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Impact of Donor and Recipient Clinical Characteristics and Hepatic Histology on Steatosis/Fibrosis Following Liver Transplantation

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Abstract

Background: Deceased donor and recipient predictors of post transplant steatosis/steatohepatitis and fibrosis are not well known.

Aim: Evaluate the prevalence and assess donor and recipient predictors of steatosis, steatohepatitis, and fibrosis in LT recipients.

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Methods: Using the ITN A-WISH multicenter study (NCT00135694), donor and recipient demographic and clinical features were collected. Liver biopsies were taken from the donor liver at transplant, and from recipients per protocol and for cause (i.e. abnormal transaminases and/or to rule out rejection) and were interpreted by a central pathologist.

Results: 183 paired donor/recipients liver biopsies at the time of transplant and post-transplant follow up (median time 582 days; average time to last biopsies was 704 days (SD ± 402 days) were analyzed. Donor steatosis did not influence recipient steatosis or fibrosis. 10/183 recipients had steatohepatitis on the last biopsy. Recipient BMI at the time of liver biopsy was the most influential factor associated with post-transplant steatosis. Both donor and recipient metabolic syndrome features were not associated with graft steatosis. Untreated HCV infection was the most influential factor associated with the development of allograft fibrosis.

Conclusions: In a large experience evaluating paired donor and recipient characteristics, recipient BMI at the time of liver biopsy was most significantly associated with post-transplant steatosis. Untreated HCV etiology influenced graft fibrosis. Thus relative to untreated HCV, hepatic fibrosis in those with steatosis/steatohepatitis is less common though long term follow up is needed to determine the course of post transplant fibrosis. Emphasis on recipient weight control is essential.

Introduction

Recurrence of native liver disease is not uncommon and occurs variably after liver transplantation (LT).[1] The frequency and magnitude of this recurrence varies among the etiologies of liver disease, and is not completely understood in the setting of nonalcoholic fatty liver disease (NAFLD) induced cirrhosis. NAFLD is the leading cause of liver disease in the Western world, and is currently the second or third most common indication for LT, depending on the region.[2,3] Given the prevalence of HCV as the leading indication for LT in the United States, much has been studied with regard to the natural history of HCV liver disease both before and after transplantation, including risk factors for recurrence and prognosis, though the same is not true for other causes of liver disease. With the advent of direct-acting antiviral (DAA) agents decreasing the incidence of end stage liver disease (ESLD) from HCV, and the concomitant rise in the obesity epidemic, NAFLD is poised to become the leading cause of transplantation in the Western world between 2020 and 2025.[4]

Despite the increasing burden of this disease, little is known about the predictors of fatty liver disease to ESLD, particularly in the post-transplantation setting. It is unclear as to which donor and/or recipient characteristics influence the development of recurrent NAFLD after LT. A few studies have endeavored to map this evolution, although robust longitudinal follow up data is lacking. These studies are retrospective in nature, and have selection bias with regard to histologic assessment. While there is data showing that severe steatosis in the donor liver is associated with increased rates of primary graft nonfunction and poorer outcomes,[5] few studies have assessed the impact of donor steatosis on short and long term outcomes,[6–9] and have mixed conclusions.

Herein, we report on the predictors of post-transplant NAFLD and fibrosis, using prospectively collected protocol and for cause liver biopsies (FCLB) from 7 different

centers as part of the ITN AWISH trial on immunosuppression withdrawal.[10] Based on the hypothesis that NAFLD is a driving cause of steatohepatitis, which in turn may lead to fibroses, we aimed to identify pre- and post-transplant risk factors in donors and recipients that might help predict the outcomes of fibrosis and steatosis/steatohepatitis. Further, in comparing the development of post transplant steatosis/steatohepatitis to recurrence of HCV fibrosis, we aimed to provide some context in understanding this relative risk, though we recognize that this framework is one of historical context given the development of effective therapies for HCV. The immune tolerance network (ITN), through the availability of a large data set and with paired donor/recipient liver biopsies, provided a unique opportunity to address the role of donor and recipient factors in the development of moderate/severe steatosis, steatohepatitis, and fibrosis in liver transplant recipients.

Materials and Methods

Patient and Study Design

The ITN study for the Gradual Withdrawal of Immune System Suppressing Drugs in Patients Receiving a Liver Transplant (A-WISH) (NCT00135694) was conducted October 2005 to September 2015 and was a prospective multicenter, open-label, randomized trial. The study was designed to assess the safety of withdrawing immunosuppressive medications in two groups of patients receiving liver transplants: those transplanted for HCV cirrhosis, and those transplanted for nonimmune, nonviral causes of liver failure. Subjects were enrolled at 7 centers in the United States (University of Pennsylvania, University of California San Francisco, University of Michigan, Northwestern University, University of Washington, Baylor University Medical Center, and University of Pittsburgh Medical Center). Inclusion criteria were cirrhosis and hepatic decompensation due to hepatitis C infection or due to nonimmune, nonviral causes. For subjects with hepatitis C infection, the presence of HCV RNA in blood was required. The last HCV patient was randomized in February 2011 and treatment with interferon was an exclusionary criterion. Other exclusion criteria included primary liver failure due to autoimmune disease or hepatitis B infection, recipient of a previous transplant, multiorgan- or split-liver transplants other than right trisegmentectomy, living donor liver transplants, recipients of deceased-after-circulatory-death donor organs or HCV-infected donor grafts, and stage III or higher hepatocellular cancer (including those detected in the explanted liver). All subjects provided written informed consent at enrollment prior to transplantation, and again at the point of assessment for randomization eligibility. The study was approved by the institutional review boards of all participating centers.

Subjects received immunosuppression with a calcineurin inhibitor, antimetabolite and corticosteroids. Corticosteroids were tapered in the 3 months following transplantation, and dual therapy was continued. Monotherapy with a calcineurin inhibitor or antimetabolite was a requirement at least 3 months prior to assessment for random assignment. Subjects were regularly assessed for evidence of allograft rejection. No sooner than one year after transplantation, eligible subjects were randomly assigned in a 4 to 1 ratio to immunosuppression withdrawal or to maintenance. Patients were considered as having taken a medication if it was prescribed for more than 6 months. Two hundred and eighty-six

patients were initially consented for the AWISH trial and 11 were excluded based on ≥ Stage III HCC in the explanted liver. Of the remaining 275 patients, 76 were removed from the study as they failed to reach the targeted monotherapy. While recipient follow up data were available in the 199 remaining subjects, donor data were missing in one case, which was excluded from analysis. An additional 15 patients were excluded from analysis, as their last biopsy was taken within 100 days of transplant, which was considered too close to transplant to assess for differences in de novo versus recurrent graft steatosis. The remaining 183 donor-recipient pairs were all included in the analysis (Figure 1).

Donor liver biopsies were obtained at the time of transplant and were assessed for baseline degree of inflammation, steatosis, and fibrosis. A scheduled post-transplant liver biopsy was obtained at the time of prerandomization assessment. All biopsies obtained specifically for the study were assessed by a central pathologist. Additional liver biopsies were performed as clinically indicated due to concern for allograft dysfunction (termed as for cause liver biopsy-FCLB). Allograft dysfunction was defined as elevated hepatic biochemical tests including AST, ALT, and/or bilirubin to higher than twice the upper limit of normal. For cause liver biopsies were sent to the central pathologist for independent analysis.

In total, 1124 liver biopsies were collected from 283 patients as part of the ITN-AWISH trial, and 896 of these are from the donor-recipient pairs that were included in this analysis. T0 biopsies (performed in the cold) and T1 biopsies (performed at reperfusion) were considered donor biopsies. Of the 896 biopsies, 182 were T0 biopsies and 183 were T1 biopsies. T0 biopsies were used as the donor baseline, except in once case where a T0 biopsy had not been collected. There were 531 post-transplant recipient biopsies collected in our group of study patients. Only one biopsy was available in 43 patients, while the remaining 140 recipients had serial post-transplant biopsies available for analysis (Figure 2).

Hepatic steatosis was graded based on the Brunt/Kleiner Scoring system, using a semi-quantitative scale of 0–3. Both macrovesicular, microvesicular, and mixed cases of hepatocyte infiltration were included, with 0 = 0 - <5% (absent), 1 = 5 - <33% (mild), 2 = 33–66% (moderate), and 3 = >66% (severe).[11] Donor graft steatosis was considered present when >5% of micro- and/or macrosteatosis was present within hepatocytes. Microvesicular steatosis was assessed on the basis of small lipid vesicles without nuclear displacement and graded as mild (<33%) or moderate/severe (≥33%). Liver Fibrosis was staged by Ishak fibrosis scale.[12]

Statistical methods

Patients were grouped as either no significant steatosis (score 0–1), or significant steatosis (score 2–3), and separately categorized as either no significant fibrosis (grade ≤2) or significant fibrosis (grade >2). The associations between individual clinical variables and severity of steatosis or fibrosis grade were tested using logistic regression. All P-values represent the results of two-sided tests. Multivariate logistic regression models were performed by including the variables significant at a nominal p-value < 0.05 in the initial univariate logistic regression analyses. Analyses were conducted using R (version 3.2.5) and NCSS 8 (NCSS, LLC. Kaysville, Utah, USA).

Results

The distributions of recipient characteristics are displayed in Table 1. The average age at transplant was 55 years (SD ± 8.55 years), and the majority were white (86%) and male (76%). The most common indication for transplant was HCV cirrhosis in 94 cases (51%), followed by alcoholic liver disease in 46 (25%), NASH cirrhosis in 25 (14%), cryptogenic cirrhosis in 11 (6%), metabolic disease in 5 (3%), and in 2 (1%) for predominantly biliary tract related indications. Average time of follow-up/last liver biopsies for this study was 704 days (SD: ± 402 days). Pretransplant diabetes mellitus, hyperlipidemia/dyslipidemia, and hypertension were present in 38.8%, 13.1%, and 40.4%, respectively. Immunosuppressive regimens were dictated by the ITN-AWISH study protocol, as summarized above.

Donor characteristics are highlighted in Table 2. The average age of donors was 42.8 years (SD: ± 17.4 years). Overall, 67.6% of donor livers had some degree of steatosis (44.5% mild, 19.8% moderate, and 3.3% severe). Donor comorbidities were present in 56.8% of cases, with diabetes mellitus present in 11.4%, hyperlipidemia/dyslipidemia in 8.8%, and hypertension (HTN) in 36.8%. Donor comorbid data were not collected as part of the AWISH trial, and missing data here were attributed to the retrospective nature of this data collection.

Assessment of donor/recipient characteristics contributing to post transplant steatosis:

The potential associations between recipient and donor characteristics and steatosis severity were tested (Table 3). In univariate analysis, indications for LT that included HCV cirrhosis, cryptogenic cirrhosis, and nonalcoholic steatohepatitis were significantly associated with moderate/severe steatosis ($p < 0.05$). Both higher recipient BMI at transplant and at biopsy were associated with higher risk of moderate/severe steatosis ($p=0.004$ and $p < 0.001$, respectively). When we evaluated the available liver biopsies at 24 ± 3 months and 36 ± 3 months, again BMI at biopsy was significantly correlated with steatosis ($p=0.04$ and $p=0.02$ respectively, Table 3). Time from LT to biopsy was also found to be moderately associated with higher risk of moderate/severe steatosis (OR=1.001, 95%CI: 1.000 – 1.002, $p=0.03$). Immunosuppression withdrawal also appeared to have a higher association with moderate/severe steatosis development (OR=2.01, 95%CI: 1.25 – 3.22, $p=0.004$).

Weight gain was associated with steatosis severity with OR (95%CI) = 1.16 (1.05 – 1.28), $p=0.005$. Given the relatively small number of patients with long term biopsies being available and multiple features being statistically significant on univariate analyses, it was felt the multivariable model data would not be robust; yet when including BMI at biopsy in the multivariate model, change in BMI became insignificant ($p=0.64$) while BMI at biopsy remained significant ($p=0.001$; data not shown). We further evaluated change in steatosis grades between last recipient biopsy and donor biopsy, and no change in severity was observed (Mean (95%CI): −0.16 (−0.34 to 0.02); $p=0.08$).

The impact of donor and recipient microvesicular steatosis on recipient steatosis was assessed and was not statistically significant. 48% (49% mild, 45% moderate/severe, 44% mixed micro/macrovesicular steatosis) of the donors and 6% of the recipient had

microvesicular steatosis and more often, the liver biopsies had mixed micro/macrovesicular steatosis rather than an isolated pattern of steatosis.

There were 26 cardiac events recorded during the study period (Table S1) and relative to those without cardiac events, moderate/severe steatosis was more common in those with cardiac events (42 % versus 20 %; OR 1.72 CI 1.11–2.66; P 0.01 (Table S2).

Impact of immunosuppression use and withdrawal on development of steatosis:

Liver biopsies were analyzed from three randomized immunosuppression withdrawal cohorts: maintenance, withdrawal, and terminated before randomization. Seventy-seven biopsies were taken from immunosuppression withdrawal patients at various stages (some in the process of withdrawal, some postwithdrawal, and some with success of withdrawal, n=9) or failed, and some from prerandomization). Tacrolimus was the most consistently used immunosuppressive medication in 96.7% of patients, followed by mycophenolate Mofetil (38.2%) and steroids (34.4%) (Table S3). Within the immunosuppression withdrawal group, the percentage of dosage (the fraction of baseline dosage) at the time of biopsies was not associated with steatosis severity (p=0.2), indicating minimal effect of IS exposure to steatosis severity.

Impact of NAFLD on development of steatosis/fibrosis:

Using a NAFLD Activity Score (NAS) score ≥4, only 10 of 183 (5.5 %) recipients had steatohepatitis on the last liver biopsy while using an NAS score of ≥5 only 7 patients had steatohepatitis. More often, NASH was associated with moderate/severe steatosis when compared to non-NASH recipients in univariate analysis (p=0.04). However, when including other clinical characteristics that also showed statistical significance in univariate analysis, such as BMI at transplant or BMI at biopsy, NASH became a nonsignificant contributor to moderate/severe steatosis (p=0.52). Further, there was no association between NASH and steatosis and fibrosis as two independent variables.

Additionally, the change in steatosis grades between last biopsy and donor biopsy was not significantly different from no steatosis stage (Mean (95%CI): −0.16 (−0.34 to 0.02); p=0.08). When stratifying recipients based on NASH, in the non-NASH group there was a significant reduction in steatosis grade from donor biopsy to last biopsy (Mean (95%CI): −0.25 (−0.43 to −0.06); p=0.01), but the increase in steatosis grade was not significant in the NASH group (Mean (95%CI): 0.41 (−0.04 to 0.88); p=0.12).

Distribution of BMI at time of liver biopsy was not significantly different in those with steatohepatitis but was significant in those with steatosis (Table 3 and Figure 3a and 3b). We evaluated steatosis grade in recipients in those who received donor organs that had grade 2 or 3 steatosis. Of note, in those who received grade 3 steatotic livers, the last recipient liver biopsy noted 50% (3/6) to be at grade 0/1.

Assessment of characteristics contributing to post transplant fibrosis:

The potential associations between recipient and donor characteristics and fibrosis stage were also tested (Table 4). Donor male gender was found to be nominally associated

with higher fibrosis stage (OR=1.47, 95%CI: 1.05 – 2.06, p=0.03). HCV cirrhosis as the indication for LT was significantly associated with increased risk of a higher fibrosis stage (OR=4.48, 95%CI: 2.83 – 7.08, p<0.001), while alcoholic liver disease and nonalcoholic steatohepatitis had an inverse association with development of fibrosis (OR=0.25, 95%CI: 0.12–0.51, p<0.001, and OR=0.38, 95%CI: 0.18–0.80, p=0.01). When we evaluated the available liver biopsies at 24 ± 3 months and 36 ± 3 months, HCV as etiology of liver disease was significantly correlated with fibrosis (p= 0.0007 and p<0.0001 respectively, Table 4). Recipient pretransplant diagnoses of hyperlipidemia/dyslipidemia and diabetes mellitus were associated with lower risk for fibrosis (p<0.05), as was BMI at the time of biopsy (p=0.04). The type of immunosuppression used, or immunosuppression withdrawal had no impact on the development of fibrosis (p>0.05).

Again recognizing the concern of the robustness of multivariable model analyses, HCV cirrhosis as the indication for LT was the only significant risk factor in development of higher post-transplant fibrosis stages (OR=4.95, 95%CI: 1.74 – 14.0, p= 0.002).

Impact of HCV on development of post transplant fibrosis:

Given the changing landscape of HCV treatment, we further analyzed the cohorts to see if factors associated with post-transplant steatosis or fibrosis were related to HCV status (Tables S4–S7). While we noted that HCV status itself was associated with the development of post-transplant fibrosis but not steatosis, there were no additional predictors of fibrosis developments within the HCV cohort. When we excluded patients with HCV and assessed for risk factors associated with post-transplant steatosis, again, on multivariate analysis, BMI at the time of transplant correlated with steatosis. There was a weak association with immunosuppression withdrawal, but caution needs to be exercised about the relatively few patients at risk.

Trends in development of steatosis/fibrosis over time:

In a subset analysis of patients with more than one biopsy post-transplant there was a trend towards development of steatosis over time across all patients, though this was not statistically significant (Figure 4). This trend was not impacted by a pretransplant diagnosis of NASH or by donor steatosis status. There was a statistically significant association in the development of fibrosis over time from transplant, which was associated with pretransplant HCV status. This was noted as early as 500 days post-transplant, and there was a positive correlation between time from transplant and increased fibrosis stage thereafter (Figure 5).

Discussion

The prevalence of NAFLD continues to rise in the general population, and while the incidence of its development into NASH cirrhosis or subsequent end-stage liver disease is unknown, it is currently the third leading indication for liver transplantation in the US after hepatitis C and alcoholic liver disease. The implication of the burden of this disease in the post-transplant patient is largely unknown, but certainly has the potential to have a significantly negative impact on survival, and has been previously associated with post-transplant cardiovascular disease and mortality over the long-term.[14,15]

Our dataset is the largest cohort representing a heterogeneous population of US patients across seven major transplant centers, wherein the proportion of primary cause for liver failure is reflective of the leading causes of end stage liver disease in the US. Furthermore, this data is unique, in that it was collected as part of a multicenter, prospective trial, and all biopsies were assessed by a central pathologist.

Overall, several important observations can be made from our analysis. Firstly, we show that only BMI at time of post-transplant biopsy was significantly associated with post-transplant steatosis. This was surprising, as previous studies, albeit with discordant observations, as well as our own intuition would have suggested that pretransplant NAFLD and donor steatosis should have had an impact on post-transplant steatosis status.[7–9,16–19] The liver, which plays a central role in lipid metabolism, has been linked with the development of NAFLD, and therefore we expected that donor genetic factors transferred to the recipient would be associated with abnormal lipid metabolism post-transplant.[20]

Both the studies by Dumortier et al. and Kim et al. found donor graft steatosis to be a significant predictor of post-transplant steatosis on multivariate analysis. That said, the patient population studied by Dumortier et al. did not include those transplanted for NASH or cryptogenic cirrhosis, and included a very large proportion of patients transplanted for ALD, bringing to question the broader applicability of their results.[7] Interestingly, analysis by Kim et al. showed that donor graft steatosis played an important role in post-transplant NAFLD in those patients undergoing transplant for ALD.[9] Taken together with the findings by Dumortier et al., this might suggest that donor steatosis may have a differential effect based on the underlying indication for transplantation. Indeed, the study by Dureja et al., which focused on patients undergoing transplant for NAFLD-related cirrhosis showed that donor steatosis had no impact on recurrent disease[8]. Our own data suggests that modifiable post-transplant factors leading to increased BMI are the driver of post-transplant steatosis, as opposed to unmodifiable donor derived factors. This was even true when we looked at the small subset of donors with severe steatosis, and found no impact on the development of post-transplant steatosis.

Further, we analyzed the frequency and impact of steatohepatitis on recipient fibrosis. Similar to the study by Dumortier et al. steatohepatitis was infrequent in our study and there was no association with fibrosis as an independent variable.[7] Additionally, we assessed the differential impact of micro- and macrosteatosis in the donor, and similar to others we saw no difference in recipient outcomes based on the donor histologic feature of microsteatosis.[6]

In our study, we found steatosis to be present in 68% of donors, which is similar to previous reports.[18,21] With the development of normothermic perfusion devices, likely more organs considered marginal for excess fatty liver content may be recovered.[22] While normothermic perfusion may improve graft steatosis, mitigating some risk of primary graft failure or long-term biliary complications, our data suggest that these organs can be used without consideration for their potential long-term impact on post-transplant NAFLD, as there is little prospective or broadly applicable data supporting this concern.

We also found that post-transplant liver steatosis, once noted, remains largely stable overtime. As with NAFLD in the general population, our concern with post-transplant patients burdened with fatty liver was that hepatic steatosis would lead to steatohepatitis, and that inflammation would eventually lead to graft dysfunction and potentially fibrosis. We, however, did not see fibrosis progression associated with hepatic steatosis. Multivariate analysis demonstrated that post-transplant fibrosis was only associated with a pretransplant diagnosis of HCV. A recent study by Galvin *et al* suggested that de novo steatosis is associated with fibrosis in the post-transplant setting, though this was strongly correlated with pretransplant HCV status.[23] De novo NASH has previously been associated with a pretransplant diagnosis of HCV,[18] and that post-transplant fibrosis associated with post-transplant NASH was uncommon.[24] Our data supports prior studies, and suggests that the burden of post-transplant disease in these patients is more strongly correlated with their pretransplant diagnosis of HCV rather than post-transplant detection of hepatic steatosis.

The unique and novel aspect of our study is the assessment of the impact of donor steatosis as well as pre- and post-transplant recipient factors on postliver transplant recipient steatosis across a large, multicenter cohort of donor-recipient pairs with prospectively collected data. Further, this analysis is unique in that we were able to concurrently look at the natural history of hepatitis C and also steatosis in those at risk for it. We demonstrated that HCV, in an untreated population, was an overriding reason for the development of fibrosis while metabolic syndrome related steatosis may have a relatively benign liver disease course over the intermediate term. This may be one of the few last opportunities to evaluate concurrently the natural history of HCV and metabolic syndrome related steatosis as successful HCV therapy, in recent times, has favorably changed the course of that disease post liver transplantation.

Another important observation was that the type of immunosuppression did not impact steatosis or fibrosis development. Immunosuppression medications have been associated with the development of metabolic syndrome, and may lead to increased morbidity and mortality in the post-transplant patient.[25] It is possible that the nature of the immunosuppression withdrawal trial from which our patient cohort was selected may bias our results diminishing the development or effects of post-transplant steatosis. That said, there did not appear to be a differential impact of any given immunosuppressive medication, and immunosuppression withdrawal did not dictate the presence or absence of post-transplant steatosis on multivariate analysis, suggesting that these medications are not the main drivers of post-transplant steatosis as previously described by Dumortier et al.[7]

There are several limitations in this analysis. Firstly, while the data were collected prospectively, it was done as part of a clinical trial aimed at addressing the impact of reduced immunosuppression regimens on patient and graft survival, and was not specifically designed to assess the natural history of post-transplant hepatic steatosis. While BMI at the time of post-transplant biopsy was the only statistically significant finding in our analysis, it is probable that our sample size is grossly underpowered to identify more subtle contribution from other known contributors to fatty liver disease. Additionally, the dataset does not include liver failure patients from autoimmune hepatitis or non-HCV viral hepatitis, and so while the majority of etiologies of cirrhosis and liver failure are included in this study, the

data may not be representative of these trends in all etiologies of liver failure. Indeed, as the landscape of liver failure continues to rapidly evolve in the era of DAAs and the obesity epidemic, this analysis will likely need to be repeated using a larger data set incorporating a higher percentage of patients presenting with NASH related liver disease.

Further limiting this study is the consideration that the impact of hepatic steatosis and steatohepatitis may manifest over a longer follow up period than the three years in which this patient cohort was followed. Thus additional analysis at later time points will be critical to identifying longer term impact of hepatic steatosis on patient and graft survival. This would also likely help us learn about co-morbidities such as cardiac disease as it impacts patient survival, given the prolonged time frame over which such complications manifest. Thirdly, this cohort may be construed as being biased given that these were not protocol liver biopsies and only selectively done when indicated, and only candidates who met inclusion criteria for an immunosuppression withdrawal study were included. Lastly, we have a cohort of untreated HCV while most cases of HCV are currently treated successfully favorably altering the natural history of this infection.

In conclusion, using the largest, most robust cohort of transplant patients with clinical data and both protocol and for-cause post-transplant liver biopsy assessment, we found that the incidence of post-transplant hepatic steatosis was only associated with post-transplant BMI at time of liver biopsy, and was not associated with the development of fibrosis. The development of post-transplant fibrosis was related to untreated HCV disease and was not influenced by steatosis suggesting that, if present, post-transplant progressive liver disease related to metabolic disease may take a longer time to evolve.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

A-WISH	Gradual Withdrawal of Immune System Suppressing Drugs in Patients Receiving a Liver Transplant
ESLD	End Stage Liver Disease
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Viral Infection
ITN	Immune Tolerance Network
LT	Liver Transplantation
NAFL	Nonalcoholic Fatty Liver

NAFLD	Nonalcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	nonalcoholic steatohepatitis

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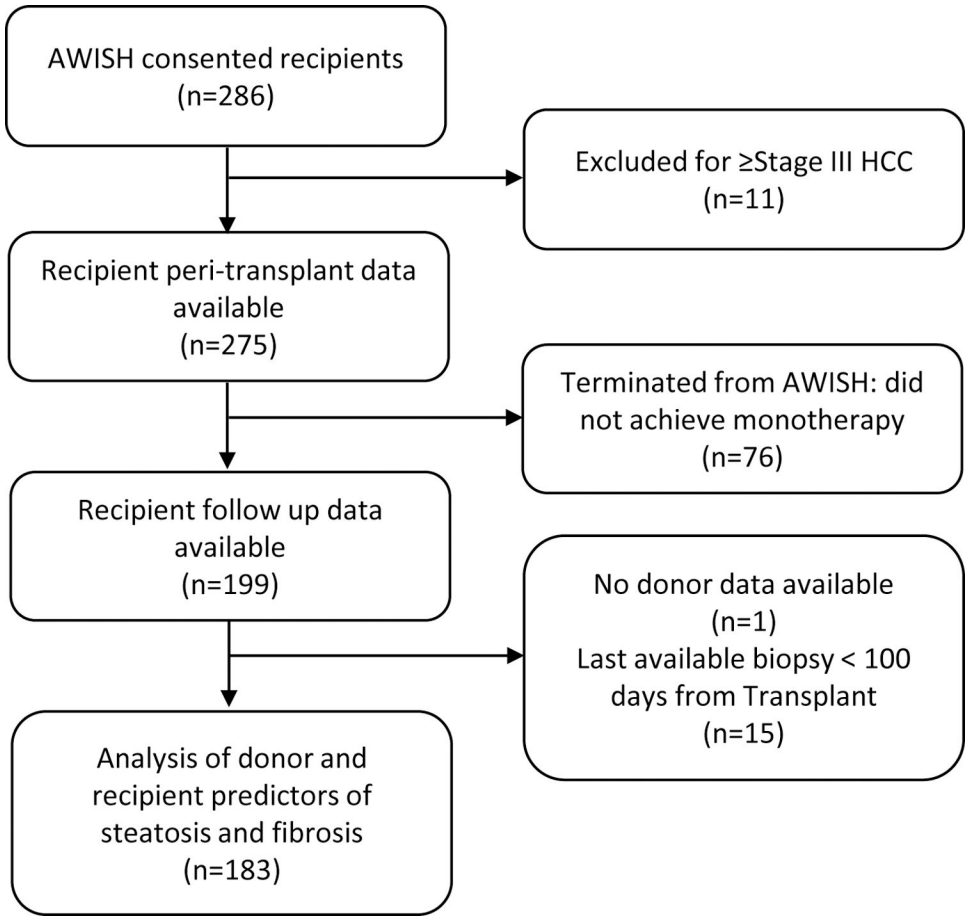


Figure 1.
Study cohort.

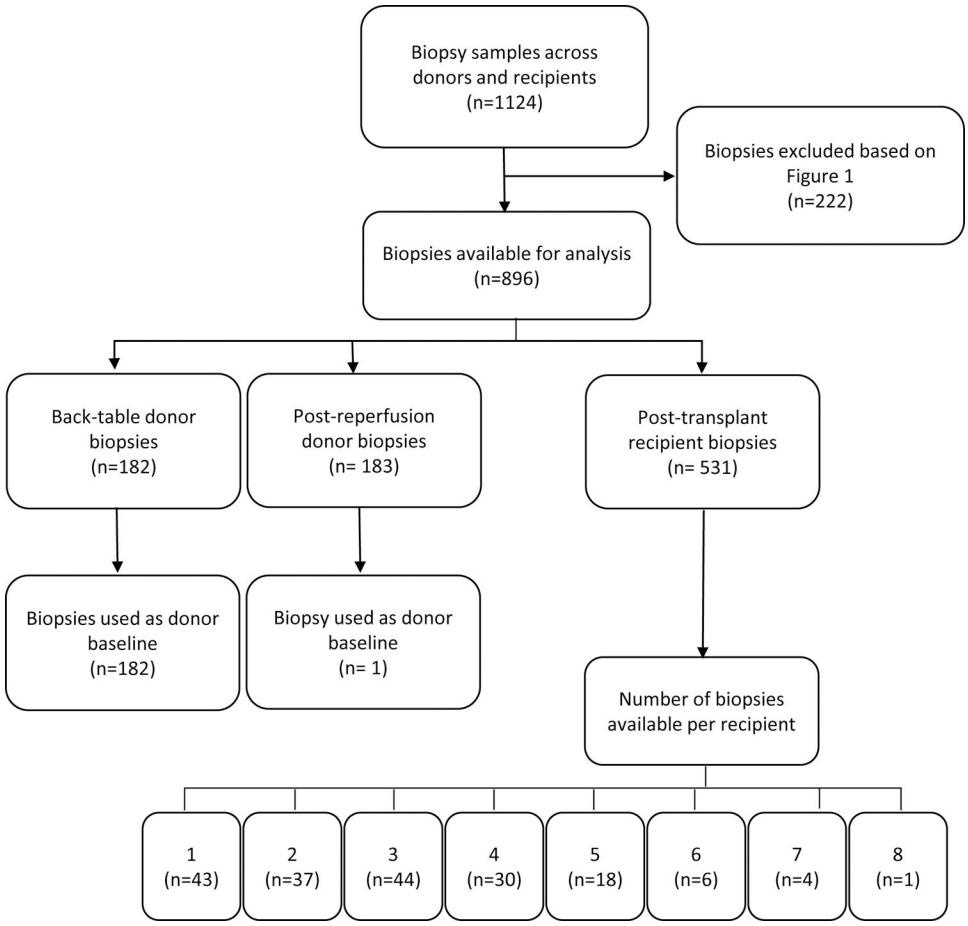
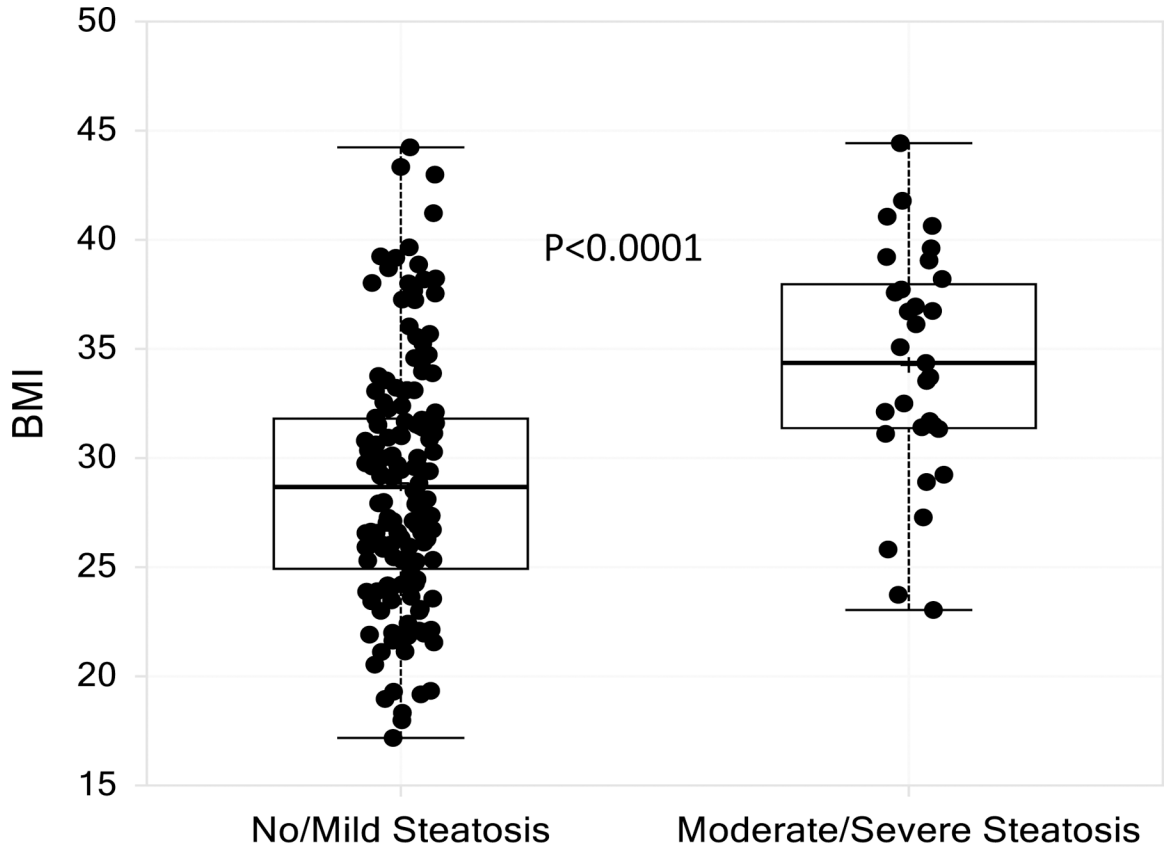


Figure 2.
Biopsies collected.



A.

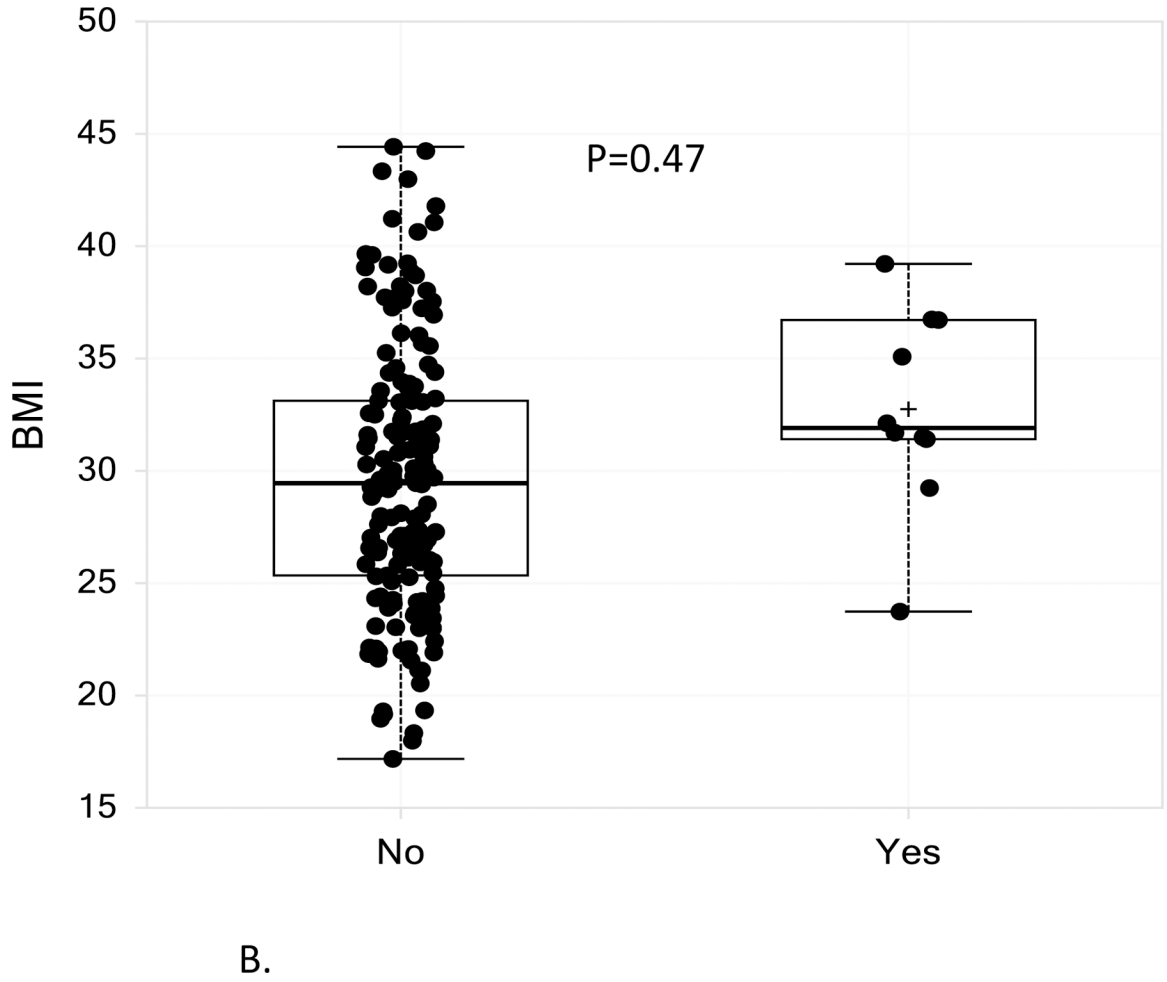


Figure 3.

A. Distribution of BMI at last biopsies by Steatosis status

B. Distribution of BMI at last biopsies by NASH status

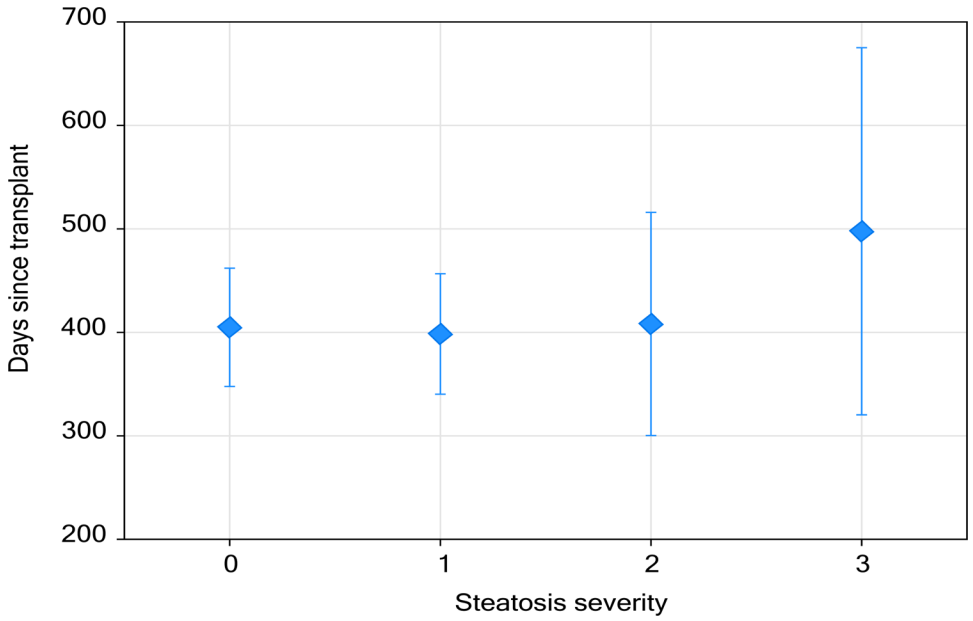


Figure 4.
Association between steatosis severity and time from transplant.

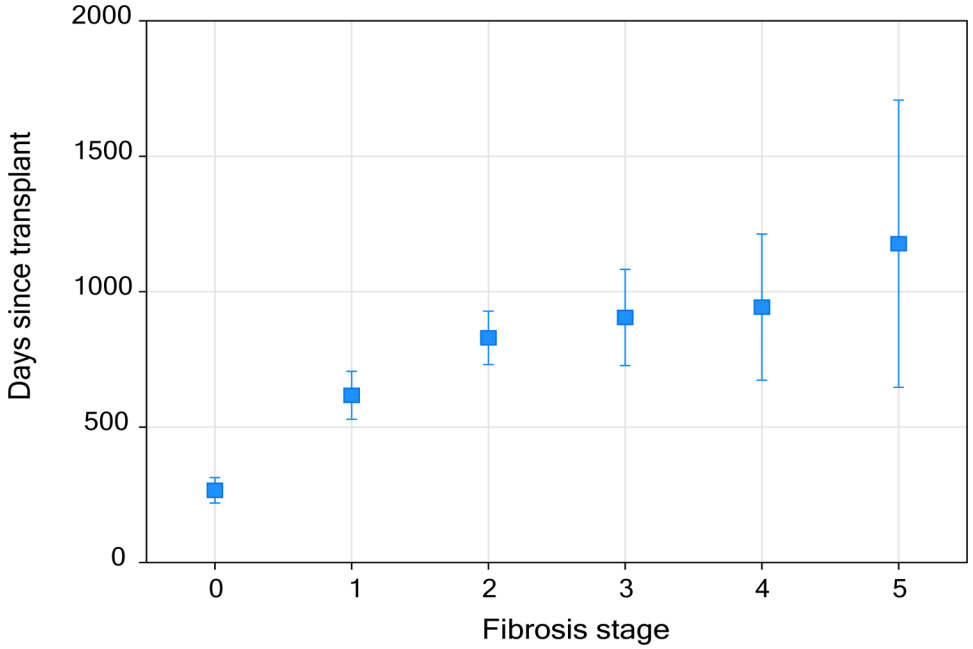


Figure 5.
Association between fibrosis stage and time from transplant.

Table 1.

Demographics and Clinical Features of Liver Transplant Recipients (n=183).

Clinical Feature	Summary
Age (mean ± SD)	55 ± 8.55
Gender (Male n, %)	139 (76%)
Race (n, %)	
African American	21 (11.5%)
Asian	3 (1.6%)
White	157 (86%)
Other	2 (1.1%)
BMI at transplant, kg/m^2 (mean ± SD)	29.7 ± 5.6
Indication for liver transplant (n, %)	
Alcoholic liver disease	46 (25%)
Cryptogenic cirrhosis	11 (6%)
Hepatitis C virus	94 (51%)
Metabolic disease	5 (3%)
Nonalcoholic steatohepatitis	25 (14%)
Other	2 (1%)
Pretransplant comorbidities (n, %)	
Diabetes mellitus	71 (38.8%)
Hypertension	74 (40.4%)
Hyperlipidemia/dyslipidemia	24 (13.1%)
Immunosuppressive regimen (≥ 6 months) (n, %)	
Steroids	63 (34.4%)
Cyclosporine	7 (3.8%)
Tacrolimus	177 (96.7%)
Azathioprine	6 (3.3%)
Mycophenolate mofetil	70 (38.2%)
Sirolimus	4 (2.2%)
Follow-up time (mean ± SD)	704 ± 402

* BMI = Body Mass Index

Table 2.

Demographics and Clinical Features of Liver Donors (n= 183).

Clinical Feature	Summary
Age (mean ± SD)	42.8 ± 17.4
Gender (Male n, %)	114 (62%)
Race (n, %)	
African American	33 (18%)
Asian	8 (4.4%)
White	126 (69%)
Other	16 (8.7%)
Hepatic steatosis (n, %)	
Mild	81 (44.5%)
Moderate	36 (19.8%)
Severe	6 (3.3%)
Comorbidities (n, %)	
Diabetes mellitus	13 (11.4%)
Hypertension	42 (36.8%)
Hyperlipidemia/dyslipidemia	10 (8.8%)
History of drinking (> 2 drinks per day)	22 (19.3%)

Table 3.

Recipient/Donor Characteristics and Steatosis (FCLB included and for overall population).

Characteristic	No / mild Steatosis	Moderate/ Severe Steatosis	OR (95%CI)	P-value
Number of Patients	152	31		
Donors				
Age (mean ± SD)	42.8 ± 17.2	43.1 ± 18.6	1.00 (0.98 – 1.02)	0.92
Gender (Male n, %)	95 (63%)	19 (61%)	0.97 (0.66 – 1.45)	0.9
Moderate/Severe hepatic Steatosis (n, %)	35 (23%)	7 (22.6%)	0.98 (0.62 – 1.56)	0.96
DM (n, %)	9 (9.6%)	4 (20%)	1.54 (0.80 – 2.93)	0.19
HTN (n, %)	34 (36%)	8 (40%)	1.08 (0.66 – 1.78)	0.74
Hyperlipidemia (n, %)	8 (8.5%)	2 (10%)	1.09 (0.48 – 2.47)	0.83
History of Drinking (>2 per day) (n, %)	19 (20%)	3 (15%)	0.83 (0.43 – 1.62)	0.59
Recipients				
Age (mean ± SD)	54.8 ± 8.7	55.9 ± 7.8	1.02 (0.97 −1.07)	0.53
Gender (Male n, %)	119 (78%)	20 (64.5%)	0.71 (0.47 – 1.08)	0.11
Indication for OLT (n, %)				
Alcoholic liver disease	37 (24.3%)	9 (29%)	1.06 (0.88 – 1.28)	0.58
Cryptogenic cirrhosis	6 (3.9%)	5 (16.1%)	2.16 (1.15 – 4.06)	0.02
HCV	85 (56%)	9 (29%)	0.57 (0.38 – 0.86)	0.008
Metabolic diseases	5 (3.3%)	0 (0%)	0.005 (0 – 1000+)	0.96
Nonalcoholic steatohepatitis	17 (11.2%)	8 (25.8%)	1.66 (1.04 – 2.67)	0.04
Other	2 (1.3%)	0 (0%)	0.01 (0 – 1000+)	0.96
BMI at LT, kg/m^2 (mean ± SD)	29 ± 5.7	32.4 ± 4.2	1.11 (1.03 – 1.19)	0.004
Pretransplant Comorbidities (n, %)				
DM	56 (37%)	15 (48%)	1.27 (0.86 – 1.87)	0.23
Hyperlipidemia/Dyslipidemia	19 (12.5%)	5 (16%)	1.16 (0.68 – 1.98)	0.59
Hypertension	60 (40%)	14 (45%)	1.12 (0.76 – 1.66)	0.56
BMI at Biopsy, kg/m^2 (mean ± SD)	28.8 ± 5.6	34.3 ± 5.3	1.18 (1.10 – 1.28)	<0.0001
BMI change, kg/m^2 (median [IQR])	−0.52[−2.2,1.2]	1.11[−0.8,5.0]	1.16 (1.05 – 1.28)	0.005
On Immunosuppression Withdrawal	16 (11%)	9 (32%)	2.01 (1.25 – 3.22)	0.004
Time from LT to Biopsy (mean ± SD)	674 ± 402	851 ± 376	1.00 (1.00 – 1.002)	0.03
Last biopsies at 24 ± 3 month				
Number of Patients	30	5		
BMI at Biopsy, kg/m^2 (mean ± SD)	29.6 ± 4.5	35.1 ± 5.2	1.22 (1.01 – 1.48)	0.04
HCV as indication for OLT (n, %)	15 (50%)	1 (20%)	0.50 (0.16 – 1.58)	0.24
Last biopsies at 36 ± 3 month				
Number of Patients	40	14		
BMI at Biopsy, kg/m^2 (mean ± SD)	31.1 ± 5.8	35.8 ± 5.5	1.16 (1.02 – 1.31)	0.02
HCV as indication for OLT (n, %)	16 (40%)	3 (21%)	0.64 (0.31 – 1.30)	0.22

Table 4.

Recipient/Donor Characteristics and Fibrosis (FCLB included and for overall population).

Characteristic	No / mild fibrosis	Fibrosis (F≥2)	OR (95%CI)	P-value
Number of Patients	122	61		
Donors				
Age (mean ± SD)	44.3 ± 17.5	39.9 ± 16.9	0.99 (0.97 – 1.00)	0.11
Gender (Male n, %)	69 (57%)	45 (74%)	1.47 (1.05 – 2.06)	0.03
Fibrosis (n, %)	0 (0%)	0 (0%)	--	--
DM (n, %)	7 (10%)	6 (13.6%)	1.19 (0.67 – 2.13)	0.55
HTN (n, %)	27 (38.6%)	15 (34.1%)	0.91 (0.61 – 1.35)	0.63
Hyperlipidemia (n, %)	7 (10%)	3 (6.8%)	0.81 (0.40 – 1.64)	0.56
History of Drinking (>2 per day) (n, %)	13 (18.6%)	9 (20.5%)	1.06 (0.66 – 1.71)	0.8
Recipients				
Age (mean ± SD)	55.2 ± 9.3	54.7 ± 6.8	0.99 (0.96 – 1.03)	0.72
Gender (Male n, %)	88 (72%)	51 (84%)	1.4 (0.95 – 2.08)	0.09
Indication for OLT				
Alcoholic liver disease (n, %)	44 (36%)	2 (3.3%)	0.25 (0.12 – 0.51)	0.0002
Cryptogenic cirrhosis (n, %)	11 (9%)	0 (0%)	0.003 (0 – 10000+)	0.93
HCV (n, %)	38 (31%)	56 (91.8%)	4.98 (3.03 – 8.17)	<0.0001
Metabolic diseases (n, %)	5 (4.1%)	0 (0%)	0.003 (0 – 10000+)	0.95
Nonalcoholic steatohepatitis (n, %)	23 (19%)	2 (3.3%)	0.38 (0.18 – 0.80)	0.01
Other (n, %)	1 (0.8%)	1 (1.6%)	1.42 (0.35 – 5.73)	0.62
BMI at LT, kg/m^2 (mean ± SD)	29.9 ± 5.7	29.3 ± 5.5	0.98 (0.93 – 1.04)	0.52
Pretransplant Comorbidities				
DM (n, %)	54 (44%)	17 (28%)	0.70 (0.50 – 0.97)	0.03
Hyperlipidemia/Dyslipidemia (n, %)	21 (17%)	3 (5%)	0.50 (0.27 – 0.93)	0.03
Hypertension (n, %)	52 (43%)	22 (36%)	0.87 (0.63 – 1.20)	0.4
BMI at Biopsy, kg/m^2 (mean ± SD)	30.4 ± 6.0	28.5 ± 5.4	0.95 (0.89 – 0.99)	0.04
On Immunosuppression Withdrawal	17 (14.2%)	6 (10%)	0.67 (0.40 – 1.13)	0.14
Time from LT to Biopsy (mean ± SD)	732 ± 392	647 ± 419	0.99 (0.99 – 1.00)	0.18
Last biopsies at 24 ± 3 month				
Number of Patients	22	13		
HCV as indication for OLT (n, %)	4 (18%)	12 (92%)	7.35 (2.32 – 23.3)	0.0007
Last biopsies at 36 ± 3 month				
Number of Patients	43	11		
HCV as indication for OLT (n, %)	8 (19%)	11 (100%)	6378 (94 – 1000+)	<0.0001