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A Canadian national retrospective chart review comparing the long term effect of cyclosporine *vs.* tacrolimus on clinical outcomes in patients with post-liver transplantation hepatitis C virus infection

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ABSTRACT

The transition from regular use of cyclosporine to the newer calcineurin-inhibitors, such as tacrolimus, has been suggested as a contributing factor to the "era effect" of worsening outcomes of post-transplant HCV recurrence. This retrospective medical chart review of 458 patients was undertaken to evaluate the role of immunosuppressant choice (cyclosporine *vs*. tacrolimus) in determining virologic response and clinical outcomes of post-liver transplant HCV infection recurrence. Our results showed that patients undergoing interferon-based treatment taking cyclosporine have significantly better odds (OR: 2.59, P = 0.043) of presenting a sustained viral response (66.7%) compared to tacrolimus (52.8%). This did not result in a significant effect on post-liver transplantation clinical events including HCV-related deaths, graft loss, fibrosing cholestatic hepatitis, hepatocellular carcinoma or graft rejection. Other variables, which showed a significant relationship with the achievement of sustained viral response included donor age (OR 0.96, P = 0.001) and HCV genotype 1 infection (OR 0.05, P < 0.001). The observed significant increase in the odds of acute/hyperacute (OR 6.49, P = 0.001) and chronic rejection (OR 10.45, P < 0.001) in the cyclosporine to tacrolimus switch group, accompanied by an increase in the odds of HCV-related death (OR 2.30, P < 0.047) compared to tacrolimus merits further study. A significant increase (P < 0.044) in new-onset diabetes mellitus with tacrolimus (28.3%) compared to cyclosporine (18.7%) was also observed. Pre-transplant diabetes mellitus was associated with a significantly increased likelihood of graft fibrosis (HR 1.95, P = 0.003).

Key words. Liver transplant. Retrospective study. Tacrolimus.

INTRODUCTION

End-stage liver disease due to chronic hepatitis C virus infection (HCV) is the leading indication for liver transplantation (LT).[1,2] HCV recurrence in the post-LT period invariably occurs and this is associated with a worsening patient and/or graft outcome.[3,4] Post-transplant HCV has an accelerated natural history with early post-transplant graft cirrhosis occurring often resulting in graft loss. Moreover, a subgroup of liver transplant recipients with HCV recurrence will develop an aggressive cholestatic variant (i.e. fibrosing cholestatic hepatitis) that is associated with accelerated graft loss and patient death.[5] As a result, the long-term survival of patients who undergo LT due to HCV is lower than that of patients who undergo LT for other indications.

A previous retrospective analysis[6] revealed an increase in the progression of HCV-related fibrosis following LT over the past 10 years compared to the previous decade referred to as an "era effect". Although the reasons for this worsening outcome in recent years are not fully understood, the replacement of cyclosporine (CsA) by newer calcineurin-inhibitors, such as tacrolimus (Tac), has been suggested as a contributing factor. Indeed, cyclosporine (CsA) has

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been shown to have an anti-viral effect on HCV *in vitro*[7] and some clinical studies have suggested that CsA may also be associated with a better response to post-transplant HCV antiviral therapy.[8] However, recent prospective studies[9,10] have failed to demonstrate a clinical benefit of CsA over Tac in terms of post-LT HCV infection but the impact of these trials have been limited by the short duration of follow up. In the absence of prospective clinical trials of adequate size and duration, other approaches are required to study whether CsA provides a benefit for recipients with HCV infection.

In Canada, CsA was the primary immunosuppressive agent used in liver transplantation until all Canadian LT centers participated in the Tac steroid reduction clinical trial[11] that began in 1996. Since then, Tac has gradually become the dominant calcineurin-inhibitor used in LT in Canada. However, there are many patients who were involved in the original Tac *vs*. CsA study who are still on long-term follow-up and others who were not enrolled but received either CsA or TAC after transplantation for HCV. These populations of patients, who received a LT between 3 and 13 years ago, represented a unique cohort in which any long-term differences between CsA and TAC could be assessed by means of an appropriately designed retrospective study.

The present national medical chart review study, comparing the long term effects of CsA *vs*. Tac, was therefore undertaken in order to evaluate the role of immunosuppressant choice in determining the virologic response and clinical outcome of post-transplant HCV infections.

In addition, some trials have shown a higher incidence of new onset diabetes mellitus (DM) with Tac treatment, as compared to CsA.[12,13] This may represent a significant clinical disadvantage in the use of tacrolimus since there is evidence that, in a non-transplantation setting, the presence of DM affects HCV progression negatively by promoting hepatic fibrosis.[14] It was therefore also of interest to assess the magnitude of this problem in the liver post-transplant setting.

MATERIAL AND METHODS

This retrospective chart review was conducted at the liver transplant programs of the University of Alberta, the University of British Columbia, the Université de Montreal, the University of Toronto and Western University (also known as the University of Western Ontario). The study protocol was reviewed and approved by the Independent Ethics Committee of each of the 5 participating Canadian LT centers.

Patient population

Patients who received a LT due to hepatitis C cirrhosis and were treated with either CsA or Tac as their initial post-transplant immunosuppressive therapy were eligible for the study. Only HCV RNA positive LT recipients who received a LT 3 to 13 years prior to study initiation (January 1, 1996 to December 31, 2006) were included in order to allow for an adequate duration of follow-up. Patients with prior transplantation of any other organ and those undergoing multi-organ transplant at time of liver transplantation were excluded from participation.

Drug therapy

CsA was the investigational therapy and Tac represented the reference therapy in this retrospective study. Regimens of each agent were as prescribed by the treating centre. Antiviral therapy for the treatment of post-transplant HCV was specifically noted.

Assessments and endpoints

Data required to meet the study objectives was extracted from the original patient charts, and entered on standardized electronic case report forms using validated software. All collected data were de-identified and patients were assigned a unique study subject identifier.

Study data included pre-transplant patient demographics and relevant medical history, including DM (defined as use of oral hypoglycemic agents and/or insulin prior to transplant), HCV and liver disease characteristics leading to transplant as well as donor information. Post-transplant information included immunosuppressant and anti-HCV treatments administered.

The primary outcome of the study was the proportion of patients with a sustained viral response (SVR) to the post-transplant HCV antiviral therapy. The SVR was defined as undetectable HCV-RNA serum levels at the 24-week follow-up period after cessation of the antiviral therapy.

Secondary endpoints included the duration of virologic response (VR), defined as the time to detectable HCV RNA in patients that had achieved undetectable HCV RNA at any time after the end of antiviral treatment, post-end of treatment HCV viral load (detectable *vs*. not detectable) and other post-

transplant outcomes including acute and chronic rejection episodes, graft loss, hepatocellular carcinoma, fibrosing cholestatic hepatitis and patient survival. Wherever post-LT biopsies were available, the histological evolution over time of graft fibrosis and graft cirrhosis was also assessed using the Metavir grading system. Finally, the incidence of post-transplantation new onset DM was determined, defined as persistent hyperglycemia requiring long-term (> 3 months) treatment with oral hypoglycemic agents and/or insulin not required prior to LT. The objectives of this study did not include the collection of adverse events.

Statistical analysis

Four treatment groups were defined: CsA, Tac, conversion from CsA to Tac and conversion from Tac to CsA. The immunosuppressant use during the period extending from the LT date to 6 months following the end of anti-viral therapy was used to determine each patient's assignment to one of the four groups. The primary statistical analyses focused on the comparison between CsA and TAC.

Results are presented as means ± standard deviation (SD). No imputation or replacement of missing values was performed as all analyses were conducted on observed cases.

Overall between-group differences were assessed for statistical significance with One Way Analysis of Variance (ANOVA) for continuous variables, and the Pearson Chi-Square statistic was used for categorical variables. Pairwise comparisons between CsA *vs.* Tac, and each switch group *vs.* Tac were performed using Fisher's exact Probability Test.

Multivariate logistic regression analysis was performed on outcome measures using the covariates that were identified as clinically relevant to the outcome. These were used to produce adjusted estimates and comparisons of the between-group difference with respect to the primary and secondary study outcomes. The covariates included: race, HCV genotype, anti-viral treatment duration, immunosuppressant treatment duration, presence of DM prior to liver transplant, and transplant donor age. The Odds Ratio (OR) and 95% confidence intervals (CI) were used as estimates of the treatment effect and to perform pairwise comparisons between CsA *vs.* Tac and each of the switch groups *vs.* Tac.

The duration of virologic response and time to relapse was described using the Kaplan Meier time to event function and the log rank statistic was used to assess the unadjusted between-group differences. Cox's proportional hazards models using the above mentioned covariates were used to produce adjusted estimates of the between-group difference with respect to duration of virologic response. The time (from LT procedure) to achieve other secondary outcomes such as acute and chronic rejection was similarly assessed.

Subgroup analyses were conducted in patients who were treated with interferon and/or ribavirin antiviral therapy (yes *vs.* no) and by HCV genotype (1 *vs.* other); these subgroup analyses were assessed for the primary endpoint (SVR) of the study.

A two-tailed P value of ≤ 0.05 was used as the significance level in all analyses.

All statistical analyses were performed using SPSS Version 12 (SPSS Inc., Chicago, IL) and SAS Version 9.2 (SAS Institute INC., Cary, NC).

RESULTS

This retrospective analysis was based on the review of 490 charts of patients who underwent LT secondary to HCV cirrhosis between January 1st, 1996 and December 31st, 2006 in 5 Canadian liver transplant centers. Thirty-two (32) charts were excluded from the final analysis for the following reasons, which are not mutually exclusive: the first immunosuppressive drug was other than CsA or Tac (19 cases), occurrence of multiple immunosuppressive drug switches (7 cases), patients received LT after December 31st, 2006 cut-off date (10 cases). The final study cohort consisted of 458 patients.

The medical and demographic characteristics of the four treatment groups at time of LT are presented in table 1. A significant overall between-group difference was observed in the mean age of patients (P = 0.043). The oldest mean age was reported in the CsA group (53 ± 8.2 yr), while patients in the CsA to Tac switch group were the youngest (50 ± 9.5 yr). Transplant procedure information including donor age and gender, and time since referral for LT as well as presence of pre-transplant DM, revealed no significant differences between the four treatment groups.

The breakdown of HCV genotypes detected in the cohort indicated that the majority of patients (69.5%) were infected with HCV genotype 1. However an overall significant between-group difference was observed (P = 0.008) with HCV genotype 1 identified in 78.8% of patients in the Tac to CsA conversion group, in 72.5% of patients receiving Tac, 64.9% of patients in the CsA group, and 59.5% of patients converted from CsA to Tac. None of the study patients were HIV positive at time of transplant.

There was a significant between-group difference ($P < 0.001$) in overall duration of post-LT immunosuppressive drug treatment with the longest treatment occurring in the CsA group (6.76 ± 4.29 yr) and the shortest occurring in the Tac group (4.98 ± 3.41 yr). Similarly, duration of antiviral therapy showed a significant overall between-group difference ($P = 0.014$) with the Tac to CsA conversion group having the longest treatment duration (2.14 ± 0.99 yr) and the Tac group presenting the shortest duration of treatment (mean of 1.21 ± 0.92 yr). Post-transplant antiviral therapy consisting of interferon/peginterferon and/or ribavirin, was administered in a significantly higher proportion of patients in the Tac

Table 1. General characteristics of study groups.

Patient study group	CsA	Tac	CsA to Tac	Tac to CsA	Between-group P-value
N	137	230	56	35	
Age (yr)	53 ± 8.2	52 ± 6.5	50 ± 9.5	51 ± 6.3	0.043*
Gender (F:M)	40:97	55:175	18:37	4:31	0.092
Race (C/O/AA/NI/other) (n)	98:26:2:2:9	191:25:1:2:10	46:3:1:1:5	30:0:1:1:3	0.074
Body weight (kg)	76.8 ± 16.84	79.9 ± 17.64	77.4 ± 18.99	84.8 ± 15.09	0.073
HCV genotype, n (%)					
1	63 (64.9)	137 (72.5)	25 (59.5)	26 (78.8)	0.008 *
2	5 (5.2)	21 (11.1)	7 (16.7)	1 (3.0)	
3	23 (23.7)	30 (15.9)	6 (14.3)	4 (12.1)	
4	6 (6.2)	1 (0.5)	4 (9.5)	2 1(6.1)	
Time to HCV diagnosis (yr)	5.94 ± 4.23	6.56 ± 5.47	5.56 ± 3.75	6.09 ± 3.77	0.509
Time since transplant referral (days)	384 ± 342.6	312 ± 311.5	323 ± 309.9	381± 350.9	0.179
Mean donor age (yr)	46 ± 17.3	42 ± 15.8	42 ± 17.9	46 ± 17.1	0.127
Donor gender (F:M)	61:74	97:132	24:33	11:24	0.540
Diabetes mellitus present before LT, n	33 (24.6)	54 (23.9)	9 (16.7)	7 (20.0)	0.636
Immunosuppressive treatment duration (yr)	6.76 ± 4.29	4.98 ± 3.41	6.45 ± 4.66	6.30 ± 3.26	< 0.001*
Antiviral treatment duration (yr)	1.66 ± 0.93	1.21 ± 0.92	1.85 ± 0.81	2.14 ± 0.99	0.014*
Post-LT antiviral treatment, n (%)	54 (39.4)	138 (60.0)	32 (57.1)	31 (88.6)	< 0.001*

F: female. M: male. C: Caucasian. O: Oriental. AA: American African/Black. NI: Native Indian. HCV: hepatitis C virus. LT: liver transplantation. CsA: cyclosporine. Tac: tacrolimus. CsA to Tac: cyclosporine to tacrolimus conversion group. Tac to CsA: tacrolimus to cyclosporine conversion group. Between-group P values are based on one-way ANOVA for continuous variables and on Pearson chi-square test for proportions. *Statistically significant results. Note: percentages are based on the number of patients with available data in each patient group.

Table 2. Incidence of sustained viral response at 24 weeks after the end of antiviral treatment.

	Statistic	CsA (n = 137)	TAC (n = 230)	CsA to TAC (n = 56)	TAC to CSA (n = 35)	Between-group P-value
SVR	n (%)	38 (66.70)	75 (52.8)	19 (63.30)	12 (40)	0.073

Multiple logistic regression analysis

Variable	Odds Ratio	95% CI Lower	95% CI Upper	P-value
Group CsA *vs.* TAC	2.59	1.02	6.50	0.043*
Donor age	0.96	0.93	0.98	0.001*
HCV genotype (type 1 *vs.* all others)	0.05	0.02	0.15	< 0.001*

SVR: sustained viral response rate. CsA: cyclosporine. Tac tacrolimus. CsA to Tac: cyclosporine to tacrolimus conversion group. Tac to CsA: tacrolimus to cyclosporine conversion group. Note: percentages are based on the number of patients with available data in each patient group. Between-group P-value is based on Pearson Chi Square Test. *Statistically significant figures. Patients without SVR include "relapsed", "non-responding" and "breakthrough" patients. Multiple logistic regression was computed using the backward conditional method. Clinically relevant covariates included in the model were: age, gender, race HCV genotype, antiviral treatment duration, diabetes mellitus prior to transplant and transplant donor age. The variable group (CsA *vs.* Tac) was forced into the model.

group (60.0%) compared to the CsA group (39.4%) (pairwise comparison: P < 0.001).

Virologic response

Table 2 summarizes the number and proportion of patients in each group with a sustained virologic response (SVR) 24 weeks after the end of antiviral treatment. The CsA group achieved the highest rate (66.7%) of SVR relative to the other treatment groups including the Tac group (52.8%). The overall between-group difference approached but did not reach statistical significance (P = 0.073). However, when adjusting for covariates by means of multiple regression analysis, a statistically significant increase in the odds of achieving SVR in the CsA group as compared to the Tac group was observed (OR 2.59, 95% CI 1.02-6.50, P = 0.043) (Table 2). It was also found that increased transplant donor age (OR: 0.96; CI: 0.93-0.98, P = 0.001) and the presence of HCV genotype 1 (OR: 0.05; CI: 0.02-0.15, P = 0.001) were associated with significantly lower odds of achieving SVR. Most of the patients in whom SVR was not achieved were in the category of non-responders (CsA: 19.3% *vs.* Tac: 32.4%), followed by relapsed patients (CsA: 12.3% *vs.* Tac: 12.0%). There was no significant difference between the four treatment groups in this respect (Table 3).

Achievement of SVR by HCV genotype for the CsA and Tac groups revealed no significant between-group

Table 3. Virologic outcome.

Virologic outcome	CsA, n (%)	Tac, n (%)	CsA to Tac, n (%)	Tac to CsA, n (%)	Between-group P-value
Sustained response	38 (66.7)	75 (52.8)	19 (63.3)	12 (40.0)	
Non-responders	11 (19.3)	46 (32.4)	7 (23.3)	15 (50.0)	0.234
Relapsed	7 (12.3)	17 (12.0)	4 (13.3)	2 (6.7)	
Breakthrough	1 (1.7)	4 (2.8)	0 (0.0)	1 (3.3)	

SVR: sustained viral response rate. CsA: cyclosporine. Tac: tacrolimus. CsA to Tac: cyclosporine to tacrolimus conversion group. Tac to CsA: tacrolimus to cyclosporine conversion group. Note: percentages are based on the number of patients with available data in each patient group. Between-group P-value is based on Pearson Chi Square Test.

Table 4. Incidence of SVR at 24 weeks after the end of antiviral treatment by HCV genotype.

HCV genotype	Patient group	SVR n (%)	Between-group P value (all groups)	Between-group (CsA *vs.* Tac) P value
Type 1	CsA	16 (51.6)		
	Tac	32 (35.2)	0.12	0.137
	CsA to Tac	8 (57.1)		
	Tac to CsA	6 (27.3)		
Type 2	CsA	3 (75)		
	Tac	13 (86.7)	0.644	0.53
	CsA to Tac	6 (100)		
	Tac to CsA	1 (100)		
Type 3	CsA	8 (88.9)		
	Tac	18 (90)	0.541	> 0.999
	CsA to Tac	2 (66.7)		
	Tac to CsA	2 (66.7)		
Type 4	CsA	3 (100)		
	Tac	1 (100)	0.446	NC
	CsA to Tac	1 (50)		
	Tac to CsA	1 (50)		

CsA: cyclosporine. Tac: tacrolimus. CsA to Tac: cyclosporine to tacrolimus conversion group. Tac to CsA: tacrolimus to cyclosporine conversion group. NC: not calculable. Between-group P value based upon Pearson chi-square. Note: percentages are based on the number of patients with available data in each patient group. Genotype 1 includes all subtypes reported in the patient chart (1a, 1b, 1c).

differences within each HCV genotype (Table 4). Although a trend towards CsA group achieving a greater SVR than the Tac group (51.6% *vs.* 35.2%, respectively) for genotype 1 virus was observed, this did not reach statistical significance (P = 0.137). A comparison between the rate of SVR achievement in HCV genotype 1 *vs.* all other genotypes (2, 3, and 4 combined) showed that patients with HCV genotype 1 have a significantly lower rate (62 patients, 39.2%) of SVR achievement compared to patients with other HCV genotypes (59 patients, 85.5%) (P < 0.001).

The overall achievement of SVR regardless of genotype, according to use of interferon and/or ribavirin is shown in table 5. Although patients in the CsA group had a higher SVR rate relative to the Tac group irrespective of using or not using antiviral therapy (65.1% *vs.* 52.0%, respectively), these differences did not reach statistical significance.

Post-transplant biopsy results

The histological progression to advanced fibrosis (Metavir score 2 or 3) or cirrhosis by treatment group is presented in table 6. The highest proportion of patients with advanced post-transplant graft fibrosis was observed in the Tac to CsA conversion group (54.3%), while the lowest proportion was observed in the Tac group (34.3%). The overall between-group difference was significant (P = 0.003). The logistic regression analysis showed that the CsA group and the CsA to TAC conversion group had significantly increased odds for advanced fibrosis by factors of 4.6 (OR 4.60, 95% CI 1.92-11.02, P = 0.001) and 2.9 (OR 2.91, 95% CI 1.09-7.76, P = 0.032), respectively, relative to the Tac group. Also, increased duration of immunosuppressant treatment (OR 0.65, 95% CI 0.57-0.73, P < 0.001) or antiviral treatment (OR 1.36, 95% CI 1.11-1.66, P = 0.003) were significantly associated with this outcome.

With respect to graft cirrhosis, a higher proportion (28.6%) of patients reaching this endpoint was reported in the Tac to CsA conversion group while the lowest proportion (12.1%) was reported in the Tac group although the between-group difference was not statistically significant (P = 0.084). Logistic regression analysis indicated that the CsA group had increased odds for cirrhosis relative to the

Table 5. Incidence of sustained viral response at 24 weeks after the end of antiviral treatment: use vs. no use of interferon/peginterferon and/or ribavirin therapy.

Interferon and/ or ribavirin	Patient group	SVR		Between-group P value (all groups)	Between-group P value (CsA *vs.* Tac)
		Yes, n (%)	No, n (%)		
Yes	CsA	28 (65.1)	15 (34.9)		
	Tac	66 (52.0)	61 (48.0)	0.133	0.157
	CsA to Tac	17 (63.0)	10 (37.0)		
	Tac to CsA	12 (40.0)	18 (60.0)		
No	CsA	10* (71.4)	4* (28.6)		
	Tac	9* (60.0)	6** (40.0)	0.810	0.700
	CsA to Tac	2 (66.7)	1 (33.3)		
	Tac to CsA	0 (0.0)	0 (0.0)		

SVR: sustained viral response rate. CsA: cyclosporine. Tac: tacrolimus. CsA to Tac: cyclosporine to tacrolimus conversion group. Tac to CsA: tacrolimus to cyclosporine conversion group. Note: percentages are based on the number of patients with available data in each patient group. Between-group P-value is based on Pearson Chi Square Test. *One patient was on antiviral medication other than interferon and/or ribavirin. **Two patients were on antiviral medication other than interferon and/or ribavirin.

Table 6. Post-transplant biopsy histological results.

Fibrosis progression	CsA n (%)	Tac n (%)	CsA to Tac n (%)	Tac to CsA n (%)	Between treatment group P-value
Advanced graft fibrosis	68 (53.1)	71 (34.3)	20 (37.7)	19 (54.3)	0.003*
Graft cirrhosis	19 (14.8)	25 (12.1)	9 (17.0)	10 (28.6)	0.084

CsA: cyclosporine. Tac tacrolimus. CsA to Tac: cyclosporine to tacrolimus conversion group. Tac to CsA: tacrolimus to cyclosporine conversion group. Note: advanced fibrosis was defined as Metavir score 2 or 3. Percentages are based on the number of patients with available data in each patient group. Between-group P-value is based on Pearson Chi Square Test. *Statistically significant figures.

TAC group (OR 2.56, 95% CI 1.00-6.52, P = 0.049). The duration of immunosuppressant treatment (OR 0.69, 95% CI 0.60-0.79, P < 0.001) or antiviral treatment (OR 1.42, 95% CI 1.15-1.74, P = 0.001) were also found to be significantly associated with progression to cirrhosis.

No statistically significant difference in either time to advanced fibrosis or time to cirrhosis was found between the four treatment groups, as assessed using Kaplan Meier survival estimates. However, the Cox proportional hazard ratio (HR) showed that increased transplant donor age was significantly associated with faster progression to advanced fibrosis (HR 1.04, 95% CI 1.02-1.05 P < 0.001) and to cirrhosis (HR 1.03, 95% CI 1.01-1.05, P = 0.005). A trend was observed for a shorter period of time to advanced fibrosis in the CsA group compared to Tac (HR 1.56, 95% CI 0.98-2.48 P = 0.059). Pre-transplant DM was also associated with faster progression to advanced fibrosis (HR 1.95, 95% CI 1.26-3.01, P = 0.003) whereas "other" race (HR 0.29, 95% CI 0.67-1.91, P = 0.013) was associated with longer time to advanced fibrosis compared to Caucasians.

Clinical outcomes

- **Post-transplant complications.** The incidence of all-cause mortality in the study population was 38.9% and no significant between-group differences were observed. The most common causes of death were non-HCV recurrence-related (65.1%) followed by HCV recurrence-related causes (32.6%) and graft loss (2.3%). There was no significant between-group difference with respect to HCV-related mortality (P = 0.322) (Table 7). However, multiple logistic regression revealed increased odds for HCV-related mortality between the CsA to Tac conversion group as compared to the Tac group (OR 2.30, 95% CI 1.01-5.22, P = 0.047). Furthermore, the duration of immunosuppressant treatment (OR 0.90, 95% CI 0.83-0.97, P = 0.010) or antiviral treatment (OR 1.04, 95% CI 1.02-1.06, P < 0.001) were significantly associated with HCV-related mortality. Graft loss was observed in 3 patients (0.7%) of which 1 occurred in the CsA group and 2 in the Tac group. The two cases in the Tac group were considered to be HCV-related. A single case of *de novo* hepatocellular carcinoma was observed in the Tac group. Few patients developed post-transplant fibrosing cholestatic hepatitis; the Tac group experienced the highest incidence (4.8%) while the Tac to CsA conversion group had none, and there was no significant between-group difference with respect to this outcome

Graft rejection episodes

- **Acute rejection.** Analysis of graft rejection by type (hyperacute/acute and chronic) indicates

Table 7. Post-transplant complications.

Clinical complication	CsA n (%)	Tac n (%)	CsA to Tac n (%)	Tac to CSA n (%)	Between-group P-value
HCV related mortality	16 (11.9)	27 (11.7)	11 (20.0)	3 (8.6)	0.322
Graft loss	1 (0.8)	2 (0.9)	0 (0.0)	0 (0.0)	0.856
De novo hepatocellular CA	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0.790
Fibrosing cholestatic hepatitis	4 (3.1)	10 (4.8)	2 (3.8)	0 (0.0)	0.541

CsA: cyclosporine. Tac: tacrolimus. CsA to Tac: cyclosporine to tacrolimus conversion group. Tac to CsA: tacrolimus to cyclosporine conversion group. Note: percentages are based on the number of patients with available data in each patient group. Between-group P-value is based on Pearson Chi Square Test.

Table 8. Graft rejection episodes.

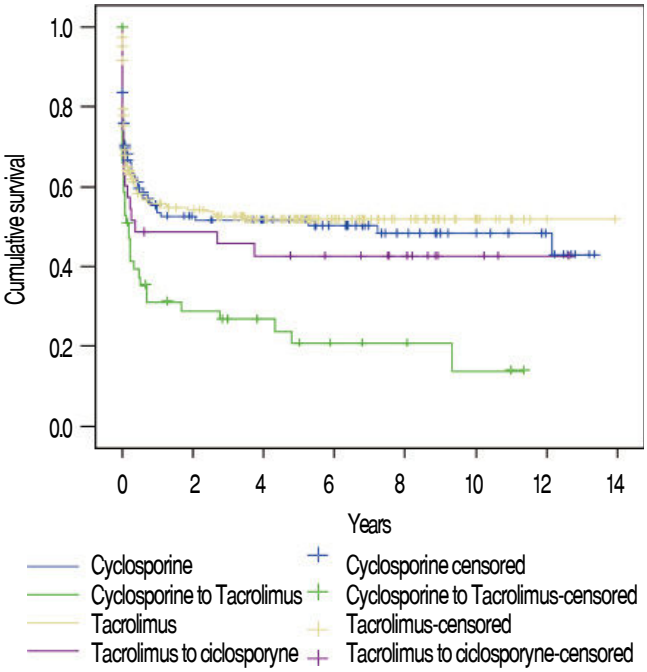
Type of rejection episode	CsA n (%)	Tac n (%)	CsA to Tac n (%)	Tac to CSA n (%)	Between-group P-value
Hyperacute/Acute rejection	65 (48.9)	103 (45.8)	41 (75.9)	20 (57.1)	P < 0.001*
Chronic rejection	5 (3.8)	6 (2.7)	11 (20.4)	1 (5.1)	P < 0.001*

CsA: cyclosporine. Tac: tacrolimus. CsA to Tac: cyclosporine to tacrolimus conversion group. Tac to CsA: tacrolimus to cyclosporine conversion group. Note: percentages are based on the number of patients with available data in each patient group. Between-group P-value is based on Pearson Chi Square Test. *Statistically significant figures.

that the majority of rejection events were acute in nature (Table 8). A significant between-group difference was observed in the incidence of acute/hyperacute rejection (P = 0.001) with the lowest incidence reported in the Tac group (45.8%), while the highest in the CsA to Tac conversion group (75.9%). There was no significant difference between the CsA and Tac groups with respect to this outcome. Logistic regression analysis indicated that patients in the two conversion groups (CsA to Tac and Tac to CsA) had significantly greater odds for acute or hyperacute rejection (OR 6.49, 95% CI 2.16-19.52, P = 0.001) and (OR 5.86, 95% CI 1.43-24.10, P = 0.014), respectively, relative to the Tac group. A longer duration of immunosuppressant treatment (OR 0.44, 95% CI 0.37-0.52, P < 0.001) and an increased donor age (OR: 0.98, 95% CI 0.96-01.00, P = 0.011) were significantly associated with decreased odds of achieving this outcome.

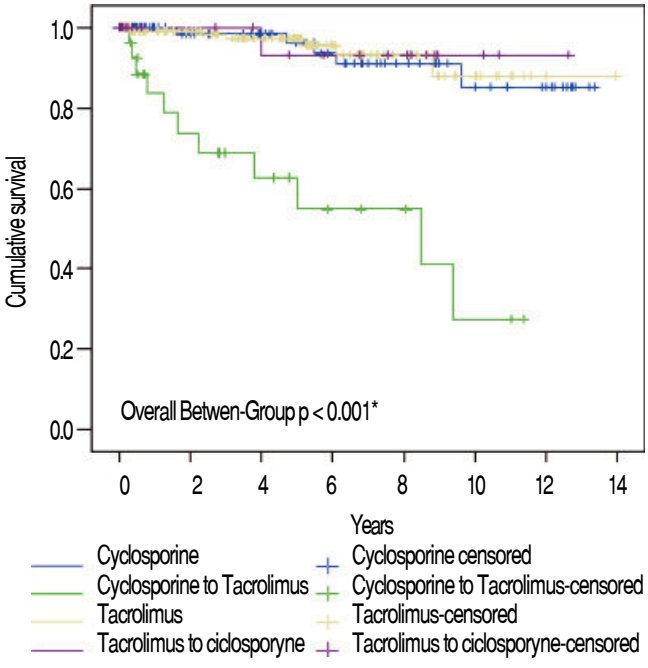
The time to reach acute or hyperacute rejection, analyzed using the Kaplan Meier method (Figure 1), revealed an overall significant difference in time to acute/hyperacute rejection between the four treatment groups (P = 0.001). Patients in the CsA to Tac conversion group had the shortest mean time to event (2.69 years) followed by the Tac to CsA conversion group (5.62 years), the CsA group (6.71 years) and the Tac group (7.37 years). The Cox proportional hazards model showed a significant difference between the CsA to Tac conversion group and the Tac group (HR 2.09, 95% CI 1.29-3.31, P 0.003). Increased transplant donor age was associated with prolonged time to acute or hyperacute rejection (HR 0.99, 95% CI 0.97-1.00, P = 0.006).

- **Chronic rejection.** Similar to the acute/hyperacute rejection, there was a significant between-group difference (P < 0.001) in the incidence of chronic rejection episodes with the lowest being observed in the Tac group (2.7%) and the highest in the CsA to TAC conversion group (20.4%) (Table 8). Here again, pairwise comparison revealed no significant differences in incidence rates between the CsA and Tac groups (OR 1.43, 95% CI 0.43-4.77, P = 0.545). Multiple regression analysis showed that patients converted from CsA to Tac had increased odds for chronic rejec-



Between-group P-value was assessed with the Log Rank test. The shortest mean time to acute/hyperacute rejection was observed in the cyclosporine to tacrolimus switch group (2.7 years).

***Figure 1**. Kaplan-Meier survival estimates: time to acute/hyperacute rejection.*



Between-group P-value was assessed with the Log Rank test. The shortest mean time to chronic rejection was observed in the cyclosporine to tacrolimus switch group (6.5 years).

***Figure 2**. Kaplan-Meier survival estimates: time to chronic rejection.*

tion relative to the TAC group (OR 10.45, 95% CI 3.61-30.27, P < 0.001). However, no significant differences were observed between either the CsA group or the Tac to CsA conversion group and the Tac group. Here also, a longer duration of immunosuppressant treatment was significantly associated with decreased odds of chronic rejection (OR: 0.87, 95% CI 0.78-0.98, P = 0.024).
The time to chronic rejection analysis using the Kaplan Meier method (Figure 2) revealed a significant difference between the four treatment groups (P < 0.001) with the shortest mean time to chronic rejection observed in the CsA to Tac conversion group (6.54 years) while patients in the Tac group showed the longest time to rejection (13.04 years). No Cox proportional hazards model could be fitted to this data since the coefficients did not converge.

Diabetes mellitus

New onset DM was defined as persistent hyperglycemia requiring long-term (> 3 months) treatment with oral hyperglycemic agents and/or insulin that was not required prior to liver transplant. One hundred and twenty four patients (27.7%) of the total cohort were identified with new onset DM in the post-transplant period and a significant between-group difference was observed for this outcome (P = 0.006). The lowest incidence of DM was reported in the CsA group (25 patients, 18.7%), followed by the Tac group (64 patients, 28.3%) and the two conversion groups (20 patients, 37.0% in the CsA to Tac and 15 patients. 44.1% in the Tac to CsA conversion groups). Pairwise comparisons revealed a statistically significant difference in the incidence of DM between the CsA and Tac groups, in favour of patients receiving cyclosporine (OR 0.58, 95% CI 0.34-0.98, P = 0.044).

DISCUSSION

The present retrospective study of this Canadian cohort of transplant recipients with recurrent HCV infection was performed to investigate whether cyclosporine conferred an advantage to tacrolimus in virological response to antiviral therapy. Other secondary outcomes studied included the development of fibrosis, graft rejection and post-transplant diabetes mellitus. While recent prospective studies limited to one to two years did not reveal a significant difference between CsA and Tac in HCV post-transplant recipients,[9,10] it is conceivable that the effects of chronic immunosuppressive therapy on progressive HCV-associated liver disease would be expected to increase with time. Accordingly, our multi-centre national study investigated the long-term effects of calcineurin inhibitors on post-transplant HCV with range of follow-up between four to fourteen years. To our knowledge, there has not been a similar study that has looked at the long-term effects. Our results provided evidence that a CsA-based regimen yielded a trend towards a higher rate of sustained viral response (66.7%) compared to patients on a Tac-based immunosuppressant regimen (52.8%) for all genotypes, resulting, on multiple logistic regression analysis, in a higher likelihood of achieving this virologic endpoint in patients treated with CsA (OR 2.6). Virus detection performed during antiviral treatment and up to 6 months after therapy also showed a slight but consistent trend toward higher proportions of patients with undetectable virus in the CsA group as compared to Tac. Our results are consistent with those of other studies who have shown in the non-transplant setting that early virologic response may be a predictor of SVR.[15] Although not statistically significant, it was of interest to note a trend towards a better SVR with CsA (51.6%) compared to Tac (35.0%) with respect to genotype 1 virus, which is the most prevalent subtype in our study but also the least responsive to antiviral treatment.

Our SVR results would appear to support results obtained in other post-transplant studies, although there were some differences. The study by Firpi, *et al.*[8] showed an SVR rate of 46% in cyclosporine-treated patients versus 27% in tacrolimus-treated patients with post-liver transplant recurrence receiving antiviral treatment, and similar rates were reported by Cescon, *et al.* (43% *vs.* 14%).[16] Although a higher overall SVR rate was observed in our study in patients treated with CsA or Tac compared to these studies, this apparent discrepancy may be related to differences in the patient populations studied and selective use of antiviral therapy. Thus, the median donor age was 45 years in our study compared to 60 years of age in the study by Cescon, *et al.*, while the proportion of patients with genotype 1 was higher in the study by Firpi, *et al.* compared to our study. Our results were most similar to those obtained by Selzner, *et al.*,[17] who achieved SVR rates of 56% with CsA *vs.* 44% in the Tac group in a population of patients that were comparable to ours in terms of genotype distribution and donor age, two important determinants of the SVR response. Several mechanisms may explain the improved virologic outcomes

observed in CsA-treated patients after HCV recurrence. A number of *in vitro* studies have demonstrated the antiviral activity of CsA against HCV.[8,18,19] Furthermore, *in vivo* studies have shown an additive effect of CsA and interferon combination treatment.[8,20] The main mechanism considered responsible for the antiviral effects of cyclosporine is the inhibition NS5B binding to cyclophilin B mediated by CsA.[21]

A significant finding of this study was the identification of patient characteristics predicting SVR achievement. Donor age has been previously shown to be a major determinant of fibrosis progression.[22,23] Our multivariate analysis showed that increased donor age and the presence of HCV genotype 1, in addition to Tac use, were associated with significantly reduced odds of achieving SVR following 6 months of antiviral treatment. These results are in agreement with recent studies[16,17,24] identifying the same predictors of SVR outcome.

The post-transplant biopsy results indicated increased odds of severe fibrosis and/or cirrhosis in the CsA group (respectively, OR 4.60 and OR 2.56) as compared to Tac treated patients, although no difference in the time to event was detected. The observed increases seem at variance with the improved SVR observed in the CsA group. However, these results include all available biopsies of the entire cohort including patients who did not receive antiviral treatment post-transplant. The non-antiviral treated patients were more numerous in the CsA group (60%) compared to the Tac group (40 %), and it is possible that this difference played a role. These observations did not translate into an increase in post-transplant clinical events as we were unable to detect any significant difference between treatment groups in the incidence of HCV-related complications including: HCV-related graft loss, HCV-related mortality, fibrosing cholestatic hepatitis, post-transplant de novo hepatocellular carcinoma and rejection episodes.

The observed difference between the higher incidence and accelerated time to event of post-transplant acute/hyperacute and chronic rejection episodes in the CsA to Tac conversion group (acute events: 75.9%, chronic events: 20.4%, OR 6.49) as compared to the tacrolimus group (acute events: 45.8%, chronic events: 2.7%, OR 10.45) was interesting. This was accompanied by a significant increase in the odds of HCV-related death (OR 2.30) in the CsA to TAC conversion group (20.0%) as compared to the Tac group (11.7%). The significance of these observations is not known but the phenomenon may warrant further investigation. We speculate that conversion of one calcineurin inhibitor agent for another may have been a surrogate marker of "graft problems" that the transplant centres attempted to rectify by switching drugs.

Our study showed a significantly higher incidence of new onset diabetes mellitus in patients treated with Tac (28.3%) compared to patients treated with CsA (18.7%) (Table 9). This is in agreement with previous clinical trials showing a higher incidence of new onset diabetes with Tac than CsA.[12] Furthermore, our analysis of post-transplant biopsy results indicated that the presence of DM prior to transplant increases the risk of a faster progression to severe fibrosis (HR 1.95). This is consistent with previous evidence that, in a non-transplantation setting, the presence of diabetes affects HCV progression negatively by promoting hepatic fibrosis and thus may represent a contributing factor in the failure to achieve a sustained response to antiviral therapy.[14] These findings are important as they suggest that diabetes mellitus may negatively impact the post-liver transplant clinical evolution.

In conclusion, our study has shown that a CsA-based immunosuppressant treatment is associated with a trend towards a higher rate of sustained viral response than tacrolimus in the treatment of post-liver transplant patients with HCV recurrence. However, this did not influence the frequency of HCV-related clinical complications, at least within the long-term follow-up period covered by our study. In addition to the type of immunosuppressant regimen, we identified donor age and viral genotype as

Table 9. Incidence of new onset diabetes mellitus.

New onset diabetes mellitus	Statistic	CsA (n = 137)	Tac (n = 230)	CsA to Tac (n = 56)	Tac to CSA (n = 35)	Between-group P value
Presence / Absence	n (%)	25/109 (18.7/81.3)	64/162 (28.3/71.7)	20/34 (37.0/63.0)	15/19 (44.1/55.9)	P = 0.006*

CsA: cyclosporine. Tac: tacrolimus. CsA to Tac: cyclosporine to tacrolimus conversion group. Tac to CsA: tacrolimus to cyclosporine conversion group. Note: percentages are based on the number of patients with available data in each patient group. Between-group P-value is based on Pearson Chi Square Test. *Statistically significant figures.

predictors of SVR. Given the retrospective nature of this study, further prospective longitudinal studies should be undertaken to better assess the long term effect of immunosuppressive choices on the clinical outcomes in liver transplant recipients with HCV infection.

ABBREVIATIONS

- **ANOVA:** analysis of variance.
- **CI:** confidence interval.
- **CsA:** cyclosporine.
- **CsA to Tac:** cyclosporine to tacrolimus switch group.
- **DM:** diabetes mellitus.
- **HCV:** hepatitis C virus.
- **HR:** hazard ratio.
- **LT:** liver transplantation.
- **OR:** odds ratio.
- **SD:** standard deviation.
- **SVR:** sustained viral response.
- **Tac:** tacrolimus.
- **Tac to CsA:** tacrolimus to cyclosporine switch group.

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CONFLICT OF INTEREST STATEMENT

- Dr. Eric Yoshida has been an investigator of clinical trials sponsored by: Merck Inc, Vertex Inc, Hoffman LaRoche Inc, Janssen Inc, Boehringer Ingelheim); Inc, Pfizer Inc, Gilead Sciences Inc, Norvartis Inc, Astellas Inc, Abbott Inc. He has received unrestricted research grants from Cangene Inc, Hoffman LaRoche Inc. He has received honoraria for lectures sponsored by Merck Canada, Hoffman LaRoche Canada, Vertex Canada, Cangene Inc, Gilead Sciences Canada.
- Dr. Leslie Lilly has been an investigator of clinical trials sponsored by: Hoffman LaRoche Inc, Norvartis Canada, Astellas Inc., Wyeth Inc and Cangene Inc. He has received honoraria for lectures sponsored by Merck Canada, Hoffman LaRoche Canada, Norvartis Canada and Astellas Canada.
- Dr. Paul Marotta has been an investigator of clinical trials sponsored by Merck Inc, Hoffman LaRoche Inc., Boehringer Ingleheim Inc., Gilead Sciences Inc., Norvartis Inc., Astellas Inc. He has received honoraria for lectures and has participated on Advisory Boards of Merck Canada, Hoffman LaRoche Canada and Vertex Canada.
- Dr. Andrew Mason has conducted research supported in part by Abbott Laboratories, Gilead Sciences and Novartis Inc.
- Dr. Marc Bilodeau has been an investigator of clinical trials sponsored by: Merck Inc., GSK, Boehringer Ingleheim Inc. He has received unrestricted research grants from Merck Canada. He has received honoraria for lectures sponsored by Novartis Inc., Merck Canada, Hoffman LaRoche Canada, Gilead Sciences Canada.
- Dr. Marc Vaillancourt is an employee of Novartis Pharmaceuticals Canada Inc.

DISCLOSURES

A preliminary analysis of this study was previously presented at the American Association for the Study of Liver Meeting in San Francisco CA in November 2011 and at the Canadian Digestive Diseases Week in Montreal PQ, Canada in March 2012.

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