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Early Withdrawal of Calcineurin Inhibitors and Everolimus Monotherapy in *de novo* Liver Transplant Recipients Preserves Renal Function

M. Masetti[a,*], R. Montalti[a], G. Rompianesi[a], M. Codeluppi[b], R. Gerring[c], A. Romano[a], B. Begliomini[d], F. Di Benedetto[a] and G. E. Gerunda[a]

[a] *Liver and Multivisceral Transplantation Center,* [b] *Division of Infectious Diseases, Azienda Ospedaliero-Universitaria di Modena-Policlinico, Modena, Italy*
[c] *Miller School of Medicine, University of Miami, Miami, FL*
[d] *Division of Anesthesiology, Azienda Ospedaliero-Universitaria di Modena-Policlinico, Modena, Italy*
***** *Corresponding author: Michele Masetti, m.masetti@ausl.bologna.it*

We designed a randomized trial to assess whether the early withdrawal of cyclosporine (CsA) followed by the initiation of everolimus (Evr) monotherapy in *de novo* liver transplantation (LT) patients would result in superior renal function compared to a CsA-based immunosuppression protocol. All patients were treated with CsA for the first 10 days and then randomized to receive Evr in combination with CsA up to day 30, then either continued on Evr monotherapy (Evr group) or maintained on CsA with/without mycophenolate mofetil (CsA group) in case of chronic kidney disease (CKD). Seventy-eight patients were randomized (Evr n = 52; CsA n = 26). The 1-year freedom from efficacy failure in Evr group was 75% versus 69.2% in CsA group, p = 0.36. There was no statistically significant difference in patient survival between the two groups. Mean modification of diet in renal disease (MDRD) was significantly better in the Evr group at 12 months (87.7 ± 26.1 vs. 59.9 ± 12.6 mL/min; p < 0.001). The incidence of CKD stage ≥3 (estimated glomerular filtration rate <60 mL/min) was higher in the CsA group at 1 year (52.2% vs. 15.4%, p = 0.005). The results indicate that early withdrawal of CsA followed by Evr monotherapy in *de novo* LT patients is associated with an improvement in renal function, with a similar incidence of rejection and major complications.

Key words: Cyclosporine, everolimus, liver transplantation, renal function, renal impairment

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Introduction

Immunosuppressive therapy based on calcineurin inhibitors (CNIs) in liver transplantation (LT) has evolved from improving short-term graft outcome and patient survival to a reduction of acute rejection rates and an improvement in other clinical endpoints associated with the risk of longer-term allograft failure.

The significance of renal dysfunction in liver transplant recipients is illustrated by a registry analysis of data on 36 849 such patients in the United States, where chronic renal failure in liver transplant recipients has a cumulative incidence of around 20% by 3 years post-LT, and is associated with a 4-fold increased risk of death (1). The major contributor to impaired renal function in a liver allograft recipient is exposure to CNIs (2,3). Indeed, renal dysfunction following LT may be of clinical impact as early as 6 months after surgery, clearly demonstrating the importance of early and proactive intervention to ameliorate or even prevent the emergence of chronic kidney disease (CKD) (4). The current clinical challenge is therefore to develop regimens that maintain high rates of efficacy while minimizing side effects.

Everolimus (Evr) (Certican Novartis; Basel, Switzerland), is a proliferation-signal inhibitor with immunosuppressive and antiproliferative activity (5).

Data from a CNIs withdrawal study in maintenance liver transplant recipients provide evidence that Evr, at doses within a monitored trough level, allows the elimination of CNIs without increasing the risk of acute rejection compared to patients who remain on CNI-based therapy. However, the same study showed that the late withdrawal of CNIs had no significant benefit on renal function, especially in patients with preswitch low creatinine clearance (6).

Reducing or eliminating the exposure of LT patients to CNIs within the first few months posttransplantation, before there is any evidence of renal dysfunction, should result in improved renal function. The purpose of this single-center, prospective, randomized, open-label, phase II trial was to evaluate whether early cyclosporine (CsA) withdrawal followed by Evr monotherapy in *de novo* liver transplant recipients leads to superior renal function while both

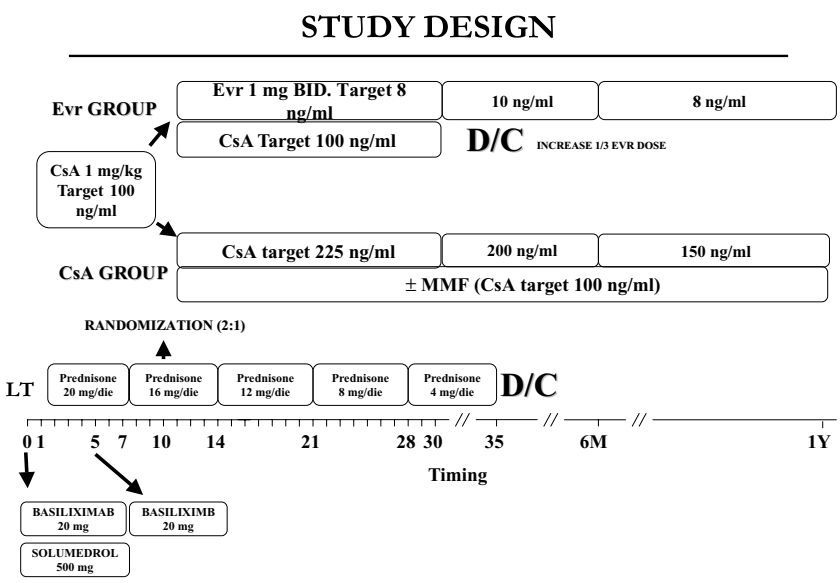

Figure 1: Study design. After a run-in period of 10 days after liver transplantation, patients were randomized in either the Evr group or the CsA group. D/C = discontinuation.

maintaining efficacy in preventing acute rejection and ensuring adequate safety.

To the best of our knowledge, this study provides the first report to examine early CNIs withdrawal followed by Evr monotherapy in *de novo* liver transplant recipients.

Patients and Methods

Study design

This was a 12-month, prospective, randomized, single-center, open-label, parallel-group study consisting of a screening period, ending on the day of randomization at 10 days posttransplantation, followed by a treatment period.

All patients received basiliximab (Simulect, Novartis, Switzerland) 20 mg intravenously (IV) on day 0 (within 6 h after reperfusion of the liver graft) and on day 5 after liver engraftment. Methylprednisolone was administered IV at a dose of 500 mg on day 0 followed by tapering of the steroid within 5 weeks (Figure 1).

All patients received prednisone (or equivalent) at the following dose: 20 mg/day in a single morning dose until day 7, 16 mg/day from day 8 to day 14, 12 mg/day from day 15 to day 21, 8 mg/day from day 22 to day 28 and 4 mg until day 35; thereafter, the drug was stopped.

The initial dose of CsA microemulsion (Neoral, Novartis) in all patients was 2 mg/kg/day, divided into two daily doses, to target a trough blood level (C_0) of 100 ± 25 ng/mL for the first 10 days.

Patients without severe postoperative complications, such as primary nonfunction, early re-LT, severe uncontrolled infection and severe surgical complications, were randomized on day 10 into one of the two following treatment groups on a 2:1 computer-based ratio:

Group 1: Early CNIs withdrawal followed by Evr monotherapy: The initial dose (day 10) of Evr was 2.0 mg/day, with the aim of achieving a C_0 level of 6–10 ng/mL. The dose was increased as appropriate on day 30, when CsA was abruptly discontinued, in order to reach and maintain an Evr target trough level of 8–12 ng/mL until the end of month 6, and 6–10 ng/mL thereafter. The dose of CsA was maintained at a target C_0 level of 100 ± 25 ng/mL until day 30.

Group 2: Standard CsA treatment: The dose of CsA was adjusted to target a C_0 level of 225 ± 25 ng/mL until day 30, then 200 ± 25 ng/mL until the end of month 6, and 150 ± 25 ng/mL thereafter. In patients with CNI-related complications, the CsA daily dose could be decreased in order to reduce the C_0 level by about 50% and to introduce mycophenolate mofetil (MMF) at a loading dose of 1 g BID followed by a maintenance dose of 500 mg TID.

Patients with severe Evr-related complication not resolved with study drug adjustment were withdrawn from the study.

Objectives

The primary objective of the study was to evaluate whether early CNIs withdrawal followed by Evr monotherapy in *de novo* liver transplant recipients would lead to superior renal function, compared to the CsA control, at 12 months posttransplantation. The primary efficacy endpoint, renal function, was assessed by estimated glomerular filtration rate (eGFR), calculated using the MDRD equation (7), at 12 months after transplantation.

The secondary objective was the incidence of CKD stage 3—defined as the eGFR as determined according to the MDRD formula <60 mL/min/1.73^2 of body surface area for at least 1 month—in accordance with the guidelines of the National Kidney Foundation (8,9), during the 12-month follow-up of the two groups. Further secondary objectives were the incidence of acute rejection and graft loss, patient survival at 6 and 12 months posttransplantation and overall morbidity.

Inclusion/exclusion criteria

Whole-organ liver transplant recipients over 18 years of age and with a maximum cold ischemia time of 12 h were included in the study.

Exclusion criteria included previous organ transplantation, HIV positivity, pregnancy or breast feeding. Recipients of multiple organ transplants, ABO-incompatible transplants, living-related or unrelated donor transplants, patients with thrombocytopenia (≤30 000/mm^3), leukopenia (≤2000/mm^3),

hypercholesterolemia (>350 mg/dL), hypertriglyceridemia (>500 mg/dL) at the time of randomization were also excluded from the study. Renal failure, defined as an eGFR ≥29 mL/min/1.73 m^2, at randomization was an additional exclusion criterion.

Baseline assessments were performed at the time of transplantation and at first administration of the study drugs and included vital signs, physical examination, electrocardiogram and laboratory tests aimed at determining drug safety (including hematology, biochemistry and urinalysis).

Safety, tolerability and efficacy

Safety of the immunosuppressive protocol was assessed by monitoring the frequency, severity and duration of adverse events throughout the study period, such as episodes of serious infection, immunosuppression-related complications and deviations from the study protocol for any reason.

Laboratory tests, including hematology, biochemistry and urinalysis, were performed on days 1, 7, 14 and 21 and at months 1, 2, 3, 6, 9 and 12 after LT.

Clinically suspected acute rejection was confirmed by biopsy and graded according to the Banff criteria (10). Liver biopsies were performed by protocol at 6 and 12 months after LT in HCV-positive recipients.

Patients were withdrawn from the study in the event of any one of the following conditions: consent withdrawal, lost to follow-up, graft loss, re-LT, discontinuation of the main immunosuppressive drug for more than 15 consecutive days or discontinuation of more than two episodes of 7 days or longer, and death.

Measurement of Evr

Evr was assayed on a TDxFLx analyzer (Abbott Diagnostic, IL) using a semi-automated Certican homogeneous fluorescence polarization immunoassay system (Innofluor Certican, Seradyn Inc., IN) as described previously (11).

Infection prophylaxis

All patients received prophylactic antibacterial treatment. Cytomegalovirus (CMV) prophylaxis, consisting of 5 mg/kg intravenous gancyclovir for the first 6 days, then 1 g TID for the next 3 months was implemented in high-risk patients such as CMV-seronegative recipients transplanted from CMV-seropositive donors.

Statistical analyses

According to the null hypothesis, there would be no difference in the mean eGFR, as defined with the MDRD formula, between the two groups. To calculate sample size, a mean creatinine clearance at 1-year follow-up of 100 ± 30 mL/min in the Evr group and 70 ± 30 mL/min in the CsA group was hypothesized. Considering a two-sided $\alpha = 0.05$ and $\beta = 0.1$, the sample size required to achieve statistical significance was 48 subjects in a 2:1 randomization, that is, 32 in the Evr group and 16 in the CsA group.

Continuous data were reported as mean ± SD and were compared using the two-sided Student's *t* test. Comparisons between groups for categorical variables were carried out using the chi-squarere test, with the Yates correction, or Fisher's exact test when appropriate. Graft and patient survival were evaluated using the Kaplan–Meier method and compared with the log-rank test. Statistical significance was set at $p < 0.05$.

The intention-to-treat (ITT) population comprised all randomized patients, and the per-protocol (PP) population comprised the randomized patients who completed the time-point follow-up before the possible drop-out date. In general terms, for the safety parameters analysis, the ITT population was used, and for the efficacy parameters analysis, the PP population after randomization. The causes of drop-out were graft loss, patient death, patient consent withdrawal and major violation of the protocol (i.e. change from the immunosuppression protocol).

Statistical analysis was done using SPSS 15.0 and Intercooled Stata 9.2 to calculate sample size.

Results

Demographics and patient pretransplant clinical conditions

Between September 2006 and November 2008, 111 patients were transplanted at our Institution, and none were lost to follow-up. During this 27-month study period, 29 patients were not enrolled due to presence of exclusion criteria, whereas 5 patients were enrolled in the study but then were not randomized at 10 days after LT due to graft dysfunction (1 patient), hepatic artery thrombosis requiring re-LT (1 patient), disseminated aspergillosis (1 patient) and cholangiocarcinoma on pathological examination of the explanted liver (1 patient). The remaining 78 patients were enrolled and regularly randomized in the protocol (Evr group, n = 52; CsA group, n = 26); those patients represent our study population. The demographic variables of the study population are summarized in Table 1.

All of the LTs were performed according to the piggy-back technique as described elsewhere (12). All recipients received a whole organ, preserved with Celsior solution, from a deceased donor. Donor characteristics are provided in Table 2.

Survival and cause of death

After an overall mean follow-up of 21.8 ± 9.2 months, the 6-month and 1-year ITT actuarial patient survival rates were, respectively, 92.3% and 90.4% in the Evr group and 92.3% and 88.5% in the CsA group ($p = 0.87$, Figure 2).

In a PP analysis, 9 patients in the Evr group died, 2 of them because of HCV recurrence 8.1, and 17.3 months after LT; 1 patient, with a well-functioning graft, died due to a pulmonary embolism 4.1 months after surgery; 1 patient died 1.3 months due to pulmonary aspergillosis; 1 patient died because of herpes pneumonia 5.6 months after LT, 1 patient died due to cerebral melanoma metastasis 30 months after LT.

Three patients in the CsA Group died, 1 due to abrupt intraabdominal hemorrhage from splenic aneurysm rupture 4 months after LT, 2 due to HCC recurrence 3.4 and 14.2 months after surgery. Causes of death occurring after withdrawal are reported in Table 3.

Renal function

The trend in MDRD in the first year after LT in the two groups is shown in Figure 3. At randomization,

Table 1: Baseline recipient demographic characteristics of study population

Patients		Evr group	CsA group	p
Gender	M/F (%)	40 (76.9)/12 (23.1)	20 (76.9)/6 (23.1)	1
Mean age	Years	53.7 ± 9.8	55.6 ± 7.4	0.38
BMI		25.1 ± 3.0	25.8 ± 2.5	0.34
MELD (at LTx)		20.4 ± 9.4	20.0 ± 9.5	0.88
Mean follow-up	Months	22.2 ± 9.1	21.1 ± 9.3	0.62
MDRD at randomization	mL/min/1.73 m^2	81.7 ± 29.5	74.7 ± 24.5	0.30
LT indication				
Viral cirrhosis	N (%)	41 (78.8)	18 (69.2)	0.41
Cholestatic cirrhosis	N (%)	6 (11.5)	3 (11.5)	
Cryptogenetic cirrhosis	N (%)	1 (1.9)	1 (3.8)	
Polycistic liver	N (%)	1 (1.9)	0 (0.0)	
Wilson disease	N (%)	0 (0.0)	2 (7.7)	
Alcoolic cirrhosis	N (%)	3 (5.8)	2 (7.7)	
HCC	N (%)	28 (53.8)	16 (61.5)	0.69
Viral status				
HCV Ab +	N (%)	24 (46.2)	10 (38.5)	0.69
HCV RNA +	N (%)	20 (38.5)	8 (30.8)	0.68
HBsAg +	N (%)	17 (32.7)	9 (34.6)	1
None	N (%)	12 (23.1)	8 (30.8)	0.42

the mean eGFR values according to MDRD were 81.7 ± 29.5 mL/min/1.73^2 and 74.7 ± 24.6 mL/min/1.73^2 in the Evr group and the control group, respectively, ($p = 0.30$).

Renal function 30 days after LT was significantly better in the Evr group, according to MDRD, than in the CsA (87.5 ± 45.1 vs. 66.6 ± 18.6 mL/min, $p = 0.03$).

The mean MDRD values at 3 months were 88.5 ± 32.3 mL/min in the Evr group and 59.7 ± 19.3 mL/min in the CsA group ($p < 0.001$). At 6 and 12 months, the mean eGFR values were, respectively, 87.8 ± 36.7 and 87.6 ± 26.1 mL/min in the Evr group versus 58.2 ± 17.9 and 59.9 ± 12.6 mL/min in the CsA group ($p < 0.001$ for the 6-months comparison, $p < 0.001$ for the 12-months comparison).

In a PP analysis, the incidence of cumulative stage 3 CKD, as defined by the National Kidney Foundation (9), was not statistically different between the two study groups at randomization and at the first month after LT; however, at the second month after LT, the incidence was higher and statistically significant in the CsA group at any follow-up time. In particular, 12 months after LT, 15.4% of the patients in the Evr group and 52.2% of those in the CsA group had developed stage 3 CKD ($p = 0.005$, Figure 4).

In 11 out of 26 patients (42.3%), the introduction of MMF was required in order to reduce the CsA trough blood level by 50%, in 10 patients due to renal impairment and in 1 due to tremor of the upper limbs. For 3 patients out of these 11, this alternate therapy was successful, the remainig 8 patients were withdrawn from the study due to the progression of renal impairment.

Table 2: Main donor characteristics of study population

Parameters		Evr group	CsA group	p
Sex	M/F (%)	36 (69.2)/16 (30.8)	15 (57.7)/11 (42.3)	0.45
Age	Years	59.3 ± 15.2	63.1 ± 19.4	0.34
Causes of death				0.67
CVA/CHI	N (%)	36 (69.2)/16 (30.8)	16 (61.5)/10 (38.4)	
Macrosteatosis				
None	N (%)	25 (48.1)	11 (42.3)	
Mild	N (%)	27 (51.9)	13 (50)	0.13
Moderate	N (%)	0	2 (7.7)	
Microsteatosis				
None	N (%)	27 (51.9)	14 (53.8)	
Mild	N (%)	23 (44.2)	11 (42.3)	0.39
Moderate	N (%)	0	1 (3.8)	
Severe	N (%)	2 (3.8)	0	
Cold ischemia time	Min	390 ± 116	377 ± 69	0.62
Warm ischemia time	Min	39.5 ± 11.2	35.5 ± 11.1	0.14

CVA = cerebral vascular accidents; CHI = closed head injury.

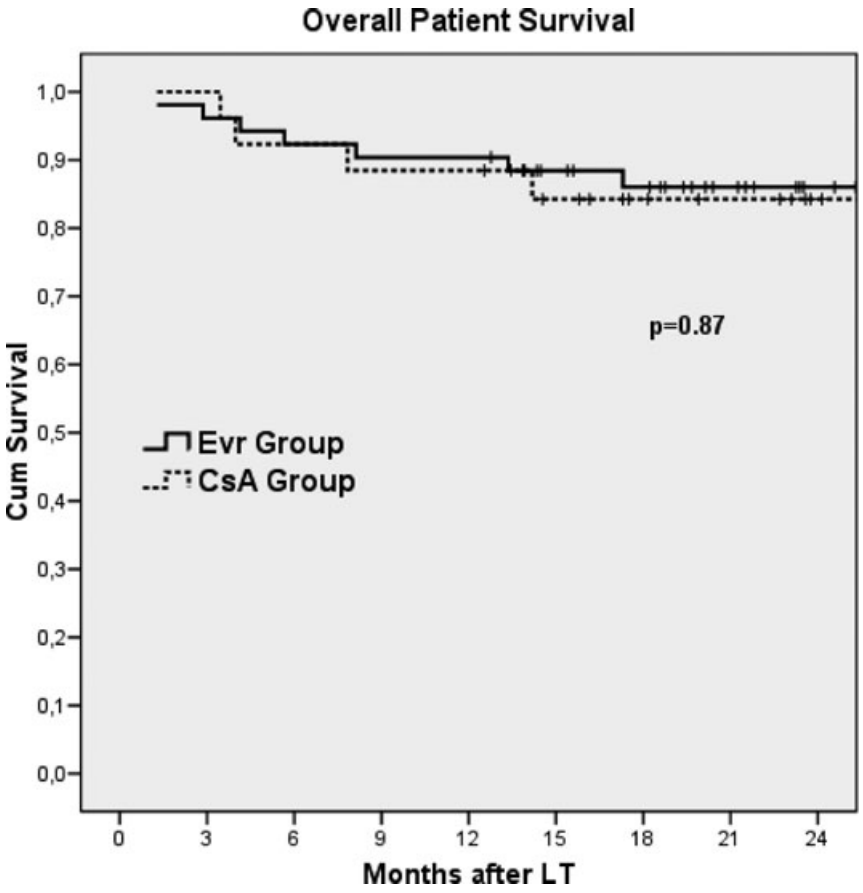

Figure 2: Intention-to-treat patient survival in the Evr and CsA groups.

Safety and tolerability (toxicity management and drug-dose adjustment)

In Table 4 are reported actual mean trough blood CsA and Evr levels in both study groups.

Adverse events observed within the first year of follow-up are summarized in Table 5. Two patients experienced aphthous-type mouth ulcers that resolved with a reduction of the daily dose. In both patients, the Evr blood levels were >17 ng/dL.

Skin disorders developed in 2 patients. During the entire follow-up incisional hernia (defined as any abdominal wall gap in the area of a postoperative scar requiring surgical

Table 3: Reason for withdrawal and subsequent follow-up of patients enrolled in the study

Reason for withdrawal	Before randomization	Status
Graft dysfunction	1 pt	Died on POD 31 due to sepsis
Hepatic artery thrombosis requiring re-LT	1 pt	Alive
Disseminated aspergillosis	1 pt	Died on POD 13 due to disseminated aspergillosis
Cholangiocarcinoma found on pathological examination on the explanted liver	1 pt	Alive
CsA group		
Renal failure	8 pts	Alive
Autoimmune hepatitis	1 pts	Died after 5.2 months due to pulmonary aspergillosis
Evr group		
Autoimmune hepatitis	1 pt	Died after 27.8 months due to cerebro vascular accident
Severe lower limb edema	5 pts	One died after 3.9 months due to recurrence HCV hepatitis
Mouth ulcer	2 pts	Alive
Re-LT (due to technical reason)	1 pt	Died after 1.8 months due to sepsis
Ascites	2 pts	Alive
Myelosuppression	3 pts	Alive

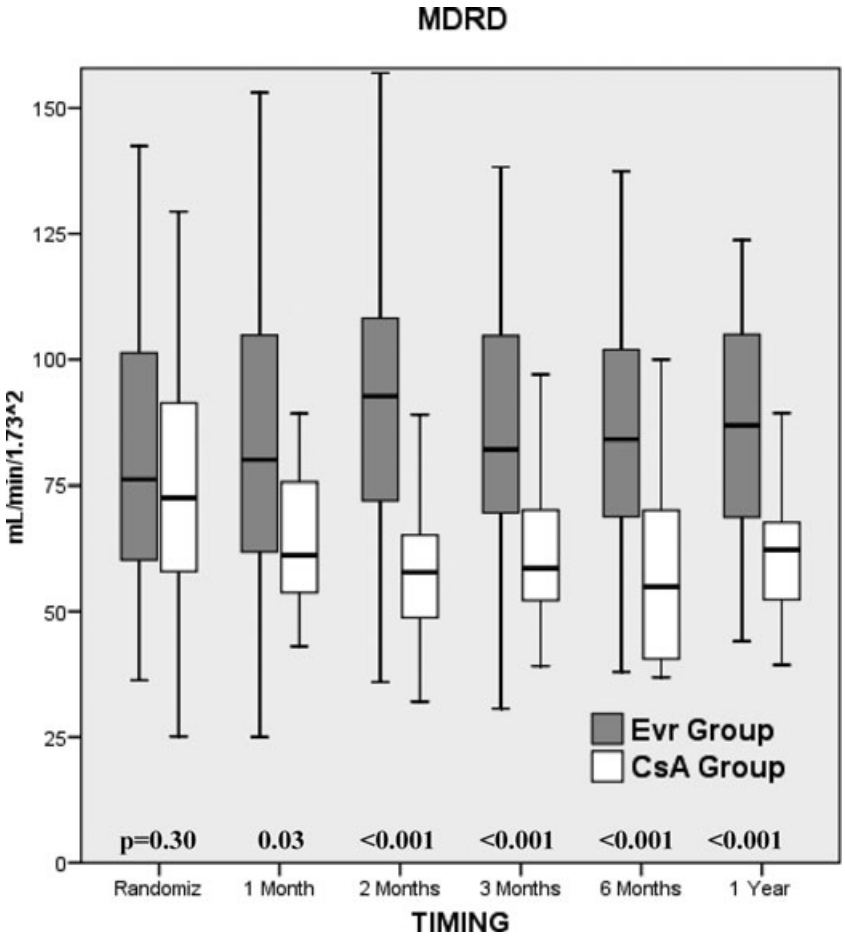

Figure 3: Glomerular filtration rate (GFR), as estimated with the MDRD formula, in the first year after liver transplantation in the Evr and CsA groups.

repair) occurred in 46.1% of patients in the Evr group compared with 26.9% in the CsA group ($p = 0.16$).

One patient (1.9%) in the Evr group experienced hepatic artery stenosis while none experienced hepatic artery thrombosis. In the CsA group, 2 patients (7.6%) had hepatic artery stenosis and there were 2 cases (7.6%) of hepatic of artery thrombosis.

During the follow-up period, 14 patients (26.9%) in the Evr group and 9 patients (34.6%) in the CsA group were withdrawn from the study ($p = 0.57$) after 9.9 ± 6.9 and

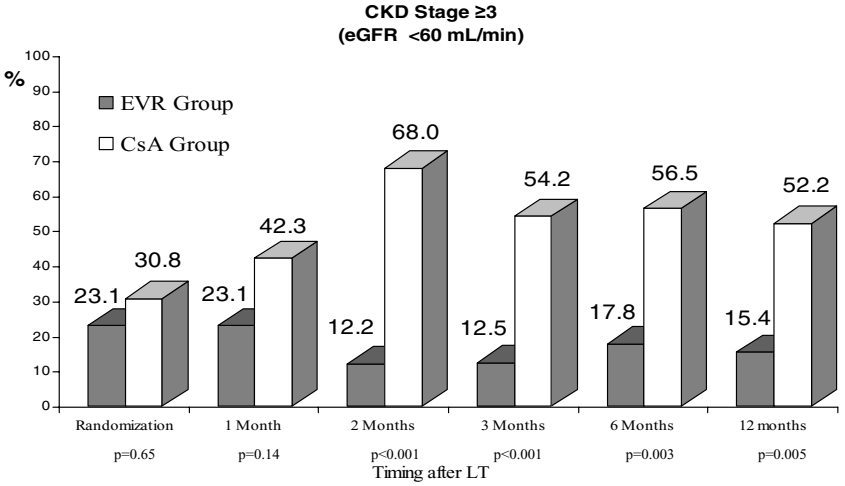

Figure 4: Per-protocol incidence of chronic kidney disease stage ≥3 (corresponding to an estimated GFR <60 mL/min) during the first year after liver transplantation in the two study groups.

Table 4: Actual mean trough blood CsA and Evr levels in both study groups

		POD 0–10	POD 10–30	POD 30–180	POD 180–365
CsA group					
Cyclosporine	ng/mL	112 ± 47	203 ± 48	180 ± 52	153 ± 46
Evr group					
Cyclosporine	ng/mL	103 ± 46	126 ± 47	–	–
Everolimus	ng/mL	–	8.8 ± 3.7	10.5 ± 3.8	9.9 ± 3

10.7 ± 8.5 months (p = 0.8), respectively. The timing from LT and the cause of withdrawal are summarized in Table 3.

All patients withdrawn from the study due to complications concerning study drugs were subsequently treated by successfully switching their immunosuppressant regimen. The freedom from efficacy failure probability is shown in Figure 5.

Acute cellular rejection

Three out of 52 (5.7%) patients enrolled in the Evr group experienced one episode of biopsy-proven acute rejection at days 40, 54 and 87, respectively; 2 of these cases were mild while the remaining 1 was moderate. In the CsA group, 2 patients out of 26 (7.7%) had a moderate BPAR (p = NS) that occurred 41 days and 8 months after LT. All of them were successfully treated with steroid pulse therapy and subsequent tapering. Moreover, 2 patients in the Evr Group experienced one episode of mild acute cellular rejection before randomization at days 7 and 9, both resolving with steroid therapy. Chronic rejection was not diagnosed in any patient during the follow-up period.

Liver-function tests and other hematological parameters

There were no clinically significant differences in liver-function test parameters, including albumin, at any of the considered time points.

After LT, hemoglobin, hematocrit, white blood cells, platelets, iron, ferritin and transferrin values did not differ significantly between the two groups at any time point. In agreement with results described in a previous, preliminary report (13), the incidences of anemia, thrombocytopenia (platelet count $\leq$50 000/mm^3) and leukopenia (white blood cell count $\leq$2000/mm^3) were not significantly different between the two groups.

Dyslipidemia

Mean cholesterol levels increased from baseline in all patients enrolled in both groups. Total cholesterol levels in patients in the Evr group were higher compared to those in the CsA group at any time during follow-up (Figure 6). This increase in cholesterol was due to change in both low-density lipoprotein and high-density lipoprotein fraction. There were also increases in mean blood triglycerides levels in both groups, but this was not statistically significant at any time point during follow-up.

Five patients out of 52 (9.6%) in the Evr group and 2 patients out of 26 (7.7%) in the CsA group required statin treatment for correction of dyslipidemia.

Infections

Thirty-seven major episodes of infection occurred in 24 patients (42.9%) in the Evr group; 28 (75.7%) of them were bacterial, 7 (18.9%) viral and 2 (5.4%) fungal. In the CsA group, 12 patients (46.1%) developed 21 major episodes of infection: 9 (42.9%) were bacterial, 7 (33.3%) viral and 5 (23.8%) fungal. The only statistically significant difference in infection between the two groups was that of fungal complications: 1 case of aspergillosis in the Evr group and 4 cases of aspergillosis and 1 case of candidemia in the CsA group (p = 0.011).

Four patients (19.2%) in the Evr group compared to 6 patients (23.1%) in the CsA group experienced CMV infection, all cases resulting from reactivation of a latent infection (p = NS). No patients in either group developed CMV disease. All episodes resolved within a period of 7–14 days with gancyclovir treatment.

Table 5: Intention-to-treat major complications after liver transplantation. Minor neurological complications were defined as tremor/headache/peripheral neuropathy

Complication	Evr group	CsA group	p
Incisional hernia	24 (46.1%)	7 (26.9%)	0.16
Biliary complications (stenosis/leak)	11 (21.1%)	8 (30.8%)	0.51
Infections	24 (46.1%)	12 (46.1%)	0.81
Minor neurological complications	3 (5.8%)	5 (19.2%)	0.11
Inferior limbs edema	5 (9.6%)	0	0.16
HCC recurrence	2/28 (7.1%)	3/16 (18.7%)	0.37
HCV recurrence	13/20 (65%)	6/8 (75%)	1
Hepatic artery stenosis/thrombosis	1 (1.9%)	4 (15.4%)	0.04

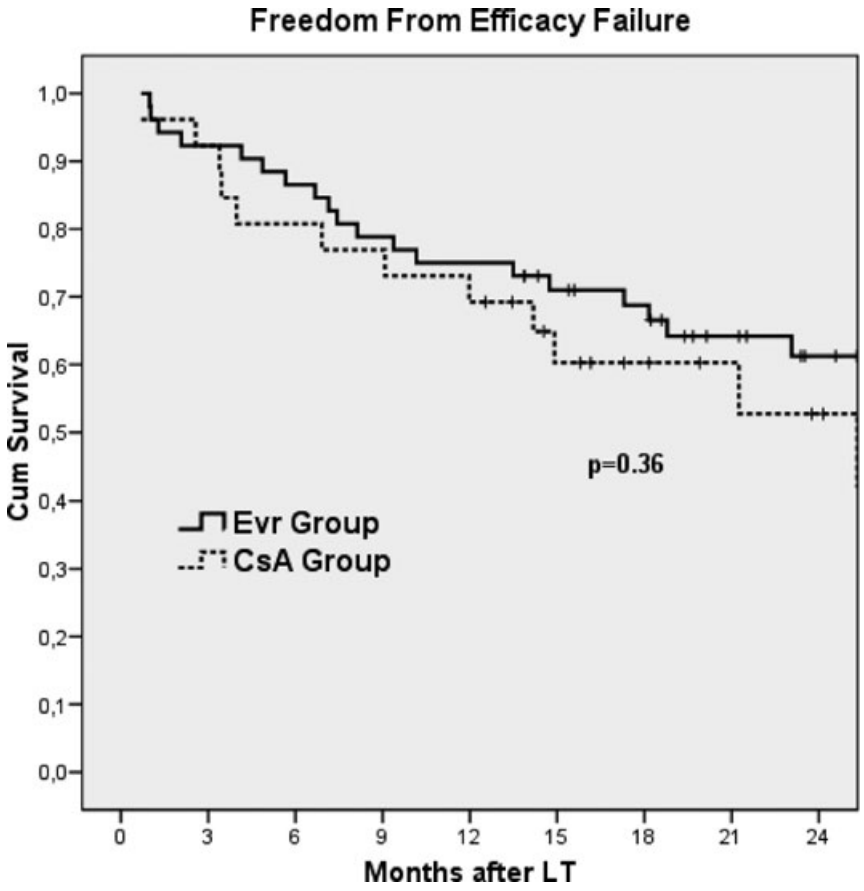

Figure 5: Freedom from efficacy failure in the Evr and CsA groups in the 78 randomized patients.

Discussion

A number of strategies have been employed to avoid CNI nephrotoxicity, including replacing CNI with MMF (14) or simply withdrawing CNIs and continuing maintenance on prednisone and azathioprine. CNI reduction or late withdrawal may be insufficient to prevent progression to end-stage renal failure when glomerular interstitial fibrosis has developed after long-term use of these drugs, or when serum creatinine levels remain high for several months (2,15,16) A potential concern with CNI withdrawal is the risk of under-immunosuppression and ensuing rejection, which has been described in several reports (6,17).

Preliminary experience regarding the use of mTOR inhibitors in maintenance LT has mainly focused on sirolimus (SRL) in the treatment of patients with CNI-related renal impairment (18–26). These studies have demonstrated that CNI minimization with SRL or conversion from CNI-based to SRL-based immunosuppression is feasible. This approach is associated with a 5–15% risk of acute rejection, with a variable degree of improvement in renal function depending on baseline creatinine clearance (23–26), concurrent non-CNI-related renal disease (25) and interval from transplantation (25,26). However, two small, prospective, randomized, single-center trials on conversion to SRL versus CNI continuation in patients with impaired renal function recently demonstrated that CNI withdrawal is associated with a significant improvement in creatinine clearance 3 months after the switch, but not at 12 months, suggesting that earlier CNI minimization is the key to preventing a posttransplant decline in renal function in LT patients (25,26).

The purpose of this study was to evaluate whether a CsA-free immunosuppression protocol with Evr monotherapy early in *de novo* LT recipients ensured better renal function within the first year of follow-up while maintaining efficacy and safety compared to a standard immunosuppression protocol using CsA, with MMF association in the case of CsA-related adverse events. The results showed that renal function was statistically better in the Evr group than in the CsA group, beginning at the first month after LT, as measured by eGFR and the MDRD formula. Moreover, the incidence of stage $\geq$3 CKD between the two groups was always statistically significant from the second to the 12th month after LT (52.2% vs. 15.4%, $p = 0.005$, in the CsA group vs. the Evr group, respectively).

Renal function in the Evr group was better than in the CsA group, even after MMF implementation and simultaneous CsA minimization in the latter, confirming a previous report

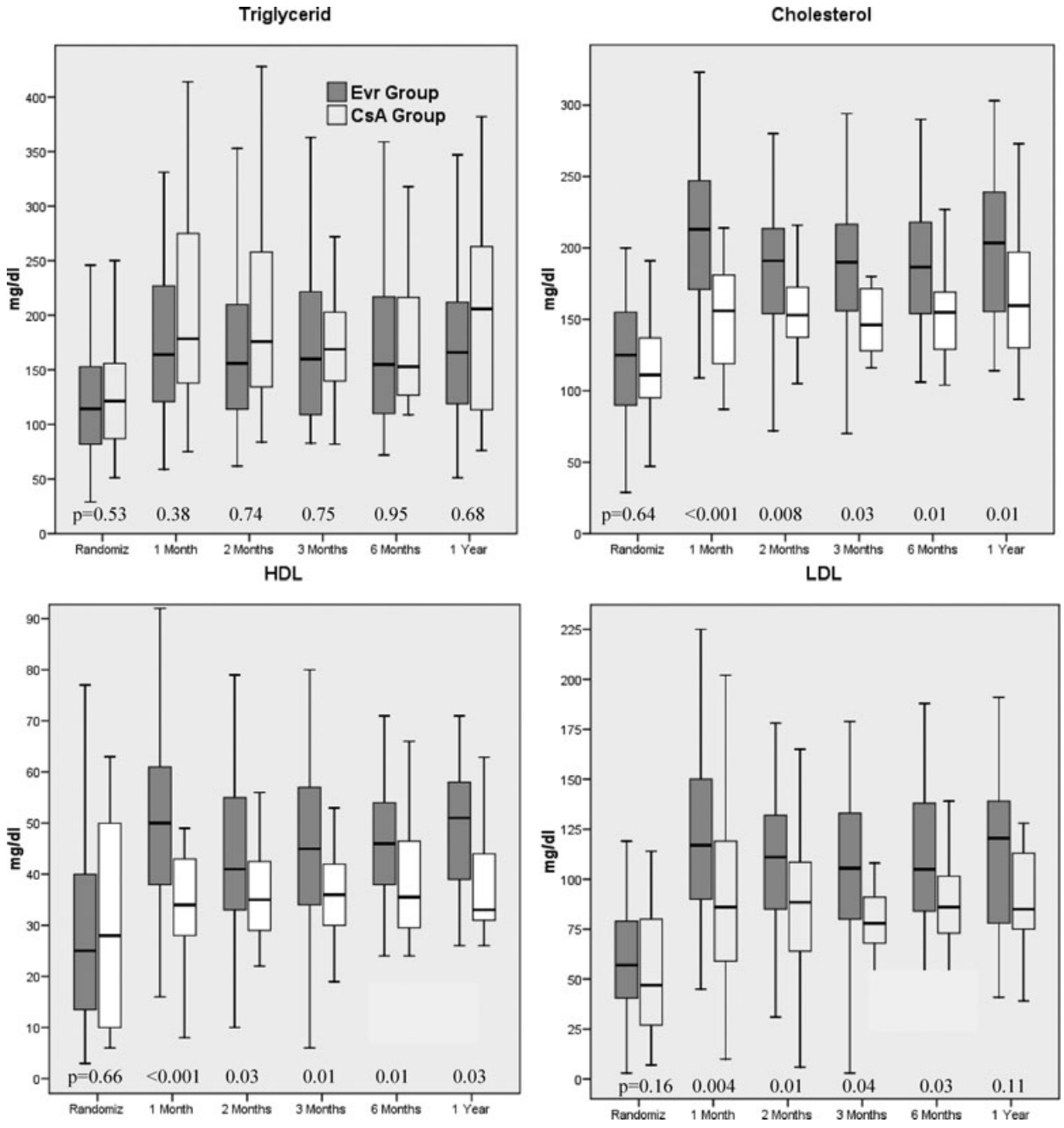

Figure 6: Lipid profiles of the two study groups during the first year after liver transplantation.

in which there was little improvement in the renal function of LT recipients after nonnephrotoxic drug implementation maintenance (27).

In light of the comparable nephrotoxicity between CsA and tacrolimus (28), it could be speculated that the same decrement in renal function between the control and study groups would have been observed if tacrolimus had been used instead of CsA.

Our data also showed that Evr monotherapy, after only 1 month of combination with CsA in *de novo* LT, maintains equal safety and efficacy as achieved with a standard CsA immunosuppressive protocol in terms of patient and graft survival and acute cellular rejection prevention.

Concerning overall morbidity, patients in the Evr group did not significantly differ in terms of anemia, leukopenia and

thrombocytopenia from those of the CsA group, nor were there any minor neurological complications. These results confirm that CNIs but not mTOR inhibitors are burdened by neurotoxicity (29). Interestingly, the incidence of episodes of infection was similar between the two groups, although the rate of fungal infections was significantly higher in the CsA group.

Administration of SRL has been associated with the development of posttransplant side effects such as hypertriglyceridemia and hypercholesterolemia (30,31). Our series of patients receiving Evr have also reflected this feature in terms of elevation in mean levels of serum cholesterol, HDL and LDL, all of which reach a statistically significant difference compared to CsA-treated patients. Interestingly, triglyceride elevation during follow-up was similar in both groups.

A higher incidence of incisional hernia was noted in patients treated with Evr than in those receiving CsA, although the difference was not statistically significant.

CsA levels required in the control group are comparable to those of several recent large clinical series, and are consistent with standard practice target CsA levels (32–34). Moreover, the mean actual CsA blood levels were at the lower limit of the range postulated in the protocol during the first year follow-up. Since these levels are not excessive, we can state that there was not any overstated nephrotoxicity from CsA in the control group (CsA group).

Another fault in the study could be the use of trough instead of peak CsA blood level measurement, since the CsA dose (in the control and the study group) was not adjusted based on blood concentration at 2 h after the dose (C_2) but rather on C_0 trough blood levels.

Like other immunosuppressive agents, mTOR inhibitors are not devoid of side effects. However, experimental and clinical studies have shown that mTOR inhibitors may help to solve some important problems related to posttransplant immunosuppression. While there are many reports on liver transplant recipients treated with SRL (drop-out rates are around 40%), there are few clinical reports utilizing Evr in the liver transplant setting, thus its role in this area needs to be evaluated.

In the most important clinical series utilizing Evr in the liver transplant setting, Levy et al. describe drop-out rates are between 46.7% and 64.5% (compared to 43.3% in placebo group, p = NS) (32). In Levy's study, the patients took Evr in association with CsA at steady doses of 1, 2 and 4 mg/day over a 1-year period without any adjustment based on trough blood level. In contrast, the drop-out rate of patients treated with Evr in our protocol was 27%. The cause of this drop-out rate is lower than in previous clinical series (32) and its causes could be multifactorial, including: Evr administration twice a day allowing daily dose adjustment improving drug exposure; Evr monotherapy and strict patient follow-up.

This study is a small clinical trial and larger studies are clearly indicated to confirm the impact on renal function of early CNI withdrawal and Evr administration after liver transplantation. However, our results do indicate that early posttransplant CNI-free immunosuppression with Evr monotherapy in *de novo* LT recipients was found to be associated with a significant improvement in renal function, with similar incidences of rejection and major complications as compared to standard CNI therapy.

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