Graft Fibrosis Over 10 to 15 Years in Pediatric Liver Transplant Recipients: Multicenter Study of Paired, Longitudinal Surveillance Biopsies

Emily R. Perito,[1] Elodie Persyn,[2] John Bucuvalas,[3] Mercedes Martinez,[4] Saeed Mohammad,[5] James E. Squires,[6] Anthony J. Demetris,[7] and Sandy Feng[8]

[1]Department of Pediatrics, Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA; [2]Institute of Liver Studies, King's College London, London, UK; [3]Department of Pediatrics, Mount Sinai Kravis Children's Hospital and Recanati/Miller Transplantation Institute, Mount Sinai Health System, New York, NY; [4]Department of Surgery, Center for Liver Diseases and Transplantation, Columbia University Irving Medical Center, New York, NY; [5]Department of Pediatrics, Siragusa Transplantation Center, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL; [6]Department of Pediatrics, University of Pittsburgh Medical Center, Children's Hospital of Pittsburgh, Pittsburgh, PA; [7]Department of Pathology, University of Pittsburgh, Pittsburgh, PA; and [8]Department of Surgery, University of California San Francisco, San Francisco, CA

Previous single-center, cross-sectional studies have reported a steep increase in the prevalence and severity of fibrosis through 10 to 15 years after pediatric liver transplantation. We report a multicenter study of paired surveillance biopsies in a contemporary cohort. Children who underwent liver transplant when younger than 6 years old and had paired surveillance liver biopsies were enrolled (n = 78, 35% girls, median 1.2 years old at transplant). A central pathologist graded inflammation, assessed rejection activity index, and staged fibrosis in the portal, sinusoidal, and perivenular compartments, allowing for calculation of the Liver Allograft Fibrosis Score (LAFSc). Analysis of variance tested associations between fibrosis progression and clinical parameters. The first biopsy, at a median 8.2 years (interquartile range, 5.9-11.6 years) after transplantation, showed absent to mild fibrosis (LAFSc 0-2) in 29%, moderate (LAFSc 3-5) in 56%, and severe (LAFSc 6-7) in 14% of patients. The second biopsy, at a median 4.7 years (IQR, 4.3-5.1 years) later, showed fibrosis progression (LAFSc increased by ≥3) in 10 (13%) and regression (LAFSc decreased by ≥3) in 4 (5%) patients. After adjusting for baseline LAFSc, younger age at transplant was the only risk factor for fibrosis progression. Although fibrosis prevalence and severity 6 to 12 years after transplant was similar to previous reports, fibrosis trajectory during the next 4 to 5 years was stable. Our data may be reassuring for children with consistently normal liver tests. A comprehensive understanding of factors determining allograft health during the very long term is essential to optimizing allograft and patient health.

Liver Transplantation 28 1051–1062 2022 AASLD.
Received September 15, 2021; accepted December 22, 2021.

Achieving a lifetime of patient and allograft health after liver transplantation is a steep challenge in children; even 20 to 25 years of posttransplant survival brings a pediatric recipient only to young adulthood. At 10 years after liver transplantation, only one-third of pediatric liver transplantation recipients attain an "ideal" outcome, including normal growth, no transplant-related medical comorbidity, and normal liver tests on a single immunosuppression medication.[1] By 25 years after transplant, almost 30% of children have died, most often from graft loss and infection but with contributions from renal, cardiovascular, and oncological morbidities, highlighting the challenge of achieving sufficient but not excessive immunosuppression.[2] Complementary studies focused on allograft health have similarly raised concerns, showing that,

Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; GGT, gamma glutamyltransferase; IQR, interquartile range; iWITH, Immunosuppression Withdrawal for Stable Pediatric Liver Transplant Recipients; iWITH-IN, iWITH ineligible; LAFSc, Liver Allograft Fibrosis Score; NA, not available; RAI, rejection activity index; RETRO, retrospective study.

at 7 to 10 years after transplant, as many as 75% of pediatric liver allografts harbor clinically silent (normal liver tests) chronic inflammation and/or moderate to severe fibrosis.[3-5] These studies highlight the need for strategies that aim to sustain both allograft and patient health over a horizon not of years but, rather, of decades.

A significant challenge to immunosuppression management for children after liver transplantation is our incomplete knowledge about the long-term costs of too much or too little immunosuppression. With either directional misstep, the erosion of allograft and patient health often occurs silently and gradually for decades. Delineation of the allograft's natural history has also been incomplete. Literature is dominated by single-center reports of retrospective cross-sectional studies, with neither standardized nor consistent scoring systems.[3-10] The weight of available evidence suggested that the prevalence and severity of inflammation and/or fibrosis increases over time.[11-13] These landmark studies were, however, based on transplants performed in the 1990s when cyclosporine-based immunosuppression regimens were common. In contrast, 2 recent articles report distinctly low rates of fibrosis for children transplanted more recently and maintained on tacrolimus monotherapy at 5 years (5%)[14] and 10 years (9%)[15] after transplantation; a third article reported that fibrosis occurred but remained mild to moderate in the majority of children through 5 and 10 years.[16]

We undertook the current study to delineate the natural history of stable liver allografts using longitudinal and, importantly, paired, nonindication biopsies collected from children undergoing liver transplantation between 2000 and 2010 with a focus on 5 to ≥15 years after transplant. We hypothesized that studying a cohort of children with strictly normal liver tests and maintained on current immunosuppression medications may provide clarity and delineate a different trajectory of allograft histopathology than earlier studies.

Address reprint requests to Sandy Feng, M.D., Ph.D., Department of Surgery, University of California San Francisco, 505 Parnassus Avenue, San Francisco, CA 94117. Telephone: 415-353-8725; Fax: (415) 353-8917; E-mail: sandy.feng@ucsf.edu

This study was supported by the National Institute of Diabetes and Digestive and Kidney Diseases (R01-DK114180).

Anthony J. Demetris consults for and received grants from Novartis. He received grants from TransMedics. Emily R. Perito received grants from Albireo and Shire.

Emily R. Perito acquired the data, contributed to data analysis and interpretation, drafted the manuscript, critically revised the manuscript, and approved the final analysis and manuscript. Elodie Persyn analyzed the data, contributed to data interpretation and manuscript drafting, critically revised the manuscript, and approved the final analysis and manuscript. John Bucuvalas and Sandy Feng conceived and designed the study, contributed to data analysis and interpretation, drafted the manuscript, critically revised the manuscript, and approved the final analysis and manuscript. Mercedes Martinez, Saeed Mohammad, and James E. Squires acquired the data, critically revised the manuscript, and approved the final analysis and manuscript. Anthony J. Demetris contributed to the study design and data analysis and interpretation, acquired the data, critically revised the manuscript, and approved the final analysis and manuscript.

Additional supporting information may be found in the online version of this article.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/lt.26409

Patients and Methods

STUDY DESIGN

We conducted a longitudinal study of 2 cohorts of stable, long-term, pediatric liver transplantation recipients, as detailed in Table 1 (R01DK114180-011). The Immunosuppression Withdrawal for Stable Pediatric Liver Transplant Recipients (iWITH)–ineligible (IN) cohort was recruited from patients who had enrolled in iWITH (NCT01638559), an immunosuppression withdrawal trial, but were ineligible to withdraw immunosuppression based on their screening biopsy.[17,18] Of 67 patients who passed the initial screening, underwent baseline data collection, and had a screening biopsy but did not complete enrollment because of inflammation and/or fibrosis beyond eligibility criteria, 43 underwent a follow-up liver biopsy per the standard of care at their center, provided assent and/or informed consent for medical record review, blood and tissue collection, and data analyses and thus comprise the iWITH-IN cohort. The retrospective study (RETRO) cohort was identified from 4 iWITH centers that performed surveillance liver biopsies as standard of care for ≥5 years such that both biopsies were done as standard of care. Institutional review board approval was obtained at all 4 centers as data collection was retrospective; assent and/or informed consent was not required as there were no study interventions (Table 1).

TABLE 1. Descriptions of iWITH-IN and RETRO Cohorts

	Cohort 1: iWITH-IN (n = 43)	Cohort 2: RETRO (n = 35)
Cohort description	Patients enrolled in iWITH (NCT01638559) but deemed ineligible to proceed with immunosuppression withdrawal secondary to screening biopsy histopathology	Patients followed at 4 iWITH centers that perform standard-of-care surveillance biopsies who met inclusion/exclusion criteria

Inclusion criteria	• <6 years of age at liver transplantation
	• Index (first) liver biopsy
	  • At <18 years of age
	  • ≥4 years after liver transplantation
	• ≥4 years between index (first) and follow-up (second) liver biopsy	

	• ALT and GGT consistently ≤50 at index (first) liver biopsy and 2 years prior • On stable calcineurin inhibitor monotherapy for 2 years • Slides available from index (first) and follow-up (second) liver biopsies • Not eligible for the iWITH trial (ie, had more than minimal fibrosis on the screening biopsy)	• ALT and GGT consistently ≤50 at index (first) liver biopsy and 1 year prior • No inclusion criteria related to immunosuppression • Slides and/or tissue block available from index (first) and follow-up (second) liver biopsies
Exclusion criteria	• Transplant for hepatitis B, hepatitis C, or autoimmune liver disease • Receipt of any non–liver transplantation (organ or cell) before or after liver transplantation • No acute or chronic rejection within 2 years prior to index (first) liver biopsy	
Year of first biopsy	2012-2014	2005-2014
Data collection	Retrospective	
Available data	• Baseline data: retrospectively collected in iWITH enrollment • Donor-specific antibody: blood collected at iWITH enrollment • Follow-up data including transplant-related diagnoses and events and detailed immunosuppression management between biopsies: retrospectively collected at follow-up (second) biopsy	• Baseline data: retrospectively collected enrollment • Follow-up data including transplant-related diagnoses and events and immunosuppression dose at time of transplant and biopsies
Biopsy assessment	Central review by iWITH pathologist with side-by-side comparison of biopsy pair digital images	
Contributing centers	Ann & Robert H. Lurie Children's Hospital of Chicago Children's Hospital of Pittsburgh Morgan Stanley Children's Hospital University of California, San Francisco Children's Healthcare of Atlanta Children's Hospital Colorado Children's Hospital of Philadelphia Cincinnati Children's Hospital C.S. Mott Children's Hospital, University of Michigan St. Louis Children's Hospital Texas Children's Hospital	

Of note, the iWITH-IN cohort by definition excluded patients with no to minimal inflammation or fibrosis who qualified for the iWITH trial, whereas the RETRO cohort included patients meeting clinical, non-biopsy-related inclusion/exclusion criteria (Table 1). iWITH-IN patients were all on stable calcineurin inhibitor monotherapy for 2 years preceding the first biopsy, as required by the iWITH trial protocol. The RETRO cohort was not restricted with respect to immunosuppression. All patient care for both cohorts, including immunosuppression management, was conducted according to each center's standard of care between the first and second surveillance biopsies. Reviewed data included pathology reports to identify rejection episodes and procedure reports of graft-related therapeutic interventions that confirmed complications such as biliary stricture and/or vascular stenosis or thrombosis.

HISTOLOGIC ASSESSMENT

All biopsies were assessed, as previously described in detail, by a single pathologist (A.J.D.).[17,18] Briefly, high-resolution, whole-slide images of formalin-fixed, paraffin-embedded and hematoxylin-eosin–stained tissue sections were scored for 48 histopathological features encompassing inflammation, fibrosis, and findings related to bile ducts and blood vessels. Inflammation

was assessed in the portal, lobular, and perivenular compartments with particular notation of interface hepatitis; each was graded as none (0), mild (1), moderate (2), or severe (3). Rejection activity index (RAI) was scored according to accepted convention based on the degree of portal inflammation, endotheliitis, and bile duct injury.[19] Fibrosis was staged as none (0), mild (1), moderate (2), or severe (3) in each of 3 compartments (portal, sinusoidal, and perivenular) and summed to calculate the composite Liver Allograft Fibrosis Score (LAFSc; range, 0-9).[20] The primary outcome was progression of fibrosis between the index and follow-up biopsies, defined as an increase in LAFSc by ≥3. In parallel, regression was defined as a decrease in LAFSc by ≤−3.

STATISTICAL ANALYSIS

Patient characteristics were compared between the iWITH-IN and RETRO cohorts. For continuous variables, a 2-sample Kolmogorov-Smirnov test was applied to compare distributions. For categorical variables, a Fisher's exact test (2 categories) or a chi-square test (>2 categories) was used. All patients were included in the descriptive analyses. Analysis of variance was performed to test the association significance between fibrosis progression and individual predictors of interest after adjusting for LAFSc at the time of index biopsy. A total of 2 patients with an LAFSc of 7 on the screening biopsy were excluded from these final analyses examining fibrosis progression because they could not, by definition, progress by ≥3 in the second biopsy as the maximum LAFSc is 9. All analyses were performed using R software (R Foundation for Statistical Computing, Vienna, Austria).[21] All authors had access to the study data and reviewed and approved the final manuscript.

Results

CHARACTERISTICS OF STUDY PATIENTS

Of the 78 patients, 27 (35%) were girls, 55 (71%) were White, and 44 (56%) received a transplant for biliary atresia. Patients were a median of 1.2 years of age (interquartile range [IQR], 0.6-2.3 years of age) at time of transplant. Among the 50 (65%) children who underwent deceased donor transplantation, 37 (74%) received a whole organ (Table 2).

The 2 cohorts were contemporaneous with respect to transplant year. iWITH-IN index biopsies were clustered, performed between 2012 and 2014, whereas RETRO cohort biopsies were dispersed between 2005 and 2014 (Fig. 1A). The median (IQR) interval between biopsies was modestly shorter for the iWITH-IN cohort than the RETRO cohort (Fig. 1B, Table 2). The 2 cohorts were similar with respect to sex, age at transplant, race/ethnic distribution, transplant indication, and donor/graft type. However, at the time of the first biopsy, the iWITH-IN patients were slightly older with a median longer interval since transplant (Table 2). The cohorts did not differ in the nature or frequency of complications, either vascular or biliary, or rejection episodes prior to the first biopsy. Use of induction immunosuppression (basiliximab or thymoglobulin) was rare in the iWITH-IN cohort (n = 1; 2%) but frequent in the RETRO cohort (n = 23; 66%).

At the first biopsy, all iWITH-IN patients were on calcineurin inhibitor monotherapy, compared with 71% of the RETRO cohort. At the second biopsy, calcineurin inhibitor monotherapy remained the most common regimen (Table 2). Finally, 72% of iWITH-IN patients exhibited class II donor-specific antibodies at the first biopsy time point[18]; no data were available at the follow-up biopsy time point nor for either biopsy time point for the RETRO cohort.

SIGNIFICANT CLINICAL EVENTS BETWEEN FIRST AND SECOND BIOPSIES

The only significant graft-related events that occurred between the 2 biopsies were biopsy-proven acute rejection episodes (6 patients), biliary stricture treated by percutaneous transhepatic bile duct dilatation (1 patient), and cholangitis (1 patient).

INFLAMMATION AND FIBROSIS SCORES FOR THE FIRST AND SECOND BIOPSIES

Inflammation in the first and second biopsies is shown in Fig. 2 and Supporting Fig. 1A, ordered by portal inflammation, followed by interface activity, lobular inflammation, and perivenular inflammation. Individual compartment and composite fibrosis scores for the first and second biopsies are shown in Fig. 2 and Supporting Fig. 1B, ordered by the LAFSc followed by portal, sinusoidal, and perivenular fibrosis. Inflammation grade was generally absent or mild and occasionally moderate for all 3 compartments; only a

TABLE 2. Patient Characteristics by Cohort: iWITH-IN and RETRO

Patient Characteristic	iWITH-IN (n = 43)	RETRO (n = 35)	*P* Value
Female sex	13 (30)	14 (40)	0.47
Age at liver transplantation, years	1.1 (0.6-2.3)	1.3 (0.6-1.9)	1.00
Race			
White	31 (72)	24 (69)	0.93
Black	5 (12)	5 (14)	
Other (Asian, Pacific Islander, Multi-Racial, not specified)	7 (16)	6 (17)	
Ethnicity			
Non-Hispanic	36 (84)	30 (86)	0.59
Hispanic	6 (14)	3 (9)	
Other (unknown or not reported)	1 (2)	2 (6)	
Liver transplantation indication*			
Biliary atresia	22 (51)	22 (63)	0.64
Acute liver failure	2 (5)	2 (6)	
Tumor	4 (9)	2 (6)	
Metabolic liver disease	14 (33)	7 (20)	
Other	1 (2)	2 (6)	
Donor and graft type			
Deceased			
Whole	20 (47)	17 (49)	0.19
Partial	10 (23)	3 (9)	
Living donor	13 (30)	15 (43)	
Induction use†			
Antithymocyte globulin or basiliximab	1 (2)	23 (66)	<0.005
Events between transplant and first biopsy			
Vascular complication	2 (5)	3 (9)	0.66
Biliary stricture	3 (7)	2 (6)	1.00
Patients with previous acute rejection			
1 episode	16 (37)	12 (34)	0.43
>1 episode	3 (7)	5 (14)	
Time since last rejection episode, years	8.3 (4.4-10.4)	6.2 (4.7-9.2)	0.87
At time of first biopsy			
Age, years	11.6 (9.0-14.3)	9.1 (6.9-11.1)	0.03
Time interval since transplant, years	9.6 (6.4-12.7)	6.3 (5.3-9.0)	0.04
ALT, U/L	25 (18-37)	22 (17-28)	0.21
GGT, U/L	15 (11-21)	14 (12-21)	0.97
Total bilirubin, mg/dL	0.6 (0.4-0.9)	0.6 (0.4-0.8)	0.97
Platelet count, $\times 10^9$/L	201 (176-248)	236 (202-276)	0.09
Class II donor-specific antibodies present	28 (72)	NA	NA
Immunosuppression			
Calcineurin monotherapy	43 (100)	25 (71)	0.002
Tacrolimus	37 (86)	23 (66)	0.06
Cyclosporine	6 (14)	7 (20)	0.55
Mycophenolate mofetil	0	0	1.00
Prednisone	0	2 (6)	0.20
Mammalian target of rapamycin inhibitor	0	5 (14)	0.02
Azathioprine	0	2 (6)	0.20

(Continues)

TABLE 2. ***Continued***

Patient Characteristic	iWITH-IN (n = 43)	RETRO (n = 35)	*P* Value
Events between biopsies			
Acute rejection	1	5	0.08
Biliary stricture/cholangitis	1	1	1.00
At time of second biopsy			
Age, years	15.6 (13.7-18.8)	14.3 (12.6-17.2)	0.28
Time interval since liver transplantation, years	13.7 (11.3-17.6)	12.6 (10.6-14.8)	0.14
Time interval since first biopsy, years	4.5 (4.3-4.8)	5.1 (4.6-6.3)	<0.005
Immunosuppression			
Calcineurin monotherapy	39 (91)	27 (77)	0.12
Tacrolimus	36 (84)	28 (80)	0.77
Cyclosporine	4 (9)	3 (9)	1.00
Mycophenolate mofetil	1 (2)	1 (3)	1.00
Prednisone	0	0	1.00
Mammalian target of rapamycin inhibitor	3 (7)	3 (9)	1.00
Azathioprine	0	2 (6)	0.20

NOTE: Data are presented as absolute number (percentage) or median (IQR).
*Metabolic liver disease includes Alagille syndrome, progressive familial intrahepatic cholestasis (any type), glycogen storage diseases, alpha-1-antitrypsin deficiency, inborn errors of metabolism, and other inherited liver diseases with identified genetic basis. Tumor includes hepatoblastoma and hepatocellular carcinoma. "Other" includes cirrhosis of cryptogenic etiology, gestational alloimmune liver disease, and secondary cholangitis.
†Data available for 42 iWITH-IN and 35 RETRO patients.

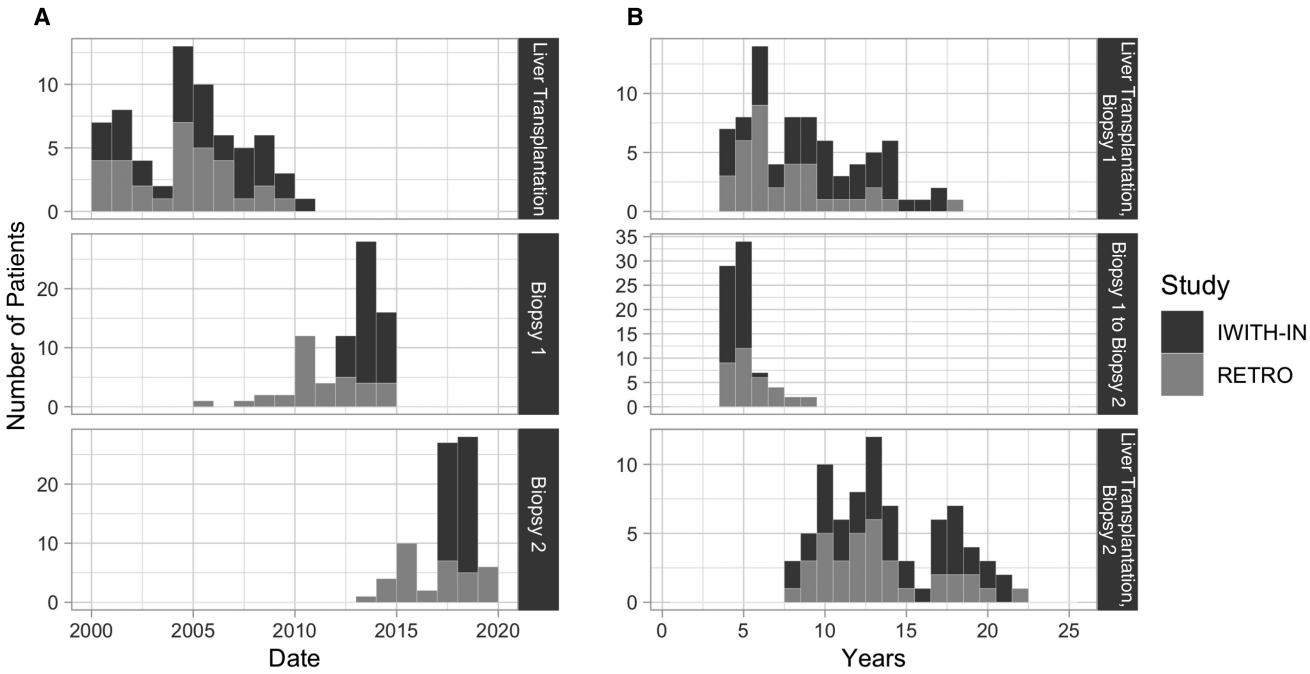

FIG. 1. Timing of and intervals between liver transplantation, biopsy 1, and biopsy 2. (A) Year of liver transplantation, biopsy 1, and biopsy 2. (B) Interval in years between liver transplantation and biopsy 1, biopsy 1 and biopsy 2, and liver transplantation and biopsy 2.

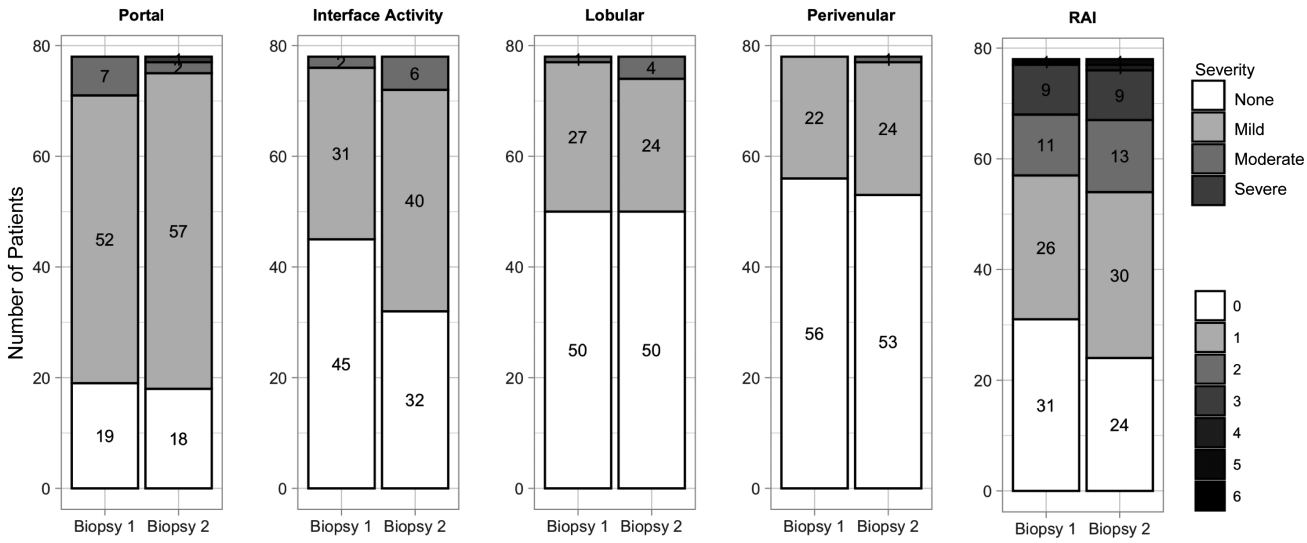

FIG. 2. Distribution of compartmental and composite inflammation grade scores for biopsy 1 and biopsy 2. The distribution of compartmental and composite inflammation scores is displayed in 5 pairs of stacked bar graphs. In each pair, the left graph shows the distribution for biopsy 1 and the right graph shows the distribution for biopsy 2. Portal, interface activity, lobular, and perivenular inflammation grade along with RAI are shown.

single patient showed severe portal inflammation in the second biopsy. Fibrosis score by compartment was most commonly mild or moderate, although severe was not rare.

Of 11 patients with LAFSc ≥6 on the index biopsy, 9 patients had LAFSc 6 and 2 patients had LAFSc 7; all had at least mild fibrosis in all 3 compartments, but only 2 had any stage 3 fibrosis (Supporting Fig. 1).

INFLAMMATION AND FIBROSIS CHANGE BETWEEN BIOPSIES

The distribution of inflammation grade in the first and second biopsies is shown in Fig. 2. The most notable change occurred with interface activity, which was present in 42% (33/78) of the first and 59% (46/78) of the second biopsies. On their second biopsy, 7 fewer patients had an RAI of 0, 4 additional patients had an RAI of 1, and 2 additional patients an RAI of 2.

The distribution of fibrosis stage for the first and second biopsies is shown in Fig. 3. In the portal and perivenular compartments, there was a shift away from the none and mild stages toward the moderate and severe fibrosis stages. This shift was not observed in the sinusoidal compartment. Between the first and second biopsies, the LAFSc distribution shifted modestly, with 4 fewer patients in the none (LAFSc 0) and mild (LAFSc 1-2) categories and 4 additional patients with high LAFSc (6-9). Among the 11 patients who had an LAFSc of ≥6 on index biopsy, 3 had no change in LAFSc in the second biopsy, 6 had an improved LAFSc (by 1-4), and only 2 had LAFSc increase from 6 to 7. The 2 who started with an LAFSc of 7 both improved, to 5 and 6. Of the 5 participants who were 15 years or farther from transplant at their index biopsy, 4 had an initial LAFSc of 2 to 5, and 1 had an LAFSc of 7. Obliterative arteriopathy was identified in the first biopsy in 1 patient and in the second biopsy in 3 patients. Bile duct loss was not seen in the first biopsy but identified in 3 patients in the second biopsy.

The change in fibrosis scores for individual patients is presented in Fig. 4. Of the 78 patients, 10 progressed (13%; 95% confidence interval [CI], 6%-20%), defined as an increase in LAFSc of ≥3. The majority (8) exhibited an increase of 3, whereas 1 each exhibited an increase of 4 and 5 (Fig. 4A). For 7 of the 10 progressors, the LAFSc of the first biopsy was 0, 1, or 2 (Fig. 4B). Of the 78 patients, 4 regressed (5%; 95% CI, 1%-13%), defined as a decrease in LAFSc of ≥3. A total of 2 patients each exhibited decreases of −3 and −4. Of the remaining 64 patients, 54 had no significant change in the LAFSc (−1, 0, or 1 change); 6 had an LAFSc increase of 2, and 4 had an LAFSc decrease

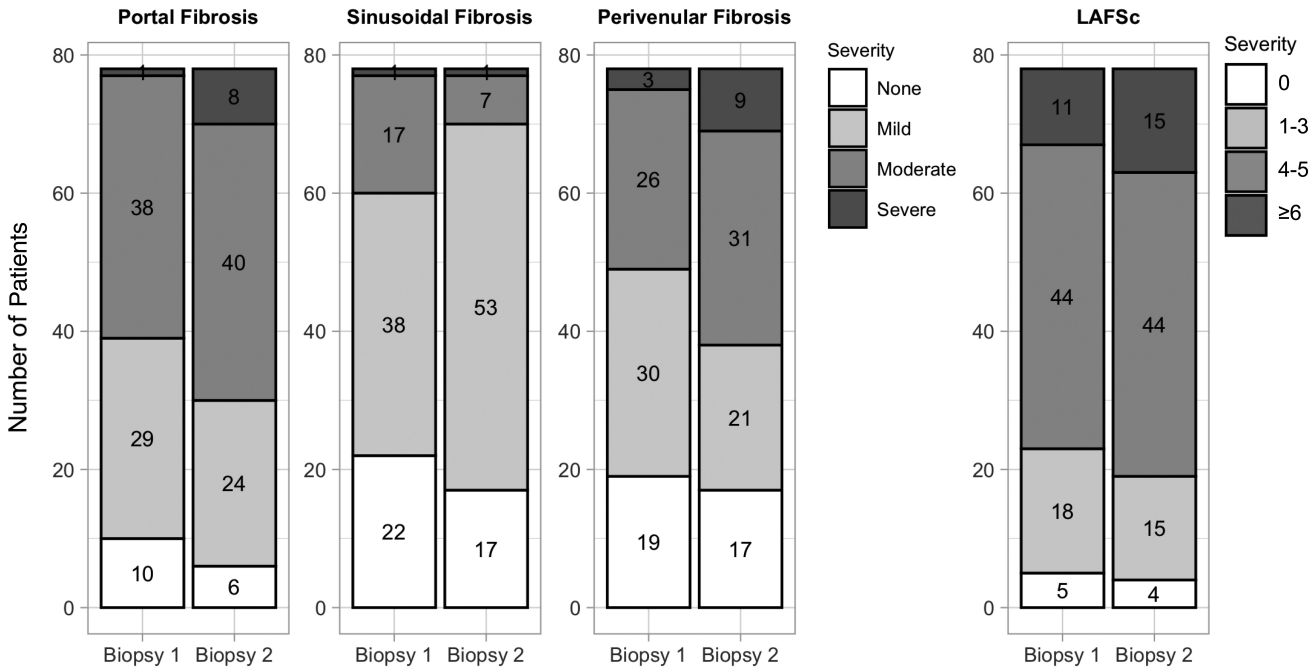

FIG. 3. Distribution of compartmental and composite fibrosis stage scores for biopsy 1 and biopsy 2. The distribution of compartmental and composite fibrosis stage scores is displayed in 4 pairs of stacked bar graphs. In each pair, the left graph shows the distribution for biopsy 1 and the right graph shows the distribution for biopsy 2. Portal, sinusoidal, and perivenular fibrosis along with the LAFSc are shown.

of −2. Of the 6 children with an LAFSc increase of 2 between biopsies, all had fibrosis increases by 1 stage in 2 compartments and remained stable in the third; 2 started with an LAFSc of 1 to 2, and 4 with an LAFSc 3 to 5 (Fig. 4). Detailed information related to the 10 progressors and 4 regressors are presented in Supporting Table 1. Of the 10 progressors, 1 exhibited obliterative arteriopathy and 1 showed bile duct loss in the second biopsy.

FACTORS ASSOCIATED WITH PROGRESSION OF LIVER FIBROSIS

Next, we explored whether demographic, clinical, or histological variables at the time of the first biopsy could predict fibrosis progression between the 2 biopsies after adjusting for LAFSc at index biopsy (Table 3). Patients who progressed were younger at transplant than those who remained stable or regressed (0.7 years [IQR, 0.6-1.2 years] versus 1.3 years [IQR, 0.6-2.5 years]). Younger age at transplant emerged as the only statistically significant association, with the odds of fibrosis progression decreasing for each year increase in age (odds ratio, 0.43 per year; 95% CI, 0.12-0.89). No other donor, graft, recipient, or transplant factors, including administration of induction immunosuppression at transplant and time interval between transplant and first biopsy, were associated with fibrosis progression. Neither biochemical markers of liver (alanine aminotransferase [ALT]) or biliary (gamma-glutamyltransferase [GGT]) injury nor histological features of the first biopsy predicted fibrosis progression. Finally, the proportion of patients who progressed was 5% (2/43) for the iWITH-IN cohort but 23% (8/35) for the RETRO cohort. However, after adjusting for LAFSc at first biopsy, the cohort (RETRO versus iWITH-IN) was not associated with fibrosis progression.

Discussion

We conducted a multicenter, longitudinal study of rigorously selected and paired surveillance biopsies to delineate the natural history of liver allograft histopathology for recently transplanted children (since 2000) managed with contemporary immunosuppression.

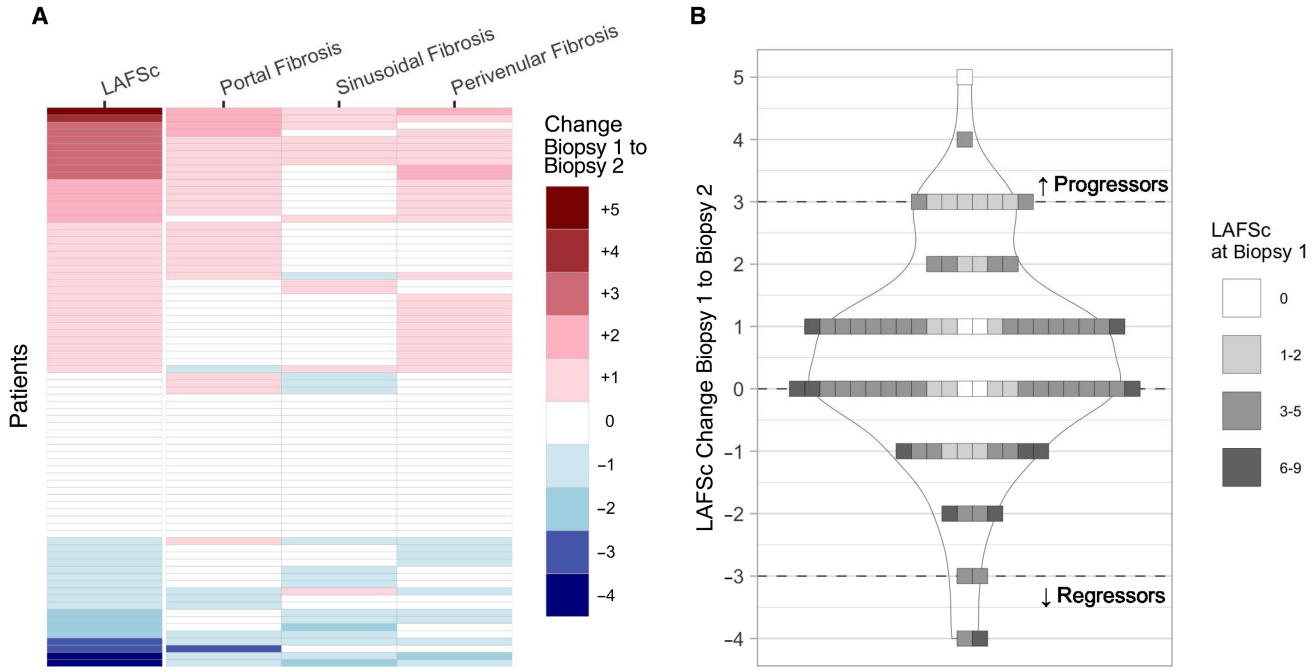

FIG. 4. Change in compartmental and composite fibrosis stage from biopsy 1 to biopsy 2. (A) Heatmap display of the change in compartmental and composite fibrosis stage from biopsy 1 to biopsy 2. Red indicates an increase, whereas blue indicates a decrease in fibrosis score over time; the intensity of color indicates the magnitude of change. Fibrosis progression is defined as an increase in LAFSc of ≥3, which was observed for 10 of 78 patients. Fibrosis regression is defined as a decrease in LAFSc of ≥3, which was observed for 4 of 78 patients. (B) Violin plot display of the change in LAFSc from biopsy 1 to biopsy 2. The color of each square corresponds to its LAFSc at biopsy 1. The position of each square corresponds to the LAFSc change over time. The progressors and regressors are shown at the top and bottom, respectively. LAFSc either did not change or changed by only +1 or −1 over time for 54 of 78 patients. For the remaining 10 patients, LAFSc increased by 2 for 6 and decreased by 2 for 4.

Many preceding single-center, cross-sectional studies have shown deteriorating allograft health leading to a high prevalence (up to 75%) of moderate to severe fibrosis at 5 to 10 years after transplant.[3-7,9,10,22,23] These trends, if persistent, would suggest that many grafts may fail during the ensuing decades. Our cohort had a similar prevalence of more advanced fibrosis at 5 to 10 years after transplant: 55% with LAFSc 4 to 6 and only 3% with LAFSc 7 to 9. However, we showed that 5 years later, the LAFSc distribution changed very modestly, with 62% having an LAFSc 4 to 6 and 5% with LAFSc 7 or higher, suggesting a stabilized rather than a progressive trajectory for children with normal aminotransferases. One recent single-center study in an Italian cohort of patients who received transplants from 2008 to 2018 similarly demonstrated a high prevalence of mild fibrosis (81% at 5 years), with fibrosis stability in the majority of children with fibrosis, including a limited subset with biopsies at both 5 and 10 years.[16]

Our study focused on children who received transplants since 2000 and were treated predominantly with tacrolimus. Previous cross-sectional studies in which most patients received transplants prior to 2000[3-7,9,10] may not generalize to current cohorts. More recent studies of children who received transplants after 2000 deliver mixed messages, with most reporting a lower[8,14,15] fibrosis prevalence, but exceptions remain.[16,23] Earlier studies often included children with aminotransferases more than 1 to 2 times the upper limit of normal,[3-7,9,16,22,23] which we strictly excluded. Instead, we focused on those with truly "normal" liver tests. This distinction may be critical, as some analyses associate the severity and frequency of elevated liver tests with fibrosis development.[8,15] Nearly all early studies used nonstandardized histological assessments by multiple pathologists. In contrast, our study benefited from a standardized assessment conducted by a single central pathologist.[24-26]

TABLE 3. Univariable Analyses of Donor, Graft, Recipient, and First Biopsy Variables for Fibrosis Progression (n = 76)*

Variables	Reference Group	Odds Ratio	95% CI
Donor and graft			
Age (n = 72; per year increment)		1.01	0.96-1.06
Male (n = 74)	Female	0.56	0.12-2.32
Living	Deceased	2.12	0.50-9.17
Whole	Partial	0.75	0.17-3.18
Recipient			
Age at transplant (per year increment)		0.43	0.12-0.89
Male	Female	0.67	0.15-3.04
Non-White (n = 73)	White	0.21	0.01-1.40
Non-Hispanic (n = 73)	Hispanic	1.11	0.05-8.9
No induction immunosuppression (n = 75)	Induction	0.29	0.05-1.66
Interval between transplant and first biopsy (per year)		0.95	0.73-1.18
ALT (U/L) at first biopsy (per 10 U/L increment)		0.75	0.32-1.14
GGT (U/L) at first biopsy (per 10 U/L increment)		0.88	0.32-1.50
Total bilirubin at first biopsy (per 1 U/L increment)		0.19	0.01-1.35
RETRO cohort	iWITH-IN	2.28	0.28-21.8
Histological features of the first biopsy			
Portal inflammation	Absent	1.82	0.37-11.51
Interface activity present	Absent	1.52	0.40-5.62
Lobular inflammation	Absent	0.63	0.09-3.10
Perivenular inflammation	Absent	0.88	0.12-4.51
RAI, per 1 point		1.13	0.55-2.03

*n = 76 unless otherwise noted; 2 patients with LAFSc 7 at screening biopsy excluded. All odds ratios adjusted for LAFSc at index biopsy.

Although the cross-sectional component of our study showed an overall prevalence of greater than mild fibrosis comparable with previous studies, the longitudinal component of our study showed fibrosis progression in a minority (13%), along with some fibrosis regression (5%). This flattened trajectory may be reassuring for liver allograft longevity. This favorable natural history may be limited to the best stratum of children with liver transplantations, those with strictly and consistently normal liver tests. Even beyond liver tests, more than half of our cohort (iWITH-IN; 55%) were eligible to enroll in a trial of immunosuppression withdrawal according to medical history, immunosuppression management, and physician assessment of ability to adhere to the constraints of a rigorous trial. Among this group of 43, only 2 had fibrosis progression. However, in the RETRO cohort, also with normal liver tests but less rigorously selected and perhaps more representative of stable long-term patients, 8 of 35 progressed. Of note, the children in the RETRO cohort were earlier after transplant, younger at biopsy, and accounted for the majority of patients with a first biopsy showing none or minimal fibrosis. Children with the least fibrosis accounted for the majority of progressors, perhaps reflecting an inherent bias for progression because mild and moderate fibrosis was the dominant overall phenotype. The difference in progression incidence between the iWITH-IN and RETRO cohorts, albeit not statistically significant, may nevertheless be instructive to inform future studies that examine long-term transplant recipient care.

Our data suggest the following hypotheses: (1) that liver allografts can be stratified by multiple clinical parameters measured over time and (2) that an individual allograft's histological trajectory might be predicted by its stratum. Among readily available metrics, ALT may best reflect the stratum for each allograft. For children with ALT consistently at the very low end of normal range to those with consistently abnormal ALT, fibrosis trajectory may vary accordingly from stable to progressive. Immunosuppression may be the only available lever to shift allografts between these strata. The

relationships are, however, complex. Low ALT and an optimally protected allograft may come at the cost of high immunosuppression exposure with attendant toxicities that adversely impact patient health. High ALT may result from low immunosuppression exposure that endangers allograft integrity and thus, indirectly, adversely impacts long-term patient health. Currently, immunosuppression decision making, driven by rudimentary clinical parameters and center-specific protocols, is unlikely to optimally negotiate these complex trade-offs. Future research efforts should elucidate clinical, histological, and/or peripheral blood biomarkers that accurately indicate allograft health and/or are predictive of allograft trajectory to guide clinicians.

A key limitation of our study is that strict eligibility criteria and modest sample size limits generalizability to all children with liver transplantations. First, in future studies, it will be important to consider similar analyses in other subsets of pediatric liver transplantation recipients—including those who received transplants at ages older than 6 years, those with recent acute or chronic rejection diagnoses, and those with more variable ALT and GGT levels over time. Second, the time interval between biopsies, approximately 5 years, is, for children, a minimum interval reflective of long-term histological evolution. Our second biopsies, however, covered a critical period between 10 and ≥15 years after transplantation, a time frame of great interest and not previously examined. A third shortcoming is the retrospective nature of some data collection, which inherently limits accuracy. As a result, we were unable to analyze liver test patterns or immunosuppression exposure between biopsies. We are, however, confident that medical record review accurately captured clinically significant events, including biopsy-proven rejection and vascular or biliary complications. We lacked data on donor-specific antibodies and auto-antibodies, on specific immunohistochemical assessment for chronic antibody-mediated rejection, and on viral or other infections that might impact development or progression of fibrosis. Fourth, we chose a relatively strict definition of fibrosis progression, LAFSc increase by ≥3 between biopsies, at the recommendation of our pathologist (A.J.D.), to exclude changes that might be within the margin of error of fibrosis staging, thereby focusing on progression most likely to be clinically significant. Finally, we acknowledge that studying children cared for according to center standard of care, although reflective of clinical reality, incurs inevitable confounders related to center-specific practices. To best mitigate center-specific bias, larger and prospective studies will be required.

In summary, our analyses expand the evidence challenging the impression that liver fibrosis progression in the first and second decades after pediatric liver transplantation is inevitable and a harbinger of clinical complications. Although only a minority of children enjoy either no fibrosis or mild fibrosis, a large majority remain relatively stable, without significant progression. Currently available clinical predictors can neither identify those with significant fibrosis nor predict those for whom fibrosis will progress. Future studies covering the full spectrum of pediatric liver allograft health should aim to delineate phenotypes along with their associated natural history of fibrosis progression. Moreover, the potential impact of "more" versus "less" immunosuppression, perhaps our only clinical lever to impact this trajectory, will need to be considered. A more granular understanding of histological trajectories and its determining factors offers the best hope to ultimately personalized decision making that will optimize outcomes for children.

Acknowledgments: The authors are grateful to the patients whose data were included, their families, and the National Institute of Allergy and Infectious Diseases and the National Institute of Diabetes and Digestive and Kidney Diseases for supporting the iWITH trial (U01-AI-100807). We greatly appreciate the contributions of the following iWITH coinvestigators and their pediatric transplant centers: University of California, San Francisco Benioff Children's Hospital (Philip Rosenthal); Children's Hospital of Pittsburgh (George Mazariegos, Veena Venkat); C.S. Mott Children's Hospital, University of Michigan (John C. Magee); Siragusa Transplantation Center, Ann & Robert H. Lurie Children's Hospital of Chicago (Estella M. Alonso); Children's Hospital of Atlanta, Emory University (Nitika A. Gupta); Texas Children's Hospital (Ryan W. Himes); Morgan Stanley Children's Hospital, Columbia University (Steven J. Lobritto); The Hospital for Sick Children, University of Toronto (Vicky L. Ng, Yaron Avitzur); The Children's Hospital of Pennsylvania (Elizabeth B. Rand), Children's Hospital Colorado, University of Colorado School of Medicine (Shikha S. Sundaram); and Washington

University School of Medicine (Yumirle P. Turmelle). We thank Katharine M. Spain from Rho, Inc., for assisting with the data.

REFERENCES

1) Ng VL, Alonso EM, Bucuvalas JC, Cohen G, Limbers CA, Varni JW, et al. Health status of children alive 10 years after pediatric liver transplantation performed in the US and Canada: report of the studies of pediatric liver transplantation experience. J Pediatr 2012;160:820-826.e823.
2) Ekong UD, Gupta NA, Urban R, Andrews WS. 20- to 25-year patient and graft survival following a single pediatric liver transplant—analysis of the United Network of Organ Sharing database: where to go from here. Pediatr Transplant 2019;23:e13523.
3) Ekong UD, Melin-Aldana H, Seshadri R, Lokar J, Harris D, Whitington PF, et al. Graft histology characteristics in long-term survivors of pediatric liver transplantation. Liver Transpl 2008;14:1582-1587.
4) Evans HM, Kelly DA, McKiernan PJ, Hubscher S. Progressive histological damage in liver allografts following pediatric liver transplantation. Hepatology 2006;43:1109-1117.
5) Fouquet V, Alves A, Branchereau S, Grabar S, Debray D, Jacquemin E, et al. Long-term outcome of pediatric liver transplantation for biliary atresia: a 10-year follow-up in a single center. Liver Transpl 2005;11:152-160.
6) Herzog D, Soglio DB, Fournet JC, Martin S, Marleau D, Alvarez F. Interface hepatitis is associated with a high incidence of late graft fibrosis in a group of tightly monitored pediatric orthotopic liver transplantation patients. Liver Transpl 2008;14:946-955.
7) Miyagawa-Hayashino A, Yoshizawa A, Uchida Y, Egawa H, Yurugi K, Masuda S, et al. Progressive graft fibrosis and donor-specific human leukocyte antigen antibodies in pediatric late liver allografts. Liver Transpl 2012;18:1333-1342.
8) Sanada Y, Matsumoto K, Urahashi T, Ihara Y, Wakiya T, Okada N, et al. Protocol liver biopsy is the only examination that can detect mid-term graft fibrosis after pediatric liver transplantation. World J Gastroenterol 2014;20:6638-6650.
9) Scheenstra R, Peeters PM, Verkade HJ, Gouw AS. Graft fibrosis after pediatric liver transplantation: ten years of follow-up. Hepatology 2009;49:880-886.
10) Briem-Richter A, Ganschow R, Sornsakrin M, Brinkert F, Schirmer J, Schaefer H, et al. Liver allograft pathology in healthy pediatric liver transplant recipients. Pediatr Transplant 2013;17:543-549.
11) Kelly D, Verkade HJ, Rajanayagam J, McKiernan P, Mazariegos G, Hubscher S. Late graft hepatitis and fibrosis in pediatric liver allograft recipients: current concepts and future developments. Liver Transpl 2016;22:1593-1602.
12) Feng S, Bucuvalas J. Tolerance after liver transplantation: where are we? Liver Transpl 2017;23:1601-1614.
13) George M, Paci P, Taner T. Significance of progressive liver fibrosis in pediatric liver transplants: a review of current evidence. World J Gastroenterol 2020;26:1987-1992.
14) Sheikh A, Chau KY, Evans HM. Histological findings in protocol biopsies following pediatric liver transplant: low incidence of abnormalities at 5 years. Pediatr Transplant 2018;22:e13212.
15) Rhu J, Ha SY, Lee S, Kim JM, Choi G-S, Joh J-W, et al. Risk factors of silent allograft fibrosis 10 years post-pediatric liver transplantation. Sci Rep 2020;10:1833.
16) Angelico R, Spada M, Liccardo D, Pedini D, Grimaldi C, Pietrobattista A, et al. Allograft fibrosis after pediatric liver transplantation: incidence, risk factors, and evolution. Liver Transpl 2022;28:280–293.
17) Feng S, Bucuvalas JB, Mazariegos GV, Magee JC, Sanchez-Fueyo A, Spain KM, et al. Efficacy and safety of immunosuppression withdrawal in pediatric liver transplant recipients: moving towards personalized management. Hepatology 2021;73:1985-2004.
18) Lai JC, Segev DL, McCulloch CE, Covinsky KE, Dodge JL, Feng S. Physical frailty after liver transplantation. Am J Transplant 2018;18:1986-1994.
19) Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 1997;25:658-663.
20) Venturi C, Sempoux C, Bueno J, Ferreres Pinas JC, Bourdeaux C, Abarca-Quinones J, et al. Novel histologic scoring system for long-term allograft fibrosis after liver transplantation in children. Am J Transplant 2012;12:2986-2996.
21) R Foundation for Statistical Computing. R: A Language and Environment for Statistical Computing [computer program]. Vienna, Austria: R Foundation for Statistical Computing; 2020.
22) Varma S, Ambroise J, Komuta M, Latinne D, Baldin P, Reding R, et al. Progressive fibrosis is driven by genetic predisposition, alloimmunity, and inflammation in pediatric liver transplant recipients. EBioMedicine 2016;9:346-355.
23) Venturi C, Sempoux C, Quinones JA, Bourdeaux C, Hoyos SP, Sokal E, et al. Dynamics of allograft fibrosis in pediatric liver transplantation. Am J Transplant 2014;14:1648-1656.
24) Banff Working Group on Liver Allograft Pathology. Importance of liver biopsy findings in immunosuppression management: biopsy monitoring and working criteria for patients with operational tolerance. Liver Transpl 2012;18:1154-1170.
25) Feng S, Bucuvalas JC, Demetris AJ, Burrell BE, Spain KM, Kanaparthi S, et al. Evidence of chronic allograft injury in liver biopsies from long-term pediatric recipients of liver transplants. Gastroenterology 2018;155:1838-1851.e1837.
26) Feng S, Ekong UD, Lobritto SJ, Demetris AJ, Roberts JP, Rosenthal P, et al. Complete immunosuppression withdrawal and subsequent allograft function among pediatric recipients of parental living donor liver transplants. JAMA 2012;307:283-293.