ABSTRACT

**Background.** Triple immunosuppression with cyclosporine, azathioprine and prednisolone is the most common regimen employed following renal transplantation. No information is available regarding its impact on the results of renal transplantation in India. The present study is an audit of a fixed-dose cyclosporine-based immunosuppressive regimen in an exclusively live-related donor transplant programme, with specific regard to graft and patient outcomes.

**Methods.** Patients transplanted over a 3-year period and receiving cyclosporine-based immunosuppression were studied. The relationship between immunosuppression and graft outcomes [rejection episodes (RE), graft function, graft survival], and patient outcomes (patient survival) was analysed in those receiving triple immunosuppression. Dosage schedules were audited. Cyclosporine trough level monitoring was employed at graft dysfunction episodes, or at dose reduction points.

**Results.** The median follow up was 14 months. Triple drug immunosuppression was used in 191 patients and double drug therapy in 26. The overall one-year patient survival rate was 91% and the corresponding graft survival rate was 90%. An audit of dosing schedules showed that over the first 6 months post-transplant, cumulatively, 20%–50% of patients received azathioprine, and 55%–60% received cyclosporine in doses below the protocol. The immunosuppressive doses (both of cyclosporine and azathioprine) in the first month were significantly related to the RE (p < 0.01) in the first month and the total number of RE in the first 6 months (p < 0.01). The other predictors were younger recipient age and older donor age. The sixth-month serum creatinine level was predicted by the donor age, the level of serum creatinine in the first month and the total number of RE in the first 6 months post-transplant. While no specific predictors of graft loss were identified in this cohort, diabetic nephropathy (p = 0.000) as the native renal disease, and the total number of RE were strongly related to patient mortality. The occurrence of ≥2 RE in the first 6 months was an independent predictor, increasing the risk of death in the first 2 years post-transplant by 2.3 (p = 0.0001, 95% CI: 1.5–3.4).

**Conclusions.** Sub-therapeutic baseline immunosuppression in the early post-transplant period predisposes to acute RE. This has an impact not only on graft function but also forms an important proximate marker of mortality, as seen in this cohort.

Thus, immunosuppressive drug dosage should be optimized and therapeutic drug level monitoring strategies should be preemptive rather than event related, especially in the early post-transplant period. While fixed-dose immunosuppressive drug schedules are widely followed, it is possible to fall short of the target unless a specific effort is made to meet and sustain schedules.


INTRODUCTION

In organ transplantation, optimal therapy implies an immunosuppressive regimen which can protect the graft from rejection with a minimum of drug toxicity and infection. Despite the introduction of many newer immunosuppressive drugs, cyclosporine (CyA) has remained the mainstay of most protocols used in renal transplantation. The most common strategy employed by many transplant units is a fixed-dose schedule, modulated by CyA trough level monitoring, and is intuitively adjusted in response to allograft rejection on the one hand and renal, hepatic or any other toxicity on the other.¹ There is little information available regarding the impact of immunosuppressive protocols on the results of renal transplantation in India.

Cost is an extremely important consideration in the context of lifelong immunosuppression in a developing country. The triple immunosuppressive regimen (or triple therapy, TT) has become the most commonly used schedule post-transplant. It decreases the use of CyA and therefore the cost by over a third as compared to both single drug and double drug therapy (DT), and carries a lower risk of nephrotoxicity² and infection.³

This retrospective study was undertaken in a patient population undergoing exclusively live-related renal transplantation, as an audit of a CyA-based, fixed-dose immunosuppressive schedule in the first 6 months post-transplant during a 3-year period. The objectives were to assess how close the actually delivered treatment was to the protocol, study the relationship of immunosuppressive doses and graft and patient outcomes, and to draw guidelines towards optimizing the immunosuppressive regimen by identifying the pitfalls in its implementation.

PATIENTS AND METHODS

Consecutive patients who had undergone live-related renal transplantation over a 3-year period and treated with CyA-based immunosuppression were included. Those patients undergoing cadaver transplantation or re-transplantation, receiving conventional immunosuppressive therapy [azathioprine (AZA) and prednisolone], and paediatric patients were excluded. Patients continued in-centre outpatient follow up for the first 6 months. Pre-transplant transfusion protocols, antirejection therapy and steroid...
therapy protocols have been described earlier. The immunosuppression protocols are outlined in Table I. Patient outcomes were analysed with respect to mortality risk, and graft outcomes in terms of rejection episodes (RE), levels of serum creatinine and graft survival. Rejection episodes, in turn, were categorized as early if occurring in the first month post-transplant. The total number of RE occurring in the first 6 months was also computed.

Acute rejection was diagnosed when there was an increase in the level of serum creatinine by at least 25% above the baseline, unexplained by urological pathology, vascular compromise, or CyA nephrotoxicity based on CyA trough levels, with a positive response to antirejection therapy, and/or histological examination where indicated. Graft loss implied a return to dialysis and/or the level of serum creatinine at 6 months post-transplant. No significant relationship to RE and graft function. Cox regression was used to relate these variables to graft and patient survival rates.

RESULTS

The total number of patients transplanted during the study period was 276. The exclusion criteria applied to 43 patients and the records of 8 were not available. Thus, the study population comprised 225 patients. However, 8 patients had either primary graft dysfunction, early CyA withdrawal or late CyA introduction, and this left 217 patients for analysis.

The mean (SD) age was 33.8 (11.8; range: 13–65) years, and 184 (82%) patients were men. The cause of renal failure was not known in 69% (159/225) who had presented with severe renal failure and had contracted kidneys; primary glomerulonephritis contributed to 18% (40/225), and non-insulin dependent diabetes to 6% (14/225). The mean (SD) donor age was 42.8 (11.4; range 20–70) years. Donors were either parents or siblings in 86% (193/225), and non-insulin dependent diabetes 6% (155/225) being haplo-matched. The mean (SD) follow up was 16.6 (8.6) months (range 2–45). Ten grafts were lost and 28 patients died during the follow up period, 25 of them with a functioning graft. The overall patient survival was 91% at 1 year and 86% at 2 years; and the corresponding graft survival rates were 90% and 81%, respectively.

Immunosuppression

Of the 217 patients analysed, TT was used in 191 (88%) patients, and DT in 26 (12%). The latter were those patients who received DT before it was phased out. Median CyA doses were lower than protocol with both DT and TT during the first 4 months. Protocol doses and median doses became equal by the fifth and sixth months post-transplant. In the case of AZA, about 50% of patients received doses lower than the protocol during the first month, 30% during the second month, and about 20% thereafter. Target doses of 1.5 mg/kg/day were reached in 20% of patients by the fifth and sixth months post-transplant.

Relationship between immunosuppressive doses and graft outcomes

Rejection episodes. Table II compares patients receiving TT with and without RE. Patients with two or more rejection episodes were significantly younger with older donors, and had a higher level of serum creatinine at 6 months post-transplant. No significant difference was appreciable within this group in the baseline immunosuppressive doses between groups with and without RE. However, all patients receiving DT, and therefore, higher baseline doses of CyA, were in the low RE group (p=0.01). The relation-
TABLE II. Comparison of patients on triple drug immunosuppression with ≤1 and ≥2 rejection episodes in the first 6 months post-transplant

<table>
<thead>
<tr>
<th>Factor</th>
<th>≤1 in 6 months</th>
<th>≥2 in 6 months</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejection episodes per patient</td>
<td>0.27 (0.4)</td>
<td>2.6 (0.75)</td>
<td>0.000</td>
</tr>
<tr>
<td>Recipient age (years)</td>
<td>34.3 (11.2)</td>
<td>29.6 (8.2)</td>
<td>0.025</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>42.0 (11.7)</td>
<td>48.6 (9.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Pre-transplant transfusion</td>
<td>3.15 (3)</td>
<td>3.3 (3.3)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Serum creatinine (mg/dl)

- First month: 1.5 (0.8) vs. 1.8 (0.9), p=0.1
- Sixth month: 1.5 (1.3) vs. 2.3 (0.85), p=0.000

Cyclosporine dose (mg/kg/day)

- First month: 7.8 (0.8) vs. 7.8 (0.4), p=0.8
- Cumulative (month.mg/kg): 25.4 (3.5) vs. 25.2 (3.3), p=0.8
- Use of diltiazem: 100.0 (60%) vs. 21 (84%), p=0.001

Azathioprine dose (mg/kg/d)*

- First month: 1.1 (0.3) vs. 1.0 (0.3), p=0.25
- Cumulative (month.mg/kg): 5.7 (1.2) vs. 5.7 (1.2), p=0.9

Graft survival

- 1 year*: 99% vs. 83%
- 2 years: 92% vs. 63%

Total grafts lost

- 6.0 (4%) vs. 3.0 (12%), p=0.12

Patient survival

- 1 year: 93% vs. 76%
- 2 years: 85% vs. 57%

Total deaths

- 17.0 (10%) vs. 8.0 (27%), p=0.01

Figures in parentheses are standard deviations unless specified

* censored for patient death

Fig 1. Cumulative frequency chart showing the proportion of patients receiving immunosuppressive drug dosages according to triple drug protocol at each follow up month

The relationship between rejection episodes per patient and actual dose of immunosuppressive drugs

<table>
<thead>
<tr>
<th>Drug dosages</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Rejection episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First</td>
<td>Total in 6 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>month</td>
<td></td>
</tr>
<tr>
<td>Triple drug therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine dose &lt;8 mg/kg</td>
<td>135</td>
<td>7.5 (0.6)</td>
<td>0.26 (0.5)</td>
</tr>
<tr>
<td>≥8 mg/kg</td>
<td>56</td>
<td>8.5 (0.7)</td>
<td>0.16 (0.3)</td>
</tr>
<tr>
<td>Azathioprine dose &lt;1 mg/kg</td>
<td>127</td>
<td>0.9 (0.1)</td>
<td>0.27 (0.5)*</td>
</tr>
<tr>
<td>≥1 mg/kg</td>
<td>64</td>
<td>1.3 (0.3)</td>
<td>0.12 (0.3)*</td>
</tr>
</tbody>
</table>

Double drug therapy

Cyclosporine dose <12 mg/kg 21 11.4 (0.5) 0.3 (0.5)* 0.33 (0.5)*
≥12 mg/kg 5 12.6 (0.5) 0* 0*

p=0.01

TABLE IV. Multiple regression analysis showing variables related to total rejection episodes in the first 6 months and the 6-month serum creatinine

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Dependent variable</th>
<th>Total rejection episodes</th>
<th>Serum creatinine at 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient</td>
<td>SE</td>
<td>p value</td>
</tr>
<tr>
<td>First-month azathioprine dose</td>
<td>-0.68</td>
<td>0.26</td>
<td>0.011</td>
</tr>
<tr>
<td>Donor age</td>
<td>0.02</td>
<td>0.006</td>
<td>0.001</td>
</tr>
<tr>
<td>Diltiazem use</td>
<td>0.42</td>
<td>0.14</td>
<td>0.002</td>
</tr>
<tr>
<td>First-month serum creatinine</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total rejection episodes in 6 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Constant</td>
<td>-0.22</td>
<td>0.4</td>
<td>0.58</td>
</tr>
<tr>
<td>R^2</td>
<td>0.12</td>
<td>0.47</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Cox regression analysis

Dependent variable: Patient follow up in months

Event: Patient death

Variable | p value | OR | 95% CI
Total rejection episodes in 6 months | 0.0001 | 2.2 | 1.5–3.4
Diabetes mellitus | 0.000 | 17.3 | 4.3–63

in the first 6 months. Those patients who received CyA doses ≥8 mg/kg/day with AZA doses ≥1 mg/kg/day, in the first month post-transplant had a rejection frequency comparable to patients receiving DT. Patients receiving DT appeared to have a lower number of mean RE [0.3 (0.4) RE per patient in 6 months, vs. 0.6 (0.9) RE per patient in 6 months, p=0.005], and lower mean levels of serum creatinine at 1 year [1.4 (0.5) vs. 1.8 (1.3) mg/dl; p=0.01]. Table IV shows the variables related to the total number of RE and the 6-month level of serum creatinine on a multiple regression analysis. The first-month AZA dose shows a significant relationship to RE; RE, in turn, is a significant predictor of graft function. Other predictors include donor age and the use of diltiazem to augment CyA levels (see below).

Graft outcomes. Of the 10 grafts lost, 9 received TT. On a Cox regression analysis, no specific variables could be identified as predictive of graft loss in this cohort.

Patient outcomes. Of 28 deaths, 25 occurred in the TT group. Patients who died were significantly older, more likely to be diabetic, showed lower mean doses of baseline immunosuppression and had significantly more RE (Table V). Cox regression
higher in those with an increased RE (Tables II and IV).

FIG 2. Actuarial patient survival in patients with ≤1 and ≥2 rejection episodes in the first 6 months post-transplant

Table V. Comparison of characteristics of survivors and non-survivors during the follow up period

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-survivors (n=28)</th>
<th>Survivors (n=189)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient age (years)</td>
<td>40.6 (12.4)</td>
<td>33 (10.6)</td>
<td>0.004</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>46.2 (11)</td>
<td>42.4 (11.5)</td>
<td>0.09</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6 (21%)</td>
<td>8 (4%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl) at 6 months</td>
<td>1.65 (0.9)</td>
<td>1.55 (0.6)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Analysis (Table IV) showed that the number of RE in the first 6 months independently related to patient death (Fig. 2). The occurrence of ≥2 RE in the first 6 months yielded a relative risk of death of 2.3 (p=0.0001; 95% CI: 1.5–3.4). On a univariate analysis, patient age >40 years yielded a 4-fold risk (p=0.004, 95% CI: 1.6–9.8), and the presence of diabetes increased the risk by 6.4 (p=0.007, 95% CI: 2.1–20.3), but both these variables were seen to be co-variates [mean (SD) age of diabetics 49.2 (5.9) v. non-diabetics 32.9 (10.6); p=0.000, Spearman’s r=0.34] and the latter formed the more important predictor on the Cox regression equation. The relationship of RE to mortality was independent of the effect of graft loss on survival.

CyA trough level monitoring and the use of diltiazem

The mean (SD) number of CyA assay levels per patient done over the first 6 months was 2 (1.5). A total of 132 patients (61%) received diltiazem and in 126 this was based on trough levels (95%). The addition of diltiazem therefore appeared to serve as a marker of low trough levels. The use of diltiazem was significantly higher in those with an increased RE (Tables II and IV).

![Graph showing Actuarial patient survival in patients with ≤1 and ≥2 rejection episodes in the first 6 months post-transplant](image)

Although CyA levels were available only in a subset of patients, the relationship to RE is shown in Table VI. Patients with RE had lower levels in the second and third post-transplant months but paradoxically higher CyA levels at 6 months.

**DISCUSSION**

In many developing countries without a firmly established cadaver transplant programme, live donors form the main organ pool. Literature has little information on the immunosuppressive protocols used in such settings, or its relationship to the results of renal transplantation. Most studies on immunosuppressive protocols have been on cadaver transplantation. There is far less literature on immunosuppressive drug trials in live-related donor transplantation, although there is a perception that a lower intensity of immunosuppression may be sufficient in the context of live-related transplantation. The rationale underlying post-transplant immunosuppression is similar in both situations—the use of relatively high drug doses during the early post-transplant period, when the risk of acute rejection is highest, tapering to a maintenance dose that is aimed at preventing rejection, while avoiding harmful drug toxicity. Fixed-dose drug protocols have been structured based on this principle. The occurrence of acute RE is a risk factor for chronic rejection and graft loss. Low CyA levels predispose to early allograft rejection. Thus, the importance of early immunosuppression has been repeatedly emphasized in transplantation literature.

The importance of AZA has probably been underestimated as exemplified by a recent editorial which stated that ‘...the benefit of AZA in maintenance regimens is not certain although it is probably not harmful’. In the pre-CyA era, the Australia and New Zealand Dialysis and Transplant Registry data showed the benefit of higher AZA doses on graft survival. After the advent of CyA, multi-centre studies did not document a positive effect of the inclusion of AZA in TT v. DT comparisons. This was thought to be a result of either inadequate dosing or the use of relatively narrow target therapeutic dose ranges. A more recent study that used higher AZA doses with TT, based on 6-thioguanine nucleotide level monitoring showed a 20% decrease in the cumulative incidence of the first RE. The relationship of lower initial AZA doses to RE in the present study underscores the contribution of AZA to immunosuppression efficacy in the context of TT in live-related transplantation. The distribution of doses employed in this cohort was probably of a threshold level of dosage needed to protect against RE, especially with the lower CyA doses as used in TT. The relative importance of AZA dosing is a pertinent observation for a developing country, where the cost of lifelong immunosuppression is the main consideration in post-transplant...
management. It is possible that the full potential of the use of AZA in post-transplant immunosuppression in our setting has not been completely exploited.

Within the TT group, CyA doses did not show a relationship to RE, probably due to the relatively narrow range of the dosage. However, the difference of the first-month doses between the DT and TT groups was sufficient to show the importance of high first-month CyA doses as used in DT to prevent or minimize RE, although this observation needs to be tempered with the awareness that a comparison of DT and TT was not an objective of this study, and patients receiving DT formed only a small number. Earlier reports have also suggested lower RE with DT.11,12

The adequacy of early immunosuppression is perhaps of paradoxically greater importance in live-related renal transplantation. Since ischaemic injury and primary graft dysfunction are uncommon consequences, the serum creatinine in the first month post-transplant is a direct reflection of the immunological insult to the graft and the intrinsic function of the donor kidney. Indeed this study, as several other reports, showed that the creatinine levels in the first month and the donor age19,24-27 were important predictors of the creatinine levels in the sixth month.26,27

While area-under-the-curve measurements may be more germane to the use of Neoral®, this remains outside the reach of routine post-transplant care in a developing country. The strategy of an event-related monitoring of CyA trough levels, in the setting of a fixed-dose drug regimen, may clarify an aetiology for, but does not diminish the predisposition to, RE. Pre-emptive monitoring against RE especially in the early post-transplant period would be more important than secondary prevention approaches. The findings here of lower CyA trough levels in the second and third months, higher trough levels in the sixth month and more common diltiazem use in patients with more RE illustrate the clinician’s response to such an approach.

Auditing the actually delivered doses for immunosuppression showed a deviation from the protocol, especially with AZA doses in the early post-transplant period. This illustrates one of the problems of a fixed-dose regimen in that it may be adhered to rigidly without ongoing adjustments to rapidly changing parameters such as body weight in the post-transplant period. The most common reasons seen in this population for differences between protocol and actual doses were a tendency to round-off doses to lower than the target rather than higher, failure to increase doses to keep up with post-transplant weight gain, and the occurrence of leucopenia.

The one-year graft survival for living donor transplantation was recently reported to be 94%.28 Kostakis et al. showed graft survivals of 87% at 3 years and patient survivals of 96% even in transplants from elderly donors.30 Graft survivals in our study are comparable, but patient survival is inferior, related to the extremely high proportion of deaths that occurred with a functioning graft (89%; 25/28). This forms a more common cause of graft loss in elderly recipients or those who receive HLA-identical kidneys.31 However, neither of these were prominent epidemiological features in this study population. Thus, live-related transplants in a developing country may be another category, where death with a functioning graft forms an important cause of graft loss. Patient survival was significantly compromised in those patients with 2 or more RE in 6 months. Graft failure formed a relatively less important cause of death and the impact of RE on patient survival was independent of its relationship to graft failure. Indeed, no direct relationship was demonstrable between RE and graft failure in this cohort. While the relationship between RE and graft survival has been extensively discussed in the literature,18,19 few studies have emphasized the independent impact that RE and therapy of rejection have on patient mortality.32-34 Jamil et al. showed that the risk of infections and lymphomas, and hence the mortality, increased with the number of antirejection therapies.32 In a tropical country where infections are prevalent, the intensity of cumulative immunosuppression is an important determinant of the risk of acquiring infections. While an analysis of the causes of mortality is beyond the scope of the present report, it is clear that the occurrence of RE forms a proximate risk factor for death.

The prevention rather than treatment of RE is, therefore, a critical goal in post-transplant immunosuppression in live-related renal transplantation in a developing country. Any fixed-dose strategy should provide adequately high immunosuppression in the early phase, and should be complemented by adequate dosage and pre-emptive drug level monitoring with, if necessary, adjunctive drugs to augment levels.

REFERENCES

FORTY-SECOND ANNUAL CONFERENCE OF THE INDIAN SOCIETY OF GASTROENTEROLOGY

The forty-second Annual Conference of the Indian Society of Gastroenterology and sister societies will be organized by the Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow between 23 and 29 November 2001. The programme includes two pre-conference symposia (on gastrointestinal motility and scientific communication, respectively on November 23), a one-day postgraduate course or CME (November 24), and an endoscopy workshop (November 28–29). For details, please write to Dr SR Naik, Department of Gastroenterology, SGPGI, Lucknow 226014 (Phone 522–440700 or 440800, Extn 2400; Fax 522–440078 or 522–440017) or visit the conference web site http://www.sgpgi.ac.in/confg2001.html.

NINTH ASIAN CONFERENCE ON DIARRHEAL DISEASES AND NUTRITION

The conference, being organized by the All India Institute of Medical Sciences and the Indian Council of Medical Research, will be held at Vigyan Bhawan, New Delhi from 28 to 30 September 2001.

The focus of the scientific programme will be on pathogen-specific burden of gastrointestinal disease, microbial genetics, enteric vaccines and non-vaccine preventive interventions, emerging gastrointestinal infections, Helicobacter pylori, environment and gastrointestinal disease, molecular epidemiology, gut inflammation, mucosal immunity, enteric nervous system, gastrointestinal pathophysiology and drug development, probiotics and prebiotics, oral rehydration, celiac disease and non-celiac chronic diarrhoeas in developing countries, exclusive breastfeeding, complementary feeding, low birth-weight and morbidity, micronutrient deficiency, diarrhoeal disease and feeding in HIV, protein–energy malnutrition and immuno-nutrition.

Last date for receipt of abstracts is 15 May 2001.

For further information visit our web site www.ascodd2001.delhi.as, or write to Professor M. K. Bhan, Conference Secretary, ASCODD2001, Room No. 3054, Academic Block, Department of Paediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, Tel. No: 6963822/6594792, 6561123, 6560110, Ext 3290; Fax: 6862663, e-mail: ascodd2001@dellhi.as