

Autoantibodies and Donor-specific Antibodies are Associated With Graft Dysfunction in Pediatric Liver Transplantation

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See “Associated With Graft Dysfunction in Pediatric Pediatric Liver Transplant: When Are They Important? What Do They Mean?” by Smith and Perito on page 643.

ABSTRACT

Objectives: Autoantibodies (AAb) and donor-specific HLA antibodies (DSA) are frequently present in pediatric liver transplant (LT) recipients. Their clinical significance remains incompletely understood. We aimed to investigate the prevalence of serum AAb and DSA in pediatric LT recipients and its correlation with patient characteristics and histological and biochemical parameters.

Methods: We retrospectively reviewed the data from 62 pediatric LT patients in follow-up at Ghent University Hospital between January 2007 and February 2018. Blood samples with AAb measurement were taken systematically, liver biopsies (LB) were performed on clinical indication.

Results: AAb were detected in 27 (43.3%) patients, with antinuclear antibodies (ANA) being the most frequently (24%) encountered AAb. There was an association between AAb positivity and female gender ($P=0.032$) and deceased donor LT ($P=0.006$). Patients with positive AAb underwent a higher number of LB during their follow-up ($P<0.001$), and an association was found with the presence of nonspecific histologic alterations ($P=0.032$) in the absence of *de novo* autoimmune hepatitis. Positive AAb were also associated with higher alkaline phosphatase ($P<0.001$), ALT ($P<0.001$), AST ($P<0.001$), γ -GT ($P=0.001$), IgG ($P=0.011$) and lower albumin ($P=0.029$). Fourteen out of 50 (28%) patients were DSA-positive, mostly anti-HLA class II. DSA positivity was associated with T-cell-mediated rejection ($P=0.019$), higher total ($P=0.033$), and direct ($P=0.012$) bilirubin and γ -GT ($P<0.001$).

Conclusions: The presence of AAb and DSA is associated with histological and biochemical parameters of graft dysfunction. Larger prospective studies are warranted to investigate the causal relationships between AAb and DSA development and outcome parameters post pediatric LT.

Key Words: antinuclear antibodies, autoantibodies, children, HLA antibodies, outcome

(JPGN 2021;72: 661–666)

Received January 15, 2020; accepted July 6, 2020.

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What Is Known

- Autoantibodies and donor-specific HLA antibodies are frequently found after pediatric liver transplantation.
- Serum autoantibody positivity in the context of autoimmune hepatitis is associated with graft dysfunction; however, the clinical significance of isolated autoantibodies positivity is unclear.
- Donor-specific HLA antibodies are detrimental in the context of kidney, lung and heart transplantation, the significance in liver transplantation remains debated.

What Is New

- Autoantibody positivity is associated with histological and biochemical parameters of graft dysfunction.
- Donor-specific HLA antibodies are associated with T-cell-mediated rejection and higher cholestatic parameters.
- There is a need for larger prospective studies to determine causal relationships.

Liver transplantation (LT) has evolved from an experimental procedure to a state-of-the-art treatment for children with end-stage liver disease. Despite this success, multiple challenges remain: scarcity of donor organs and minimization of long-term complications of immunosuppression.

De novo autoimmune hepatitis (AIH) is an important cause of graft dysfunction after pediatric LT (1). The presence of serum autoantibodies (AAb), such as antinuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA) in the absence of autoimmune liver disease is common in children after LT with a reported prevalence ranging from 24% to 75% (2–5).

Supplemental digital content 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.jpgn.org).

Carolien Bonroy and Ruth M.L. De Bruyne equally contributed to the study as co-last authors.

The authors report no conflicts of interest.

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DOI: 10.1097/MPG.0000000000002913

The detrimental effects of donor-specific HLA antibodies (DSA) in kidney, lung, and heart transplantation are well known. Where the liver was initially believed to be relatively resistant to DSA-mediated damage, recent studies have questioned this hypothesis (6–12). On the basis of the currently existing data, the role of AAb and DSA in pediatric liver transplantation remains incompletely understood (2–5,13–15).

In this study, we aimed to determine the prevalence of serum AAb and DSA in a cohort of pediatric LT recipients and investigate its correlation with patient characteristics and clinical outcome in terms of histological and biochemical parameters.

METHODS

Patients and Data

Sixty-two patients in follow-up at Ghent University Hospital between January 2007 and February 2018 after pediatric LT were included in the study. Data and patient characteristics (sex, median age at the time of LT, median follow-up after LT, transplant indication, donor type [deceased/living], immunosuppressive regimen, need for retransplantation, and death) were retrospectively collected from electronic patient records. Serum AAb results and histological and biochemical parameters were registered at different time points (before LT and 1, 3, 5, 10, 15 years after LT). All histological data were collected in each patient. We did not perform protocol liver biopsies (LB) during the study period but LB was performed as soon as there were any clinical, biochemical, or radiological abnormalities compatible with graft dysfunction. We defined the following histological parameters: need for LB, number of LB, T-cell-mediated rejection (TCMR), chronic rejection (CR), histological signs of biliary obstruction, *de novo* AIH, idiopathic posttransplant hepatitis (IPTH), steatosis and nonspecific histological changes that were not diagnostic for another condition. Nonspecific histological alterations were defined as the presence of mild, for example, periportal or perisinusoidal fibrosis and/or mild ductular reaction and/or mild portal or lobular inflammation in the absence of other histological abnormalities. The included biochemical parameters were: alkaline phosphatase (AP), ALT, AST, bilirubin (direct and total), γ -GT, albumin, platelet count, IgG, EBV, and CMV DNA. These parameters were measured simultaneously with the AAb titers at each patient's annual review and these data were clustered throughout the statistical analyses. The study was approved by the ethics committee of Ghent University Hospital. Until July 2003, the immunosuppressive protocol consisted of 10 mg/kg methylprednisolone perioperatively and maintenance immunosuppression with cyclosporin and prednisolone. From July 2003 onwards, basiliximab was added as induction immunosuppression and cyclosporin was replaced by tacrolimus.

Autoantibodies

Data on the following AAb were collected at each time point: ANA, ASMA, anti-soluble liver antigen antibodies (ASLA), anti-liver cytosol type 1 antibodies (anti-LC1), and anti-liver kidney microsomal antibodies (anti-LKM). Detection of ANA was conducted via indirect immunofluorescence (IIF) with a polyspecific IgG-conjugate on HEp-2000 cells using a 1 in 40 serum dilution (Immuno Concepts, Sacramento, CA). Detection of ASMA and anti-LKM was conducted via IIF on Rat-Liver Kidney Stomach slides. Analyses before January 25, 2016 were conducted with a serum dilution of 1:20 with the kit of Inova (NovaLite Rat Liver, Kidney, Stomach; Inova, S. Diego, USA). Analyses after this date were conducted with a serum dilution of 1 : 40 with the kit of Menarini Diagnostics (Antibody Detection IFA Rat Kindey/Stomach/Liver, A. Menarini Diagnostics, Florence, Italy). AAb titers were expressed by intensity scale of values up to 5+ in

relation to a negative control. Borderline results were considered negative for statistical analysis. Detection of ASLA and anti-LC1 was conducted via the Euroline Liver Profile lineblot kit conforming the manufacturer's instructions. Results were digitized using a calibrated flatbed and accompanying software (Euroimmun, Lübeck, Germany). Cut-off values as proposed by the manufacturer were applied. Global AAb positivity was defined as at least 1 positive AAb-measurement during follow-up time.

Donor-specific HLA antibodies

Fifty out of 62 patients were screened for DSA at the Belgian Red Cross-Flanders in the Histocompatibility and Immunogenetics Laboratory (HILA). HLA antibody evaluation was performed with Immucor LIFECODES LifeScreen Deluxe kits. A positive screening for the presence of circulating HLA antibodies was followed by HLA antibody identification with Immucor LIFECODES LSA (Luminex Single Antigen) kits. All tests were performed and interpreted according to the manufacturer's instructions. HLA genotyping was performed with Immucor LIFECODES HLA-SSO (sequence-specific oligonucleotides, intermediate resolution) or Olerup HLA-SSP (sequence-specific primers, low resolution) kits.

DSA were assessed by comparing the HLA antibodies to the HLA type of the patient and donor. DSA screening was only implemented in our institution from 2016 onwards. We defined DSA positivity based on the latest available DSA result in the follow-up period of the study.

Statistics

Statistical analysis was performed using IBM SPSS Statistics (version 25). The following tests were used: Chi²-test or Fisher exact test (categorical variables), Mann-Whitney *U* test (continuous variables), McNemar test (paired tests). z-scores were used for normalization in case of parameters with age-dependent (bilirubin, albumin, IgG) and age- and sex-dependent (AP, ALT, AST, GT, platelet count) reference values. A *P* value <0.05 was considered statistically significant.

RESULTS

Characteristics and Liver Biopsies

The patient characteristics are shown in Table 1. TCMR was present in 20 (32%), CR in 7 (11%), histological signs of biliary obstruction in 16 (25%), *de novo* AIH in 4 (6,5%), IPTH in 5 (8,1%), steatosis in 12 (19%) patients, and nonspecific histological changes in 25 (40%) patients. In the latter group, the isolated finding of mild fibrosis, mild inflammatory changes, and mild ductular reactions was present in respectively, 2, 6, and 1 patients. The rest (16/25) of this group had a mixed pattern of these mild histologic abnormalities.

Autoantibodies

Twenty-seven out of 62 (43%) patients were considered positive for at least one AAb during the follow-up period. ANA was most frequently found (in 15 patients, 24%), followed by ASMA (in 13 patients, 21%). ASLA and anti-LC1 were both detected in 2 patients (3.2%). None of the patients had anti-LKM. The association between age at time of LT and positive AAb was near significant ($P=0.05$), with patients in the AAb-positive group being younger (median age 1.3 years; range 0.1–9.8 years versus median age 2.7 years; range 0.3–17.1 years). AAb positivity was associated with female sex ($P=0.032$). Fifteen out of

TABLE 1. Patient characteristics, n = 62

Median age at LT in years (range)	1.9 (0.1–17.1)
Median follow-up after LT in years (range)	9.6 (1–20)
Male/female, n	37/25
Transplant indication, n	
Biliary atresia	24
IEM	10
Inherited disorders	8
ALF (non-AI)	5
AILD	4
Tumor and other	11
Transplant type, n	
Deceased, split	36
Deceased, full graft	14
Living related	12
Retransplantation	6
Death	3
Immunosuppression, n	
Tacrolimus	54
Cyclosporin	8
MMF	18
Steroids	6

AILD = autoimmune liver disease; ALF (non-AI) = acute liver failure (not because of proven autoimmune liver disease); IEM = inherited inborn errors of metabolism; LT = liver transplant; MMF = mycophenolate mofetil; n = number.

25 (60%) of the girls were AAb-positive, compared with 12 of the 37 (32%) boys. We found an association between positive AAb and deceased donor LT ($P = 0.006$) (Fig. 1A). There was a correlation between the need for LB and the presence of AAb: 26 out of 52 (50%) patients who had at least one LB were positive for AAb, compared with 1 out of 10 (10%) of the patients who did not undergo LB ($P = 0.033$). Patients with positive AAb underwent a

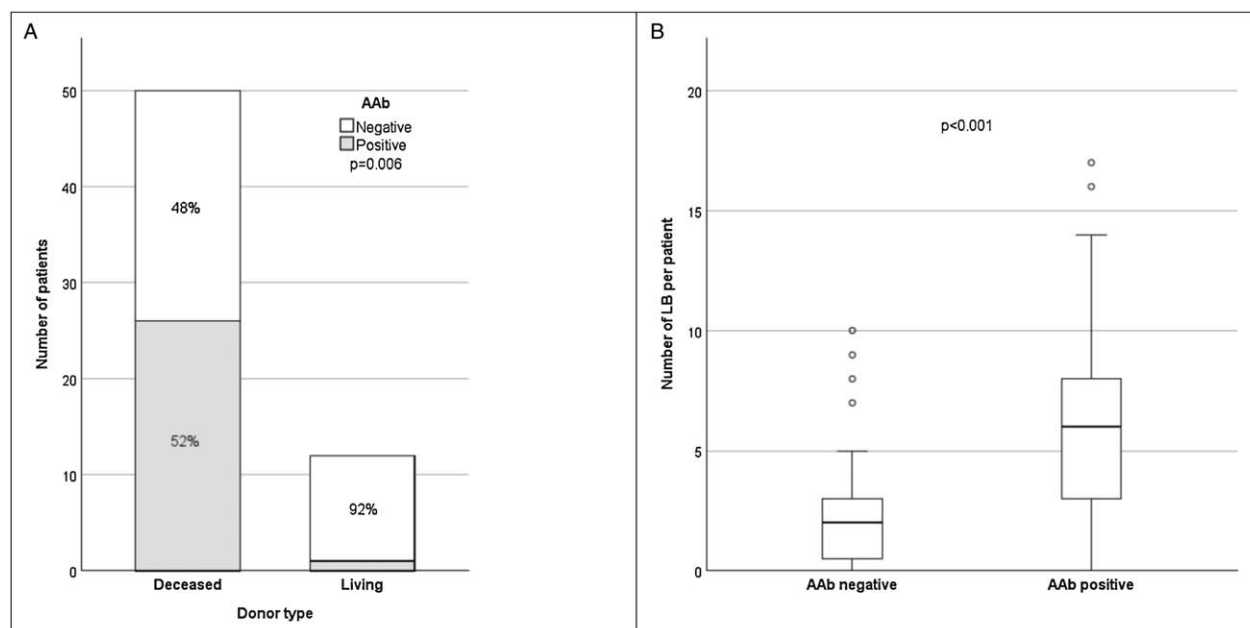
higher number of LB during their follow-up compared with patients without AAb ($P < 0.001$): a median of 6 (range 0–17) versus 2 (range 0–10) biopsies (Fig. 1B). In the absence of *de novo* AIH, an association was found between positive AAb and the presence of nonspecific histologic alterations ($P = 0.032$). Outside the context of *de novo* AIH, positive AAb were associated with higher AP ($P < 0.001$), ALT ($P < 0.001$), AST ($P < 0.001$), γ -GT ($P = 0.001$), and lower albumin ($P = 0.029$). After exclusion of patients with pre-existing autoimmune liver disease, an association was found between AAb positivity and IgG ($P = 0.011$). For the following variables, no association with AAb positivity was found: median follow-up after LT, transplant indication, immunosuppressive regimen, TCMR, biliary obstruction, steatosis, direct and indirect bilirubin, platelet count, and EBV DNA.

For the following parameters, no reliable statistical analysis could be performed because of small numbers: death, CR, IPH, *de novo* AIH, ASLA, anti-LC1, and CMV DNA.

Subanalysis of data on individual AAb (ANA and ASMA) is reported in Table 1, Supplemental Digital Content, <http://links.lww.com/MPG/B943>. Analyses with different cut-offs for AAb-positivity are displayed in Table 2, Supplemental Digital Content, <http://links.lww.com/MPG/B944>. The time course of the AAb results in relation to the biopsy results is displayed in Table 3, Supplemental Digital Content, <http://links.lww.com/MPG/B945>. Of note, although AAb titers fluctuated in the majority of patients, AAb positivity seems a persistent finding in the patients who underwent a liver biopsy. None of these patients had lost AAb positivity at the end of follow-up.

Donor-specific HLA antibodies

Fourteen out of 50 (28%) patients were positive for DSA, all but one were anti-HLA class II. Characteristics of the DSA and MFI are displayed in Table 4, Supplemental Digital Content, <http://links.lww.com/MPG/B946>. DSA positivity was associated with



AAb=autoantibodies; LB=liver biopsy.

FIGURE 1. Relation between autoantibody positivity, donor type and number of liver biopsy sampling. (A) Association between AAb positivity and deceased donors (χ^2 test, $P = 0.006$); (B) Association between AAb and number of LB (Mann-Whitney U -test, $P < 0.001$). AAb = autoantibody; LB = liver biopsy.

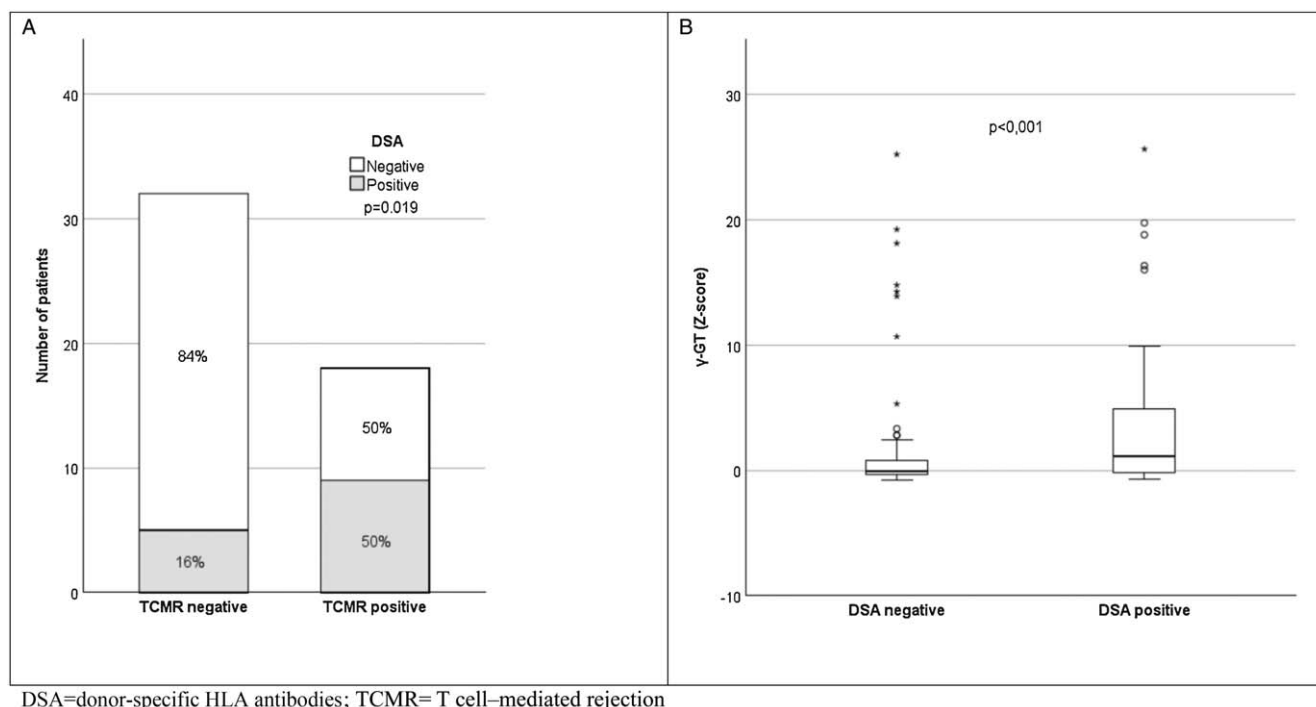


FIGURE 2. Relation between donor-specific HLA antibodies, T-cell-mediated rejection and γ -GT. (A) Association between DSA and TCMR (χ^2 test, $P=0.019$); (B) association between DSA and γ -GT (expressed as z-scores) (Mann-Whitney U -test, $P<0.001$). DSA = donor-specific HLA antibodies; TCMR = T-cell-mediated rejection.

TCMR ($P=0.019$) (Fig. 2A), higher total bilirubin ($P=0.033$), direct bilirubin ($P=0.012$), and γ -GT ($P<0.001$) (Fig. 2B). For the following variables, no association with DSA was found: median age at LT, median follow-up after LT, sex, transplant indication, donor type (deceased/living), immunosuppressive regimen, need for LB, number of LB, biliary obstruction, nonspecific histological changes, steatosis, AP, ALT, AST, indirect bilirubin, platelet count, albumin, IgG, and EBV DNA.

For the following variables, statistical analysis was unreliable because of small numbers: death, CR, IPTH, *de novo* AIH, and CMV DNA.

DISCUSSION

AAb positivity, outside the context of *de novo* autoimmune hepatitis, is a frequent finding in children after LT. In our cohort, AAb were positive in 43%, where reported prevalence range between 16% and 75% (2–4,16,17). ANA and ASMA were most frequently detected. The clinical relevance of these AAb is still debated. Several authors found an association between AAb positivity and histological allograft abnormalities, such as CR (3,5) and chronic hepatitis and fibrosis (14,15,18) in children. In two other studies, however, no such association was observed (16,17).

Solid organ transplant patients are at risk for the development of *de novo* autoimmune diseases, allergic disorders, and graft-specific alloantibodies (19). T-follicular helper (Tfh) cells have been shown to play an important role in the pathogenesis of autoimmune disease (20–23). We previously observed a marked increase in circulating Tfh cells in pediatric liver transplant patients compared with healthy controls (24). The immunosuppressive treatment might affect the balance between Tfh and other T-helper cells, hence influencing humoral immunity. We found that children transplanted at a younger age more often developed AAb. Other

authors did not establish this relation (2,17). This could be because of a differential effect of the immunosuppressive regimens on the immature immune system (24).

We found an association between AAb positivity and female sex. No similar reports are available in pediatric LT recipients. In two studies in healthy children, no difference in prevalence of ANA between boys and girls was found (25,26). It is, however, known that patients with autoimmune diseases, like chronic autoimmune hepatitis, have a female predominance (27).

We are the first to report a positive association between AAb and deceased donor LT. Deceased donor LT implies a longer cold ischemia time and higher risk for reperfusion injury compared with living donor LT (28). As a consequence of reperfusion injury, the recipient can be exposed to donor or self-antigens presented by (either donor or recipient) antigen-presenting cells. Regulatory T cells play a primary role in controlling adaptive immune responses and maintaining tolerance to self-antigens and harmless non-self-antigens (29). Natural and induced regulatory CD4⁺ T cells are decreased in pediatric liver transplant patients (30), potentially leading to the development of autoreactive T-cell clones and antibodies.

In our patient cohort, AAb positivity was associated with biochemical signs of graft dysfunction (higher AP, ALT, AST, γ -GT, lower albumin). In one study, a similar association was observed (2), but was not confirmed by others (3,4,17). Furthermore, patients with positive AAb more often underwent a LB and required a higher number of LB per person. The causal relationship between AAb positivity and graft dysfunction is, however, yet to be established. AAb could indeed lead to graft injury when they are directed against components of the hepatic cell surface or when they form soluble immune complexes that precipitate in the liver tissue (31). On the other hand, graft injury (e.g., ischemia or biopsy sampling) on itself and the subsequent increased exposure to self-antigens could trigger AAb development as a secondary

phenomenon (32). Future prospective studies will allow us to further solve this question. Of note, isolated AAb positivity in the absence of other signs of graft dysfunction or elevated IgG was not an indication for LB in our hands.

We detected DSA in 14 out of 50 (28%) patients whereas the reported prevalence ranges from 14% to 55.6% (8–11,17,33,34). In line with other reports, class II DSA were more frequently found than class I DSA (8–11,17,34–37). HLA class II, however, has only limited expression in the microvasculature of the liver, whereas HLA class I is expressed in all hepatocytes. At times of rejection and AIH, endothelial cells can upregulate the expression of HLA class II and the expression of HLA class II on hepatocytes can be induced when there is inflammation (38).

Evidence is growing that DSA could be of significance in LT. In our cohort, DSA class II positivity was associated with biochemical signs of cholestasis (higher bilirubin and γ -GT) as well as histological signs of TCMR. An association between DSA positivity and higher bilirubin was also reported by Miyagawa-Hayashino et al (9); other authors did not confirm this finding (8,11,12,17,33,37). Schluckebier et al (39) also found that patients with DSA had a significantly higher rate of TCMR than patients without DSA.

Discrepant results on the relationship between DSA positivity and histological alterations have been published. In analogy with our study, several authors reported a relationship between DSA positivity and histological signs of T-cell-mediated rejection (8,9,40) whilst others did not (10,17). Most authors, except Dao et al (37), have found a positive association between allograft fibrosis and DSA positivity (9,33,35,41,42).

This retrospective study has its limitations. We cannot exclude that the relatively small sample size in our study influenced statistical analysis and has reduced the chance of detecting some other true associations. Moreover, no serial protocol biopsies or C4d-immunostaining were performed, whereas in other studies the latter has already given valuable extra information (9,37). Finally, we should be careful when comparing study results as cut-off titers for AAb detection and techniques for DSA detection are variable. Hence, it might be difficult to generalize outcomes from different studies.

In conclusion, in this study, we showed that the presence of AAb and DSA is associated with histological injury and graft dysfunction (cholestasis). Future prospective studies on bigger cohorts including the results of regular protocol biopsies are warranted to further investigate causal relationships between AAb and DSA development and outcome parameters post pediatric LT. On the basis of our findings, we advise to monitor AAb and DSA on a yearly base in pediatric LT recipients and if possible to also obtain pre-transplant data. The latter might allow us to design recommendations for differential disease monitoring and treatment strategies depending on the (auto)antibody status.

Acknowledgments: The technical assistance of Bada Tandir, Vicky Mortier, Josie Servaes, Karen Nys, and Elke Lecocq is greatly acknowledged.

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