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Long-term Outcome of Asymptomatic Patients With Graft Fibrosis in Protocol Biopsies After Pediatric Liver Transplantation

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Background. The histological prevalence of allograft fibrosis in asymptomatic children after liver transplantation (LT) is well documented. However, long-term graft and patient survival remain unclear. This retrospective multicenter study aims to determine the prevalence of allograft fibrosis and analyze the long-term outcome for patients transplanted in childhood. **Methods.** We reviewed clinical data of children who had undergone 10-y protocol liver biopsies. We excluded patients with autoimmune hepatitis, primary sclerosing cholangitis, hepatitis B or C, and retransplantation. In total, 494 patients transplanted in childhood across 12 international transplant centers were included. We evaluated the development of fibrosis by comparing the results with biopsies obtained 5 and 15 y post-LT. Histological findings were correlated with graft and patient survival up to 20 y post-LT. **Results.** In the 10-y biopsies, periportal or pericentral fibrosis was observed in 253 patients (51%), 87 (18%) had bridging fibrosis, 30 (6%) had cirrhosis, and 124 (25%) had no fibrosis. The prevalence and stage of graft fibrosis significantly progressed from 5 to 10 y. At 10 y, the severity of fibrosis correlated significantly with inflammation. Patients with graft cirrhosis in the 10-y biopsy were more likely to die or require retransplantation subsequently ($P = 0.027$). **Conclusions.** At 10 y post-LT, most patients transplanted in childhood developed fibrosis, based on the protocol liver biopsies. Although mild-to-moderate graft fibrosis did not largely affect patient or graft survival up to 20 y post-LT, this progressive fibrosis finding has substantial implications for developing cirrhosis and portal hypertension in adult care.

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INTRODUCTION

Over the last decades, pediatric liver transplantation (LT) has become a standard procedure for children with end-stage liver disease. Over time, the patient and graft survival after LT has significantly improved. A recent report of the European Liver Transplant Registry documented an increase in 5-y graft survival from 65% in children transplanted before 2000 to 75% transplanted between 2000 and 2009.[1] Furthermore, reports by high-volume pediatric transplant centers in the United States, Europe, and Japan documented a 20-y patient and graft survival of 69% to 80% and 53% to 64%, respectively.[2-5]

Most long-term LT recipients transplanted in childhood enjoy a satisfactory quality of life without biochemical evidence of allograft dysfunction.[6] However, single-center studies of protocol liver allograft biopsies have demonstrated a high prevalence of unexplained graft inflammation and fibrosis in biopsies obtained >1 y post-LT in clinically asymptomatic children who have (near) normal liver biochemistry.[7-20] Subclinical chronic graft injury is likely more relevant for transplanted children, who should have a considerably longer life expectancy than adult LT patients. However, the extent to which subclinical fibrosis and inflammation impact long-term patient and graft survival remains uncertain.

The Graft Injury Group (GIG), founded in 2015 with a registry grant from European Association for the Study of the Liver, is now a research working group of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition, focusing on long-term outcomes after pediatric LT and mechanisms of chronic graft injury. Here, we report a retrospective, multicenter cohort study on the development of graft fibrosis and inflammation, based on protocol liver biopsies, in patients transplanted in childhood and the long-term outcomes.

PATIENTS AND METHODS

Study Design

Participating GIG centers submitted their data on LT in children performed between 1985 and 2009 to our retrospective registry. The requirement of approval by institutional review committees for participation in the study was institute-dependent and obtained if required. Depending on the participating centers' regulations, the study was reviewed by the institutional review committees of the participating centers and was granted IRB approval. Patients who underwent a protocol liver biopsy at 10 y (±2 y) were identified. The following exclusion criteria were then applied:

- Pretransplant autoimmune hepatitis, primary sclerosing cholangitis, liver disease, hepatitis B, or hepatitis C
- Multiorgan transplant
- Retransplant between 7 d post-LT and the date of the 10-y protocol biopsy
- Alanine aminotransferase >50 IU/L at the time of the 10-y protocol biopsy

For the included patients, data were collected at the time of LT, as well as at the follow-up appointments at 5 y (±24 mo), 10 y (±24 mo), and 15 y (±36 mo) post-LT. At each follow-up visit, biopsy findings were recorded, as well as details of immunosuppression, biochemistry, and clinical data. In addition, any episodes of rejection or biliary or vascular complications that had occurred before the 10-y follow-up were noted. For survival outcomes, any retransplant or death dates were recorded, with patients being censored at their most recent contact with the clinic as of March 2020. In most cases, follow-up was continued up to 20 y post-LT, even after the transition to adult care.

Biopsies and Histological Assessment

Protocol liver biopsies, at least 10 y post-LT, were standard procedures at each GIG center. Liver biopsies at 10 y and, if available, at 5 y were included. At 15 y, all biopsies were included. In all cases, liver biopsies were performed using the standard approach at each participating center and were assessed by local pathologists, many of whom were members of the GIG consortium. Inflammation was quantified retrospectively as none, mild, moderate, or severe. Classification of fibrosis was on a 4-point ordinal scale, as developed at a consensus meeting of the GIG, with categories of none, periportal or pericentral fibrosis, bridging fibrosis, and cirrhosis. Examples of biopsies demonstrating each level of fibrosis are shown in Figure 1.

Statistical Analysis

The distribution of the severity of fibrosis was compared between the 5-, 10-, and 15-y biopsy assessments.

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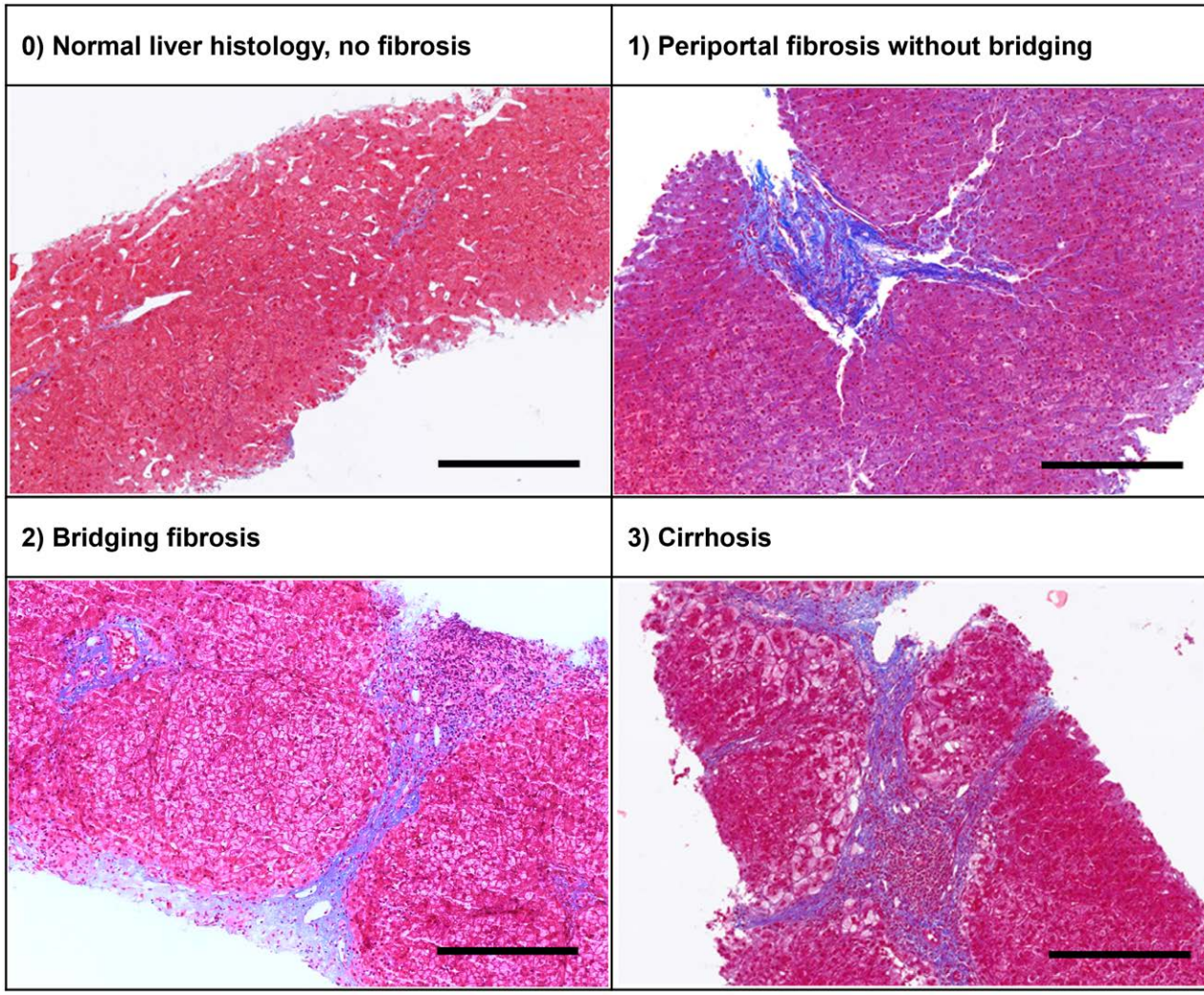

FIGURE 1. Four-point scale for retrospective assessment of liver allograft fibrosis.

The initial analysis was based on a repeated-measures approach, using Friedman test to account for the paired nature of repeated biopsies in the same patient. However, this was a complete-cases analysis, hence only included those patients with biopsies at all 3 time points. A sensitivity analysis was also performed using a Kruskal-Wallis test, including all available biopsy data. Post hoc pairwise comparisons were performed in each case, wherein the overall effect was significant. Correlations between inflammation and fibrosis were then assessed using Spearman's (rho) correlation coefficients.

Comparisons were made between those patients with versus without fibrosis at 10 y. Nominal variables were analyzed using Fisher's exact or χ^2 tests for factors with 2 or >2 categories. Normally distributed variables were reported as mean ± SD, with *P* values from independent *t* tests. Otherwise, variables were reported with medians and interquartile ranges (IQRs) with *P* values from the Mann-Whitney U test. In addition, we performed a multivariable binary logistic regression analysis with a backward-stepwise selection of possible factors associated with fibrosis in the 10-y biopsy.

For analysis of survival outcomes, follow-up commenced at the time of the 10-y biopsy. Outcome rates were estimated using Kaplan-Meier curves, with associations with the degree of fibrosis assessed using a log-rank test.

All analyses were performed using IBM SPSS 22 (IBM Corp. Armonk, NY), with $P < 0.05$ indicating statistical significance. Patients with missing data were excluded from the analysis of the affected factors, and the numbers of cases included in each calculation are reported throughout.

RESULTS

Study Cohort

Twelve centers from Europe, the United States, and Australasia contributed to our study and provided data for N = 494 patients meeting the inclusion criteria (median: 19/center; range, 5–165). The median age at transplant was 19.8 mo (IQR, 9.9–44.6), and the most common underlying liver disease was biliary atresia (62.6%). Further details of the demographics of the cohort are reported in Table 1, and details of immunosuppression regimens are reported in Table 2. This found a remarkably high prevalence of low-dose steroids at 5, 10, and 15 y post-LT (42.4%, 55.4%, and 53.3%, respectively).

In addition to the 10-y biopsy required for study inclusion, 263 patients (53%) had a 5-y biopsy, and 125 patients

TABLE 1.
Demographics

Variable	N	Statistic
Age at transplant, mo	494	19.8 (9.9–44.6)
Sex, % male	494	255 (51.6)
Indication for transplant	494	
Biliary atresia		309 (62.6)
Other cholestatic diseases		51 (10.3)
Metabolic		60 (12.1)
Malignancy		18 (3.6)
Acute liver failure		34 (6.9)
Others		22 (4.5)
Donor type	494	
Living donation		65 (13.2)
Deceased		429 (86.8)
Graft type	494	
Whole		165 (33.4)
Split		165 (33.4)
Reduced		164 (33.2)
Donor CMV serostatus, % positive	442	207 (46.8)
Recipient CMV serostatus, % positive	477	183 (38.4)
CIT, min	475	524 ± 223
Year of transplant	494	1999 (1993–2004)
Induction monoclonal antibody	494	
None		359 (72.7)
Basiliximab		78 (15.8)
Daclizumab		39 (7.9)
Antithymoglobulin		18 (3.6)
Induction IV steroids	448	147 (32.8)
Outcomes before 10-y biopsy		
Histologically proven rejection	483	228 (47.2)
Biliary complication	461	66 (14.3)
Vascular complication	478	64 (13.4)

Data are reported as n (%), mean ± SD, or as median (interquartile range), as applicable.
CIT, cold ischemia time; CMV, cytomegalovirus; IV, intravenous.

(25%) had a 15-y biopsy. The 369 patients who did not have a 15-y biopsy included 4 patients who underwent a biopsy at 15 y, which was insufficient for examination and 83 who attended a follow-up clinic in the pediatric transplant center at 15 y but did not undergo a biopsy at this time. A further 4 patients died and 2 were retransplanted after <15 y, and so did not attend the 15-y follow-up clinic. In 110 patients, the index LT was <15 y from the date of data collection, meaning these were not yet due for a 15-y follow-up biopsy. Most remaining patients were followed up at other centers, including adult transplant centers, and we retrieved data on patient and graft survival over >15 y (N = 108 patients), meaning that only 58 were lost to follow-up before 15 y.

Fibrosis on Biopsy

Among the 494 asymptomatic patients, 75% (N = 370) showed features of fibrosis on their 10-y protocol biopsy, with periportal/pericentral fibrosis in 51% (N = 253), bridging fibrosis in 18% (N = 87), or cirrhosis in 6% of cases (N = 30) (Table 3). Only 25% of patients (N = 124) were found to have no fibrosis in their 10-y biopsy.

On complete-cases analysis of patients with biopsies at 5, 10, and 15 y (N = 98; 20%), the degree of fibrosis was found to differ significantly between the 3 time points ($P < 0.001$, Figure 2A). Post hoc pairwise comparisons identified a significant progression of fibrosis between the 5-y biopsy and both the 10-y ($P = 0.004$) and 15-y ($P < 0.001$) biopsies. However, no significant change between the 10- and 15-y biopsies was detected ($P = 0.491$). As a sensitivity analysis, an unpaired analysis was also performed to include all available biopsies for the overall cohort of 494 patients, which returned consistent results (Figure 2B).

The Interaction Between Inflammation and Fibrosis

We also assessed inflammation in the protocol biopsies, which was described as mild or moderate graft inflammation with a predominantly portal distribution in most cases (Table 3). Further data to score graft hepatitis were not available. A significant correlation was observed between the degree of fibrosis at 5 and 10 y in the patients with biopsies performed at both time points (N = 263; rho = 0.380, $P < 0.001$, Figure 3A). The degree of fibrosis at 10 y was not significantly correlated with the degree of inflammation at 5 y (rho = 0.064, $P = 0.302$, Figure 3B), suggesting that inflammation at 5 y was not a significant predictor

TABLE 2.
Immunosuppression at 5-, 10-, and 15-y follow-up

	Discharge		5-y follow-up		10-y follow-up		15-y follow-up	
	N	n (%)	N	n (%)	N	n (%)	N	n (%)
Immunosuppression	494		346		492		150	
Tacrolimus monotherapy		186 (37.7)		188 (54.3)		219 (44.5)		58 (38.7)
Cyclosporine monotherapy		132 (26.7)		77 (22.3)		139 (28.3)		28 (18.7)
Tacrolimus combination		44 (8.9)		32 (9.2)		39 (7.9)		10 (6.7)
Cyclosporine combination		131 (26.5)		22 (6.4)		31 (6.3)		8 (5.3)
MMF or azathioprine only		0 (0.0)		19 (5.5)		48 (9.8)		39 (26.0)
Others		1 (0.2)		7 (2.0)		13 (2.6)		3 (2.0)
Off immunosuppression		0 (0.0)		1 (0.3)		3 (0.6)		4 (2.7)
Maintenance low-dose prednisolone	493	347 (70.4)	344	146 (42.4)	491	272 (55.4)	150	80 (53.3)

Combination, combination therapy with calcineurin inhibitor plus azathioprine or MMF; MMF, mycophenolate mofetil.

TABLE 3.

Biopsy findings and selected blood markers at 5-, 10-, and 15-y post-LT

	5-y follow-up		10-y follow-up		15-y follow-up	
	N	Statistic	N	Statistic	N	Statistic
Biopsy performed	494	263 (53.2)	494	494 (100.0)	494	125 (25.3)
Years from transplant to biopsy	263	5.0 (5.0–5.3)	494	10.1 (9.9–10.4)	125	15.0 (15.0–15.3)
Inflammation	263		494		125	
None		133 (50.6)		253 (51.2)		69 (55.2)
Mild		103 (39.2)		206 (41.7)		49 (39.2)
Moderate		25 (9.5)		35 (7.1)		7 (5.6)
Severe		2 (0.8)		0 (0.0)		0 (0.0)
Fibrosis	263		494		125	
None		121 (46.0)		124 (25.1)		25 (20.0)
Periportal/pericentral		114 (43.3)		253 (51.2)		61 (48.8)
Bridging		23 (8.7)		87 (17.6)		37 (29.6)
Cirrhosis		5 (1.9)		30 (6.1)		2 (1.6)
Blood markers						
Auto AB, % positive	227	54 (23.8)	390	104 (26.7)	100	18 (18.0)
ANA, % positive	227	41 (18.1)	390	75 (19.2)	101	17 (16.8)
SMA, % positive	226	16 (7.1)	386	49 (12.7)	99	1 (1.0)
LKM, % positive	226	2 (0.9)	385	2 (0.5)	99	2 (2.0)
Platelets, G/L	237	257 ± 86	385	227 ± 71	192	215 ± 65
Albumin, g/L	224	41.5 ± 4.4	363	42.1 ± 4.1	175	43.1 ± 3.7
Bilirubin, μmol/L	244	9 (6–13)	392	10 (7–13)	195	11 (8–18)
GGT, IU/L	236	16 (12–21)	380	16 (12–21)	184	19 (14–30)
APRI	224	0.36 (0.29–0.55)	369	0.33 (0.25–0.43)	178	0.31 (0.24–0.46)
IgG, g/L	242	11.8 ± 3.7	431	12.1 ± 3.2	138	11.9 ± 3.4

Data are reported as n (%), mean ± SD, or as median (interquartile range), as applicable.
AB, antibody; ANA, antinuclear antibody; APRI, aspartate aminotransferase to platelet ratio; GGT, gamma-glutamyl transferase; LKM, anti–liver-kidney microsomal antibody; LT, liver transplantation; SMA, alpha-smooth muscle actin antibody.

of future fibrosis. Conversely, levels of fibrosis and inflammation at 10 y were significantly correlated (rho = 0.141, $P = 0.002$, Figure 3C).

Predictors of Fibrosis at 10 Years

None of the demographic factors considered differed significantly between those with and those without fibrosis on the 10-y biopsy (Table 4). Also, vascular or biliary complications were not associated with a higher prevalence of graft fibrosis (Table 4). However, significant associations between immunosuppression and fibrosis rates were observed (Figure 4A). Specifically, patients on primary immunosuppression with cyclosporine were significantly more likely to have fibrosis in the 10-y biopsy than patients on tacrolimus at discharge after transplantation (79% versus 70%, $P = 0.034$) and 10 y post-LT (81% versus 74%, $P = 0.020$). Furthermore, patients with histologically proven rejection before their 10-y biopsy were significantly more likely to have fibrosis (79.8% versus 71.8%, $P = 0.044$). However, rates of fibrosis were higher for those with maintenance low-dose prednisolone at discharge after transplantation according to the local protocols (77.8% versus 68.0%, $P = 0.024$).

The study did not include a protocol for the immunosuppressive regimen or therapy augmentation based on the biopsy results. But, analyzing the complete-cases cohort (N = 98), we observed a tendency for centers to adjust immunosuppression based on biopsy findings. For example, for the subgroup of patients that were not on low-dose prednisolone at the time of their 5-y biopsy, the likelihood of commencing prednisolone before the subsequent biopsy increased from 14% (7 of 51) in those with no inflammation on the 5-y biopsy, to 24% (14 of 59) and 47% (8 of 17) in those with mild and moderate-to-severe inflammation, respectively ($P = 0.011$). Despite the maintenance or introduction of low-dose steroids, the graft fibrosis progressed from 5 to 10 y.

Analysis of liver function tests and other blood tests (Table 5) revealed significantly higher gamma-glutamyl transferase and alanine aminotransferase levels at 5 y in those with fibrosis at 10 y; however, all were within the normal range. The prevalence of positive autoantibodies was not associated with graft fibrosis in the 10-y protocol biopsy (Table 5).

Of interest, rates of fibrosis at 10 y declined significantly with the year of LT, from 78.9% in those transplanted pre-1990 to 69.8% in those from 2005 onward ($P = 0.023$, Figure 4B). This was likely a reflection of changes in immunosuppression regimes over time, with all patients with LT before 1990 being on cyclosporine at discharge, whereas those from 2005 onward, almost exclusively received tacrolimus (98.3%). This was confirmed by a multivariable binary logistic regression analysis, which considered all factors in Table 4 for inclusion. After backward-stepwise variable selection, only the 10-y immunosuppression was found to be a significant independent predictor of graft fibrosis in the 10-y biopsy, with an odds ratio of 2.15 (95% CI, 1.21-3.80; $P = 0.009$) for cyclosporine versus tacrolimus.

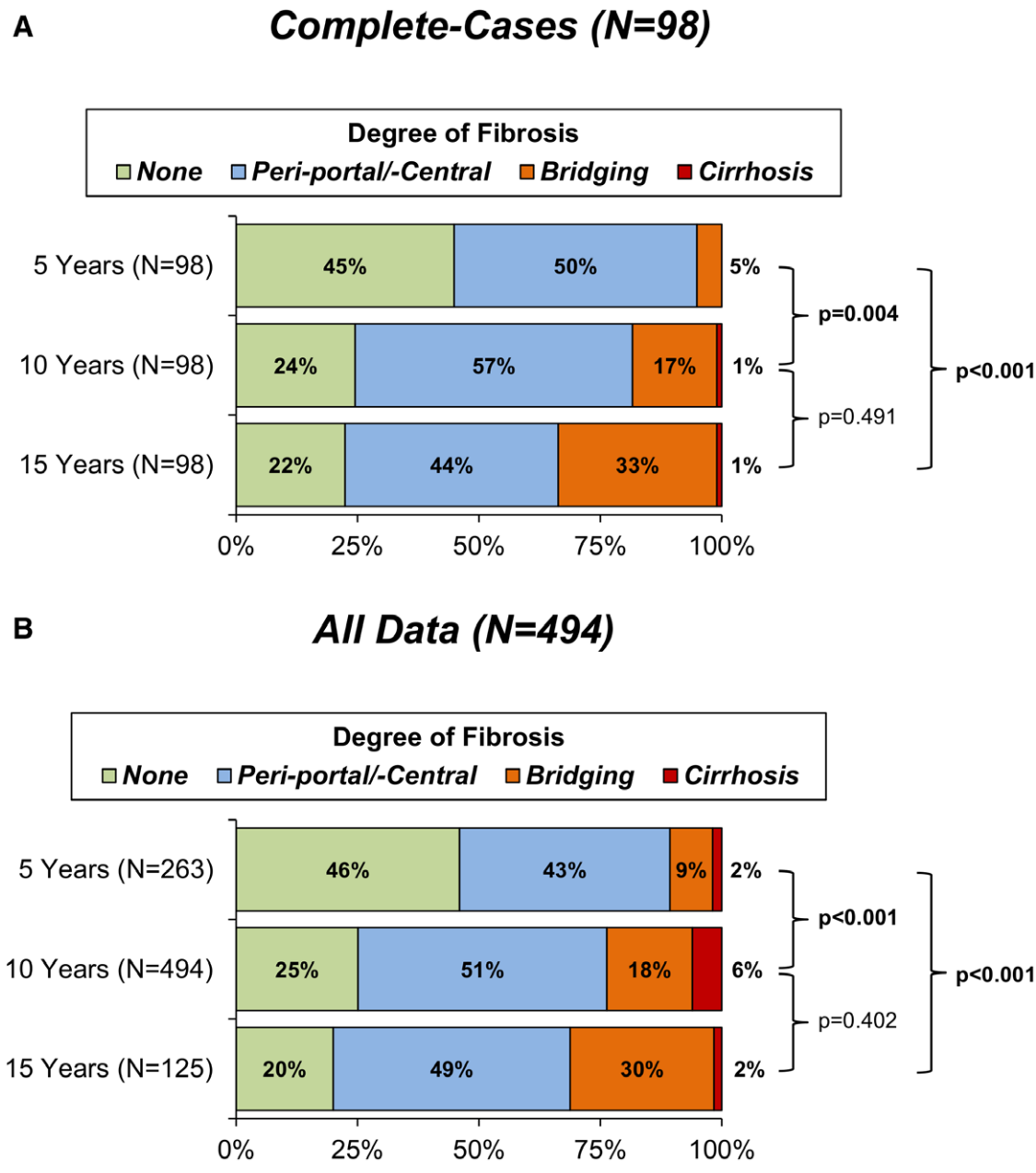

FIGURE 2. Evolution of liver allograft fibrosis over time. A, Complete-cases analysis, which only includes those patients with biopsies at all 3 follow-up times (N = 98) and found the degree of fibrosis to differ significantly between the 3 time points (Freidman test; $P < 0.001$). B, This represents a sensitivity analysis using all available biopsy data, which were analyzed using an unpaired approach (Kruskal-Wallis test; $P < 0.001$). *P* values on both plots are for post hoc pairwise comparisons, and bold *P* values are significant with a $P < 0.05$.

Long-term Graft Outcomes

Patients were followed up for a median of 77 mo (IQR, 22–126) after their 10-y biopsy, during which period 9 patients died and 10 were retransplanted (2 died after retransplantation). At 10 y after the 10-y biopsy, about 20 y post-LT, the Kaplan-Meier estimated patient survival rate was 97.5%, with a retransplant rate of 3.2%. The composite outcome of retransplant-free survival differed significantly with the degree of fibrosis on the 10-y biopsy ($P = 0.027$; Figure 5). Of patients with cirrhosis at the 10-y biopsy, 8% died or were retransplanted within the subsequent 5 y, compared with 2%, 1%, and 2% of those with no fibrosis, periportal/pericentral, or bridging fibrosis, respectively. After 20 y of follow-up, the difference between the groups appeared smaller, although formal analysis of this effect was not possible due to the low event rate.

DISCUSSION

This retrospective multicenter study aimed to evaluate the prevalence of graft fibrosis and its impact on long-term outcomes after LT in childhood. We observed relevant graft fibrosis in 10-y protocol liver biopsies in 75% of patients, despite all children having normal/near-normal liver biochemistry. Further, we documented an increase in the prevalence and grade of fibrosis from 5 to 10 y post-LT. After the 10-y biopsy, the severity of liver fibrosis did not progress significantly.

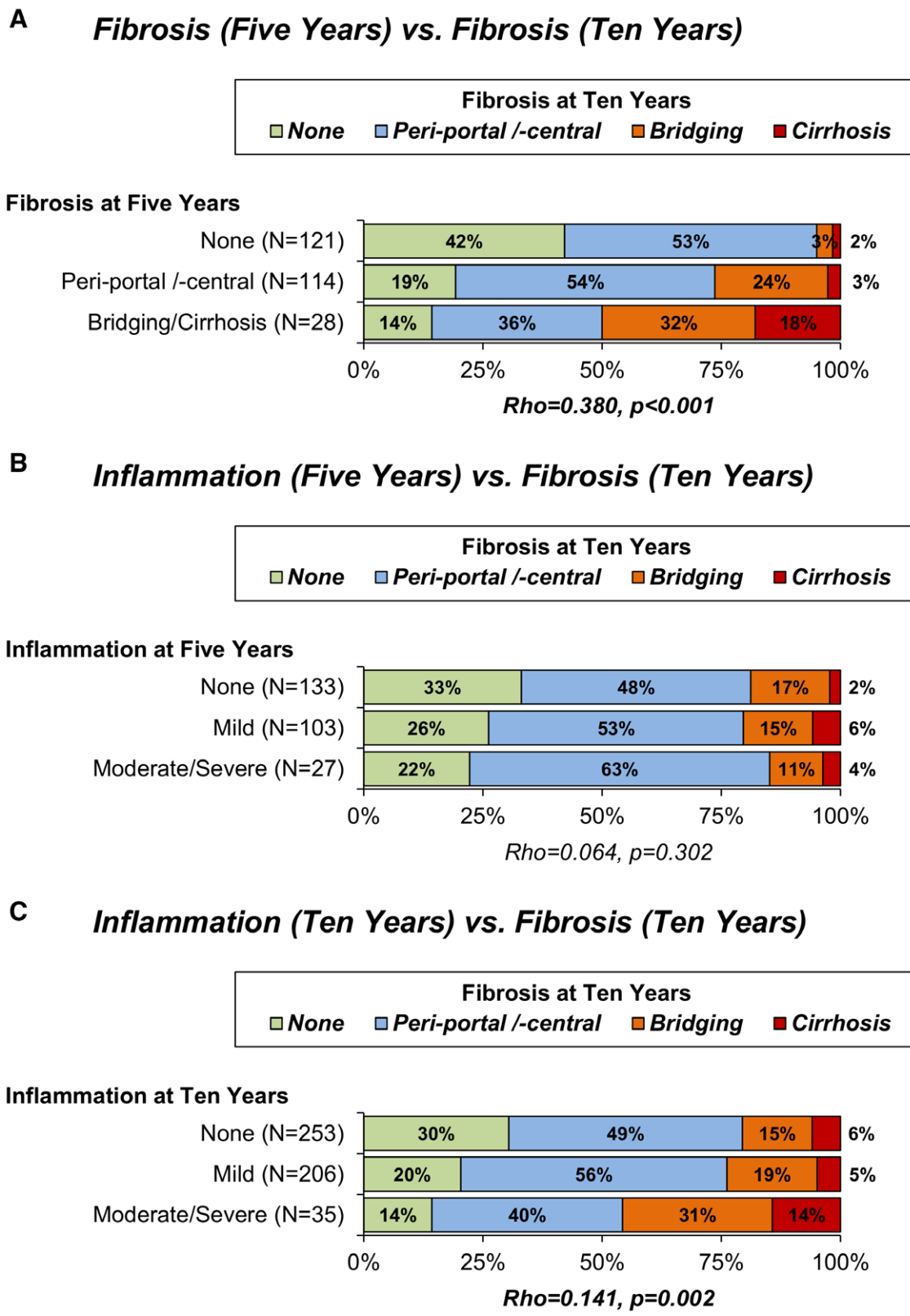

FIGURE 3. Interplay between inflammation and fibrosis. A, Correlation between fibrosis in 5-y biopsies and fibrosis in 10-y biopsies. B, Interplay between inflammation at 5 y and fibrosis at 10 y. C, Interplay between inflammation at 10 y and fibrosis at 10 y. Correlations were assessed using Spearman's (rho) correlation coefficients. Bold values are significant at $P < 0.05$.

Most children receive LT during their first years of life, ideally, these patients must benefit from a functioning allograft for >80 y. Factors associated with the transplant procedure, such as cold ischemia time and biliary or vascular complications, have been described to be related to graft fibrosis.[15,21,22] However, in our study, demographic

TABLE 4.

Risk factors for graft fibrosis in 10-y liver biopsy

		Evidence of fibrosis at 10 y		
	N	No (N = 124)	Yes (N = 370)	*P*
Age at transplant, mo	494	20.2 (10.4–53.9)	19.5 (9.7-43.7)	0.532
Sex, % male	494	71 (57.3)	184 (49.7)	0.177
Indication for transplant	494			0.796
Biliary atresia		80 (64.5)	229 (61.9)	
Cholestasis		10 (8.1)	41 (11.1)	
Metabolic		16 (12.9)	44 (11.9)	
Others		18 (14.5)	56 (15.1)	
Donor type, % deceased	494	104 (83.9)	325 (87.8)	0.283
Graft type	494			0.536
Whole		46 (37.1)	119 (32.2)	
Split		41 (33.1)	124 (33.5)	
Reduced		37 (29.8)	127 (34.3)	
Donor CMV serostatus, % positive	442	58 (50.4)	149 (45.6)	0.386
Recipient CMV serostatus, % positive	477	52 (43.7)	131 (36.6)	0.192
CIT, min	475	503 ± 229	531 ± 220	0.243
Year of transplant	494	2000 (1994-2005)	1997 (1992-2004)	**0.023**
Induction monoclonal antibody	494			0.130
None		81 (65.3)	278 (75.1)	
Basiliximab		27 (21.8)	51 (13.8)	
Daclizumab		10 (8.1)	29 (7.8)	
Antithymocyte globulin		6 (4.8)	12 (3.2)	
Histologically proven rejection, <10 y[a]	483	46 (39.0)	182 (49.9)	**0.044**
Biliary complication, <10 y[a]	461	16 (14.0)	50 (14.4)	1.000
Vascular complication, <10 y[a]	478	17 (14.8)	47 (12.9)	0.638
Immunosuppression, discharge	494			**0.034**
Tacrolimus		70 (56.5)	160 (43.2)	
Cyclosporine		54 (43.5)	209 (56.5)	
Others/none		0 (0.0)	1 (0.3)	
Immunosuppression, 5 y	346			0.614
Tacrolimus		65 (63.7)	155 (63.5)	
Cyclosporine		27 (26.5)	72 (29.5)	
Others/none		10 (9.8)	17 (7.0)	
Immunosuppression, 10 y	492			**0.020**
Tacrolimus		68 (55.3)	190 (51.5)	
Cyclosporine		32 (26.0)	138 (37.4)	
Others/none		23 (18.7)	41 (11.1)	
Low-dose prednisolone, discharge	494	77 (62.1)	270 (73.0)	**0.024**
Low-dose prednisolone, 5 y	344	38 (37.6)	108 (44.4)	0.281
Low-dose prednisolone, 10 y	491	60 (48.8)	212 (57.6)	0.094

Nominal variables are reported as n (column %), with *P* values from Fisher exact tests or χ^2 tests, for factors with 2 or >2 categories, respectively. Continuous variables are reported as median (interquartile range), with *P* values from Mann-Whitney U tests, or as mean ± SD, with *P* values from independent samples *t* tests. Bold *P* values are significant at $P < 0.05$.

[a]Before the 10-y biopsy.

CIT, cold ischemia time; CMV, cytomegalovirus.

and transplant-related factors were not associated with the evolution of graft fibrosis in the 10-y protocol biopsy. Instead, we found that the prevalence of fibrosis correlated with the degree of inflammation in the 10-y biopsy (Figure 3C) and the incidence of biopsy-proven rejection before the 10-y protocol biopsy.

The etiology of silent graft hepatitis and its impact on graft fibrosis progression are incompletely understood. However, previous findings, including positive autoantibodies, donor-specific HLA antibodies (DSAs), and the histologic pattern of inflammation, indicate that a distinct form of subclinical alloimmune reaction contributes to evolving graft hepatitis.[23-25] In the pediatric population, recurrence of underlying liver disease is a rare cause of late graft dysfunction. In this study, we excluded patients with autoimmune liver disease or viral hepatitis so that alloimmune determinants may play a significant role in chronic graft injury. Novel immunosuppressive regimens, that is, tacrolimus instead of cyclosporine on discharge and 10 y after transplantation, were associated with less liver fibrosis in a 10-y protocol biopsy. However, these changes could be associated with the evolution of immunosuppressive regimen and be an era effect (Table 4 and Figure 4A). Further, steroids per protocol on discharge

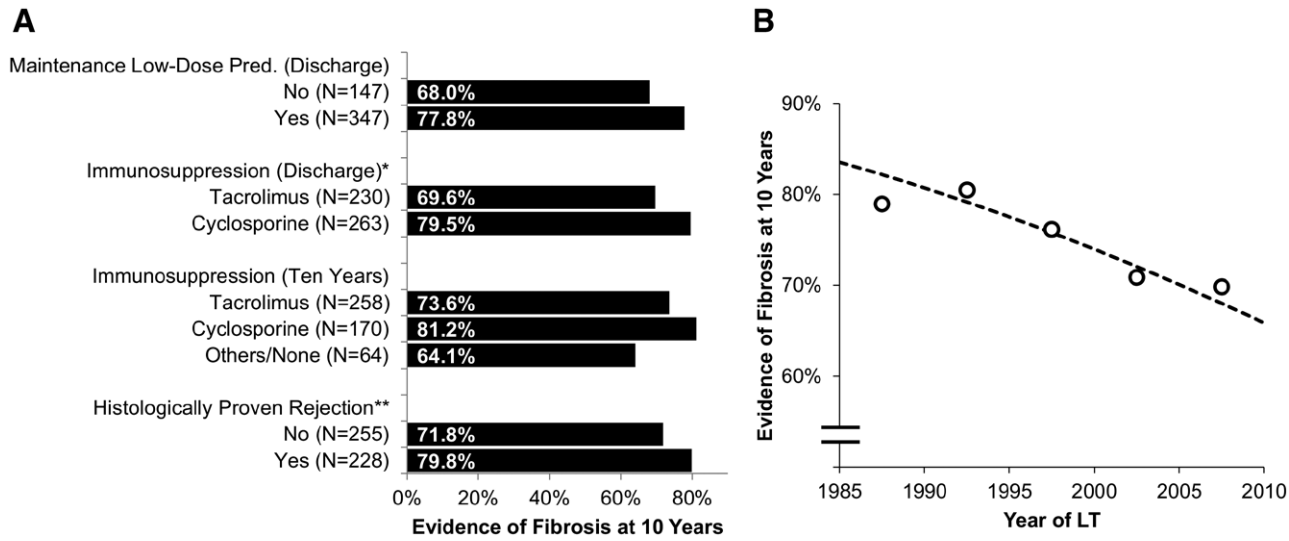

FIGURE 4. Predictors of fibrosis at 10 y. Only those factors found to be significant in Table 4 are included in the plots in A. B, Points represent the observed rates within 5-y intervals, and the broken line is from a binary logistic regression model, with the year of LT as a continuous covariate. *Excludes the others/none group, as there was only a single case. **Before the 10-y biopsy. LT, liver transplantation.

TABLE 5.

Predictors of graft fibrosis in 10-y liver biopsy

		Evidence of fibrosis at 10 y		
	N	No (N = 124)	Yes (N = 370)	*P*
AB positive, 5 y	227	14 (20.3)	40 (25.3)	0.499
AB positive, 10 y	390	23 (23.0)	81 (27.9)	0.361
ANA positive, 5 y	227	11 (15.9)	30 (19.0)	0.708
ANA positive, 10 y	390	17 (17.0)	58 (20.0)	0.559
SMA positive, 5 y	226	4 (5.8)	12 (7.6)	0.781
SMA positive, 10 y	386	14 (14.0)	35 (12.2)	0.727
LKM positive, 5 y	226	0 (0.0)	2 (1.3)	1.000
LKM positive, 10 y	385	1 (1.0)	1 (0.3)	0.445
Platelets, 5 y, G/L	237	265 ± 93	254 ± 84	0.363
Platelets, 10 y, G/L	385	233 ± 83	224 ± 66	0.294
Albumin, 5 y, g/L	224	41.3 ± 5.6	41.6 ± 4.0	0.678
Albumin, 10 y, g/L	363	42.7 ± 4.1	41.9 ± 4.0	0.113
Bilirubin, 5 y, µmol/L	244	9 (6–13)	9 (7–13)	0.789
Bilirubin, 10 y, µmol/L	392	9 (7–13)	10 (7–13)	0.646
GGT, 5 y, IU/L	236	14 (10–20)	16 (13–22)	**0.033**
GGT, 10 y, IU/L	380	15 (11–20)	16 (12–21)	0.387
ALT, 5 y, IU/L	251	21 (16–29)	24 (18–35)	**0.021**
ALT, 10 y, IU/L	393	20 (17–27)	20 (16–27)	0.908
AST, 5 y, IU/L	238	35 (31–46)	37 (32–48)	0.300
AST, 10 y, IU/L	373	30 (24–34)	30 (25–35)	0.285
APRI, 5 y	224	0.36 (0.24–0.56)	0.36 (0.29–0.55)	0.407
APRI, 10 y	369	0.32 (0.24–0.40)	0.33 (0.26–0.44)	0.148
IgG, 5 y, g/L	242	11.8 ± 3.5	11.8 ± 3.8	0.925
IgG, 10 y, g/L	431	12.1 ± 2.9	12.1 ± 3.3	0.857

Nominal variables are reported as n (column %), with *P* values from Fisher exact tests. Continuous variables are reported as median (interquartile range), with *P* values from Mann-Whitney U tests, or as mean ± SD, with *P* values from independent samples *t* tests. Bold *P* values are significant at $P < 0.05$.

AB, antibody; ALT, alanine transaminase; ANA, antinuclear antibody; APRI, aspartate aminotransferase to platelet ratio; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; IgG, immunoglobulin G; LKM, anti–liver-kidney microsomal antibody; SMA, alpha-smooth muscle actin antibody.

after transplantation were associated with a higher prevalence of graft fibrosis 10 y post-LT. Therefore, the impact of intensified immunosuppression on fibrosis progression remains unclear.

Overall, we observed a significant decrease in the prevalence of graft fibrosis with the year of LT, suggesting that exogenous factors influence its occurrences, such as adaptations in transplant techniques, management of

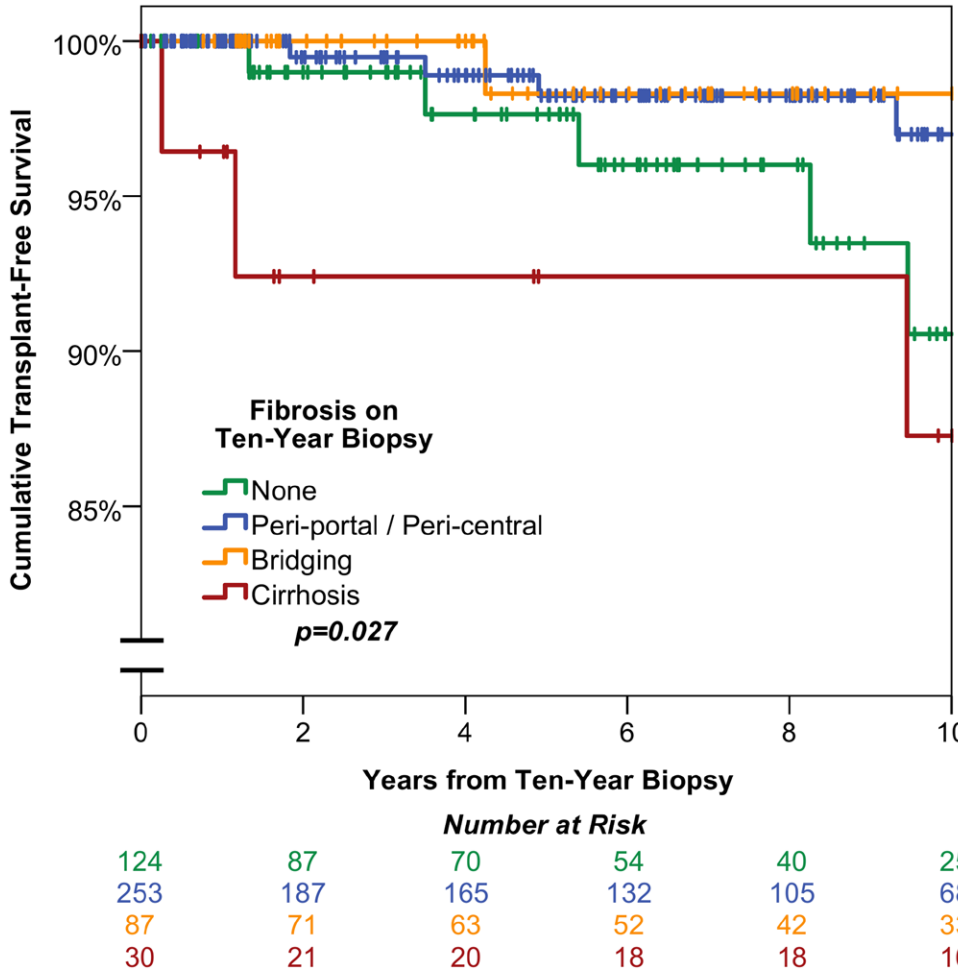

FIGURE 5. Transplant-free survival by the degree of fibrosis in the 10-y biopsy. The *P* value is from a log-rank test. The x-axis is truncated at 10 y, because of the declining numbers of patients at risk.

complications, and adaptations of the immunosuppressive regimen. Thus, the introduction of tacrolimus had the largest impact on improving graft fibrosis rates, as this was the only significant independent predictor in the multivariable analysis of graft fibrosis in 10-y biopsies.

Although previous publications documented an association between graft fibrosis and inflammation and positive autoantibodies,[10] this was not confirmed in this multicenter study. Other centers found an association between DSA class II and chronic graft hepatitis and fibrosis.[26-28] Chronic graft injury correlates with a higher prevalence of DSA.[20,23] Unfortunately, additional immunological markers, for example DSA, were not available in our cohort because of the long-term follow-up with patients transplanted >20 y ago. Recent studies in adult[29] and pediatric[23] LT demonstrated that graft hepatitis with portal inflammation and interface activity and varying degrees of fibrosis was associated with a specific transcriptional pattern associated with T-cell-mediated rejection. Based on those findings, a treatment modification through intensifying immunosuppression appears to be justified upon the detection of graft hepatitis and progressive fibrosis. Some groups suggest that graft inflammation and, less convincingly, graft fibrosis could improve with an intensification of immunosuppression.[12,13] Based on our present data with long-term follow-up, however, we feel that there is not enough evidence to support the concept that adaptations or increases of the immunosuppressive medication decrease the occurrence and severity of graft fibrosis in general. For example, in our study, the application of steroids did not affect the progression of graft fibrosis from 5 to 10 and 15 y post-LT. Prospective interventional studies are required to observe whether changes or intensification of immunosuppressive medication can prevent or treat chronic graft injury.

Patients with portal, perivenular, or even bridging fibrosis in 10-y protocol biopsies stayed stable without increased risk of graft loss for the following 10 y. Only the 30 patients with cirrhosis at 10 y (prevalence 6%) had a significantly decreased graft survival (Figure 5). A recent study in highly selected patients with normal surveillance biopsies at entry demonstrated moderate fibrosis in 56% of patients at a median of 8.2 y, associated with a younger age at transplant. The majority had stable fibrosis after the 6- to 12-y biopsy, but no mortality data were included.[30] The data in our large international cohort with a long-term follow-up show that early abnormalities of liver histology including subclinical graft cirrhosis can impact the long-term outcome. These patients, asymptomatic at 10-y biopsy, underline the value of protocol biopsies to detect patients at risk of unfavorable outcomes. Furthermore, the impact of

the documented high prevalence of graft fibrosis in general on the long-term outcome of patients as adults after transplantation in childhood is entirely unknown. It has to be emphasized that in this cohort of asymptomatic patients, 24% and 31% of the 10-y and 15-y biopsies, respectively, contain moderate-to-severe fibrosis. As the fibrotic process has been shown to be progressive, these patients enter adulthood with a suboptimal graft, which possibly harbors an ongoing injurious process. Although data on adherence to immunosuppressive medication were not documented in our retrospective study, transition from pediatric to adult clinical care is known to be a critical time that can be associated with medical nonadherence[31] and an increased risk of graft failure. Therefore, individualized care and consideration including special transition programs should be given to this vulnerable group of patients.

Although our study presents the largest multicenter cohort of children with longitudinal and cross-sectional graft histology data after LT with long-term follow-up, there are some limitations. Primarily, it is a retrospective analysis focusing on 10-y-protocol biopsies assessed by several different pathologists working at the 12 participating centers. A retrospective, multicenter study of this nature in which biopsies were assessed by several different observers over a long time period has potential problems with observer variability and standardization of the assessments made. The GIG has recently published an article proposing a standardized approach for the histological assessment of late posttransplantation biopsies from pediatric liver allograft recipients.[32] It is intended that the schema described in this study will be used to provide a comprehensive standardized assessment of changes in late biopsies obtained from children enrolled prospectively into centers participating in GIG studies. However, for the purposes of the current retrospective study, we concluded that such an approach would not be logistically feasible and decided instead to adopt a pragmatic, simplified approach using previously described and validated methods for assessing fibrosis and inflammation in late posttransplant biopsies.[10] We believe that the data obtained concerning late graft fibrosis in this large multicenter study are thus valid, and provide novel insights into the pathogenesis and natural history of late graft fibrosis in pediatric liver allograft recipients. Furthermore, patients retransplanted, having died, or lost to follow-up before the 10-y protocol biopsy were not included, which may have given rise to a selection bias.

In conclusion, graft fibrosis is highly prevalent in long-term pediatric LT survivors. A relevant proportion of patients developed progressive fibrosis despite immunosuppression, including steroids. Further, the vast majority of patients with graft fibrosis stayed stable with surprisingly high graft survival, at least during the second decade after pediatric LT. However, protocol biopsies are the only tool to detect subclinical graft hepatitis and fibrosis and to identify patients at risk of progressive disease. However, decisions on the augmentation of immunosuppressive treatment must be made case by case. Currently, there is not enough evidence to recommend on intensification of immunosuppressive treatment to counteract graft fibrosis development in general. Future prospective studies are, therefore, needed to evaluate the impact of treatment interventions on graft fibrosis as well as studies on the long-term outcome of patients transplanted in childhood entering their adulthood with a fibrotic graft. In the meantime, this study has highlighted the need for careful transitioning and follow-up in adult services to detect the potential development of cirrhosis and the necessity for retransplantation early.

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