest as a 'rejection episode' with associated clinical and histological signs and symptoms. In this case, the increased variability in CyAC_b seen immediately prior to a rejection episode may be the result, rather than the cause, of this increase in underlying 'activity'.

There are a number of practical implications which arise from the above finding. In particular, CyA*D* could be administered by intravenous infusion rather than orally during the first few post-operative days and when the patient is at high risk of rejection. This procedure is practised in some transplant groups at other centres, and may help to produce stable CyAC_b when bioavailability and absorption kinetics are very variable. In addition, it would be useful to develop new formulations of CyA with improved and more consistent oral pharmacokinetics.

The other variable found to be associated with the

risk of rejection in the present study was the occurrence of a rejection episode in the previous 5 days. This result is probably due to the short-term protective effect of both the i.v. methylprednisolone and the reducing-dose oral steroids used to treat rejection. This finding emphasizes the efficacy of the steroid therapy, and implies that rejection may be influenced by other immunosuppressants besides CyA. In particular, all the patients also received azathioprine as part of their triple therapy, which could influence the risk of rejection. However, this source of variation was largely accounted for by the inclusion of a separate parameter for each patient in the regression model, and so should not confound the findings of this study.

The importance of the patient effects included in the logistic regression model should also be emphasized. As just mentioned, these parameters account for all remaining sources of explainable variation between patients that are not described by the other parameters in the model. Such sources include differences in age, sex, primary diagnosis, tissue type matching, concomitent drug therapy and so on. The significant improvement in model fit due to the inclusion of these patient parameters suggests that many of these characteristics have an important influence on the short-term risk of rejection. Furthermore, the additional improvement in model fit due to the inclusion of a patient by time interaction implies that those characteristics which pre-dispose a patient to early lung rejection are not necessarily the same as those associated with later rejection episodes.

In summary, the present study implies that a negative log linear relationship between $CyAC_b$ and the risk of lung rejection exists, but only during the first few weeks following transplantation. After this early period, the $CyAC_b$ maintained in HL transplant patients may be approaching the top of the concentration-effect curve, so that no further increase in immunosuppressive effect can be expected. Consequently, a population therapeutic threshold for $CyAC_b$ could not be identified in this transplant group. Instead, patient-specific characteristics were found to exert a large influence on rejection, and a more detailed study is required to investigate which of the many sources of between-patient differences are of importance. There also appears to be a link between CyA and rejection which relates to the daily variability in $CyAC_b$. Since this variation cannot be explained entirely by changes in $CyAD$, it seems reasonable to attribute it to underlying pharmacokinetic instability. Whilst further studies with more subjects are needed to confirm the findings of the present analysis, this observation seems reasonable on considering the complex nature of the factors influencing rejection. Patients are regularly diagnosed as experiencing rejection and either infection or nephrotoxicity concomitantly, suggesting that the traditional concept of a therapeutic 'window' for CyA is too simplistic. A more realistic model would be to define a dynamic system in which equilibrium represents the therapeutic goal, and periods of unstable activity correspond to an elevated risk of adverse clincial events such as lung rejection.

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