Severe cholestasis during the first year post-transplant predicts primary sclerosing cholangitis recurrence

By

Bishoi H. G. Aziz

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

Department of Medicine
University of Alberta

Abstract

Background Primary Sclerosing Cholangitis (PSC) is a chronic cholestatic disease of unknown etiology, whose only definitive management is liver transplantation. One of the dilemmas that PSC patients face is the recurrence of the disease in the graft, which may shorten the organ's life expectancy due to failure. To date, there is little consensus on how PSC recurrence (rPSC) affects the graft"? Our research team implicated rPSC as an inducer of early cholestasis during the first three months post-transplant.

Aim 1) Demonstrate the link between early cholestasis and rPSC and/or graft survival post-transplant; 2) Investigate the effect of rPSC on graft survival.

Methodology We constructed a retrospective cohort for patients who received liver transplant for PSC in the University of Alberta transplant center between 1985 and 2019. The database included several characteristics of both the recipients and the donors, time to rPSC and graft loss, graft loss reasons and liver enzymes collected pretransplant, at 1, 3, 6, 9, and 12 months following transplantations. We collaborated with a multicenter team in the United Kingdom (UK) to obtain an external validation cohort. Predictors of rPSC were assessed using a Cox regression and the results were validated using the UK cohort. After that, the rPSC effect on graft survival was investigated using a semi-Markov model as a time-dependent covariate. The end point for the latter was graft loss through death or retransplant due to failure.

Results The recurrence rate from 158 PSC cases in our database was 30.4% over 34 years. In comparison, the recurrence rate in the UK database was 13.8% over 20 years. To predict the occurrence of rPSC, we assessed the clinical and lab variables with a Cox regression. An increase in Alkaline Phosphatase (ALP) was significantly associated with an rPSC incidence in both univariate and multivariable analyses. It remained significant at all time points starting from one month post-transplant (HR=1.3, p =0.027). Also, patients with severe cholestasis three months post-transplant were at a higher risk of contracting rPSC (HR=2.41, p =0.046).

We found that rPSC negatively impacted the graft survival (HR=8.63, p <0.0001). The multivariable semi-Markov analysis showed that severe cholestasis was an independent predictor of graft survival in the models at all time points (HR=4.77, p =0.021).

Conclusion Early cholestasis within three months following Liver transplant (LT) is predictive of rPSC. Patients who developed rPSC were more likely to have their graft fail than those who did not. Cholestasis is also a predictor of graft loss independent from the rPSC effect. The reason for the early inflammation and disease recurrence is complex; however, early inflammation and disease recurrence is also consistent with the course of the infectious hepatic diseases.

Preface

This thesis is an original intellectual work, the result of a collaboration between a research team led by Dr. Andrew Mason at the University of Alberta and Dr. Reena Ravikumar and Emmanuel Tsochatzis from the UK, the latter two of whom provided the UK database to validate the results.

The data was collected by Shawn Wasilenko and Dr. Ellina Lytvyak, who refined it and performed preliminary statistical analysis. The preliminary manuscript was written by Beverley Kok.

Bettina Hansen offered guidance on using the Semi-Markov model mentioned in chapters 2 & 3. Matthew Pietrosanu and Hussain Syed assisted me on using R software to code the Semi-Markov model. I designed the study analysis with the assistance of Dr. Mason. I refined the final data and wrote the literature review (Chapter 1), performed the statistical analysis (Chapter 2), and documented the final results (Chapter 3). I also did the data interpretation (Chapter 4) with the help of Dr. Mason and Dr. Aldo Montano-Loza

The preliminary results of this study were abstracted as “Post-transplant cholestasis within 1-year predicts PSC recurrence. S. Wasilenko, E. Lytvyak, A. Montano-Loza, A. Mason. CDDW Annual Meeting Can J Gastro Hep 2016:31;A23”

The research project in this thesis was conducted after obtaining ethics approval from the University of Alberta, Human Research Ethics Board, Pro00085859.

Dedication

"After these things the word of the Lord came unto Abram in a vision, saying Fear not, Abram: I am thy shield, and thy exceeding great reward." (Genesis 15:1)

*

*

*

To GOD, with whom I struggle every day to grasp his light despite my limitations, I dedicate this significant milestone of my life to you as a continuity of my whole life dedication to you.

Amen

Acknowledgements

First, I would like to thank my mentor, Dr. Andrew Mason, for all the dedication he shown me. He helped me along the road of academic growth and maturation with a balance of guidance, pushing me to become independent. He helped me to hone my critical thinking during our discussions and to acquire invaluable skills that I will use for throughout my career and in my personal life. His greatest gift was his patience with me as I experienced the cultural shock of immigration. I will always remember and be grateful for that support.

I would also like to thank my committee members, Dr. Aldo Montano-Loza and Bettina Hansen, for the teaching and guidance they offered during my research, and the encouragement they gave me with each step I took.

I want to acknowledge the commitment of Dr. Ellina Lytvyak for the team, which was apparent in making her expertise in database design, analysis and problem solving available for me to learn and remove the obstacles. I also want to thank Jaehyeon Koo, Ryan Ahn and Dr. Bavan Sangarasivam for their hard work.

I had the honor of receiving support from the Faculty of Medicine and Dentistry, which granted me the 75th Anniversary Graduate Student Award. This recognition gave me positive energy, which helped me to embark on the difficult new road of my academic studies.

And I wish to add that I wouldn't have succeeded during my master's degree studies if it weren't for the help and love of my family and friends. First and foremost, I want to express my appreciation for my wife, Dr. Shery Faheem, for her unconditional love and dedication that led her to cross oceans and leave everything behind for me. I keep learning from your altruism and selfless devotion. Thanks for being in my life.

As well, I want to thank my mother, Mary Henin, for believing in me; my father, Dr. Hany Seif, who taught me perseverance; and Dr. Essam Kamel, my godfather, who is mentoring my personal development and has trained me to improve my resilience.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Meaning
AIH	Autoimmune Hepatitis
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CCA	Cholangiocarcinoma
CD	Crohn's Disease
CI	Confidence Interval
CRC	Colorectal Cancer
DM	Diabetes Mellitus
EMR	Electronic Medical Records
ERCP	Endoscopic Retrograde Cholangiopancreatography
GGT	Gamma-Glutamyl Transferase
HLA	Human Leukocyte Antigen
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HR	Hazard Ratio
IBD	Inflammatory Bowel Diseases
ICU	Intensive Care Unit
IL	Interleukins
INR	International Normalized Ratio

IPSCSG	International Primary Sclerosing Cholangitis Study Group
LLN	Lower Limit of Normal
MELD	Model for End-Stage Liver Disease
MMF	Mycophenelate Mofetil
MRCP	Magnetic Resonance Cholangiopancreatography
n	Sample Size
NHSBT	National Health Service Blood and Transplant
OTTR	Organ Transplant Tracking Record
PRI	Preservation-Reperfusion Injury
PSC	Primary Sclerosing Cholangitis
PVT	Portal Vein Thrombosis
REB	Research Ethics Board
rPSC	Recurrent PSC
SD	Standard Deviation
UC	Ulcerative Colitis
UDCA	Ursodeoxycholic Acid
UK	United Kingdom
ULN	Upper Limit of Normal

Chapter 1: Introduction

1.1 Intro to Primary Sclerosing Cholangitis (PSC)

Primary Sclerosing Cholangitis (PSC) is a chronic inflammatory disease affecting the large extrahepatic and/or small intrahepatic biliary ducts causing chronic cholestasis. The disease usually progresses to liver cirrhosis and liver failure. Patients without other comorbidities may undergo liver transplantation, which is currently the only cure. Diagnosing PSC depends on the distinctive cholangiographic strictures and beading appearance of the extrahepatic biliary system using MRCP or ERCP[1], [2] *(Figure 1.1)*. PSC is a diagnosis made in the absence of genetic disease, surgery, choledocholithiasis, biliary cancer and chemotherapy. Histopathology doesn't offer additive help in the disease diagnosis except for small duct PSC [3], [4]; an intrahepatic subtype of PSC that doesn't have an identifiable imaging stigma. Moreover, PSC can occur with the hepatitis picture of Autoimmune Hepatitis (AIH) in a condition called PSC/AIH overlap syndrome [5], [6].

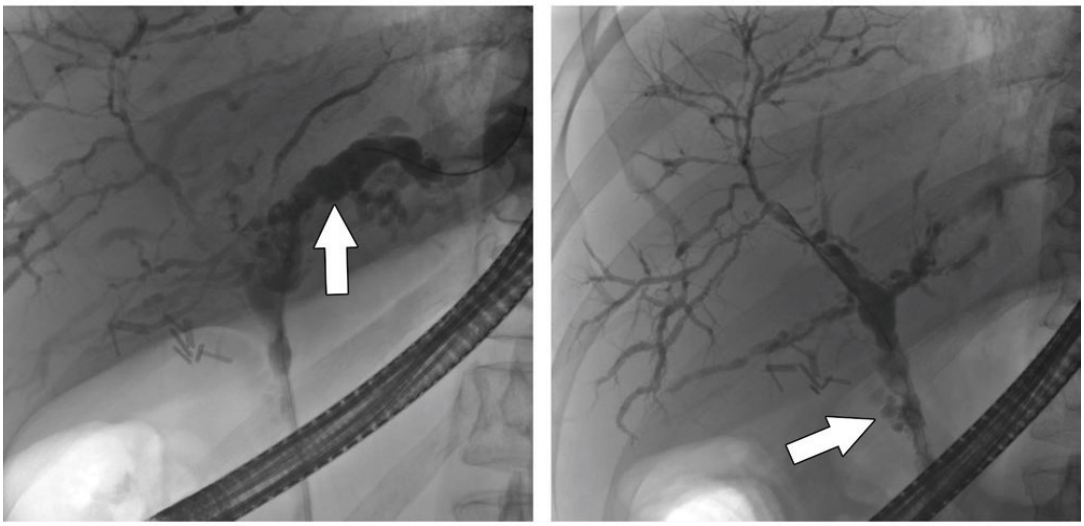

Figure 1.1: ERCP showing biliary duct irregularities of PSC[7]

1.2 Etiology

PSC occurs in the absence of identifiable causes of biliary injury and fibrosis such as long-standing biliary obstruction or infections [8]. However, the etiology of PSC is still an enigma. Autoimmune inflammation is implicated in the disease process, which is evident in the high prevalence of inflammatory bowel disease (IBD) in PSC patients [9] and in the detection of several autoantibodies in PSC [10] patients' serum, the most prevalent of which is the perinuclear antineutrophil cytoplasmic antibody [11]. Nevertheless, steroids or other immunomodulatory drugs usually fail to improve disease outcomes [12], [13]. Although genetic factors related mainly to HLA have been found to be a risk factor for PSC [14]–[16], the genetic component of the disease accounts for less than 10% of the risk [14]. This suggests that environmental exposure plays a critical role. Indeed, studies have shown that around 40% of PSC patients have retroviral antibodies in their sera [17].

1.3 PSC Epidemiology

PSC is a rare disease with a very low incidence (0-1.3 per 100,000 population/year) and prevalence rates that vary depending on the geographical distribution (0-16.2 per 100,000 population) [9], [18]–[22], [22]–[26]. PSC is considered an orphan disease and traditionally the pharmaceutical industry has been reluctant to invest in clinical trials to find new management strategies. The largest epidemiological study performed to date, by the International Primary Sclerosing Cholangitis Study Group (IPSCSG),

found that two-thirds of patients affected by PSC are males late in their fourth decade of life [27].

1.4 Outcomes

The IPSCSG study found that the progression rate to liver transplantation or death for more than 50% of PSC patients was approximately 15 years from the time of diagnosis [27]. Cholangicarcinoma (CCA) and hepatic failure are the most common causes of death (58% and 30% respectively)[28]. Risk factors for worse prognosis remain controversial and include subjects with ulcerative colitis (UC), males, and older aged patients [9], [27], [29]–[31].

1.5 Management

1.5.1 Medical management

Given the idiopathic cause of PSC, it is hard to develop a medication that targets the disease pathology. The issue is complicated by being an orphan disease that needs several years of follow up for events to occur[32]. Accordingly, few drugs have been used to treat PSC. The most widely used was Ursodeoxycholic acid (UDCA), which yielded contradictory results in terms of improving survival [33], [34]. This originally resulted in experts discouraging against the routine use of UDCA to treat PSC [35], [36]. Immunosuppressive medications have been tried but either lacked efficacy or produced side effects. However, there may be a role for immunosuppression in patients with the PSC/AIH overlap syndrome to treat the AIH component of disease [5], [37], [38].

1.5.2 Interventional management

Physicians usually need to manage complications that arise during the course of the disease. This may include the use of ERCP for dominant strictures [39], [40], antibiotics and drainage for recurrent bacterial cholangitis [35], radiation therapy with or without liver transplantation for CCA [41], and colonoscopy and colectomy to manage dysplasia and colorectal cancer (CRC) [35], [42]. Hepatic failure and portal hypertension are treated in the same way as in other chronic liver conditions. With the absence of medical therapy to alter the disease progression, liver transplantation remains the only definitive management for PSC [35]. Indeed, PSC is the primary etiology in 4% of transplant recipients [43]

1.6 PSC recurrence post-transplant

1.6.1 rPSC diagnostic criteria

PSC patients usually experience excellent outcomes after liver transplantation, with 5-year graft survival rates ranging from 95.4%-75% [44]–[46]. With this high probability prolonged survival following transplantation, patients are more likely to develop recurrent PSC (rPSC) in the allograft *(Figure 1.2)*, which was first described in a case series in 1988 [47], and was later confirmed by subsequent reports [48], [49]. rPSC increases with time [50] with a 5-year recurrence probability of around 14% [51].

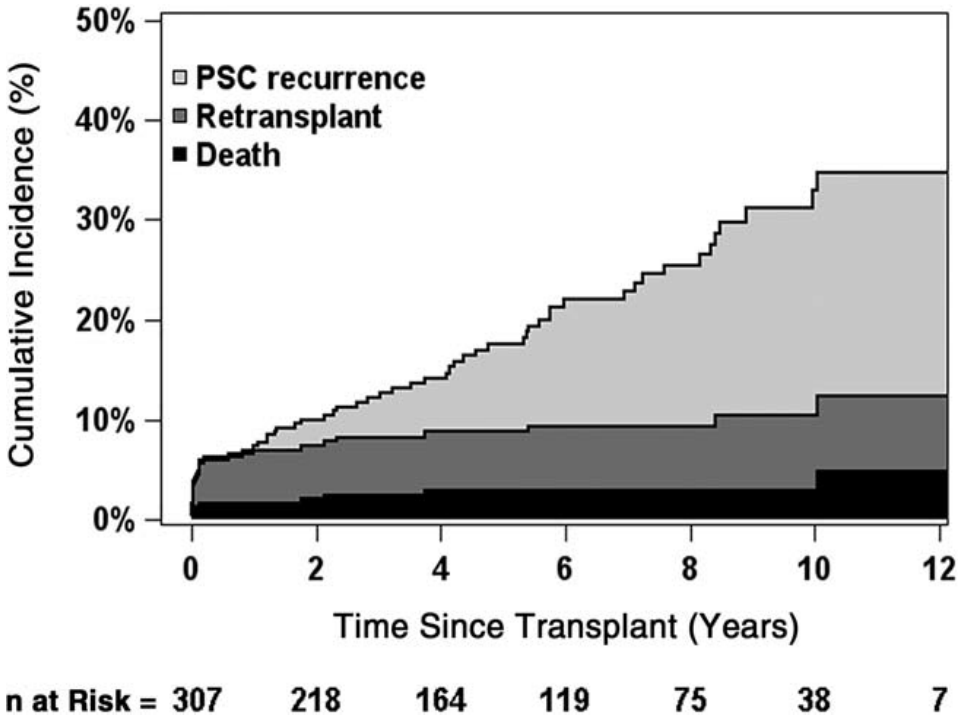

Figure 1.2: *Incidence rate of PSC recurrence, retransplant and death post-transplant for PSC [52]*

According to the Mayo Clinic guidelines, rPSC can be documented 90 days after transplantation because biliary strictures that occur during the first 90 days are considered to arise as a result of complication from surgery. When diagnosing rPSC, it is important to exclude other causes of secondary sclerosing cholangitis, such as hepatic artery thrombosis, ductopenic rejection and ABO incompatibility [53].

1.6.2 Risk factors for rPSC

To date, there is no agreement in the literature on the risk factors for rPSC [54]. Several covariates have been debated [55]: Moderate to severe IBD post-transplant, CCA prior to transplant, colectomy, higher donor age, higher model for end-stage liver disease

(MELD) score at the time of transplantation and HLA mismatch [50]–[52], [56]–[60], [60]–[63]. Indeed, cholestasis within the first three months following the transplant is suspected to be a precursor of possible rPSC [62]. However, until now, there has been no formal study to investigate the liver biochemistry profile to identify rPSC after a transplant. Similarly, researchers have not reached a consensus on whether rPSC impacts graft survival [51], [61].

As liver transplantation is the most effective tool accessible to clinicians to manage PSC, it is important to identify the true effect of disease recurrence on the graft. It is even more important to learn whether the simple biochemical tests performed shortly after the liver transplant can predict disease recurrence in the long run. In one such analysis, cholestasis as early as the third month post-transplant *(Figure 1.3)* was associated with the development of rPSC [62].

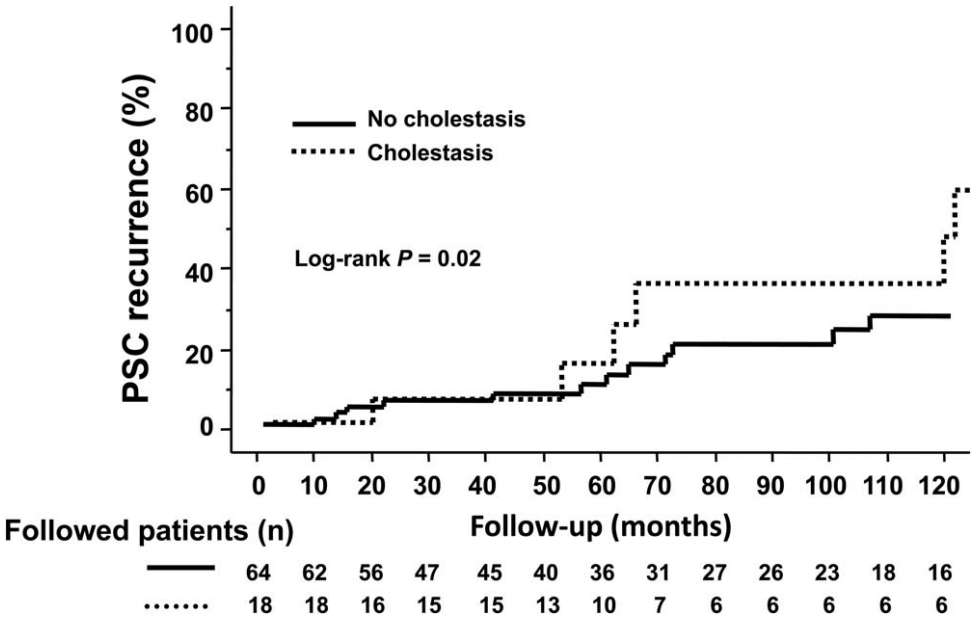

Figure 1.3: *Incidence rate of PSC recurrence according to cholestasis at 3rd month post-transplant [62]*

When compared with other chronic liver diseases requiring transplantation, we noticed a similarity with an infectious disease process. Recurrent HCV [63], for instance, occurs very soon after transplantation and this is associated with elevation of the liver enzymes within the first months following transplantation. In contrast, recurrent NAFLD and AIH can take 10 years to develop, with elevated enzymes occurring several years after the transplant[64], [65].

1.7 Study goals

- To estimate the frequency of PSC recurrence over time and the risk factors in liver transplant recipients at the University of Alberta Hospital Liver Transplant program.
- To assess the effect of rPSC on graft survival.
- To evaluate the usefulness of biochemistry early post-transplant at different time points in predicting rPSC, and to validate the findings using a multicenter database from the United Kingdom (UK).

1.8 Hypothesis

We hypothesize that recurrent PSC behaves like infectious disease process following liver transplantation. To address the hypothesis, we will evaluate whether patients who develop early cholestasis following liver transplantation are at greater risk of developing rPSC.

1.9 Study Implications

The findings of this investigation will evaluate the burden of disease recurrence on the morbidity and mortality of patients receiving a liver transplant for PSC. The study will also shed the light over a possible infectious disease process linked with the development of rPSC.

This study may pave the way for more extensive cohort studies to validate the results. This in turn, may guide clinicians accordingly for post-operative care of PSC transplant recipients.

Chapter 2: Methodology

2.1 Ethics approval

This study was conducted according to the Declaration of Helsinki for medical research involving human subjects. It was approved by the University of Alberta health research ethics board (HREB). Also, we received ethical approval from the National Health Service Blood and Transplant (NHSBT) and the National Information Governance Board in the UK to perform this multicenter study.

2.2 Study population

2.2.1 Inclusion criteria

We abstracted the clinical data for all liver transplant recipients with a diagnosis of PSC at the University of Alberta transplant center in Edmonton, Canada. This data came from the Organ Transplant Tracking Record (OTTR), the electronic medical record (EMR) used to manage the data for the transplant service. We evaluated data from July 1985 through January 2019. PSC was diagnosed before transplant based on typical cholangiographic structuring and beading. Patients received a diagnosis of recurrent PSC (rPSC) based on the cholangiographic finding of non-anastomotic strictures in the biliary system in patients with a cholestatic biochemical profile [53].

2.2.2 Exclusion criteria

a) Graft loss during the first 90 days after receiving the liver transplant [66]; b) concomitant other organ transplantation; c) unresolved biliary anastomotic stricture disease; d) confounding diagnoses such hepatic artery thrombosis or stenosis, ABO-incompatibility or ductopenic rejection; e) unknown dates or reasons for major events, and unavailable labs.

We obtained an external cohort from the UK to validate the usefulness of the biochemical labs in predicting rPSC. The data was collected from Jan. 1st, 1990 to Dec. 31st, 2010. The cohort included the patients from six of the seven national liver transplant centers. The same inclusion and exclusion criteria used for the Canadian cohort were applied to the UK cohort.

2.3 Data collected

2.3.1 Clinical data

To construct the Canadian cohort, the EMR was checked retrospectively to obtain the data for the first liver transplantation related to the recipient: gender, age at transplant, ethnicity, transplant from ICU, recipient comorbidity of diabetes mellitus, and the presence and type (Crohns's disease vs. ulcerative colitis) of inflammatory bowel diseases (IBD).

Clinical data related to the operation included the transplant date, donor age and gender, cold ischemic time, warm ischemic time, type of the received liver (deceased vs.

living donor), and type of the biliary anastomosing surgery done (Roux-en-Y vs. End-to-End anastomosis).

Additionally, charts were examined for the follow-up after the transplant to obtain the immunosuppression regimen during the first year following the surgery; the diagnosis of acute and chronic rejection; the method and date of the diagnosis of rPSC; and the graft loss reason, date, and type (death vs. retransplant).

The UK cohort included the date of transplant, the diagnosis and date of recurrence, graft loss diagnosis, date, and the type of graft loss (death vs. retransplant).

2.3.2 Biochemistry lab tests

We collected the following lab tests pretransplant in the Canadian cohort: albumin, creatinine, INR, and liver biochemistry and enzymes (total bilirubin, ALT, AST, ALP, GGT) and calculated the model for end-stage liver disease (MELD) score at the time of transplantation. Whereas pre-transplant labs weren't available in the UK cohort. Post-transplant, labs were obtained at the following time points: 1 month, 3 months, 6 months, 9 months and 1 year for the Canadian cohort; and at 1 year only for the UK cohort. These lab tests included total bilirubin, ALT, AST, ALP, and GGT. The latter were not available in the UK cohort, and the availability was limited in the Canadian cohort.

The labs were analyzed in two ways. First, labs were analyzed as continuous variables and expressed either as times to ULN or LLN. Next, patients were assigned based on whether or not they had developed severe or mild cholestasis using the previous definitions in the literature. ALP > 2xULN or the combination of both abnormally elevated ALP and total bilirubin were determined mild cholestasis, while ALP > 3xULN, or total bilirubin > 100 μmol/L is considered to be severe cholestasis [62], [66], [67].

2.4 Post-transplant immunosuppression regimens

The immunosuppression protocol at the University of Alberta consists of induction and maintenance regimens. Following transplant, the patient is maintained on tacrolimus and mycophenelate mofetil (MMF) for one year. After that the MMF is withdrawn. Sirolimus is usually substituted for tacrolimus in patients with renal toxicity. The liver transplant program at the University of Alberta doesn't use long-term low-dose prednisolone as a maintenance immunosuppressive. In contrast, the UK programs administer a tapering prednisolone dose over the first three months, and then maintains the patients on tacrolimus for immunosuppression.

2.5 Statistical analyses

2.5.1 Descriptive analysis

For the purpose of this investigation, I used STATA/IC 16.1 for windows for data description analysis and to perform the time-to-event analysis for the PSC recurrence as the event of question. I also used R version 4.0.4 to perform the multi-state model

analysis for the graft survival, as I will describe later in this section. A $p < 0.05$ was considered significant.

For descriptive analysis, non-normal variables are described as median and range, while normal variables are described as mean ± standard error of the mean. To analyze continuous data, I used the command *(summarize v1, detail)*. Categorical variables are described as a percentage and analyzed using the *(tab)* command.

2.5.2 Cox regression analysis for rPSC predictors

A time-to-event analysis was performed to detect the predictors of rPSC. First, the data were declared to be survival-time data using the command *(stset tvar, failure(event=1))*. The event was defined as having rPSC diagnosis. Patients were censored at the time of their last visit. Univariate Cox regression analyses were done for all the variables. Variables with $p < 0.1$ were then entered in multivariable Cox regression analyses. Several models were built based on the time point of the labs. In each time point, two models were built: one with ALP and/or total bilirubin as continuous variables, and the other one with severe and/or mild cholestasis as categorical variables replacing the ALP and/or total bilirubin. A Cox regression was calculated using the command *(stcox)*. The proportionality hazard assumption was tested for all models using Schoenfeld and scaled Schoenfeld residuals. Variables with $p < 0.005$ means that they violate the proportionality assumption. The test was computed using the command *(quietly stcox varlist, schoenfeld(sch*) scaledsch(sca*))* followed by the command *(stphtest, detail)*.

Kaplan-Meier curves were graphed for categorical variables that remained significant in a multivariate analysis using the command *(sts graph, by(catvar))*.

2.5.3 The relationship between rPSC and graft survival using multi-state analysis

To assess the effect of rPSC on graft survival, I performed a multi-state model analysis in the form of an illness-death model. In this model, all patients started at the same state (state 1 = post-transplant. They could then move to an intermediate state (state 2 = rPSC). At the end, they could move to an absorbing state (state 3 or absorbing state=graft-loss due to death or retransplant) either from the intermediate state or directly from the first state *(Figure 2.1)*. Deceased patients were considered to have graft loss if they died with a failed graft. Patients were censored at the time of their last visit if they didn't experience the third state.

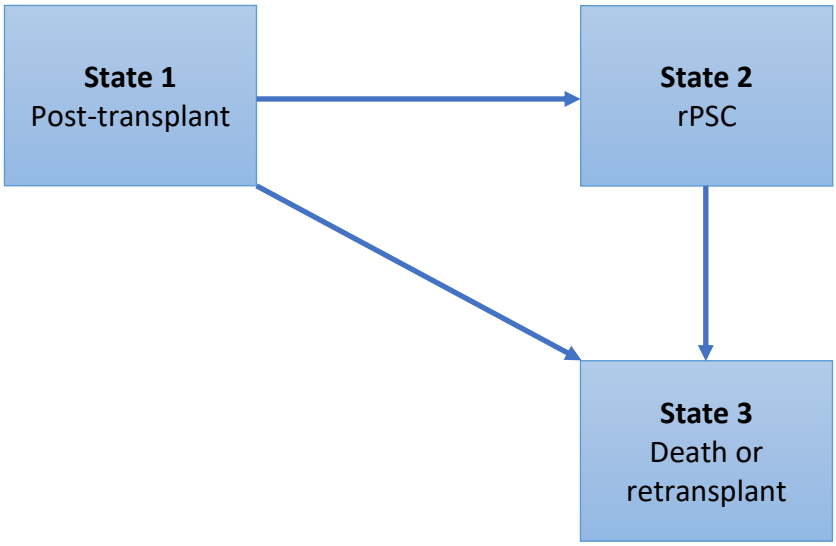

***Figure 2.1**: Illness-death model*

I first performed a univariate Cox regression to decide on the variables that modified the transition between states. Variables with $p < 0.05$ were then incorporated with the rPSC binary variable as a time-dependent variable in the multivariable multi-state model analyses. Several models were built in the same manner as the previously explained time-to-event analysis for the rPSC event. In other words, several multivariable semi-Markov models were built according to the different time points and to the cholestasis variables.

The multi-state models were computed in R software using *(p3state.msm)* package [62], [63]. This package computes an overall time-dependent Cox model which includes rPSC as a time-dependent covariate. This was performed using the (TDCM) option. It also computed a semi-Markov model using the (CSMM) option, which gives the effect of the different covariates on the transition between the three states.

Chapter 3: Results

3.1 Cohorts' Characteristics

3.1.1 Characteristics of the Canadian database

We collected data retrospectively from July 1985 to January 2019 for patients at the University of Alberta transplant center who had a liver transplant due to PSC. Of the 158 patients who met the inclusion and the exclusion criteria for the study, three were dropped from the semi-Markov analysis that tested the effect of rPSC on graft survival, because the graft loss reason wasn't known.

Around 74% of the patients were males. Their median age at transplant was 41.8 years (n= 158, range= 6.02 - 71.99). The patients were followed until the first graft loss for median of 7.49 years (n= 158, range= .22 - 23.09). The proportion of patients in the database who suffered from IBD was 82.3% (130/158). Almost three-quarters of those patients were diagnosed with UC (Table 3.1). Additionally, labs were collected pre-transplant and at different time points post-transplant: 1, 3, 6, 9, and 12 months *(Table 3.2)*.

Table 3.1: *A comparison between the clinical characteristics of the Canadian and UK databases.*

		Canada (n= 158)		UK (n= 549)	
		Median/% (n)	**Range**	**Median/% (n)**	**Range**
Sex	**Female: Male**	41:117 (158)		_	_
Age at Transplant		41.8 (158)	6.02 - 71.99	_	_
Ethnicity	**Caucasian**	95.7% (134/140)		_	_
	Other*	4.3% (6/140)		_	_
Primary Diagnosis	**PSC**	88.6% (140/158)		_	_
	PSC-AIH	11.4% (18/158)		_	_
Transplant Year			1985 - 2019		1990 - 2010
Follow-up Duration		7.49 (158)	0.22- 23.09	10.1 (549)	0.13 - 26.19
Transplants Number	**1**	89.2% (141/158)		_	_
	2	12.7% (20/158)		_	_
	3	3.2% (5/158)		_	_
Transplant from ICU		18.6% (26/140)		_	_
Recipient DM		18.5% (29/157)		_	_
IBD	**Total**	82.3% (130/158)		_	_
	UC	75.4% (98/130)		_	_
	Crohn's	24.6% (32/130)		_	_
	Colectomy	17.7% (23/130)		_	_
Donor Type	**Deceased**	73.5% (114/155)		_	_
	Living	26.5% (41/155)		_	_
Donor Sex	**Male**	63.5% (94/148)		_	_
	Female	36.5% (54/148)		_	_
Sex Match		66.9% (99/148)		_	_
Surgery Type	**Roux-en-Y**	92.6% (138/149)		_	_
	End-to-end	7.4% (11/149)		_	_
		Immunosuppression			
Tacrolimus		85.14%(126/148)		_	_
Sirolimus		22.3% (33/148)		_	_
MMF		54.05%(80/148)		_	_
Cyclosporine		15.54% (23/148)		_	_
Recurrence Rate		30.4% (48/158)		13.8% (76/549)	_
Time to PSC Recurrence (y)		7.2(48)	0.77 - 18.44	5.2 (76)	0.13 - 14.93
Rejection		42.3% (66/156)		_	_
Graft Loss	**Total**	26.5% (41/155)		29.7% (163/549)	_
	Deceased	55.1% (23/41)		65.0% (106/163)	_
	Retransplant	43.9% (18/41)		35.0% (57/163)	_
Graft-Loss Reason	**rPSC**	43.9% (18/41)		_	_
	Sepsis	14.6% (6/41)		_	_
	Chronic Rejection	9.8% (4/41)		_	_
	Other †	31.7% (13/41)		_	_

Time to Graft Loss	8.18 (41)	0.28 - 20.47	5.5 (163)	0.29 - 18.9
Cold-Ischemia Time (min)	232 (138)	14 - 843	_	_
Warm-Ischemia Time (min)	54 (123)	23 - 322	_	_
Donor Age (y)	35.4 (142)	3.6 - 73	_	_

LLN= lower limit of normal / ULN= upper limit of normal.
** Other ethnicities include Aboriginal, Asian Indian, Middle Eastern, Black, Filipino.*
† Other etiologies of graft-loss include portal vein thrombosis (PVT), hepatocellular carcinoma (HCC), colorectal cancer (CRC), cholangeocarcinoma, breast cancer, hepatic artery thrombosis, early rejection, multiple myeloma.
‡ Albumin is the only continuous variable that is normally distributed. It is reported as mean ± standard deviation (SD).

Table 3.2: *Biochemistry profile of the Canadian and UK database*

	Labs Pre-Transplant			
Albumin x LLN ǂ	0.91 (119)	0.20	_	_
Creatinine x ULN	0.69 (139)	0.37- 1.34	_	_
INR	1.3 (116)	0.9 - 3.7	_	_
MELD	19 (75)	6 - 40	_	_
AST x ULN	3.03 (115)	0.45 - 23.4	_	_
ALT x ULN	1.54 (96)	0.36 - 8.68	_	_
GGT x ULN	2.67 (47)	0.3 - 43.27	_	_
ALP x ULN	2.41 (117)	0.18 - 15.09	_	_
Total Bilirubin x ULN	5.58 (140)	0.25 - 54.6	_	_
Mild Cholestasis	15.9% (20/126)		_	_
Severe Cholestasis	77.0% (97/126)		_	_
	Labs 1-Month Post-Transplant			
AST x ULN	0.75 (118)	0.23 - 14.45	_	_
ALT x ULN	0 .68 (90)	0.18 - 22.68	_	_
GGT x ULN	0.89 (12)	0.19- 8.31	_	_
ALP x ULN	1.21 (118)	0.29 - 9.08	_	_
Total Bilirubin x ULN	1.15 (118)	0.25 - 29.25	_	_
Mild Cholestasis	33.1% (39/118)		_	_
Severe Cholestasis	19.5% (23/118)		_	_
	Labs 3-Month Post-Transplant			
AST x ULN	0.71 (110)	0.35 - 7.78	_	_
ALT x ULN	0.68 (85)	0.22 - 8.44	_	_
GGT x ULN	1.06 (35)	0.2 - 13.2	_	_
ALP x ULN	1.01 (112)	0.38 - 11.57	_	_
Total Bilirubin x ULN	0.52 (112)	0.1 - 11.35	_	_
Mild Cholestasis	10.7% (12/112)		_	_
Severe Cholestasis	12.5% (14/112)		_	_
	Labs 6-Month Post-Transplant			
AST x ULN	0.68 (108)	0.22 - 6.14	_	_
ALT x ULN	0.72 (84)	0.24 - 9.2	_	_
GGT x ULN	0.52 (44)	0.19 - 10.5	_	_
ALP x ULN	1.15 (109)	0.29- 10.52	_	_
Total Bilirubin x ULN	0.55 (107)	0.1 - 5.6	_	_
Mild Cholestasis	14.68% (16/109)		_	_
Severe Cholestasis	12.8% (14/109)		_	_
	Labs 9-Month Post-Transplant			
AST x ULN	0.78 (102)	0.25 - 3.28	_	_
ALT x ULN	0.68 (75)	0.22 - 6.14	_	_
GGT x ULN	0.5 (39)	0.09 - 9.57	_	_

ALP x ULN	1.08 (103)	0.42 - 10.96	_	_
Total Bilirubin x ULN	0.6 (103)	0.1 - 7.05	_	_
Mild Cholestasis	12.6% (13/103)		_	_
Severe Cholestasis	9.7% (10/103)		_	_
	Labs 1-Year Post-Transplant			
AST x ULN	0.73 (102)	0.3 - 4.4	0.6 (321)	0.15 - 9.2
ALT x ULN	0 .64 (85)	0.2 - 3.76	0.6 (251)	0.1 - 7.68
GGT x ULN	0.41 (39)	0.07 - 7.21	_	_
ALP x ULN	1.02 (103)	0.38 - 5.83	1.18 (496)	0.27 - 35.58
Total Bilirubin x ULN	0.55 (101)	0.05 - 13.25	0.55 (495)	0.15 - 47.55
Mild Cholestasis	12.6% (13/103)	_	17.3% (86/496)	_
Severe Cholestasis	10.7% (11/103)	_	14.7% (73/496)	_

The PSC recurrence rate was 30.4% (48/158), with a median (n) time-to-recurrence in years= 7.2 (48). The overall 1, 5, 10- and 15-year probability of recurrence = 1%, 12%, 25%, and 58% respectively *(Figure 3.1)*.

After excluding the three patients with unknown reasons for graft-loss, a total of 26.5% (41/155) of patients experienced graft loss over the follow-up period either by death (55.1% (23/41)) or retransplant (43.9% (18/41)). The main reason for losing the graft was rPSC (43.9% (18/41)) *(Table 3.1)*. Patients were followed up for a median of 8.81 years (n= 41, range= 0.28 - 20.47) before losing their graft, with 1-, 5-, 10- and 15-year overall survival probability= 97%, 89%, 78% and 62% respectively *(Figure 3.2)*.

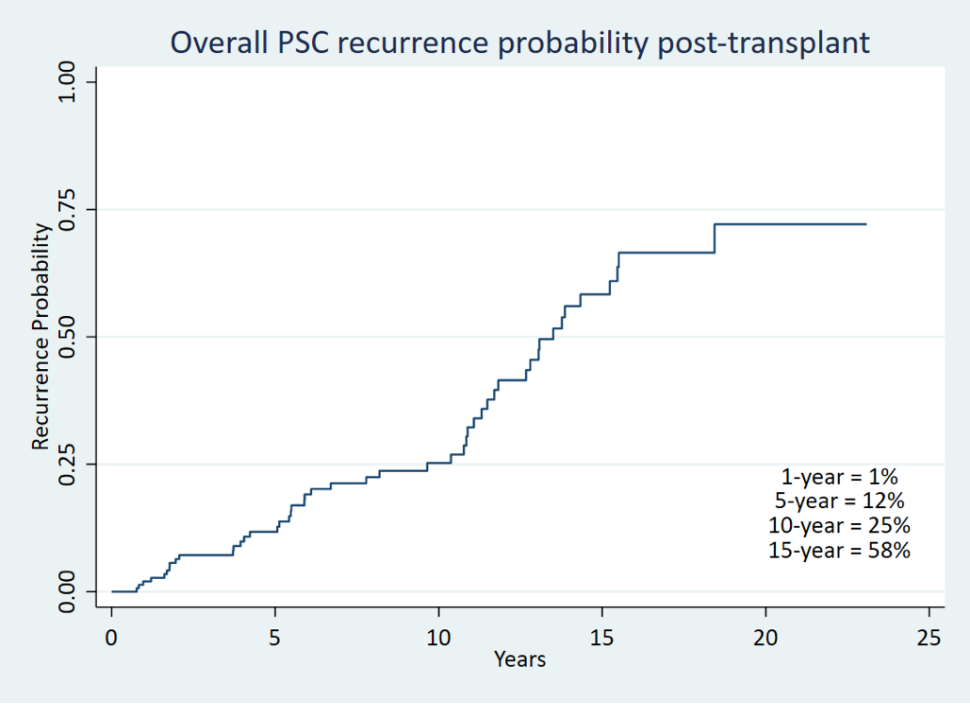

Figure 3.1: *A Kaplan-Meier curve showing the overall rPSC probability post-transplant at the University of Alberta transplant center.*

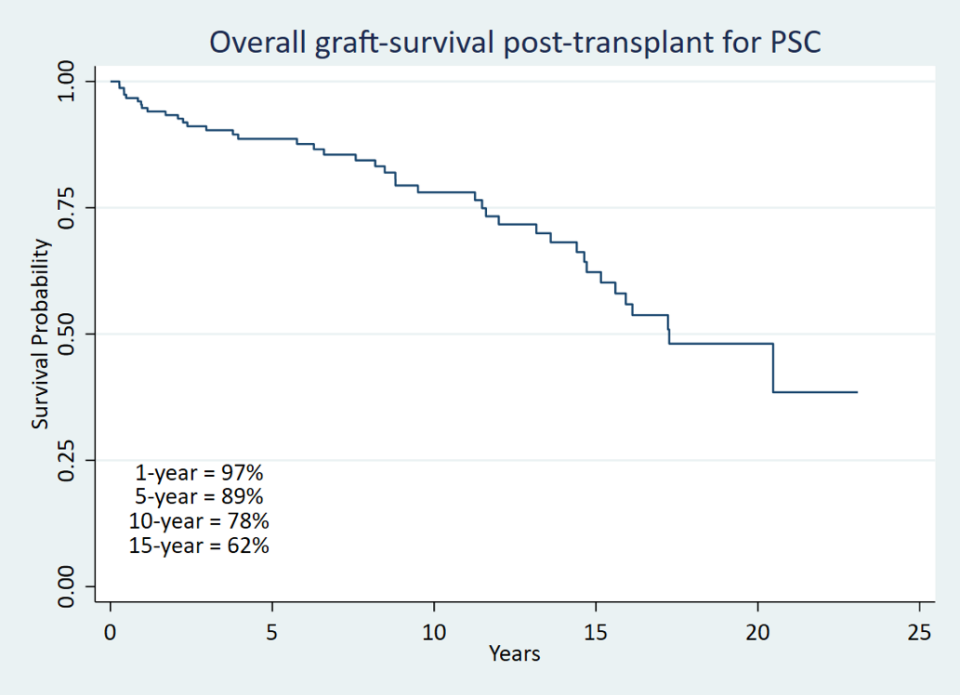

Figure 3.2: *A Kaplan-Meier curve showing the overall graft survival post-transplant at the University of Alberta transplant center.*

3.1.2 Characteristics of the UK validation database

Data were obtained from a multicenter PSC transplant cohort derived from six of the seven liver transplant centers from the UK to validate the biochemical predictors of rPSC. Data were collected from 549 patients who received their first transplant for PSC meeting the relevant inclusion and the exclusion criteria. A total of 13.8% (76/549) developed rPSC in their graft after a median of 5.2 years (n= 76, range = 0.13 - 14.93). The lower percentage of recurrence may be attributable to the shorter follow up period in the UK cohort. Notably, the UK database lacked recipient and the donor information, and the labs were collected only one-year post-transplant, whereas the Alberta database contained more extensive detail *(Table 3.1, 3.2)*.

3.2 Predictors of rPSC

3.2.1 Clinical predictors in the univariate analysis

We tested the covariates using a Cox regression analysis in the Alberta cohort. In a univariate analysis, transplantation from the intensive care unit (ICU) was significantly associated with a higher risk of recurrence (HR = 2.66, p= 0.046, 95% CI = 1.02 - 6.98), and coming from a Caucasian background was protective against rPSC (HR = 0.27, p = 0.033, 95% CI = 0.08 - 0.90). However, the latter was excluded from the multivariable analysis because there was not a sufficient number of non-Caucasians (n=6) for analysis.

The calendar year of transplantation wasn't associated with rPSC but was used in the multivariate Cox model for having p <0.1 (HR = 1.05, p = 0.055, 95% CI = 1 - 1.11). To determine the relationship between rPSC and the year of the transplant, we dichotomized the study period into two separate time periods ranging from 1985 – 2002 vs. 2003 – 2019 *(Figure 3.3)*; but no significant difference in outcomes were observed (HR = 0.61, p = 0.138, 95% CI = 0.32 - 1.17). Furthermore, neither IBD, colectomy nor other clinical features were associated with the risk of rPSC. These features included gender, age at first transplant, diabetes mellitus (DM) diagnosis in the recipient, deceased vs. living donor, cold and warm ischemic time, type of anastomosis used, donor age, donor sex, recipient-donor sex match, rejection, or immunosuppression regimen during the first year after the transplantation *(Table 3.3)*.

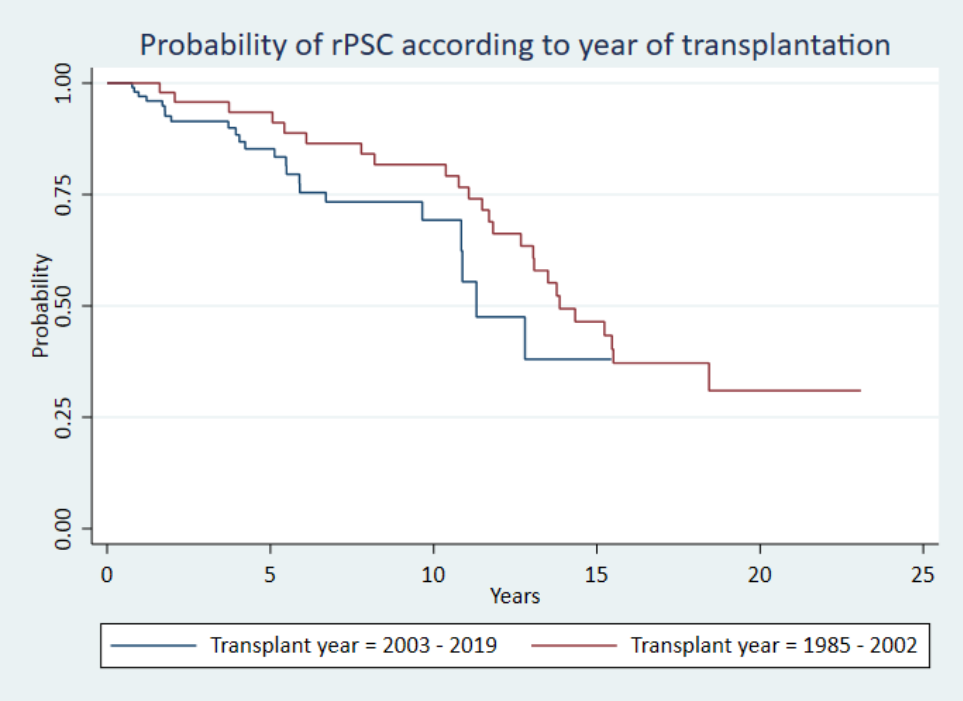

Figure 3.3: *A Kaplan-Meier curve showing the relationship between rPSC and transplant year.*

3.2.2 Lab predictors in univariate analysis

An elevated bilirubin was the only biochemical predictor pre-transplant that was found to be associated with the development of rPSC post-transplant by univariate Cox analysis; however, the impact was light (HR = 1.03, p = 0.013, 95% CI = 1.01 - 1.06). Whereas, an elevated bilirubin at 6 and 9 months post-transplant had a much stronger effect on the development of rPSC over the course of the follow-up (HR = 1.79, p = 0.02, 95% CI = 1.09 - 2.93) and (HR = 2.15, p < 0.0001, 95% CI = 1.43 - 3.23), respectively. As early as one month post-transplant, ALP was able to predict the recurrence of PSC (HR = 1.26, p = 0.016, 95% CI = 1.04 - 1.52). This association was observed at all time points evaluated until the end of the first year after the surgery. Having mild cholestasis one month after the transplantation also raised the risk of suffering from rPSC (HR = 2.14,

Table 3.3: *Univariate Cox regression of the relationship between the clinical variables and rPSC*

	Non-recurrent PSC (n = 110)		Recurrent PSC (n = 48)				
	Median/%(n)	**Range**	**Median/%(n)**	**Range**	**HR**	**95% CI**	**p-value**
Recipient Sex (Female: Male)	(28: 82)		(13: 35)		0.92	0.49 - 1.75	0.805
Age at Transplant	40.89 (110)	6.02 - 71.99	43.06 (48)	14.84 - 64.34	1.01	0.99 - 1.03	0.599
Ethnicity (non-Caucasian: Caucasian)	(3: 90)		(3: 44)		0.27	0.08 - 0.90	0.033
IBD	78.2% (86/110)		91.7% (44/48)		1.84	0.66 - 5.14	0.243
Colectomy	18.4% (16/87)		15.9% (7/44)		0.90	0.40 - 2.03	0.808
Transplant Year					1.05	1.00 - 1.11	0.055
Transplant Year Categorical (early vs. late)	(26: 84)		(25: 23)		0.61	0.32 - 1.17	0.138
Overlap Syndrome: PSC	(13: 97)		(5: 43)		2.08	0.82 - 5.31	0.124
Transplant from ICU	21.3% (20/94)		13.0% (6/46)		2.66	1.02 - 6.98	0.046
Recipient Diagnosis with DM	15.6% (17/109)		25% (12/48)		1.24	0.64 - 2.39	0.519
Donor Type (Living: Deceased)	(34: 74)		(7 :40)		0.95	0.41 - 2.20	0.912
Donor Age	34 (98)	3.6 - 67	40 (44)	8 - 73	1.02	1.00 - 1.-04	0.108
Donor Sex (Female: Male)	(40: 63)		(14: 31)		1.36	0.72 - 2.58	0.348
Sex Match	64.1% (66/103)		73.3% (33/45)		1.24	0.64 - 2.40	0.523
Cold Ischemia Time (min)	180 (99)	14 - 843	287 (39)	20 - 835	1.00	1.00 -1.00	0.454
Warm Ischemia Time (min)	56 (87)	23 - 322	51.5 (36)	36 - 153	1.01	1.00 - 1.02	0.185
Surgery (Roux: End-to End)	(7: 96)		(4: 42)		1.10	0.39 - 3.08	0.854
Rejection	38.0% (41/108)		52.1% (25/48)		1.13	0.64 - 2.00	0.669
			Immunosuppression				
Tacrolimus	89.3% (92/103)		75.6% (34/45)		0.88	0.44 - 1.76	0.717
Sirolimus	22.3% (23/103)		22.2% (10/45)		0.68	0.34 - 1.38	0.286
Cyclosporine	11.7% (12/103)		24.4% (11/45)		0.90	0.45 - 2.18	0.77
MMF	62.1% (64/103)		35.6% (16/45)		1.15	0.60 - 1.81	0.679

p = 0.041, 95% CI = 1.03 - 4.44). However, severe cholestasis three and six months after the surgery was predictive of rPSC (HR = 2.81 p = 0.011, 95% CI = 1.27 - 6.23), and (HR = 2.55 p = 0.026, 95% CI = 1.12 - 5.81), respectively *(Figures 3.4, 3.5)*.

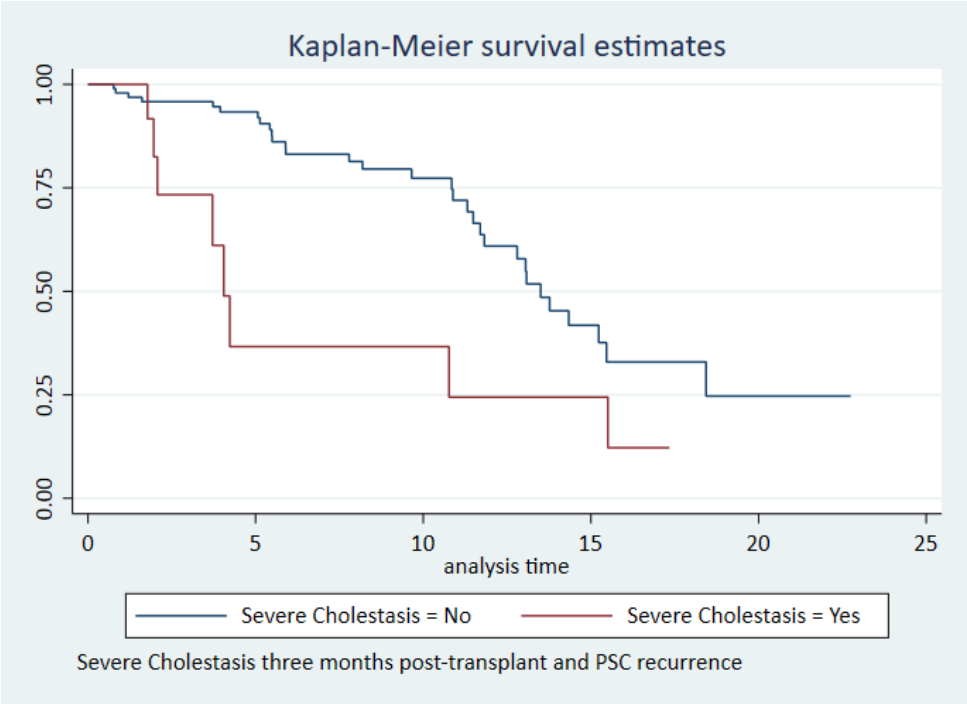

Figure 3.4: *A Kaplan-Meier curve shows the relationship between developing rPSC and having severe cholestasis 3 months post-transplant using Alberta cohort.*

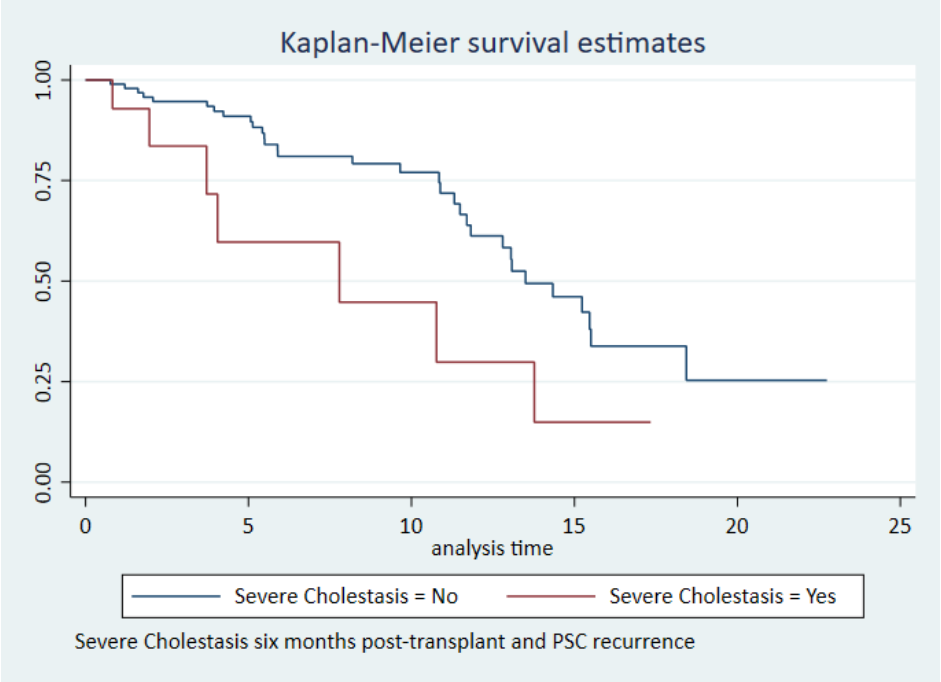

Figure 3.5: *A Kaplan-Meier curve shows the relationship between developing rPSC and having severe cholestasis 6 months post-transplant using Alberta cohort.*

In contrast, only a trend was observed for severe cholestasis to predict rPSC at 9 and 12 months but because the p value remained <0.1 at these time points, severe cholestasis was kept in the multivariable model.

In addition to cholestasis, elevation of AST and ALT following surgery were also linked with rPSC, suggesting that early development of hepatitis and cholestasis were risk factors for recurrent disease. Indeed, elevated AST at 1 month (HR = 1.64, p = 0.008, 95% CI = 1.14 - 2.37) and 3 months (HR = 1.31, p = 0.36, 95% CI = 1.02 - 1.68), and the HR of ALT at one month (HR = 1.63, p = 0.004, 95% CI = 1.16 - 2.29) all provided increased risk. We also tested GGT at the different time points, but it wasn't included in the multivariable analyses due to the limited availability of GGT data in the cohort *(Table 3.4)*.

Table 3.4: *Univariate Cox regression of the labs during the first year post-transplant and rPSC*

	Non-recurrent PSC (n = 110)		Recurrent PSC (n = 48)				
	Median/%(n)	**Range**	**Median/%(n)**	**Range**	**HR**	**95% CI**	**p-value**
			Pre-Transplant				
Albumin x LLN*	0.91 (79)	0.19	0.93 (40)	0.21	0.95	0.20 - 4.39	0.943
Creatinine x ULN	0.68 (99)	0.23 - 3.04	0.71 (40)	0.36 - 2.26	1.44	0.68 - 3.04	0.34
INR	1.3 (81)	0.9 - 3.7	1.2 (35)	0.9 - 2.1	0.61	0.23 - 1.65	0.329
MELD	22 (46)	6 - 40	16 (29)	6 - 38	1.02	0.98 - 1.07	0.353
AST x ULN	2.95 (77)	0.45 - 23.4	3.2 (38)	0.88 - 7.25	0.97	0.87 - 1.07	0.509
ALT x ULN	1.44 (63)	0.36 - 8.68	1.98 (33)	0.38 - 6.2	0.97	0.78 - 1.21	0.798
GGT x ULN	2.91(36)	0 .3 - 10.87	2.11 (11)	0.7 - 43.27	1.06	1.00 - 1.12	0.04
ALP x ULN	2.5 (78)	0.18 -9.12	2.4 (39)	0.58 - 15.09	1.00	0.90 - 1.12	0.932
Total Bilirubin x ULN	5,.15 (99)	0.25 - 52.8	6.2 (41)	0.25 - 54.6	1.03	1.01 - 1.06	0.013
Mild Cholestasis	15.1% (13/86)		17.5% (7/40)		0.94	0.42 - 2.13	0.883
Severe Cholestasis	77.9% (67/86)		75.0% (30/40)		1.010	0.49 - 2.07	0.978
			1 Month Post-Transplant				
AST x ULN	0.7 (79)	0.23 - 14.45	0 .8 (39)	0.23 - 5.83	1.64	1.14 - 2.37	0.008
ALT x ULN	0.62 (65)	0.18 - 22.68	0.9 (25)	0.26 - 3.74	1.63	1.16 - 2.29	0.004
GGT x ULN	0.59 (8)	0.19- 1.5	5.36 (4)	0.7 - 8.31	1.64	1.04 - 2.57	0.032
ALP x ULN	1.22 (79)	0.43 - 9.08	1.13 (39)	0.29 - 8.66	1.26	1.04 - 1.52	0.016
Total Bilirubin x ULN	1.15 (79)	0.25 - 29.25	1.25 (39)	0.25 - 11.75	1.09	0.95 - 1.24	0.226
Mild Cholestasis	34.2% (27/79)		30.8% (12/39)		2.14	1.03 - 4.44	0.041
Severe Cholestasis	16.5% (13/79)		25.6% (10/39)		1.70	0.82 - 3.51	0.152
			3 Months Post-Transplant				
AST x ULN	0.66 (71)	0.35 - 4.88	0.78 (39)	0.4 - 7.78	1.31	1.02 - 1.68	0.036
ALT x ULN	0.64 (62)	0.22 - 8.44	0.9 (23)	0.22 - 8.24	1.15	0.96 - 1.37	0.14
GGT x ULN	1.05 (26)	0.2 - 6.59	0.49 (9)	0.26 - 13.2	1.23	1.00 - 1.52	0.048
ALP x ULN	0.96 (73)	0.38 - 5.62	1.15 (39)	0.39 - 11.57	1.22	1.07 - 1.40	0.002
Total Bilirubin x ULN	0.5 (73)	0.1 - 11.35	0.6 (39)	0.2 - 6.9	1.18	0.96 - 1.44	0.117
Mild Cholestasis	11.0% (8/73)		10.3% (4/39)		1.46	0.51 - 4.17	0.481
Severe Cholestasis	8.2% (6/73)		20.5% (8/39)		2.81	1.27 - 6.23	0.011
			6 Months Post-Transplant				
AST x ULN	0.73 (69)	0.28 - 3.63	0.88 (39)	0.4 - 3.9	1.32	0.91 - 1.91	0.143
ALT x ULN	0.68 (60)	0.24 - 9.2	0.9 (24)	0.34 - 2.58	1.04	0.76 - 1.42	0.795
GGT x ULN	0.49 (34)	0.19 - 10.5	1.06 (10)	0.19 - 3.41	1.02	0.67 - 1.54	0.931
ALP x ULN	0.99 (70)	0.37 - 6.69	1.19 (39)	0.29 - 10.52	1.32	1.13 - 1.54	<0.0001
Total Bilirubin x ULN	0.5 (68)	0.1 - 5.6	0.55 (39)	0.1 - 3.95	1.79	1.09 - 2.93	0.02
Mild Cholestasis	12.9% (9/70)		17.9% (7/39)		1.31	0.57 - 2.98	0.522
Severe Cholestasis	10.0% (7/70)		17.9% (7/39)		2.55	1.12 - 5.81	0.026

			9 Months Post-Transplant				
AST x ULN	0.73 (63)	0.33 - 3.28	0.875 (39)	0.25 - 2.83	1.64	0.90 - 2.96	0.104
ALT x ULN	0.58 (53)	0.22 - 6.14	0.74 (22)	0.4 - 2.78	1.25	0.82 - 1.90	0.295
GGT x ULN	0.45 (28)	0.09 - 9.57	0.74 (11)	0.23 - .5	1.00	0.77 - 1.29	0.999
ALP x ULN	1.02 (64)	0.42 - 6.2	1.21 (39)	0.48 - 10.96	1.20	1.04 - 1.39	0.012
Total Bilirubin x ULN	0.6 (64)	0.1 - 4.6	0.65 (39)	0.1 - 7.05	2.15	1.43 - 3.23	<0.0001
Mild Cholestasis	14.1% (9/64)		10.3% (4/39)		1.20	0.42 - 3.42	0.73
Severe Cholestasis	7.8% (5/64)		12.8(5/39)		2.51	0.97 - 6.47	0.057
			1 Year Post-Transplant				
AST x ULN	0.63 (63)	0.3 - 4.4	0.9 (39)	0.38- 3.9	1.13	0.80 - 1.59	0.496
ALT x ULN	0.57 (58)	0.2 - 3.76	0.9 (27)	0.5 - 2.58	1.28	0.87 - 1.88	0.206
GGT x ULN	0.36 (30)	0.07 - 7.21	1.21 (9)	0.26 - 2.87	1.15	0.84 - 1.57	0.392
ALP x ULN	0.97 (64)	0.38 - 4.74	1.15 (39)	0.4 - 5.83	1.45	1.15 - 1.84	0.002
Total Bilirubin x ULN	0.55 (62)	0.05 - 13.25	0.55 (39)	0.15 - 7.65	0.98	0.83 - 1.14	0.765
Mild Cholestasis	9.4% (6/64)		17.9% (7/39)		1.46	0.64 - 3.31	0.368
Severe Cholestasis	7.8% (5/64)		15.4% (6/39)		2.39	1.00 - 5.74	0.051

** Albumin is normally distributed. It is reported as mean ± standard deviation (SD).*
ULN = upper limit of normal

3.2.3 Predictors of rPSC in multivariable analysis

As described earlier, we used all the variables with p <0.1 in several multivariable Cox regression models depending on the time point at which the labs were collected. For each time point, two models were built depending on the type of cholestasis indicators: either ALP and total bilirubin as continuous variables or mild and severe cholestasis.

In those multivariable models, only indicators of biliary cholestasis posed a risk for recurring PSC in the graft post-transplant. In other words, total bilirubin pre-transplant had a weak but significant association with rPSC (HR = 1.004, p = 0.009, 95% CI = 1.00 - 1.001). However, that association increased at 9-month timepoint (HR = 1.929, p = 0.002, 95% CI = 1.28 - 2.90). Similarly, patients with higher ALP were more likely to be diagnosed with rPSC at any time from one month following the surgery (HR = 1.304, p = 0.027, 95% CI = 1.03 - 1.65) up to 12 months (HR = 1.429, p = 0.003, 95% CI = 1.13 - 1.81).

Using the categorical indicators of cholestasis, only severe cholestasis at 3 months (HR = 2.41, p = 0.046, 95% CI = 1.02 - 5.70), and at 6 months (HR = 2.54, p = 0.028, 95% CI = 1.11 - 5.83) was associated with increased risk of rPSC. Severe cholestasis at 9 and 12 months maintained the trend of increasing the risk for rPSC. All the multivariable models met the proportional hazard assumption needed for a Cox regression *(Table 3.5)*.

We validated these findings using the UK database, which showed that one year post-transplant, a higher ALP (HR = 1.423, p <0.0001, 95% CI = 1.06 - 1.23), and severe cholestasis (HR = 3.141, p <0.0001, 95% CI = 1.85 - 5.34) *(Figure 3.6)* were both significantly associated with rPSC *(Table 3.6)*.

These findings indicate that early pathological changes in the bile ducts signals subsequent disease recurrence and suggests a hypothesis that early inflammation may progresses to a chronic state.

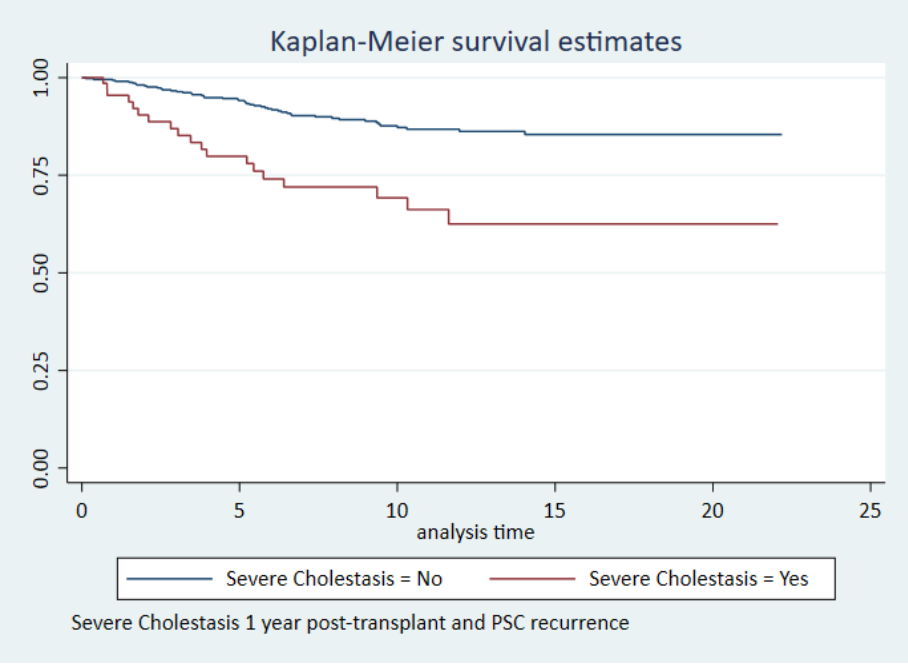

Figure 3.6: *A Kaplan-Meier curve shows the relationship between developing rPSC and having severe cholestasis 12 months post-transplant using the UK validation cohort.*

Table 3.5: *Multivariate Cox regression models for predicting rPSC using Alberta cohort data*

Models with Continuous Chole.				Models with Categorical Chole.			
Model at Pre-Transplant (n = 125)							
	HR	**p Value**	**95% CI**				
Transplant from ICU	2.424	0.137	0.75 - 7.78				
Transplant Year	0.984	0.683	0.91 - 1.06				
Total Bilirubin x ULN	1.035	0.014	1.01 - 1.06				
Model at 1 Month Post-Transplant (n = 87)				***Model at 1 Month Post-Transplant (n = 87)***			
	HR	**p Value**	**95% CI**		**HR**	**p Value**	**95% CI**
Transplant from ICU	1.706	0.4	0.49 - 5.92	**Transplant from ICU**	1.94	0.278	0.58 - 6.46
Transplant Year	0.951	0.281	0.87 - 1.04	**Transplant Year**	0.94	0.204	0.86 - 1.03
AST x ULN	1.394	0.318	0.73 - 2.67	**AST x ULN**	1.67	0.125	0.87 - 3.21
ALT x ULN	1.155	0.631	0.64 -2.08	**ALT x ULN**	1.25	0.462	0.69 - 2.26
ALP x ULN	1.304	0.027	1.03 - 1.65	**Mild Cholestasis**	1.84	0.228	0.68 - 4.95
Model at 3 Months Post-Transplant (n = 107)				***Model at 3 Months Post-Transplant (n = 107)***			
	HR	**p Value**	**95% CI**		**HR**	**p Value**	**95% CI**
Transplant from ICU	1.647	0.443	0.46 - 5.89	**Transplant from ICU**	1.58	0.477	0.45 - 5.59
Transplant Year	1.011	0.799	0.93 - 1.10	**Transplant Year**	1.02	0.581	0.94 - 1.11
AST x ULN	1.182	0.285	0.87 - 1.61	**AST x ULN**	1.23	0.148	0.93 - 1.64
ALP x ULN	1.176	0.033	1.01 - 1.36	**Severe Cholestasis**	2.41	0.046	1.02 - 5.70
Model at 6 Months Post-Transplant (n = 105)				***Model at 6 Months Post-Transplant (n = 107)***			
	HR	**p Value**	**95% CI**		**HR**	**p Value**	**95% CI**
Transplant from ICU	1.013	0.985	0.26 - 3.99	**Transplant from ICU**	1.56	0.490	0.44 - 5.44
Transplant Year	1.030	0.463	0.95 - 1.12	**Transplant Year**	1.03	0.528	0.95 - 1.11
ALP x ULN	1.246	0.018	1.04 - 1.49	**Severe Cholestasis**	2.54	0.028	1.11 - 5.83
Total Bilirubin x ULN	1.291	0.35	0.76 - 2.21				
Model at 9 Months Post-Transplant (n = 102)				***Model at 9 Months Post-Transplant (n = 102)***			
	HR	**p Value**	**95% CI**		**HR**	**p Value**	**95% CI**
Transplant from ICU	1.294	0.699	0.35 - 4.78	**Transplant from ICU**	1.76	0.378	0.50- 6.20
Transplant Year	1.036	0.396	0.96 - 1.12	**Transplant Year**	1.02	0.549	0.95 - 1.11
ALP x ULN	1.197	0.029	1.02 - 1.41	**Severe Cholestasis**	2.41	0.071	0.93 - 6.24
Total Bilirubin x ULN	1.929	0.002	1.28 - 2.90				
Model at 12 Months Post-Transplant (n = 102)				***Model at 12 Months Post-Transplant (n = 102)***			
	HR	**p Value**	**95% CI**		**HR**	**p Value**	**95% CI**
Transplant from ICU	1.649	0.449	0.45 -6.03	**Transplant from ICU**	1.91	0.312	0.54 - 6.73
Transplant year	1.028	0.515	0.95 - 1.12	**Transplant year**	1.02	0.559	0.94 - 1.11
ALP x ULN	1.429	0.003	1.13 - 1.81	**Severe Cholestasis**	2.34	0.058	0.97 - 5.64

Table 3.6: *Results validation using UK cohort data.*

Analysis with Continuous Chole.				Analysis with Categorical Chole.			
	HR	**p Value**	**95% CI**		**HR**	**p Value**	**95% CI**
ALP x ULN at 12 Months Post-Transplant	1.14	<0.0001	1.06 - 1.23	**Severe Cholestasis at 12 Months Post-Transplant**	3.14	<0.0001	1.85 - 5.34

Chole. = Cholestasis, ULN = upper limit of normal, n is sample size depending on lab availability

3.3 Effect of rPSC on graft survival using multi-state model

3.3.1 Clinical predictors in univariate analysis

After excluding three patients with unknown cause of graft loss, the total number of patients available for analysis was 155. We performed a univariate Cox regression to determine the variables to be used further to build the multivariable multi-state models. Variables with $p<0.1$ were used in the multivariable models.

Notably, patients with IBD had improved graft survival (HR = 0.36, p = 0.004, 95% CI = 0.18 - 0.73). This was mostly related to UC (HR = 0.50, p = 0.035, 95% CI = 0.27 - 0.95) rather than CD (HR = 1.00, p = 0.993, 95% CI = 0.44 - 2.28). We kept only the UC without IBD in the multivariable models to prevent collinearity. Colectomy wasn't associated with graft survival (HR = 1.51, p = 0.276, 95% CI = 0.72 - 3.17).

Tacrolimus seems to have had a protective effect against graft loss (HR = 0.36, p = 0.005, 95% CI = 0.18 - 0.73), in contrast to cyclosporine, which posed a significant risk to graft viability (HR = 2.25, p = 0.025, 95% CI = 1.11 - 4.57). Sirolimus and MMF weren't associated with graft survival.

Other variables with a $p<0.1$ that were used to build the model included transplant year (HR = 0.96, p = 0.055, 95% CI = 0.91 - 1.00) and donor age (HR = 1.02, p = 0.062, 95% CI = 1.00 - 1.04). We analyzed the following clinical features and found that they were not related to graft loss: sex, age at transplant, ethnicity, overlap syndrome, transplant from ICU, recipient with DM, receiving a graft from deceased vs. living donor, donor

sex, donor-recipient sex match, cold and warm ischemic time, and type of the anastomosis used *(Table 3.7)*. Testing the timing of PSC recurrence in univariate analysis wasn't possible because it is a time-dependent continuous variable.

Table 3.7: *Univariate Cox regression for variables related to graft loss*

	HR	95% CI	p-value
Sex	1.12	0.53 - 2.35	0.769
Age at Transplant	0.99	0.97 - 1.01	0.464
Ethnicity (Non-Caucasian: Caucasian)	0.44	0.10 - 1.88	0.269
IBD	0.36	0.18 - 0.73	0.004
Ulcerative Colitis	0.50	0.27 - 0.95	0.035
Crohn's Disease	1.00	0.44 - 2.28	0.993
Colectomy	1.51	0.72 - 3.17	0.276
Transplant Year	0.96	0.91 - 1.00	0.055
Overlap	0.70	0.17 - 2.93	0.630
Transplant from ICU	1.33	0.44 - 4.03	0.617
Diabetes Recipient	1.53	0.77 - 3.02	0.221
Donor Type (Deceased: Living)	1.06	0.43 - 2.60	0.894
Donor Age	1.02	1.00 - 1.04	0.062
Donor Sex	0.65	0.34 - 1.26	0.206
Sex Match	0.94	0.46 - 1.91	0.860
Cold Ischemia (min)	1.00	1.00 - 1.00	0.136
Warm Ischemia (min)	1.01	1.00 - 1.02	0.114
Surgery (End-to End: Roux-En-Y)	1.13	0.35 - 3.70	0.837
Immunosuppression			
Tacrolimus	0.36	0.18 - 0.73	0.005
Sirolimus	1.05	0.48 - 2.26	0.908
MMF	1.42	0.69 - 2.91	0.342
Cyclosporine	2.25	1.11 - 4.57	0.025

3.3.2 Lab predictors in univariate analysis

None of the lab tests obtained prior to transplantation were found predictive of graft survival. However, the presence of cholestasis able to predict graft survival as early as the first month post-transplant: ALP (HR = 1.35, p = 0.001, 95% CI = 1.13 - 1.62), total bilirubin (HR = 1.18, p <0.0001, 95% CI = 1.10 - 1.27), mild cholestasis (HR = 2.29, p = 0.038, 95% CI = 1.05 - 5.00), and severe cholestasis (HR = 2.22, p = 0.039, 95% CI = 1.04 - 4.75). Higher ALP and severe cholestasis remained associated with a higher risk of

graft loss at all time points until 12 months post-transplant, whereas total bilirubin remained associated with higher probability of graft loss until 9 months post-transplant. Mild cholestasis was also significant at the 9-month time point only (HR = 3.10, p = 0.017, 95% CI = 1.23 - 7.85).

There was also a direct relationship between AST after one month of the surgery and graft loss (HR = 1.36, p = 0.002, 95% CI = 1.12 - 1.64), and this correlation was maintained for up to six months post-transplant. In contrast, ALT was only significant one month after the surgery (HR = 1.21, p = 0.004, 95% CI = 1.06 - 1.37). Although GGT was significant at some time points, it wasn't involved further in the multivariable models due to missing data *(Table 3.8)*.

Table 3.8: *Univariate Cox regression for the labs related to graft loss*

	HR	p Value	95% CI
Labs Pre-Transplant			
Albumin x LLN	0.37	0.256	0.06 - 2.07
Creatinine x ULN	0.99	0.977	0.40 - 2.43
INR	1.02	0.963	0.43 - 2.40
MELD	1.02	0.295	0.98 - 1.07
AST x ULN	1.01	0.765	0.92 - 1.12
ALT x ULN	0.95	0.748	0.72 - 1.27
GGT x ULN	1.05	0.167	0.98 - 1.11
ALP x ULN	0.94	0.402	0.82 - 1.08
Total Bilirubin x ULN	1.02	0.236	0.99 - 1.05
Mild Cholestasis	0.50	0.254	0.15 - 1.65
Severe Cholestasis	1.69	0.286	0.64 - 4.45
Labs 1 Month Post-Transplant			
AST x ULN	1.36	0.002	1.12 - 1.64
ALT x ULN	1.21	0.004	1.06 - 1.37
GGT x ULN	1.42	0.121	0.91 - 2.21
ALP x ULN	1.35	0.001	1.13 - 1.62
Total Bilirubin x ULN	1.18	<0.0001	1.10 - 1.27
Mild Cholestasis	2.29	0.038	1.05 - 5.00
Severe Cholestasis	2.22	0.039	1.04 - 4.75
Labs 3 Months Post-Transplant			
AST x ULN	1.26	0.059	0.99 - 1.61
ALT x ULN	1.12	0.319	0.90 - 1.38
GGT x ULN	1.24	0.018	1.04 - 1.49
ALP x ULN	1.22	0.003	1.07 - 1.40
Total Bilirubin x ULN	1.24	0.023	1.03 - 1.49
Mild Cholestasis	1.53	0.434	0.53 - 4.39
Severe Cholestasis	3.88	0.001	1.72 - 8.76
Labs 6 Months Post-Transplant			

AST x ULN	1.43	0.094	0.94 - 2.19
ALT x ULN	1.03	0.871	0.70 - 1.53
GGT x ULN	1.28	0.040	1.01 - 1.62
ALP x ULN	1.32	<0.0001	1.13 - 1.55
Total Bilirubin x ULN	2.45	<0.0001	1.64 - 3.66
Mild Cholestasis	2.02	0.111	0.85 - 4.81
Severe Cholestasis	3.34	0.006	1.42 - 7.88
	Labs 9 Months Post-Transplant		
AST x ULN	1.38	0.312	0.74 - 2.55
ALT x ULN	1.04	0.923	0.50 - 2.14
GGT x ULN	1.23	0.136	0.94 - 1.62
ALP x ULN	1.26	0.001	1.10 - 1.44
Total Bilirubin x ULN	2.11	<0.0001	1.52 - 2.94
Mild Cholestasis	3.10	0.017	1.23 - 7.85
Severe Cholestasis	4.43	0.001	1.78 - 11.00
	Labs 1 Year Post-Transplant		
AST x ULN	1.08	0.747	0.67 - 1.74
ALT x ULN	1.20	0.486	0.72 - 1.98
GGT x ULN	0.95	0.861	0.50 - 1.78
ALP x ULN	1.45	0.004	1.12 - 1.88
Total Bilirubin x ULN	0.98	0.876	0.81 - 1.20
Mild Cholestasis	1.21	0.699	0.46 - 3.20
Severe Cholestasis	2.77	0.040	1.05 - 7.30

3.3.3 Multivariable time-dependent Cox model

We analyzed predictors of graft loss and abnormal lab tests at differing time points following liver transplantation using rPSC as a time-dependent covariate. Not surprisingly, we found that patients who developed rPSC were at a significant risk of losing their grafts. This risk remained consistent all the time points. The risk of losing the graft to rPSC using the continuous labs at 1-month time point was (HR = 8.63, p <0.0001, 95% CI = 2.80- 26.66). Moreover, ALP and severe cholestasis were independent predictors of graft loss as early as one month following transplantation (HR = 1.29, p = 0.039, 95% CI = 1.01 - 1.65), and (HR = 4.77, p = 0.021, 95% CI = 1.27 - 17.93) respectively. Both variables remained significant at time points until 12 months, except for ALP, which became a trend only at the 6-month time point.

At the 6-month time point, total bilirubin was significantly associated with rPSC (HR = 1.76, p = 0.035, 95% CI = 1.04 - 2.99), which remained significant at the 9-month time point (HR = 1.74, p = 0.047, 95% CI = 1.01 - 3.02). Additionally, patients with mild cholestasis very early at 1-month time point were at a higher risk of graft loss (HR = 7.81, p = 0.001, 95% CI = 2.25 - 27.09). The risk was also significant 9 months after the surgery (HR = 3.64, p = 0.014, 95% CI = 1.29 - 10.25).

Of note, tacrolimus appeared to be protective against graft loss after adjusting for other variables using labs at 6-month time point (HR = 0.06, p = 0.037, 95% CI = 0.004 - 0.84) *(Table 3.9)*.

Again, these findings align with the previous results that an early inflammatory process predominantly affects the biliary system. This process progresses to reach hyper bilirubinemia. This may be a cholestasis condition that predisposes rPSC, or indicates an early disease (rPSC) process.

Table 3.9: *Multivariate time-dependent Cox models predicting graft failure post-transplant for PSC*

Models with Continuous Chole.				Models with Categorical Chole.			
Model at 1 Month Post-Transplant (n= 112)				**Model at 1 Month Post-Transplant (n= 112)**			
	HR	**95% CI**	**p value**		**HR**	**95% CI**	**p value**
Donor Age	1.01	0.981 - 1.05	0.418	**Donor Age**	1.02	0.98 - 1.05	0.352
Ulcerative Colitis	0.36	0.125 - 1.04	0.059	**Ulcerative Colitis**	0.41	0.14 - 1.21	0.106
Tacrolimus	1.47	0.072 30.07	0.802	**Tacrolimus**	0.25	0.02 - 2.91	0.266
Cyclosporine	4.79	0.168 - 137.10	0.360	**Cyclosporine**	0.58	0.05 - 7.25	0.675
Transplant Year	1.00	0.894 - 1.12	0.992	**Transplant Year**	0.96	0.86 - 1.08	0.512
AST x ULN	0.98	0.461 - 2.10	0.968	**AST x ULN**	1.15	0.53 - 2.50	0.716
ALT x ULN	0.85	0.509 - 1.41	0.524	**ALT x ULN**	1.04	0.63 - 1.71	0.873
ALP x ULN	1.29	1.013 - 1.65	0.039	**Severe Cholestasis**	4.77	1.27 - 17.93	0.021
Total Bilirubin x ULN	1.14	0.993 - 1.31	0.062	**Mild Cholestasis**	7.81	2.25 -27.09	0.001
PSC Recurrence	8.63	2.796 - 26.66	<0.0001	**PSC Recurrence**	6.73	2.20 - 20.61	0.001
Model at 3 Months Post-Transplant (n= 144)				**Model at 3 Months Post-Transplant (n= 144)**			
	HR	**95% CI**	**p value**		**HR**	**95% CI**	**p value**
Donor Age	1.01	0.988 - 1.03	0.380	**Donor Age**	1.02	0.99 - 1.04	0.171
Ulcerative Colitis	0.48	0.212 - 1.07	0.073	**Ulcerative Colitis**	0.48	0.22 - 1.05	0.067
Tacrolimus	0.65	0.035 - 12.02	0.772	**Tacrolimus**	0.56	0.04 - 9.00	0.686
Cyclosporine	2.19	0.111 - 43.21	0.606	**Cyclosporine**	1.82	0.10 - 31.57	0.681
Transplant Year	1.03	0.941 - 1.13	0.506	**Transplant Year**	1.04	0.94 - 1.14	0.436
AST x ULN	0.89	0.623 - 1.28	0.544	**AST x ULN**	0.85	0.63 - 1.16	0.306
ALP x ULN	1.28	1.088 - 1.51	0.003	**Severe Cholestasis**	5.70	2.01 - 16.17	0.001
Total Bilirubin x ULN	1.11	0.839 - 1.47	0.461	**PSC Recurrence**	8.19	3.12 - 21.51	<0.0001
PSC Recurrence	7.62	2.888 - 20.09	<0.0001				
Model at 6 Months Post-Transplant (n= 139)				**Model at 6 Months Post-Transplant (n= 141)**			
	HR	**95% CI**	**p value**		**HR**	**95% CI**	**p value**
Donor Age	1.00	0.979 - 1.03	0.803	**Donor Age**	1.01	0.98 - 1.03	0.659
Ulcerative Colitis	0.58	0.249 - 1.34	0.202	**Ulcerative Colitis**	0.66	0.29 - 1.51	0.322
Tacrolimus	0.06	0.004 - 0.84	0.037	**Tacrolimus**	0.05	0.003 - 0.76	0.031
Cyclosporine	0.19	0.011 - 3.19	0.248	**Cyclosporine**	0.20	0.01 - 2.90	0.237
Transplant Year	1.03	0.922 - 1.14	0.632	**Transplant Year**	1.04	0.94 - 1.14	0.461
AST x ULN	0.69	0.243 - 1.95	0.481	**AST x ULN**	1.28	0.69 - 2.40	0.436
ALP x ULN	1.35	0.958 - 1.90	0.087	**Severe Cholestasis**	3.18	1.02 - 9.94	0.046
Total Bilirubin x ULN	1.76	1.042 - 2.99	0.035	**PSC Recurrence**	5.90	2.43 - 14.31	<0.0001
PSC Recurrence	5.43	2.163 - 13.65	<0.0001				
Model at 9 Months Post-Transplant (n= 137)				**Model at 9 Months Post-Transplant (n= 137)**			
	HR	**95% CI**	**p value**		**HR**	**95% CI**	**p value**
Donor Age	0.99	0.968 - 1.02	0.542	**Donor Age**	1.00	0.973 - 1.02	0.806
Ulcerative Colitis	0.53	0.226 - 1.26	0.151	**Ulcerative Colitis**	0.63	0.273 - 1.43	0.267

Tacrolimus	1.69	0.082 - 35.02	0.734
Cyclosporine	9.17	0.423 - 198.76	0.158
Transplant Year	1.09	0.983 - 1.20	0.104
ALP x ULN	1.39	1.150 - 1.67	0.001
Total Bilirubin x ULN	1.74	1.008 - 3.02	0.047
PSC Recurrence	6.02	2.377 - 15.24	<0.0001

Tacrolimus	0.24	0.018 - 3.33	0.291
Cyclosporine	1.21	0.077 - 18.87	0.894
Transplant Year	1.06	0.960 - 1.18	0.239
Severe Cholestasis	7.83	2.50 - 24.49	0.000
Mild Cholestasis	3.64	1.293 - 10.25	0.014
PSC Recurrence	6.52	2.67 - 15.90	<0.0001

Model at 12 Months Post-Transplant (n= 137)			
	HR	**95% CI**	**p value**
Donor Age	1.00	0.973 - 1.02	0.765
Ulcerative Colitis	0.67	0.293 - 1.53	0.340
Tacrolimus	0.18	0.014 - 2.46	0.201
Cyclosporine	0.76	0.052 - 11.02	0.840
Transplant Year	1.06	0.958 - 1.17	0.256
ALP x ULN	1.43	1.055 - 1.95	0.021
PSC Recurrence	6.76	2.70 - 16.95	<0.0001

Model at 12 Months Post-Transplant (n= 137)			
	HR	**95% CI**	**p value**
Donor Age	1.00	0.976 - 1.02	0.955
Ulcerative Colitis	0.76	0.331 - 1.72	0.506
Tacrolimus	0.22	0.016 - 2.95	0.251
Cyclosporine	1.01	0.065 - 15.78	0.992
Transplant Year	1.07	0.969 - 1.18	0.182
Severe Cholestasis	3.21	1.027 - 10.03	0.045
PSC Recurrence	7.05	2.861 - 17.37	<0.0001

3.3.4 Multivariable semi-Markov Cox model analyses for factors affecting transition probabilities

In addition to the overall models that include rPSC as a time-dependent covariate, we analyzed the effects of the clinical features and abnormal hepatic biochemistry on the development of graft loss for the patient groups experienced rPSC and those who didn't separately using multiple Cox models between the states (Figure 2.1). Accordingly, we tested the effects of the covariates on losing the graft (state 3) in separate patient groups: those who developed rPSC (moving from state 2 to state 3), and those who lost their graft without experiencing rPSC (state 1 to state 3 transition). In this analysis, the time spent before developing rPSC is taken into account when analyzing the state 2 to state 3 transition. Both abnormal ALP and severe cholestasis at 3 months post-transplant were associated with higher risk of graft loss for patients with rPSC: (HR = 1.52, p = 0.003, 95% CI = 1.149 - 2.01) and (HR = 6.15, p = 0.043, 95% CI = 1.062 - 35.66), respectively. The relationship with graft loss and either increased ALP or severe cholestasis was maintained at 9- and 12-month time points but not observed at 6 months. Moreover, increasing total bilirubin nine months after the surgery was associated with a higher risk of graft loss in patients with rPSC (HR = 3.18, p = 0.026, 95% CI = 1.150 - 8.80 *(Table 3.10)*.

These associations disappeared in the patient's group with no rPSC *(Table 3.11)*. This may indicate that cholestasis imposes a risk for graft failure on top of the rPSC effect. In other words, there is a possibility that the higher the cholestasis level in patients who suffer from rPSC, the greater the risk that those patients will lose their graft.

Nevertheless, the models assessing the transition between state 2 (rPSC) and state 3 (graft loss) should be interpreted carefully due to the small sample size used to analyze that transition.

Apart from cholestasis, rPSC patients who took tacrolimus had a better prognosis than those who didn't in terms of graft survival. This protective effect appears in the 3- and 6-month time points (HR = 0.01, p = 0.004, 95% CI = 0.001 - 0.22) and (HR = 0.02, p = 0.008, 95% CI = 0.001 - 0.35) respectively using continuous labs, although the drug didn't have that protective effect for patients who didn't suffer from rPSC. Cyclosporine may probably have a protective effect on the graft as well for patients with rPSC but not on patients without rPSC. This effect appears in the model using labs at 3-month time point (HR = 0.03, p = 0.029, 95% CI = 0.001 - 0.70) *(Table 3.10)*.

Of interest, UC appears to be the only protective factor against graft loss in the patients who didn't experience rPSC. However, that appeared only in the model using the labs at 1 month post-transplant (HR = 0.20, p = 0.033, 95% CI = 0.048 - 0.88) *(Table 3.11)*.

Table 3.10: *Multivariate semi-Markov models for variables effects on graft loss for patients with rPSC (state 2 to state 3 transition)*

Models with Continuous Chole.			
Model at 1 Month Post-Transplant (n= 25)			
	HR	**95% CI**	**p value**
Donor Age	0.97	0.906-1.04	0.375
Ulcerative Colitis	0.70	0.105 - 4.66	0.711
Tacrolimus	0.00	0.000 - Inf.	1.000
Cyclosporine	0.00	0.000 - Inf.	1.000
Transplant Year	1.18	0.963 - 1.45	0.109
AST x ULN	0.09	0.00 - 24.69	0.400
ALT x ULN	0.93	0.077 - 11.29	0.956
ALP x ULN	1.44	0.883 - 2.35	0.143
Total Bilirubin x ULN	1.68	0.771 - 3.68	0.191
Model at 3 Months Post-Transplant (n= 39)			
	HR	**95% CI**	**p value**
Donor Age	0.99	0.959 - 1.03	0.663
Ulcerative Colitis	0.45	0.125 - 1.64	0.227
Tacrolimus	0.01	0.001 - 0.22	0.004
Cyclosporine	0.03	0.001 - 0.70	0.029
Transplant Year	1.02	0.881 - 1.18	0.777
AST x ULN	0.60	0.315 - 1.16	0.131
ALP x ULN	1.52	1.149 - 2.01	0.003
Total Bilirubin x ULN	1.95	0.963 - 3.95	0.063
Model at 6 Months Post-Transplant (n= 39)			
	HR	**95% CI**	**p value**
Donor Age	1.00	0.969 - 1.04	0.876
Ulcerative Colitis	0.50	0.126 - 1.97	0.321
Tacrolimus	0.02	0.001 - 0.35	0.008
Cyclosporine	0.05	0.002 - 1.50	0.085
Transplant Year	1.00	0.830 - 1.21	0.972
AST x ULN	0.67	0.121 - 3.69	0.644
ALP x ULN	1.40	0.815 - 2.41	0.222
Total Bilirubin x ULN	1.90	0.895 - 4.01	0.095
Model at 9 Months Post-Transplant (n= 39)			
	HR	**95% CI**	**p value**
Donor Age	0.99	0.957 - 1.02	0.476
Ulcerative Colitis	0.48	0.144 - 1.58	0.227
Tacrolimus	35.37	0.080 - 15696.81	0.252
Cyclosporine	189.74	0.314 - 114724.80	0.108

Models with Categorical Chole.			
Model at 1 Month Post-Transplant (n=25)			
	HR	**95% CI**	**p value**
Donor Age	1.01	0.969 - 1.06	0.573
Ulcerative Colitis	1.29	0.147 - 11.22	0.821
Tacrolimus	0.00	0.000 - Inf.	1.000
Cyclosporine	0.00	0.000 - Inf.	1.000
Transplant Year	1.20	0.989 - 1.46	0.064
AST x ULN	0.27	0.006 - 12.63	0.503
ALT x ULN	0.52	0.062 - 4.37	0.549
Severe Cholestasis	15.81	0.941 - 265.97	0.055
Mild Cholestasis	2.77	0.363 - 21.08	0.326
Model at 3 Months Post-Transplant (n= 39)			
	HR	**95% CI**	**value**
Donor Age	1.00	0.969 - 1.04	0.842
Ulcerative Colitis	0.47	0.138 - 1.62	0.233
Tacrolimus	0.02	0.001 - 0.34	0.007
Cyclosporine	0.06	0.003 - 1.07	0.056
Transplant Year	1.03	0.877 - 1.21	0.731
AST x ULN	0.94	0.65 - 1.36	0.738
Severe Cholestasis	6.15	1.062 - 35.66	0.043
Model at 6 Months Post-Transplant(n= 39)			
	HR	**95% CI**	**p value**
Donor Age	1.00	0.963 - 1.03	0.862
Ulcerative Colitis	0.81	0.230 - 2.85	0.741
Tacrolimus	0.02	0.001 - 0.36	0.008
Cyclosporine	0.04	0.002 - 1.15	0.061
Transplant Year	0.99	0.841 - 1.18	0.945
AST x ULN	1.92	0.642 - 5.72	0.244
Severe Cholestasis	1.47	0.219 - 9.91	0.690
Model at 9 Months Post-Transplant (n= 39)			
	HR	**95% CI**	**p value**
Donor Age	1.00	0.970 - 1.03	0.971
Ulcerative Colitis	0.48	0.145 - 1.60	0.232
Tacrolimus	0.10	0.006 - 1.73	0.115
Cyclosporine	0.47	0.023 - 9.69	0.625

Transplant Year	1.06	0.926 - 1.22	0.393	**Transplant Year**	1.03	0.891 - 1.18	0.716
ALP x ULN	1.45	1.10 - 1.92	0.009	**Severe Cholestasis**	7.52	1.405 - 40.20	0.018
Total Bilirubin x ULN	3.18	1.150 - 8.80	0.026	**Mild Cholestasis**	5.16	1.012 - 26.28	0.048
Model at 12 Months Post-Transplant (n= 39)				**Model at 12 Months Post-Transplant (n= 39)**			
	HR	**95% CI**	**p value**		**HR**	**95% CI**	**p value**
Donor Age	0.99	0.960 - 1.02	0.543	**Donor Age**	0.99	0.965 - 1.03	0.726
Ulcerative Colitis	0.75	0.235 - 2.40	0.629	**Ulcerative Colitis**	0.91	0.273 - 3.04	0.880
Tacrolimus	0.08	0.006 - 1.13	0.062	**Tacrolimus**	0.14	0.009 - 2.21	0.163
Cyclosporine	0.18	0.011 - 2.94	0.231	**Cyclosporine**	0.43	0.022 - 8.23	0.575
Transplant Year	1.02	0.884 - 1.8	0.759	**Transplant Year**	1.04	0.900 - 1.20	0.586
ALP x ULN	1.56	1.009 - 2.42	0.045	**Severe Cholestasis**	5.95	1.218 - 29.40	0.028

Inf = Infinity
State 2= rPSC
State 3= graft loss

Table 3.11: *Multivariate semi-Markov models for variables effects on graft loss for patients without rPSC (State 1 to state 3 transition)*

Models with Continuous Chole.			
Model at 1 Month Post-Transplant (n= 88)			
	HR	**95% CI**	**p value**
Donor Age	1.03	0.979 - 1.08	0.254
Ulcerative Colitis	0.20	0.048 - 0.88	0.033
Tacrolimus	0.72	0.003 - 199.83	0.909
Cyclosporine	3.66	0.008 - 1748.95	0.680
Transplant Year	0.95	0.819 - 1.11	0.541
AST x ULN	2.12	0.712 - 6.30	0.177
ALT x ULN	0.59	0.285 - 1.20	0.145
ALP x ULN	1.10	0.674 - 1.78	0.714
Total Bilirubin x ULN	1.11	0.851 - 1.44	0.451
Model at 3 Months Post-Transplant (n= 106)			
	HR	**95% CI**	**p value**
Donor Age	1.03	0.991 - 1.07	0.132
Ulcerative Colitis	0.36	0.115 - 1.11	0.074
Tacrolimus	1.76	0.158 - 19.59	0.647
Cyclosporine	3.81	0.318 -45.62	0.291
Transplant Year	0.99	0.868 - 1.14	0.938
AST x ULN	0.38	0.047 - 3.11	0.368
ALP x ULN	1.12	0.731 - 1.71	0.610
Total Bilirubin x ULN	0.90	0.357 - 2.24	0.814
Model at 6 Months Post-Transplant (n= 101)			
	HR	**95% CI**	**p value**
Donor Age	1.01	0.967 - 1.05	0.780
Ulcerative Colitis	0.46	0.138 - 1.56	0.215
Tacrolimus	0.00	0.00 - Inf.	0.998
Cyclosporine	0.00	0.00 - Inf.	0.998
Transplant Year	1.03	0.897 - 1.18	0.696
AST x ULN	0.70	0.183 - 2.69	0.605
ALP x ULN	1.24	0.753 - 2.04	0.398
Total Bilirubin x ULN	1.76	0.803 - 3.88	0.158
Model at 9 Months Post-Transplant (n= 99)			
	HR	**95% CI**	**p value**
Donor Age	1.00	0.960 - 1.04	0.959
Ulcerative Colitis	0.43	0.125 - 1.50	0.186
Tacrolimus	0.00	0.00 - Inf.	0.999
Cyclosporine	0.00	0.00 - Inf.	0.999
Transplant Year	1.07	0.928 - 1.23	0.359

Models with Categorical Chole.			
Model at 1 Month Post-Transplant (n= 88)			
	HR	**95% CI**	**p value**
Donor Age	1.06	0.987 - 1.13	0.112
Ulcerative Colitis	0.13	0.026 - 0.66	0.014
Tacrolimus	0.07	0.003 - 1.69	0.103
Cyclosporine	0.20	0.006 - 6.76	0.369
Transplant Year	0.89	0.745 - 1.07	0.217
AST x ULN	3.45	1.086 - 10.96	0.036
ALT x ULN	0.58	0.293 - 1.16	0.123
Severe cholestasis	1.59	0.155 - 16.21	0.697
Mild cholestasis	12.13	1.942 - 75.77	0.008
Model at 3 Months Post-Transplant (n= 106)			
	HR	**95% CI**	**value**
Donor Age	1.03	0.992 - 1.07	0.126
Ulcerative Colitis	0.36	0.114 - 1.12	0.076
Tacrolimus	1.54	0.150 - 15.71	0.718
Cyclosporine	3.01	0.240 - 37.65	0.393
Transplant Year	0.99	0.867 - 1.14	0.925
AST x ULN	0.27	0.031 - 2.31	0.231
Severe Cholestasis	3.43	0.559 - 21.04	0.183
Model at 6 Months Post-Transplant (n= 101)			
	HR	**95% CI**	**p value**
Donor Age	1.01	0.967 - 1.05	0.727
Ulcerative Colitis	0.48	0.145 - 1.61	0.236
Tacrolimus	0.00	0.00 - Inf.	0.998
Cyclosporine	0.00	0.00 - Inf.	0.998
Transplant Year	1.04	0.918 - 1.17	0.559
AST x ULN	0.92	0.353 - 2.42	0.872
Severe Cholestasis	3.94	0.758 - 20.52	0.103
Model at 9 Months Post-Transplant (n= 99)			
	HR	**95% CI**	**p value**
Donor Age	1.00	0.955 - 1.04	0.872
Ulcerative Colitis	0.57	0.167 - 1.94	0.367
Tacrolimus	0.00	0.00 - Inf.	0.998
Cyclosporine	0.00	0.00 - Inf.	0.998
Transplant Year	1.06	0.923 - 1.23	0.394

ALP x ULN	1.30	0.967 - 1.75	0.082	**Severe Cholestasis**	4.35	0.822 - 22.97	0.084
Total Bilirubin x ULN	1.15	0.258 - 5.11	0.856	**Mild Cholestasis**	3.55	0.782 - 16.12	0.101

Model at 12 Months Post-Transplant (n= 99)				**Model at 12 Months Post-Transplant (n= 99)**			
	HR	**95% CI**	**p value**		**HR**	**95% CI**	**p value**
Donor Age	1.01	0.968 - 1.05	0.726	**Donor Age**	1.01	0.970 - 1.05	0.649
Ulcerative Colitis	0.49	0.146 - 1.62	0.240	**Ulcerative Colitis**	0.48	0.145 - 1.62	0.239
Tacrolimus	0.00	0.00 - Inf.	0.998	**Tacrolimus**	0.00	0.00 - Inf.	0.998
Cyclosporine	0.00	0.00 - Inf.	0.999	**Cyclosporine**	0.00	0.00 - Inf.	0.999
Transplant Year	1.05	0.915 - 1.21	0.477	**Transplant Year**	1.05	0.912 - 1.20	0.505
ALP x ULN	1.20	0.730 - 1.98	0.471	**Severe Cholestasis**	1.27	0.156 - 10.28	0.824

Inf = Infinity
State 1= didn't experience rPSC post-transplant
State 3= graft loss

Chapter 4: Discussion

4.1 Introduction

PSC is a rare and chronic condition of unknown etiology. In the absence of known etiology or effective treatment, liver transplantation remains the only definitive management for end stage liver disease in patients with PSC [36]. Accordingly, investigation of post-transplant recurrence may provide insight into the disease process and help to identify the factors that limit graft performance.

4.2 Disease severity prior to transplant and rPSC risk

Our findings suggest that some parameters of disease severity prior to transplant put patients at increased risk of recurrent disease. We found that patients who received a transplant after being in the ICU were at greater risk of developing rPSC. However, the multivariable analysis did not support this finding. Another finding suggested by our analysis was that higher bilirubin pre-transplant resulted in increased risk of getting rPSC.

4.3 Is infection the cause of disease recurrence?

We found that cholestasis, as early as the first month post-transplant, is closely associated with recurrent PSC in the allograft. The cholestatic risk was observed over a one-year follow-up and progressed over time to include hyper-bilirubinemia. Also, increased AST put patients at a higher the risk of losing the graft because of rPSC.

It is worth noting that early inflammatory indicators of disease recurrence are recorded in chronic infectious hepatic diseases such as HCV, in which early biochemical elevation of liver enzymes in the first few months following liver transplantation is associated with recurrence [64]. This isn't the case with other non-infectious diseases in which the liver enzymes are elevated when the disease recurs within 10 years of transplantation and the recurrence is proven histologically with normal enzymes [65].

It is well known that, early post-transplant, some patients suffer from anastomotic biliary stricture [53]. Such biliary stasis together with lowered immunity can be a good environment for infectious microorganisms to induce biliary inflammation that would result in biliary scarring and disease recurrence in susceptible individuals.

However, infection isn't the only etiology of early inflammation. Preservation-reperfusion injury (PRI) induces early elevation in liver enzymes as a result of inducing both innate and adaptive immune pathways to the graft [70]. Such an early priming immune activity could play a role in intensive auto-recognition of a possible unknown implicated biliary antigen, resulting in a disease recurrence in the liver graft *(Figure 4.1)*.

4.4 Possible confounders

Both tacrolimus and cyclosporine emerged as possible modifiers for the PSC effect on survival, improving long-term prognosis. At the first glance, this appears to contradict the findings in the literature that immunosuppressives don't have a therapeutic effect on

PSC. Nevertheless, it is hard to compare our findings to the therapeutic performance of the different studied drugs. Most of the drug trials that involved

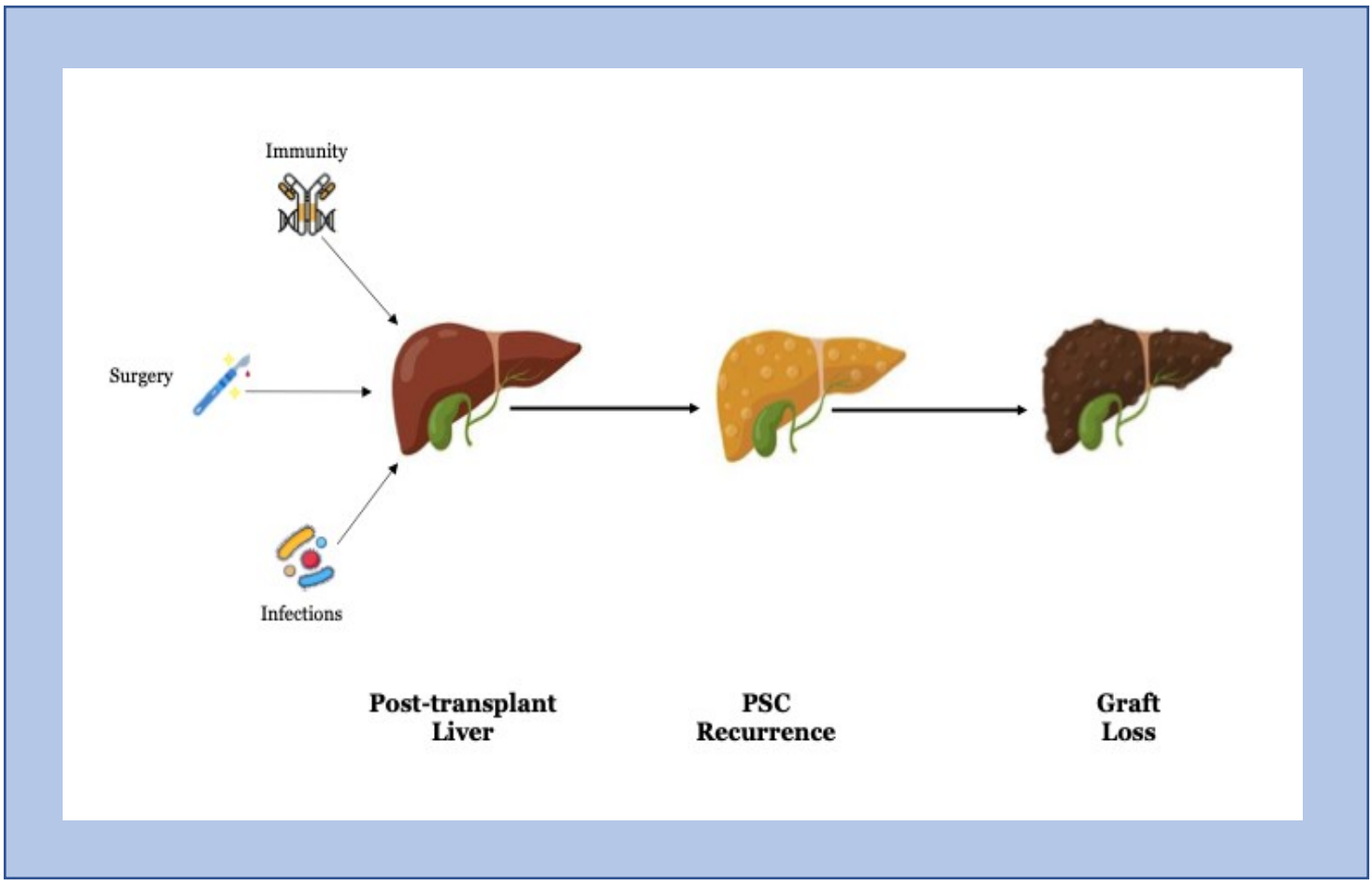

Figure 4.1: *The complex factors that may play a role in the rPSC disease process*

PSC spanned a short duration (months to couple of years) and involved a small sample size. Those factors limit the chances of finding a true effect. Additionally, the endpoint of those studies was mostly biochemical improvement, as it is impractical to follow patients with a rare disease over a long period to record events [32]. Moreover,

tacrolimus was found to improve ALP in couple of studies [71], [72], which is consistent with our findings.

Also, UC appeared to be protective against graft loss in univariate analysis. Using the 1-month labs, it appeared to be significantly protective in a multivariable semi-Markov model but only when transitioning directly to graft loss without having rPSC. However, this finding should be viewed carefully. A recent study by IPSCSG showed that UC is actually associated with worse prognosis in PSC. The reason for this discrepancy is that the IPSCSG included UC as a time-dependent covariate, which is probably the most appropriate approach. Unfortunately, this approach was difficult to use in our study given that we were already including another time-dependent variable (rPSC). Also, the IPSCSG study included a huge sample size, which wasn't available for our study [27].

4.5 Study limitations

This study was as a retrospective observational study and as such was prone to human error in the data collection and limited in terms of data availability. Also, our sample size wasn't large enough, which restricted our ability to detect significance in some models, especially the semi-Markov state 2 to state 3 transition. Moreover, the validation cohort missed several clinical data and didn't include labs at more detailed earlier time points. However, the follow-up duration in the cohort was long, which is strengthens our ability to detect events.

4.6 Future directions

We showed that the hepatic biochemistry profile during the first year is linked to developing rPSC. Our findings need to be validated with a large multicenter database that have hepatic biochemical labs at different time points during the first year. The database also should include relevant clinical characteristics (e.g., recipient and donor characteristics) to adjust for. A detailed history of the immunosuppression regimen post-transplant should be studied to draw reliable conclusions for clinical use.

Additionally, researchers should look deeper into the relationship between ethnicity and disease burden. There is a need for epidemiological studies from ethnically diverse populations to have the true PSC rates, and to study the possible environmental exposures between the different groups. Researchers can also investigate the genetic differences between PSC patients from the different backgrounds to identify their genetic disparity and its link to the disease process.

Also, basic-science researchers need to test our hypothesis that rPSC follows an infectious model in the wet lab. This can be done by examining PSC explant samples for the presence of different infections. Bile samples in addition to patients' blood samples pre- and post-transplant can also be examined for the presence of suspected microorganisms.

Regarding the clinical applications, experts should revise the notion that rPSC can only be diagnosed after 3 months of transplantation. We proved that abnormal ALP from the

first month post-transplant points out the possibility of rPSC. Accordingly, clinicians should offer the proper imaging investigation and follow-up for patients as soon as they develop abnormal ALP to early detect rPSC and slow the disease process.

4.7 Conclusion

Our study showed that cholestasis early in the first year post-transplant was linked to the recurrence of PSC in the liver graft. This is mostly due to an early infectious process. We also found that patients who develop rPSC are at a significantly high risk of losing their graft due to failure. The higher the level of cholestasis in patients with rPSC, the greater the risk of graft loss.

Bibliography

[1] M. Dave, B. J. Elmunzer, B. A. Dwamena, and P. D. R. Higgins, “Primary sclerosing cholangitis: meta-analysis of diagnostic performance of MR cholangiopancreatography,” *Radiology*, vol. 256, no. 2, pp. 387–396, Aug. 2010, doi: 10.1148/radiol.10091953.

[2] J. A. Talwalkar, P. Angulo, C. D. Johnson, B. T. Petersen, and K. D. Lindor, “Cost-minimization analysis of MRC versus ERCP for the diagnosis of primary sclerosing cholangitis,” *Hepatol. Baltim. Md*, vol. 40, no. 1, pp. 39–45, Jul. 2004, doi: 10.1002/hep.20287.

[3] V. J. Desmet, “Histopathology of chronic cholestasis and adult ductopenic syndrome,” *Clin. Liver Dis.*, vol. 2, no. 2, pp. 249–264, viii, May 1998, doi: 10.1016/s1089-3261(05)70006-4.

[4] E. Björnsson *et al.*, “The natural history of small-duct primary sclerosing cholangitis,” *Gastroenterology*, vol. 134, no. 4, pp. 975–980, Apr. 2008, doi: 10.1053/j.gastro.2008.01.042.

[5] G. V. Gregorio *et al.*, “Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study,” *Hepatol. Baltim. Md*, vol. 33, no. 3, pp. 544–553, Mar. 2001, doi: 10.1053/jhep.2001.22131.

[6] M. Rabinovitz, A. J. Demetris, C. F. Bou-Abboud, and D. H. Van Thiel, “Simultaneous occurrence of primary sclerosing cholangitis and autoimmune chronic active hepatitis in a patient with ulcerative colitis,” *Dig. Dis. Sci.*, vol. 37, no. 10, pp. 1606–1611, Oct. 1992, doi: 10.1007/BF01296509.

[7] P. Khoshpouri *et al.*, “Imaging Features of Primary Sclerosing Cholangitis: From Diagnosis to Liver Transplant Follow-up,” *Radiogr. Rev. Publ. Radiol. Soc. N. Am. Inc*, vol. 39, no. 7, pp. 1938–1964, Dec. 2019, doi: 10.1148/rg.2019180213.

[8] R. Abdalian and E. J. Heathcote, “Sclerosing cholangitis: a focus on secondary causes,” *Hepatol. Baltim. Md*, vol. 44, no. 5, pp. 1063–1074, Nov. 2006, doi: 10.1002/hep.21405.

[9] E. Toy, S. Balasubramanian, C. Selmi, C.-S. Li, and C. L. Bowlus, “The prevalence, incidence and natural history of primary sclerosing cholangitis in an ethnically diverse population,” *BMC Gastroenterol.*, vol. 11, p. 83, Jul. 2011, doi: 10.1186/1471-230X-11-83.

[10] J.-R. Hov, K.-M. Boberg, and T.-H. Karlsen, “Autoantibodies in primary sclerosing cholangitis,” *World J. Gastroenterol.*, vol. 14, no. 24, pp. 3781–3791, Jun. 2008, doi: 10.3748/wjg.14.3781.

[11] B. Terjung *et al.*, “Diagnostic accuracy of atypical p-ANCA in autoimmune hepatitis using ROC- and multivariate regression analysis,” *Eur. J. Med. Res.*, vol. 9, no. 9, pp. 439–448, Sep. 2004.

[12] R. Olsson *et al.*, “Colchicine treatment of primary sclerosing cholangitis,” *Gastroenterology*, vol. 108, no. 4, pp. 1199–1203, Apr. 1995, doi: 10.1016/0016-5085(95)90220-1.

[13] H. van Hoogstraten, “Budesonide or prednisone in combination with ursodeoxycholic acid in primary sclerosing cholangitis: a randomized double-blind pilot study,” *Am. J. Gastroenterol.*, vol. 95, no. 8, pp. 2015–2022, Aug. 2000, doi: 10.1016/S0002-9270(00)01059-5.

[14] X. Jiang and T. H. Karlsen, “Genetics of primary sclerosing cholangitis and pathophysiological implications,” *Nat. Rev. Gastroenterol. Hepatol.*, vol. 14, no. 5, pp. 279–295, May 2017, doi: 10.1038/nrgastro.2016.154.

[15] D. Ellinghaus *et al.*, “Genome-wide association analysis in primary sclerosing cholangitis and ulcerative colitis identifies risk loci at GPR35 and TCF4,” *Hepatol. Baltim. Md*, vol. 58, no. 3, pp. 1074–1083, Sep. 2013, doi: 10.1002/hep.25977.

[16] T. Folseraas *et al.*, “Extended analysis of a genome-wide association study in primary sclerosing cholangitis detects multiple novel risk loci,” *J. Hepatol.*, vol. 57, no. 2, pp. 366–375, Aug. 2012, doi: 10.1016/j.jhep.2012.03.031.

[17] A. L. Mason *et al.*, “Detection of retroviral antibodies in primary biliary cirrhosis and other idiopathic biliary disorders,” *The Lancet*, vol. 351, no. 9116, pp. 1620–1624, May 1998, doi: 10.1016/S0140-6736(97)10290-2.

[18] J. E. Berdal, J. Ebbesen, and A. Rydning, “[Incidence and prevalence of autoimmune liver diseases],” *Tidsskr. Den Nor. Laegeforening Tidsskr. Prakt. Med. Ny Raekke*, vol. 118, no. 29, pp. 4517–4519, Nov. 1998.

[19] K. M. Boberg, E. Aadland, J. Jahnsen, N. Raknerud, M. Stiris, and H. Bell, “Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population,” *Scand. J. Gastroenterol.*, vol. 33, no. 1, pp. 99–103, Jan. 1998, doi: 10.1080/00365529850166284.

[20] T. R. Card, M. Solaymani-Dodaran, and J. West, “Incidence and mortality of primary sclerosing cholangitis in the UK: a population-based cohort study,” *J. Hepatol.*, vol. 48, no. 6, pp. 939–944, Jun. 2008, doi: 10.1016/j.jhep.2008.02.017.

[21] J. G. C. Kingham, N. Kochar, and M. B. Gravenor, “Incidence, clinical patterns, and outcomes of primary sclerosing cholangitis in South Wales, United Kingdom,” *Gastroenterology*, vol. 126, no. 7, pp. 1929–1930, Jun. 2004, doi: 10.1053/j.gastro.2004.04.052.

[22] B. Lindkvist, M. Benito de Valle, B. Gullberg, and E. Björnsson, “Incidence and prevalence of primary sclerosing cholangitis in a defined adult population in Sweden,” *Hepatol. Baltim. Md*, vol. 52, no. 2, pp. 571–577, Aug. 2010, doi: 10.1002/hep.23678.

[23] T. L. Ang, K. M. Fock, T. M. Ng, E. K. Teo, T. S. Chua, and J. Y.-L. Tan, “Clinical profile of primary sclerosing cholangitis in Singapore,” *J. Gastroenterol. Hepatol.*, vol. 17, no. 8, pp. 908–913, Aug. 2002, doi: 10.1046/j.1440-1746.2002.02835.x.

[24] G. G. Kaplan, K. B. Laupland, D. Butzner, S. J. Urbanski, and S. S. Lee, “The burden of large and small duct primary sclerosing cholangitis in adults and children: a population-based analysis,” *Am. J. Gastroenterol.*, vol. 102, no. 5, pp. 1042–1049, May 2007, doi: 10.1111/j.1572-0241.2007.01103.x.

[25] D. Byron and G. Y. Minuk, “Clinical hepatology: profile of an urban, hospital-based practice,” *Hepatol. Baltim. Md*, vol. 24, no. 4, pp. 813–815, Oct. 1996, doi: 10.1002/hep.510240410.

[26] A. Escorsell, A. Parés, J. Rodés, J. A. Solís-Herruzo, M. Miras, and E. de la Morena, “Epidemiology of primary sclerosing cholangitis in Spain. Spanish Association for the Study of the Liver,” *J. Hepatol.*, vol. 21, no. 5, pp. 787–791, Nov. 1994, doi: 10.1016/s0168-8278(94)80240-8.

[27] T. J. Weismüller *et al.*, “Patient Age, Sex, and Inflammatory Bowel Disease Phenotype Associate With Course of Primary Sclerosing Cholangitis,” *Gastroenterology*, vol. 152, no. 8, pp. 1975-1984.e8, Jun. 2017, doi: 10.1053/j.gastro.2017.02.038.

[28] J. J. W. Tischendorf, H. Hecker, M. Krüger, M. P. Manns, and P. N. Meier, “Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: A single center study,” *Am. J. Gastroenterol.*, vol. 102, no. 1, pp. 107–114, Jan. 2007, doi: 10.1111/j.1572-0241.2006.00872.x.

[29] U. Navaneethan, P. G. K. Venkatesh, B. A. Lashner, B. Shen, and R. P. Kiran, “The impact of ulcerative colitis on the long-term outcome of patients with primary sclerosing cholangitis,” *Aliment. Pharmacol. Ther.*, vol. 35, no. 9, pp. 1045–1053, May 2012, doi: 10.1111/j.1365-2036.2012.05063.x.

[30] J. Fevery *et al.*, “Patients with large-duct primary sclerosing cholangitis and Crohn’s disease have a better outcome than those with ulcerative colitis, or without IBD,” *Aliment. Pharmacol. Ther.*, vol. 43, no. 5, pp. 612–620, Mar. 2016, doi: 10.1111/apt.13516.

[31] J. Kumagai *et al.*, “Clinical characteristics and outcomes of primary sclerosing cholangitis and ulcerative colitis in Japanese patients,” *PLOS ONE*, vol. 13, no. 12, p. e0209352, Dec. 2018, doi: 10.1371/journal.pone.0209352.

[32] C. Y. Ponsioen *et al.*, “Surrogate endpoints for clinical trials in primary sclerosing cholangitis: Review and results from an International PSC Study Group consensus process,” *Hepatol. Baltim. Md*, vol. 63, no. 4, pp. 1357–1367, Apr. 2016, doi: 10.1002/hep.28256.

[33] U. Beuers *et al.*, “Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebo-controlled trial,” *Hepatol. Baltim. Md*, vol. 16, no. 3, pp. 707–714, Sep. 1992, doi: 10.1002/hep.1840160315.

[34] K. D. Lindor *et al.*, “High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis,” *Hepatology*, vol. 50, no. 3, pp. 808–814, Sep. 2009, doi: 10.1002/hep.23082.

[35] R. Chapman *et al.*, “Diagnosis and management of primary sclerosing cholangitis,” *Hepatology*, vol. 51, no. 2, pp. 660–678, Feb. 2010, doi: 10.1002/hep.23294.

[36] M. H. Chapman *et al.*, “British Society of Gastroenterology and UK-PSC guidelines for the diagnosis and management of primary sclerosing cholangitis,” *Gut*, vol. 68, no. 8, pp. 1356–1378, Aug. 2019, doi: 10.1136/gutjnl-2018-317993.

[37] A. Floreani *et al.*, “Clinical course and outcome of autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome,” *Am. J. Gastroenterol.*, vol. 100, no. 7, pp. 1516–1522, Jul. 2005, doi: 10.1111/j.1572-0241.2005.41841.x.

[38] K. M. Boberg, T. Egeland, and E. Schrumpf, “Long-term effect of corticosteroid treatment in primary sclerosing cholangitis patients,” *Scand. J. Gastroenterol.*, vol. 38, no. 9, pp. 991–995, Sep. 2003, doi: 10.1080/00365520310005172.

[39] M. Kaya *et al.*, “Balloon dilation compared to stenting of dominant strictures in primary sclerosing cholangitis,” *Am. J. Gastroenterol.*, vol. 96, no. 4, pp. 1059–1066, Apr. 2001, doi: 10.1111/j.1572-0241.2001.03690.x.

[40] A. A. Gaing, J. M. Geders, S. A. Cohen, and J. H. Siegel, “Endoscopic management of primary sclerosing cholangitis: review, and report of an open series,” *Am. J. Gastroenterol.*, vol. 88, no. 12, pp. 2000–2008, Dec. 1993.

[41] G. J. Gores, D. M. Nagorney, and C. B. Rosen, “Cholangiocarcinoma: is transplantation an option? For whom?,” *J. Hepatol.*, vol. 47, no. 4, pp. 455–459, Oct. 2007, doi: 10.1016/j.jhep.2007.07.003.

[42] A. H. Kartheuser, R. R. Dozois, N. F. LaRusso, R. H. Wiesner, D. M. Ilstrup, and C. D. Schleck, “Comparison of surgical treatment of ulcerative colitis associated with primary sclerosing cholangitis: ileal pouch-anal anastomosis versus Brooke ileostomy,” *Mayo Clin. Proc.*, vol. 71, no. 8, pp. 748–756, Aug. 1996, doi: 10.4065/71.8.748.

[43] M. Berenguer *et al.*, “Characteristics, trends and Outcomes of Liver Transplantation for Primary sclerosing cholangitis in female vs male patients: An analysis from the European Liver Transplant Registry,” *Transplantation*, vol. Publish Ahead of Print, Nov. 2020, doi: 10.1097/TP.0000000000003542.

[44] R. Adam *et al.*, “Evolution of liver transplantation in Europe: report of the European Liver Transplant Registry,” *Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc.*, vol. 9, no. 12, pp. 1231–1243, Dec. 2003, doi: 10.1016/j.lts.2003.09.018.

[45] R. Kashyap *et al.*, “Living donor and deceased donor liver transplantation for autoimmune and cholestatic liver diseases--an analysis of the UNOS database,” *J.*

Gastrointest. Surg. Off. J. Soc. Surg. Aliment. Tract, vol. 14, no. 9, pp. 1362–1369, Sep. 2010, doi: 10.1007/s11605-010-1256-1.

[46] I. W. Graziadei *et al.*, "Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis," *Hepatol. Baltim. Md*, vol. 30, no. 5, pp. 1121–1127, Nov. 1999, doi: 10.1002/hep.510300501.

[47] J. Lerut *et al.*, "Intrahepatic bile duct strictures after human orthotopic liver transplantation. Recurrence of primary sclerosing cholangitis or unusual presentation of allograft rejection?," *Transpl. Int. Off. J. Eur. Soc. Organ Transplant.*, vol. 1, no. 3, pp. 127–130, Oct. 1988.

[48] R. F. Harrison, M. H. Davies, J. M. Neuberger, and S. G. Hubscher, "Fibrous and obliterative cholangitis in liver allografts: evidence of recurrent primary sclerosing cholangitis?," *Hepatol. Baltim. Md*, vol. 20, no. 2, pp. 356–361, Aug. 1994.

[49] R. Sheng, W. L. Campbell, A. B. Zajko, and R. L. Baron, "Cholangiographic features of biliary strictures after liver transplantation for primary sclerosing cholangitis: evidence of recurrent disease," *AJR Am. J. Roentgenol.*, vol. 166, no. 5, pp. 1109–1113, May 1996, doi: 10.2214/ajr.166.5.8615253.

[50] E. Alabraba *et al.*, "A re-evaluation of the risk factors for the recurrence of primary sclerosing cholangitis in liver allografts," *Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc.*, vol. 15, no. 3, pp. 330–340, Mar. 2009, doi: 10.1002/lt.21679.

[51] J. Campsen *et al.*, "Clinically recurrent primary sclerosing cholangitis following liver transplantation: A time course," *Liver Transpl.*, vol. 14, no. 2, pp. 181–185, Feb. 2008, doi: 10.1002/lt.21313.

[52] F. D. Gordon *et al.*, "Recurrent primary sclerosing cholangitis in the Adult-to-Adult Living Donor Liver Transplantation Cohort Study: Comparison of risk factors between living and deceased donor recipients," *Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc.*, vol. 22, no. 9, pp. 1214–1222, Sep. 2016, doi: 10.1002/lt.24496.

[53] I. W. Graziadei *et al.*, "Recurrence of primary sclerosing cholangitis following liver transplantation," *Hepatol. Baltim. Md*, vol. 29, no. 4, pp. 1050–1056, Apr. 1999, doi: 10.1002/hep.510290427.

[54] A. J. Montano-Loza, R. A. Bhanji, S. Wasilenko, and A. L. Mason, "Systematic review: recurrent autoimmune liver diseases after liver transplantation," *Aliment. Pharmacol. Ther.*, vol. 45, no. 4, pp. 485–500, Feb. 2017, doi: 10.1111/apt.13894.

[55] I. C. Steenstraten *et al.*, "Systematic review with meta-analysis: risk factors for recurrent primary sclerosing cholangitis after liver transplantation," *Aliment. Pharmacol. Ther.*, vol. 49, no. 6, pp. 636–643, Mar. 2019, doi: 10.1111/apt.15148.

[56] M. Peverelle, S. Paleri, J. Hughes, P. De Cruz, and P. J. Gow, "Activity of Inflammatory Bowel Disease After Liver Transplantation for Primary Sclerosing Cholangitis Predicts

Poorer Clinical Outcomes," *Inflamm. Bowel Dis.*, vol. 26, no. 12, pp. 1901–1908, Nov. 2020, doi: 10.1093/ibd/izz325.

[57] L. Lindström *et al.*, "Risk factors and prognosis for recurrent primary sclerosing cholangitis after liver transplantation: a Nordic Multicentre Study," *Scand. J. Gastroenterol.*, vol. 53, no. 3, pp. 297–304, Mar. 2018, doi: 10.1080/00365521.2017.1421705.

[58] D. Morioka *et al.*, "Impact of human leukocyte antigen mismatching on outcomes of living donor liver transplantation for primary biliary cirrhosis," *Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc.*, vol. 13, no. 1, pp. 80–90, Jan. 2007, doi: 10.1002/lt.20856.

[59] P. Manousou *et al.*, "Primary biliary cirrhosis after liver transplantation: influence of immunosuppression and human leukocyte antigen locus disparity," *Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc.*, vol. 16, no. 1, pp. 64–73, Jan. 2010, doi: 10.1002/lt.21960.

[60] J. E. Guy, P. Qian, J. A. Lowell, and M. G. Peters, "Recurrent primary biliary cirrhosis: peritransplant factors and ursodeoxycholic acid treatment post-liver transplant," *Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc.*, vol. 11, no. 10, pp. 1252–1257, Oct. 2005, doi: 10.1002/lt.20511.

[61] J. Alexander, J. D. Lord, M. M. Yeh, C. Cuevas, R. Bakthavatsalam, and K. V. Kowdley, "Risk factors for recurrence of primary sclerosing cholangitis after liver transplantation," *Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc.*, vol. 14, no. 2, pp. 245–251, Feb. 2008, doi: 10.1002/lt.21394.

[62] A. L. Mason and A. J. Montano-Loza, "Systematic investigation of elevated cholestatic enzymes during the third posttransplant month," *Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc.*, vol. 19 Suppl 2, pp. S23-30, Nov. 2013, doi: 10.1002/lt.23742.

[63] J. F. Gallegos-Orozco *et al.*, "Natural history of post-liver transplantation hepatitis C: A review of factors that may influence its course: Natural History of Post-LT Hepatitis C," *Liver Transpl.*, vol. 15, no. 12, pp. 1872–1881, Dec. 2009, doi: 10.1002/lt.21954.

[64] S. M. Malik, M. E. deVera, P. Fontes, O. Shaikh, E. Sasatomi, and J. Ahmad, "Recurrent disease following liver transplantation for nonalcoholic steatohepatitis cirrhosis: Recurrent Nash Following It For Nash Cirrhosis," *Liver Transpl.*, vol. 15, no. 12, pp. 1843–1851, Dec. 2009, doi: 10.1002/lt.21943.

[65] J.-C. Duclos-Vallée *et al.*, "A 10 year follow up study of patients transplanted for autoimmune hepatitis: histological recurrence precedes clinical and biochemical recurrence," *Gut*, vol. 52, no. 6, pp. 893–897, Jun. 2003, doi: 10.1136/gut.52.6.893.

[66] A. J. Montano-Loza *et al.*, "Factors Associated With Recurrence of Primary Biliary Cholangitis After Liver Transplantation and Effects on Graft and Patient Survival,"

Gastroenterology, vol. 156, no. 1, pp. 96-107.e1, Jan. 2019, doi: 10.1053/j.gastro.2018.10.001.

[67] A. Corbani and A. K. Burroughs, “Intrahepatic Cholestasis After Liver Transplantation,” *Clin. Liver Dis.*, vol. 12, no. 1, pp. 111–129, Feb. 2008, doi: 10.1016/j.cld.2007.11.001.

[68] L. Meira-Machado and J. Roca-Pardiñas, “**p3state.msm** : Analyzing Survival Data from an Illness-Death Model,” *J. Stat. Softw.*, vol. 38, no. 3, 2011, doi: 10.18637/jss.v038.i03.

[69] L. Meira-Machado, J. de Uña-Alvarez, and C. Cadarso-Suárez, “Nonparametric estimation of transition probabilities in a non-Markov illness-death model,” *Lifetime Data Anal.*, vol. 12, no. 3, pp. 325–344, Sep. 2006, doi: 10.1007/s10985-006-9009-x.

[70] A. Hann, D.-C. Osei-Bordom, D. A. H. Neil, V. Ronca, S. Warner, and M. T. P. R. Perera, “The Human Immune Response to Cadaveric and Living Donor Liver Allografts,” *Front. Immunol.*, vol. 11, p. 1227, Jun. 2020, doi: 10.3389/fimmu.2020.01227.

[71] J. A. Talwalkar, A. A. Gossard, J. C. Keach, R. A. Jorgensen, J. L. Petz, and R. N. K. D. Lindor, “Tacrolimus for the treatment of primary sclerosing cholangitis,” *Liver Int.*, vol. 27, no. 4, pp. 451–453, May 2007, doi: 10.1111/j.1478-3231.2007.01441.x.

[72] D. H. Van Thiel *et al.*, “Tacrolimus (FK 506), a treatment for primary sclerosing cholangitis: results of an open-label preliminary trial,” *Am. J. Gastroenterol.*, vol. 90, no. 3, pp. 455–459, Mar. 1995.