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Hepatic Steatosis at One Year is an Additional Predictor of Subsequent Fibrosis Severity in Liver Transplantation Recipients with Recurrent Hepatitis C Virus

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Abstract

Recurrent hepatitis C virus (HCV) is the most common cause of graft loss among HCV-infected liver transplant (LT) recipients. Diabetes (DM) has been associated with increased rates of fibrosis progression but whether steatosis affects post-LT outcomes, independent of DM, is unclear. Using a retrospective cohort of HCV-infected LT recipients, we determined the prevalence of hepatic steatosis and evaluated the relationship between steatosis on index biopsy at one year post-LT (± 6 months) and severity of subsequent fibrosis. A total of 152 HCV LT recipients were followed for a median follow-up after the index biopsy of 2.09 (range 0.13–6.17) years, with a median number of biopsies/patient after the index biopsy of 2 (range 1–6). Steatosis ($\geq 5\%$) was present in 45 (29.6%) individuals in the 1-year index biopsy post-LT; steatosis was mild (grade 1) in 80%. In multivariate analysis, presence of steatosis at 1-year post-LT was positively associated with genotype 3 (OR= 3.60, $p=0.02$), older donor age (OR= 1.03, $p=0.04$), and pre-LT hypertension (OR= 3.29, $p=0.009$). At 3 years post-LT, the cumulative rate of significant fibrosis (F2–4 Ludwig-Batts scale) was 49% in those with steatosis at 1 year versus 24% in those without steatosis ($p=0.003$). In multivariable analysis, steatosis at 1-year was an independent predictor of subsequent F2–4 fibrosis (OR= 2.20, $p=0.008$). Steatosis was a stronger predictor of fibrosis in the setting of sirolimus use (HR 9.38, 95% CI 1.37, 64.16; $p=0.02$). We conclude that steatosis is frequent in the early post-LT period, and that steatosis within the 1 year post-LT is a marker of higher risk of fibrosis progression in HCV-infected patients.

INTRODUCTION

End-stage liver disease (ESLD) resulting from chronic infection with hepatitis C virus (HCV) is the leading indication for liver transplantation (LT).¹ In HCV-infected liver transplantation (LT) recipients, recurrent HCV disease is the most frequent cause of graft loss,² and identification of potentially modifiable factors that may attenuate the risk of recurrent cirrhosis is important. In the non-transplant setting, individuals with diabetes mellitus (DM) are more likely to have advanced fibrosis on liver biopsy, as compared to those who are not diabetic^{3,4} and in liver transplant recipients, insulin resistance, on the basis of either a diagnosis of diabetes mellitus (DM) or HOMA-IR of >2.5 , has been associated with a greater risk of advanced fibrosis.^{5,6} The relationship between HCV and DM in the post-transplant setting is likely to be bidirectional, as HCV infection has been demonstrated to be a significant risk factor for the development of new-onset, persistent DM following LT.^{7,8} It is not clear whether improvement in the control of DM or insulin resistance will reduce the risk of fibrosis progression and graft loss.

Steatosis has been shown to be independently associated with insulin resistance, as well as with fibrosis severity in HCV patients.³ In a cross-sectional study of nondiabetic HCV-infected individuals, steatosis severity was correlated with fibrosis severity, independent of age, HCV activity, and HCV viral load.⁹ Analysis of a large, multicenter sample of HCV-infected individuals revealed that steatosis, age, and HCV activity, but not DM, were all independently associated with fibrosis.¹⁰ In the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) trial, a study of HCV patients with advanced fibrosis, fibrosis progression was associated with less steatosis, but biopsies assessing steatosis severity and fibrosis severity were obtained at the same time, making it difficult to draw any causal inferences.¹¹ Because most of the available studies evaluating the association between steatosis and fibrosis are cross-sectional in nature, it is difficult to determine if steatosis is causally or temporally related to fibrosis severity.

Steatosis alone without inflammation is not thought to impart a risk of development of clinically significant fibrosis in non-HCV-infected individuals,¹² although few individuals have been followed long-term with sequential liver biopsies.^{13,14} The presence and natural progression of steatosis in LT recipients is less well understood. LT recipients with chronic HCV infection are frequently biopsied as part of routine clinical follow-up thereby providing a unique opportunity to study the effect of steatosis on subsequent fibrosis. Therefore, the aim of our study was to determine the prevalence of hepatic steatosis in LT recipients with HCV, and whether hepatic steatosis within the first year post-LT predicts subsequent fibrosis severity.

METHODS

Study design

Adults who had undergone LT for HCV from March 2002 through December 2007 at our center and had a liver biopsy available at one year (± 6 months) post-LT were eligible for inclusion in this retrospective cohort study. Patients were excluded if they had HIV infection, prior solid organ transplantation, or undetectable HCV RNA post-LT. The study was approved by the University of California San Francisco Committee on Human Research. Data sources included the United Network for Organ Sharing/Organ Procurement and Transplantation Network (UNOS/OPTN) database, the UCSF Liver Transplant database, and electronic medical records.

At our center, patients with HCV undergo annual biopsies to assess disease severity and when clinically indicated for abnormal liver tests. For this study, the index liver biopsies were evaluated by a single pathologist who was unaware of the clinical status of the patients. Biopsies were assessed for the presence of macrovesicular steatosis (5% considered present), as well as HCV necroinflammatory activity and fibrosis stage using Ludwig-Batts criteria.¹⁵ If steatosis was present on the index liver biopsy, steatosis severity was graded as grade 1 = 5–33%, grade 2 = 34–66%, grade 3 = 67–100% and the nonalcoholic steatohepatitis activity score determined (range of NASH activity score = 0–8).¹⁶ Subsequent biopsies, that occurred approximately annually, were evaluated for fibrosis stage only. For this study, significant fibrosis was defined as Ludwig-Batts stage 2 (scale 0–4). Subjects were followed until they achieved the study endpoint of significant fibrosis or until the end of the follow-up period. Donor biopsies are not routinely performed at our center, but the biopsies available were reviewed for the degree of macro- and microvesicular steatosis.

The presence of diabetes mellitus (DM), hypertension (HTN), and hyperlipidemia (HL) was determined using chart review, with the conditions defined as present if medications were used to treat DM, HTN, or HL. These conditions were deemed present pre-LT if either chart

documentation of the condition existed or if medications to treat the conditions were used. Donor variables were obtained from OPTN/UNOS sources and donor biopsy information was not routinely obtained. Donor risk index (DRI) was calculated.¹⁷ Immunosuppression information was obtained by chart review. The usual immunosuppression regimen at our center is tacrolimus, mycophenolate mofetil, and prednisone. Tacrolimus target levels were 8–12 ng/dL in the first year, and 6–8 ng/dL thereafter. Mycophenolate mofetil doses were tapered from 2–3 grams per day to 0.5–1 gram per day over the first year and weaned off in the majority of patients thereafter. Prednisone doses were tapered to 5 mg daily by the end of first month. Cyclosporine was used if patients were intolerant of tacrolimus. Sirolimus was used as alternative to tacrolimus primarily for renal-sparing effects. The exposure to prednisone, tacrolimus, cyclosporine, and sirolimus was determined by calculating the proportion of the total follow-up period during which the subject were treated with a specific medication. For example, if an individual were treated with prednisone from the time of LT until 3 years post-LT, with a total follow-up period of 5 years until the achievement of F2 fibrosis, the prednisone use was calculated to be $3/5 = 0.6$.

Statistical analysis

Categorical variables were analyzed using the chi square or Fischer exact test. Continuous variables were compared using the Wilcoxon rank sum test. Individuals with steatosis at index liver biopsy were compared to those without steatosis on index liver biopsy, with the primary outcome of interest being subsequent significant fibrosis (fibrosis score F2-4). Predictors of steatosis were evaluated using logistic regression. Time to significant fibrosis was assessed by Kaplan-Meier methods with steatosis and non-steatosis groups compared using log-rank test. Predictors of significant fibrosis were assessed using Cox regression models. Covariates evaluated as predictors of significant fibrosis and/or steatosis included metabolic factors (DM, HTN, HL, and body mass index), HCV-related factors (genotype, HCV activity on index liver biopsy, receipt of HCV antiviral treatment), and transplant-related factors (MELD, donor age, cold ischemia time, immunosuppressant medications, episodes of rejection treated with pulse-dosed corticosteroids and/or anti-lymphocyte therapy, treated cytomegalovirus infection). Covariates with p values of ≤ 0.1 in univariable models and/or were clinically relevant were considered for inclusion in the multivariable models. Stata v11.0 (College Station, TX) was used for data analysis.

RESULTS

Cohort and biopsy characteristics

Of the 293 HCV-infected transplant recipients meeting inclusion criteria, 152 patients had biopsies available for review. Reasons for exclusion from the study were lack of index liver biopsy between 6–18 months post-LT ($n=45$), lack of follow-up liver biopsy after index liver biopsy ($n=74$), and presence of at least stage 2 fibrosis on index liver biopsy ($n=22$). Seventeen of the 45 subjects without index biopsies had undergone liver biopsy within 6–18 months post-LT, but these biopsies were done at other centers and were not available for review by our pathologist and 42 of the 74 subjects without follow-up biopsies were on post-LT HCV treatment resulting in an omitted annual protocol biopsy.

Excluded subjects did not differ significantly from included subjects with regards to recipient age, gender, race, MELD, HCV genotype, or donor age (data not shown). The median post-LT follow-up for the study cohort was 2.97 (range 0.55–6.57) years and the median time from index liver biopsy to study endpoint (either significant fibrosis or end of follow-up period) was 2.09 (range 0.13–6.17) years. The median total number of biopsies per person (after the index biopsy at 1 year) was 2 (range 1 to 6). Index liver biopsies had a

median length of 2.5 cm (IQR 1.7–3.1), and contained a median of 8 (IQR 6–11) portal tracts.

Thirty-six recipients had pre-LT donor biopsies performed. A smaller proportion of the steatosis group than the no steatosis group had had donor biopsies performed (13 vs. 28%; $p=0.06$). Most donor biopsies demonstrated at least 5% steatosis, with only one donor having >30% steatosis. The proportion of donors with at least 5% macrovesicular steatosis was similar between patients with and without steatosis at one year post-LT (9% vs. 14%; $p=0.14$).

The steatosis and no steatosis groups were similar except for a trend toward older donor age (45 vs. 39 years; $p=0.11$), a significantly lower median body mass index (BMI) at the time of listing for LT (25 vs. 28 kg/m²; $p=0.04$), higher prevalence of pre-LT HTN (40% vs. 23%, $p=0.03$), and a higher proportion with HCV genotype 3 infection (26% vs 12%, $p=0.06$) in the steatosis group (Table 1). Similar proportions of both groups had pre-LT DM (9% vs. 17%, $p=0.20$) and hyperlipidemia (2% vs. 1%, $p=0.53$). The two groups were similar with regards to time from LT to index biopsy, and AST, ALT and creatinine levels at the time of index biopsy. However, a greater proportion of patients with steatosis on index biopsy had stage 1 (vs. 0) fibrosis on this index biopsy, as compared with the group without steatosis (62 vs. 43%), but this difference was not statistically significant ($p=0.09$).

Prevalence and Factors Associated with Steatosis

Steatosis was present on the index liver biopsy in 45 (29.6%) subjects. Biopsies with steatosis were mostly grade 1 (80%), with a small proportion with grade 2 (13%) or grade 3 (7%) steatosis. Only one subject had a NASH activity score greater than 4, but did not meet histologic criteria for a diagnosis of NASH. The presence of post-LT steatosis was positively associated with genotype 3 (OR 3.60, 95% CI), donor age (OR 1.03, 95% CI), and pre-LT HTN in multivariable models (OR 3.29 95% CI) (Table 2).

The proportion of obese (BMI ≥ 30 kg/m²) individuals at the time of LT listing tended to be lower in the steatosis than no steatosis group (20% vs. 39%; $p=0.09$). The prevalence of pre-LT obesity was also similar in the HCV genotype 3 and non-genotype 3-infected recipients (10% vs. 13%; $p=0.9$). At the time of index liver biopsy, a numerically but not statistically greater proportion of the steatosis group had developed new-onset DM than in the no steatosis group (22% vs. 12%; $p=0.11$). Similar proportions of the steatosis and no steatosis groups had developed new-onset HTN (24% vs. 31%; $p=0.43$) and HL (4% vs. 2%; $p=0.37$). By the end of the follow-up period, similar proportions of the steatosis and no steatosis groups had developed new-onset DM (26% vs. 18%, $p=0.39$), HTN (59% vs. 54%, $p=0.64$), and HL (12% vs. 9%, $p=0.56$) after the index biopsy.

Steatosis as a Predictor of Fibrosis Severity

The unadjusted cumulative rate of significant fibrosis (stage 2–4) in transplant recipients with steatosis vs. without steatosis was 49% vs. 24% two years after the index liver biopsy, and was 72% vs. 45% four years after the index biopsy (Figure 1). Significant fibrosis was seen in similar proportions of individuals with grade 1 vs. grade 2 or 3 steatosis at two years after the index liver biopsy (51% vs. 47%, logrank $p=0.87$). Steatosis was a significant predictor of F2–4 fibrosis (HR 2.09, 95% CI 1.26, 3.47) in univariable and multivariable models (HR 2.63, 95% CI 1.49, 4.63) (Table 3). Other variables independently associated with an increased risk of significant fibrosis were new-onset diabetes, new-onset hypertension, HCV activity on index liver biopsy, sirolimus use, and treated CMV infection. Post-LT HCV treatment was associated with a decreased rate of significant fibrosis (HR 0.45, 95% CI: 0.25, 0.83). In a sensitivity analysis including subjects with at least stage 2

fibrosis on index biopsy (n=22), steatosis remained a significant predictor of F2-4 fibrosis in the multivariable model (HR 2.03, 95% CI 1.26, 3.28). A larger proportion of this subgroup had an HCV activity 3, as compared with the rest of the cohort (29% vs. 10%, p=0.03).

Because steatosis is known to be associated with HCV genotype 3 infection, we chose to evaluate our multivariable model for predictors of F2-4 fibrosis in individuals not infected with HCV genotype 3. Steatosis persisted as an independent predictor of F2-4 fibrosis (HR 2.92, 95% CI 1.61, 5.28), with other factors associated with F2-4 fibrosis being HCV activity on index biopsy and new-onset hypertension post-LT, and receipt of anti-HCV therapy being protective against F2-4 fibrosis.

Limited interactions were evaluated. Evaluation of the interaction between sirolimus use and the presence of steatosis revealed that sirolimus use in the presence of steatosis was associated with a 9.38-fold greater risk of F2-4 fibrosis, compared with sirolimus use in the absence of steatosis (95% CI 1.37, 64.16). While indications for use of sirolimus were similar between the steatosis and no steatosis groups, and included rejection, renal failure, and malignancy, those who were treated with sirolimus had a higher proportion of at least one episode of treated rejection than those who were not treated with sirolimus (47% vs. 26%, p=0.02). No interaction between DM and steatosis was identified.

DISCUSSION

Steatosis is a frequent finding in HCV-infected liver transplant recipients, with about one-third demonstrating at least mild steatosis at approximately one year post-LT. Three factors were found to be associated with steatosis. HCV genotype 3 was an expected finding since this genotype is associated with “virologic” steatosis.¹⁸ The association between donor age and steatosis at one-year post-LT is new and may indicate that donor factors rather than host factors are important in determining the early presence of steatosis on post-LT liver biopsy. As expected, post-LT steatosis was associated with some comorbidities of the metabolic syndrome, since nonalcoholic fatty liver disease (NAFLD) is felt to be the hepatic manifestation of the metabolic syndrome.¹⁹ Pre-LT HTN as a predictor of steatosis at index liver biopsy may be an indicator of individuals who have the propensity to develop the metabolic syndrome, particularly since HTN is generally an infrequent finding in patients with ESLD due to high levels of circulating vasodilatory substances.²⁰ The lack of association between DM and steatosis was somewhat surprising, although in the non-LT setting, not all studies have demonstrated an independent relationship between insulin resistance and steatosis.²¹ Because of a low frequency of hyperlipidemia both pre- and post-LT, we were unable to detect an association between this condition and other comorbidities of the metabolic syndrome or with steatosis.

Importantly, we found that post-transplant steatosis was a strong and independent predictor of significant fibrosis. Our ability to detect a “dose-response” with greater degrees of steatosis was limited by the small proportions of individuals with grades 2 and 3 steatosis. The association between steatosis and fibrosis was present, despite adjustment for metabolic factors such as pre-existing or new-onset DM and HTN. Previous studies evaluating the relationship between steatosis and fibrosis have been limited by small sample size and/or lack of adjustment for metabolic confounders such as DM.^{22, 23} As has been documented in multiple prior studies, DM (both pre-existing and new-onset) was associated with increased risk of fibrosis progression in individuals infected with HCV.^{3, 24} Our findings contrast with those reported by Veldt et al⁶, likely due to differences in study populations, analytic methods and endpoints between the two studies. Veldt et al reported a higher prevalence of mild to moderate steatosis (39%) than our study but they evaluated steatosis at any time from 4 months to years post-transplantation. Our evaluation of steatosis was at a single early

time point post-LT to specifically determine the temporal relationship between steatosis and subsequent fibrosis. Additionally, the Veldt study examined diabetes and insulin resistance at 4 months post-LT whereas our study focused on pre-LT DM as a predictor of outcomes.

HTN alone has not previously been associated with fibrosis progression in HCV-infected individuals, and the pathophysiologic basis for promotion of fibrosis development is unclear. While antihypertensive medications such as angiotensin receptor blockers have been shown to improve inflammation and fibrosis in individuals with NASH, it is unclear if this phenomenon would apply to HCV-infected LT recipients.²⁵ Comorbidities of the metabolic syndrome, such as hypertension, may correlate with a more severe systemic inflammatory state.²⁶ Altered levels of several different adipocytokines (e.g., adiponectin, TNF- α , plasminogen activator inhibitor type 1) have been observed in individuals with the metabolic syndrome, as well as in those with NAFLD, and may mediate hepatocellular damage in the setting of steatosis.^{27–29}

Our finding of an association between sirolimus use and risk of significant fibrosis among patients with steatosis was unexpected. Animal studies indicate that sirolimus has antifibrotic properties, and suggest this drug may have potential benefit in recurrent fibrotic diseases such as HCV.^{29–31} However, hypertriglyceridemia is a known adverse effect of sirolimus,³² which may contribute to the risk of hepatic steatosis, though a direct association between sirolimus and hepatic steatosis has not been reported. Finally, because individuals who were treated with sirolimus had more episodes of treated rejection, sirolimus treatment may be a surrogate marker for treated acute rejection that involved more use of corticosteroids, although our sample size limited such subgroup analyses. It is possible that rejection severe enough to warrant the addition of sirolimus to the immunosuppressant regimen is a factor that is strong enough may drive fibrosis progression, overriding any potential antifibrotic effects of sirolimus, particularly if steatosis is present. Alternatively, individuals who are treated with sirolimus may possess unmeasured factors that make them more susceptible to both the development of steatosis and advanced fibrosis.

A potential limitation in our study is the lack of a donor liver biopsy for all subjects. However, donor age may be used as a surrogate for hepatic steatosis, since increasing age is associated with higher prevalence of hepatic steatosis.¹¹ Importantly, the prevalence of donor DM and median donor BMI were similar between steatosis groups, suggesting no obvious differences in potential risks of steatosis between donor groups. Finally, the existing data regarding donor steatosis and HCV recurrence and fibrosis progression is mixed, possibly related to differences in categorization of steatosis^{33–34}.

Our use of single center data may limit the generalizability of the results, particularly in centers with different immunosuppression strategies. Because we excluded individuals with greater than fibrosis stage 2 on the index biopsy and those without biopsies during the first 6–18 months post-LT, we may have excluded those with both the most severe disease, as well as those with mild disease. Also, because our classification of DM, HTN, and HL were based on the use of medications to treat these conditions, it is likely that the true prevalence of these conditions is higher than we observed. However, because we would expect this misclassification to be random and evenly distributed between the steatosis and no steatosis groups, the relationship between steatosis and fibrosis progression should not be affected. Finally, given the challenges in distinguishing 5% steatosis from that slightly more or less than 5% by microscopy, other methods of assessment such as digital quantification may be more useful, at least in the research setting³⁵. However, the relevance of such methods in clinical practice has not been established.

In conclusion, we have shown that steatosis is a frequent finding in HCV-infected transplant recipients and its presence early post-LT predicts a greater risk of subsequent fibrosis progression. Moreover, we found that the association between steatosis and risk of significant fibrosis remained even after adjusting for other components of the metabolic syndrome. Thus, a finding of steatosis early post-LT should serve as a marker of the need for closer histological follow-up and possible consideration of early antiviral intervention. An important future area of research is to determine if modification of the metabolic syndrome and/or steatosis can reduce the HCV transplant recipients' risk of fibrosis progression.

Abbreviations

BMI	Body mass index
DM	Diabetes mellitus
DRI	Donor risk index
ESLD	End-stage liver disease
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HL	Hyperlipidemia
HTN	Hypertension
IQR	Interquartile range
LT	Liver transplantation
MELD	Model for End-stage Liver Disease
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis

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Figure 1.

A larger proportion of the steatosis group had developed F2-4 fibrosis, compared to the no steatosis group (log rank $p = 0.003$).

Table 1

Characteristics of the Study Cohort

	Steatosis on Index Biopsy (n = 45)	No Steatosis on Index Biopsy (n = 107)	p value
Age, median years (IQR)	54 (51–60)	55 (51–59)	0.89
Male (%)	33 (73)	80 (75)	0.85
Black race (%)	3 (7)	14 (13)	0.25
MELD at LT, median (IQR)	21 (15–27)	20 (17–26)	0.88
HCC (%)	26 (50)	54 (45)	0.55
Listing BMI, median kg/m ² (IQR)	25 (24–27)	28 (25–32)	0.04
Pre-LT DM (%)	4 (9)	18 (17)	0.20
Pre-LT HTN (%)	18 (40)	24 (23)	0.03
Pre-LT hyperlipidemia (%)	1 (2)	1 (1)	0.53
Donor risk index (IQR)	1.45 (1.25, 1.68)	1.42 (1.23, 1.73)	0.99
Donor age, median (IQR)	45 (32–52)	39 (26–51)	0.11
Cold ischemia time, median hours (IQR)	8 (7–10)	9 (7–10)	0.35
Donor diabetes (%)	3 (7)	6 (6)	0.73
Donor BMI, median kg/m ² (IQR)	26 (22–30)	27 (24–31)	0.33
HCV genotype 3 (%)	10 (26)	10 (12)	0.06
HCV activity, median (IQR)	2 (1–2)	2 (1–2)	0.38
Post-LT HCV treatment (%)	18 (40)	38 (36)	0.60
Treated CMV infection (%)	3 (7)	10 (9)	0.59
Episode of acute rejection (%)	9 (20)	37 (35)	0.12
Biliary complication (%)	6 (13)	20 (19)	0.42
Time to index biopsy, median years (IQR)	0.96 (0.6–1.1)	0.89 (0.7–1.1)	0.58
AST at index biopsy, median U/L (IQR)	91 (49–130)	80 (39–138)	0.51
ALT at index biopsy, median U/L (IQR)	112 (77–187)	109 (50–196)	0.45
Creatinine at index biopsy, median mg/dL (IQR)	1.2 (1.0–1.4)	1.2 (1.0–1.4)	0.85

* IQR=interquartile range; MELD=Model for Endstage Liver Disease; HCC=hepatocellular carcinoma; BMI=body mass index; DM=diabetes mellitus; HTN=hypertension; HCV=hepatitis C virus; CMV=cytomegalovirus

Table 2

Independent predictors of steatosis at 1 year post-LT

Variable	<i>Univariable</i>	<i>Multivariable</i>
	OR (95% CI)	OR (95% CI)
Genotype 3	2.52 (0.95, 6.68)	3.60 (1.24, 10.47)
Pre-LT HTN	2.28 (1.08, 4.82)	3.29 (1.34, 8.03)
Donor age	1.02 (0.99, 1.05)	1.03 (1.00, 1.06)

Variables evaluated but not significantly associated in multivariable models with steatosis included DM, recipient age, race, BMI, calcineurin inhibitor use, sirolimus use, and anti-HCV medication use.

Table 3

Predictors of Significant Fibrosis (F2-4) in HCV-Infected LT Recipients

	<i>Univariable</i> HR (95% CI)	<i>Multivariable</i> HR (95% CI)	
		All subjects (n=152)	Non-genotype 3 (n=132)
Steatosis	2.09 (1.26, 3.47)	2.63 (1.49, 4.63)	2.92 (1.61, 5.28)
CMV infection	2.97 (1.46, 6.04)	3.88 (1.72, 8.77)	--
HCV activity	1.69 (1.15, 2.49)	1.77 (1.17, 2.67)	1.93 (1.17, 3.18)
Post-LT HCV treatment	0.66 (0.39, 1.13)	0.45 (0.25, 0.83)	0.31 (0.15, 0.65)
New-onset DM	1.87 (1.03, 3.39)	2.03 (1.01, 4.08)	--
New-onset HTN	1.65 (0.98, 2.78)	1.97 (1.14, 3.39)	2.69 (1.46, 4.98)
Sirolimus exposure	1.85 (0.81, 4.24)	3.13 (1.23, 7.96)	--

Variables evaluated in multivariate models, but were not significantly associated with significant fibrosis included donor risk index, donor diabetes, HCV genotype, 1 episode of treated rejection, or hyperlipidemia.