Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

A prospective randomized trial comparing tacrolimus and steroids with tacrolimus monotherapy in liver transplantation: the impact on recurrence of hepatitis C

Carlos Margarit,[1] Itxarone Bilbao,[1] Lluis Castells,[2] Iñigo Lopez,[1] Leonor Pou,[3] Elena Allende[4] and Alfredo Escartin[1]

1 Liver Transplantation Unit, Department of General Surgery, Hospital Vall Hebrón, Universidad Autónoma Barcelona, Barcelona, Spain
2 Hepatology Unit, Department of Internal Medicine, Hospital Vall Hebrón, Universidad Autónoma Barcelona, Barcelona, Spain
3 Department of Biochemistry, Hospital Vall Hebrón, Universidad Autónoma Barcelona, Barcelona, Spain
4 Department of Pathology, Hospital Vall Hebrón, Universidad Autónoma Barcelona, Barcelona, Spain

Keywords
hepatitis C virus patients, immunosuppression, liver transplantation, steroid-free, tacrolimus.

Correspondence
Carlos Margarit MD, PhD, Liver Transplantation Unit, Hospital General Vall Hebrón, 08035 Barcelona, Spain. Tel.: 34 932746113; fax: 34 932746112; e-mail: cmargarit@vhebron.net

Received: 3 November 2004
Revision requested: 25 March 2005
Accepted: 31 August 2005

doi:10.1111/j.1432-2277.2005.00217.x

Summary

The aim of this prospective randomized trial was to study the efficacy and safety of tacrolimus monotherapy (TACRO) and compare it with our standard treatment of tacrolimus plus steroids (TACRO + ST) after liver transplant (LT). Furthermore, the impact of steroid-free immunosuppression on outcome of hepatitis C virus (HCV) was analysed. Between 1998 and 2000, 60 patients (mean age: 57 years) were included in the study and randomized to receive TACRO ($n = 28$) or TACRO + ST ($n = 32$). Indication for LT was postnecrotic cirrhosis in all cases (58.3% were HCV-positive). Mean follow-up was 44 months. Survival, incidence of rejection, infection and side-effects were compared between the two groups. In patients with HCV infection, incidence and severity of acute hepatitis C, long-term outcome of recurrent hepatitis C and survival were studied in an intention-to-treat analysis or in the real group analysis (real-TACRO versus real-TACRO + ST). Patient survival at 1, 3 and 5 years, tacrolimus pharmacokinetics, incidence of rejection infections and side-effects were similar. In patients with HCV, the incidence and severity of graft hepatitis C tended to be lower in TACRO (47%) compared with TACRO + ST (67%) ($P = $ NS), and also in real-TACRO (42%) compared with real-TACRO + ST (61%) ($P = $ NS). A poor outcome considered as evolution to cirrhosis at 3 years was observed in one (9%) living patient in real-TACRO and nine (45%) in real-TACRO + ST ($P = 0.04$). Patient survival at 1, 3 and 5 years was 92%, 92% and 73% for real-TACRO and 78%, 61% and 51% for real TACRO + ST ($P = 0.07$). Steroid-free immunosuppression appears to be safe and efficacious. The main advantage of this regimen could be in HCV patients, as recurrence of hepatitis in the graft was less severe in the group of patients in whom steroids could be avoided completely.

Introduction

Steroids, which cause important side-effects, are still used for induction as well as for maintenance immunosuppression in the majority of transplant centres [1]. With the introduction of cyclosporin, steroid dosage could be successfully reduced or even withdrawn in some individuals, particularly children who were likely to suffer severe side-effects such as growth retardation [2,3]. Few investigators have been willing to risk induction therapy free of steroids [4–6] because of their extremely low cost, familiarity with their management and their effectiveness in

control of rejection. However, early cessation of steroid therapy at 3 months post-liver transplantation (LT) [7,8], or as early as 15 days post-LT [9], has demonstrated a reduction in and better control of side-effects such as arterial hypertension, hypercholesterolaemia, diabetes and other complications. The next logical step was to try to avoid steroids from the beginning. Several pilot studies, and few randomized trials, have explored this possibility with mixed results [4–14]. Other studies explored a steroid-free regimen including a calcineurin inhibitor with mycophenolate mofetil (MMF) [6], rapamicine [10], rabbit antithymocyte globulin plus MMF [2] or MMF and daclizumab [14]. The incidence of rejection with these associations was lower, between 8.5% and 28%, than with calcineurin inhibitor monotherapy. Therefore, the question remains as to whether a short course of steroids in the peritransplant period is safe or an unnecessary risk.

Hepatitis C viral (HCV) cirrhosis is currently the main indication for LT; unfortunately, recurrence of HCV infection in the graft post-LT is universal. Ten to twenty per cent of patients develop severe cholestatic hepatitis with a high proportion of early graft failure soon after LT, whereas around 40% of patients present cirrhosis at 5 years post-transplant. Consequently, long-term patient survival in hepatitis C patients is lower than other indications [15–17]. Among other factors, the severity of graft hepatitis and poor outcome after LT have been related to steroid administration for prophylaxis and treatment of rejection.

Objectives

The main purpose of the present prospective randomized trial was to assess the efficacy and safety of tacrolimus monotherapy without steroids in LT, and compare the results with our standard treatment of tacrolimus plus steroids. Efficacy was assessed by (i) patient and graft survival at 1, 3 and 5 years, (ii) incidence and cause of early and follow-up mortality and (iii) incidence and severity of rejection during the first 3 months post-transplant. Safety was assessed by the rate of infectious complications and side-effects such as renal insufficiency, arterial hypertension, diabetes, dyslipaemia, neurotoxicity and other complications during the first 3 months post-transplant. The second important objective was to study the impact of steroid-free immunosuppression on the outcome of HCV recurrence post-LT.

Patients and methods

Over a 2-year period between October 1998 and September 2000, a total of 82 adult patients underwent LT in our Unit. Institutional review board approval for the study was obtained and all patients provided written informed consent before enrolment into each of the groups with 1:1 randomization. Of the potential patients available over the recruitment period, 22 liver transplant recipients were excluded. The reasons for exclusion were combined liver–kidney transplant ($n = 5$), re-transplants ($n = 6$), renal failure pretransplant ($n = 4$), fulminant liver failure ($n = 3$) and pre-LT oral consumption of steroids ($n = 1$). Three patients were excluded after randomization because of perioperative death ($n = 2$) and positive cross-match ($n = 1$). Finally, 60 patients were randomized to receive tacrolimus alone (TACRO) or tacrolimus plus steroids (TACRO + ST), just before the surgical procedure.

Patients

Patient demographic characteristics and indications for LT are presented in Table 1. Mean age of patients was 57 years; 50% were over 60 years of age, and there was a male predominance. All patients suffered from end-stage liver cirrhosis or hepatocellular carcinoma (HCC) in cirrhosis. The main aetiology of cirrhosis was HCV infection (58%), with or without HCC.

Donors

The mean age of the patients was 43 years and mean cold ischaemia time was 8 h.

Operation

Recipient hepatectomy with inferior vena cava preservation was performed in all cases. Choledocho-choledochostomy without T-tube was performed in 63.5% of cases. A 500 mg steroid bolus was administered to all patients during the anhepatic phase before liver reperfusion.

Immunosuppression and drug measurements

Tacrolimus treatment was started at the end of the operation and administered through a nasogastric tube at 0.05 mg/kg body weight every 12 h in both groups. Tacrolimus dosage was set to achieve a trough level between 10 and 15 ng/ml over the first few weeks and between 8 and 12 ng/ml thereafter. Tacrolimus trough levels were measured daily during the hospital stay and at each outpatient visit. Pharmacokinetic studies were performed on days 3 and 9 post-LT. Whole blood samples were taken at 1, 2, 4 and 6 h postdose and predose for calculation of C_{min}, C_{max} and area under the curve (AUC_{0-12}) of tacrolimus. In patients randomized to TACRO + ST, a steroid taper was instituted starting at 100 mg b.i.d. of methylprednisolone

Table 1. Characteristics of recipients, donors, surgery and post-transplant complications.

Characteristic	TACRO ($n = 28$)	TACRO + ST ($n = 32$)	*P*-value
Recipient			
Mean age	57 ± 7	56 ± 8	NS
Patients over 60 years	15 (54)	14 (44)	NS
Male/female	18/10	25/7	NS
Diagnosis			
HCV	20 (71)	15 (47)	0.05
ETOH	5	11	
HBV	3	2	
Cryptogenic	0	2	
Haemochromatosis	0	2	
HCC over cirrhosis	13 (46)	11 (34)	
Child–Pugh A/B/C (%)	18–28–54	12–37–51	NS
Donor			
Mean age (years)	42 ± 18	45 ± 19	NS
Graft steatosis	3 (11)	11 (35)	0.037
Surgery			
Mean RBC units	6.3 ± 5	5.7 ± 4	NS
Cold ischaemia time(min)	498 ± 118	481 ± 120	NS
Post-transplant			
Mod/severe ischaemic injury	7 (25)	7 (22)	NS
Mean post-op stay (days)	26 ± 14	36 ± 17	NS
Re-transplantation (%)	0	5 (15)	NS
Mortality	7	9	
Early (<3 months)			
PDF	1	2	
Sepsis-MOF	2	1	
Late (>3 months)			
HCV recurrence	3	3	
HCC recurrence	1	0	
De novo tumour	0	2	
HAT-retx	0	1	
Patient survival (%)			
1, 3 and 5 years	85, 81, 66	84, 78, 73	NS
Graft survival (%)			
1, 3 and 5 years	85, 81, 66	75, 60, 60	NS

Percentage values are given in parentheses.
HCV, hepatitis C virus; ETOH, cirrhosis due to alcohol; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; RBC, red blood cells; PDF, primary dysfunction; MOF, multiorgan failure; HAT, hepatic artery thrombosis.

post-LT day 1 and decreasing to 20 mg/day by day 6. Patients were weaned off prednisone, if possible, within 3 months post-LT.

Diagnosis and treatment of rejection

Acute rejection episodes were diagnosed by alteration in liver function tests and confirmed by liver biopsy. The Banff 97 criteria [18] were used for histopathological diagnosis. Ultrasound examination was used to rule out technical complications. Acute rejection was treated by increasing tacrolimus doses if levels were under 15 ng/ml, together with steroid therapy. A 3-day 500 mg i.v. bolus of methylprednisolone was administered followed by a steroid taper from 200 to 20 mg/day over 6 days in cases of severe rejection. When liver graft dysfunction persisted despite steroid pulses, administration of 1–2 g/day of MMF was started. Orthoclone monoclonal antibodies against T lymphocytes was not used in any case. Severe acute rejection was defined when there was an incomplete response to steroid treatment and other immunosuppressive drugs, such as MMF or rapamicine, were necessary to control rejection. Refractory rejection was recorded when no response was obtained, and re-transplantation or death were derived from rejection.

Definition of side-effects

Renal insufficiency was classified as mild (creatinine >1.5 mg/dl), moderate (creatinine >3 mg/dl) and severe (need for dialysis or extrarenal depuration) [19]. Arterial hypertension was recorded if treatment with drugs was required to control blood pressure values. Diabetes was defined as the need for insulin for more than 1 week, when the patient was not receiving total parenteral nutrition or i.v. glucose infusion. Hypercholesterolaemia and hypertriglyceridaemia were defined as values over 300 and 280 mg/dl, respectively, in two consecutive analyses separated by at least 1 week. Only bacterial, viral and fungal infections that required active treatment were considered as infections.

Hepatitis C viral infection

Viral load and genotype were evaluated pretransplantation. HCV RNA levels were measured at 1 week, 2 weeks, 1 month, 3 months, 6 months, 9 months and 1 year post-transplantation. Complete data were available in 31 of 35 HCV-positive patients: 17 in the TACRO and 14 in the TACRO + ST groups. Results are expressed as $\log_{10}$ HCV RNA copies/ml serum [20]. Diagnosis of recurrent hepatitis C in the graft was based on elevation of liver enzymes together with histologic confirmation from liver biopsy tissue. Mild hepatitis was recorded for patients with transaminase concentrations <400 IU, total bilirubin (TB) <2 mg/dl and with no general symptoms such as malaise, fever and tenderness of the right upper abdomen. Moderate hepatitis was recorded when transaminases were >400, TB >2 but <10 mg/dl and no symptoms. Severe hepatitis was considered when TB >10 mg/dl and with symptoms. The clinical progress of HCV-positive patients at 3 years, or at the end of follow up, was monitored using the measurement of liver enzymes, liver biopsy and clinical symptoms. Liver function was considered normal

when transaminases and total bilirubin were lower than twofold normal values. Liver biopsies were performed when clinically indicated (alteration of liver function tests or at time of death) and protocol liver biopsies were performed to almost all HCV patients at 3 years. Histology grading according to the Ishak K classification system was used [21]. Chronic hepatitis grading score (scale of 0–18) representing necro-inflammatory activity was the sum of piecemeal necrosis score (0–4), confluent necrosis score (0–6), focal lytic necrosis, apoptosis and focal inflammation score (0–4) and portal inflammation score (0–4). Chronic hepatitis fibrosis score (0–6) was based on the extent of fibrosis and the development of cirrhosis. At the close of the study, 'good outcome' was considered if the patient had normal liver function or chronic active hepatitis shown on liver biopsy, and 'poor outcome' if the patient had compensated or decompensated cirrhosis or death related to the HCV recurrent cirrhosis. Risk factors for poor outcome of HCV recurrence were analysed.

Data analysis

The SPSS software program was used throughout. Student's *t*-test or the Mann–Whitney *U*-test were used for quantitative data and Pearson's chi-square or Fisher's exact test for categorical data. Differences were considered statistically significant when the $P < 0.05$. Data are shown as the mean ± SD, or as percentages. The Kaplan–Meier method was used for survival analysis. Demographic data of recipient and donor and surgery-related factors were compared to assess comparability of the two treatment groups (TACRO and TACRO + ST). End points between groups were compared to evaluate efficacy and safety. To evaluate the impact of a steroid-free protocol in hepatitis C-positive patients, this subgroup of patients was analysed independently and special attention paid to incidence and severity of rejections, recurrence of hepatitis C in the graft and toxicities directly related to immunosuppression. In HCV-positive patients, both treatment groups were compared as intention-to-treat (TACRO versus TACRO + ST) and as real groups: patients who received steroids either for protocol or for rejection (real-TACRO + ST) and those who received no steroids at all during follow up (real-TACRO). A Cox regression analysis of risk factors for poor outcome of HCV recurrence was performed, including recipient, donor, surgery, immunosuppression and postoperative factors.

Results

Randomization assigned 28 patients to the TACRO group and 32 patients to the TACRO + ST group. Demographic characteristics, clinical indications, donor characteristics and surgical variables are presented in Table 1. No differences were found between treatment groups except for a higher incidence of graft steatosis in TACRO + ST and of HCV-positive patients in the TACRO group. Primary graft dysfunction secondary to ischaemic–reperfusion injury that could affect tacrolimus metabolism and pharmacokinetic parameters was similar in both groups. The majority of technical complications and re-interventions were of biliary origin.

Patient and graft outcome

Actuarial survival at 1, 3 and 5 years was 85%, 81% and 66% in the TACRO group and 84%, 78% and 73% in the TACRO + ST group. Seven deaths occurred in the TACRO group: three early (<3 months) and four late deaths (three of them because of hepatitis C recurrence after 1 year). There were nine deaths in the TACRO + ST group: three early and six late deaths (three of them because of hepatitis C recurrence, two in the first year post-LT and one after 1 year). Mean follow-up was 44 months (range: 3–60) and all patients have been followed up for more than 4 years. Five re-transplants in four patients were performed during follow up, all of which were in the TACRO + ST group, and were the result of primary non-function (in two patients), hepatic artery thrombosis (two re-transplants in the same patient) and HCV recurrence (in one patient).

Incidence of rejection

Incidence of acute rejection and severe acute rejection was similar in TACRO versus TACRO + ST (39% vs. 32% and 14% vs. 9%, P = NS; see Table 2). Rejection with low tacrolimus levels was found in one patient of the TACRO group and two patients of the TACRO + ST group. Seven patients presented severe acute rejection and received a mean accumulative MMF dose of 13 g/patient in the TACRO group versus 20 g/patient in the TACRO + ST group. Steroid withdrawal in these seven patients was possible in the sixth month for the TACRO group and in the fourth month for the TACRO + ST group.

Incidence of infections, toxicity and other side-effects

During the first 3 months post-LT, 26 episodes of infections occurred in the TACRO group (62% bacterial, 23% viral and 15% fungal) and 30 in the TACRO + ST group (40% bacterial, 37% viral and 23% fungal); (P = NS). All cytomegalovirus (CMV) infections appeared in patients of the TACRO + ST group: positive CMV antigenaemia was

Table 2. Rejections, infections and toxicity comparing TACRO versus TACRO + ST over the first 3 months post-transplant.

Event	TACRO ($n = 28$)	TACRO + ST ($n = 32$)	*P*-value
Rejections			
Patients with acute rejection	11 (39.3)	10 (32.3)	NS
Number of episodes	12	11	
Methyl-prednisolone (g)/episode	1.5	1.5	NS
Steroid-sensitive rejection	8 (28.57)	8 (25)	NS
Rejection requiring MMF treatment	4 (14.28)	3 (9.37)	NS
Refractory rejection and chronic rejection	0	0	
Patients without steroids 3, 6, 12 months (%)	64, 78, 93	19, 44, 86	
Conversion to MMF, neoral	2 (7)	3 (9.3)	
Episodes of Infections (3 months)	28	34	NS
Bacterial/viral/fungal (%)	62/23/15	40/37/23	
Side-effects and toxicity			
Pre-LT renal failure	3 (11)	4 (12.5)	NS
Renal insufficiency	20 (71.4)	17 (53)	NS
Mild/moderate/severe (dialysis)	14/0/6	10/2/5	
Pre-LT arterial hypertension	3 (10.7)	6 (18.8)	NS
De novo arterial hypertension	1 (3.5)	3 (9.6)	NS
Pre-LT diabetes	6 (21.4)	5 (15.6)	NS
De novo diabetes mellitus	2 (7.1)	6 (18.7)	NS
Dyslipaemia	5 (17.8)	4 (14.2)	NS
Neurologic complications	9 (32)	10 (31.3)	NS
Diarrhoea	4 (14.2)	5 (15.6)	NS

Percentage values are given in parentheses.

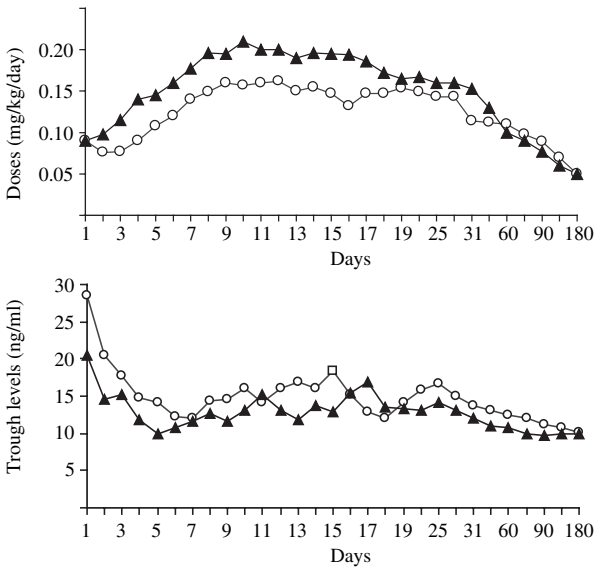
found in three patients and only one patient developed CMV disease with pneumonitis and sepsis. All episodes of fungal infection were the result of *Candida*.

No statistically significant differences were found regarding side-effects and toxicity, although trends towards a higher incidence of renal insufficiency were observed in the TACRO group and arterial hypertension and *de novo* diabetes mellitus in the TACRO + ST group. With regard to neurological complications, three patients in each group had tremors.

Conversion to MMF ($n = 3$) because of renal insufficiency, rapamicine ($n = 1$) due to refractory ascites plus renal insufficiency, or neoral ($n = 1$) due to intestinal problems, was required in two patients in TACRO group and three patients in the TACRO-ST group.

Pharmacokinetic measurements

Pharmacokinetic studies were available for 21 patients in the TACRO and 24 in the TACRO + ST study groups (Fig. 1). A trend was observed towards higher trough levels of tacrolimus with lower doses in the monotherapy group, probably because of steroid induction on cytochrome P450 system. However, tacrolimus doses, trough levels and AUC were similar in both groups. Comparison of tacrolimus pharmacokinetic profile between patients with and without rejection showed no significant differences.



P = ns	3rd day			9th day		
	Cmin	Cmax	AUC (0–12 h)	Cmin	Cmax	AUC (0–12 h)
○ Tacrolimus (range)	13.1 (3.9–31)	22 (8–54.4)	205.3 (80–479)	12.3 (8.8–21)	33.5 (14–58)	217.2 (130–353)
▲ Tacro + steroids (range)	12 (5.2–20)	22.5 (11–51.2)	183 (107–367)	13.2 (4.4–24)	31.5 (8.2–66)	229.6 (62–416)

Figure 1 Comparison between mean doses and trough levels in TACRO and TACRO + ST during the first 6 months post-transplant. C_{min} (ng/ml), C_{max} (ng/ml) and $AUC_{0-12\ h}$ (ng h/ml) at third and ninth days post-transplant.

Hepatitis C virus recurrence

Thirty-five patients were HCV-positive: 20 were randomized to the TACRO and 15 to the TACRO + ST groups (see Table 3). Three patients in the TACRO group and one patient in the TACRO + ST group died within 3 months post-transplant from unrelated causes and were excluded from the analysis because the main end point was to evaluate the evolution of HCV graft re-infection. Mean age was 59 ± 6 years and distribution of HCC over cirrhosis were similar in both groups. Incidence of acute rejection, CMV infection and side-effects was similar in both groups. Postoperative mortality and patient and graft survival showed no statistically significant differences. All re-transplantations were performed in the TACRO + ST group.

Table 3. Demographic characteristics, incidence of rejection, infections, side-effects and mortality in hepatitis C virus patients.

	TACRO	TACRO + ST	*P*-value
Demographic characteristics (*n*)	20	15	
Early deaths <3 months excluded (*n*)	17	14	
Viral load pre-LT HCV-RNA	5 ± 1.2	4.7 ± 1.3	NS
1 week	4.6 ± 2	5.4 ± 1.6	
2 weeks	4.6 ± 2	5.4 ± 2.3	
1 month	5.2 ± 2	5.5 ± 2	
3 months	5.4 ± 2	6 ± 2	
6 months	5.7 ± 2.2	5.8 ± 1.8	
9 months	5.8 ± 1.7	5.5 ± 2	
12 months	5.6 ± 1.2	5.9 ± 2	
Postoperative outcome			
Acute rejection	8 (42)	7 (46)	NS
Severe AR requiring MMF	3	0	
Recurrence of HCV in graft (*n*)	17	14	
Acute graft hepatitis C	9 (53)	10 (71)	NS
Mild (AST <400, TB <2)	7 (41)	5 (36)	
Moderate (AST >400, TB>2)	2 (12)	3 (21)	
Severe (TB >10 + symptoms)	0	2 (14)	
No hepatitis	8 (47)	4 (29)	
IFN-ribavirin treatment	1	3	
Graft status at 3 years (*n*)	17	14	
Mean follow-up in months	43 ± 12	39 ± 15	NS
(a) Normal liver function test	6 (35)	5 (36)	
(b) Chronic active hepatitis	7 (41)	3 (21)	
(c) Compensated cirrhosis	1 (6)	2 (15)	NS
(d) Decompensated cirrhosis	0	1 (7)	
(e) HCV-related death	3 (18)	3 (21)	
a + b (good outcome)	13 (76)	8 (57)	NS
c + d + e (poor outcome)	4 (24)	6 (43)	
Histology findings (*n*)	14	11	
Piecemeal necrosis score	1.77 ± 0.93	2 ± 1.18	NS
Confluent necrosis score	2.46 ± 0.88	2.55 ± 1.21	NS
Focal lytic necrosis/inflam.	2.31 ± 0.63	2.27 ± 0.90	NS
Portal inflammatory score	1.85 ± 0.69	1.91 ± 0.94	NS
Fibrosis score	2.77 ± 2	3.27 ± 1.8	NS
Survival			
Patient survival (%)			
1, 3, 5 years	89, 83, 61	81, 61, 61	NS
Re-transplantation			
Technical	0	2	
HCV recurrence	0	1	

Percentage values are given in parentheses.

Complete viral load and genotype were available in 31 of the 35 HCV-positive patients: 17 in the TACRO group and 14 in the TACRO + ST group. All patients were HCV genotype 1b, except one patient who was type 1a, and one who was type 1a-b. Quantification of HCV RNA pre-LT and throughout the first year was similar in both groups when analysed on an intention-to-treat basis.

Clinical hepatitis C recurrence in the graft showed a trend towards a higher incidence in TACRO + ST with 10 patients (71%) compared with nine patients (53%) in the TACRO group (P = NS). Graft status at 3 years, as assessed by liver function, clinical symptoms and histological findings, showed no statistically significant differences. Protocol liver biopsies were performed in all but six cases (three in each group), the reason being the excellent clinical status and normal liver enzymes during follow up. Hence, with respect to histological findings, there were 14 patients in the TACRO group and 11 patients in the TACRO + ST group. A good outcome was observed in 76% of patients in the TACRO group versus 57% in the TACRO + ST group (P = NS) (Table 3).

A comparison was then made between the 12 patients who received no steroids at all (real-TACRO) and the 23 patients who received steroids (real-TACRO-ST) either from the beginning (randomization assignment, $n = 15$) or to treat rejection ($n = 8$). (Table 4). Viral load was lower for real-TACRO and statistically different from the period of 2 weeks to 3 months post-transplant. Recurrence of hepatitis C virus in the graft showed a trend towards a lower incidence in patients with no steroids at all: five patients (45%) in real-TACRO, all of whom were classified as mild hepatitis, compared with 14 (70%) in real-TACRO-ST classified as mild ($n = 7$), moderate ($n = 5$) and severe ($n = 2$). These differences were not statistically significant ($P = 0.06$). Graft status at 3 years or at the end of the follow-up period indicated more cases of poor outcome: nine patients (45%) in the real-TACRO + ST group developed cirrhosis or HCV-related death compared with one (9%) in the real-TACRO group ($P = 0.046$). The six patients who died as a result of HCV recurrence belonged to the real-TACRO + ST group. With respect to histological findings, necro-inflammatory activity did not differ. The only statistically significant difference ($P = 0.005$) was observed in fibrosis score: 1.78 ± 1 ($n = 9$) vs. 3.73 ± 1.94 ($n = 15$). Patient survival at 1, 3 and 5 years was 92%, 92% and 73% for real-TACRO versus 78%, 61% and 51% for real-

Table 4. Graft hepatitis analysed as 'real' groups receiving and not receiving steroids.

	Real-TACRO ($n = 12$)	Real-TACRO + ST ($n = 23$)	*P*-value
Viral load RNA-VHC			
Pre-LT	4.7 ± 1.5	4.9 ± 1.5	NS
1 week	4.1 ± 2.3	4.1 ± 1.6	NS
2 weeks	3.6 ± 2	5.6 ± 1.8	0.02
1 month	4.2 ± 2.2	5.8 ± 1.8	0.05
3 months	4.6 ± 2	6.1 ± 1.8	0.05
6 months	4.8 ± 2.8	6.1 ± 1.6	NS
9 months	5.2 ± 2.1	5.8 ± 1.8	NS
12 months	5.3 ± 1.6	5.9 ± 1.7	NS
Recurrence of HCV (*n*)	11	20	
(Early deaths excluded) (*n*)	1	3	
Graft hepatitis C	5 (45)	14 (70)	NS
Mild/mod/severe	5/0/0	7/5/2	0.06
IFN–ribavirin treat.	1	3	
Follow-up at 3 years (*n*)	11	20	
Follow-up (months)	42.9 ± 10.5	37.9 ± 12	NS
Progress of HCV			
(a) Normal liver function test	4 (36)	7 (35)	NS
(b) CAH	6 (55)	4 (20)	
(c) Compensated cirrh.	1 (9)	2 (10)	
(d) Decomp. cirrhosis	0	1 (5)	
(e) HCV-related death	0	6 (30)	
a + b (good outcome)	10 (91)	11 (55)	0.046
c + d + e (poor outcome)	1 (9)	9 (45)	
Histology findings (*n*)	9	15	
Piecemeal	1.67 ± 1.12	2 ± 1	NS
Confluent necrosis	2.33 ± 1	2.60 ± 1	NS
Focal lytic necrosis	2.44 ± 0.73	2.20 ± 0.77	NS
Portal inflammatory	1.67 ± 0.71	2 ± 0.85	NS
Fibrosis score	1.78 ± 1	3.73 ± 1.94	0.005
Patient survival (%)			
1, 3, 5 years	92, 92, 73	78, 61, 51	0.07

Percentage values are given in parentheses.

Table 5. Risk factors for poor outcome for HCV recurrence in graft (univariate analyses).

Factors	Good outcome ($n = 21$)	Poor outcome ($n = 10$)	*P*-value
Donor			
Mean age (years)	40 ± 24	48.5 ± 16	NS
>60 years	6 (29)	4 (40)	NS
Graft steatosis	2 (9.5)	6 (60)	0.004
Recipient			
Mean age (years)	58 ± 5	62 ± 6	NS
Age >60 years	10 (48)	9 (90)	0.049
HCC	10 (48)	5 (50)	NS
HBV	3 (14)	0	NS
Pre-LT viral load	4.6 ± 1.2	6 ± 0.4	0.06
Viral load >5	7 (47)	8 (100)	0.022
Surgery			
Ischaemic time >10 h	3 (14)	0	NS
Postoperation			
Mod/sev ischaemic injury	3 (15.8)	4 (36)	NS
Acute rejection	7 (33)	5 (50)	NS
Severe acute rejection	2 (10)	1 (10)	NS
Intent-to-treat groups			
TACRO	13 (62)	4 (40)	NS
TACRO + ST	8 (38)	6 (60)	
Real groups			
Real TACRO	10 (47)	1 (10)	0.055
Real TACRO + ST	11 (53)	9 (90)	
Intent-to-treat groups – according to rejection			
TACRO no rejection	10 (48)	1 (10)	NS
TACRO + rejection	3 (14)	3 (30)	
TACRO + ST no rejection	4 (19)	4 (40)	
TACRO + ST + rejection	4 (19)	2 (20)	

Percentage values are given in parentheses.
Good outcome: patients with normal liver function test, no clinical evidence of HCV recurrence on the graft and no clinical signs of cirrhosis plus patients with chronic active hepatitis on the graft but no clinical signs of cirrhosis. Poor outcome: Patients with cirrhosis on the graft and clinical signs of compensated or decompensated cirrhosis or patients who had died or had been retransplanted because of HCV recurrence on the graft.

TACRO + ST, almost reaching statistical significance ($P = 0.07$).

Risk factors for poor outcome of HCV recurrence
Recipient age over 60 years, graft steatosis, pre-LT viral load higher than 5 ($\log_{10}$ HCV RNA copies/ml) and treatment with steroids (either at baseline or subsequently) were found to be statistically significant for poor outcome of HCV recurrence by univariate analysis (Table 5).

Discussion

In our experience, immunosuppression regimen with tacrolimus in monotherapy after LT is feasible and safe. Patient survival, acute rejection rates and immune graft loss were similar to our standard regimen of tacrolimus and steroids. Sixty per cent of patients in the study group never received steroids in the post-LT period whereas 40% were treated with steroids for rejection. Steroid withdrawal was started at 3 months post-LT in the TACRO + ST group, and at 10 months of follow-up 90% of patients in both groups were free of steroids. The incidence of rejection in our study was lower than in previous studies with monotherapy with either neoral or tacrolimus [4,5], probably because of the high proportion of older patients, all of whom had postnecrotic cirrhosis mainly of alcohol or HCV origin. It is well known that senior and alcoholic patients have lower immunoreactivity and a lower incidence of acute rejection. This protocol may not be applicable to younger patients with autoimmune or cholestatic diseases who have higher

inmunoreactivity and, consequently, suffer more frequent and severe rejection episodes. In terms of safety, we could not demonstrate a significant benefit of avoiding steroids, probably because of the low number of patients included in the study. However, a trend was observed towards less *de novo* arterial hypertension and diabetes in the monotherapy group, whereas renal insufficiency was higher in the TACRO group.

Concerning pharmacokinetics studies, in the TACRO + ST group, higher doses of tacrolimus were required to reach the same blood levels achieved with tacrolimus alone. This finding confirms the increase in tacrolimus metabolism due to cytochrome P450 3A4 iso-enzyme (CYP3A4) induction by steroids, and shown in the report of van Duijnhoven *et al.* [22].

HCV subgroup of patients

Recurrent hepatitis C viral infection is universal post-LT. Almost half the patients present acute graft hepatitis early after LT and 90% develop chronic active hepatitis with evolution to cirrhosis in 30% of cases at 5 years post-LT [23]. Evolution to liver failure and death is very rapid once these patients present decompensated cirrhosis. Results obtained with retransplantation for recurrent HCV infections are poor. Recent data from UNOS and the Spanish Liver Transplant Registry have shown lower patient and graft survival in HCV patients [16,17,24]. Moreover, reports suggested that graft outcome of LT for HCV may have deteriorated in recent years. Hence, the subgroup of hepatitis C patients was evaluated independently to assess the potential benefit of a steroid-free immunosuppression regimen.

In our experience, there was a tendency for the subgroup of HCV patients to have more acute rejections diagnosed than HCV-negative patients. Difficulty in liver biopsy interpretation between acute rejection and HCV hepatitis, or the coexistence of both, has sometimes led to patients with recurrent hepatitis C being treated for rejection with steroid boluses. TACRO and TACRO + ST groups had similar demographic characteristics and acute rejection rates. Analysis of HCV recurrence in the graft showed a lower, although not significant, incidence of graft hepatitis in the TACRO monotherapy group as well as a higher proportion of patients with good outcome. However, these differences did not reach statistical significance, probably because of the scant number of patients studied.

Although further grouping down patients into real-TACRO and real-TACRO + ST leaves only few patients in each group, we were interested in knowing the evolution of graft hepatitis in the patients who never received steroids and compared this group with the remaining patients who received steroids either for protocol or to treat acute rejection episodes. Significantly lower levels of hepatitis C viraemia were found during the early weeks and months in patients without steroids, thereby confirming the findings of Garcia Retortillo *et al.* [25] and Boker *et al.* [26]. This may have been the reason for the lower incidence of acute graft hepatitis and its milder course in all 45% of steroid-free patients who presented it. We observed that a cut-off point in viral load pre-LT of $>\log_{10} 5$ was predictive of a poor outcome. At 12 months, viral loads in our study were similar in both groups, probably because at that time point most of the patients in both groups were free of steroids and received the same amount of immunosuppression. In contrast to our findings, Papatheodoridis *et al.* [15] reported higher levels of HCV RNA at 3 months in patients with single initial therapy compared with those receiving double therapy. However, a correlation was found between viral load at 12 months, duration of steroid treatment and extent of fibrosis. All these reports indicate a correlation between viral load and the amount of immunosuppression.

After a mean follow up of 44 months, evolution of HCV graft infection was significantly better in the steroid-free group. Only one patient presented a poor outcome (compensated cirrhosis), whereas the rest had good outcome (normal liver function or mild chronic active hepatitis). These findings were confirmed by a significantly lower fibrosis score in the liver biopsy study. Nevertheless, the possible effects of steroids on HCV recurrence and fibrosis remain controversial. A recent study by Fasole *et al.* [27] defended the beneficial effect of steroids on avoiding fibrosis in graft HCV recurrence. The hypothesis of Berenguer [28] is that the immune system becomes reconstituted following steroid withdrawal and dose reduction in calcineurin inhibitors and immune-mediated liver damage may then occur, together with progression to severe forms of chronic hepatitis.

There are many factors which influence the outcome of graft hepatitis C re-infection. Liver graft quality is extremely important: suboptimal donor liver either due to steatosis, old donor age, fibrosis, etc. results in important ischaemic–reperfusion injury, activation of inflammatory processes, greater susceptibility to acute rejection and probably more severe HCV graft re-infection. Recipient age over 60 years appeared to be significant in our study as well as in other studies [16,29–33]. In a previous study [34] concomitant CMV hepatitis was found to aggravate the evolution of hepatitis C; no case of CMV hepatitis was found in the present study. The link between anti-rejection therapy and worsening of hepatitis C seems very clear. Many authors and ourselves have demonstrated an association between more aggressive recurrence of hepatitis and evolution to fibrosis and cirrhosis and episodes of treated rejection [31,35,36].

Finally, patient survival was much better in real-TACRO, although the low number of patients in each group did not allow to reach statistical significance. Altogether, we can affirm that outcome of HCV graft infection was better in patients who could be maintained without steroids after LT. All HCV-related deaths occurred in the group receiving steroids from the beginning, or during follow-up. This study is one of the few prospective and randomized trial of immunosuppression regimen based on tacrolimus in monotherapy since the first post-transplant day, and although it was not originally designated to look at hepatitis C this is the first prospective randomized study of monotherapy regimen and recurrent hepatitis C infections. Another strong point of the trial is the long-term follow-up presented in patients without steroids. Other authors have reported differences in recurrent hepatitis but not long-term evolution of the graft. The few number of patients enrolled and the lack of periodically protocol biopsies in HCV patients are the weak points of the study. However, we should be able to learn about these findings and in fact, this study has prompt us to change our standard immunosuppressive regimen in HCV positive patients, which is based at the moment on tacrolimus plus MMF.

In summary, (i) a steroid-free immunosuppression protocol based on tacrolimus monotherapy is safe in a cohort of senior patients transplanted mainly for alcoholic or viral cirrhosis. Patient and graft survival and acute rejection and steroid-resistant rejection rates were similar to those of our standard tacrolimus short-term steroid protocol. (ii) No significant benefit was obtained in terms of side-effects of this steroid-free protocol although a trend was observed towards less hypertension and diabetes but more nephrotoxicity. (iii) The principal benefit could be in patients transplanted for HCV cirrhosis, as a trend towards lower incidence and milder course of acute recurrent HCV hepatitis and better long-term outcome was found in the group without steroids. (iv) This benefit was more evident in patients receiving no steroids at all after LT. Therefore, the key of future protocols for HCV patients would be a more effective steroid-free immunosuppression to achieve acute rejection rates of <10%; consequently, more than 90% of HCV LT patients will be steroid-free.

Acknowledgements

The study was supported, in part, by a grant from Fujisawa GM.

References

1. Billingham RE, Krohn PL, Medawar PB. Effect of cortisone on survival of skin homografts in rabbits. *Br J Med* 1951; **1**: 1157.
2. Eason JD, Loss GE, Blazek J, Nair S, Mason AL. Steroid-free liver transplantation using rabbit antithymocyte globulin induction: results of a prospective randomised trial. *Liver Transpl* 2001; **7**: 693.
3. Margarit C, Martinez-Ibañez V, Tormo R, Infante D, Iglesias J. Maintenance immunosuppression without steroids in pediatric liver transplantation. *Transplant Proc* 1989; **21**: 2230.
4. Rolles K, Davidson BR, Burroughs AK. A pilot study of immunosuppressive monotherapy in liver transplantation: tacrolimus versus microemulsified cyclosporin. *Transplantation* 1999; **68**: 1195.
5. Tisone G, Angelico M, Palmieri G, *et al.* A pilot study on the safety and effectiveness of immunosuppression without prednisone after liver transplantation. *Transplantation* 1999; **67**: 1308.
6. Ringe B, Braun F, Schutz E, *et al.* A novel management strategy of steroid-free immunosuppression after liver transplantation: efficacy and safety of tacrolimus and mycophenolate mofetil. *Transplantation* 2001; **71**: 508.
7. Reding R. Steroid withdrawal in liver transplantation. Benefits, risks and unanswered questions. *Transplantation* 2000; **70**: 405.
8. Keteman NM. Steroid-free immunosuppression: balancing efficacy and toxicity. *Liver Transpl* 2001; **7**: 698.
9. Stegall MD, Wachs ME, Everson GT, *et al.* Prednisone withdrawal 14 days after liver transplantation with mycophenolate. *Transplantation* 1997; **64**: 1755.
10. Trotter JF, Wachs M, Bak T, Trouillot T, Stolpman N, Everson GT. Liver transplantation using sirolimus and minimal cortico-steroids (3-day taper). *Liver Transpl* 2001; **7**: 343.
11. Lerut JP. Avoiding steroids in solid organ transplantation. *Transpl Int* 2003; **16**: 213.
12. Lucey MR. Changing perspectives on the role of corticosteroids after liver transplantation. *Liver Transplant Surg* 1999; **5**: S58.
13. Margarit C, Rimola A, Gonzalez-Pinto I, *et al.* Efficacy and safety of oral low-dose tacrolimus treatment in liver transplantation. *Transpl Int* 1998; **11**(Suppl. 1): S260.
14. Figueras J, Bernardos A, Prieto M, *et al.* Steroid-free regimen with daclizumab, mycophenolate mofetil, and tacrolimus in liver transplant recipients. *Transplant Proc* 2002; **34**: 1511.
15. Papatheodoridis GV, Barton SG, Andrew D, *et al.* Longitudinal variation in hepatitis C virus (HCV) viraemia and early course of HCV infection after liver transplantation for HCV cirrhosis: the role of different immunosuppression regimens. *Gut* 1999; **45**: 427.
16. Charlton M. The impact of advancing donor age on histologic recurrence of hepatitis C infection: The perils of ignored maternal advice. *Liver Transpl* 2003; **9**: 535.
17. Adam R, Cailliez V, Majno P, *et al.* Normalised intrinsic mortality risk in liver transplantation: European Liver

Transplant Registry Study. *The Lancet* 2000; **356**: 621. Available at: http://www.eltr.org/publi/publications
18. Demetris AJ. Banff schema for grading liver allograft rejection. An International Consensus Document. *Hepatology* 1997; **75**: 3.
19. Bilbao I, Charco R, Balsells J, *et al.* Risk factors for acute renal failure requiring dialysis after liver transplantation. *Clin Transplant* 1998; **12**: 123.
20. Martell M, Gomez J, Esteban JI, *et al.* High-throughput real-time reverse transcription-PCR quantitation of hepatitis C virus RNA. *J Clin Microbiol* 1999; **37**: 327.
21. Ishak K. Baptista A, Bianchi L, *et al.* Histological grading and staging of chronic hepatitis. *J. Hepatol* 1995; **22**: 696.
22. van Duijnhoven EM, Boots JMM, Christiaans MHL, Stolk L, Undre NA, van Hooff JP. Increase in tacrolimus trough levels after steroid withdrawal. *Transpl Int* 2003; **16**: 712.
23. Prieto M, Berenguer M, Rayon JM, *et al.* High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. *Hepatology* 1999; **29**: 250.
24. Forman LM, Lewis JD, Berlin JA, *et al.* The association between hepatitis C infection and survival after liver transplantation. *Gastroenterology* 2002; **122**: 889.
25. Garcia-Retortillo M, Forns X, Feliu A, *et al.* Hepatitis C virus kinetics during and immediately after liver transplantation. *Hepatology* 2002; **35**: 680.
26. Boker KHW, Dalley G, Bahr MJ, *et al.* Long-term outcome of hepatitis C virus infection after liver transplantation. *Hepatology* 1997; **25**: 203.
27. Fasole CG, Netto GJ, Thomas M, *et al.* Corticosteroid (CS) maintenance and progression of hepatitis C histologic recurrence (HHCVR) in liver transplant recipients (OLT) at one and two years (Y) post transplant (TX): a dose-dependent benefit?. *American Transplant Congress* 2003; 558 (abstract).
28. Berenguer M. Natural history of recurrent hepatitis C. *Liver Tanspl* 2002; **8**: S14.
29. Ghobrial RM, Steadman R, Gornbein J, *et al.* A 10-year experience of liver transplantation for hepatitis C: analysis of factors determining outcome in over 500 patients. *Ann Surg* 2001; **234**: 384.
30. Sreekumar R, Gonzalez-Koch A, Maor-Kendler Y, *et al.* Early identification of recipients with progressive histologic recurrence of hepatitis C after liver transplantation. *Hepatology* 2000; **32**: 1125.
31. Berenguer M, Crippin J, Gish R, *et al.* A model to predict severe HCV-related disease following liver transplantation. *Hepatology* 2003; **38**: 34.
32. Burak KW, Kremers WK, Batts KP, *et al.* Impact of cytomegalovirus infection, year of transplantation, and donor age on outcomes after liver transplantation for hepatitis C. *Liver Transpl* 2002; **8**: 362.
33. Wali MH, Heydtmann M, Harrison RF, Gunson BK, Mutimer DJ. Outcome of liver transplantation for patients infected by hepatitis C, including those infected by genotype 4. *Liver Transpl* 2003; **9**: 796.
34. Otero J, Gavalda J, Murio JE, *et al.* Cytomegalovirus disease as a risk factor for graft loss and death after orthotopic liver transplantation. *Clin Infect Dis* 1998; **26**: 865.
35. Sheiner PA. Immunosuppression modifications in hepatitis C. *Curr Opin Organ Transplant* 2001; **6**: 327.
36. Gregory TE. Impact of immunosuppressive therapy on recurrence of hepatitis C. *Liver Transpl* 2002; **8**(Suppl. 1): S19.