

## Everolimus immunosuppression reduces the serum expression of fibrosis markers in liver transplant recipients

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### Abstract

**AIM:** To evaluate the expression of serum fibrosis markers in liver transplantation (LT) recipients on everolimus monotherapy compared to patients on an anti-calceinurin regimen.

**METHODS:** This cross-sectional case-control study included LT patients on everolimus monotherapy (cases) (E) ( $n = 30$ ) and matched controls on an anti-calceinurin regimen (calcineurin inhibitors, CNI), paired by etiology of liver disease and time since LT ( $n = 30$ ). Clinical characteristics, blood tests and elastography were collected. Serum levels of transforming growth factor- $\beta$  (TGF- $\beta$ ), angiotensin-1, tumor necrosis factor (TNF), platelet derived growth factor, amino-terminal propeptide of type III procollagen (P $\text{III}$ NP), hyaluronic

acid (HA), VCM-1 (ng/mL), interleukin (IL)-10, interferon-inducible protein 10 (IP-10), vascular endothelial growth factor and hepatocyte growth factor (HGF) (pg/mL) were determined by enzyme-linked immunosorbent assay. Expression of these markers between E and CNI was compared. Stratified analysis was done according to factors that may influence liver fibrosis. Variables are described with medians (interquartile range) or percentages.

**RESULTS:** A total of 60 patients [age: 59 (49-64), hepatitis C virus (HCV):  $n = 21$  (35%), time from LT: 73 mo (16-105)] were included. Patients had been on everolimus for a median of 15 mo. No differences in inflammatory activity, APRI test or liver elastography were found between the groups. No significant differences were observed between the groups in serum levels of P $\text{III}$ NP, metalloproteinase type = 1, angiotensin, HGF, IP-10, TNF- $\alpha$ , IL-10 and vascular cell adhesion molecule. Patients on E had a lower expression of TGF- $\beta$  [E: 12.7 (3.7-133.6), CNI: 152.5 (14.4-333.2),  $P = 0.009$ ] and HA [E: 702.89 (329.4-838.2), CNI: 1513.6 (691.9-1951.4),  $P = 0.001$ ] than those on CNI. This difference was maintained in the stratified analysis when recipient age is more than 50 years (TGF- $\beta$ 1:  $P = 0.06$ ; HA:  $P = 0.005$ ), in patients without active neoplasia (TGF- $\beta$ 1,  $P = 0.009$ ; HA:  $P = 0.01$ ), according to time since LT (> than 5 years, TGF- $\beta$ 1:  $P = 0.001$ ; HA:  $P = 0.002$ ), related to previous history of biliary complications (HA:  $P = 0.01$ ) and HCV recurrence (HA:  $P = 0.004$ ). Liver transplant recipients with everolimus monotherapy had less serum expression of TGF- $\beta$  y HA than matched patients with anti-calceinurins. This difference remains when classifying patients according to donor age and time since LT. Due to the small sample size, when examining patients with a prior history of biliary complications or recurrent HCV, the difference was non-significant but trends towards the lower expression of TGF- $\beta$ 1 in the everolimus group. Mammalian target of rapamycin (mTOR) plays a role in the transformation of quiescent hepatocellular stellate cell to their active

profibrotic state, and experimental models have demonstrated the potential activity of mTOR inhibition in attenuating fibrogenesis.

**CONCLUSION:** This study supports a possible role of everolimus in liver fibrosis modulation after LT in a clinical setting and suggests that tailoring immunosuppression could avoid fibrosis progression in the allograft.

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**Key words:** Everolimus; Rapamycin; Liver fibrosis; Mammalian target of rapamycin; Transplantation

**Core tip:** This study tries to approach the possible antifibrotic effect of everolimus, a mammalian target of a rapamycin inhibitor, in the clinical setting. Some studies in animal models suggest that it could also have an antifibrotic effect. The main conclusion of this study is that liver transplantation recipients with everolimus monotherapy had less serum expression of transforming growth factor- $\beta$  and hyaluronic acid than matched patients with anti-calceinurins that play an important role in liver fibrosis. The study offers the rationale for much needed future randomized controlled trials that evaluate the modulation of post-transplant fibrosis.

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## INTRODUCTION

Liver transplantation (LT) is the definitive treatment for end-stage liver disease. However, the outcome of a liver transplant can be compromised by allograft dysfunction due to fibrosis, which can even lead to cirrhosis. Approximately 75% of liver biopsies conducted in long-term LT survivors in whom liver tests are anomalous show significant histopathological abnormalities<sup>[1,2]</sup>. Fibrosis in the graft may be due to the recurrence of native disease [especially recurrent hepatitis C virus (HCV)], hepatotoxicity, *de novo* disease, non-alcoholic steatohepatitis, chronic rejection and/or vascular and biliary complications.

Strategies designed to prevent the progression of fibrosis in the allograft include the specific treatment of native disease<sup>[3,4]</sup> and/or stricter control of factors that can accelerate this fibrosis<sup>[5]</sup>. In addition, tailoring the immunosuppressive regime has been proposed as a strategy to regulate fibrogenesis in the post-transplant period. In HCV patients, measures such as avoiding the use of adjuvant pulse steroids for acute rejection and slow withdrawal of low-dose steroids beyond 12 mo have been proposed to avoid any immune-mediated graft injury that could induce an inflammatory and fibrogenic response<sup>[6-8]</sup>.

However, the results of a meta-analysis indicate no differences in mortality, graft survival, rejection, fibrosing cholestatic hepatitis or severe fibrosis related to the use of the calcineurin inhibitors, cyclosporine and tacrolimus at 1 year of follow-up<sup>[9]</sup>.

For prophylaxis against rejection in kidney transplant patients, new immunosuppressors known as mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) have been recently introduced<sup>[10]</sup>. Small observational studies have described their use<sup>[11]</sup>, particularly in patients with renal failure<sup>[12-14]</sup> and in those who develop post-transplant neoplasia<sup>[15,16]</sup>. mTOR is a serine/threonine kinase that plays an important role in cell proliferation, stellate cell activation, protein synthesis [synthesis of interleukins interleukin (ILs) and transforming growth factor- $\beta$  (TGF- $\beta$ )]<sup>[17,18]</sup>, angiogenesis<sup>[19]</sup> and cell metabolism (hypoxia inducible factor)<sup>[20]</sup>.

Due to the role played by mTOR in key steps of fibrogenesis, mainly reducing proliferation and activating hepatic stellate cell (HSC) and portal fibroblasts<sup>[21]</sup>, it has been proposed that inhibition of this molecule could alleviate liver fibrosis in the graft. In effect, a recent study conducted on bile duct-ligated (BDL) cirrhotic rats showed that the mTOR inhibitors, sirolimus and everolimus, reduced liver fibrosis compared to the effects of calcineurin inhibitors (CNI) after 5 wk of treatment<sup>[22]</sup>.

The aim of this study was to compare serum levels of mediators of liver fibrosis in liver transplant patients under immunosuppressive regimes based on everolimus (E) with those based on calcineurin inhibitors.

## MATERIALS AND METHODS

This cross-sectional study was conducted over the period April to October 2010. All consecutive patients who underwent liver transplantation between 1995 and 2010 under everolimus immunosuppression alive at the time of the study were enrolled. Patients were matched with control LT patients undergoing calcineurin inhibitor treatment according to liver disease etiology and time since LT. Exclusion criteria for cases and control patients were acute rejection in the previous 6 mo, uncontrolled infection or antiviral treatment, or unresolved biliary complications.

Everolimus (Certican®, Novartis Pharma Schweiz AG, Bern, Switzerland) is approved for prophylaxis against rejection in *de novo* renal transplant recipients<sup>[10]</sup>, for management of malignancy (chemotherapy resistant kidney cancer, subependymal giant cell astrocytoma and neuroendocrine neoplasm)<sup>[20]</sup> and for use in drug-eluting coronary stents<sup>[23]</sup>. However, this drug has also been used off-label in liver and lung transplantation patients<sup>[24-27]</sup>. At our center, the use of everolimus in LT recipients is approved in situations such as renal dysfunction or adverse events like neurotoxicity due to CNI, development of *de novo* malignancies, recurrence of hepatocellular carcinoma, and the presence of predictors of a high risk of hepatocellular carcinoma recurrence in the explanted liver (satellitosis, vascular infiltration and multinodularity disease)<sup>[28,29]</sup>. Con-

traindications for the use of everolimus are a prior history of hepatic artery thrombosis, proteinuria greater than 800 mg/d and/or surgery in the previous 4 wk<sup>[29,30]</sup>.

### Everolimus dosing and switching

An initial dose of 0.5-0.75 mg bid E was administered and then increased 0.5 mg weekly to obtain a trough level of 3-8 ng/mL. Tacrolimus and cyclosporine were tapered by 15%-25% of the usual dose every 2 wk until complete withdrawal. The overlap period between both drugs in the E group treatment was a median of 1 or 2 mo<sup>[31-33]</sup> before monotherapy was achieved. In patients who were started on everolimus, steroids were given according to the usual schedule, and then progressively tapered and withdrawn by month 12 after liver transplantation. Trough levels of everolimus, hematological and lipid profiles, renal and liver function tests and proteinuria were monitored weekly until stable levels of the drug were achieved<sup>[34]</sup>.

### Clinical and laboratory variables

Information was compiled on patient demographics, etiology of cirrhosis, LT surgical variables, postoperative period and laboratory data. The immunosuppression regime data recorded were present dose and blood levels, time of administration, and combined treatment with corticosteroids and/or mycophenolate.

Laboratory tests were performed to determine transaminases, cholestasis enzymes and simple validated fibrosis scores (APRI)<sup>[35-37]</sup>. Additionally, 20 mL of blood were obtained to determine serum biomarkers of fibrosis that had been identified in previous studies<sup>[38-42]</sup>. We determined serum markers that could be correlated with late liver fibrosis by enzyme-linked immunosorbent assay, including those linked to matrix deposition such as hyaluronic acid (Echelos, Bioscience Inc.), amino-terminal propeptide of type III procollagen (P3NP; Cusabio, Bionova), those linked to matrix degradation such as tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1; Ray-Biotech, Bionova), growth factors like angiopoietin (Ray-Biotech, Bionova), hepatocyte growth factor (HGF; Ray-Biotech, Bionova), platelet derived growth factor (PDGF; RayBiotech, Bionova) and finally, inflammatory markers that participate in the fibrogenesis-like TGF- $\beta$ 1 (Dialclone, Bionova), adiponectin and leptin, IP-10 (interferon-inducible protein 10 calcineurin inhibitor; Dialclone, Bionova), tumor necrosis factor alpha (TNF- $\alpha$ ; Dialclone, Bionova), interleukin 10 (IL-10; Dialclone, Bionova) and *vascular* cell adhesion molecule (VCAM; Cusabio, Bionova).

Liver stiffness was measured by a trained nurse or physician by transient elastography using a Fibroscan instrument (Echosens, Paris, France). Measurements in which 10 acquisitions were achieved, with a success rate of at least 60% and an interquartile range lower than 30 were considered valid<sup>[43,44]</sup>.

### Definitions

The following definitions were made: (1) early allograft dysfunction<sup>[45]</sup>: one or more of the following postop-

erative findings: bilirubin > 10 mg/dL, INR > 1.6 on postoperative day or alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2000 IU/mL within the first 7 postoperative days; (2) previous acute rejection: a histological picture compatible with rejection or in cases of an abnormal liver test, conversion to a normal test result after reaching optimal serum levels of immunosuppressants<sup>[1]</sup>; (3) chronic rejection: a compatible histological picture<sup>[1]</sup>; (4) biliary tract disease: anastomotic and non-anastomotic biliary strictures detected on imaging showing biochemical expression. Resolution of strictures was defined as a non-requirement for endoscopic, radiological or surgical treatment of the stricture in the 6 months before inclusion; (5) uncontrolled neoplasia: a remission time under 2 years; (6) HCV recurrence: histological indicators of inflammation or fibrosis in patients with HCV viremia detected in protocol biopsies at 6 and 12 mo; and (7) arterial hypertension, diabetes mellitus or dyslipidemia: defined according to the criteria established by the European Society of Hypertension and the International Diabetes Federation<sup>[46,47]</sup>.

## RESULTS

Sixty LT patients were recruited for the study (30 on everolimus and 30 on CNI). The demographic characteristics of the participants are provided in Table 1. Patients were predominantly men of median age 60 (49-64) years in the everolimus group and 54 (46-60) years in the control CNI group. The most common cause of liver disease that led to transplantation was alcoholic liver cirrhosis. Median time since LT was approximately 6 years (IQR 16.7-106.4 mo) for both groups. No difference in donor age or in the proportion of patients with early allograft dysfunction was observed.

The median time of everolimus treatment was 15 (5-29) mo and the median time of the initial dose of everolimus given from the time of LT was 2.7 years (0.7-8.3). This is because the main indication to use everolimus in our center was developing neoplasia *de novo* (85.71%). Monotherapy with everolimus was achieved in 25 patients (83.3%). Of the 5 patients on combination therapy (everolimus plus CNI), 1 patient was under cyclosporine treatment and 4 patients received tacrolimus. These patients did not tolerate monotherapy with everolimus immunosuppression. Most patients in the CNI group were receiving tacrolimus (24 patients) and had been under CNI treatment for a median time of 72 (17-108) mo. Approximately 25% of patients in the CNI group were given concomitant mycophenolate mofetil to minimize adverse effects linked to CNI treatment, while only one patient in the everolimus group received this drug. No differences in the proportions of patients under concomitant steroid treatment were observed between the two groups (Table 2).

No differences between treatment groups were detected in: HCV recurrence, previous episodes of acute, chronic rejection and biliary complications, proportion of patients with diabetes mellitus, arterial hypertension,

**Table 1** Demographic and clinical characteristics of patients before liver transplantation

	Everolimus patients ( <i>n</i> = 30)	CNI patients ( <i>n</i> = 30)	<i>P</i> value
Male	24 (80)	24 (80)	1.000
Age (yr)	46 (44-60)	51 (44-59)	0.760
Etiology of liver disease			
EtOH	16 (53.3)	13 (43.3)	0.541
HCV	11 (36.7)	10 (33.3)	
HBV	0	2 (6.7)	
Autoimmune	1 (3.3)	2 (6.7)	
Hemochromatosis	1 (3.3)	2 (6.7)	
Cholestatic disorders	1 (3.3)	0	
Cryptogenic	0	1 (3.3)	
Time from LT (mo)	75 (16-113)	72 (17-108)	0.859
Indication for LT			
HCC	2 (6.7)	4 (13.3)	0.041
Decompensated cirrhosis	16 (53.3)	21 (70)	
Decompensated cirrhosis and HCC	12 (40)	4 (13.3)	
Acute liver Failure	0	1 (3.3)	
Donor age (yr)	55 (34-72)	49 (31-63)	0.521
Early allograft dysfunction	5 (16.7)	2 (6.7)	0.212

Categorical variables are expressed as absolute *n* (%), continuous variables are expressed as medians and interquartilic range. CNI: Calcineurin inhibitors; HCV: Hepatitis C virus; HBV: Hepatitis B virus; LT: Liver transplantation; HCC: Hepatocarcinoma.

obesity or dyslipidemia. Patients in the everolimus group had higher serum levels of cholesterol, a well-known side effect of the drug. As expected, given the accepted local indications for everolimus treatment, patients in this group had a greater proportion of neoplasms and hepatocellular carcinoma outside the Milan criteria (data not shown) at the time of the study.

Although bilirubin levels were higher in the CNI group (*P* = 0.002), no differences were observed in transaminase levels (AST and ALT), GGT or in the proportion of patients with hyperbilirubinemia. Similarly, no differences in APRI or elastography were detected between the groups.

No significant differences were observed between the groups in serum levels of PIIIINP, TIMP-1, angiopoietin, HGF, IP-10, TNF- $\alpha$ , IL-10 and VCAM (Table 3). Interestingly, patients on everolimus showed a markedly lower expression of TGF- $\beta$ 1 [12.7 (3.7-133.6) ng/mL *vs* 152.5 (IQR 14.4-333.2) ng/mL; *P* = 0.009] (Figure 1A). TGF- $\beta$ 1 is the most potent stimulus for hepatic fibrogenesis through activation of hepatic stellate cells<sup>[48]</sup>. Furthermore, patients on everolimus showed the lower expression of hyaluronic acid [702.89 (329.4-838.2) ng/mL *vs* 1513.6 (691.9-1951.4) ng/mL; *P* = 0.001] (Figure 1B), an essential component of the extracellular matrix (ECM) mostly synthesized by hepatic stellate cells<sup>[49]</sup>.

To determine whether the results could be influenced by other factors, markers were compared in different patient subgroups by univariate analysis (Table 4). First of all, we examined the expression of fibrosis markers in patients without active neoplasia given the uneven distribution of neoplasia between groups. Other patient

**Table 2** Clinical characteristics of patients after liver transplantation

	Everolimus patients ( <i>n</i> = 30)	CNI patients ( <i>n</i> = 30)	<i>P</i> value
Age	60 (49-64.5)	54 (46.5- 60.5)	0.756
BMI (kg/m <sup>2</sup> )	26.2 (23.3-28.2)	27.9 (25.2-31.3)	0.211
Dyslipemia	10 (33.3)	12 (40)	0.789
Diabetes mellitus	9 (30)	13 (43.3)	0.284
HTA	18 (60)	18 (60)	1.000
Neoplasia			
HCC	8 (26.7)	1 (3.3)	0.026
Solid non-hepatic neoplasia	2 (6.7)	0	
Skin neoplasia	4 (13.3)	1 (3.3)	
Hematological neoplasia	2 (6.7)	0	
Recurrent HCV	11 (36.7)	10 (33.3)	1.000
AST (IU/L)	23 (18-53)	35 (25-66)	0.081
ALT (IU/L)	43 (18- 65)	24 (18-62)	0.260
Bilirubin (mg/ dL)	0.5 (0.37-0.7)	0.85 (0.5-1.1)	0.002
Cholesterol (mg/ dL)	188.5 (167-220.75)	158 (141.25-178.25)	0.002
Acute rejection	7 (23.3)	9 (30)	0.771
Chronic rejection	2 (6.7)	1 (3.3)	1.000
Biliary complications	10 (33.3)	12 (40)	0.789
APRI	0.74 (0.48-2)	0.47 (0.34-1.4)	0.135
Liver stiffness (kPa)	7.6 (5.1-8.6)	8.4 (5.6-10.7)	0.134
Concomitant steroids	6 (20)	6 (20)	1.000
Concomitant Mycophenolate mofetil	1 (3.3)	8 (26.7)	0.026

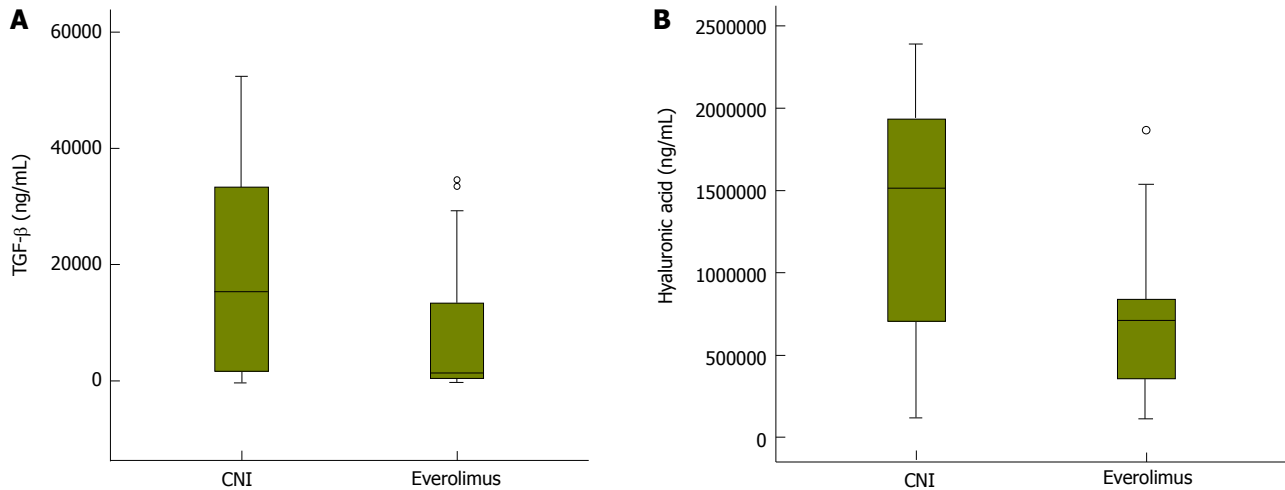
Categorical variables are expressed as absolute *n* (%), continuous variables are expressed as medians and interquartilic range. HCV: Hepatitis C virus; HBV: Hepatitis B virus; LT: Liver transplantation; HCC: Hepatocarcinoma; HTA: Arterial hypertension; APRI: APRI score.

subsets were established according to time since LT (> than 5 years), recipient age (> 50 years), previous history of biliary complications and HCV recurrence. The differences observed between TGF- $\beta$ 1 and hyaluronic acid expression in the main everolimus and CNI groups persisted in our analysis by subgroups. This difference was statistically significant when classifying patients according to donor age and time since LT. However, due to the small sample size, when examining patients with a prior history of biliary complications or recurrent HCV, the difference emerged as a non-significant trend towards the lower expression of TGF- $\beta$ 1 in the everolimus group. Although there were differences in the use of mycophenolate mofetil among both groups, the results described before remained when we compared both groups excluding patients who were receiving mycophenolate mofetil.

## DISCUSSION

In this study we show that LT patients on everolimus therapy have lower serum levels of TGF- $\beta$ 1 and hyaluronic acid than patients matched for disease etiology and time since LT receiving CNI. TGF- $\beta$ 1 is the most significant inducer of the synthesis of extracellular matrix proteins (collagen and glycosaminoglycans such as





**Figure 1** Box plots of serum transforming growth factor- $\beta$  (A) and serum Hyaluronic acid (B) in patients under calcineurin inhibitors or everolimus regime. TGF- $\beta$ : Transforming growth factor- $\beta$ ; CNI: Calcineurin inhibitors.

**Table 3** Serum levels of liver fibrosis mediators

	Everolimus patients (n = 30)	CNI patients (n = 30)	P value
VCAM (ng/mL)	68.25 (25.98-135.17)	58.88 (35.30-115.52)	0.668
P11NP (ng/mL)	172.4 (119.75-1195.90)	879.40 (140.10-1555.15)	0.193
IP-10 (pg/mL)	86.01 (51.10-210.91)	79.61 (59.2-172.64)	0.669
HGF (pg/mL)	225.17 (163.30-320.17)	205.53 (152.59-297.86)	0.363
Angiopoietin (ng/mL)	26.97 (19.58-32.25)	30.108 (24.60-38.8)	0.122
TNF- $\alpha$ (ng/mL)	41.12 (38.35-44.8)	42.70 (40.20-45.07)	0.435
IL-10 (pg/mL)	8.52 (6.07-9.23)	8.66 (6.95-9.40)	0.856
TGF- $\beta$ (ng/mL)	12.7 (3.7-133.6)	152.5 (14.4-333.2)	0.009
HA (ng/mL)	702.89 (329.4-838.2)	1513.6 (691.9-1951.4)	0.001
PDGF (ng/mL)	1.5630 (1.4663-1.6369)	1.5630 (1.4616-1.6369)	0.720

Variables are expressed as medians and interquartilic range. VCAM: Vascular cell adhesion molecule; P11NP: Amino-terminal propeptide of type III procollagen; IP10: Interferon-inducible protein 10 calcineurin inhibitor; TNF- $\alpha$ : Transforming necrosis factor alpha; TGF: Tissue growth factor; HGF: Hepatocyte growth factor; HA: Hyaluronic acid; PDGF: Platelet derived growth factor.

hyaluronic acid) by hepatic stellate cells, and also regulates many proteins involved in their turnover including matrix metalloproteinases (MMP) and their inhibitors (TIMP)<sup>[48,50-52]</sup>.

MTOR signaling includes several steps in the transformation of quiescent HSC to their active profibrotic state<sup>[53]</sup>. Although some studies have addressed the modulation of liver fibrosis in patients on CNI, no study has assessed the role of mTOR inhibitors in fibrogenesis in a clinical setting.

The potential role of mTOR inhibition in attenuating fibrogenic pathways has been examined in experimental models of cirrhosis. After bile duct ligation- and thioacetamide induced cirrhosis, low dose rapamycin led to

**Table 4** Stratified analysis according to factors that could influence liver fibrosis

	TGF- $\beta$ (ng/mL)	P value	HA (ng/mL)	P value
All patients				
E (n = 30)	12.7 (3.7-133.6)	0.009	702.89 (329.4-838.2)	0.001
CNI (n = 30)	152.5 (14.4-333.2)		1513.6 (691.9-1951.4)	
Free of neoplasia				
E (n = 29)	11.1 (3.2-22.4)	0.005	754.8 (351.3-837)	0.030
CNI (n = 22)	137.5 (14.4-333.2)		1296.7 (703.7-1936.1)	
Time from LT > 5 yr				
E (n = 17)	16.5 (7.6-264.6)	0.010	462.0 (351.3-770.3)	0.002
CNI (n = 16)	296.8 (125.4-337.1)		1084.7 (523.7-1665.8)	
Donor age > 50 yr				
E (n = 12)	14.0 (6.0-67.1)	0.06	910.0 (589.2-1510.8)	0.005
CNI (n = 15)	96.0 (14.5-297.1)		1897.0 (1519-2136.5)	
Biliary complications				
E (n = 12)	20.6 (7.6-265)	0.110	516.75 (235.6-1079.4)	0.010
CNI (n = 10)	272.3 (16.5-403.4)		1545.37 (1085.8-1888.7)	
Recurrent HCV				
E (n = 10)	6.5 (1.6-15.3)	0.260	914.55 (768.8-1513.6)	0.410
CNI (n = 11)	14.5 (6.1-225)		1991.17 (1532.4-2168.9)	

Variables are expressed as medians and interquartilic range. TGF- $\beta$ : Transforming growth factor- $\beta$ ; HA: Hyaluronic acid; E: Everolimus; CNI: Calcineurin inhibitors.

the reduced accumulation of ECM-producing cells, ECM components, reduced interstitial MMP-2 activity and a reduced spleen weight as an indicator of portal hypertension than in vehicle-treated cirrhotic rats<sup>[54]</sup>. Higher doses of rapamycin in the BDL rats gave rise to a reduction in HSC activation and proliferation as well as a reduced capacity of other cells to transition to myofibroblasts<sup>[21]</sup>. Lastly, mTOR inhibitors have been noted to reduce liver fibrosis up to 70% and portal pressure up to 50% in BDL rats compared to CNI-treated rats. Furthermore, in mTOR inhibitor-treated rats, the clinical manifestation of portal hypertension was lessened, as indicated by factors such as the development of ascites.

In the context of LT, one of the main causes of fibrosis is recurrent hepatitis C. The activation of HSC has been correlated not only with the fibrosis stage, but

also with the rate of liver fibrosis progression<sup>[55]</sup>. In a retrospective clinical study, the use of sirolimus compared to CNIs was associated with a trend towards diminished disease activity and fibrosis in serial biopsies, although no differences were observed in incidence and time to recurrence of HCV<sup>[56]</sup>.

No differences in the extent of fibrosis as measured by transient elastography and APRI score were detected, although our study was not designed to assess this factor. Elastography has not been validated in long-term liver grafts and its sensitivity to determine fibrosis is probably not comparable to the use of direct molecular markers of fibrogenesis. The limitations of our study include those inherent to its cross-sectional design, which precludes establishing a temporal relationship between drug initiation and serum levels of fibrosis markers. In addition, it has been well established that different etiologies of liver disease produce different fibrosis patterns. Unfortunately, our sample size was insufficient to determine the effect of everolimus according to the etiology of liver disease. Also, serum biomarker expression could be influenced by factors secondary to the inflammatory response or to other forms of chronic visceral damage. To avoid this bias, patients with acute conditions were not included and the influence of other chronic conditions was assessed by examining different patient subgroups.

In conclusion, patients under everolimus therapy show reduced serum expression of fibrosis markers such as TGF- $\beta$ 1 and hyaluronic acid compared to patients matched for LT etiology and time since LT under a CNI immunosuppressive regimen. The results of this study provide direction for future studies designed to address the issue of modulating post-transplant fibrosis using individualized immunosuppression strategies.

## COMMENTS

### Background

The outcome of liver transplant may be conditioned by allograft dysfunction associated with the development of fibrosis which can even lead to cirrhosis. Tailoring immunosuppression has been postulated to have a role in fibrosis progression. Mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) have been introduced for prophylaxis against rejection in transplant patients and, because of their antiangiogenic, antiproliferative and antifibrotic properties, it has been postulated that they could modulate liver fibrosis in liver transplant (LT) grafts.

### Research frontiers

Low dose rapamycin can reduce accumulation of extracellular matrix (ECM)-producing cells (extracellular matrix), ECM components, interstitial matrix metalloproteinases (MMP)-2 activity (metalloproteinases) and a reduced spleen weight as an indicator of portal hypertension in cirrhotic rats. Experimental models have demonstrated the potential activity of mTOR inhibition in attenuating fibrosis but there is no evidence in a clinical setting. The hotspot of this article is the study about the impact of everolimus immunosuppression in serum levels of liver mediator fibrosis expression in a clinical practice.

### Innovations and breakthroughs

mTOR signaling includes several steps in the transformation of quiescent hepatic stellate cell (HSC) to their active profibrotic state. Higher doses of rapamycin in rats give rise to a reduction in HSC activation and proliferation as well as a reduced capacity of other cells to transition to myofibroblasts. These rats had reduced liver fibrosis up to 70% and portal pressure up to 50% compared to calcineurin inhibitors-treated rats. Clinical manifestation of portal hypertension

like ascites development was lessened in mTOR inhibitor-treated. Due to the potential role mTOR in key steps of fibrogenesis, mainly reducing proliferation and activating hepatic stellate cell and portal fibroblasts, it has been proposed that inhibition of this molecule could alleviate liver fibrosis in the graft. In this study we show that liver transplant patients on everolimus therapy have lower serum levels of transforming growth factor- $\beta$  (TGF- $\beta$ ) 1 and hyaluronic acid than patients matched for disease etiology and time since LT receiving calcineurin inhibitors. TGF- $\beta$ 1 is the most significant inducer of the synthesis of extracellular matrix proteins (collagen and glycosaminoglycans such as hyaluronic acid) by hepatic stellate cells, and also regulates many proteins involved in their turnover including MMP and their inhibitors (TIMP).

### Applications

The study results suggest that mTOR inhibitors could modulate fibrosis progression in liver grafts. Although this is not a prospective study, the results support the need to investigate the role of an immunosuppression regime in fibrosis development after liver transplantation.

### Terminology

MTOR: MTOR is a serine/threonine kinase that plays an important role in cell proliferation, stellate cell activation, protein synthesis (synthesis of interleukins and transforming growth factor beta), angiogenesis and cell metabolism (hypoxia inducible factor).

### Peer review

This is a good descriptive study in which the authors compare serum liver fibrosis expression between both immunosuppression regimes (anti-calcineurin vs everolimus). The results of this study are interesting and provide direction for future studies designed to address the issue of modulating post-transplant fibrosis using individualized immunosuppression strategies in clinical practice.

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