

## Effect of hepatitis C infection on tacrolimus doses and blood levels in liver transplantation recipients

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Accepted for publication 30 March 2005

### SUMMARY

**Background:** In our cohort of patients with hepatitis-C virus, which is the most common indication for liver transplantation, we have noted higher relative blood levels of tacrolimus compared to patients without hepatitis-C virus.

**Aim:** To verify this observation and determine its clinical significance, we performed a comparison of doses and blood levels of tacrolimus in hepatitis-C virus and non-hepatitis-C virus liver transplantation recipients.

**Methods:** Tacrolimus dose and trough level, as well as mean alanine aminotransferase, for all patients transplanted at our center with a deceased donor between 1/1995 and 12/1999 with hepatitis-C virus were recorded at monthly intervals during the first 24 months following transplantation and compared to patients without hepatitis-C virus.

**Results:** The tacrolimus levels for hepatitis-C virus and non-hepatitis-C virus patients were not significantly different at any of the monthly intervals, except month 9. In addition, the overall mean tacrolimus levels for

hepatitis-C virus and non-hepatitis-C virus patients were not significantly different ( $P = \text{ns}$ ). However, the mean tacrolimus dose (mg/kg) was significantly higher for hepatitis-C virus patients at 12, 15, 18, 21 and 24 months,  $P < 0.01$ . The total mean tacrolimus dose in hepatitis-C virus patients was lower during year one by 39% ( $P = 0.018$ ) and by 73% ( $P = 0.001$ ) during year two. The total difference in cost of tacrolimus (for year one and two) administered to hepatitis-C virus patients was \$4920,  $P = 0.03$ . The serum alanine aminotransferase was significantly higher in hepatitis-C virus patients at each monthly interval except month 1,  $P \leq 0.01$ .

**Conclusions:** Liver transplant recipients with hepatitis-C virus require significantly lower oral doses of tacrolimus to achieve the same blood levels compared to non-hepatitis-C virus patients. This difference may result in a significant reduction in the cost of tacrolimus in hepatitis-C virus patients. The most likely explanation for these findings is decreased hepatic clearance of tacrolimus caused by mild hepatic injury from recurrent hepatitis-C virus.

### INTRODUCTION

Hepatitis C virus (HCV) is the most common indication for liver transplantation. Approximately half of liver transplantation recipients in the United States are

infected with HCV at the time of transplantation. Following transplantation virtually all patients develop recurrent HCV infection, but the severity of hepatitis is variable.<sup>1</sup> Approximately 20% of recipients progress to cirrhosis within 5 years of transplantation and another 20% develop recurrent disease. In the remaining 60% of patients, mild chronic hepatitis occurs without rapid progression to cirrhosis. Recurrent chronic hepatitis and cirrhosis negatively impacts the long-term survival of

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HCV-infected recipients; the long-term mortality of recipients with HCV is 23% higher than patients without HCV.<sup>2</sup>

Hepatitis C may also impair the metabolic function of the liver. As a result, clearance of drugs metabolized by the liver, such as tacrolimus and ciclosporin, may be reduced in patients with recurrent HCV. In fact, several studies have found higher relative levels of calcineurin inhibitors in renal transplantation and hepatic transplantation recipients infected with HCV. Tuncer *et al.* reported ciclosporin trough levels in 18 renal transplant recipients who converted HCV antibody following transplantation.<sup>3</sup> After conversion of HCV antibody, ciclosporin trough levels were 59% higher in HCV antibody-positive than in HCV antibody-negative patients (220.8 ng/dL vs. 137.9 ng/dL, respectively,  $P < 0.05$ ). Before HCV antibody conversion, ciclosporin doses were similar, but after conversion the HCV antibody-positive patients required 37% less ciclosporin (2.05 mg/kg/day vs. 2.80 mg/kg/day,  $P < 0.05$ ). Manzanares *et al.* reported tacrolimus trough levels in four renal transplant recipients who were HCV antibody-positive.<sup>4</sup> The mean dose was the same for HCV and non-HCV patients (0.26 mg/kg/day,  $P = \text{N.S.}$ ), but initial levels were 47% higher in HCV (21.2 ng/mL) than non-HCV patients (14.4 ng/mL,  $P < 0.05$ ). Four years after transplantation, the trough levels of tacrolimus were not different, but the tacrolimus oral dose/kg in HCV patients (0.03 mg/kg) was half that of non-HCV patients (0.06 mg/kg,  $P < 0.05$ ). Latorre *et al.* reported doses and levels of ciclosporin ( $n = 12$ ) and tacrolimus ( $n = 14$ ) in 26 renal transplant patients with HCV infection.<sup>5</sup> At 1 year, the levels of tacrolimus and ciclosporin were similar, but the doses required in HCV patients were significantly lower by one-third compared to patients without HCV. In a large study which randomized renal transplant recipients to ciclosporin or tacrolimus at transplantation, the doses and levels were reported in 30 HCV patients.<sup>6</sup> There was a trend towards administration of lower dose of tacrolimus and ciclosporin in the HCV patients. In the tacrolimus group, HCV-positive patients ( $n = 15$ ) received a mean dose of 0.103 mg/kg compared with 0.122 mg/kg in HCV-negative patients ( $P = \text{N.S.}$ ). In the ciclosporin patients, HCV patients ( $n = 15$ ) received a mean dose of 3.06 mg/kg, compared with 3.65 mg/kg in HCV-negative patients ( $P = \text{N.S.}$ ).

The data on tacrolimus levels and doses in HCV-infected liver transplantation recipients are very

limited. In a small case series of liver transplantation recipients, the dose of tacrolimus in seven HCV patients was compared with 13 non-HCV patients.<sup>7</sup> Up to the third week after transplantation, dosing requirements were the same in both groups. Thereafter, tacrolimus requirements decreased sharply in HCV patients, stabilizing at about 20% of the value in non-HCV recipients. At our centre, we have noted that HCV-infected liver transplantation recipients require lower relative doses of tacrolimus than patients without HCV. Therefore, we performed this study to confirm this clinical observation, describe its clinical significance and impact on cost of tacrolimus maintenance therapy.

## METHODS

Liver transplantation recipients transplanted at our centre (the University of Colorado Health Sciences Center in Denver) between January 1995 and December 1999 were included in this retrospective analysis which was approved by the Colorado Multiple Institutional Review Board. The immunosuppression protocol used at our centre has evolved over time. From January 1995 until December 1998 patients received alternating assignment to tacrolimus or ciclosporin and 14-day prednisone taper with mycophenolate mofetil. From January 1999 until December 1999 the same immunosuppressive regimen was used without mycophenolate mofetil. From January 2000 until current, patients received alternate assignment to tacrolimus or ciclosporin and 3-day corticosteroid taper with sirolimus (6 mg loading dose followed by 2 mg/day). However, all patients receiving sirolimus were excluded from analysis, because of a possible drug interaction with tacrolimus because these drugs are cleared through the same pathway. In addition, all living donor liver transplantation recipients were excluded from analysis as we have previously shown that these patients may have impaired clearance of tacrolimus.<sup>8</sup> Patients were also excluded if they were administered medications known to alter the hepatic metabolism of tacrolimus, e.g. fluconazole, phenytoin, etc. Tacrolimus was administered orally or via nasogastric tube at 0.05 mg/kg/day b.d. in the immediate post-operative period. Oral doses of tacrolimus were adjusted based on the following target levels: 10–15 ng/mL for month 1, 8–12 ng/mL for month 2 and 5–8 ng/mL, thereafter.<sup>9</sup> The following data were recorded for each patient at monthly intervals

1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21 and 24: total daily dose of tacrolimus, trough level of tacrolimus and serum alanine aminotransferase (ALT) on the last day of the monthly interval. Body weight was also recorded for each patient. The cost of tacrolimus was based on a retail price of \$3.09/mg (<http://www.drugstore.com>) Statistical differences between each patient group were performed using chi-square or Student's *t*-test where appropriate.

## RESULTS

Patient demographics are depicted in Table 1. There was a trend (not statistically significant) towards a greater proportion of male HCV patients. Therefore, there was also a trend (not statistically significant) towards higher body weight in HCV patients. There was a significantly higher proportion of white non-HCV patients compared with white HCV patients ( $P = 0.03$ ). There were no African-Americans in either group. Figure 1 depicts the tacrolimus trough levels at each

Table 1. Patient demographics

	HCV	Non-HCV	<i>P</i> -value
N	31	35	
Age	49.6	47.1	N.S.
Male (%)	68	46	0.07
Weight (kg)	75.0	68.7	0.08
Race/ethnicity			
White	22 (71%)	32 (91%)	0.03
Hispanic	7 (23%)	3 (9%)	N.S.
Asian	2 (6%)	0	N.S.
African-American	0	0	N.S.

HCV, hepatitis C virus; non-HCV, no hepatitis C virus.

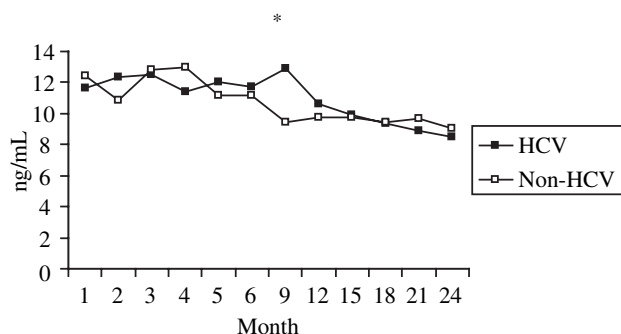


Figure 1. Tacrolimus level: HCV vs. non-HCV. HCV, hepatitis C virus; non-HCV, no hepatitis C virus; m, month post-transplantation ( $*P < 0.05$ ).

monthly interval. At each of the monthly intervals except month 9, the tacrolimus levels were not significantly different. The overall mean tacrolimus level for HCV patients (11.1 ng/mL) was not significantly different for non-HCV patients (10.7 ng/mL,  $P = N.S.$ ). Figure 2 shows the tacrolimus total daily dose (expressed as mg tacrolimus/kg body weight) for HCV and non-HCV patients at each monthly interval. The daily dose of tacrolimus administered to HCV patients was significantly lower in HCV (vs. non-HCV patients) at months 12, 15, 18, 21 and 24. In Figure 3 the level/dose ratio (expressed as ng/mL per mg/kg) for tacrolimus is shown for HCV and non-HCV patients. The level/dose ratio for HCV patients was significantly higher by nearly twofold at months 5–21. The mean serum ALT values are depicted in Figure 4. At each monthly interval after month 1, the mean ALT was significantly higher in HCV patients compared with non-HCV patients by approximately two- to threefold ( $P \leq 0.01$ ).

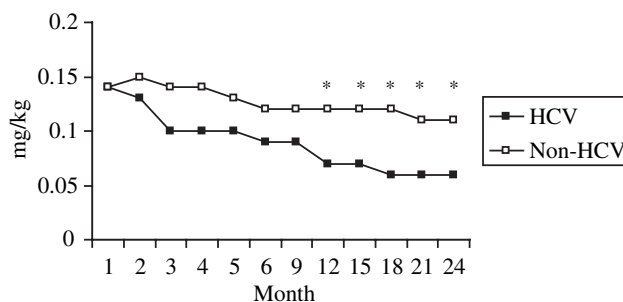


Figure 2. Tacrolimus dose: HCV vs. non-HCV. HCV, hepatitis C virus; non-HCV, no hepatitis C virus; m, month post-transplantation ( $*P < 0.05$ ; 'dose' is expressed as mg tacrolimus/kg body weight).

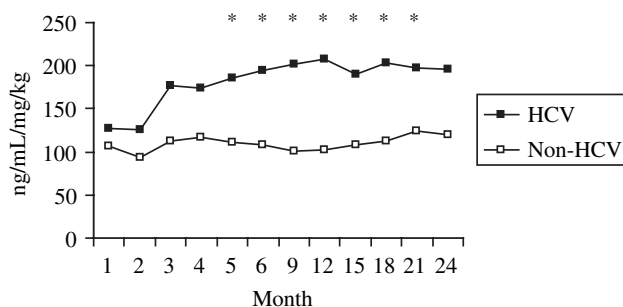


Figure 3. Tacrolimus level/dose: HCV vs. non-HCV. HCV, hepatitis C virus; non-HCV, no hepatitis C virus; m, month post-transplantation ( $*P < 0.05$ ; 'level/dose' is expressed as ng/mL per mg tacrolimus/kg body weight).

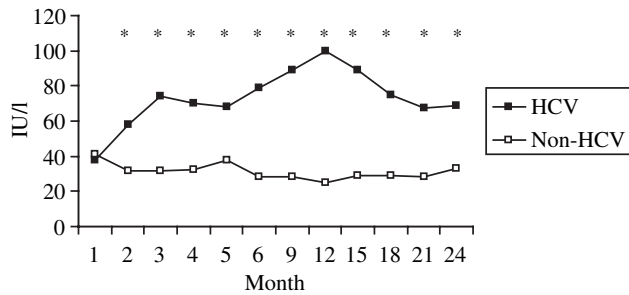


Figure 4. ALT: HCV vs. non-HCV. ALT, alanine aminotransferase; HCV, hepatitis C virus; non-HCV, no hepatitis C virus; m, month post-transplantation (\* $P < 0.05$ ); IU/L, international units/L.

Table 2. Mean tacrolimus dose and cost

	HCV	Non-HCV	Difference (%)	<i>P</i> -value
Dose (mg/kg)				
Year 1	0.101	0.140	39	0.018
Year 2	0.064	0.111	73	0.001
Total	0.085	0.128	51	0.004
Cost (\$)				
Year 1	5784	7778	34	0.03
Year 2	4519	7446	65	0.003
Total	10 304	15 224	48	0.03

HCV, hepatitis C virus; non-HCV, no hepatitis C virus.

Data based on \$3.09 per mg tacrolimus.

Tacrolimus cost is calculated by mean dose (mg) of tacrolimus multiplied by \$3.09 which is the retail cost of tacrolimus (<http://www.drugstore.com>).

Table 2 shows the total yearly dose (expressed at mg tacrolimus/kg body weight) during year 1 and year 2 for HCV and non-HCV patients. The total yearly dose for tacrolimus was significantly lower by 39% in HCV patients at year 1, by 73% for year 2 ( $P = 0.001$ ). The total difference in tacrolimus dose for year 1 and year 2 combined was 51% ( $P = 0.004$ ). As depicted in Table 2, the total difference in cost of tacrolimus in HCV patients for years 1 and 2 combined was 48% or \$4920 lower ( $P = 0.03$ ).

## DISCUSSION

The HCV patients require significantly lower doses of tacrolimus to achieve the same blood levels as non-HCV patients. The most likely explanation for this finding is an alteration in the pharmacokinetics of tacrolimus caused by HCV (see Figure 5). Following oral administration, tacrolimus is absorbed into enterocytes and

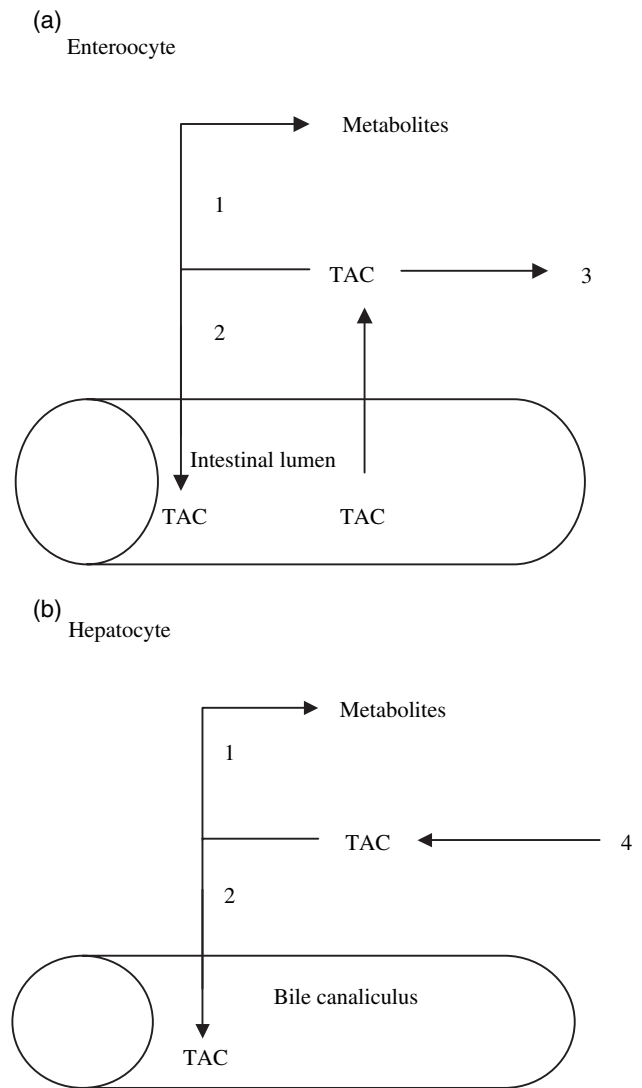


Figure 5. Pharmacokinetic of tacrolimus. TAC, tacrolimus; 1, metabolism by cytochrome P450<sub>3A4</sub>; 2, transport out of cell via P-glycoprotein; 3, tacrolimus travels to hepatocyte via the portal vein; 4, tacrolimus arrives at the hepatocyte via the portal vein.

metabolized by intestinal cytochrome P450. However, the catalytic activity of intestinal cytochrome P450 is relatively small, compared with hepatic metabolism. The biotransformation rate of tacrolimus in enterocytes (54 pmol/min/mg) is only about half of the hepatic activity (96 pmol/min/mg).<sup>10</sup> As a result, alterations in intestinal metabolism of tacrolimus are unlikely to be as clinically relevant as changes in the hepatic cytochrome P450 system.<sup>11</sup> The concentration of tacrolimus in the enterocyte is also regulated by P-glycoproteins, a family of transporter proteins encoded by the multidrug-resistant (MDR) gene and expressed in a variety of

tissue including enterocytes and hepatocytes.<sup>12, 13</sup> P-glycoproteins reduce the intracellular concentration of specific drugs, including calcineurin inhibitors, via transport into the extracellular space. In the enterocyte, a fraction of the absorbed tacrolimus is transported from the intracellular space back into the intestinal lumen. The remaining tacrolimus travels to the liver via the portal vein where it is metabolized by the microsomal cytochrome P450<sub>3A4</sub> system. In addition, tacrolimus may be excreted from the hepatocyte into the bile canaliculus by P-glycoproteins expressed on the hepatocyte membrane.

The higher systemic levels of tacrolimus may be due to one (or more) of the following mechanisms: increased intestinal absorption, decreased intestinal cytochrome P450 metabolism, decreased intestinal excretion by P-glycoprotein, decreased volume of distribution, decreased hepatic cytochrome P450 activity and/or decreased hepatic P-glycoprotein excretion. The intestinal absorption of tacrolimus could be enhanced by HCV. However, to our knowledge, there is no evidence of HCV altering tacrolimus absorption. HCV could inhibit the activity of intestinal cytochrome P450 and intestinal P-glycoprotein (see below). However, because most of the inflammation caused by HCV occurs in the liver, the effect of HCV on intestinal P-glycoprotein activity seems unlikely. The volume of distribution of tacrolimus could be lower in HCV patients. However, in our cohort, the HCV patients were 6.3 kg heavier than non-HCV recipients. Therefore, the volume of distribution in the HCV patients is likely to be larger than non-HCV. The heavier body weight in the HCV patients is due to the greater proportion of men that are diagnosed with HCV compared with non-HCV patients.

Therefore, the most likely explanation for higher systemic levels of tacrolimus in the HCV patients is impaired hepatic clearance of tacrolimus which could be caused by reduced activity of hepatic cytochrome P450<sub>3A4</sub> and/or impaired function of hepatic P-glycoprotein. In the HCV patients, the reduction in tacrolimus dose and rise in the level/dose ratio occurred at about the same time as the increase in ALT, i.e. month 3. This is indirect evidence that as hepatic inflammation from recurrent HCV increases, the clearance of tacrolimus decreases. Other studies have found reduced clearance of calcineurin inhibitors in patients with liver dysfunction. Bekersky *et al.* studied pharmacokinetics of tacrolimus in six patients with cirrhosis compared with normal controls.<sup>14</sup> The half-life of tacrolimus was nearly twofold

longer in patients with cirrhosis (60.6 h) compared with controls (34.2 h,  $P = 0.06$ ). Similar results are reported with ciclosporin. Ptachcinski *et al.* administered intravenous ciclosporin to eight patients with liver failure and found that the clearance was approximately half that of healthy volunteers.<sup>15</sup> Abu-Elmagd *et al.* measured tacrolimus levels in liver transplantation recipients with graft dysfunction (total serum bilirubin  $>2$  mg/dL) immediately after transplant compared to patients with no graft dysfunction (total serum bilirubin  $<2$  mg/dL).<sup>16</sup> The patients with liver dysfunction received a slightly lower mean tacrolimus dose (0.15 mg/kg) compared with normals (0.2 mg/kg). However, tacrolimus levels in patients with liver dysfunction were approximately twofold higher. Another study measured tacrolimus oral dose and levels in liver transplantation recipients with significantly impaired hepatic function (mean ALT = 89, mean serum bilirubin = 11.8 mg/dL) and recipients with normal liver function. The tacrolimus level/dose ratio in patients with impaired function was over threefold higher (2.6) compared to patients with normal function (0.82).<sup>17</sup>

The HCV infection itself can impair metabolic activity of the liver, even in the absence of significant hepatic inflammation or fibrosis. Herold *et al.* performed quantitative liver function tests in 367 HCV non-transplantation patients including the aminopyrine breath test which measures demethylation of aminopyrine by the cytochrome P450 microsomal enzyme system.<sup>18</sup> Patients were categorized based on the histological stage of fibrosis. Compared with control patients, the rate of aminopyrine demethylation was lower in hepatitis C patients, including patients with minimal fibrosis (stage 1). Demethylation was significantly lower in patients with minimal inflammation (grade 1) compared with normal control patients. These results demonstrate that hepatitis C infection itself may also impair cytochrome P450 activity independent of overall hepatic dysfunction. The mechanism by which HCV infection reduces cytochrome P450 activity is unknown. However, one possibility is the negative effect of inflammatory cytokines, produced in response to HCV, on cytochrome P450. Numerous studies have found that HCV infection is associated with increased hepatic and systemic levels of many inflammatory cytokines.<sup>19</sup> Specifically, the levels of tumour necrosis factor (TNF)- $\alpha$ ,<sup>20</sup> transforming growth factor (TGF)- $\beta$ ,<sup>21</sup> nitric oxide, interleukin (IL)-2, -4, -6 and -10<sup>22, 23</sup> are increased in patients with HCV. Some of these cytokines reduce the activity of cytochrome P450

including: TNF- $\alpha$ ,<sup>24</sup> nitric oxide,<sup>25</sup> and IL-6<sup>24, 26</sup> and TGF- $\beta$ .<sup>27–29</sup>

As described above, calcineurin inhibitor clearance is also regulated by hepatic P-glycoproteins which transport tacrolimus from the hepatocyte into the bile canaliculus. Many of the cytokines which are elevated in hepatitis C also decrease the expression and activity of P-glycoprotein.<sup>30, 31</sup> Inflammatory cytokines decrease the activity of P-glycoprotein which could, in turn, increase the systemic levels of calcineurin inhibitors. IL-6 decreases expression of P-glycoprotein and the MDR gene which encodes the protein.<sup>32</sup> TNF- $\alpha$  suppresses *mdr1b* gene (which encodes P-glycoprotein) expression.<sup>33–35</sup>

There are two clinical implications of the findings in this report. First, compared to patients without HCV, liver transplant recipients infected with HCV require significantly lower doses of tacrolimus to achieve the same blood level. As most experienced clinicians have discovered, tacrolimus levels increase in patients with hepatic dysfunction. This is most evident in patients with rapidly progressive hepatic failure. In this setting, the oral dose of tacrolimus must be substantially reduced to maintain appropriate levels. However, in most patients with recurrent hepatitis C, the progression of hepatic dysfunction is more indolent, often evolving over many months. The results of our analysis suggest that clinicians may need to monitor tacrolimus levels over time more carefully in patients with mild recurrent HCV. The differences in tacrolimus dose and level/dose ratio were minimal in the first few months after transplantation (see Figures 2 and 3). However, during the second year (months 12–24) the tacrolimus dose was reduced by twofold in the HCV patients and the corresponding level/dose ratio was twofold higher. Secondly, the reduction the overall dose of tacrolimus in the HCV-infected patients was associated with a substantial reduction in the cost of tacrolimus. The difference in cost between HCV and non-HCV patients was most apparent during the second year after transplantation. Between months 12 and 24, the cost of tacrolimus in non-HCV patients was 65% higher than HCV patients. Whether this difference extends beyond 24 months is unknown. However, we anticipate that as long as recurrent HCV is present in the transplanted graft, the dose requirements for the patient will remain substantially lower. In fact, we have noted that eradication of HCV by interferon therapy after liver transplantation is associated with a reduction in tacrolimus levels by approximately 30%.<sup>36</sup> In some

patients, the drop in tacrolimus levels following HCV eradication may contribute to the development of acute cellular rejection.

A substantial number of patients at our centre have received ciclosporin-based immunosuppression. With ciclosporin, we have also observed similar results as tacrolimus; there was a significant reduction (40%) in the total dose (expressed at mg ciclosporin/kg body weight) in HCV-infected recipients compared with non-HCV (data not shown). However, the HCV-infected patients receiving ciclosporin are substantially heavier (mean difference in body weight, 17 lbs) than HCV-infected patients on tacrolimus. Therefore, the cost-benefits of the reduction in dose/kg are, in part, offset by the higher body weights in the ciclosporin patients (who require a higher total dose because of increased body weight).

There are several problems with our study. First, the data in this report were collected retrospectively which is not as reliable as a prospective study. In addition, the tacrolimus dose for each time interval was estimated based on the dose administered on the last day of each time interval. However, we doubt if this has a significant impact on the results, because the greatest differences in tacrolimus doses occurred more than 3 months after transplantation when changes in oral doses were relatively small and infrequent. The cost of tacrolimus was calculated based on the prescribed dose, not actual payment of for the drug. Finally, because of the negative impact of immunosuppression on the progression of post-transplantation hepatitis, we consciously attempted to administer less immunosuppression to hepatitis C patients. This practice could potentially contribute to the finding that hepatitis C patients receive less tacrolimus. However, if this were the primary cause for our findings, then one would expect lower tacrolimus levels and no difference in the level/dose ratio in HCV patients, neither of which was found as shown in Figures 1 and 3, respectively.

In conclusion, our results demonstrate that liver transplantation recipients with HCV achieve relatively higher blood levels of tacrolimus compared to patients without hepatitis C. As a result, HCV patients receive 51% less oral tacrolimus during the first 2 years after transplantation which translates into a cost savings of nearly \$5000. The most likely explanation for this observation is impaired clearance of tacrolimus due either to reduced activity of cytochrome P450 and/or P-glycoprotein caused by recurrent hepatitis.

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