OPEN

HLA-DR Mismatch and Black Race Are Associated With Recurrent Autoimmune Hepatitis After Liver Transplantation

Marshall McCabe IV, MD,[1] Natalia Rush, MD,[2] Craig Lammert, MD,[1] Kavish R. Patidar, MD,[1] Lauren Nephew, MD,[1] Romil Saxena, MD,[2] Burcin Ekser, MD,[3] James Salven, MS,[4] Chandrashekhar Kubal, MD, PhD,[3] and Marwan Ghabril, MD[1]

Background. The predictors of recurrent autoimmune hepatitis (R-AIH) after liver transplantation (LT) are heterogeneous with limited data to guide immunosuppression, with little data on impact of race. **Aims.** To describe the incidence, predictors, and outcomes of R-AIH. **Methods.** We studied patients undergoing LT for AIH during 2000–2017 at our center. Liver biopsies were performed for clinical indications. R-AIH was defined using clinical and histologic criteria. **Results.** Among 75 patients undergoing LT for AIH (mean age 45 ± 16, 65% female individuals, 19% Black), 71 (95%) received antithymocyte globulin induction with tacrolimus-based immunosuppression. R-AIH developed in 20 (27%) patients at a median interval of 313 d (interquartile range, 155–1205). R-AIH was associated with level 2 HLA-DR mismatch (hazard ratio, 3.6; (95% confidence interval, 1.3-9.9; $P = 0.01$) and Black race (hazard ratio, 4.5; 95% confidence interval, 1.8-11.8; $P = 0.002$)] in the multivariable analysis. R-AIH developed in 62% of patients with level 2 HLA-DR mismatch on single-agent immunosuppression but in <20% of patients with no or 1 HLA-DR mismatch regardless of maintenance immunosuppression. R-AIH developed in 8 (57%) of 14 Black patients (71% on single-agent and 43% on dual-agent maintenance immunosuppression). Patient and graft survival were not impacted by R-AIH over a median follow-up of 8.3 y (interquartile range, 3–12). **Conclusions.** High-level HLA-DR mismatch and Black recipient race are associated with an increased risk of R-AIH. Immunosuppression did not predict R-AIH, but higher rates of disease recurrence with single-agent maintenance immunosuppression with these risk factors were observed and may guide maintenance immunosuppression in LT for AIH.

(*Transplantation Direct* 2021;00: e713; doi: 10.1097/TXD.0000000000001160. Published online 10 June, 2021.)

Received 9 March 2021.

Accepted 30 March 2021.

[1] Department of Gastroenterology and Hepatology, Indiana University School of Medicine, Indianapolis, IN.

[2] Department of Pathology, Indiana University School of Medicine, Indianapolis, IN.

[3] Department of Transplant Surgery, Indiana University School of Medicine, Indianapolis, IN.

[4] Department of Biostatistics, Indiana University School of Medicine, Indianapolis, IN.

The authors declare no funding or conflicts of interest.

M.M., N.R., C.L., R.S., B.E., C.K., and M.G. participated in study design, data collection and analysis, article preparation, and final approval. K.R.P., L.N., and J.S. participated in data analysis, article preparation, and final approval.

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.transplantationdirect.com).

Correspondence: Marwan Ghabril, MD, Department of Gastroenterology and Hepatology, Indiana University School of Medicine, 702 Rotary Cir, Suite 225, Indianapolis, IN 46202. (mghabril@iu.edu).

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001160

INTRODUCTION

Autoimmune hepatitis (AIH) is estimated to recur in approximately 27% (10%–50%) of patients undergoing liver transplantation (LT) for AIH despite potent and sustained immunosuppression.[1-3] Despite heterogeneity in the definition of and described risk factors for recurrent autoimmune hepatitis (R-AIH), patient and graft survival are not significantly impacted.[3] However, predicting disease recurrence and tailoring immunosuppression to mitigate associated risk are important considerations in patients undergoing LT for AIH.

Reported risk factors for R-AIH include younger age, HLA-DR status of the recipient and donor and DR3 mismatch, acute rejection, and re-LT for R-AIH.[1-3] These risk factors have not been found consistently in all reported series, and the aggregated experience from multiple LT centers examining outcomes of LT for AIH and R-AIH has been invaluable.[2,3] Such heterogeneity may arise from differing approaches and institutional protocols for immunosuppression post-LT. The majority of published data on R-AIH relates to experiences with predominance of cyclosporine-based immunosuppression,[2] with only a few recent series reflecting largely tacrolimus-based immunosuppression.[4] Approach of our center to

immunosuppression in LT includes induction with rabbit antithymocyte globulin (rATG) since 2001 and additionally rituximab since 2004, with initial tacrolimus-based immunosuppression.[5] Our aim was to describe the rate of R-AIH and outcomes in our center and to describe the factors associated with R-AIH in the cohort of LT recipients with AIH at our center.

PATIENTS AND METHODS

This study was approved by the institutional review board at Indiana University School of Medicine. We examined all-patients receiving their first LT for AIH during 2000–2017 at our center. We included patients with at least 6-mo follow-up post-LT and a history of pre-LT AIH based on elevated antinuclear antibody (ANA) or smooth muscle antibody (ASMA) titers, increased IgG level >1.5 upper limit of normal, negative viral serologies, absence of alcohol abuse, and compatible histology on pre-LT or explant finding review. Patients with AIH and overlapping liver disease, such as primary biliary cholangitis or primary sclerosing cholangitis, or those undergoing multiorgan transplant were excluded. Demographic and clinical characteristics of LT recipients were reviewed, as well as any documented noncompliance with medications. The HLA-DR 3,4 and overall DR mismatch at LT were determined. Induction, initial and subsequent immunosuppression, was determined. Immunosuppression induction and maintenance therapy of our center evolved during the study period and as previously described.[5] rATG was not used in 2000. Between 2001 and 2005, induction consisted of rATG and after 2005, it consisted of rATG and a dose of rituximab. The initial maintenance immunosuppression agent used was tacrolimus.

Patients were followed from LT until last follow-up in 2018 if alive, retransplant or death, irrespective of R-AIH. Post-LT liver biopsies were performed for clinical indications and not per protocol. Indications for liver biopsy were persistent transaminitis in the absence of identified viral, biliary, or vascular disease. Acute (cell-mediated) and chronic rejection of the liver allograft were diagnosed according to consensus recommendations.[6-8] Testing for antibody-mediated rejection with donor-specific antibodies and c4d staining of liver biopsies were not routinely performed in the cohort. Atypical, plasma-rich rejection could also not be differentiated from R-AIH; however, per the updated Banff Criteria, this entity can only be diagnosed in patients without underlying AIH.[8]

Definition of Recurrent Autoimmune Hepatitis

There are no universally accepted diagnostic criteria for R-AIH, but the most uniformly reported criteria include abnormal liver biochemistries, compatible histology and absence of viral hepatitis (A, B, C, cytomegalovirus), cell-mediated rejection, or other competing forms of liver injury, noted in all methodologies reviewed here.[4,9-19] Hepatitis E serologies were not tested in the cohort. Other criteria are less consistently used, including autoantibodies or elevated immunoglobulin (Ig)/globulins (41% of methodologies reviewed) and response to oral steroids or increased immunosuppression (18% of methodologies reviewed).[4,9-19]

In this cohort and analysis, R-AIH was clinically diagnosed based on (1) sustained elevation in liver enzymes, (2) histological features of portal or lobular lymphoplasmacytic hepatitis with or without interface hepatitis, and (3) absence of histological features of cell-mediated rejection (including central perivenulitis), biliary obstruction, viral hepatitis, granulomatous disease, alcohol use, or suspected drug-induced liver injury. These criteria reflect those used by multiple studies.[9,10,13-17,20-22] While not uniformly used for the clinical diagnosis of R-AIH, here we examined elevations of ANA or ASMA titers and increased IgG or gamma-globulin levels when available and, additionally, the biochemical transaminase response to oral steroid (no patients received intravenous steroids [prednisone initial dose 40–60 mg daily with taper]) in patients with clinically diagnosed R-AIH. We also calculated the simplified criteria score for AIH in cases with evaluable data.[23]

Analysis

Baseline patient and transplant related factors were compared in patients with and without R-AIH. Categorical variables were compared using the chi-square of Fisher exact test, and continuous variables with nonparametric methods using the Mann-Whitney test. Clinical outcomes were compared in patients with and without R-AIH, including histologic outcomes (advanced fibrosis) and patient and graft survival. Immunosuppression induction and maintenance therapy at discharge from LT hospitalization and at the time of R-AIH (in cases of R-AIH) or last follow-up (in cases of no R-AIH) were compared, as were rates of acute and chronic rejection, biliary and vascular complications, and de novo malignancy. Cause of death was determined when possible for all deaths. We assessed the association of clinical factors with R-AIH using univariable Cox proportional hazard regression, and factors achieving a P value <0.1 were entered into a multivariable (1 step) model. Finally, for descriptive purposes, we explored any observed interplay of immunosuppression maintenance and incidence of R-AIH in patients with or without categorical risk factors identified by the multivariable modeling. Analyses were performed using Stata SE 15 (College Station, TX). All comparisons were 2 sided, with significance at a P value <0.05.

RESULTS

Of 2305 patients undergoing their first LT during the study period, 105 patients had a reported indication of AIH. However, 12 of the 105 patients were excluded for non-AIH diagnoses on review of clinical and or explant findings (fatty or cryptogenic liver disease 7, drug-induced liver injury 3, primary biliary cholangitis 1, and alpha-1-antitrypsin deficiency 1). Additionally, we excluded 9 patients with overlapping or other liver diseases (primary biliary cholangitis 5, primary sclerosing cholangitis 3, hepatitis C infection 1), 7 for early mortality (within 6 mo without R-AIH), and 2 for multiorgan transplants.

The study cohort comprised 75 patients who underwent LT for AIH, mean age 45 ± 16 y (range, 16–69), and 45 (65%) female individuals. During a median follow-up period of 8.3 y (interquartile range [IQR], 3.2–12.1), 20 patients (27%) developed graft dysfunction with R-AIH based on histologic features and exclusion of competing causes. The mean Ishak activity scores at the time of R-AIH was 9.3 ± 3.3 (score components A-interface hepatitis 2.7 ± 1.3, B-confluent necrosis 2 ± 1.9, C-focal necrosis/inflammation 2.4 ± 1, and D-portal

inflammation 2.3 ± 1.2). Titers for ANA or ASMA at the time of recurrence and IgG levels were obtained in 6 and 5 patients, respectively, and were abnormal consistent with a clinical diagnosis of R-AIH. All patients with R-AIH also had a response to steroids and or additional immunosuppression, while none were treated with intravenous steroids. The mean evaluable simplified AIH score was 7 (range, 6–8), suggesting consistency with probable or definite AIH, though this scoring system is not validated in LT. The simplified AIH scores pre-LT were 7.7 ± 0.5 in those with R-AIH and 7.4 ± 0.9 in those without R-AIH ($P = 0.4$).

In patients with R-AIH, disease recurred at a median interval of 485 d (IQR, 169–1338), with an incidence of 14% by 1 y, 21% by 3 y, 27% by 5 y, and 32% by 10 y. Baseline demographic, clinical, and transplant factors were compared in patients with and without R-AIH (Table 1). Patients with R-AIH were younger, more frequently Black, and with recipient-donor level 2 HLA-DR mismatch (mismatch at 2 DR loci versus 1 locus or no locus mismatch). Of note, level 2 HLA-DR mismatch was observed in similar proportions of White (46%) and Black (57%) patients ($P = 0.5$).

Immunosuppression

All but 4 patients received antithymocyte globulin induction, and all patients received tacrolimus-based immunosuppression initially. Immunosuppression at discharge from LT hospitalization, 1 and 5 y post-LT, and at the time of R-AIH (in cases of R-AIH) or last follow-up (in cases of no R-AIH) were compared (Table 2). Patients with R-AIH were more frequently receiving 3 immunosuppressive agents initially, but rates of early steroid use were low in both groups. There were no differences in the agents used, multiple agent therapy or steroid use at 4 mo, 1, or 5 y post-LT. However, when immunosuppression was examined in patients with R-AIH at the time of disease recurrence, they were more frequently on a single immunosuppressive agent compared with the last documented maintenance regimen in patients without R-AIH.

Liver Transplant Complications

Liver transplant complications were compared in patients with and without R-AIH (Table 3). Acute cellular rejection was more common in patients with R-AIH, particularly late acute cellular rejection (>1 y post-LT), as was chronic rejection. De novo malignancies were observed more frequently in patients without (25%) versus with (10%) R-AIH ($P = 0.12$), with a trend for more frequent malignancies excluding squamous and basal cell skin cancer in 20% versus 5%, respectively ($P = 0.08$). Patients without R-AIH and these latter malignancies included 4 patients with posttransplant lymphoproliferative disorder, 2 with colon cancer, 2 with melanoma, and 1 each with lung, bladder, prostate, and metastatic squamous cell cancer of the head and neck. One patient with R-AIH developed posttransplant lymphoproliferative disorder. All but 2 patients with de novo malignancy were maintained on multiple immunosuppressive agents at the time of developing de novo malignancy.

TABLE 1.

Baseline demographic and clinical characteristics and donor risk index in patients with and without recurrent autoimmune hepatitis

	R-AIH (n = 20)	No R-AIH (n = 55)	*P*
Age at transplant	35.3 ± 11.8	45 ± 15.3	0.001
Female gender	13 (65)	36 (65)	0.9
Body mass index (kg/m^2)	27.2 ± 5.1	27.6 ± 6.3	0.9
Race			
White	12 (60)	48 (87)	0.015
Black	8 (40)	6 (11)	
Other	None	1 (2)	
Cirrhosis on explant	15 (75)	48 (87)	0.2
HLA-DR4[a]			
Recipient	3 of 17 (18)	10 of 48 (21)	0.5
Donor	3 of 20 (15)	17 of 55 (31)	0.13
Mismatch	4 of 17 (23)	20 of 48 (42)	0.15
HLA-DR mismatch level[a]			
None or 1 mismatch	6 of 17 (35)	27 of 48 (56)	0.1
2 mismatches	11 of 17 (65)	21 of 48 (44)	

Data are shown as mean ± SD or number (percentage of patients with available data) unless specified as otherwise.
[a]HLA-DR loci of the recipient were missing in 10 patients (9 underwent transplant before 2003), and only 1 recipient and 1 donor had HLA-DR3 limiting any meaningful analysis.
IQR, interquartile range; R-AIH, recurrent autoimmune hepatitis.

TABLE 2.

A comparison of immunosuppression, acute and chronic rejection, biliary complications, and de novo malignancy in patients with and without recurrent autoimmune hepatitis

	R-AIH (n = 20)	No R-AIH (n = 55)	*P*
Immunosuppression induction			
No antithymocyte globulin	2 (10)	2 (4)	0.9
Antithymocyte globulin	4 (20)	17 (31)	
Antithymocyte globulin and rituximab	14 (70)	36 (65)	
Immunosuppression at discharge from liver transplant hospitalization			
Main immunosuppressive agent used			
Tacrolimus	17 (85)	52 (94)	0.2
Cyclosporine	2 (10)	3 (7)	
Sirolimus	1 (1)	None	
Second agent used			
Mycophenolate mofetil	9 (45)	21 (38)	0.4
Azathioprine	1 (5)	4 (7)	0.6
Prednisone	5 (25)	15 (27)	0.5
Two or more agents used	11 (55)	33 (60)	0.4
Immunosuppression at autoimmune hepatitis recurrence or last follow-up if no disease recurrence			
Main immunosuppressive agents used			
Tacrolimus	16 (80)	44 (80)	1
Cyclosporine	2 (10)	3 (5)	0.5
Sirolimus	1 (5)	7 (13)	0.3
Mycophenolate mofetil (alone)	None	1 (2)	0.6
Second agent used			
Mycophenolate mofetil	10 (50)	33 (60)	0.4
Azathioprine	1 (5)	6 (11)	0.4
Prednisone	None	4 (8)	0.17
Two or more agents used at R-AIH or last follow-up[a]	10 (50)	39 (70)	0.08

Data are shown as number (percentage).
[a]Three of 49 patients on multiple agents were on 3 agents; otherwise this reflects dual-agent regimens.
R-AIH, recurrent autoimmune hepatitis.

TABLE 3.

A comparison of liver transplant complications including acute and chronic rejection and biliary and vascular complications in patients with and without recurrent autoimmune hepatitis

	R-AIH (n = 20)	No R-AIH (n = 55)	*P*
Acute cellular rejection			
At any interval post-LT	11 (55)	15 (73)	0.03
Occurring >1 y post-LT	5 of the 11 (45)	2 of the 15 (14)	0.06
Chronic rejection	5 (25)	2 (4)	0.01
Biliary complications[a]	14 (70)	30 (55)	0.2
Vascular complications[b]			
Hepatic artery	4 (20)	2 (4)	0.11
Hepatic vein	1 (5)	2 (4)	
Portal vein	2 (10)	2 (4)	

Data are shown as number (percentage).
[a]Biliary strictures responding to stenting, with 3 patients undergoing surgical revision of the biliary anastomosis.
[b]Vascular complications include thrombosis or stenosis of the respective vessels.
LT, liver transplantation; R-AIH, recurrent autoimmune hepatitis.

TABLE 4.

The baseline clinical factors associated with recurrent autoimmune hepatitis after liver transplantation on univariable and multivariable Cox proportional hazard analyses

	Univariable analysis			Multivariable analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age	0.95	0.92-0.98	0.002	0.96	0.92-0.99	0.02
Black race (reference White)	4.3	1.7-10.6	0.002	4.4	1.6-11.9	0.004
Level 2 HLA-DR mismatch (reference none or 1 mismatch)	2.5	0.92-6.8	0.07	3.3	1.2-9.4	0.02
Female gender	1	0.4-2.6	0.9			
Fulminant disease at transplant (reference cirrhosis)	1.9	0.7-5.3	0.2			
Immunosuppression induction (reference no thymoglobulin)						
Thymoglobulin	0.4	0.1-2.2	0.3			
Thymoglobulin and rituximab	0.8	0.2-3.7	0.8			
Dual agents at initial immunosuppression (reference single agent)	0.97	0.4-2.3	0.9			
Prednisone use in initial immunosuppression	0.4	0.15-1.3	0.13			
Recipient HLA-DR 4 status (reference negative)	0.8	0.2- 2.7	0.7			
Donor HLA-DR4 status (reference negative)	0.4	0.12-1.4	0.14			

CI, confidence interval; HR, hazard ratio.

Patient and Graft Survival

The median interval from LT to last available biopsy was 2.6 y (IQR, 6 mo–7.8 y), which was not significantly different in patients with and without R-AIH. Patients with R-AIH had more frequent advanced fibrosis (F3 or F4) (26%) than those without R-AIH (4%) and less frequent absence of fibrosis (F0) (39%) than those without R-AIH (77%) ($P = 0.02$). Six patients with R-AIH progressed to ≥F3 fibrosis, with 1 re-LT and 1 liver-related death.

Remembering that patients who died within 6 mo of LT were excluded, there were no differences in 1-, 5-, and 10-y patient survival in patients with R-AIH (100%, 82%, and 82%) and patients without R-AIH (96%, 90%, and 82%), respectively ($P = 0.2$). There were also no differences in 1-, 5-, and 10-y graft survival in patients with R-AIH (100%, 82%, and 82%) and patients without R-AIH (94%, 91%, and 83%), respectively ($P = 0.13$).

Despite similar patient and graft survival the causes of death differed between patients with and without R-AIH. In all, 5 (62%) of 8 deaths in patients with R-AIH were due to immune injury-related graft loss (2 attributed to R-AIH and 3 attributed to rejection) compared with none of the deaths in patients without R-AIH. In contrast, 6 (55%) of 11 deaths in patients without R-AIH were due to de novo malignancy, compared with none of the deaths in patients with R-AIH. Deaths due to infection and other causes were similar in patients with and without R-AIH (Table S1, SDC, http://links.lww.com/TXD/A327).

Factors Associated With Recurrent AIH

The factors associated with R-AIH were analyzed by univariable and multivariable Cox proportional hazard regression analyses (Table 4). Younger age, Black recipient race, and level 2 HLA-DR mismatch were independently associated with R-AIH (Figure 1A and B). The results were similar in a post hoc model adjusting for factors not known at baseline, including the use of multiple versus single immunosuppressive agents at the time of R-AIH or last follow-up in patients without R-AIH. Additional sensitivity analyses were performed to examine the predictors of R-AIH while excluding the 4 patients who did receive rATG induction. The findings were similar (Black race: HR, 4.9; 95% confidence interval [CI], 1.7-13.6; $P = 0.003$; level 2 HLA-DR mismatch: HR, 3.4; 95% CI, 1.2-10.3; $P = 0.03$; age: HR, 0.96; 95% CI, 0.9-1; $P = 0.05$). Further excluding patients not receiving rituximab induction, the results were similar although *P* values were larger with the smaller subgroup analyzed (Black race: HR, 4.7; 95% CI, 1.5-14.5; $P = 0.008$; level 2 HLA-DR mismatch: HR, 3.1; 95% CI, 1-9.7; $P = 0.05$; age: HR, 0.99; 95% CI, 0.9 3-1; $P = 0.1$).

For descriptive purposes, we explored any observed interplay of immunosuppression maintenance (single versus multiple agents) and incidence of R-AIH in patients with or without the binary risk factors identified by the multivariable modeling (Black recipient race versus White and level 2 HLA-DR mismatch versus none or level 1). The highest rates of R-AIH were observed with single-agent immunosuppression in Black recipients (71%) and in level 2 HLA-DR mismatch (61%) (Table 5). There was a trend for lower R-AIH (0.6-fold reduction) with multiple versus single-agent immunosuppression in level 2 DR mismatch and a smaller and nonsignificant reduction (0.39-fold) in Black recipients. Finally, rates of R-AIH were not increased and were numerically similar (range, 17%–26%) with single or multiple agent immunosuppression in White recipients or those with no or level 1 DR mismatch.

With the lack of clear definition of R-AIH in the field and among studies, at our center, we examined the impact of study definition of R-AIH on the analysis of predictors of disease recurrence. Autoantibodies were available and elevated in 11 of 20 patients with R-AIH. IgG levels were available and elevated in 5, and both autoantibodies and IgG levels in only 3 of the patients with R-AIH. We examined the frequency of

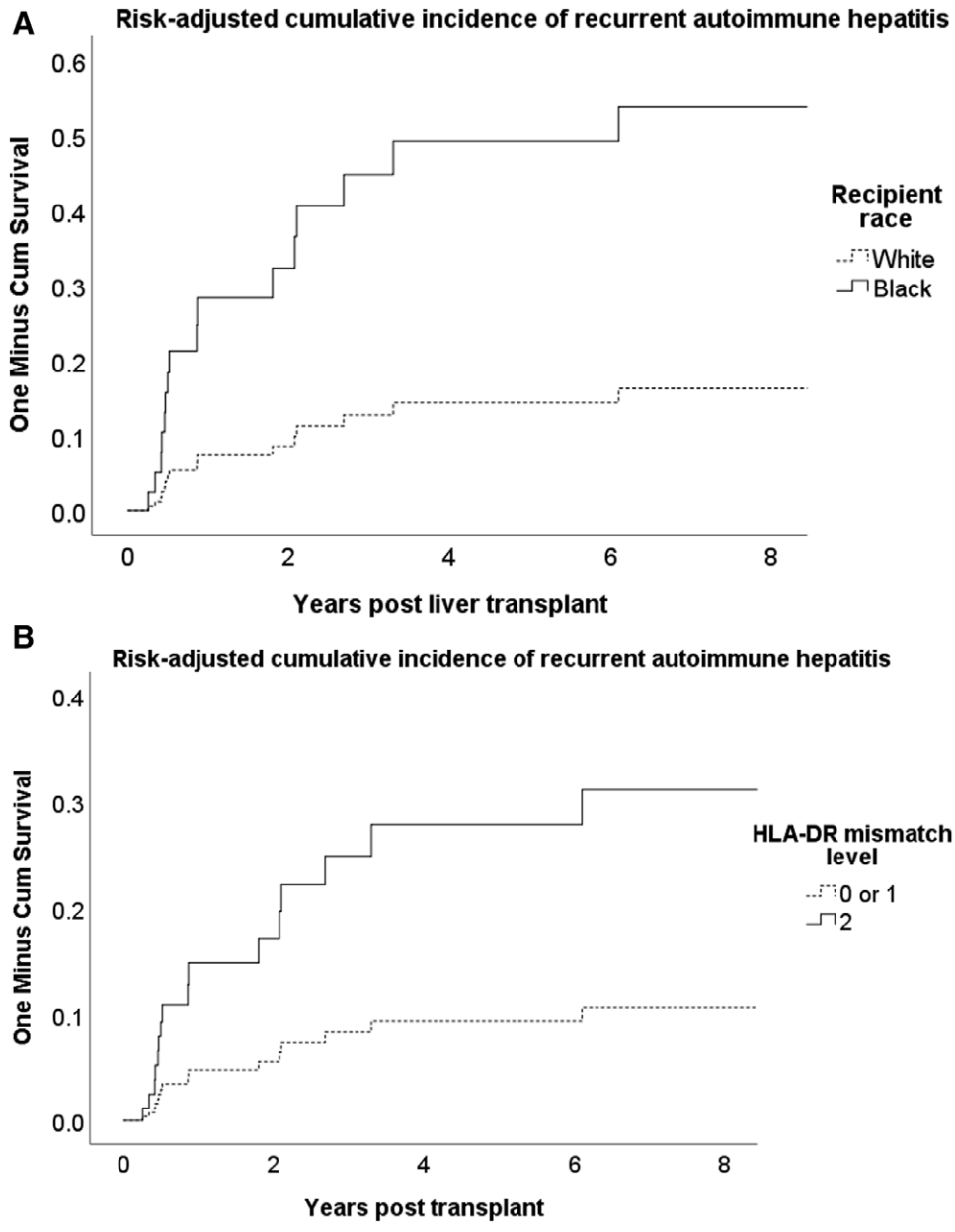

FIGURE 1. The risk-adjusted cumulative incidence of recurrent autoimmune hepatitis per recipient race (A) and HLA-DR mismatch (B).

valid autoantibody titers in the identified high- and low-risk groups. They were available in similar proportions of Black (4 of 8) versus White (5 of 12) patients or evaluable patients with level 2 HLA-DR mismatch (5 of 11) versus 1 or none (3 of 6) with R-AIH. The number of patients with available Ig levels was too small to allow for a sensitivity analysis. However, we performed a post hoc multivariable Cox proportional hazard model while excluding patients with R-AIH, but missing autoantibody titers at disease recurrence and results were unchanged. The hazard ratios (HRs) were 0.96 for age (95% CI, 0.9-1.00; $P = 0.08$), 5.4 for Black race (95% CI, 1.3-22.6; $P = 0.02$), and 4.8 for level 2 HLA-DR mismatch (95% CI, 1.1-21.4; $P = 0.04$).

DISCUSSION

This is a relatively large cohort of patients with AIH undergoing LT with predominantly rATG and tacrolimus-based immunosuppression, with low rates of steroid use. Despite this, we observed a similar rate of R-AIH (27%) compared with other studies (28%), albeit at a shorter median time to recurrence of 16 mo contrasting with 30 mo (range, 12–60) in other studies.[4,13,16-18,21,24-28] While this suggests that strong immunosuppression induction with rATG did not apparently prevent or delay risk of disease recurrence, the numbers of patients not receiving rATG was very small and no conclusions can be drawn on the impact of rATG induction on risk of R-AIH.

Important findings included the independent associations of Black race (relative to White) and level 2 HLA-DR mismatch (relative to no or 1 locus mismatch) with R-AIH. The observation of more aggressive behavior of AIH post-LT in Black patients may not be surprising. AIH is known to be more aggressive among Black patients, despite medical therapy.[29,30] However, to our knowledge, there have been

TABLE 5.

Rates of recurrent autoimmune hepatitis post–liver transplantation dichotomized by the identified risk factors of Black recipient race (vs White) and level 2 (vs no or level 1) HLA-DR mismatch, with single-agent vs multiple-agent immunosuppression

Immunosuppression	High risk factor	Low risk factor	
	R-AIH in Black recipients (n = 14)	R-AIH in White recipients (n = 60)	*P* comparing Black vs White recipients with single or multiple agent immunosuppression
Single agent	5 of 7 (71%)	5 of 19 (26%)	0.04
Multiple agents	3 of 7 (43%)	7 of 41 (17%)	0.12
P comparing singe vs multiple agents within same race recipients	0.3	0.3	
	R-AIH in level 2 HLA-DR mismatch (n = 32)	R-AIH in no or level 1 HLA-DR mismatch (n = 33)	*P* comparing HLA-DR mismatch category with single or multiple agent immunosuppression
Single agent	5 of 8 (62%)	2 of 12 (17%)	0.03
Multiple agents	6 of 24 (25%)	4 of 21 (19%)	0.6
P comparing single vs multiple agents within HLA-DR mismatch category	0.05	0.9	

Data are shown as number (%).
R-AIH, recurrent autoimmune hepatitis.

little to no data describing increased risk of disease recurrence in Black patients undergoing LT for AIH. We observed that Black recipients had twice the rate of R-AIH compared with Whites regardless of stratification by single or multiple agent immunosuppression. Three of 7 Black patients on multiple agent immunosuppression still developed R-AIH, highlighting the need for closer monitoring and potentially more potent immunosuppression in Black patients with AIH undergoing LT.

As for level 2 HLA-DR mismatch, a biologic explanation is less clear. However, level 2 mismatch was noted in approximately half of the study cohort and may impact a large proportion of patients undergoing LT. We observed a 3-fold higher risk of R-AIH in patients with high risk (level 2) mismatch on single compared with multiple agents, but relatively low and numerically similar rates of R-AIH in single and multiple agents in patients with no or level 1 mismatch. Without intending to overstate these observations, they appear to lend plausibility of high-level DR mismatch as a risk factor for R-AIH, as are findings of increased R-AIH with DR mismatch in a study from the National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database.[31] Interestingly, 5 of 7 patients developing R-AIH in another series also had level 2 HLA-DR mismatch (examination of tables).[12] Ultimately, heterogeneity in risk factors for R-AIH among reporting centers is well recognized, and one cannot exclude associations of other risk factors not identified in the analysis due to sample characteristics and size limitations. The increased risk of R-AIH with younger patient age that we observed has been previously described and adds validation to the cohort.

Although we noted a trend for R-AIH developing more frequently in patients on a single rather than 2 or more immunosuppressive agents, this was not associated with R-AIH in the post hoc risk-adjusted analysis. Admittedly this subanalysis was limited by sample size, particularly when examining Black recipients. Yet exceptionally high rates of R-AIH were observed in high-risk patients (Black and level 2 HLA-DR mismatch) on single-agent immunosuppression. In other words, single-agent immunosuppression may be insufficient and deleterious for risk of R-AIH in recipients with those risk factors.

In contrast, we observed higher rates of de novo malignancy in patients without R-AIH most of whom were on multiple agents. De novo malignancy is a recognized risk of immunosuppression, particularly in older patients and with multiple agents.[32] This observation may suggest a potential of overimmunosuppression in those patients developing malignancies. Our data suggest that older recipients are at lower risk for AIH; furthermore, there was no observed benefit of multiple-agent immunosuppression for lower R-AIH in White recipients or in those with no or level 1 DR mismatch.

These contrasting considerations are most starkly demonstrated in the causes of death among patients with and without R-AIH. Although R-AIH was not associated with differences in patient or graft survival, patients with R-AIH had more immune-mediated causes of death and graft loss including R-AIH and rejection, while those without R-AIH had more de novo malignancy-related deaths. These observations underscore the need for a personalized approach to achieving the optimal balance of risk and benefit with immunosuppression in patients undergoing LT for AIH.

These data lead us to speculate that the benefits of multiple-agent immunosuppression in preventing R-AIH may be important in Black recipient and or cases of level 2 HLA-DR mismatch. Conversely, single-agent regimens may be adequate to minimize the risk of R-AIH and potentially mitigate the risk of overimmunosuppression in White LT recipients with low-risk HLA-DR mismatch. Of note, the majority of multiple agent immunosuppression in this cohort reflected dual-agent regimens, and the comparisons reported largely reflect single- versus dual-agent therapies. Ultimately, more data are needed to corroborate these associations and examine the impact of differing immunosuppression regimens and protocols on R-AIH and complications such as de novo malignancy.

The diagnosis of R-AIH differs between studies, and there are no sanctioned diagnostic criteria, particularly in the context of retrospective observational studies. This warrants discussion, and we were careful in our methods to collate the diagnostic criteria reported in the literature. The clinical

criteria that were most common among studies included histology findings, transaminitis, and absence of competing causes of liver injury including rejection.[4,9-19] In clinical experience of our center, R-AIH was diagnosed based on those salient features as well. We also included the response to oral steroid (no intravenous steroids) therapy to lend support for the clinical diagnosis made in the course of clinical care. Autoimmune serologies and IgG levels *were also* diagnostic *when* available. These data lend support for the validity of describing risk factors and outcomes in those patients with missing IgG levels or ANA/ASMA titers as having R-AIH when they met the salient clinical and treatment response features. The sensitivity analysis excluding patients without autoantibody titers at the time of R-AIH did not alter the factors associated with disease recurrence. More broadly, a definition of R-AIH that demands the availability of elevated autoantibodies and IgG levels would negate or exclude multiple studies[9,10,13-17,20-22] our study included. Enforcing such a limitation would limit much of our ability to understand the risk of recurrent disease in an area that already suffers from limited patient numbers. However, one must acknowledge the challenge posed by the absence of diagnostic criteria for R-AIH. This highlights the need for explicit description, transparency, and consideration of the diagnostic criteria used for R-AIH when contributing or inclusively interpreting the literature.

The study has a number of limitations including the retrospective nature and small sample size inherent to this area of research. That said, this report represents one of the largest cohorts of LT for AIH and points to viable and prevalent risk factors for disease recurrence. Despite this, the study may have missed important factors that are associated with R-AIH due to limited sample size. The lack of a clear definition of R-AIH in the field is also notable, but we broadly collated multiple methodologies and used the key clinical criteria common to all examined studies, including biochemical and histologic disease with exclusion of competing causes. We demonstrated validity of these criteria with steroid treatment response and with the simplified diagnostic criteria when antibody or Ig data were available. Liver biopsies were performed for clinical indications and not per protocol, and biochemically silent R-AIH may have been missed, although there was a long follow-up in the cohort (median >8 y) and causes of death or graft failure were examined closely. Also, testing for antibody-mediated rejection with donor-specific antibodies and c4d staining of liver biopsies were not performed in the cohort with the majority of biopsies diagnosing R-AIH predating the guidelines to establish that diagnosis.[8] Atypical, plasma rich rejection could also not be differentiated from R-AIH; however, per the updated Banff Criteria, this entity can only be diagnosed in patients without underlying AIH.[8] Hepatitis E serologies were not routinely tested in the cohort of patients with R-AIH. Finally, the findings from this single-center study may lack wider applicability without validation with strict definitions of R-AIH.

In summary, R-AIH in our cohort occurred in 27% of patients, and early recurrence was not apparently mitigated by potent immunosuppression induction with rATG. Patient or graft survival was not impacted by R-AIH, but the contrasting causes of death in patients with and without R-AIH suggest a potential need for a personalized approach to immunosuppression in this population. Younger age, Black race (an expected but novel description post-LT), and high-level HLA-DR mismatch were independently associated with risk of R-AIH and may serve as useful considerations to guide single- versus multiple-agent maintenance immunosuppression. Additional data are needed to corroborate these and other reported risk factors in the ongoing effort to optimize outcomes for this unique population of LT recipients.

REFERENCES

1. Beal EW, Black SM, Michaels A. Autoimmune hepatitis in the liver transplant graft. *Clin Liver Dis*. 2017;21:381–401.
2. Gautam M, Cheruvattath R, Balan V. Recurrence of autoimmune liver disease after liver transplantation: a systematic review. *Liver Transpl*. 2006;12:1813–1824.
3. Montano-Loza AJ, Bhanji RA, Wasilenko S, et al. Systematic review: recurrent autoimmune liver diseases after liver transplantation. *Aliment Pharmacol Ther*. 2017;45:485–500.
4. Krishnamoorthy TL, Miezynska-Kurtycz J, Hodson J, et al. Longterm corticosteroid use after liver transplantation for autoimmune hepatitis is safe and associated with a lower incidence of recurrent disease. *Liver Transpl*. 2016;22:34–41.
5. Mangus RS, Fridell JA, Vianna RM, et al. Immunosuppression induction with rabbit anti-thymocyte globulin with or without rituximab in 1000 liver transplant patients with long-term follow-up. *Liver Transpl*. 2012;18:786–795.
6. Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology*. 1997;25:658–663.
7. Demetris A, Adams D, Bellamy C, et al. Update of the International Banff Schema for Liver Allograft Rejection: working recommendations for the histopathologic staging and reporting of chronic rejection. An international panel. *Hepatology*. 2000;31:792–799.
8. Demetris AJ, Bellamy C, Hübscher SG, et al. 2016 comprehensive update of the Banff working group on liver allograft pathology: introduction of antibody-mediated rejection. *Am J Transplant*. 2016;16:2816–2835.
9. Ayata G, Gordon FD, Lewis WD, et al. Liver transplantation for autoimmune hepatitis: a long-term pathologic study. *Hepatology*. 2000;32:185–192.
10. Cárdenas Ramírez B, Padilla-Machaca PM, Mantilla Cruzatti O, et al. [Autoimmune hepatitis recurrence after liver transplantation: 15 years experience in Peru]. *Rev Gastroenterol Peru*. 2018;38:44–48.
11. Dbouk N, Parekh S. Impact of pretransplant antinuclear antibody and antismooth muscle antibody titers on disease recurrence and graft survival following liver transplantation in autoimmune hepatitis patients. *J Gastroenterol Hepatol*. 2013;28:537–542.
12. Duclos-Vallée JC, Sebagh M, Rifai K, et al. A 10 year follow up study of patients transplanted for autoimmune hepatitis: histological recurrence precedes clinical and biochemical recurrence. *Gut*. 2003;52:893–897.
13. González-Koch A, Czaja AJ, Carpenter HA, et al. Recurrent autoimmune hepatitis after orthotopic liver transplantation. *Liver Transpl*. 2001;7:302–310.
14. Heffron TG, Smallwood GA, Oakley B, et al. Autoimmune hepatitis following liver transplantation: relationship to recurrent disease and steroid weaning. *Transplant Proc*. 2002;34:3311–3312.
15. Molmenti EP, Netto GJ, Murray NG, et al. Incidence and recurrence of autoimmune/alloimmune hepatitis in liver transplant recipients. *Liver Transpl*. 2002;8:519–526.
16. Montano-Loza AJ, Mason AL, Ma M, et al. Risk factors for recurrence of autoimmune hepatitis after liver transplantation. *Liver Transpl*. 2009;15:1254–1261.
17. Reich DJ, Fiel I, Guarrera JV, et al. Liver transplantation for autoimmune hepatitis. *Hepatology*. 2000;32(Pt 1):693–700.
18. Vogel A, Heinrich E, Bahr MJ, et al. Long-term outcome of liver transplantation for autoimmune hepatitis. *Clin Transplant*. 2004;18:62–69.
19. Yusoff IF, House AK, De Boer WB, et al. Disease recurrence after liver transplantation in Western Australia. *J Gastroenterol Hepatol*. 2002;17:203–207.
20. Campsen J, Zimmerman MA, Trotter JF, et al. Liver transplantation for autoimmune hepatitis and the success of aggressive corticosteroid withdrawal. *Liver Transpl*. 2008;14:1281–1286.
21. Narumi S, Hakamada K, Sasaki M, et al. Liver transplantation for autoimmune hepatitis: rejection and recurrence. *Transplant Proc*. 1999;31:1955–1956.

22. Wright HL, Bou-Abboud CF, Hassanein T, et al. Disease recurrence and rejection following liver transplantation for autoimmune chronic active liver disease. *Transplantation*. 1992;53:136–139.
23. Yeoman AD, Westbrook RH, Al-Chalabi T, et al. Diagnostic value and utility of the simplified International Autoimmune Hepatitis Group (IAIHG) criteria in acute and chronic liver disease. *Hepatology*. 2009;50:538–545.
24. Milkiewicz P, Hubscher SG, Skiba G, et al. Recurrence of autoimmune hepatitis after liver transplantation. *Transplantation*. 1999;68:253–256.
25. Prados E, Cuervas-Mons V, de la Mata M, et al. Outcome of autoimmune hepatitis after liver transplantation. *Transplantation*. 1998;66:1645–1650.
26. Puustinen L, Boyd S, Arkkila P, et al. Histologic surveillance after liver transplantation due to autoimmune hepatitis. *Clin Transplant*. 2017;31:e12936.
27. Ratziu V, Samuel D, Sebagh M, et al. Long-term follow-up after liver transplantation for autoimmune hepatitis: evidence of recurrence of primary disease. *J Hepatol*. 1999;30:131–141.
28. Renz JF, Ascher NL. Liver transplantation for nonviral, nonmalignant diseases: problem of recurrence. *World J Surg*. 2002;26:247–256.
29. Verma S, Torbenson M, Thuluvath PJ. The impact of ethnicity on the natural history of autoimmune hepatitis. *Hepatology*. 2007;46:1828–1835.
30. de Boer YS, Gerussi A, van den Brand FF, et al; Dutch Autoimmune Hepatitis Study Group. Association between Black race and presentation and liver-related outcomes of patients with autoimmune hepatitis. *Clin Gastroenterol Hepatol*. 2019;17:1616–1624.e2.
31. Balan V, Ruppert K, Demetris AJ, et al. Long-term outcome of human leukocyte antigen mismatching in liver transplantation: results of the National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. *Hepatology*. 2008;48:878–888.
32. Bhat M, Mara K, Dierkhising R, et al. Immunosuppression, race, and donor-related risk factors affect de novo cancer incidence across solid organ transplant recipients. *Mayo Clin Proc*. 2018;93:1236–1246.