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Original article

The Effect of Sirolimus Immunosuppression on Cardiovascular Outcomes in Liver Transplantation

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ABSTRACT

Introduction: Non-alcoholic steatohepatitis (NASH) is a rising cause of liver transplantation and is linked to higher rates of cardiovascular complications. The aim of this study was to evaluate the risk of post-transplant cardiac events in patients with NASH that were exposed to sirolimus (SRL) vs. calcineurin-inhibitor (CNI) immunosuppression.

Methods: We retrospectively reviewed all adult liver transplant recipients at our institution between 2002 and 2020. Subjects were grouped based on immunosuppressive regimen. We also analyzed the subgroup of patients with NASH as the primary indication for transplant, as well as a non-NASH subpopulation. The primary outcome measure was risk of major adverse cardiovascular events (MACE) post-transplant. Comparisons between groups were conducted with chi-squared tests. Univariate Cox regression and multivariate time-dependent Cox regression models were used to analyze the relationship between immunosuppression and MACE risk.

Results: 803 liver transplant patients met criteria for study inclusion. Of these, 169 patients had NASH as their primary indication for liver transplant. 18 % of the study population received SRL immunosuppression post-transplant, and the remainder received only CNI immunosuppression. Post-transplant MACE occurred in 32.65 % of patients on SRL compared to 10.27 % in patients on CNI immunosuppression ($p =< 0.001$). Without taking development of post-transplant CKD into account, our study showed a significantly higher risk of MACE with SRL immunosuppression in both the non-NASH cohort (HR 1.67, $p = 0.036$) and the NASH cohort (HR 2.48, $p = 0.037$. However, when accounting for post-transplant CKD, our analysis of the Non-NASH and NASH cohorts did not show a significantly greater risk of post-transplant MACE with SRL compared to CNI immunosuppression.

Conclusions: Our analysis shows that in both the NASH and non-NASH cohorts, liver transplant patients on sirolimus did not have a significantly higher risk of developing cardiovascular disease after transplant compared to immunosuppression with calcineurin inhibitors.

Introduction

As the incidence of obesity, diabetes, and metabolic syndrome increase throughout the world, the incidence of non-alcoholic steatohepatitis (NASH) has risen as well. NASH, a progressive liver disease that can lead to liver fibrosis, cirrhosis, and hepatocellular carcinoma, has become a common indication for liver transplant. In fact, it is the fastest growing cause for liver transplantation (LT) in the United States with a 114 % and 80 % expansion in liver transplant waitlist registration due to NASH for men and women, respectively, from 2004 to 2016 [1]. In addition to the risk of end-stage liver disease, NASH is linked to higher rates of cardiovascular complications [2]. With more patients being transplanted for NASH, the need to address cardiovascular outcomes in liver transplant recipients has become ever more vital. LT in and of itself is associated with an increased risk of major adverse cardiovascular events (MACE) compared to the general population [3]. With a focus on NASH in particular, previous studies have shown that patients undergoing transplant for NASH had significantly higher risk of developing cardiovascular events post-transplant than other etiologies of pre-transplant liver disease. Specifically, a prior study showed that patients transplanted for NASH had a 15.3 and 19.3 % chance of developing an adverse cardiovascular event at 1- and 3-years post-transplant,

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respectively, compared to a 4.5 % and 10.1 % risk in patients transplanted for all other etiologies [4].

With both the growing incidence of transplant recipient metabolic risk factors and the significant risk of post-transplant cardiovascular mortality, there has recently been greater consideration of the metabolic profile of immunosuppressive medications used in LT. The two most common immunosuppressive medication classes used in LT are calcineurin inhibitors (CNIs) and mammalian target of rapamycin (MTOR) inhibitors. Currently, calcineurin inhibitors such as tacrolimus and cyclosporine are more commonly utilized than MTOR inhibitors such as Sirolimus due to their superiority at preventing organ rejection. CNIs are associated with adverse effects of renal injury, weight gain, diabetes, and metabolic syndrome [5], all of which are associated with increased risk of developing cardiovascular disease.

Sirolimus (SRL), an mTOR inhibitor, has been used for immunosuppression in organ transplantation since its FDA approval for renal transplantation in 1999 [5]. Its use in LT was initially minimal due to an FDA black box warning regarding hepatic artery thrombosis, but there has been renewed interest in sirolimus in LT, especially in patients with hepatocellular carcinoma, renal insufficiency, and hepatitis C [6]. SRL is associated with pronounced hypertriglyceridemia and can be seen in up to 40 %−75 % of patients taking the drug [5]. This can lead to clinician avoidance of SRL given that dyslipidemia is associated with increased risk of developing cardiovascular disease in the general population [7]. Although dyslipidemia is an accepted adverse effect of SRL, multiple in vitro and animal studies have paradoxically shown that mTOR inhibition may in fact have an overall net anti-atherosclerotic effect. Mechanistically, mTOR inhibition has been shown to decrease atherosclerosis through improving endothelial function, inhibition of smooth-muscle proliferation, activation of lipolysis, and decreasing lipid accumulation in atherosclerotic plaques [8]. As such, SRL has been used in drug-eluting coronary stents to great effect, and these have been shown to reduce incidence of adverse cardiovascular events and in-stent restenosis compared to bare metal coronary stents [9].

Despite cardiovascular disease being a leading cause of post-transplant mortality, there is a paucity of data addressing cardiovascular disease outcomes in liver transplant patients on sirolimus immunosuppression. In two prior retrospective studies, it was found that there was no significant difference in the risk of cardiovascular disease events between patients on SRL versus CNI immunosuppression [10–11]. However, these studies only followed patients to a maximum of ten years after starting immunosuppression and had an average of 4–5 years of post-transplant follow up, which may be too short of a follow up period to adequately assess development of cardiovascular disease. These studies also did not specifically analyze patients with different underlying precipitants of LT such as NASH, which would be associated with a higher risk for post-transplant cardiovascular events.

The aim of this study was to evaluate the risk of post-transplant cardiovascular events in patients with NASH that were exposed to SRL vs. CNIs. We hypothesized that patients who were transplanted for NASH and were exposed to SRL immunosuppression would have a lower risk of developing major adverse cardiovascular events based on the aforementioned in vitro data on a potential anti-atherosclerotic and cardioprotective effect of mTOR inhibition.

Material/methods

Study Design

This was a single-center study conducted at an urban, mid-sized liver transplant center in Philadelphia that has been transplanting patients since 1984. Retrospective chart review of the Epic™ electronic medical record (EMR) and legacy EMR databases was conducted to identify all adult liver transplant recipients seen at our institution from January 1st, 2002 (start of MELD-era) to December 31st, 2020 ($N = 1165$). Based on our center's practice pattern, the number of patients on SRL prior to 2002 was minimal. Exclusion criteria included age less than 18 years old at time of transplant ($n = 17$), combined liver-kidney transplant ($n = 135$), other multi-organ transplantation ($n = 2$), post-transplant survival <30 days ($n = 21$), repeat organ transplant ($n = 30$), and patients without available data on immunosuppressive medications ($n = 188$). Of the latter, a vast majority of these patients captured on the data query were transplanted at other institutions and not followed longitudinally at our institution. Specific variables abstracted from the EMR included demographic factors such as age, race, and gender. We collected information on liver disease etiology and immunosuppressive medication data. We also obtained data on pre-existing cardiovascular risk factors such as hypertension, hyperlipidemia, diabetes, chronic kidney disease, smoking status, and BMI, as well as development of post-transplant cardiovascular events. The above data were gathered via clinician notes, ICD-10 diagnosis codes, and medication orders. Institutional review board approval was obtained from our institution.

The primary objective of this study was to compare the development of major adverse cardiovascular events (MACE) between SRL and CNI immunosuppressive medication groups. We further investigated MACE risk specifically in subjects with NASH as their primary indication for liver transplant. Subjects were split into two cohorts by primary indication for transplantation: a NASH cohort and a non-NASH cohort. Within each cohort we grouped subjects into the SRL and CNI immunosuppressive groups. The SRL group contained all subjects with any exposure to SRL at some point post-transplant. The CNI group contained subjects who were never exposed to immunosuppression other than CNIs.

A major adverse cardiovascular event (MACE) was defined as myocardial infarction (MI), percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), stroke, development of congestive heart failure (CHF), or death from cardiovascular disease. Secondary outcomes included incidence of metabolic syndrome complications post-transplant. Specifically, the diagnosis of chronic kidney disease (CKD) was identified in subjects based on the ICD-10 code N18.9.

At our institution, all patients receive IL-2 induction with basiliximab post-transplant. Per standard immunosuppression protocol, transplant recipients are then subsequently managed with triple immunosuppression with a calcineurin inhibitor, mycophenolate mofetil, and prednisone. Patients are then transitioned off prednisone within 30 days and mycophenolate within 90 days. Ongoing management continues with CNI or mTOR-inhibitor monotherapy. In the early post-transplant period, SRL immunosuppression is avoided due to its association with increased risk of hepatic artery thrombosis and impaired wound healing. After this immediate post-transplant period, a common indication for transition from CNI to SRL includes progressive chronic kidney disease (CKD) from CNI toxicity. Other less common indications include recurrent HCC or CNI neurotoxicity.

Statistical Analysis

Comparisons between groups were conducted with chi-squared tests (two-sided Fisher's exact test) for the categorical variables of race, sex, liver transplant indication, pre-transplant cardiovascular risk factors, and post-transplant outcomes, while *t*-tests were used to analyze continuous variables of age and BMI at transplant. Both univariate and multivariate time-dependent Cox regression models were used to analyze the relationship between types of immunosuppression and development of MACE. Variables in the univariate Cox models with p-values <0.25 were adjusted for as covariates in the multivariate Cox regression. Using data specifying duration on each immunosuppressive drug, we were able to construct time-dependent Cox proportional hazard regression models analyzing the relationship between types of immunosuppression and MACE risk. We addressed potential confounders in our model, specifically post-transplant development of metabolic risk factors such as CKD, HTN, DM, and HLD. These potential

confounders were examined in our time-dependent Cox regression model and were defined as a confounder if there was >10 % change in hazard ratio. Only one confounder was tested at a time due to a limited number of events. Although post-transplant MACE was our primary interest, we also considered post-transplant deaths from other causes as competing risk events in our Cox regression model.

Spearman correlation was used to investigate a possible time-dependent relationship between duration of SRL exposure and time to post-transplant MACE. The significance level for all tests was set in advance at 0.05. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA) and IBM SPSS Statistics for Windows, Version 21.0.

Results

Of the 803 liver transplant recipients that met criteria for study inclusion, 147 (18.3 %) patients received SRL immunosuppression at some point during their post-transplant course. The remainder of study subjects received only CNI immunosuppression. There were 169 patients in the NASH cohort, and of these 26 (15.4 %) patients were exposed to SRL. There were 634 patients in the non-NASH cohort, and of these 121 (19.1 %) patients were exposed to SRL. The mean post-transplant follow-up time was 1930 days for the CNI group and 1997 days for the SRL group ($p = 0.67$). Within the SRL group, the mean duration of SRL therapy per patient was 1601 days (range: 30 to 7703). These patients were also exposed to CNI therapy, and the mean duration of CNI therapy per patient was 1615 days (range: 88 to 6301). Within the CNI group, the mean duration of CNI therapy per patient was 1930 days (range: 58 to 6652).

Patients on SRL were significantly more likely to be male (80.27% vs. 66.77 %, $p =< 0.01$) and more likely to have a diagnosis of chronic kidney disease (CKD) prior to liver transplant (16.33% vs. 9.15 %, $p = 0.02$, Table 1). There were no significant differences between the CNI and SRL groups regarding other pre-transplant cardiovascular risk factors, such as hypertension, hyperlipidemia, diabetes, smoking status, race, age, and BMI. The majority of patients in the study received liver transplant for an indication of HCV (42 %), followed by NASH (21 %), and alcohol-associated liver disease (21 %, Table 2). Importantly, there was no significant difference in the incidence of patients transplanted for NASH between the CNI and SRL group (21.8% vs. 17.7 %, $p = 0.31$).

There were significant differences in post-transplant incidence of both MACE and metabolic disease between the CNI and SRL groups. Out of the total study population of 803 patients, 15.28 % developed MACE post-transplant. Of the patients that received only CNI immunosuppression, 10.97 % developed post-transplant MACE. In contrast, MACE occurred in 34.53 % of patients on SRL ($p =< 0.01$, Table 3). Patients on SRL had a significantly higher risk of all post-transplant MACE outcomes including MI, requirement of PCI/CABG, stroke, and CHF (see Table 3). Similarly, post-transplant incidence of all collected metabolic risk factors including diabetes, hyperlipidemia, hypertension, CKD, and ESRD was also increased in the SRL group (see Table 3).

To further analyze the data, we first constructed univariate Cox proportional hazard regression models of MACE development with a single covariate in the non-NASH and NASH cohorts. These results are shown in Table 4. Due to the dynamic nature of the immunosuppression variable, it was not included in the static univariate analysis. Subsequently, we then constructed a time-dependent multivariate Cox

Table 2
Indications for liver transplant.

Liver Disease	n	%
Non-NASH	634	78.95 %
NASH	169	21.05 %
HCV	335	41.72 %
EtOH	170	21.12 %
PSC	33	4.11 %
HCC	25	3.11 %
PBC	21	2.62 %
Autoimmune Hepatitis	21	2.62 %
Drug	14	1.74 %
HBV	12	1.49 %

Table 1
Pre-transplant characteristics.

	Total Population		Immunosuppressive Group				p-value
			CNI		SRL		
	$N = 803$		$N = 656$		$N = 147$		
	Mean	SD	Mean	SD	Mean	SD	
Age	56.58	9.84	56.46	10.24	57.14	7.83	0.37
BMI[1]	29.12	5.96	29.03	5.89	29.81	6.50	0.39
	N	**%**	**N**	**%**	**N**	**%**	**p-value**
Male gender	556	69.24 %	438	66.77 %	118	80.27 %	<0.01
Active smoking	107	13.33 %	82	12.50 %	25	17.01 %	0.18
Diabetes	134	16.69 %	106	16.16 %	28	19.05 %	0.39
Hyperlipidemia	117	14.57 %	96	14.63 %	21	14.29 %	0.91
Hypertension	320	39.85 %	259	39.48 %	61	41.50 %	0.71
Chronic kidney disease	84	10.46 %	60	9.15 %	24	16.33 %	0.02
Myocardial Infarction	17	2.12 %	12	1.83 %	5	3.40 %	0.22
PCI[2]	20	2.49 %	14	2.13 %	6	4.08 %	0.24
CABG[3]	10	1.25 %	8	1.22 %	2	1.36 %	0.89
Coronary artery disease	38	4.73 %	30	4.57 %	8	5.44 %	0.67
Stroke	21	2.62 %	16	2.44 %	5	3.40 %	0.57
Congestive heart failure	9	1.12 %	8	1.22 %	1	0.68 %	0.58
Race	**N**	**%**	**N**	**%**	**N**	**%**	**p- value**
Caucasian	623	77.58 %	504	76.83 %	119	80.95 %	0.59
African-American	98	12.20 %	82	12.50 %	16	10.88 %	
Hispanic	39	4.86 %	32	4.88 %	7	4.76 %	
Asian/PI	40	4.98 %	36	5.49 %	4	2.72 %	
Other	3	0.37 %	2	0.30 %	1	0.68 %	
Indication for transplant							
Non-NASH	634	78.95 %	513	78.20 %	121	82.31 %	0.31
NASH	169	21.05 %	143	21.80 %	26	17.69 %	

[1] Body Mass Index - Missing BMI data for $n = 289$ CNI group, $n = 97$ SRL group.
[2] Percutaneous Coronary Intervention.
[3] Coronary Artery Bypass Grafting.

Table 3
Post-transplant outcomes.

	Total population	Immunosuppressive group				p-value
		CNI		SRL		
	$N = 803$	$N = 656$		$N = 147$		
Diagnosis occurring after liver transplant	**N**	**% = *n*/N*[1]**	**n**	**% = *n*/N***	**n**	**% = *n*/N***	
Major adverse cardiac events (MACE)							
Overall MACE	116	15.28 %	68	10.97 %	48	34.53 %	<0.01
Myocardial infarction	31	3.94 %	14	2.17 %	17	11.97 %	<0.01
PCI	22	2.81 %	10	1.56 %	12	8.51 %	<0.01
CABG	15	1.89 %	8	1.23 %	7	4.83 %	0.01
Stroke	37	4.73 %	25	3.91 %	12	8.45 %	0.03
Congestive heart failure	53	6.68 %	31	4.78 %	22	15.07 %	<0.01
Other diagnoses							
Diabetes	145	21.67 %	108	19.64 %	37	31.09 %	0.03
Hyperlipidemia	140	20.41 %	91	16.25 %	49	38.89 %	<0.01
Hypertension	275	56.96 %	207	52.14 %	68	79.07 %	0.02
Chronic kidney disease	300	41.72 %	224	37.58 %	76	61.79 %	<0.01
Coronary artery disease	48	6.27 %	25	3.99 %	23	16.55 %	<0.01
ESRD[2]	36	4.48 %	20	3.05 %	16	10.88 %	<0.01

[1] N* represents the number of subjects that did not have pre-existing diagnosis prior to transplant.
[2] End-Stage Renal Disease.

regression model for MACE risk. In the Non-NASH cohort, based on the univariate Cox regression results, we further looked at the relationship between types of immunosuppression and MACE by adjusting for age, BMI, smoking status, pre-transplant diabetes, pre-transplant hypertension, pre-transplant CKD, pre-transplant MI, pre-transplant PCI, post-transplant HLD, and post-transplant CKD because their p-values in the univariate model were less than 0.25. Similarly, in the NASH cohort, age, gender, pre-transplant diabetes, pre-transplant hypertension, pre-transplant HLD, pre-transplant CKD, pre-transplant CAD, pre-transplant MI, pre-transplant CAGB, pre-transplant HCC, and post-transplant CKD were accounted for as covariates for the multivariate model. In both the NASH and non-NASH cohort we also decided to investigate the effect of post-transplant CKD on MACE risk because a common indication to change from CNI to SRL immunosuppression is the development of renal insufficiency. We conducted our multivariate analysis both with and without post-transplant CKD as a covariate.

Without taking into account development of post-transplant CKD, our initial time-dependent multivariate analysis of MACE risk in the non-NASH cohort showed that patients exposed to SRL had a 67 % greater risk of developing MACE post-transplant than patients on CNI (HR 1.67, 95 % CL = [1.03, 2.70], $p = 0.04$, Table 5). In addition to SRL exposure, pre-transplant history of smoking, DM, and HTN were other strong predictors of MACE development post-transplant in the non-NASH group. In the multivariate analysis of the NASH cohort, there was an even greater MACE risk with a 148 % increase in MACE events with SRL compared to CNI (HR 2.48, 95 % CL = [1.06, 5.82], $p = 0.04$, Table 5). In patients with NASH on SRL, age and male gender were significant predictors of post-transplant MACE, but exposure to SRL remained the strongest predictor with a 2.48-fold higher risk of developing post-transplant MACE compared to CNI.

We then adjusted our analysis to account for potential post-transplant confounders, including the development of post-transplant metabolic risk factors such as CKD, HTN, HLD, and DM. In both the non-NASH and NASH cohorts, post-transplant HTN, HLD, and DM did not lead to a >10 % change in HR for development of post-transplant MACE, and therefore were not considered confounders. However, development of post-transplant CKD was associated with >10 % change in HR of post-transplant MACE, so was considered a confounder. When taking post-transplant CKD into account, our adjusted multivariate analysis of MACE risk in the non-NASH cohort did not show a statistically significant greater risk of developing post-transplant MACE in patients exposed to SRL compared to patients on CNI (HR 1.52, 95 % CL = [0.94, 2.46], $p = 0.09$, Table 6). In the multivariate analysis of the NASH cohort, after adjusting for post-transplant CKD, there was also no statistically significant increase in post-transplant MACE risk with SRL compared to CNI (HR 2.06, 95 % CL = [0.85, 4.99], $p = 0.11$, Table 6). In the non-NASH cohort, post-transplant CKD was the strongest predictor of the development of post-transplant MACE. Post-transplant CKD was a strong predictor in the NASH cohort, but ultimately gender was the strongest predictor of post-transplant MACE with a HR of almost 6 (Table 6). Diagnosis of post-transplant CKD was associated with a 211 % greater chance of developing post-transplant MACE in the non-NASH cohort (HR 2.11, CL = [1.41, 3.18], $p =< 0.01$, Table 6), and a 290 % greater chance in the NASH cohort (HR 2.90, CL = [1.36, 6.18], $p =< 0.01$, Table 6).

We then also considered non-cardiac death as a competing risk. Considering non-cardiac death, the risk of post-transplant MACE in the non-NASH cohort was not significantly increased in the SRL group compared to the CNI group (HR 1.53, CL = [0.95, 2.48], $p = 0.08$, Table 7). Non-NASH patients on SRL were found to have a significantly increased risk of non-cardiac death (HR 1.68, CL = [1.09, 2.60], $p = 0.02$, Table 7). In the NASH cohort MACE risk was similarly not increased with SRL (HR 1.83, CL = [0.76, 4.38], $p = 0.18$, Table 7). For the NASH cohort, the SRL group had a non-significant trend towards higher risk of non-cardiac death (HR 3.00, CL = [0.99, 9.11], $p = 0.05$, Table 7).

In the non-NASH cohort median time to MACE was 1903 days in the SRL group and 1363 days in the CNI group. In the NASH cohort, median time to MACE was 2104 days in the SRL group and 568 in the CNI group. Spearman's rank correlation was computed to assess the relationship between SRL exposure duration and post-transplant time to MACE. There was a statistically significant, moderately positive correlation between the two variables in the overall study population ($r_s = 0.57$, $p =< 0.01$, Table 7) and the non-NASH cohort ($r_s = 0.59$, $p =< 0.01$, Table 8). In the NASH cohort, there was a moderate positive correlation ($r_s = 0.57$, $p = 0.11$, Table 8) that was not statistically significant. Graphical representation of this is shown in Fig. 1.

Discussion

Our analysis shows that liver transplant patients on sirolimus did not have a significantly higher risk of developing cardiovascular disease after transplant compared to immunosuppression with calcineurin inhibitors. There was also no statistically significant difference between SRL and CNI immunosuppression regarding post-transplant MACE risk in patients with NASH as their indication for liver transplant. The risk of post-transplant MACE in our SRL population seems to be driven by the development of post-transplant CKD, as after adjusting for post-transplant CKD in the multivariate analysis the significant differences in MACE were no longer evident. When not accounting for development

Table 4
Univariate cox regression on hazard of MACE.

Parameter	Hazard ratio	95 % hazard ratio confidence limits		p-value
Non-NASH cohort				
Age at transplant*	1.02	0.99	1.05	0.06
BMI at transplant*	1.03	0.98	1.10	0.25
Gender (Female vs. Male)	0.78	0.48	1.27	0.32
Race				
African-American vs. white	1.17	0.66	2.08	0.59
Asian/PI vs. white	0.40	0.10	1.62	0.20
Hispanic vs. white	0.50	0.12	2.04	0.34
Smoking at transplant (Yes vs. No)*	1.50	0.90	2.50	0.12
Pre-transplant DM vs. no pre-transplant DM*	2.12	1.14	3.95	0.02
Pre-transplant HLD vs. no pre-transplant HLD	0.99	0.50	1.98	0.98
Pre-transplant HTN vs. no pre-transplant HTN*	1.95	1.27	2.98	0.01
Pre-transplant CKD vs. no pre-transplant CKD*	1.51	0.82	2.78	0.19
Pre-transplant CAD vs. no pre-transplant CAD	1.27	0.40	4.02	0.69
Pre-transplant MI vs. no pre-transplant MI*	2.53	0.80	8.03	0.12
Pre-transplant PCI vs. no pre-transplant PCI*	2.13	0.78	5.82	0.14
Pre-transplant CABG vs. no pre-transplant CABG	1.74	0.24	12.54	0.58
Pre-transplant stroke vs. no pre-transplant stroke	1.28	0.40	4.06	0.67
Pre-transplant CHF vs. no pre-transplant CHF	1.64	0.23	11.85	0.62
Pre-transplant HCC vs no pre-transplant HCC	0.82	0.53	1.28	0.38
Post-transplant CKD vs. no post-transplant CKD*	1.96	1.28	3.00	<0.01
Post-transplant DM vs. no post-transplant DM	1.01	0.62	1.64	0.96
Post-transplant HTN vs. no post-transplant HTN	0.86	0.56	1.33	0.51
Post-transplant HLD vs. no post-transplant HLD*	2.12	1.37	3.27	<0.01
NASH cohort				
Age at transplant*	1.04	1.01	1.08	0.01
BMI at transplant	0.96	0.89	1.04	0.35
Gender (Female vs. Male)*	0.24	0.080	0.70	0.01
Race				
African-American vs. white	0.00	0.00	.	0.99
Asian/PI vs. white	0.94	0.12	7.30	0.95
Hispanic vs. white	1.57	0.37	6.71	0.54
Smoking at transplant (Yes vs. No)	1.05	0.24	4.49	0.95
Pre-transplant DM vs. no pre-transplant DM*	1.88	0.83	4.26	0.13
Pre-transplant HLD vs. no pre-transplant HLD*	2.08	0.86	4.99	0.10
Pre-transplant HTN vs. no pre-transplant HTN*	1.77	0.80	3.89	0.16
Pre-transplant CKD vs. no pre-transplant CKD*	2.47	1.04	5.85	0.04
Pre-transplant CAD vs. no pre-transplant CAD*	2.48	0.84	7.29	0.10
Pre-transplant MI vs. no pre-transplant MI*	3.55	0.82	15.39	0.09
Pre-transplant PCI vs. no pre-transplant PCI	1.97	0.46	8.39	0.36
Pre-transplant CABG vs. no pre-transplant CABG*	3.66	0.86	15.60	0.08
Pre-transplant Stroke vs. no pre-transplant stroke	0.00	0.00	.	0.99
Pre-transplant CHF vs. no pre-transplant CHF	0.00	0.00	.	0.99
Pre-transplant HCC vs no pre-transplant HCC*	2.71	0.92	7.97	0.07
Post-transplant CKD vs. no post-transplant CKD*	2.116	0.99	4.53	0.05
Post-transplant DM vs. no post-transplant DM	1.152	0.48	2.74	0.75
Post-transplant HTN vs. no post-transplant HTN	1.169	0.55	2.51	0.69
Post-transplant HLD vs. no post-transplant HLD	1.207	0.49	2.99	0.69

* Significant variables used in corresponding multivariate analysis.

Table 5
Time-dependent multivariate cox regression on hazard of MACE.

Parameter	Hazard ratio	95 % hazard ratio confidence limits		p-value
Non-NASH cohort				
SRL vs. CNI	1.67	1.03	2.70	0.04
Smoking at transplant (Yes vs. No)	1.68	1.04	2.71	0.03
Pre-transplant DM vs. no pre-transplant DM	1.94	1.09	3.45	0.03
Pre-transplant HTN vs. no pre-transplant HTN	1.92	1.27	2.90	<0.01
NASH cohort				
SRL vs. CNI	2.48	1.06	5.82	0.04
Age at transplant	1.05	1.01	1.09	0.01
Gender (Female vs. Male)	0.20	0.07	0.59	<0.01

Note: Only variables that were statistically significant in the multivariate model are displayed.

Table 6
Time-dependent multivariate cox regression on hazard of MACE with post-transplant risk factors.

Parameter	Hazard ratio	95 % hazard ratio confidence limits		p-value
Non-NASH cohort				
SRL vs. CNI	1.52	0.94	2.46	0.09
Pre-transplant DM vs. no pre-transplant DM	1.83	1.03	3.26	0.04
Pre-transplant HTN vs. no pre-transplant HTN	1.85	1.22	2.80	<0.01
Post-transplant CKD vs others (no CKD or pre-transplant CKD)	2.11	1.41	3.18	<0.01
NASH Cohort				
SRL vs. CNI	2.06	0.85	4.99	0.11
Age at transplant	1.05	1.01	1.09	0.01
Gender (Female vs. Male)	5.99	2.06	17.45	<0.01
Post-transplant CKD vs others (no CKD or pre-transplant CKD	2.90	1.36	6.18	<0.01

Note: Only variables that were statistically significant in the multivariate model are displayed.

of post-transplant CKD, patients on SRL did have significantly higher risk of developing post-transplant MACE in both the non-NASH and NASH cohorts. SRL immunosuppression was associated with significantly increased post-transplant rates of other metabolic diseases aside from the anticipated rapamycin-associated hyperlipidemia. These risks included hypertension, diabetes, HLD, CKD, ESRD, and CAD. Interestingly, in the non-NASH cohort, SRL exposure was associated with significantly increased risk of non-cardiac death. In the NASH cohort, an association between non-cardiac death and SRL exposure fell just short

Table 7
Comparing time-dependent multivariate cox regression on hazard of MACE and non-cardiac death.

Parameter	Hazard ratio of MACE	95 % Hazard ratio confidence limits		p-value	Hazard ratio of non-cardiac death	95 % hazard ratio confidence limits		p-value
Non-NASH cohort								
SRL vs. CNI	1.53	0.95	2.48	0.08	1.68	1.09	2.60	0.02
Pre-transplant DM vs. no pre-transplant DM	1.86	1.04	3.32	0.04	0.89	0.45	1.79	0.75
Pre-transplant HTN vs. no pre-transplant HTN	1.77	1.17	2.66	<0.01	0.90	0.61	1.31	0.58
Post-transplant CKD vs. others (no CKD or pre-transplant CKD)	2.15	1.43	3.23	<0.01	0.82	0.57	1.19	0.29
Post-transplant HLD vs. others (no HLD or pre-transplant HLD)	1.78	1.17	2.71	<0.01	0.77	0.49	1.21	0.25
NASH Cohort								
SRL vs. CNI	1.83	0.76	4.38	0.18	3.00	0.99	9.11	0.05*
Age at transplant	1.04	1.01	1.08	0.02	1.04	0.99	1.09	0.13
Gender (Female vs. Male)	6.06	2.09	17.58	<0.01	0.65	0.24	1.77	0.40
Post-transplant CKD vs. others (no CKD or pre-transplant CKD)	3.07	1.46	6.48	<0.01	0.92	0.34	2.46	0.87

* Considering rounding, this p-value is not statistically significant.

Table 8
Spearman correlation between days on SRL and time to post-transplant MACE.

	Spearman correlation	p-value
Total	0.57	<0.01
Non-NASH	0.59	<0.01
NASH	0.57	0.11

of statistical significance (HR 3.00, CL = [0.99, 9.11], $p = 0.05$, Table 7). Reasons for the increased rates of non-cardiac death are unclear, but could be linked to CKD-related mortality or vascular events. Another contributor could be the increased metabolic complications in the SRL group post-transplant, and perhaps a difference in focus on mitigation of metabolic risk factors in non-NASH vs. NASH populations. Further study will be needed.

Immediately post-transplant, most patients are managed with CNI immunosuppression, but development of CNI-associated renal toxicity is a common reason for transition to SRL. Therefore, the high percentage of SRL patients having developed post-transplant CKD is expected. CKD is independently associated with increased risk of cardiovascular disease [10], and development of post-transplant CKD was the most significant predictor of development of post-transplant MACE in our multivariate analysis. Surprisingly, post-transplant HLD was not a predictor of MACE in the NASH group, but post-transplant HLD and CKD were predictors of MACE in the non-NASH group. Bias to monitor and manage NASH patients' metabolic profiles more stringently post-transplant, due to higher perceived cardiovascular risk, may explain this discrepancy [11]. Patients on SRL had relatively similar pre-transplantation cardiovascular risk factors compared to patients on CNI. The only statistically significant differences were that a larger proportion of the SRL group was male (80.27% vs. 66.77 %, $p =< 0.01$, Table 1) and had more patients with pre-existing CKD (16.33% vs. 9.15 %, $p = 0.02$, Table 1). Male gender and CKD are independent risk factors for cardiac disease, but our multivariate analyses took these into account.

Prior studies evaluating the relationship between SRL and cardiovascular disease in liver transplant did not find a significant difference in the incidence of cardiovascular disease with SRL use compared to CNI [12,13]. Similar to previous reports, our analysis was unable to show a statistically significant difference in MACE outcomes. While our study had a similar average post-transplant follow up time compared to previous investigations, our study population was larger and spanned 18 years of liver transplant data. This lends itself to diversification of our study population as over the past decade the American obesity epidemic has exploded. Also, over recent years, liver transplantation has evolved to transplanting sicker recipients with higher MELD and at older ages, which may account for the high rates of CKD noted in our study. Our study is also unique in that we specifically analyzed the NASH subpopulation, but we did not find a significantly greater risk of post-transplant cardiovascular events compared to patients with other indications for liver transplant. Finally, this study did not rely on patient-supplied surveys to report cardiovascular events and metabolic risk factors [10], but instead took advantage of EMR systems and chart review tools that may not have existed at the time of the previous investigations.

Our study does have limitations. Our study relied on ICD-10 diagnosis codes for data gathering. In doing so, certain diagnoses such as chronic kidney disease, diabetes mellitus, and hypertension were treated in a binary fashion as "yes/no", whereas in clinical practice these diseases exist on a spectrum from mild to severe. Specifically, it would be interesting in future studies to compare MACE risk with SRL use with cohorts stratified into early vs. late-stage CKD. Another result of relying on ICD-10 diagnosis codes is that we did not review imaging data to assess pre-transplant cardiovascular disease or structural heart disease. Another limitation to our study is that we did not collect data on the background reason for deciding on a specific immunosuppressive agent, nor the reasons for switching immunosuppressants. The decision-making behind switching immunosuppressive agents is complex and could involve other variables beyond the common factors of renal insufficiency or presence of HCC in the explanted liver. We were limited by a lack of data regarding the number of patients on sirolimus prior to the study period's start in 2002. However, based on our center's practice pattern and the small number of patients utilizing sirolimus in the first few years of our study period, it is unlikely that there were an appreciable number of patients on sirolimus pre-2002 that would significantly affect our results. Given the retrospective nature of this study, there was missing data regarding pre-transplant and post-transplant BMI; however, our study otherwise had a complete data set regarding other metabolic factors between the two groups and similar incidence of NASH in SRL and CNI groups. As discussed above, most patients initially receive CNI immunosuppression immediately post-transplant. A possible limitation is that in our SRL group these patients still on average had spent approximately an equal total time on CNI immunosuppression and SRL immunosuppression. However, this approach ultimately mirrors a real-world study. Finally, our study results may have been limited by small sample size of patients on SRL in the NASH group.

When accounting for post-transplant CKD there was no statistically significant difference in MACE risk between SRL and CNI, but our data does note a significantly higher incidence of other post-transplant metabolic diseases (HTN, DM, HLD) in SRL-treated patients. This may create a higher additive risk of post-transplant MACE. It is possible that a higher-powered study with a larger study population and longer follow

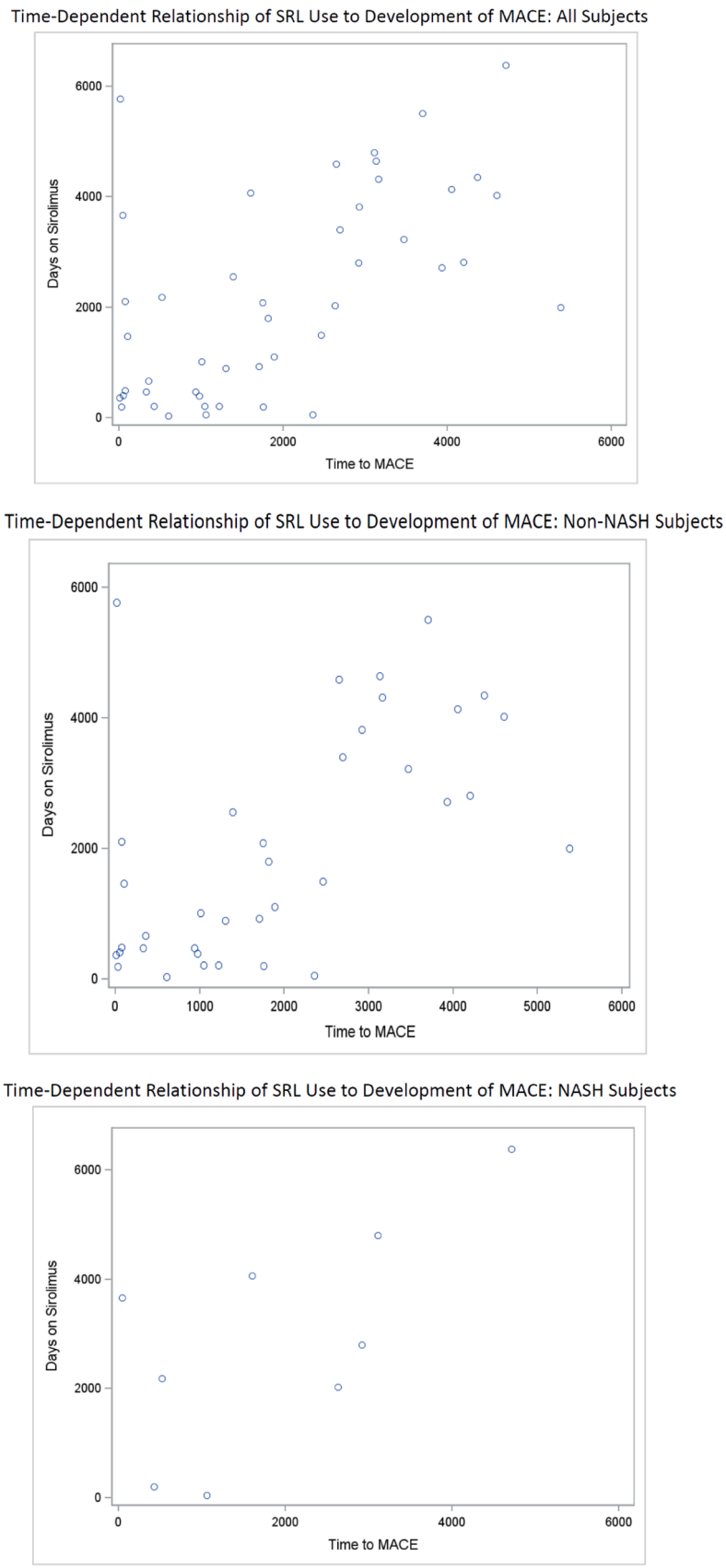

Fig. 1. Time-dependent relationship of SRL use to development of MACE.

up time may be able to further elucidate any significant differences in cardiovascular risk between SRL and CNI immunosuppression. The prior in vitro and animal studies that suggested a net anti-atherosclerotic effect of SRL immunosuppression may not be generalizable in the real-world liver transplant population. With such a marked association between post-transplant CKD and post-transplant cardiovascular events, our findings may warrant closer screening for cardiovascular conditions and more aggressive management of metabolic profiles in patients that develop CKD post-transplantation. Our finding of increased risk of non-cardiac death in patients on SRL is concerning as well and warrants further investigation.

CRediT authorship contribution statement

Ho Jason: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Breslin Zachary:** Formal analysis, Data curation. **Lally Lauren:** Data curation. **Halegoua-DeMarzio Dina:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Tholey Danielle:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Cholankeril G, Wong RJ, Hu M, et al. Liver transplantation for nonalcoholic steatohepatitis in the US: temporal trends and outcomes. Dig Dis Sci 2017;62:2915–22.

[2] Baratta F, Pastori D, Angelico F, Balla A, Paganini AM, Cocomello N, Ferro D, Violi F, Sanyal AJ, Del Ben M. Nonalcoholic fatty liver disease and fibrosis associated with increased risk of cardiovascular events in a prospective study. Clin Gastroenterol Hepatol 2020;18(10):2324–2331.e4. https://doi.org/10.1016/j.cgh.2019.12.026. Epub 2019 Dec 27. PMID: 31887443.

[3] Kim NG, Sharma A, Saab S. Cardiovascular and metabolic disease in the liver transplant recipient. Best Pract Res Clin Gastroenterol 2020;46-47:101683. https://doi.org/10.1016/j.bpg.2020.101683. Epub 2020 Sep 12. PMID: 33158470.

[4] Albeldawi M, Aggarwal A, Madhwal S, Cywinski J, Lopez R, Eghtesad B, Zein NN. Cumulative risk of cardiovascular events after orthotopic liver transplantation. Liver Transpl 2012;18(3):370–5. https://doi.org/10.1002/lt.22468. PMID: 22140067.

[5] Geissler EK, Schlitt HJ. Immunosuppression for liver transplantation. Gut 2009;58:452–63.

[6] Geissler EK, Schnitzbauer AA, Zülke C, et al. Sirolimus use in liver transplant recipients with hepatocellular carcinoma: a randomized, multicenter, open-label phase 3 trial. Transplantation 2016;100(1):116–25. https://doi.org/10.1097/TP.0000000000000965.

[7] Mozaffarian D. Executive summary: heart disease and stroke statistics—2016 update. Circulation 2016;133(4):447–54. https://doi.org/10.1161/CIR.0000000000000366.

[8] Kurdi Ammar, et al. MTOR inhibition and cardiovascular diseases. Transplantation 2018;102(2S). https://doi.org/10.1097/tp.0000000000001693.

[9] Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, Colombo A, Schuler G, Barragan P, Guagliumi G, Molnàr F, Falotico R, RAVEL Study Group. Randomized study with the sirolimus-coated Bx velocity balloon-expandable stent in the treatment of patients with de novo native coronary artery lesions. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. N Engl J Med 2002;346(23):1773–80. https://doi.org/10.1056/NEJMoa012843. PMID: 12050336.

[10] Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, Jafar TH, Heerspink HJL, Mann JF, Matsushita K, Wen CP. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. The Lancet 2013;382(9889):339–52. https://doi.org/10.1016/s0140-6736(13)60595-4.

[11] Charlton M, Levitsky J, Aqel B, O'Grady J, Hemibach J, Rinella M, Fung J, Ghabril M, Thomason R, Burra P, Little EC, Berenguer M, Shaked A, Trotter J, Roberts J, Rodriguez-Davalos M, Rela M, Pomfret E, Heyrend C, Gallegos-Orozco J, Saliba F. International Liver Transplantation Society Consensus Statement on Immunosuppression in Liver Transplant Recipients. Transplantation, 2018;102(5):727–43. https://doi.org/10.1097/TP.0000000000002147.

[12] Mckenna GJ, Trotter JF, Klintmalm E, Ruiz R, Onaca N, Testa G, Klintmalm GB. Sirolimus and cardiovascular disease risk in liver transplantation. Transplant J 2013;95(1):215–21. https://doi.org/10.1097/tp.0b013e318279090c.

[13] Weick A, Chacra W, Kuchipudi A, Elbatta M, Divine G, Burmeister C, Moonka D. Incidence of cardiovascular and cerebrovascular events associated with sirolimus use after liver transplantation. Transplant Proc 2015;47(2):460–4. https://doi.org/10.1016/j.transproceed.2014.11.036.