ORIGINAL ARTICLE

Recipient risk factors for acute cellular rejection after orthotopic liver transplant - a single-center, retrospective study

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SUMMARY

The use of model for end-stage liver disease (MELD) score for liver allocation has resulted in transplanting sicker patients. As such, it is unclear whether the risk factors and severity of acute cellular rejection (ACR) have changed. To identify ACR characteristics where average MELD score at transplant is higher than previously published studies. This is a single-center, retrospective study designed to assess risk factors associated with ACR after adult orthotopic liver transplant (OLT) using a steroid sparing regimen. This study included 174 OLT patients transplanted from 2008 to 2013 at a single tertiary care center. Recipient demographics, preoperative clinical, and laboratory data were recorded for each transplant. Univariate and multivariate regression analyses were performed to identify variables that are significant predictors for ACR. The median MELD at transplantation was 29.5. The average time from transplant to ACR diagnosis was 283.9 days and a majority of ACR episodes were mild to moderate. Serum creatinine, primary sclerosing cholangitis etiology, and tacrolimus use were significant predictors for ACR ($P < 0.05$). This study confirmed a change in timing and severity of ACR in the MELD era. Recipient characteristics may affect the risk for developing ACR and should be considered when managing immunosuppression.

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Introduction

Acute cellular rejection (ACR) after adult orthotopic liver transplant (OLT) remains a significant cause of morbidity in the era of immunosuppressive therapy, with rates ranging from 10 to 40% while on triple therapy immunosuppressive protocols [1,2]. ACR is mediated by recipient T-cell activation against donor alloantigens and is dependent on the host inflammatory microenvironment to initiate and recruit effector cells at the time of transplant [3,4]. Additionally, ischemia–reperfusion injury and rapid neutrophilic infiltrate result in innate immune system activation and graft inflammation [5]. Modern immunosuppressive regimens for OLT attenuate this response by blunting T-cell immune response in combination with anti-inflammatory effect of corticosteroids. Recipients may differ in their intrinsic abilities to activate inflammatory and immune pathways, so immunosuppressant therapies would be better served if individually tailored based on ACR risk.

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Previous studies have consistently identified age, underlying liver disease, and creatinine, as well as other factors as possible predictors of ACR, as demonstrated by Table S1 [1,6-13]. In the pre-MELD era, a landmark study of large 762-patient Liver Transplant Database (LTD) was used to identify risk factors associated with rejection from 1990 to 1995 [7]. It was found that age, underlying liver disease and creatinine are significant predictive factors for rejection and have been consistent across multiple studies [7,8,12]. Of the described underlying liver disease, fulminant hepatitis, hepatitis B virus (HBV), and autoimmune hepatitis (AIH) had more frequent instances of rejection, possibly due to the pro-inflammatory state in this population. Heterogeneity of the study population was apparent as the rates of rejection differed based on the immunosuppression protocol [7]. Other studies suggest that underlying primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and hepatitis C virus (HCV) have a higher risk of ACR [1,11]. Additionally, creatinine> 2 mg/dl was associated with significantly lower rates of rejection, suggesting that renal dysfunction may suppress a robust immune response. However, this study used the LTD, which aggregated data from multiple centers that employ various immunosuppressive therapies.

In a single-center retrospective study from 2007 to 2010, Wang et al. compared the characteristics of ACR group versus non-ACR in 110 consecutive patients [14]. In patients with average MELD of 18–19, the authors identified age as a risk factor for ACR. The most consistent risk factors for ACR were found to be PSC/PBC etiology, young donor age, and renal function. In support of the first risk factor, Berlakovich et al. found that PBC was associated with more acute rejection episodes and increased rejection severity, suggesting that the recipient inflammatory milieu may be an important factor in rejection mediation [15].

The use of MELD score for liver allocation has resulted in transplanting sicker patients. As such, it is unclear whether the risk factors and severity of ACR have changed so we aimed to identify site-specific characteristics of ACR in our cohort of liver transplant patients during the similar periods of time when the landmark papers regarding risk for ACR were performed.

Materials and methods

Recipient and donor demographics, preoperative clinical and laboratory findings, and cytomegalovirus (CMV) immune status data were collected for 215 consecutive OLT patients from January 2008 to June 2013. The following exclusion criteria were applied: death within 6 months (most commonly due to sepsis and multiorgan failure), patients with histological findings of antibody-mediated rejection (during this period of time, C4d was not available), and retransplantation. None of the excluded deaths were due to ACR. After exclusion criteria were applied, 174 patients remained to be analyzed. Our analysis did not include patients after 2013 in order to avoid confounding factors that may affect transplant practice due to personal turnover after 2013 in our program, multiple changes in the regulatory policies that affected organ transplant allocation in the United States (use of MELD score versus MELD-Na, regional allocation of the organs), and introduction of direct antiviral agents for HCV with downstream effect on the type of liver disease-causing liver failure and indication for liver transplant.

Liver biopsies were only performed in the setting of suspicion for rejection based on the follow-up blood test results during the standard care and at the discretion of the transplant hepatologist and surgeon. Protocol biopsies were not routinely performed at specific time intervals for this cohort of patients.

ACR was diagnosed and graded based on pathology reports of our program's expert liver pathologist using Rejection Activity Index as per BANFF schema. Findings of acute rejection were identified by the presence of mixed inflammatory infiltrate within the portal triad, endotheliitis/venular inflammation, and destructive or nondestructive nonsuppurative cholangitis of the interlobular bile duct epithelium. These features were quantified in severity on a scale of 0 to 3 (0 none, 1 mild, 2 moderate, 3 severe). The three scores were added to determine Rejection Activity Index [4,16-19]. Timing of 6 months from liver transplant was considered the cutoff between early versus late ACR.

The management of ACR was generally at the discretion of the transplant physician, which warranted 3 days of high-dose steroids for moderate/severe cases (500–1000 mg methylprednisolone/day) and optimization of maintenance immunosuppression for mild cases of ACR (with the goal to achieve tacrolimus levels of 8–10 ng/ml). A calculated MELD score was derived from the last set of laboratory tests prior to transplant, without adjustments for dialysis or exception points for tumor size [20].

There were some quality improvement adjustments to the immunosuppression protocol between January 2008 and March 2013; therefore, the type of immunosuppressive regimens can be broken down into 4 different time periods (Table S2 with detailed changes over time of the

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protocol). Our center is steroid sparing center, with a protocol for discontinuation of steroids by day 5, and all patients received induction with either a polyclonal antibody or IL-2 receptor antagonist unless the underlying disease was hepatitis C from 2010 to 2013.

Univariate and multivariate analysis was applied for each variable using STATA v 13.0 software and $P < 0.05$ was used as threshold for significance. A multistep regression was performed in which variables with insignificant *P*-values (>0.2) were eliminated sequentially until a linear model with good fit was established. However, if clinically indicated, additional risk factors previously reported as associated with ACR were included in the multivariate logistic regression to find independent risk factors. The variables in this linear model were also fitted into a logistical regression because the variable ACR is a binary term.

Results

Characteristics of study population

Recipient demographics, main clinical findings as well as donor demographics and CMV status of donor/recipient are presented in Table 1. Patients in this study were predominantly male, 50% white Caucasians, with median age of 57. The primary indication for liver transplant was decompensated HCV cirrhosis (46.1%), as expected before introduction of direct antiviral agents in clinical practice. Median MELD score at transplantation was 29.5 and 32.8% had concurrent hepatocellular carcinoma. Of the 174 patients analyzed, 73% had ascites and 70% had overt porto-systemic hepatic encephalopathy. Twenty-five cases (14.3%) of simultaneous liver–kidney transplants were included, most commonly due to hepato-renal syndrome or concomitant end-stage renal disease.

Timing and severity of ACR

The overall incidence of biopsy-proven ACR was 26.4%. In our cohort, the average time from transplant to ACR was 283 days (approximately 9 months), with only 17% (8/47) of rejection episodes taking place prior to 6 weeks (Figure 1). Based on this timing, the majority of ACR cases were late ACR and mild in severity (56.5%, 26/46). When analyzed by case–control approach, patients diagnosed with ACR were more likely have PSC as the cause of their liver cirrhosis and were less likely to have undergone combined liver–kidney transplant (Table 1). No differences of demographics, clinical, and laboratory characteristics were identified between patients with early and late ACR (Table S3).

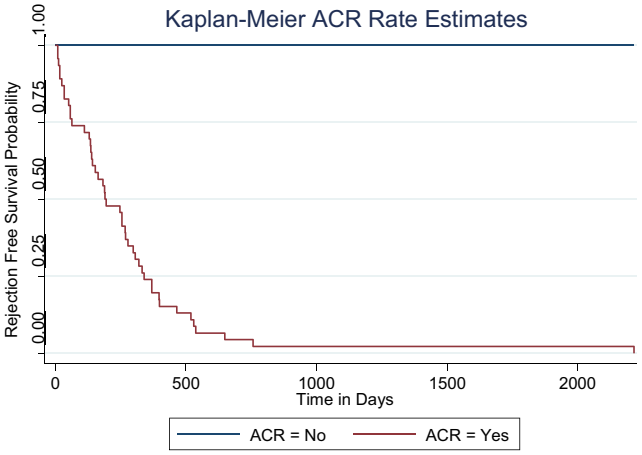

Figure 1 Acute Cellular Rejection Kaplan-Meier Survival Estimates.

The impact of immunosuppressive regimen on ACR rates

Next, we were interested to compare the rates of ACR during the four time periods in which changes to immunosuppression periods were made. There was one period timeframe with less ACR (07/2008–04/2009). (Tables S1 and S4) correlated with administration of daclizumab as induction for nearly all recipients (96.2%). In spite of this, there was no statistical difference in ACR rates (*P* 0.103) when broken down into the different immunosuppression protocols utilized and after ANOVA testing was performed.

Risk factors for ACR

Etiology of end-stage liver disease may impact immune response of the recipient so the top underlying liver pathologies were examined for association with ACR, including alcoholic liver disease, HCV, hepatocellular carcinoma (HCC), nonalcoholic steatohepatitis (NASH) and PSC/PBC. In a univariate analysis, creatinine, PSC, simultaneous liver–kidney transplant, and use of tacrolimus were found to be associated with higher rates of ACR (Table 2). Additional data analysis was done excluding patients with simultaneous liver–kidney transplant: PSC maintains statistical significance for ACR prediction while, as expected, creatinine was not. PSC etiology was positively correlated with both presence and severity of ACR: the average Banff of this group

Table 1. Recipients and donor characteristics

	Overall (n = 174)	No Acute Cellular Rejection (n = 128)	Acute Cellular Rejection (n = 46)	P value
	Recipient demographics			
Age (years), median (IQR)	57 (12)	57 (12)	57 (11)	0.868
Gender				
Female, *n* (%)	66 (37.9)	47 (36.7)	19 (41.3)	0.582
Male, *n* (%)	108 (62.1)	81 (63.3)	27 (58.7)	
Race				
White, *n* (%)	86 (49.4)	67 (52.3)	19 (41.3)	0.210
Hispanic, % (*n*)	42 (24.1)	32 (25.0)	10 (21.7)	
Black, *n* (%)	33 (19.0)	20 (15.6)	13 (28.3)	
Asian, *n* (%)	10 (5.8)	6 (4.7)	4 (8.7)	
Other, *n* (%)	3 (1.7)	3 (2.3)	0 (0)	
HCC, *n* (%)	56 (32.2)	43 (33.6)	13 (28.3)	0.507
Underlying liver disease				
HCV, *n* (%)	81 (46.6)	56 (43.8)	25 (30.9)	0.216
Alcohol, *n* (%)	54 (31.0)	38 (29.7)	16 (34.8)	0.522
NASH, *n* (%)	21 (12.1)	18 (14.1)	3 (6.5)	0.178
HBV, *n* (%)	11 (6.3)	10 (7.8)	1 (2.2)	0.178
Fulminant failure, *n* (%)	10 (5.8)	7 (5.5)	3 (6.5)	0.792
Autoimmune hepatitis, *n* (%)	6 (3.5)	4 (3.1)	2 (4.4)	0.697
PBC, *n* (%)	5 (2.9)	4 (3.1)	1 (2.2)	0.741
PSC, *n* (%)	7 (4.0)	2 (1.6)	5 (10.9)	0.006*
Cryptogenic cirrhosis, *n* (%)	8 (4.6)	7 (5.5)	1 (2.2)	0.360
Alpha 1 antitrypsin, *n* (%)	4 (2.3)	3 (2.3)	1 (2.2)	0.947
Sarcoidosis, *n* (%)	1 (0.6)	1 (0.8)	0 (0)	0.548
Amyloidosis, *n* (%)	2 (1.2)	2 (1.6)	0 (0)	0.394
Dialysis, *n* (%)	47 (27.0)	36 (28.1)	11 (23.9)	0.581
Ascites, *n* (%)	127 (73.0)	96 (75.0)	31 (67.4)	0.319
Encephalopathy, *n* (%)	122 (70.1)	87 (70.0)	35 (76.1)	0.302
Creatine (mg/dl), median (IQR)	1.55 (1.8)	1.6 (2.3)	1.3 (1.17)	0.047*
BUN (mg/dl), median (IQR)	20 (24)	22 (24.0)	17.5 (24.0)	0.117
T-bilirubin, median (IQR)	9.1 (16.3)	6.9 (16.3)	11.1 (18.4)	0.323
AST, median (IQR)	64 (70)	63 (76)	68.5 (68)	0.616
ALT, median (IQR)	37.5 (40.0)	37.5 (34.5)	38.5 (41)	0.887
INR, median (IQR)	2.2 (1.3)	2.2 (1.25)	2.4 (1.5)	0.183
PT, median (IQR)	25 (12.5)	24.6 (11.4)	26.5 (12.9)	0.137
Albumin, mean (SD)	2.6 (1.2)	2.7 (1.1)	2.5 (1.2)	0.323
Calculated MELD*, median (IQR)	29.5 (19.1)	29.3 (19.8)	29.9 (11.4)	0.859
Child Pugh, median (IQR)	11 (3)	11 (3)	11 (3)	0.446
Simultaneous Liver–Kidney, *n* (%)	25 (14.4)	23 (18.0)	2 (4.4)	0.024*
CMV Positive, *n* (%)	130 (74.7)	94 (73.4)	36 (78.3)	0.519
CMV High Risk (D+/R-)	28 (16.1)	20 (15.6)	8 (17.4)	0.780
CMV Moderate Risk (D+/R + or D-/R+)	130 (74.7)	94 (73.4)	36 (78.3)	0.519
CMV Low Risk (D-/R-)	15 (8.6)	13 (10.2)	2 (4.4)	0.229
CMV Unknown Risk (Donor Status Unknown)	1 (0.6)	1 (0.78)	0 (0)	0.548
	Donor demographics			
Age (years), median (IQR)	45.5 (25.5)	45 (43.3)	48 (43.8)	0.680
Gender				
Female, *n* (%)	53 (41.1)	37 (40.7)	16 (42.1)	0.879
Male, *n* (%)	76 (58.9)	54 (59.3)	22 (57.9)	

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Table 1. Continued.

	Overall (n = 174)	No Acute Cellular Rejection (n = 128)	Acute Cellular Rejection (n = 46)	P value
Race				
White, n (%)	83 (68.0)	63 (73.3)	20 (55.6)	0.138
African American, n (%)	26 (26.3)	17 (19.8)	9 (25.0)	
Hispanic, n (%)	10 (8.2)	5 (5.8)	5 (13.9)	
Asian, n (%)	3 (2.5)	1 (1.2)	2 (5.6)	
CMV Positive, n (%)	92 (52.9)	63 (49.2)	29 (63.0)	0.268

The symbol "*" was to denote statistically significant p-values

Table 2. Result of univariate analysis

	No acute cellular rejection (n = 128)	Acute cellular rejection (n = 46)	Odds ratio (95% CI)	P value
Age (yrs), median (IQR)	57 (12)	57 (11)	1.00 (0.96–1.04)	0.921
Gender (female), n (%)	47 (36.7)	19 (41.3)	0.82 (0.41–1.64)	0.583
SLK, n (%)	23 (18.0)	2 (4.4)	0.21 (0.05–0.92)	0.038*
Race (Black), n (%)	20 (15.6)	13 (28.3)	2.13 (0.96–4.73)	0.064
HCC, n (%)	43 (33.6)	13 (28.3)	0.78 (0.37–1.63)	0.507
HBV, n (%)	10 (7.8)	1 (2.2)	0.26 (0.03–2.11)	0.208
HCV, n (%)	56 (43.8)	25 (30.9)	1.53 (0.78–3.01)	0.218
Alcohol, n (%)	38 (29.7)	16 (34.8)	1.10 (0.54–2.27)	0.788
NASH, n (%)	18 (14.1)	3 (6.5)	0.43 (0.12–1.52)	0.189
PBC, n (%)	4 (3.1)	1 (2.2)	0.69 (0.07–6.33)	0.742
PSC, n (%)	2 (1.6)	5 (10.9)	7.68 (1.44–41.11)	0.017*
Dialysis, n (%)	36 (28.1)	11 (23.9)	0.80 (0.37–1.75)	0.582
Ascites, n (%)	96 (75.0)	31 (67.4)	0.67 (0.33–1.44)	0.320
Encephalopathy, n (%)	87 (70.0)	35 (76.1)	1.50 (0.69–3.25)	0.304
Creatinine, median (IQR)	1.6 (2.3)	1.3 (1.17)	0.72 (0.54–0.95)	0.020*
Calculated MELD, median (IQR)	29.3 (19.8)	29.9 (11.4)	1.00 (0.98–1.03)	0.805
Child Pugh, median (IQR)	11 (3)	11 (3)	1.07 (0.922–1.25)	0.354
Fulminant, n (%)	7 (5.5)	3 (6.5)	1.21 (0.30–4.87)	0.793
Time Period (07/2008-04/2009)	22 (17.2)	4 (8.7)	0.46 (0.15–1.41)	0.174
Tacrolimus, n (%)	109 (85.2)	33 (71.7)	0.44 (0.20–0.99)	0.047*
Mycophenolate, n (%)	117 (94.4)	41 (89.1)	0.77 (0.25–2.35)	0.648
Sirolimus, n (%)	9 (7.0)	4 (8.7)	1.26 (0.37–4.30)	0.713
Prednisone, n (%)	20 (15.6)	9 (19.6)	1.31 (0.55–3.14)	0.539
Donor Age (yrs), median (IQR)	45 (43.3)	48 (43.8)	1.00 (0.98–1.03)	0.863
Donor Gender (female), n (%)	37 (40.7)	16 (42.1)	0.94 (0.44–2.03)	0.879
Donor Race (white), n (%)	63 (73.3)	20 (55.6)	1.26 (0.64–2.48)	0.504

The symbol "*" was to denote statistically significant p-values

was 5.5, higher than the average Banff of 4 in the ACR group.

Next, we performed multivariant analysis by stepwise elimination of nonsignificant variables with additional correction of previously reported risk factors for ACR from other studies (see Methods). Finally, our model was built using nine variables: age, gender, race, combined liver–kidney status, HCV, NASH, PSC, dialysis, creatinine, time period (07/2008–04/2009), and use of tacrolimus. In this multivariate logistic regression analysis, PSC, creatinine, and use of tacrolimus were confirmed to be associated with ACR (Table 3) The impact of simultaneous liver–kidney transplants as well as the need for renal replacement was accounted for in the multivariate logistic regression and did not have an effect of the ACR risk in this model. Opposite than PSC etiology, creatinine level was found to be negatively correlated with ACR development. Recipient race was not

Table 3. Result of multivariate analysis

	No acute cellular rejection (*n* = 128)	Acute cellular rejection (*n* = 46)	Odds ratio (95% CI)	*P* value
Age (yrs), median (IQR)	57 (12)	57 (11)	0.99 (0.95–1.03)	0.638
Gender (female), *n* (%)	47 (36.7)	19 (41.3)	0.84 (0.37–1.90)	0.678
Race (Black), *n* (%)	20 (15.6)	13 (28.3)	0.81 (0.63–1.04)	0.093
SLK, *n* (%)	23 (18.0)	2 (4.4)	0.36 (0.06–2.14)	0.263
HCV, *n* (%)	56 (43.8)	25 (30.9)	2.05 (0.87–4.81)	0.099
NASH, *n* (%)	18 (14.1)	3 (6.5)	1.08 (0.25–4.73)	0.922
PSC, *n* (%)	2 (1.6)	5 (10.9)	10.88 (1.57–75.22)	0.016*
Dialysis, *n* (%)	36 (28.1)	11 (23.9)	2.94 (0.80–10.81)	0.104
Creatinine, median (IQR)	1.6 (2.3)	1.3 (1.17)	0.63 (0.40–0.99)	0.046*
Tacrolimus, *n* (%)	109 (85.2)	33 (71.7)	0.36 (0.14–0.90)	0.029*
Time Period (07/2008-04/2009)	22 (17.2)	4 (8.7)	1.46 (0.95–2.24)	0.083

The symbol "*" was to denote statistically significant p-values

Table 4. Rate of ACR decreases with increasing creatinine

Percentile	Cr	Rate of ACR	*P*-value
10	0.7	37.5%	0.2939
25	0.9	34.9%	0.1492
50	1.6	31.8%	0.1064
75	2.7	30.1%	0.0566
90	4.5	28.8%	0.0344

reached statistical significance based on our model. Moreover, when we performed similar analysis after exclusion of liker kidney transplant recipients, we obtained identical result (Table S6).

To further characterize the effect of creatinine on ACR rates, values of creatinine were divided into 10th, 25th, 50th, 75th, and 90th percentiles to determine differences in the rates of ACR. A standard t-test was performed and there was a statistical difference starting at the 75th percentile or creatinine of 2.7 mg/dl (Table 4), meaning that patients with a creatinine higher than 2.7 mg/dl at transplantation have a lower risk for ACR.

Discussion

Our study sought to validate the risk factors of ACR in the changing landscape of liver transplantation characterized by transplanting sicker patients with higher MELD score and the use of steroid sparing regimens. Overall, the national reported distribution for patients on the waiting list was 92% with MELD score less than or equal to 18 and 8% with MELD score greater than 18 before MELD was implemented [21]. After MELD score was introduced for liver allocation, average MELD score at transplantation in the United States has been variable and depends on the regional populational size of organ procurement organizations, number of transplant centers in each region and length of waiting list. In our study period, 66% patients had a MELD at transplant higher than 21, as compared to national percentage of 43.9% with MELD higher than 21. [22] The study population was much more critically ill compared to the national average, with only 26% (46/178) patients with MELD score less than or equal to 18, and an average MELD at transplant higher than 25 in our previous UNOS region. Based on our knowledge, this is the first single-center retrospective study looking into risk factors for ACR in a center where patients had average MELD more than 25 at transplantation during the study period. Moreover, this study aimed validation at the single-center level of previous risk factors for ACR in two large UNOS databases during the predirect antiviral era [1,11].

There are some additional differences in our study recipient population compared to the pre-MELD Liver Transplant Database (LTD) used to explore the risk factors for ACR [7]. The LTD is a database of 762 liver transplants that took place between 1990 and 1995 in three centers: Mayo Clinic, University of Nebraska Medical Center and University of California, San Francisco. When compared with this cohort, our recipients include fewer Caucasians (49.4% versus 79.8%) and older average age (55.3 versus 48.7 years). These changes may

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reflect a shift in the patient population receiving LT since the implementation of the MELD score, or it may reflect the demographics differences of the allocated region.

The average time to ACR in this study was 283 days, well outside of the 4 to 6-weeks window, with half of ACR episodes occurring greater than 6 months after transplantation. This observation could be due to the fact that ACR was documented based on biopsies done in the setting of a clinical suspicion of rejection, rather than routinely. Another alternative explanation is that late acute rejection (> 6 months) may be secondary to a different mechanism than early acute rejection and that immunosuppression noncompliance may be implicated in the former [2].

In the pre-MELD studies looking into ACR risk factors, most episodes of ACR occurred during the first 4–6 weeks post OLT [2,23]. In our study population, initiation of immune response against allograft is beyond the previous 4 to 6-week window period predicted by studies performed before MELD era. This observation may have clinical impact regarding the care and frequency with which we are monitoring for ACR in these patients, most particularly in those who are 6–12 months post-transplant. Further investigation is required to determine if this is due to a delay in the recovery of the immune system of high MELD patients causing longer time to recognition the allograft or due to other related mechanisms. Additionally, it is important to note that the majority of the ACR episodes found in this study were mild to moderate on the confirmatory liver biopsy.

PSC is a significant predicator of ACR, as found by multivariate analysis, concordant with previous studies suggesting that PSC transplants are associated with more frequent and severe episodes of ACR [1,9,11]. Five out of the six PSC patients in our study had an episode of ACR, supporting a consistently high risk of ACR in this specific population, despite that the incidence of rejection after liver transplant has been declining [24,25]. PSC is a chronic fibrosing cholangitis of autoimmune etiology and common cause of end-stage liver disease in young population. The specific hyperactive immune response predisposition in PSC seems to be responsible for its association with high rates of ACR [24,25]. A larger-sized study with more PSC patients would add to this observation and provide additional granular data regarding ACR risk factors in this population. Unexpectedly, AIH does not increase the risk of ACR in our study population, although it is unclear why, especially in the setting of a similar autoimmune milieu as PSC. One of the possible reasons for why we observed a low risk of ACR in this subset is maintenance on low-dose prednisone when compared with PSC patient who were weaned off.

Our study confirmed that creatinine level is negatively associated with ACR, similar to the previous study by Weisner et al. using the LTD cohort [7]. This suggests that renal dysfunction in the setting of end-stage liver disease may be associated with decreased immune response, though the mechanism by which this occurs remains unclear. The need for dialysis did not have a correlation to ACR, which would imply that creatinine alone predicts an abnormal immune response that is not corrected by the initiation of renal replacement therapy. Further investigation is required to determine if patients with creatinine higher than 2.7 mg/dl at the time of transplant would benefit from reduced immunosuppression. If there is no statistical difference between standard immunosuppression protocol and a reduced immunosuppression regimen, it may be beneficial to minimize immunosuppression, thereby decreasing the risk of infection post-transplant.

Although development of direct antiviral agents improved outcome of HCV after liver transplant, the relationship between HCV etiology of end-stage liver disease and ACR is less clear [26-29]. HCV-positive status was not found to have an increased risk of ACR by our group. This is in opposite with the findings of Levitsky et al, who found that HCV was associated with a higher rate of acute cellular rejection in the UNOS database with patients transplanted before the wide use of the direct antiviral agents era [11]. However, outcome of our analysis in our center is similar to Tanaka et al. and Dogan et al., both of whom found that HCV was not associated with an increased risk of rejection [13,30]. The first study was done also on the UNOS database using time spans marked by type of HCV treatment available: interferon based, interferon + direct antiviral, and direct antiviral agents and probable what is clinically relevant for our time that the risk of ACR in HCV patients in the setting of new treatment should not be major clinical problem.

The use of tacrolimus was confirmed to be protective for ACR in our population, finding that is similar with the result of the landmark study published by Wisner et al. in the pre-MELD era [31].

Our study has some weaknesses that should be considered when translating the results to other populations. First of all, the monitoring for compliance with

immunosuppression regimen was based only on the trough levels obtained during the clinic visits and directed by transplant physician and pharmacist. Secondly, our study was focused only on recipient risk factors and did not look into the complex impact of post-transplant events on ACR risk (e.g., CMV infection). Additionally, our study population enrolled patients that exclusively received a steroid sparing immunosuppression regimen with some variability in standard immunosuppression protocol during the 5-year study period due to need for continuous quality improvement measures, changing the landscape of end-stage liver disease etiology as indications for liver transplant and trend in immunosuppression for liver transplant that included the decreasing use of IL-2 receptor blockers.

Nevertheless, despite these weaknesses, the study reflects the real-life clinical transplant hepatology practice in the United States and supports the need for close monitoring of immunological outcomes by periodic outcome quality measures, with the aim of fine-tuning immunosuppression and optimizing outcomes after liver transplant.

Conclusion

This study sought to identify risk factors for ACR in the current MELD inflation and MELD-based liver transplant allocation. The majority of ACR were diagnosed greater than 6 weeks after LT. PSC was associated with a higher risk of ACR post-transplant while elevated serum creatinine greater than 2.7 mg/dl was found to have a protective effect against ACR for patients post-transplant. This confirms a change in the pattern of ACR timing in MELD era and serves to emphasize the need for close monitoring of liver allograft for late acute cellular rejection 6–12 months after OLT.

Authorship

DC and ML designed the study, collected and analyzed the data and wrote the manuscript. DG interpreted and wrote the manuscript. KG analyzed the data and wrote the manuscript. GG confirmed pathologic diagnosis and contributed to manuscript. JH interpreted and wrote the manuscript. CA designed the study, interpreted the data and wrote the manuscript.

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Conflict of interest

The authors have declared no conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Studies exploring risk factors for acute cellular rejection in liver transplant recipients.

Table S2 Detailed immunosuppression protocols

Table S3 Early versus Late ACR Recipient Risk Factors Comparison.

Table S4 Rates of ACR with changes to immunosuppression induction protocol.

Table S5 Result of univariate analysis after exclusion of liver kidney transplants.

Table S6 Result of multivariate analysis after exclusion of liver kidney transplants.

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