

High Frequency of Concomitant Food Allergy Development and Autoantibody Formation in Children Who Have Undergone Liver Transplantation

Timo Käppi, MD,¹ Hardis Rabe, PhD,² Christine Lingblom, PhD,² Bill Hesselmar, MD, PhD,³ Carola Kullberg-Lindh, MD, PhD,¹ Agnes E. Wold, MD, PhD,² Christine Wennerås, MD, PhD,² and Robert Saalman, MD, PhD¹

Background. Allergy and other immune-mediated diseases are more frequently reported in children who have undergone liver transplantation. Furthermore, autoantibodies are also prevalent, suggesting a state of immune dysregulation in these patients. Whether or not these processes occur simultaneously in the same individual has not been studied previously. **Methods.** A cohort of 43 children who had undergone liver transplantation for nonautoimmune liver disease at median age of 1.3 years was investigated for allergy and autoimmune disease. Sensitization to food and inhalant allergens was assessed, and autoantibodies were measured. **Results.** The prevalence of food allergy was 26% and that of respiratory allergy was 23%, whereas 33% and 26% of the subjects were sensitized to food and inhalant allergens, respectively. Autoimmune disease (ie, autoimmune hepatitis) occurred in a single individual (2%), whereas autoantibodies were present in 44% of the children. Food allergy and autoantibodies occurred concomitantly in 19% of the children, which was almost twice the frequency expected by chance (11%, $P = 0.04$). Respiratory allergy and the presence of autoantibodies were unrelated (12% concurrence versus the expected 10%, $P = 0.73$). In the logistic regression analysis, autoantibody formation was associated with discontinued immunosuppression and food allergy, with odds ratios of 13 ($P = 0.01$) and 7.1 ($P = 0.03$), respectively. **Conclusions.** In contrast to respiratory allergy, food allergy and autoantibody formation occurred together in the same children who underwent liver transplantation at a frequency higher than would be expected by chance. This may reflect an underlying immune dysregulation that impairs immune tolerance to both food allergens and autoantigens.

(*Transplantation* 2019;103: 2338–2346)

INTRODUCTION

Liver transplantation is an established therapy with excellent survival rates for children with acute fulminant liver failure and end-stage chronic liver disease.¹ However, 1 of the long-term complications of liver transplantation that is increasingly observed in this patient group is morbidity that is related to dysregulation of the immune system. These manifestations typically develop within a few years of the

transplantation² and include food allergy, which affects up to 37% of children who have received a liver transplant,^{3–11} as well as other immune-mediated disorders, such as de novo autoimmune hepatitis with a prevalence of 2%–6%^{12–17} and de novo inflammatory bowel disease.^{18–20} Moreover, up to 75% of children who receive a liver transplant develop autoantibodies, such as anti-nuclear antibody (ANA) and smooth muscle antibody (SMA),^{12,21–24} although it appears

Received 7 December 2018. Revision received 14 March 2019.

Accepted 25 March 2019.

¹ Department of Pediatric Gastroenterology, Hepatology and Nutrition, Institute of Clinical Sciences, The Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden.

² Department of Infectious Diseases, Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden.

³ Department of Pediatric Allergy and Pulmonology, Institute of Clinical Sciences, The Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

T.K. and R.S. participated in the performance of the research, research design, data analysis and interpretation, and writing of the article. C.W. and A.W. participated in research design, data interpretation, and writing of the article.

H.R. and C.L. participated in data analysis and interpretation. C.K.-L. and B.H. participated in revising the manuscript critically for important intellectual content. All authors approved the final version of the article.

The authors declare no conflicts of interest.

The study was financed by grants from the Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (ALF-70420), the Professor Lars-Erik Gelin Memorial Foundation for Transplant Research, and the Queen Silvia Children's Hospital Research Fund, Gothenburg, Sweden (The Märtha and Gustaf Ågren Foundation).

Correspondence: Timo Käppi, MD, Department of Pediatric Gastroenterology, Hepatology and Nutrition, Queen Silvia Children's Hospital, 416 85 Göteborg, Sweden. (timo.kappi@vgregion.se).

Copyright © 2019 Wolters Kluwer Health, Inc. All rights reserved.

ISSN: 0041-1337/19/10311-2338

DOI: 10.1097/TP.0000000000002751

the presence of such antibodies is seldom associated with autoimmune disease in this patient group.²¹⁻²³

Although food allergies are frequently observed in children after liver transplantation, data regarding respiratory allergies are scarce.^{2,4,5} Furthermore, the prevalence of sensitization to food allergens has not been studied extensively,^{3,25} and to our knowledge, no study has addressed sensitization to inhalant allergens in pediatric liver transplant recipients.

Previous studies of immune-mediated diseases occurring in children who have undergone liver transplantation have focused on either allergic diseases or autoimmunity and autoantibody formation. Consequently, it is not known if these clinical entities are likely or unlikely to affect the same individuals. We hypothesized that these disease manifestations would appear concurrently in the same individuals as a result of a dysregulated immune system in children following liver transplantation.^{26,27}

The aims of this cross-sectional cohort study of children who had undergone liver transplantation were to investigate: (1) the prevalences of clinical allergy and sensitization to food and inhalant allergens; (2) the prevalences of autoimmune/immune-related disorders and autoantibody formation; and (3) the frequencies with which clinical allergy/sensitization and autoimmune disorders/autoantibody formation occurred simultaneously in the same patient.

PATIENTS AND METHODS

Study Design

The study was designed as a cross-sectional study involving a total of 43 pediatric liver transplant recipients (28 boys, 15 girls) with age range of 1.8–18 years (median: 8.9 y) who were enrolled at the transplant unit of Queen Silvia Children's Hospital, Sahlgrenska University Hospital, Gothenburg, Sweden, in the period of August 2014 to May 2017. Recruitment of subjects took place at the yearly follow-up visit, at which time point all the blood samples were drawn. The exclusion criteria were age >17 years at transplantation, follow-up shorter than 1 year after transplantation, or any preexisting autoimmune disease before transplantation. One patient who had autoimmune sclerosing cholangitis was excluded based on the latter criterion, whereas another patient declined to participate in the study. Written informed consent was obtained from all study participants and their parents. The study was approved by the Regional Ethical Review Board of Gothenburg, Gothenburg, Sweden (Dnr 366-14).

Patients' Characteristics

The clinical characteristics of the patients are listed in Table 1. The most common indication for liver transplantation was biliary atresia. Two of the children in the cohort had been retransplanted due to graft-related, long-term complications, both of them 5 years after the primary transplantation. All of the patients had received tacrolimus as primary maintenance immunosuppression, with the exception of 1 patient who had undergone allogeneic hematopoietic stem cell transplantation with the same donor before the liver transplantation and who was judged to have developed immunologic tolerance to the donor. At the time of this analysis, however, tacrolimus

immunosuppression had been discontinued in an additional 8 of the study patients due to high levels of Epstein-Barr virus in the blood. In total, 9 patients (21%) had been off immunosuppressive therapy for a median time of 5.4 years (range, 1.4–12 y). None of the patients had developed the posttransplantation lymphoproliferative disease.

Immunosuppression and Liver Biopsy Protocols

The standard immunosuppressive protocol consisted of methylprednisolone and either anti-thymocyte globulin (transplantations performed before 2012) or basiliximab (transplantations performed in 2012 and later) given as induction therapy, followed by tacrolimus monotherapy as maintenance immunosuppression. The tacrolimus trough levels were targeted at 10–15 ng/mL for the first 2 weeks posttransplantation and were subsequently tapered to 3–5 ng/mL during the first 3 months posttransplantation if no acute rejection had occurred. Long-term immunosuppression was designed to ensure tacrolimus trough levels at around 3 ng/mL.

Routine liver biopsies were performed 1, 5, and 10 years after transplantation. Additional biopsies were performed when liver disease was clinically suspected, eg, because of persistently elevated levels in liver function tests and increased total serum IgG levels.

Clinical and Laboratory Parameters

To obtain a clinical diagnosis of allergy, the patient's medical records were reviewed, and the parents (and when appropriate for age, the patient) were interviewed using a structured questionnaire. This included information as to the time of onset of the allergic disease, symptoms and their duration, as well as responses to treatment.

Food allergy was defined as an immediate- (start within 2 hours) or late-onset reaction after ingestion of the suspected food. The reaction should improve and not recur

TABLE 1.

Clinical characteristics of the patients in the cohort

	N = 43
Sex	
Male	65% (28)
Female	35% (15)
Current age in y, median (range)	8.9 (1.8–18)
Age at the first transplantation in y, median (range)	1.3 (0.1–15)
No. of y since the first transplantation, median (range)	5 (1–17)
Indications for liver transplantation	
Extrahepatic biliary atresia	28% (12)
Hepatoblastoma	23% (10)
Metabolic liver disease	14% (6)
Congenital hepatic fibrosis	9% (4)
Acute liver failure	5% (2)
Cryptogenic cirrhosis	5% (2)
Other	16% (7)
Current immunosuppressive therapy	
Tacrolimus monotherapy	72% (31)
Tacrolimus + mycophenolate mofetil	2% (1)
Tacrolimus + everolimus + prednisolone	2% (1)
Cyclosporine + prednisolone	2% (1)
Discontinued immunosuppression	21% (9)

once the food was eliminated from the diet. The diagnosis was supported by a positive open food challenge (except in cases of severe allergic reaction) or demonstration of allergen-specific serum IgE to a panel of food allergens (fx5; Phadia AB/Thermo Fisher Scientific, Uppsala, Sweden). However, the latter criteria were not considered mandatory.

Respiratory allergy included allergic rhinoconjunctivitis and allergic asthma triggered by inhalant allergens. Allergic rhinoconjunctivitis was defined as symptoms involving the eyes or nose during the past year that occurred seasonally (pollen) or after exposure to animal dander. The diagnosis was supported by the presence of allergen-specific serum IgE to the specific inhalant allergen(s) (Phadiatop; Phadia AB/Thermo Fisher Scientific), although a positive test was not mandatory. Allergic asthma was defined as a doctor's diagnosis of asthma triggered by inhalant allergen and the need for any asthma medication during the past year. Sensitization to the triggering inhalant allergen was also required for the diagnosis. The asthma diagnosis could either be documented in hospital records or reported by a parent.

Sensitization was defined as a positive response to food allergens (cow's milk, egg, cod, soy, peanut, and wheat) analyzed by fx5 (Phadia AB) or to inhalant allergens (cat, dog, horse, birch, timothy, mugwort, *Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae*), as measured by Phadiatop (Phadia AB) with a cutoff level of ≥ 0.35 kU/L for a single antigen.

Autoantibodies analyzed in the present study included ANA, antimitochondrial antibody (AMA), SMA, antineutrophil cytoplasmic antibody (ANCA), and antibodies directed against soluble liver antigen (SLA). The cutoff levels employed were as follows: $\geq 1:20$ for ANCA; $\geq 1:80$ for SMA and AMA; $\geq 1:200$ for ANA; and ≥ 20 RU/mL for SLA. The levels of ANA, AMA, SMA, and ANCA were analyzed by indirect immunofluorescence-accredited tests (Department of Clinical Immunology, Sahlgrenska University Hospital, Gothenburg, Sweden), while antibodies to SLA were analyzed using a commercial immunoblot test with enzyme-conjugated antibodies to human IgG (Euroline Profile Autoimmune Liver Diseases DL 1300-1601-3G/4G; Euroimmun, Luebeck, Germany).

Statistical Analysis

Because of the limited sample sizes and nonnormal distribution of the variables, nonparametric tests were used, and the data are presented as medians with ranges. For comparison of continuous variables, the nonpaired Mann-Whitney *U*-test was used. Fisher's exact test was used for comparison of dichotomous parameters. Two-tailed $P < 0.05$ were considered statistically significant. Logistic regression was applied, including covariates that showed $P \leq 0.1$ in the univariate analysis. Adjusted odds ratios (aOR) were calculated with 95% confidence intervals (CIs). For all statistical analyses, the IBM SPSS Statistics version 22 software (IBM Corp., New York, NY) was used.

RESULTS

Prevalences of Allergy and Sensitization

Almost every second child suffered from allergy to either food and/or inhalant allergens ($N = 18$, 42%). Food

allergy was present in a quarter ($N = 11$, 26%) of the children, 8 of whom had developed allergy after liver transplantation, at a median time of 2 years (range, 1–12 y) after the procedure. Detailed clinical data for the patients with food allergy are given in Table 2. An additional 4 children had experienced transient food allergy with onset after transplantation but developed tolerance by the time of this study. The most common food-allergic symptom was angioedema, and cow's milk was the most frequent triggering food allergen (Table 2).

Respiratory allergy occurred in about every fourth child in the cohort ($N = 10$, 23%), with all but 1 case having presented after liver transplantation. Three patients (7%) had both food and respiratory allergies. Notably, both food allergy and respiratory allergy affected mainly children 6 years of age and older (Figure 1A).

Almost half of the children were sensitized to 1 or more food and/or inhalant allergens ($N = 19$, 44%). Every third child ($N = 14$, 33%) was sensitized to food allergens, and a quarter of the subjects ($N = 11$, 26%) were sensitized to inhalants. Overall, 14% ($N = 6$) of the children were sensitized to both food and inhalant allergens. Detailed data for the sensitized patients are given in Table S1 (SDC, <http://links.lww.com/TP/B733>). The specific food allergens and the percentages of children sensitized to them were cow's milk, 25%; egg, 16%; wheat, 9%; peanut, 9%; soy, 7%; and fish, 2%. Corresponding percentages for inhalant allergens were timothy, 19%; birch, 12%; house dust mite, 12%; cat, 7%; dog, 5%; mugwort, 5%; and horse, 0%.

Whereas sensitization to food was maximal in children in the 6.0–11.9 years age group, sensitization to inhalants increased with age (Figure 1B). Overall, 4/14 (29%) patients who were sensitized to a specific food antigen reacted clinically to that food, whereas 5/11 (45%) children sensitized to any of the tested inhalant allergens exhibited a respiratory allergy.

Current allergy tended to be less common in children who were transplanted due to biliary atresia compared with the remainder of the cohort (25% versus 48%, $P = 0.19$), and fewer children in this patient group were sensitized to any allergen (17% versus 55%, $P = 0.02$). The groups were of similar age (median age, 9.3 y versus 8.9 y) and differed only with regard to age at transplantation—children with biliary atresia were transplanted at a median age of 0.8 years (range, 0.2–5 y), as compared with a median age of 2.8 years (range, 0.1–15 y) for the other indications ($P = 0.006$). In the entire cohort, however, neither age at transplantation nor discontinued immunosuppression were significantly associated with the development of either allergy or sensitization.

Other Immune-mediated Disorders Occurring After Transplantation

One child (2%) in the cohort developed de novo autoimmune hepatitis after transplantation. Another child who was transplanted for acute liver failure of unknown cause was diagnosed with celiac disease at the time of transplantation. No cases of de novo inflammatory bowel disease or immune cytopenia were diagnosed. Furthermore, 5 (12%) patients had been diagnosed with a recently described condition, termed giant papillae tongue disorder. This disorder affects the tongue and orofacial region in children

TABLE 2.
Clinopathological characteristics of children with food allergy after liver transplantation

Patient code	Sex	Current age (y)	Age at LTx (y)	Indication	Time from LTx (y)	Immunosuppression	Triggering food	s-IgE specific for triggering food (kU/L)	Type of food allergic reaction	Start in relation to LTx	Provocation performed	Other concurrent atopy
Current food allergy with onset after LTx												
A	M	6.1	1.1	AHF	5	Tacrolimus	Cow's milk	14	Immediate- (pruritus) and late-onset (eczema)	2 y post-LTx	Yes	Nonallergic asthma ^a
B	M	17.9	1.1	EHBA	17	Tacrolimus	Beans	nd	Immediate-onset (angioedema)	12 y post-LTx	Yes	No
C	M	11.3	0.3	Agenesis syndrome	11	Tacrolimus	Hazelnuts	nd	Immediate-onset (anaphylaxis)	2 y post-LTx	No	No
D	F	13.7	1.7	SOS	12	None ^b	Cow's milk	0.36	Late-onset (abdominal pain)	5 y post-LTx	Yes	No
							Cow's milk, egg, soy, wheat, peanuts	<0.35 (for all triggering foods)	Late-onset (abdominal pain, diarrhea)	1st y post-LTx	Yes	No
E	M	17.8	3.8	HBL	14	Tacrolimus	Soy	<0.35	Immediate-onset (angioedema)	10 y post-LTx	Yes	Nonallergic asthma ^a , eczema
F	F	11.2	6.2	Hyperoxaluria	5	Tacrolimus	Date plum	nd	Eosinophilic esophagitis	14 y post-LTx	Yes	No
G	F	7.8	3.8	Glycogenosis	4	Tacrolimus	Pistachio nuts	nd	Immediate-onset (urticaria)	1st y post Ltx	Yes	No
H	M	8.9	0.9	CHF	8	Tacrolimus	Fish	0.63	Immediate-onset (angioedema)	1st y post-LTx	Yes	Eczema
									Immediate-onset (bronchial obstruction, abdominal pain)	2 y post-LTx	Yes	Allergic asthma, ARC (pollen)
Current food allergy with onset before Ltx												
I	M	2.7	0.7	HBL	2	Tacrolimus	Cow's milk	<0.35	Late-onset (abdominal pain, diarrhea)	3 mo before LTx	Yes	Eczema
J	M	16.2	15.2	CHF	1	Tacrolimus	Peanuts	8.9	Immediate-onset (bronchial obstruction, urticaria)	Several y before LTx	Yes	ARC (pollen, cat)
K	M	1.8	0.8	HBL	1	Tacrolimus	Cow's milk	<0.35	Late-onset (abdominal pain, diarrhea)	3 mo before LTx	Yes	ARC (pollen, dog)
Transient ^c food allergy with onset after Ltx												
L	F	13.5	0.5	EHBA	13	Tacrolimus	Egg	<0.35	Immediate-onset (angioedema)	4 y post-Ltx	Yes	ARC (cat)
M	M	15.8	5.0	HBL	11	Tacrolimus	Hazelnuts	nd	Immediate-onset (angioedema)	2 y post-Ltx	No	ARC (pollen)
N	F	13.2	0.2	EHBA	13	Tacrolimus	Fish	<0.35	Immediate-onset (angioedema)	2 y post-Ltx	Yes	No
O	F	11.4	0.4	EHBA	11	Tacrolimus	Egg	<0.35	Immediate-onset (angioedema)	2 y post-Ltx	Yes	Eczema

^aAsthma without clinical identification of the triggering allergen.
^bBefore liver transplantation undergone with allogeneic stem cell transplantation from the same donor.
^cPatient had developed tolerance by the time of the analysis.
AHF, acute hepatic failure; ARC, allergic rhinoconjunctivitis; CHF, congenital hepatic fibrosis; EHBA, extrahepatic biliary atresia; F, female; HBL, hepatoblastoma; LTx, Liver transplantation; M, male; nd, no data; SOS, sinusoidal obstruction syndrome.

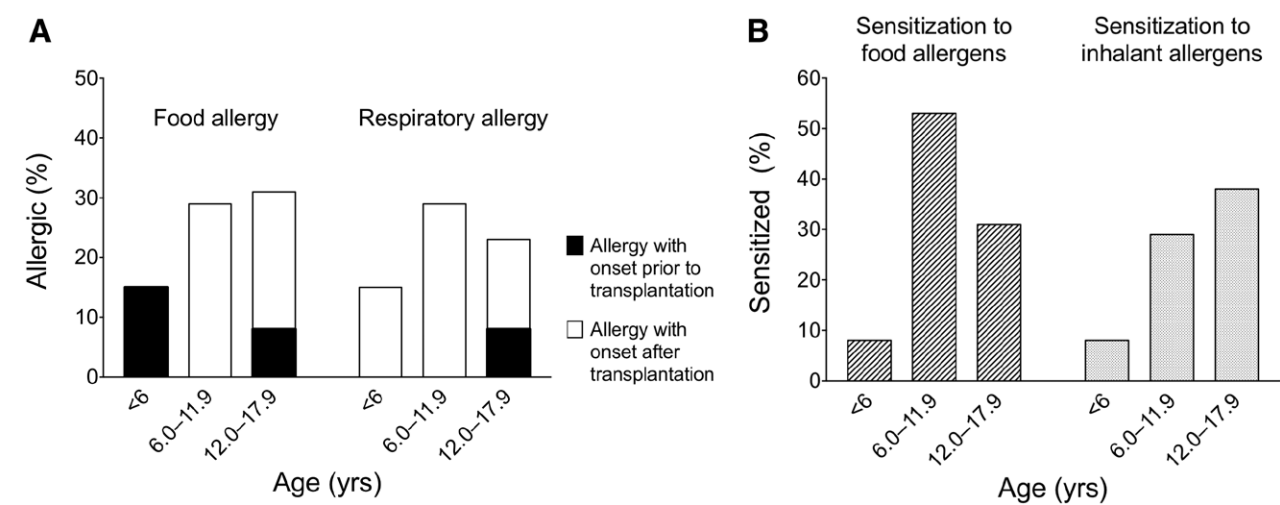


FIGURE 1. Frequency of current food and respiratory allergies (A) and sensitization to food and inhalant allergens (B) in the cohort of 43 children who underwent liver transplantation divided into the following age groups: younger than 6 y (N = 13), 6.0–11.9 y (N = 17), and 12.0–17.9 y (N = 13).

who have undergone solid-organ transplantation, and it is postulated to represent an immune-mediated disorder.^{28,29} However, all these children had recovered from the disorder by the start of the study.

Prevalence of Autoantibodies

Autoantibodies were detected in the sera of 19 children (44%). The most common autoantibody specificities were SMA (N = 7, 16%), ANA and ANCA (both, N = 6, 14%), and SLA (N = 5, 12%). No subject tested positive for AMA. The majority of the children (14/19, 74%) were positive for a single type of autoantibody, whereas 5 children had 2 different autoantibody specificities. In about half of the cases (9/19, 47%), it was possible to establish that the patients had been negative for autoantibodies up to 6 months before the transplantation.

The presence of autoantibodies was unrelated both to the age at transplantation and to the current age of the subjects. Antibodies were detected in the different age groups at the following rates: <6 years, 38%; 6.0–11.9 years, 38%; and 12.0–17.9 years, 46%. However, autoantibodies were significantly less common in children who had been transplanted for biliary atresia than in those transplanted for other indications (17% versus 55%, *P* = 0.04). In contrast, autoantibodies were detected significantly more often in those children for whom immunosuppression had been discontinued than in those on immunosuppressive therapy (78% versus 35%, *P* = 0.03).

Cooccurrence of Autoantibodies and Allergy or Sensitization

Children with an ongoing food allergy were more likely to have autoantibodies than children without a food allergy (73% versus 34%, *P* = 0.04) (Figure 2). Similarly, 64% of the children who were sensitized to food allergen(s) had autoantibodies in their sera, as compared to 35% of those who exhibited no sensitization to foods (*P* = 0.10). In contrast, neither respiratory allergy nor sensitization to inhalant allergens was associated with an increased occurrence of autoantibodies (50% versus 42%, *P* = 0.73 and 55% versus 41%, *P* = 0.50, respectively).

To clarify further the potential interactions between the development of autoantibodies and the presence of food allergy and food sensitization, a logistic regression analysis was performed using the presence of autoantibodies as a dependent (outcome) variable (Table 3). As independent (explanatory) variables, variables with *P* ≤ 0.1 in the univariate analyses were tested, ie, current food allergy, current food sensitization, discontinued immunosuppression, and transplantation due to extrahepatic biliary atresia. In the crude analysis, discontinued immunosuppressive therapy was the strongest predictor of carrying autoantibodies, followed by food allergy, whereas sensitization to food antigens did not reach significance (Table 3). Being transplanted due to biliary atresia was a negative predictor of autoantibody development (OR, 0.17; *P* = 0.04) (Table 3). In the adjusted model, discontinued immunosuppressive therapy remained a significant positive predictor of having

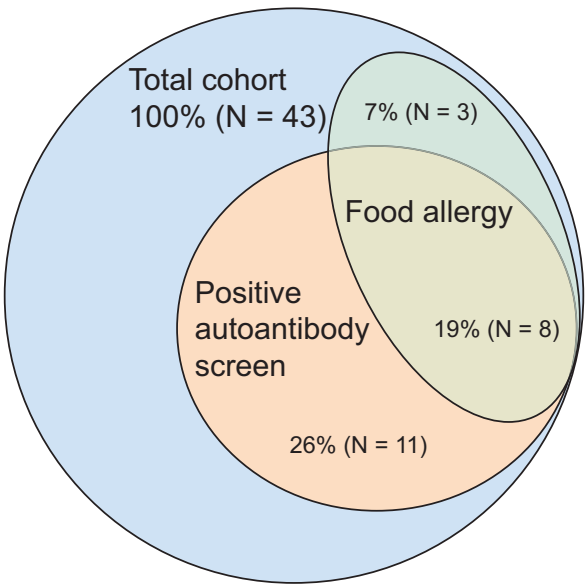


FIGURE 2. Relationship between current food allergy and positive autoantibody screen in the cohort of 43 children who received liver transplants. The areas are proportional to the numbers of cases.

TABLE 3.

Factors associated with autoantibody formation in children after liver transplantation, as determined by logistic regression analysis

Variable	Crude OR	95% CI	P
Discontinued immunosuppression	6.4	1.2-36	0.03
Current food allergy	5.1	1.1-23	0.04
Current food sensitization	3.4	0.90-13	0.10
Extrahepatic biliary atresia	0.17	0.03-0.88	0.04
	Adjusted OR ^a	95% CI	P
Model 1			
Discontinued immunosuppression	13	1.7-95	0.01
Current food allergy	7.1	1.3-39	0.03
Extrahepatic biliary atresia	0.19	0.03-1.3	0.10
Model 2			
Discontinued immunosuppression	11	1.4-84	0.02
Current food sensitization	4.3	0.91-20	0.07
Extrahepatic biliary atresia	0.17	0.03-1.1	0.07

^aAdjusted for shown variables included in each model.
CI, confidence interval; OR, odds ratio.

autoantibodies in the serum (aOR, 13; 95% CI, 1.7-95, $P = 0.01$), as did current food allergy (aOR, 7.1; 95% CI, 1.3-40; $P = 0.03$). In contrast, neither food sensitization nor extrahepatic biliary atresia was a statistically significant explanatory variable in the adjusted model.

DISCUSSION

In this cross-sectional study, we have investigated a cohort of 43 pediatric recipients of liver transplants for the prevalence of food and respiratory allergies, sensitization to food and inhalant allergens, as well as autoantibodies and autoimmune disorders at a median follow-up time of 5 years (range, 1–17 y) posttransplantation. In line with the results of previous studies,²⁻⁴ food allergy was found to be common in the present cohort. Similarly, sensitization to food allergens was detected in every third child. Furthermore, while almost half of the patients carried autoantibodies, clinical autoimmune disease occurred in only a single patient.

To the best of our knowledge, the current study is the first one to investigate the putative relationship between food allergy and autoantibodies at the level of the individual patient. We found that patients who had a current food allergy were more likely to test positive for autoantibodies. This suggests the presence of an underlying immune dysregulation that results in increased immune activation, both against environmental allergens and self-antigens. This immune dysregulation may reflect the effects on the immune system of the chronic liver disease present before transplantation, immaturity of the immune system, and/or immunosuppressive treatment.^{26,27} The liver receives the blood that drains from the gastrointestinal tract through the portal vein and is exposed to a continuous load of dietary antigens and microbial components. Furthermore, the liver is considered to play a critical role in the development of tolerance to food antigens and autoantigens.^{30,31} Tolerance to inhalant allergens may be controlled mainly by the lymphatic tissue surrounding the pharynx.³² This may explain the observed discrepancy in the frequencies

of food and respiratory allergies after pediatric liver transplantation, ie, the risk for food allergy is substantially increased, whereas that for respiratory allergy seems to be unaffected.

The mechanisms underlying the immune-related manifestations after pediatric liver transplantation have been scarcely studied.³³⁻³⁵ Changes in T-cell subsets have been suggested to be of importance and have been implicated in the immunosuppression. Although the evidence is limited,^{36,37} it has been speculated that calcineurin inhibitors (in particular, tacrolimus) are more suppressive of T-helper type 1 than type 2 cells, shifting the balance toward Th2-type responses, and thereby potentially favoring the development of allergic diseases.^{38,39} Moreover, calcineurin inhibitors have been reported to decrease the proportion of regulatory T cells in the T-cell population,⁴⁰ a finding that has also been noted in children after liver transplantation.^{33,35} In addition, a small-scale study has reported reduced levels of interleukin-10 in pediatric liver transplant recipients, indicating a functional defect in regulatory T-cell activity.³⁴ Therefore, the above-mentioned changes may be of importance, as regulatory T cells are considered to be involved both in the development of allergic disease⁴¹ and in controlling the B cell-mediated production of the autoantibodies associated with the development of autoimmune diseases.⁴²

Given the above background, an interesting (and seemingly contrasting) finding in our study was that autoantibody formation was significantly more common among the subgroup of 9 patients who were not receiving immunosuppressive therapy at the time of inclusion in the study. Furthermore, we could not demonstrate any link between discontinued immunosuppressive therapy and the occurrence of allergy or sensitization, an unexpected finding in view of the claim that tacrolimus increases the risk of developing allergy,^{4,39,43} as well as sensitization.^{44,45} However, it should be noted that with the exception of 1 patient, all these children had initially been on maintenance immunosuppressive therapy with tacrolimus. Although it has been proposed that tacrolimus in combination with other factors (eg, chronic liver disease present before transplantation, immaturity of the immune system in a young child, and/or immunomodulatory effects of viral infections) contributes to the induction of immune dysregulation,^{2,4,25} one can speculate that this impact on the immune system occurs primarily in the early phase after pediatric liver transplantation. In contrast, later in the course, the immunosuppression may merely hamper the misguided immune responses that result from the immune dysregulation established earlier. This theory should be explored further in a longitudinal study.

The prevalence of food allergy in the present study was several-fold higher than the latest prevalences reported for the general Scandinavian pediatric population, eg, 1.2% at the age of 6 years in Danish children⁴⁶ and 1.4% at the age of 12 years in Swedish children.⁴⁷ Furthermore, the high frequency of food allergy occurrence in the older age groups in our cohort is clearly different from that seen in the general pediatric populations in developed countries, where food allergy is more common in younger children.^{48,49}

One-quarter of the children suffered from respiratory allergy. The prevalence and increase in prevalence with age found in our cohort reflect the findings for the general

pediatric population in Sweden, eg, asthma in 6%–7% of children at 7–8 years of age and in 6%–10% of children at 12–14 years of age.^{50,51} The corresponding percentages for allergic rhinitis are 13%–15% and 13%–20%, respectively.^{50,51}

Only 1 other study from Canada has reported the actual prevalences of food and respiratory allergies in pediatric liver transplant recipients.² Similar to the results of our study, food allergy was found to be more prevalent in children who received liver transplants than in the general pediatric population of North America. In that study, food allergy, asthma, and allergic rhinitis were seen in 15%, 9%, and 4.5% of children, respectively, although children with a history of allergy before transplantation were excluded (unlike in our study), which may explain in part the higher prevalence rates found in our cohort. Taken together, our results lend important additional support to the concept that pediatric recipients of liver transplants have a substantially increased risk of developing a food allergy. In contrast, our data indicate that the prevalence of respiratory allergy in such patients is similar to that seen in the general pediatric population.

Most of the previous studies have focused exclusively on new-onset food allergy after pediatric liver transplantation and reported the cumulative incidence rather than the prevalence.^{3–11,52} In our cohort, 12 patients had new-onset food allergy at some point posttransplantation, giving a cumulative incidence of 28%. This is in line with the results of studies with a similar follow-up period (ie, median time from transplantation of ≥ 5 y), which have reported cumulative incidences in the range of 27%–38%.^{3,4} One of the previous studies also reported the cumulative incidence of asthma or allergic rhinitis as being 6%,⁴ which is a surprisingly low value compared to our results and the prevalence in the general pediatric population.

Regarding sensitization, one-third of the children in our cohort were sensitized to food allergens, and every fourth child was sensitized to inhalant allergens, the majority of these being of school age. A large Swedish population-based birth cohort study has demonstrated that sensitization to both food and inhalant allergens in the general pediatric population varies with age.⁵³ That study employed the same test panels as we used in our study, and sensitization to food was seen in 17%, 20%, and 14% of children at the age of 4, 8, and 12 years, respectively. The corresponding values for sensitization to inhalants were 16%, 26%, and 45%, respectively. Thus, the prevalence of sensitization to foods seen in school-age children in our cohort is at least twice that in the general Swedish pediatric population, while the prevalence of sensitization to inhalant allergens is similar to that seen in the general pediatric population.

Few other studies have reported sensitization data for children after liver transplantation or provided a comparison to the general pediatric population.^{3,25} Brown et al²⁵ found that 36% of the children <3 years of age were sensitized to cow's milk, egg, and/or peanut 1 year after transplantation. Another study reported that sensitization to cow's milk, egg, wheat, and/or peanut was found in 24% of children who underwent liver transplantation, during a median follow-up of 5.8 years posttransplantation, although, in dissimilarity to our study, children with allergy before liver transplantation were excluded from this study.³ To our knowledge, there are no previous studies

reporting data on sensitization to inhalant allergens after liver transplantation in children.

Few patients in our cohort had immune-mediated disorders other than allergy, with 1 child suffering from de novo autoimmune hepatitis and another being diagnosed with celiac disease. This is consistent with previous studies reporting de novo autoimmune hepatitis in 2%–6%,^{12–17} de novo inflammatory bowel disease in 0.9%–4.3%,^{2,19,20} and immune cytopenia in 1.8%–4%^{2,54} of children after liver transplantation. In contrast, autoantibodies were detected in almost half of the children in our cohort, which is in line with previous studies showing autoantibodies in 13%–75% of children after liver transplantation.^{12,21–24,55} For comparison, the estimated prevalence of corresponding autoantibodies in the general pediatric population is <10%.^{56,57} The broad range in the prevalence of autoantibodies reported among children who received liver transplants may reflect differences in the panels of autoantibodies analyzed and/or cutoff levels for such assays.

The clinical significance of autoantibody formation in this patient group is unclear, since both in our study and in previous studies^{21–23} autoantibodies were associated with concomitant autoimmune disease (ie, autoimmune hepatitis) in <5% of the patients. Nevertheless, one cannot exclude the possibility that these patients have an increased long-term risk of developing autoimmune and other immune-mediated diseases.

Children who had been transplanted for biliary atresia had significantly lower prevalences of autoantibodies and sensitization than children who were transplanted due to other reasons. This is surprising and partly in conflict with previous reports that have associated young age at transplantation with an increased probability to develop allergy and other immune-mediated disorders after transplantation.^{2,3,9,11} Our finding needs to be interpreted with caution due to the small sample size and the fact that biliary atresia did not reach a significant association with autoantibody formation in the logistic regression model.

One of the main strengths of our study is its broad approach, comprising detailed data on posttransplantation allergy and sensitization, as well as on other immune-mediated diseases and autoantibodies. Furthermore, practically the entire eligible patient population of children who received liver transplants at our center was included. A limitation may be the relatively small size of our cohort.

Immune-mediated disorders that occur after liver transplantation, such as food allergy, can have a substantial impact on the quality of life of the affected individuals.^{58,59} The clinical implication of this study is the need for heightened awareness regarding these disorders.

In summary, this study shows that in contrast to respiratory allergy, food allergy and autoantibody formation occurred together in the same children who underwent liver transplantation at a frequency higher than would be expected by chance. This may reflect an underlying immune dysregulation that impairs immune tolerance to both food allergens and autoantigens in these children.

ACKNOWLEDGMENTS

We thank Eva Karlsson, RN, for coordination of the clinical visits and the collection and the registration of the clinical data.

REFERENCES

- Pham YH, Miloh T. Liver transplantation in children. *Clin Liver Dis*. 2018;22:807–821.
- Marcus N, Amir AZ, Grunebaum E, et al. De novo allergy and immune-mediated disorders following solid-organ transplantation-prevalence, natural history, and risk factors. *J Pediatr*. 2018;196:154–160.e2.
- Lee Y, Lee YM, Kim MJ, et al. Long-term follow-up of de novo allergy in pediatric liver transplantation—10 yr experience of a single center. *Pediatr Transplant*. 2013;17:251–255.
- De Bruyne R, Dullaers M, Van Biervliet S, et al. Post-transplant food allergy in children is associated with liver and not with renal transplantation: a monocentric comparative study. *Eur J Pediatr*. 2013;172:1069–1075.
- Shroff P, Mehta RS, Chinen J, et al. Presentation of atopic disease in a large cohort of pediatric liver transplant recipients. *Pediatr Transplant*. 2012;16:379–384.
- Levy Y, Davidovits M, Cleper R, et al. New-onset post-transplantation food allergy in children—is it attributable only to the immunosuppressive protocol? *Pediatr Transplant*. 2009;13:63–69.
- Mitsui M, Shoda T, Natsume O, et al. Factors associated with development of food allergy in young children after liver transplantation: a retrospective analysis of 10 years' experience. *J Allergy Clin Immunol Pract*. 2017;5:1698–1706.
- Arikan C, Kilic M, Tokat Y, et al. Allergic disease after pediatric liver transplantation with systemic tacrolimus and cyclosporine a therapy. *Transplant Proc*. 2003;35:3039–3041.
- Ozbek OY, Ozcay F, Avci Z, et al. Food allergy after liver transplantation in children: a prospective study. *Pediatr Allergy Immunol*. 2009;20:741–747.
- Topal E, Çatal F, Selimoğlu MA, et al. Acquired atopic disease after liver transplantation in children; similarities to and differences from adults: a preliminary study. *Eur J Gastroenterol Hepatol*. 2014;26:1055–1059.
- Wisniewski J, Lieberman J, Nowak-Węgrzyn A, et al. De novo food sensitization and eosinophilic gastrointestinal disease in children post-liver transplantation. *Clin Transplant*. 2012;26:E365–E371.
- Andries S, Casamayou L, Sempoux C, et al. Posttransplant immune hepatitis in pediatric liver transplant recipients: incidence and maintenance therapy with azathioprine. *Transplantation*. 2001;72:267–272.
- Kerkar N, Hadzić N, Davies ET, et al. De-novo autoimmune hepatitis after liver transplantation. *Lancet*. 1998;351:409–413.
- Spada M, Bertani A, Sonzogni A, et al. A cause of late graft dysfunction after liver transplantation in children: de-novo autoimmune hepatitis. *Transplant Proc*. 2001;33:1747–1748.
- Gupta P, Hart J, Millis JM, et al. De novo hepatitis with autoimmune antibodies and atypical histology: a rare cause of late graft dysfunction after pediatric liver transplantation. *Transplantation*. 2001;71:664–668.
- Hernandez HM, Kovarik P, Whittington PF, et al. Autoimmune hepatitis as a late complication of liver transplantation. *J Pediatr Gastroenterol Nutr*. 2001;32:131–136.
- Venick RS, McDiarmid SV, Farmer DG, et al. Rejection and steroid dependence: unique risk factors in the development of pediatric posttransplant de novo autoimmune hepatitis. *Am J Transplant*. 2007;7:955–963.
- Verdonk RC, Dijkstra G, Haagsma EB, et al. Inflammatory bowel disease after liver transplantation: risk factors for recurrence and de novo disease. *Am J Transplant*. 2006;6:1422–1429.
- Wörns MA, Lohse AW, Neurath MF, et al. Five cases of de novo inflammatory bowel disease after orthotopic liver transplantation. *Am J Gastroenterol*. 2006;101:1931–1937.
- Fernandes MA, Braun HJ, Evason K, et al. De novo inflammatory bowel disease after pediatric kidney or liver transplant. *Pediatr Transplant*. 2017;21:e123835.
- Chen CY, Ho MC, Wu JF, et al. Development of autoantibodies after pediatric liver transplantation. *Pediatr Transplant*. 2013;17:144–148.
- Avitzur Y, Ngan BY, Lao M, et al. Prospective evaluation of the prevalence and clinical significance of positive autoantibodies after pediatric liver transplantation. *J Pediatr Gastroenterol Nutr*. 2007;45:222–227.
- Richter A, Grabhorn E, Helmke K, et al. Clinical relevance of autoantibodies after pediatric liver transplantation. *Clin Transplant*. 2007;21:427–432.
- Riva S, Sonzogni A, Bravi M, et al. Late graft dysfunction and autoantibodies after liver transplantation in children: preliminary results of an Italian experience. *Liver Transpl*. 2006;12:573–577.
- Brown C, Haringman N, Davies C, et al. High prevalence of food sensitisation in young children with liver disease: a clue to food allergy pathogenesis? *Pediatr Allergy Immunol*. 2012;23:771–778.
- Needham JM, Nicholas SK, Davis CM. Food allergies developing after solid organ transplant. *Pediatr Transplant*. 2015;19:827–835.
- Kerkar N, Yanni G. "De novo" and "recurrent" autoimmune hepatitis after liver transplantation: a comprehensive review. *J Autoimmun*. 2016;66:17–24.
- Vivas AP, Bomfin LE, Costa WI Jr, et al. Oral granulomatosis-like lesions in liver-transplanted pediatric patients. *Oral Dis*. 2014;20:e97–102.
- Saalman R, Sundell S, Kullberg-Lindh C, et al. Long-standing oral mucosal lesions in solid organ-transplanted children—a novel clinical entity. *Transplantation*. 2010;89:606–611.
- Yang R, Liu Q, Grosfeld JL, et al. Intestinal venous drainage through the liver is a prerequisite for oral tolerance induction. *J Pediatr Surg*. 1994;29:1145–1148.
- Jenne CN, Kubes P. Immune surveillance by the liver. *Nat Immunol*. 2013;14:996–1006.
- Hesselmar B, Sjöberg F, Saalman R, et al. Pacifier cleaning practices and risk of allergy development. *Pediatrics*. 2013;131:e1829–e1837.
- Elwegger T, Gruber S, Geiger C, et al. Impact of systemic immunosuppression after solid organ transplantation on allergen-specific responses. *Allergy*. 2011;66:271–278.
- Nahum A, Brenner A, Granot E. Cytokine profile of food-allergic post-liver transplant children is identified by high levels of IL-5 and low IL-10 secretion from patients' peripheral blood mononuclear cells. *Pediatr Transplant*. 2015;19:716–721.
- De Bruyne R, Gevaert P, Van Winckel M, et al. Raised immunoglobulin A and circulating T follicular helper cells are linked to the development of food allergy in paediatric liver transplant patients. *Clin Exp Allergy*. 2015;45:1060–1070.
- Weimer R, Melk A, Daniel V, et al. Switch from cyclosporine A to tacrolimus in renal transplant recipients: impact on th1, th2, and monokine responses. *Hum Immunol*. 2000;61:884–897.
- Sugiyama M, Funachi M, Yamagata T, et al. Predominant inhibition of th1 cytokines in New Zealand black/white F1 mice treated with FK506. *Scand J Rheumatol*. 2004;33:108–114.
- Lacaille F, Laurent J, Bousquet J. Life-threatening food allergy in a child treated with FK506. *J Pediatr Gastroenterol Nutr*. 1997;25:228–229.
- Lykavieiris P, Frauger E, Habes D, et al. Angioedema in pediatric liver transplant recipients under tacrolimus immunosuppression. *Transplantation*. 2003;75:152–155.
- Zeiser R, Nguyen VH, Beilhack A, et al. Inhibition of CD4+CD25+ regulatory T-cell function by calcineurin-dependent interleukin-2 production. *Blood*. 2006;108:390–399.
- Palomares O, Akdis M, Martin-Fontecha M, et al. Mechanisms of immune regulation in allergic diseases: the role of regulatory T and B cells. *Immunol Rev*. 2017;278:219–236.
- Sakaguchi S, Yamaguchi T, Nomura T, et al. Regulatory T cells and immune tolerance. *Cell*. 2008;133:775–787.
- Lebel MJ, Chapdelaine H, Paradis L, et al. Increase in de novo food allergies after pediatric liver transplantation: tacrolimus vs. cyclosporine immunosuppression. *Pediatr Transplant*. 2014;18:733–739.
- Granot E, Yakobovich E, Bardenstein R. Tacrolimus immunosuppression—an association with asymptomatic eosinophilia and elevated total and specific IgE levels. *Pediatr Transplant*. 2006;10:690–693.
- Gruber S, Tiringier K, Dehlink E, et al. Allergic sensitization in kidney-transplanted patients prevails under tacrolimus treatment. *Clin Exp Allergy*. 2011;41:1125–1132.
- Eller E, Kjaer HF, Høst A, et al. Food allergy and food sensitization in early childhood: results from the DARC cohort. *Allergy*. 2009;64:1023–1029.
- Winberg A, West CE, Strinnholm Å, et al. Assessment of allergy to milk, egg, cod, and wheat in Swedish schoolchildren: a population based cohort study. *PLoS One*. 2015;10:e0131804.
- Sicherer SH, Sampson HA. Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol*. 2014;133:291–307; quiz 308.
- Hill DA, Spergel JM. The atopic march: critical evidence and clinical relevance. *Ann Allergy Asthma Immunol*. 2018;120:131–137.
- Bjerg A, Sandström T, Lundbäck B, et al. Time trends in asthma and wheeze in Swedish children 1996–2006: prevalence and risk factors by sex. *Allergy*. 2010;65:48–55.
- Ballardini N, Kull I, Lind T, et al. Development and comorbidity of eczema, asthma and rhinitis to age 12: data from the BAMSE birth cohort. *Allergy*. 2012;67:537–544.
- Noble C, Peake J, Lewindon PJ. Increase in de novo allergies after paediatric liver transplantation: the Brisbane experience. *Pediatr Transplant*. 2011;15:451–454.

53. Wickman M, Asaranoj A, Tillander H, et al; MeDALL consortium. Childhood-to-adolescence evolution of IgE antibodies to pollens and plant foods in the BAMSE cohort. *J Allergy Clin Immunol*. 2014;133:580–582.
54. Schoettler M, Elisofon SA, Kim HB, et al. Treatment and outcomes of immune cytopenias following solid organ transplant in children. *Pediatr Blood Cancer*. 2015;62:214–218.
55. Hadzic NS, Srinivasan R, Rela M, et al. De novo autoimmune hepatitis after liver transplantation in children: a single center experience. *Hepatology*. 2003;38(suppl):202.
56. Kasapçopur O, Ozbakir F, Arisoy N, et al. Frequency of antinuclear antibodies and rheumatoid factor in healthy turkish children. *Türk J Pediatr*. 1999;41:67–71.
57. Martini A, Lorini R, Zanaboni D, et al. Frequency of autoantibodies in normal children. *Am J Dis Child*. 1989;143:493–496.
58. Lieberman JA, Sicherer SH. Quality of life in food allergy. *Curr Opin Allergy Clin Immunol*. 2011;11:236–242.
59. DunnGalvin A, Dubois AE, Flokstra-de Blok BM, et al. The effects of food allergy on quality of life. *Chem Immunol Allergy*. 2015;101:235–252.