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

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Background. Deceased donor and recipient predictors of posttransplant steatosis/steatohepatitis and fibrosis are not well known. Our aim was to evaluate the prevalence and assess donor and recipient predictors of steatosis, steatohepatitis, and fibrosis in liver transplantation recipients. **Methods.** Using the immune tolerance network 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 (ie, abnormal transaminases and to rule out rejection) and were interpreted by a central pathologist. **Results.** One hundred eighty-three paired donor/recipients liver biopsies at the time of transplant and posttransplant follow-up (median time 582 d; average time to last biopsies was 704 d [SD ± 402 d]) were analyzed. Donor steatosis did not influence recipient steatosis or fibrosis. Ten of 183 recipients had steatohepatitis on the last biopsy. Recipient body mass index at the time of liver biopsy was the most influential factor associated with posttransplant steatosis. Both donor and recipient metabolic syndrome features were not associated with graft steatosis. Untreated hepatitis C viral (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 body mass index at the time of liver biopsy was most significantly associated with posttransplant 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 posttransplant fibrosis. Emphasis on recipient weight control is essential.

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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

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region.[2,3] Given the prevalence of hepatitis C viral (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 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 posttransplantation 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 are lacking. These studies are retrospective in nature and have selection bias with regard to histologic assessment. Although there are 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 posttransplant NAFLD and fibrosis, using prospectively collected protocol and for-cause liver biopsies (FCLBs) from 7 different centers as part of the ITN-A-WISH 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 pretransplant and posttransplant risk factors in donors and recipients that might help predict the outcomes of fibrosis and steatosis/steatohepatitis. Further, in comparing the development of posttransplant steatosis/steatohepatitis to recurrence of HCV fibrosis, we aimed to provide some context in understanding this relative risk, although 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 2 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 before 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 mo following transplantation, and dual therapy was continued. Monotherapy with a calcineurin inhibitor or antimetabolite was a requirement at least 3 mo before assessment for random assignment. Subjects were regularly assessed for evidence of allograft rejection. No sooner than 1 y after transplantation, eligible subjects were randomly assigned in a 4:1 ratio to immunosuppression withdrawal or to maintenance. Patients were considered as having taken a medication if it was prescribed for >6 mo. Two hundred eighty-six patients were initially consented for the A-WISH trial and 11 were excluded based on ≥stage III hepatocellular carcinoma in the explanted liver. Of the remaining 275 patients, 76 were removed from the study as they failed to reach the targeted monotherapy. Although recipient follow-up data were available in the 199 remaining subjects, donor data were missing in 1 case, which was excluded from analysis. An additional 15 patients were excluded from analysis, as their last biopsy was taken within 100 d 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 posttransplant 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 because of concern for allograft dysfunction (termed as FCLB). Allograft dysfunction was defined as elevated hepatic biochemical tests including AST, ALT, and bilirubin to higher than twice the upper limit of normal. FCLBs were sent to the central pathologist for independent analysis.

In total, 1124 liver biopsies were collected from 283 patients as part of the ITN-A-WISH 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 1 case in which a T0 biopsy had not been collected. There were 531 posttransplant recipient biopsies collected in our group of study patients. Only 1

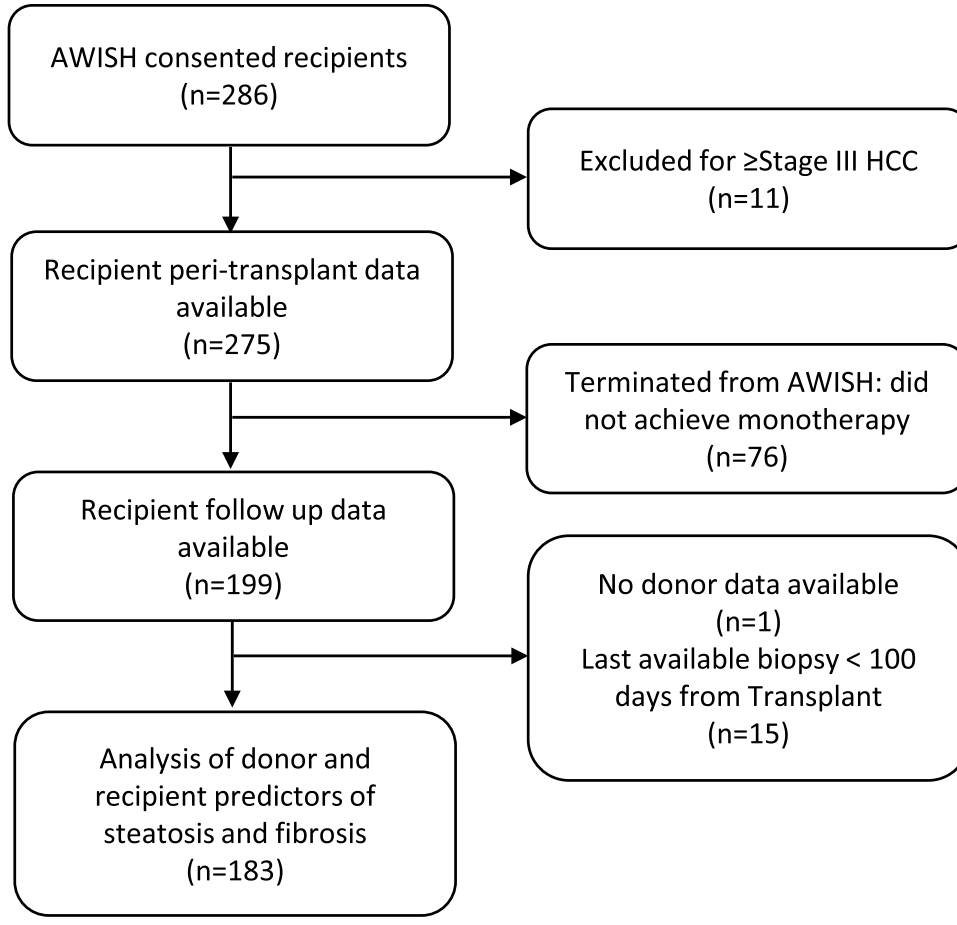

FIGURE 1. Study cohort. AWISH, Gradual Withdrawal of Immune System Suppressing Drugs in Patients Receiving a Liver Transplant; HCC, hepatocellular carcinoma.

biopsy was available in 43 patients, whereas the remaining 140 recipients had serial posttransplant biopsies available for analysis (Figure 2).

Hepatic steatosis was graded based on the Brunt/Kleiner Scoring system, using a semiquantitative 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 microsteatosis and 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* represent the results of 2-sided tests. Multivariate logistic regression models were performed by including the variables significant at a nominal $P < 0.05$ in the initial univariate logistic regression analyses. Analyses were conducted using R (version 3.2.5) and NCSS 8 (NCSS, LLC. Kaysville, UT).

RESULTS

The distributions of recipient characteristics are displayed in Table 1. The average age at transplant was 55 y (SD ± 8.55 y), 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 d (SD ± 402 d). 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-A-WISH study protocol, as summarized above.

Donor characteristics are highlighted in Table 2. The average age of donors was 42.8 y (SD ± 17.4 y). 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 A-WISH trial, and missing data here were attributed to the retrospective nature of this data collection.

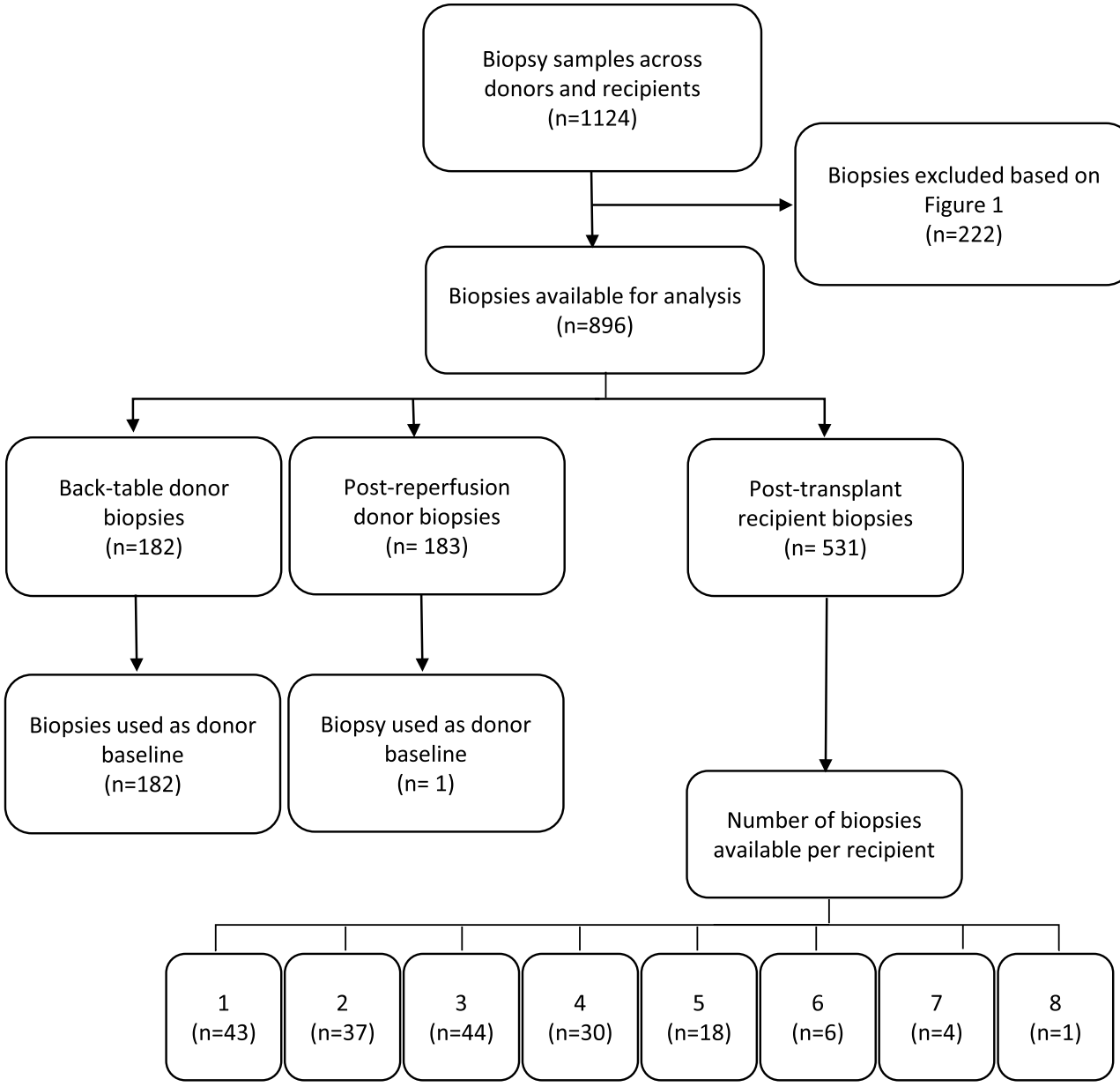

FIGURE 2. Biopsies collected.

Assessment of Donor/Recipient Characteristics Contributing to Posttransplant 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 body mass index (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 mo and 36 ± 3 mo, 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 ($P = 0.64$), whereas 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 [–0.34-0.02]; $P = 0.08$).

The impact of donor and recipient microvesicular steatosis on recipient steatosis was assessed and was not statistically significant. Forty-eight percent (49% mild, 45% moderate/severe, 44% mixed microvesicular/

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 mo) (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 d)	22 (19.3%)

macrovesicular steatosis) of the donors and 6% of the recipient had microvesicular steatosis and more often, the liver biopsies had mixed microvesicular/macrovesicular steatosis rather than an isolated pattern of steatosis.

There were 26 cardiac events recorded during the study period (Table S1, SDC, http://links.lww.com/TP/C131) and relative to those without cardiac events, moderate/severe steatosis was more common in those with cardiac events (42% versus 20%; OR, 1.72; 95% CI, 1.11-2.66; $P = 0.01$ [Table S2, SDC, http://links.lww.com/TP/C131)].

Impact of Immunosuppression Use and Withdrawal on Development of Steatosis

Liver biopsies were analyzed from 3 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, SDC, http://links.lww.com/TP/C131). 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 score ≥4, only 10 of 183 (5.5 %) recipients had steatohepatitis on the last liver biopsy while using an NAFLD Activity Score 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 2 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, B). 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 of 6) to be at grade 0/1.

Assessment of Characteristics Contributing to Posttransplant Fibrosis

The potential associations between recipient and donor characteristics and fibrosis stage were also tested (Table 4).

TABLE 3.

Recipient/donor characteristics and steatosis (FCLB included and for overall population)

Characteristic	No/mild steatosis	Moderate/severe steatosis	OR (95% CI)	*P*
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 d) (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 mo				
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 mo				
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

BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; FCLB, for-cause liver biopsy; HCV, hepatitis C viral; HTN, hypertension; LT, liver transplantation; OLT, orthotopic liver transplantation.

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; $P < 0.001$), whereas 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 mo and 36 ± 3 mo, 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 posttransplant fibrosis stages (OR = 4.95; 95% CI, 1.74-14.0; $P = 0.002$).

Impact of HCV on Development of Posttransplant Fibrosis

Given the changing landscape of HCV treatment, we further analyzed the cohorts to see if factors associated with posttransplant steatosis or fibrosis were related to HCV status (Tables S4–S7, SDC, http://links.lww.com/TP/C131). Although we noted that HCV status itself was associated with the development of posttransplant 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 posttransplant steatosis, again, on

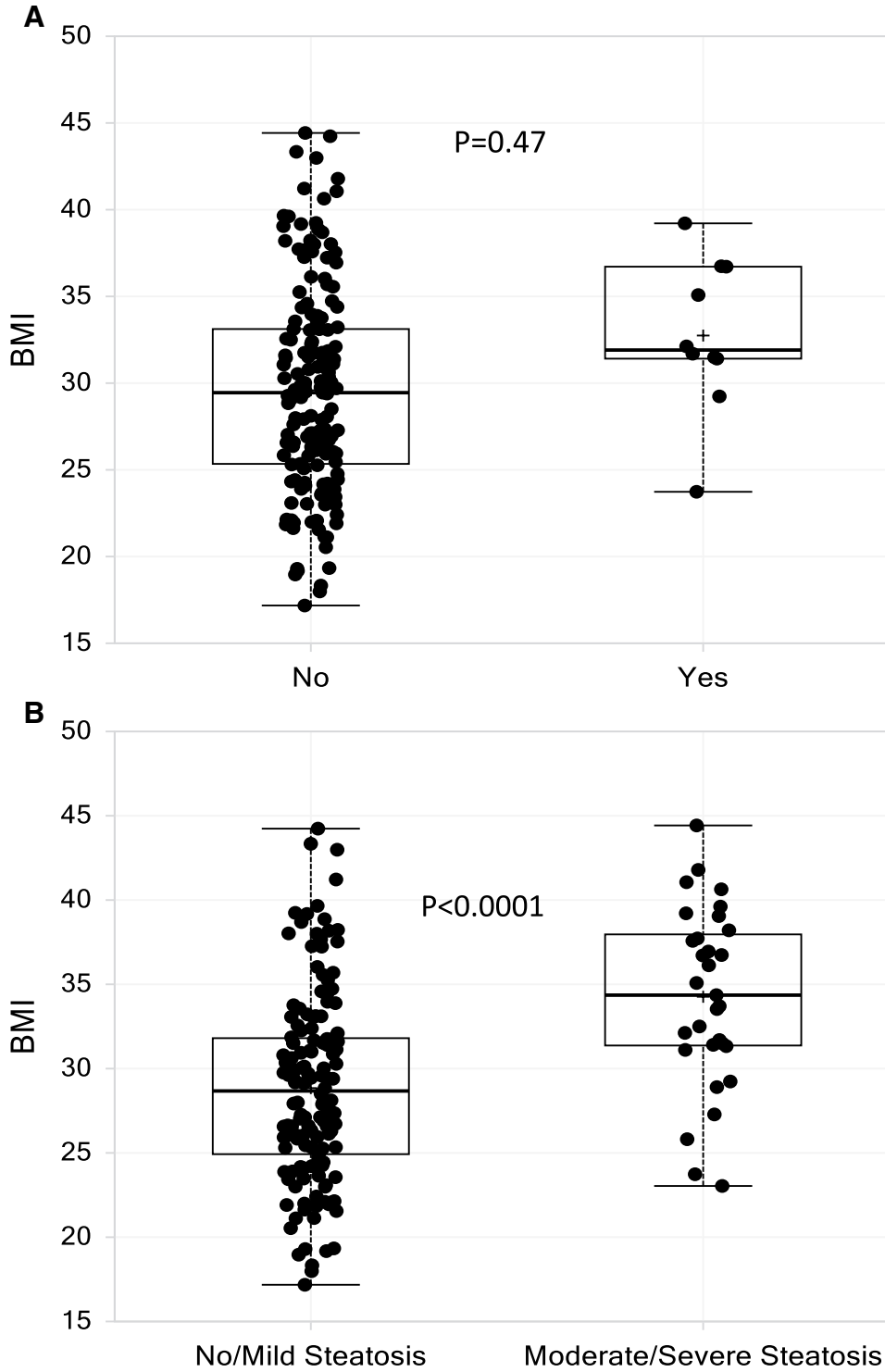

FIGURE 3. Distribution of BMI at last biopsy by steatosis and steatohepatitis status. BMI, body mass index.

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 >1 biopsy posttransplant, there was a trend toward development of steatosis over time across all patients, although 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 d posttransplant, and there was a positive correlation between time from transplant and increased fibrosis stage thereafter (Figure 5).

TABLE 4.

Recipient/donor characteristics and fibrosis (FCLB included and for overall population)

Characteristic	No/mild fibrosis	Fibrosis (F≥2)	OR (95% CI)	*P*
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 d) (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 (0-10 000+)	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 (0-10 000+)	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 mo				
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 mo				
Number of patients	43	11		
HCV as indication for OLT (n, %)	8 (19%)	11 (100%)	6378 (94-1000+)	<0.0001

BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; FCLB, for-cause liver biopsies; HCV, hepatitis C viral; HTN, hypertension; LT, liver transplantation; OLT, orthotopic liver transplantation.

DISCUSSION

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

Our dataset is the largest cohort representing a heterogeneous population of US patients across 7 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 United States. Furthermore, these data are unique, in that they were 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. First, we show that only BMI at time of posttransplant biopsy was significantly associated with posttransplant 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 posttransplant 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 posttransplant.[20]

Both the studies by Dumortier et al and Kim et al found donor graft steatosis to be a significant predictor of posttransplant 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 posttransplant NAFLD in those patients

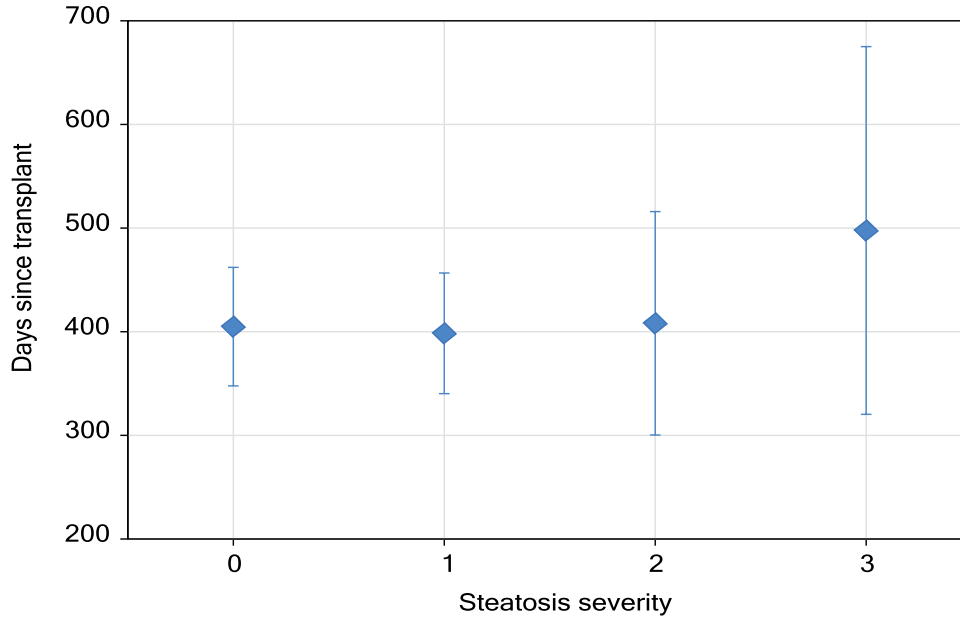

FIGURE 4. Association between steatosis severity and time from transplant.

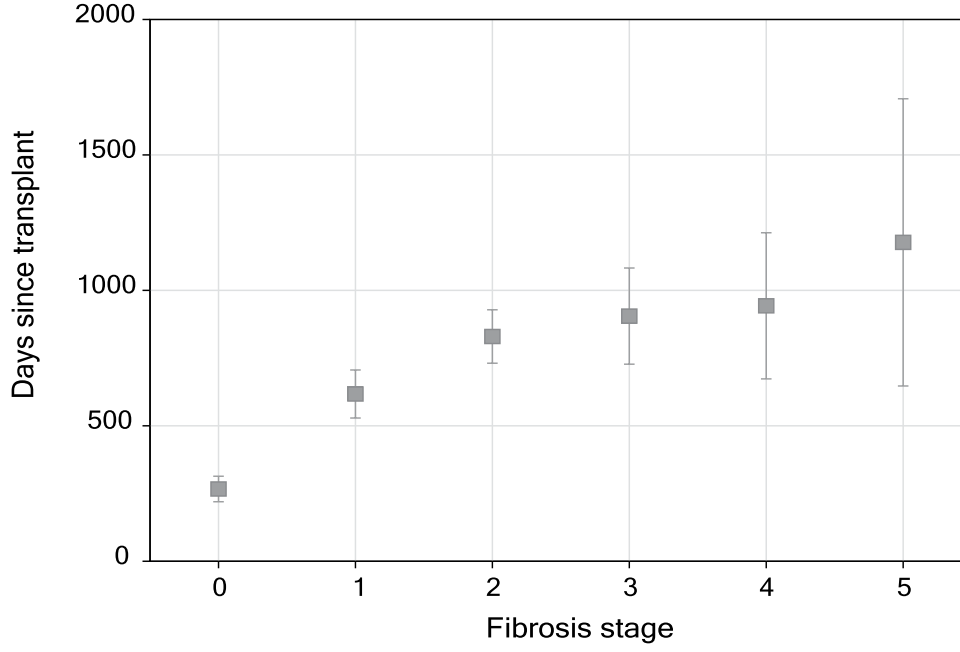

FIGURE 5. Association between fibrosis stage and time from transplant.

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 suggest that modifiable posttransplant factors leading to increased BMI are the driver of posttransplant 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 posttransplant 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 microsteatosis 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] Although 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 posttransplant NAFLD, as there is little prospective or broadly applicable data supporting this concern.

We also found that posttransplant liver steatosis, once noted, remains largely stable over time. As with NAFLD in the general population, our concern with posttransplant 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 posttransplant 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 posttransplant setting, although 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 posttransplant fibrosis associated with posttransplant NASH was uncommon.[24] Our data support prior studies and suggest that the burden of posttransplant disease in these patients is more strongly correlated with their pretransplant diagnosis of HCV rather than posttransplant detection of hepatic steatosis.

The unique and novel aspect of our study is the assessment of the impact of donor steatosis as well as pretransplant and posttransplant 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, whereas 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 LT.

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 posttransplant 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 posttransplant 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 posttransplant steatosis on multivariate analysis, suggesting that these medications are not the main drivers of posttransplant steatosis as previously described by Dumortier et al.[7]

There are several limitations in this analysis. First, although 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 posttransplant hepatic steatosis. Although BMI at the time of posttransplant 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 although 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 dataset 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 3 y 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 comorbidities such as cardiac disease as it impacts patient survival, given the prolonged time frame over which such complications manifest. Third, 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. Finally, we have a cohort of untreated HCV, whereas 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 posttransplant liver biopsy assessment, we found that the incidence of posttransplant hepatic steatosis was only associated with posttransplant BMI at time of liver biopsy and was not associated with the development of fibrosis. The development of posttransplant fibrosis was related to untreated HCV disease and was not influenced by steatosis suggesting that, if present, posttransplant progressive liver disease related to metabolic disease may take a longer time to evolve.

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