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Hepatic steatosis after pediatric liver transplant

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Abstract

Rationale—Hepatic steatosis develops after liver transplant in 30% of adults, and non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in non-transplanted children. However, post-transplant steatosis has been minimally studied in pediatric liver transplant recipients. We explored the prevalence, persistence, and association with chronic liver damage of hepatic steatosis in these children.

Results—In this single-center study of pediatric patients transplanted 1988–2015 (n=318), 31% of those with any post-transplant biopsy (n=271) had 1 biopsy with steatosis. Median time from transplant to first biopsy with steatosis was 0.8 months (IQR 0.3–6.5) and to last biopsy with steatosis was 5.5 months (IQR 1.0–24.5). 85% of patients with steatosis also had for-cause biopsies without steatosis. All available for-cause biopsies were re-evaluated (n=104); Of 9 biopsies that could be interpreted as NASH/Borderline NASH, with steatosis plus inflammation or ballooning, 8 also had features of cholestasis or rejection. Among 70 patients with surveillance biopsies 3.6–20.0 years post-transplant, only 1 overweight adolescent had a biopsy with NAFLD (grade 1 steatosis, mild inflammation, no ballooning or fibrosis)—despite a 30% prevalence of overweight/obesity in the cohort and 27% with steatosis on previous for-cause biopsy. Steatosis on preceding for-cause biopsy was not associated with portal (p=0.49) or perivenular fibrosis (p=0.85) on surveillance biopsy.

Conclusions—Hepatic steatosis commonly develops early post-transplant in children and adolescents, but it rarely persists. Biopsies that did have steatosis with NASH characteristics were

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all for-cause, mostly in patients with NAFLD risk factors and/or confounding causes of liver damage. Prospective studies that follow children into adulthood will be needed to evaluate if and when hepatic steatosis presents a long-term risk for pediatric liver transplant recipients.

Keywords

liver transplantation; non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; children; long-term outcomes

INTRODUCTION

As 10-year survival after pediatric liver transplant now exceeds 80%,⁽¹⁾ long-term graft and patient health is key to optimizing outcomes. In contrast to adults, most children are transplanted for liver diseases that do not recur after transplant. For children, threats to long-term graft health include rejection, vascular and biliary issues, and chronic liver diseases like viral hepatitis or non-alcoholic fatty liver disease (NAFLD).

NAFLD is the most common cause of chronic liver disease in U.S. children. ⁽²⁾ Obesity, insulin resistance, and metabolic syndrome are strongly associated with NAFLD. We have recently reported that long-term survivors after pediatric liver transplant have a similar high prevalence of obesity—approximately 30%—and metabolic syndrome—approximately 15%—as do non-transplanted peers.⁽³⁾ In adults who undergo liver transplant, at least 30% develop significant steatosis post-transplant, with risk increased by post-transplant obesity and metabolic syndrome, and by pre-transplant steatotic disease.⁽⁴⁾ Fibrosis frequently develops in adults with post-transplant NAFLD, and it can progress rapidly.⁽⁵⁾

Post-transplant steatosis has not been well-studied in pediatric liver transplant recipients. ^(6,7) The possibility of steatosis-associated chronic liver damage has not been an analytical focus in descriptions of long-term liver graft histology.^(8,9) Steatosis prevalence has been reported in 10–43% of pediatric liver transplant surveillance biopsies in cross-sectional studies. But none of these studies evaluates longitudinally whether steatosis persists, should be classified as NAFLD or related to other conditions, or contributes to long-term graft damage.^(10–13)

In this analysis, we explored the prevalence and persistence of steatosis after pediatric liver transplant, and investigated whether steatosis might be associated with chronic graft damage.

METHODS

We analyzed single-center data from (1) retrospective review of records on pediatric liver transplant recipients and (2) from a cross-sectional study on a subset of the cohort conducted September 2012–August 2016. All patients at our center receive induction with corticosteroids post-transplant and are weaned off as tolerated within 2–4 months. All remain on a calcineurin-inhibitor (tacrolimus or cyclosporine) long-term and receive mycophenolate mofetil for 0.75–2 years post-transplant. This study was approved by the UCSF Committee on Human Research (CHR 10–01363, 12–10290, 14–13939); participants

in the cross-sectional study gave consent/assent prior to participation, and were consented for review of medical records.

Hepatic steatosis in for-cause biopsies—retrospective analysis

Our initial cohort for retrospective analysis included all patients transplanted at UCSF between January 1988 and June 2015, <21 years of age at transplant. Original pathology reports on liver biopsies for all 318 patients were reviewed to identify patients with steatosis in any post-transplant biopsy. (FIGURE 1) “For-cause” biopsies were performed for clinical suspicion of rejection, hepatitis, or other pathology. Eleven biopsies were not available for review, primarily because they had been returned to a referring center. An additional 24 were not assessable due to faded stains. 22 of 24 were performed before 1996.

Available for-cause biopsies with steatosis noted on original pathology report were re-read by a single liver pathologist (RR). As steatosis and its significance has been minimally studied after pediatric liver transplant, and many of these biopsies occurred before NAFLD/NASH were well-characterized entities in children, the research read was a blinded re-interpretation of whether NAFLD or NASH-like pathology was present. To standardize the interpretation and consider whether previously undetected NAFLD/NASH might be present, we utilized the NASH Clinical Research Network (CRN) histologic scoring system to evaluate steatosis severity and for other NASH features (14): steatosis (grade 0 [<5% macrovesicular], grade 1 [5%–33%], grade 2 [34%–66%], and grade 3 [>66%]), portal inflammation (none, mild, more than mild), lobular inflammation (none, <2 foci/hpf, 2–4 foci/hpf, >4 foci/hpf), ballooning degeneration (none, few, many), and fibrosis (stage 0, stage 1a [mild perisinusoidal], stage 1b [moderate perisinusoidal], stage 1c [portal/periportal fibrosis only], stage 2 [zone 3 and periportal], stage 3 [bridging fibrosis], and stage 4 [cirrhosis]). In addition, biopsies were classified as “NASH” or “Borderline NASH” based on the pathologist’s overall assessment of the biopsy, and “Not NASH” if diagnostic features of NASH were absent.(14)

Data on demographics, transplant, outcomes, anthropometrics, and medications were collected from the electronic medical record. Explant data was missing in most cases (72 of 84 with steatosis, 158 of 187 with no steatosis reported).

Hepatic steatosis in surveillance biopsies—retrospective analysis

In August 2013, our center instituted standard-of-care surveillance liver biopsies starting 3–5 years post-transplant.(9,13) Additional patients underwent surveillance liver biopsies in the NIH-NIDDK immunosuppression withdrawal trials (NCT01638559; NCT00320606). All surveillance biopsies were interpreted by a single pathologist (KJ), per our center’s standardized protocol; steatosis grading and NASH diagnosis was again based on the NASH CRN system.(14) These reads were extracted retrospectively from the medical record.

Hepatic steatosis screening—cross-sectional study

We enrolled 83 pediatric liver transplant recipients, all included in the retrospective analysis, in a cross-sectional study of post-transplant metabolic syndrome and its consequences—including NAFLD. Participants were <21 years old at transplant, 8–30 years at study visit,

1 year from last liver transplant, on a stable immunosuppressive regimen. All had anthropometrics and 2-hour glucose tolerance testing done according to NHANES 2011 protocols (http://www.cdc.gov/nchs/nhanes/nhanes2011-2012/manuals11_12.htm)

To assess for hepatic steatosis, subjects underwent liver ultrasound during the study visit using a Logiq E9 ultrasound machine (General Electric, Milwaukee WI) with a 6 MHz curved transducer. Study ultrasounds were interpreted by a single pediatric radiologist (AP) and over-read by a second radiologist (VF); any discrepancies in interpretation were resolved by group consensus of the two radiologists. All recorded images were evaluated for: (1) hepatic parenchymal echogenicity; (2) relative echogenicity of the liver to right kidney; (3) deep attenuation of the sound beam; and (4) relative echogenicity of the portal triads and walls of the hepatic vessels compared with background liver.(20,21).(15,16) Overall assessment of hepatic steatosis was based on these characteristics.

Classification of anthropometrics—retrospective and cross-sectional data

For subjects younger than 18 years at measurement, BMI percentile for age and gender was calculated based on 2000 CDC growth chart data.(17) BMI percentile 85th–94th percentile for age and gender was overweight, and 95th percentile obese. In subjects 18 years, overweight was BMI 25–29.9kg/m² and obese BMI 30 kg/m².

Statistical analysis

Analysis focused on descriptive statistics. Median and interquartile range was used to describe distribution for non-normally distributed variables, mean and standard deviation for those with normal distributions. All data was collected in a REDCap database and analyzed using Stata 14 (College Station, TX).

RESULTS

Evolution, and resolution, of hepatic steatosis in for-cause biopsies

Our retrospective cohort included 318 pediatric liver transplant recipients. At least 1 for-cause biopsy was done in 85%, and 64% had >1 (range 2–36). (FIGURE 1) Eighty-four children had at least one for-cause biopsy with steatosis noted on the original pathology report. In these 84, 22% of all for-cause biopsies (n=624) were reported to have steatosis. Eight patients had 15 biopsies each. The remaining 76 patients had a median 4.5 biopsies each, IQR 3.0–8.0. No patients were transplanted for NASH.

Figure 2 demonstrates the timing of biopsies with steatosis in these 84 patients; most of the biopsies with steatosis were done within 2 months post-transplant. Median time between transplant and first for-cause biopsy with steatosis was 0.8 months (IQR 0.3–6.5 months). Median time to last for-cause biopsy with steatosis was 5.5 months post-transplant, IQR 1.0–24.5 months. Of the patients with steatotic biopsies, 85% also had for-cause biopsies without steatosis.

Of the 50 patients with steatosis in only 1 biopsy, median time between transplant and steatotic biopsy was 0.8 months (IQR 0.3–13.3). Among those with >1 for-cause biopsy (n=46), 87% had steatosis resolution in subsequent liver biopsies. Only one of these 50

biopsies was classified as NASH on the original pathology read; it was in an overweight patient 9.8 years post-transplant, with grade 2 macrovesicular steatosis and inflammation, no significant fibrosis, and no other post-transplant biopsies in our system. (TABLE 2)

Of the 23 patients with steatosis in 2 for-cause biopsies, 69% had resolution documented by subsequent biopsies. The median time to 1st biopsy with steatosis was 0.2 months post-transplant (IQR 0.5–4.9) and to 2nd biopsy was 2.3 months post-transplant (IQR 0.7–15.2). Three of these 23 patients had biopsies with steatosis and NASH features. Two of the 3 were within 1 month post-transplant; steatosis resolved in both patients. The third had a biopsy with steatosis grade 1 and NASH features at 2 months after she was re-transplanted for idiopathic cirrhosis. At 14 months after her second transplant, the patient had another biopsy with steatosis grade 3 and similar inflammation, but no ballooning. (TABLE 2)

Of the 7 patients with steatosis in 3 for-cause biopsies, median time to 1st steatotic biopsy was 1.1 month post-transplant (IQR 0.3–7.7) and to 3rd was 11.7 months (IQR 2.9–64.8). 71% had resolution in subsequent biopsies without known recurrence. One progressed to NASH by 5.5 years post-transplant. A second had subsequent biopsies that showed features of acute and chronic rejection with persistent steatosis of varying severity. (TABLE 2)

Four subjects had steatosis in 4 for-cause biopsies. All 4 had the first steatotic biopsy within 1.2 months post-transplant. Two had within 1 month post-transplant while recovering from preservation injury, both had improved but not resolved steatosis at last biopsy—6 and 9 months post-transplant. A third, transplanted at age 1 for biliary atresia, had grade 2 steatosis while on corticosteroids that resolved, but then recurred as persistent grade 1 steatosis through 2 years post-transplant. The fourth had progressive familial intrahepatic cholestasis Type 1 (PFIC1), as detailed below. None had features of NASH like ballooning or characteristics inflammation.

Steatosis in patients with progressive familial intrahepatic cholestasis (PFIC)

Six of the patients were transplanted for confirmed PFIC1 or low-GGT PFIC. PFIC1 has been linked to hepatic steatosis in previous case series. (18) Three of the 6 PFIC patients had post-transplant steatosis. One 14 year old had grade 1 steatosis on biopsy 1 month after living-related transplant, and then normal AST/ALT with no later biopsies. A second developed grade 2 macrovesicular steatosis without inflammation or fibrosis 2 years after living-related transplant. A third had 4 for-cause biopsies had grade 1–3 macrovesicular steatosis over 11 years of follow-up, with mild portal inflammation and eventually stage 2 pericellular fibrosis. This patient had several episodes of acute cellular rejection treated with corticosteroid pulses, and he was the only PFIC patient to suffer from chronic diarrhea and malnutrition. The 3 other PFIC patients had no available biopsies >16 months post-transplant.

Characteristics of for-cause biopsies with steatosis

A single liver pathologist re-evaluated all available for-cause biopsies with steatosis (n=104, on 68 patients) to evaluate for other characteristics of NASH. The pathologist was blinded to clinical data. Of biopsies adequate for review (FIGURE 1), 20% had grade 3 steatosis, 23% had grade 2, 34% grade 1, and the remaining 23% had grade 0 (<5%). Only 1 biopsy had

exclusively microvesicular fat; this was a 1 month post-transplant biopsy in a child who received a whole, deceased donor liver for maple syrup urine disease. At 3 months, her for-cause biopsy had grade 2 macrovesicular fat. Biopsies at 6 months and 1 year showed no steatosis.

90% of the reviewed biopsies with steatosis did not have any features suggestive of NASH. The 7 patients that did have a biopsy which met criteria for NASH/Borderline NASH on pathology re-evaluation are detailed in Table 2. Of 4 biopsies that met criteria for NASH; two had been diagnosed with NASH on the original pathology read. All four had ballooned hepatocytes and at least grade 2 steatosis. Five biopsies had findings that could be interpreted as Borderline NASH. Three of these 5 had steatosis with hepatocellular ballooning; all 5 had features of other types of liver damage (TABLE 2). All 7 patients were Latino or Caucasian, and 80% of those with data available were overweight/obese.

Four of these seven patients had steatosis resolution on subsequent biopsies. (FIGURE 1, TABLE 2) Two of three who were more than 3 years post-transplant also had features of chronic rejection and chronic corticosteroid exposure. The last patient had 1 biopsy with NASH as an obese adult; she died from unrelated causes. One of the seven required re-transplant, because of hepatitis B recurrence. Fifteen of the biopsies with steatosis also met criteria for acute rejection on the research read.

Hepatic steatosis in surveillance biopsies

Surveillance biopsies were available on 70 patients. (TABLE 1) 85% were on tacrolimus and 15% on cyclosporine. One patient was on prednisone, 1mg daily. Patients had liver enzymes in the normal range, and almost 1/3 were overweight/obese. (TABLE 1) No PFIC patients had a surveillance biopsy.

Only one surveillance biopsy had significant steatosis (>5%); it was grade 1. This was a female transplanted for biliary atresia with a living donor; biopsy was 8 years post-transplant. The biopsy had mild portal and lobular inflammation but no ballooning or fibrosis. Ultrasound on the same day was read as no steatosis. At biopsy, her BMI was 97th percentile, and she was on tacrolimus with AST and ALT <35IU/L. She had an additional surveillance biopsy 13 years post-transplant, with no steatosis and no fibrosis. Her BMI remained 97th percentile, and she was off immunosuppression. Her only previous for-cause biopsy was 6 days post-transplant, with no steatosis.

Four additional patients had surveillance biopsies with very mild steatosis, in <5% of hepatocytes. Two had previous for-cause biopsies with steatosis, also <5%. Of interest, both had glucose intolerance (oral glucose tolerance test with 2 hour blood glucose > 140mg/dL), but neither had diabetes or previous insulin requirement. One was an obese, Latino adolescent male with insulin resistance. The second was a normal weight Caucasian adolescent female transplanted for a urea cycle disorder. The other two with <5% steatosis on surveillance biopsy had previous for-cause biopsies without steatosis. Again, one was an obese Latino adolescent male with insulin resistance. The last was a normal weight Caucasian adolescent female. All 4 had minimal lobular inflammation with no portal or

interface inflammation, no portal fibrosis, and no hepatocellular ballooning. One had mild perivenular fibrosis.

Of the 70 patients with surveillance biopsies, 62 had previous for-cause biopsies: 27% had steatosis on at least one for-cause biopsy. There was no correlation between portal fibrosis on surveillance biopsy and steatosis on preceding for-cause biopsies ($p=0.49$). Of the patients with previous steatosis, 71% had no portal fibrosis, 6% had stage 1, and 24% had stage 2–3. Among patients without previous steatosis, 73% had no portal fibrosis, 13% had stage 1 and 13% had stage 2–3. Perivenular fibrosis on surveillance biopsy also had no association with steatosis on previous for-cause biopsies ($p=0.85$, chi-squared). Perivenular fibrosis was moderate/severe in 18% of those with previous steatosis and 13% without previous steatosis, and mild in 35% and 42% respectively.

Ultrasound screening for hepatic steatosis

Fifty-six patients with surveillance biopsies also participated in a cross-sectional study of post-transplant metabolic syndrome, (3) during which they underwent abdominal sonography for NAFLD screening. In addition, 27 patients without surveillance biopsies participated in the research screening.

One subject was judged to have moderate hepatic steatosis on screening ultrasound (1.2%). She had a for-cause liver biopsy confirming NASH 3 months prior to study visit and no surveillance biopsies. She was the only subject with a biopsy read as NASH (TABLE 2) in the cross-sectional study. All four subjects with steatosis $<5\%$ on surveillance biopsy had ultrasounds read as no steatosis.

Eleven subjects (12%) had ultrasounds reported as mild hepatic steatosis. Of these, 4 had surveillance liver biopsies within 3 months; none had steatosis but all 4 had mild portal inflammation and 3 had minimal lobular inflammation. Two had mild perivenular fibrosis, and one had moderate/severe perivenular and portal fibrosis. Two of the 11 had previous for-cause biopsies with no steatosis (1.5 years, 7.5 years prior to study visit), but no biopsies within 1 year of study visit. The other six patients had no post-transplant liver biopsies. The 11 subjects with mild hepatic steatosis on research ultrasound were more likely to be overweight or obese (55%) than the study patients without hepatic steatosis (22%, $p=0.02$), but there were no differences in ALT (median 38, IQR 21–56, vs. median 29, IQR 21–46, $p=0.85$). Two PFIC patients participated in the cross-sectional study; both had ultrasounds read as no steatosis.

DISCUSSION

Hepatic steatosis was common within the first months post-transplant, but not in the long-term, in our cohort of pediatric liver transplant recipients. The majority of steatosis detected was mild, seen in conjunction with other forms of liver damage, and subsequently resolved. A small minority of patients had biopsies that met criteria for NASH; all had elevated serum transaminases leading to for-cause biopsies.

This analysis follows a sizeable cohort through long-term follow-up, providing novel, longitudinal insight into hepatic steatosis prevalence and persistence in this population. Given the retrospective nature of most of our data collection, and lack of surveillance biopsies in all patients, this is not a definitive account of steatosis incidence or natural history after pediatric liver transplant. Most for-cause biopsies with steatosis were within 1–3 months post-transplant, when patients are still on corticosteroids and conditions like preservation injury or vascular complications may still be manifest. (19) In the steatotic biopsies that met criteria for NASH on blinded review, it is key to note that features of rejection—acute and chronic—were common. It is possible that a clinically reviewing pathologist would have labeled the steatosis as related to another diagnosis.

But given the relatively high prevalence of steatosis early post-transplant, and the high prevalence of obesity in our cohort, the lack of persistent steatosis and NASH was striking. In non-transplanted patients, NAFLD is seen in 30% of overweight/obese children (2) and 60–95% of those with metabolic syndrome. (20) In our surveillance biopsy cohort, we identified steatosis in only 5% of overweight/obese subjects and NASH in none. Only 1.8% biopsied patients had steatosis with NASH characteristics that persisted >1 year post-transplant. Only 10% of our for-cause biopsies with steatosis, totaling less than 1% of all for-cause biopsies performed in our cohort, met criteria for NASH.

All biopsies that had steatosis and met criteria for NASH were for-cause, performed on patients with elevated transaminases. All 7 patients were Latino or Caucasian, and 80% of those with data available were overweight/obese. However, most also had features of other liver injury types, including rejection and cholestasis. In the surveillance biopsies with any hepatic steatosis, characteristics associated with NAFLD were also common: all were Latino or Caucasian, 4 adolescents, 3 of 5 overweight/obese, and 3 with abnormal glucose tolerance. The one other study that examined post-transplant NAFLD and metabolic syndrome in children also suggested a connection. Of their 7 patients with post-transplant metabolic syndrome, 6 had steatosis. (10) Thus, suspicion for NASH or NAFLD should be highest in our patients with traditional NAFLD risk factors.

In our cross-sectional study, ultrasound was not reliable as a screen for mild hepatic steatosis. Although ultrasound is known to have limited sensitivity for steatosis, our data suggests that it may also have limited specificity in the liver transplant population—as 4 patients with ultrasound suggestive of steatosis had none on biopsy. We identified only 1 patient with moderate steatosis. It is possible obesity in some patients contributed to the interpretation of ultrasound echogenicity as mild steatosis, as obesity prevalence was higher in these patients. Even if the patients with mild steatosis on ultrasound and without confirmatory biopsy did have steatosis, this would amount to a prevalence of 6–10%—again despite a 27% prevalence of overweight/obesity.

The four previous pediatric liver transplant surveillance biopsy studies that describe steatosis all report higher prevalence than in our cohort. None followed steatosis evolution over time. Kosola et al. reported the highest prevalence: 43% in surveillance biopsies (n=56) done 3–22 years after transplant. (10) Sixty-five percent of their patients were on low-dose corticosteroids, but other clinical characteristics were similar.

Of note, the histologic description of steatosis in Kosola et al's cohort was quite different. 80% of their biopsies were read as microvesicular steatosis and 20% mixed micro/macrovesicular. Of those with macrovesicular steatosis, all had grade 1 with no other NASH features. (21) Neither inflammation nor fibrosis was associated with steatosis. (10, 21) Thus, they similarly describe a very low prevalence of biopsies meeting NASH criteria, and no clear evidence of long-term impact on the graft.

Venturi et al. identified steatosis in 27% of surveillance biopsies (n=71), done at 0.5 and 7 years post-transplant. Most of their patients were also on corticosteroids. Their data suggested, as did ours, that significant steatosis may be accompanied by ALT elevation. (11) In a German cohort of 60 children, 17% had grade 1 steatosis on surveillance biopsies 1–17 years post-transplant. Most were on low-dose corticosteroids. (13) Finally, in a Japanese study of 59 surveillance biopsies 0.2–15 years post-transplant; 10% had mild steatosis. (12)

In adults after liver transplant, NASH is a risk factor for progression to bridging fibrosis and cirrhosis. We did not find a correlation between steatosis in for-cause biopsies and fibrosis in later surveillance biopsies. The Finnish and Japanese studies also identified no association between steatosis and fibrosis in their cross-sectional biopsies. (12,21) Prospective evaluation with repeated surveillance biopsies will be required to definitively judge the role of hepatic steatosis in long-term graft health for children.

We also examined whether steatosis was associated with specific transplant indications. Our previous systematic review identified only 9 case reports/series, reporting on 19 patients, focused on post-transplant steatosis in children. All of these cases cited specific risk factors: post-transplant thrombosis or preservation injury, pre-transplant NASH, panhypopituitarism, or PFIC. (7) We did see steatosis in 50% of our patients transplanted for low-GGT PFIC, which has been reported previously (18,22) Interestingly, impaired FIC1 mutation, as seen in PFIC1, is thought to downregulate the farnesoid X receptor (FXR). (23) FXR agonists have showed promise for treatment of NASH in adults.

Important questions remain unanswered by this analysis. Although we suspect that factors like preservation injury, post-transplant corticosteroids, and nutritional supplementation contributed to early steatosis, we could not define the exact contribution of each in this retrospective study. Our center does not routinely biopsy the graft immediately preceding transplant or at reperfusion, so we had could not report on donor-derived steatosis. Further delineation of donor versus host risk factors is an important topic for future research, given how common NAFLD is in the general population. Follow-up of long-term histology in livers split between adults and children would be one fascinating research strategy.

The major limitations of this study are the reliance on retrospective, for-cause biopsies and lack of surveillance biopsies in all patients. It is possible that these limitations caused us to underestimate steatosis and NASH prevalence. Not all patients underwent surveillance biopsy, so we cannot definitively rule out steatosis or NASH with normal liver enzymes. However, given our very low prevalence of steatosis also on ultrasound, it seems unlikely that we missed many cases of severe steatosis. Chronic elevation of serum AST or ALT, as

seen in active NASH, would trigger a for-cause liver biopsy; we thus suspect that we did not miss a substantial number of NASH cases.

Further research is needed on risk factors for and outcomes associated with persistent hepatic steatosis and NASH in pediatric liver transplant recipients. As knowledge from long-term monitoring and surveillance biopsies builds, we will gain more insight into the clinical importance of these conditions in pediatric patients.

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Abbreviations

ACR	Acute cellular rejection
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CDC	Centers for Disease Control
CRN	Clinical Research Network
DDLT	Deceased-donor liver transplant
FXR	Farnesoid X receptor
GGT	Gamma-glutamyl transpeptidase
HPF	High power field
IQR	Interquartile range
LRLT	Living-related liver transplant
LT	Liver transplant
MMF	Mycophenolate mofetil
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis

NHANES	National Health and Nutrition Examination Survey
PFIC	Progressive familial intrahepatic cholestasis
PRED	Prednisone
TAC	Tacrolimus

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FIGURE 1.

Retrospective analysis of hepatic steatosis in post-transplant liver biopsies of pediatric liver transplant recipients, detailing samples available for evaluation and histologic outcomes for patients in the cohort. Surveillance biopsies are shaded grey; all other biopsies described were for-cause. Characteristics of patients with biopsies that met criteria for NASH are detailed in Table 2. *Eleven biopsies were not available for review, primarily because they had been returned to a referring center. An additional 24 were not assessable due to faded stains. 22 of 24 were performed before 1996.

FIGURE 2.

Post-transplant time course of for-cause biopsies with steatosis in pediatric liver transplant recipients. Each horizontal gray line corresponds to one of the 84 patients with any for-cause steatotic biopsy, and each X represents one biopsy with steatosis. Each patient's biopsies are connected by a black line. The grey dashed vertical line marks 2 months post-transplant; the majority of biopsies with steatosis occurred early after transplant, and few patients had persistent steatosis long-term.

Table 1

Pediatric liver transplant recipient retrospective and cross-sectional cohort demographics

	Steatosis in for-cause biopsies (n=84)	No steatosis in for-cause biopsies (n=187)	p *	Surveillance biopsy (n=70)	Post-transplant metabolic syndrome study (n=83)
Female	52%	48%	0.46	43%	42%
Latino	27%	24%	0.54	23%	34%
Race					
White	54%	52%		49%	45%
Black	4%	7%		6%	7%
Asian	7%	6%		12%	12%
Other/unknown	36%	43%		33%	36%
Age at transplant, years[†]	2.0 (0.8–10.0)	1.8 (0.7–10.4)		0.96 (0.6–2.7)	1.7 (0.7–6.9)
Year of transplant					
1998–2000	63%	50%	0.04	32%	31%
2000–2013	37%	50%		67%	69%
Primary diagnosis[‡]					
Biliary atresia	37%	36%		48%	35%
Metabolic disease	21%	22%		23%	23%
Cholestatic disease	12%	11%	0.31	13%	15%
Acute liver failure	17%	10%		9%	10%
Tumor	4%	3%		1%	2%
Other	10%	18%		6%	15%
Living donor	35%	24%	0.06	62%	71%
Transplant type					
Whole	38%	52%		41%	49%
Split (deceased)	27%	24%	0.06	21%	23%
Split (living)	35%	24%		38%	28%
Liver-kidney transplant	6%	4%	0.44		5%
Number of post-transplant biopsies	5 (3–11)	3 (1–6)	<0.001	3 (1–4)	3 (1–4)
Deceased as of 8/2016	12%	8%	0.24	0	0

	Steatosis in for-cause biopsies (n=84)	No steatosis in for-cause biopsies (n=187)	p *	Surveillance biopsy (n=70)	Post-transplant metabolic syndrome study (n=83)
Characteristics at time of surveillance biopsy or study visit					
Age, years	NA	NA	NA	12.1 (8.8–16.6)	15.6(11.9–18.5)
Years post-transplant	NA	NA	NA	9.4 (6.1–13.0)	10.9 (6.5–15.3)
ALT (IU/L)	NA	NA	NA	28 (21–42)	30 (21–45)
Overweight/obese	NA	NA	NA	30%	27%

* p represents chi-squared comparison of groups in first three table columns. NA = not applicable

[†]Continuous variables r as median (interquartile range)

[‡]Metabolic liver disease includes alpha-1-antitrypsin deficiency, Crigler-Najjar syndrome, cystic fibrosis, glycogen storage disease, inborn errors in bile acid metabolism, neonatal hemochromatosis, primary hyperoxaluria, tyrosinemia, urea cycle defects, Wilson's disease. Cholestatic conditions include Alagille syndrome, Byler disease, progressive intrahepatic cholestatic syndromes, total parenteral nutrition cholestasis, sclerosing cholangitis, and idiopathic cholestasis. Other includes congenital hepatic fibrosis, Budd-Chiari syndrome, autoimmune hepatitis cirrhosis, drug toxicity, hepatitis C cirrhosis, and unknown cirrhosis.

Pediatric liver transplant recipients with post-transplant biopsies meeting criteria for non-alcoholic steatohepatitis (NASH)

Table 2

ID	Age at transplant(s): type and indication	For-cause liver biopsies that met criteria for NASH						At biopsy		Clinical course after biopsy meeting NASH criteria
		time post- transplant	Steatosis grade	Inflammation	Ballooning	Fibrosis stage, type	Other biopsy findings	Obesity	Meds	
Latino female	11m: DDLT, split -Biliary atresia f/u to age: 4y	1y	0	Mild lobular Mod portal	None	None	ACR	BMI 99 th %ile	Tac	-Chronic EBV hepatitis -Concern for chronic rejection -No later biopsies
		2y	2	Mild lobular Mod portal	None	3: Bridging	ACR	BMI 99 th %ile	Tac	
		3y	0	Mild portal No lobular	None	2: Peri-sinusoidal + Portal	Duct damage No ACR	BMI 99 th %ile	Tac Pred 10mg/kg	
White male	1.1y: DDLT, split -Alpha-1 antitrypsin deficiency f/u to age: 24.6y	1m	2	Mild lobular Mild portal	Few	1: Portal	Mild ACR, Cholestasis	NA	CSA AZA Pred	-No steatosis on biopsies 1y, 2y, 4y, 23y post-transplant
Latino female	1.4y: LRLT -Idiopathic ALF 5.9y: DDLT, whole -Idiopathic cirrhosis f/u to age: 13.9y	2m after 2 nd LT	1	Mod lobular Mild portal	Few	None	Duct damage	No	Pred 5mg Tac MMF	-No later biopsies -AST/ALT normal on Tac, MMF
		14m after 2 nd LT	3	Mild lobular Mild portal	None	None	Possible chronic rejection	No	TPN Pred 5mg Tac MMF	
White male	2.3y: DDLT, split + kidney -Primary hyperoxaluria f/u to age: 5.3y	1m	2	None	Few	None	Cholestasis	BMI 99 th %ile	TPN Pred 5mg Tac, MMF	-No later biopsies -AST/ALT normal on Tac/MMF
		2m	2	Mild lobular No portal	Many	None	Mild duct damage, cholestasis	BMI 99 th %ile	Tac MMF	
		6m	1	None	None	None	Duct damage	BMI 99 th %ile	Tac MMF	
Latino female	13.1y: DDLT, whole -Hepatitis B with ALF19.7y: DDLT -Retransplant, HepatitisB f/u to age: 39y	0.5m after 1 st LT	2	Mild lobular No portal	Few	None	Cholestasis Preservation injury	NA	CSA Pred 40mg	-No steatosis on biopsies 3.5, 4, 5y after 1 st transplant -No steatosis after re-transplant
		3.1y after 1 st LT	Mild (unable to grade)	Mod lobular Mild portal	Few	NA	ACR	NA	CSA Pred 5mg	
		3m, 19m, 3y	3	No lobular Mild portal	None	1: Peri-sinusoidal	None	BMI 85 th %ile	Tac MMF (at 3, 19m)	
Latino female	16.8y: DDLT, whole -ALF (isoniazid) f/u to age: 23.3y	5.5y	3	Mild lobular Mild portal	Few	1a: Peri-sinusoidal	Mild ACR	BMI 25	Tac ω3	-Chronic rejection despite steroids, Tac, MMF, Everolimus - Moderate steatosis on study ultrasound
Latino female	20.0y: DDLT, whole -Primary hyperoxaluria f/u to age: 24y	9.8y	2	No lobular Mild portal	Few	None	None	BMI 27	Tac Pred 5mg	-No later biopsies -Deceased

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ACR acute cellular rejection; ALF acute liver failure; AZA azathioprine; BMI Body mass index; CSA cyclosporine; DDLT deceased donor liver transplant; F/U follow-up; LRLT living-related liver transplant; m months; MMF mycophenolate mofetil; NA Not available; Pred prednisone; Tac tacrolimus; y years