ORIGINAL ARTICLE

Impact of steroid-avoidance immunosuppression on long-term outcome after liver transplantation for HCV cirrhosis : the need for well documented long-term follow-up

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Abstract

Aim : study impact of steroid avoidance on HCV recurrence after transplantation.

Methods and material : 35 HCV pats, being part of prospective, randomized, double-blind, placebo-controlled study comparing Tacrolimus (TAC)-Placebo (PLAC) (n = 14) to TAC-short-term (2 mo) low-dose steroid (STER) (n = 21), had 5 years follow-up. Primary endpoint was 1 and 5 years survival ; secondary (composite) endpoint comprised HCV related cirrhosis, re-transplantation (re-LT) and death.

Results : 1 and 5-years survival were 93% and 75% in TAC-PLAC group ; 91% and 66% in TAC-STER group (p 0.38). Two (14.3%) TAC-PLAC pats died due to HCV cirrhosis at 54 and 72 mo ; 7 (33%) TAC-STER pats died due to cholestatic hepatitis at 5.8 and 9 mo, to cirrhosis at 18, 22, 34, 73 and 79 mo (p 0.20). Composite endpoint at 5 years didn't show advantage in favor of TAC-PLAC patients (5/14 [35.7%] vs. 9/21 [42.8%] pts, p.0.69). Early biopsies seemed more favorable in TAC-PLAC pats ; at 5 years results were identical for both groups. Only 1 (7.1%) TAC-PLAC and 2 (9.5%) TAC-STER pats needed rejection treatment.

Conclusion : immunosuppression using steroid avoidance or short-term use had similar outcomes. Well documented long-term follow-up, including biopsies, is necessary in order to make conclusions in relation to impact of steroid use on outcome of HCV liver recipients. (Acta gastroenterol. belg., **2012**, 75, 411-418).

Key words : liver transplantation, HCV infection, steroid avoidance, immunosuppression, minimal immunosuppression, disease recurrence.

Abbreviations

AZA	azathioprine
CSR	corticosteroid-sensitive rejection
ELTR	European Liver Transplant Registry
HCV	hepatitis C viral infection
IS	immunosuppresion
MMF	mycophenolate mofetil
pts	patients
PLAC	placebo
(re)LT	liver (re)transplantation
S	degree of fibrosis
ST	cortico-steroids
STAV	steroid avoidance
STWD	steroid withdrawal
TAC	tacrolimus

Introduction

Hepatitis C viral cirrhosis (HCV) represents the main indication for adult liver transplantation (LT). During the period 1968-June 2008 17% of recipients collected in the European Liver Transplant Registry (ELTR) were transplanted because of HCV-related disease ; 27% of patients transplanted because of liver cirrhosis had a HCV infection (1). Unfortunately, most of these liver recipients present an allograft re-infection. The natural evolution of the HCV allograft infection is more rapid and aggressive in the immunocompromized patient (2-7). Indeed many recipients die or need a liver re-transplantation (re-LT) because of HCV induced allograft failure within five years after transplantation (4). Different donor and recipient factors have been identified to be responsible for this negative evolution ; immuno-suppression (IS) is one of them (4). The influence of minimal and steroid avoidance IS on the evolution of the viral allograft recurrent disease has been very rarely studied in a detailed way. The aim of the study of a small, well documented, series was to examine the long-term outcome of HCV viral disease under a tacrolimus (TAC) monotherapy and steroid (almost) avoidance (STAV) regimen.

Material and methods

This paper deals with a pre-planned subgroup analysis of thirty-five genotype 1b HCV positive patients out of a, previously published, large prospective, randomized, double-blind, placebo-controlled study comparing TAC (Prograft®, Astellas, JPN) -Placebo (TAC-PLAC) (n = 14) to TAC-short-term (2 mo) low-dose steroid (TAC-STER) (n = 21) IS in adult LT (8). During the period January 2000-May 2005, all HCV patients were included in the study, irrespective of their physical and

immunological condition. This HCV patient cohort represented 20.8% of the total cohort of 168 patients included in the original study. Ten TAC-PLAC and twelve TAC-STER patients had a hepatocellular cancer (HCCA). The patients were randomized into both IS schemes at the end of surgery using serially numbered, sealed and opaque envelopes. Both groups had similar characteristics except for cold ischemia time (Table 1). Three other HCV recipients who needed mycophenolate mofetil (MMF) (Cellcept®, Roche, CH) within the first six post-LT months because of renal insufficiency were excluded from the analysis.

In all patients a vena cava preservation technique was applied without use of veno-venous bypass, the graft implantation was done using a large latero-lateral cavo-caval anastomosis (9).

All patients had TAC-based IS, with levels adjusted according to the clinical situation. The first TAC dose was given 12 hours after the end of surgery. The dose was then progressively increased in order to reach trough whole-blood levels (determined by monoclonal fluorescence assay) around 6 to 8 ng/ml. Low-dosage TAC level was defined as a level below 6 ng/ml. Steroid and placebo were administered in identical plastic containers containing a similar number of identical, opaque capsules. Their number, corresponding to a reducing dose that covered a post-LT period of 64 days, were prepared by an independent pharmacist. In order to counteract ischemia-reperfusion injury all patients received 1000 mg of hydrocortisone (Solucortef®, Upjohn Pharmacia, S) ; 400 mg during the intervention, followed by 200 mg during the first three days. In the TAC-STER group methylprednisolone (MP) treatment (Medrol®-Upjohn-Pharmacia, S) was started at day 4 at a dose of 16 mg. Steroid or placebo were tapered from day 21 onwards. Every 14 days, steroid or placebo were reduced by 4 mg in order to be stopped in all patients at day 64, independently of any previously occurring immunological event. The total equivalent dose of MP in the TAC-STER group amounted up to 834 mg. From the sixth post-transplant month onwards MMF or azathioprine were eventually introduced in case of occurrence of nephro- and neurotoxicity.

All patients received identical intraoperative and postoperative care as well as histological follow-up (8). Biochemical and histological scores, expressed as mean ± SD, were calculated in order to diagnose rejection and its need for treatment. These scores were calculated at post-LT day 7, the time at which the incidence of rejection is highest. Progressive rise in total bilirubin and peripheral blood eosinophilia, absolute eosinophilia count above 600 mm³ and progressive lowering of platelets during day 5 to 7 post-LT were each scored 0 to 1. In order to avoid interference with these variables, no blood products and no other medications, were administered within the first post-LT week. A biochemical score of > 2 was considered significant (8). Biopsies, carried out at day 7, at 6 months, yearly and when clinically indi-

cated, were read blindly by two experienced transplant pathologists. The day 7 biopsy was graded according to the Banff score (10). Moderate or severe histological rejection was recorded if the score was ≥ 6 or ≥ 8. Treatment of early cellular rejection was considered *only* if biochemical (> 2) and histological (≥ 6) scores were present simultaneously (8). The clinical and pathological observance of rejection was followed strictly in order to avoid unnecessary use of high dose steroid boluses and anti-lymphocytic serum. Steroid avoidance (STAV) IS matters in this context as it eliminates the possibility of breakthrough rejection or sudden destabilization of liver function (12-14). Corticosteroid-sensitive rejection (CSR) was treated with 3 to 5 oral or IV boluses of 200 mg MP.

HCV re-infection was classified according to Ludwig taking into account portal (P) and lobular (L) infiltration as well as degree of fibrosis (S). These three parameters were scored from 1 to 4. S1 : means no fibrosis, S2 : beginning fibrosis, S3 : fibrosis and S4 : cirrhosis. S3 and S4 stages were considered together when analysing severity of disease progression (11). Due to lack of financial support, HCV-RNA dynamics were determined at baseline and at 12 months in six TAC-PLAC and seven TAC-STER patients only. HCV viral loads were measured by the COBAS AMPLICOR HCV MONITOR test, v2.0 (Roche Molecular Systems, Branchburg, NJ, USA).According to the manufacturer, the dynamic range of quantification is 600 to 700,000 HCV RNA UI/mL.

If the clinical evolution allowed to do so, HCV antiviral treatment, consisting of α-interferon (Pegasys®, Roche, CH or Pegintron®, Shering Plough, USA) and ribavirin (Rebetol®, Roche, CH or Copegus, Shering Plough, USA), was avoided as much as possible during the first post-transplant year based on the following arguments : (a) avoidance of interference with the 'natural' evolution of HCV under minimal IS ; (b) avoidance of possible triggering of auto-immune hepatitis or rejection ; (c) postponement of treatment to a later period at which this therapy is easier and more efficient and (d) finally compliance with the desire of many patients to postpone this, many times already experienced, cumbersome treatment until good recuperation from transplant surgery. Antiviral HCV treatment was given only in case rapid progression of the viral recurrence was shown between the 6 and 12 months biopsies.

Follow-up at 5 years from date of LT was complete for all patients. There were no dropouts or withdrawals in either intervention group. Following the practice of the ELTR, early and late events were divided into those occurring before and after the third post-LT month.

Primary endpoints of the study were graft and patient survival rates at 3, 12 and 60 months. Secondary endpoints were the progression of HCV allograft re-infection from 12 to 60 months and a composite HCV endpoint taking into consideration development of cirrhotic stage (Ludwig S4 score), re-LT and fatal outcome due to HCV re-infection.

Table 1. — **Patient characteristics of thirty-five HCV allograft recipients**

Characteristics		TAC PLACEBO n = 14	(%)	TAC STEROIDS n = 21	(%)	p
Recipient gender M/F		6/8		6/15		0.38
Recipient age (yrs)		56(44-69)		60 (41-70)		0.68
Hepatocellular cancer		10	(71%)	12	(57%)	0.67
Diabetes mellitus						
	pre-LT	1	(7.1%)	6	(28.5%)	0.20
	post-LT	8	(57.1%)	6	(28.5%)	0.09
Alcohol use		1	(7.1%)	2	(9.5%)	0.65
MELD	median	14 (8-30)		13(7-34)		
	mean	*15.3 ± 6.3*		*15.2 ± 7.9*		*0.97*
	< 15	8	(57.1%)	11	(52.3%)	0.78
	≥ 15	6	(42.8%)	10	(47.6%)	0.53
CHILD A / B		10	(71%)	14	(66.6%)	0.77
	C	4	(28.4%)	7	(33.3%)	0.53
UNOS 1-2		1	(7.1%)	6	(28.5%)	0.12
	3-4	13	(92.8%)	15	(71.4%)	0.13
Ischemia time						
Cold	median	707.5 (477-1100)		616 (57-1125)		0.07
	mean	*702 ± 166*		*548 ± 276*		
Warm	median	42.5 (27-57)		44 (17-570)		0.75
	mean	*41.6 ± 8.1*		*66.4 ± 116*		
Donor age (yrs)		47 (15-68)		43(12-78)		0.68
Donor age > 50 yrs		5	(35.5%)	9	(42.8%)	0.67
Donor gender M/F		5/9		9/12		0.67
CMV D+ / R-		1	(7.1%)	1	(4.7%)	0.65
Surgical technique						
	Whole liver / variants	11/3	(52.4%)	14/7	(66.6%)	0.44
	Living donor	0	(0%)	3	(14.2%)	0.20
Highest post-LT AST (UI/L)		1089 (326-4064)		874 (269-2789)		0.12
	mean	*1399 ± 966*		*927 ± 693*		
Biliary complications		1	(7.1%)	6	(28.5%)	0.20
Post-transplant infection (pts)						
	≤ 3mo	7	(50%)	13	(61.9%)	0.48
	> 3-12 mo	3	(21.4%)	8	(38%)	0.46
	≤ 12 mo	9	(64.2%)	18	(85.7%)	0.22
Rejection (all corticosensitive)						
	≤ 3mo	1	(7.1%)	2	(9.5%)	0.65
	> 3 ≤ 12 mo	0	(0%)	0	(0%)	1.00
Re-transplantation < 5 years						
	Total	1	(7.1%)	4	(18%)	0.32
	HCV recurrence	1	(7.1%)	4	(18%)	0.32
				(1 combined HCV and ischemic type biliary lesion)		

The results were analysed primarily according to the type of study medication (TAC-PLAC vs. TAC-STER). Continuous variables were expressed as mean, median and range, and were compared between groups by the Student's t-test for parametric variables and the Mann-Whitney U test for non parametric variables. Categorical variables were analysed with Pearson's chi-square test or Fischer's exact test as indicated. Survival rates were estimated by Kaplan-Meier method and compared by the log-rank test. A p value < 0.05 was considered as the criterion of statistical significance. The analyses were performed using the SPSS 16.0 (SPSS Inc., Chicago, IL) statistical software. There was no power calculation for the here reported subgroup of HCV patients ; such calculation was only done in relation to the whole group of 168 patients, being the subject of a previous publication (8).

The institutional review board approved the study. Informed written consent was obtained from all patients or the next of kin before LT. All patients, health care providers and outcome assessor teams were blinded until the 12-month analysis was complete ; afterwards the reading of the pathology slides was continued blindly. This investigator-driven study was designed, initiated and managed by the senior author (JL). The authors adhered to the guidelines of the Consolidated Standards on Reporting Trials (CONSORT).

Results

Three months, one and five year patient survival rates were 100% ; 93% and 76% in TAC-PLAC group and 100% ; 91% and 66% in TAC-STER group (p 0.38) (Fig. 1).

Three months, one and five year graft survival rates were 100%, 93% and 69% in the TAC-PLAC group and 95.2%, 91% and 61% in the TAC-STER group (p 0.46) (Fig. 2).

Two TAC-PLAC patients died due to HCV cirrhosis at 54 and 72 months. The former patient also presented with an abdominal PTLD, the latter patient had re-LT at 29 months because of recurrent HCV cirrhosis. He
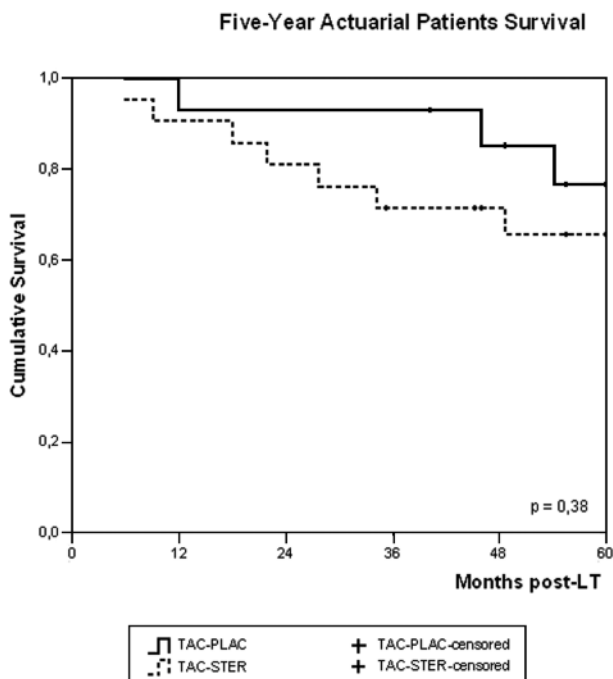
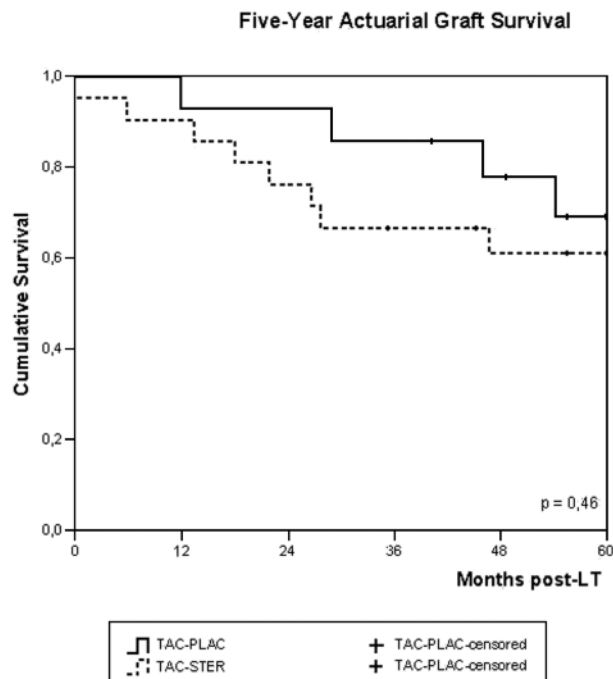Fig. 1. — Patient survival after LT for HCV related cirrhosis

Fig. 2. — Graft survival after LT for HCV related cirrhosis

finally died of recurrent HCV 43 months later. One patient died due to recurrent HCCA at 12 months and one patient died of de novo unspecified gynaecological tumor at 46 months.

Seven TAC-STER group patients died due to HCV recurrent disease : two died at 5.8 and 9 months due to cholestatic hepatitis and five died due to HCV cirrhosis at 22, 34, 73 and 79 months ; one patient who died at 18 months of recurrent HCV also presented a PTLD. One patient died at 48.2 months due to pulmonary embolism occurring two months after re-LT done because of HCV cirrhosis. One patient died due to recurrent HCCA at 28 months and one died due to stroke at 75 months.

One TAC-STER patient had re-LT at 26 mo for recurrent HCV ; he is doing very well 37 months later.

In total two (14.3%) of 14 TAC-PLAC patients and seven of 21 (33%) TAC-STER patients died of HCV recurrence during the study period (p 0.20).

When taking into consideration the composite HCV endpoint consisting of cirrhotic stage and both re-LT and death due to recurrent allograft disease there was no advantage in favour of TAC-PLAC patient group at five years (5/14 [35.7%] vs. 9/21 [42.8%] pts ; p.0.67).

The low (8.5%) incidence of rejection is explained by the careful observation of clinical, biochemical and histological evolution of the patients as described. Only one (7.1%) TAC-PLAC needed treatment for a CSR at day 15. Two (9.5%) TAC-STER patients needed anti-rejection treatment : one patient was urgently re-transplanted at day 7 because of hemorrhagic allograft necrosis ; the second patient developed a CSR at day 25 due to a dras-

tic reduction of TAC therapy made necessary because of severe neurotoxicity. This patient died later due to a stroke. No patient presented with a ductopenic rejection.

Antiviral treatment was applied during the first post-transplant year in 2 (14.3%) of 14 TAC-PLAC patients and in 3 (14.3%) of 21 TAC-STER patients. Two TAC-STER patients were treated because of development of cholestatic hepatitis ; the three other patients were treated based on progression of their Ludwig scores. In total four (28.5%) TAC-PLAC and eight (38.1%) TAC-STER patients received antiviral treatment during the study period. This treatment consisted in all but one patients of a combination of α-interferon and ribavirin ; one TAC-STER patient got α-interferon only. In two TAC-STER patients antiviral treatment had to be interrupted because of severe side-effects. One out of four TAC-PLAC patients and two out of eight TAC-STER patients had a sustained response to antiviral treatment.

The histological evolution of the HCV allograft re-infection at one, three and five years post-LT is shown in table 2. There was no statistically significant difference between both patient groups at all studied time points. Only two (5.7%) of the 35 patients had a normal biopsy at 12 months. The two lethal cholestatic hepatitis were observed in the TAC-STER group. Although early biopsies seemed to indicate a more favourable outcome in TAC-PLAC patients, evolution of biopsies at four and five years became almost identical between both patient groups. There was no correlation between the results of the 6 month biopsy and the later evolution towards cholestatic hepatitis and (decompensated) liver cirrhosis ; indeed seven patients had S1 score ; two had S2

Table 2. — **Histological findings following Ludwig on protocol biopsies in HCV allograft recipients**

Group	TAC-PLACEBO	TAC-STEROIDS	
At one year	**14**	**21****	**0.27°**
Normal	0	2 (9.5%)	
Aspecific hepatitis	2 (14.3%)	0	
S1	7 (50%)	8 (38.1%)	
S2	5 (35.7%)	8 (38.1%)	
S3	0	1 (4.8%)	
S4	0	0	
Fibrosing cholestatic hepatitis	0	2** (9.5%)	
At three years*	**13**	**15 SURVIVORS**	**0.42°**
S1	7 (53.8%)	7 (46.7%)	
S2	1 (7.7%)	1 (6.6%)	
S3	4 (30.8%)	4 (26.7%)	
S4	0	3 (20%)	
Not available	1* (7.7%)	0	
At five years	**11**	**14 SURVIVORS**	**0.67°**
S1	5 (45.5%)	4 (28.6%)	
S2	2 (18.2%)	3 (21.5%)	
S3	1 (9.1%)	5 (35.8%)	
S4	3 (27.2%)	1 (7.1%)	
Not available	1* (9.1%)	1* (7.1%)	
(re-LT at 28.9 mo ;	(re-LT at 26 mo)		
	- died at 72 mo)	- doing well at 5 yrs)	

* biopsies of retransplanted patients are considered as not available.
** two patients died of cholestatic hepatitis at 5.6 and 9 months ; their Ludwig scores in the 6 mo biopsy were S1 and S2.
° statistical evaluation comparing each time S1-S2 and S3-S4.

score and S3 score. The median TAC levels were similar in both groups throughout the whole study period (Table 3). Baseline HCV-RNA levels at day 0 were similar between the two groups [8.2 (0.6-3370) in TAC-PLAC vs. 702 (0.6-3060) × 10³ UI/ml. The HCV-RNA levels markedly raised, in the absence of any antiviral treatment, 12 months post-LT in five of six TAC-PLAC and four of seven TAC-STER tested patients ; there was however no significant difference between both groups [2920 (20.6-7800) in TAC-PLAC vs. 3040 (500-19000) × 10³ UI/ml in TAC-STER patients ; p 0.49].

The specificities of the antiviral and immunosuppressive treatments going along with the biopsy findings during the study period are listed in table 3.

Discussion

In HCV liver recipients, allograft re-infection is the rule (2-4). During the last years different viral (pre-transplant viral load, genotype and quasi-species), donor (age with a cut off value varying from 40 to 50 years, gender and graft steatosis), host (HLA type, HLA-DR match, immune genetic background, immune status, race, gender, age, diabetes, alcohol use, viral co-infection eg ; CMV, antiviral treatment and last but not least immunosuppressive therapy) and surgical (warm ischemia time) factors all have been identified to have an impact on the severity of viral disease recurrence (4,6,7,15-18). The impact of IS on HCV recurrence is a major topic of debate especially since Berenguers' reports in 2000 and 2002 indicating a more aggressive behavior of HCV reinfection during recent years, possibly linked to re-

inforced IS schemes (16,19). High dose steroid boluses and anti-lymphocytic antibodies were clearly shown to hasten viral disease progression (4). The type of calcineurin inhibitor (tacrolimus versus cyclosporine) and the type of anti-metabolite (MMF versus azathioprine) seems to impact in a minor way on disease evolution (20-23). The use of steroids and the modality of steroid administration in contrast are said to be more relevant (14,18,24-25). Three studies addressed the impact of steroid withdrawal (STWD) on HCV allograft recurrence. Berenguer and Vivarelli reported respectively that STWD after more than 6 months (late STWD), and steroid taper over a period of 24 months (slow STWD or taper) were associated with less severe HCV recurrence (26-27). Humar however showed in a historical control study that histological recurrence was significantly lower after rapid (6 days) steroid discontinuation (28). Nine STAV studies were done in relation to outcome of LT in HCV patients (29-38). Unfortunately only one study, comparing triple and quadruple drug induction IS, was done in a placebo-controlled and double blind fashion (29). In most studies recipients were on triple, TAC based, IS. TAC monotherapy was considered in two study arms only (31,36). Except for the two Barcelona studies (31,34-35), results were disappointing in relation to HCV recurrence. The influence of IS on the post-transplant evolution of HCV-RNA load was reported in seven of these studies. HCV-RNA levels were higher (2×), lower (2×), higher and lower depending on time lapse between LT and RNA monitoring (1×) and stable (2×) (30-34,36,38). Lower viral replication correlated only in Margarits' steroid-free TAC monotherapy study, including with a milder HCV-recurrence. The results of

Table 3. — **Antiviral and immunosuppressive treatments of HCV allograft recipients**

Group	TAC-PLACEBO n = 14	TAC-STEROIDS n = 21	
Antiviral treatment			
Pre-LT	7	10	0.89
Post-LT	4	8	0.56
Treatment ≤ 12 mo	2	3	
Treatment initiation	13.7 ± 9	20.5 ± 22.2	0.74
Treatment duration	12.7 ± 3.3	7.1 ± 5.2	0.41
Response	1	2	ns
Rejection treatment			
≤ 3 mo	1	2	ns
> 3 ≤ 12 mo	0	0	
Immunosuppression			
At 6 mo TAC-MONO	14/14	20/21	ns
TAC-MMF		1	
At 12 mo TAC-MONO	14/14	17/19	0.22
TAC-AZA/MMF		2	
TAC-level	4.4 (3.3-11.4)	5.9 (4.3-23.1)	0.26
At 24 mo TAC-MONO	12/13	14/17	0.45
TAC-AZA/MMF	1	3	
At 36 mo TAC-MONO	12/13	13/15	0.27
TAC-AZA/MMF	1	24	
TAC-level	3.5 (2.0-5.1)	3.5 (2.9-4.5)	0.90
At 48 mo TAC-MONO	11/12	13/15	0.57
TAC-AZA/MMF	1	2	
At 60 mo TAC-MONO	10/10 (dose-spacing* 7)	9/14 (dose-spacing 3)	0.12
TAC-MMF	0	3	ns
MMF	0	1	ns
MMF-RAPA	0	1	ns
TAC-level	3.7 (2.0-8.3)	3.5 (0.0-7.0)	0.24

AZA : azathioprine ; MMF : mycophenolate mofetil ; RAPA : rapamycin.
*dose-spacing means absence of daily intake of tacrolimus monotherapy.

viral kinetics, although controlled in a small patient group, go in the same direction. Histological findings in the 3 years biopsy (done in 83% of patients) did however not show significant differences between TAC monotherapy and TAC-STER patient groups. Fibrosis score and development of cirrhosis was significantly lower only in 12 patients who never received steroids in the TAC monotherapy arm including for treatment of rejection (called 'real' TAC monotherapy) [9% vs. 46%] in 23 patients having TAC-3 month steroid treatment and in TAC monotherapy patients treated with steroids because of rejection (p 0.046) (31).

Llado showed in a prospective randomized, open label, multi-centre Spanish study, including 89 patients, comparing Neoral® (Novartis,CH) and Simulect® (Novartis,CH) to Neoral, Simulect and 3 months steroids that early (6 months) and 'late' (2 years) histological outcome of HCV allograft infection was significantly better under steroid free IS in relation to lobular activity only (p 0.01) but not in relation to fibrosis (34-35). Segevs' recent meta-analysis (which didn't include the recent Llado report) showed that HCV recurrence was lower with steroid avoidance IS (rr 0.90, p 0.03) but no single study in this meta-analysis reached statistical significance on its own (24).

The here presented study challenges the results of the two Barcelona studies. Our study has several features which are of major importance when judging results of

LT in HCV recipients : (a) it is the only reported analysis done in the context of a prospective randomized double-blind, placebo controlled, study analyzing the evolution of hepatitis C using minimal IS. ; (b) the interpretation of all, biochemical, clinical, as well as histological, results was done in a blinded matter during the first post-transplant year and the biopsy reading was further done blindly up to the fifth post-LT year ; (c) all patients had a minimal follow-up of five years including yearly protocol biopsies and (d) the results were not 'contaminated' by aggressive antiviral and anti-rejection treatment, especially during the first post-transplant year. It is indeed well documented that aggressive anti-rejection treatment substantially triggers viral replication. The incidence of rejection in the Margarit and Llado studies were 44% and 19% respectively in contrast to 8.5% in our study. The great importance of long-term follow-up can be taken from table 2. Indeed the apparent, but statistically not significant, benefit of steroid-avoidance IS seen in the first year biopsies was waved away during the further, well documented, histological follow-up. After the five years time span the histological picture of viral allograft recurrence became indeed identical in both study groups. The composite endpoint of the study, taking into account development of cirrhosis and re-transplantation and/or death due to HCV allograft re-infection, judged to be a better tool to evaluate the impact of IS on the outcome of these recipients, was also similar in both study arms.

When interpreting these results one should be aware that this lack of difference could be due to the small number of patients (type II error). The major information about minimal IS in HCV recipients relates however to the safety of this approach. Indeed no allograft was lost due to immunological reasons and the need for treatment of rejection was very low. This safety should also be seen in the context of the major morbidity and mortality related to post-transplant antiviral treatment. The minimal IS approach might therefore represent a progress in this difficult and many times discouraging field of LT. The optimal treatment of the HCV recipient could indeed first consist of a minimization IS followed, after one year, when liver function is stabilized, by an effective antiviral treatment, done under strict histological guidance (39-41) completed with repetitive HCV-RNA levels (42-44). The result of the liver biopsy at one year combined with viral kinetics has been said to identify those patients prone to develop progressive recurrent disease (41). Such combined monitoring could therefore be of great help not only to avoid unnecessary reinforcement of IS but also to allow better targeting of anti-viral therapy (44).

The obtained results in both the Brussels and Barcelona studies, all including a small number of recipients, appeal for larger prospective randomized, (and if possible) double-blind, placebo controlled and well documented studies. Double-blind analysis is fundamental in these recipients as differentiation of rejection from viral re-infection is many times very difficult, especially during the early post-LT period. Unjustified reinforcement of IS for a supposed rejection, using boluses of steroids or anti-lymphocytic sera, is many times the first step to trigger viral replication and to speed up the disease progression making thereby a correct interpretation of results and of the real influence of the immunosuppressive schemes on viral disease recurrence hazardous. The advantage of steroid avoidance IS must also be seen in this context as this strategy eliminates another major confounding factor, the breakthrough rejection, leading frequently to administration of high-dose steroid boluses (14).

The ideal immunosuppressive strategy for HCV patients is clearly not yet determined as demonstrated by the contradictory results of different IS trials. Further well conducted studies are badly needed in order to identify for the best possible IS in the HCV recipients. The combination of 'appropriate IS scheme(s), better and safer antiviral therapies (13,45-46) and optimization of different donor and surgical variables such as donor age, graft steatosis and ischemia times (3,4,6,7) are necessary to improve the outcome of these recipients. But whatever measure or immunosuppressive scheme is taken, it cannot be stressed enough that long-term, well documented histologic follow-up of these liver recipients is a must in order to avoid early and many times misleading conclusions in relation to the impact of any IS scheme on the outcome of HCV patients (47).

Acknowledgements

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