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# Associations of vitamin D with hepatobiliary malignancy and liver transplantation in patients with primary sclerosing cholangitis

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**ASSOCIATIONS OF VITAMIN D WITH HEPATOLOBILIARY MALIGNANCY  
AND LIVER TRANSPLANTATION IN PATIENTS WITH PRIMARY  
SCLEROSING CHOLANGITIS**

by

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B.A., Grinnell College, 2017

Submitted in partial fulfillment of the  
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Master of Science

2021

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**ABSTRACT**

Primary Sclerosing Cholangitis (PSC) is a progressive cholestatic liver disease with outcomes that include hepatobiliary malignancy and liver transplantation. The pathogenesis of PSC is incompletely understood and, as a result, few markers of disease progression have been identified. Vitamin D is associated with the development and treatment of multiple cancers as well as the progression of inflammatory bowel disease, making it a possible candidate as a biomarker associated with PSC outcomes. In this study, we retrospectively and prospectively collected complete laboratory results and outcome datapoints on 179 patients with PSC to determine the association between total 25(OH)-vitamin D levels, vitamin D supplementation, and both hepatobiliary malignancy and liver transplantation. Through survival analysis, we found that history of vitamin D supplementation was significantly associated with increased hepatobiliary malignancy-free and liver transplantation-free survival ( $p=0.025$  and  $p=0.042$ , respectively). These results indicate that vitamin D is a promising factor associated with the progression of PSC to transplantation and malignancy. Future studies on this registry cohort as it increases in size and age may provide more conclusive data on the relationship between vitamin D and PSC.

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## LIST OF ABBREVIATIONS

ACR .....	Acute Cellular Rejection
AIH .....	Autoimmune Hepatitis
ALP .....	Alkaline Phosphatase
ASC.....	Autoimmune Sclerosing Cholangitis
BMI.....	Body Mass Index
CCA .....	Cholangiocarcinoma
ERCP.....	Endoscopic Retrograde Cholangiopancreatography
HCC .....	Hepatocellular Carcinoma
HDAC7 .....	Histone Deacetylase 7
IBD.....	Inflammatory Bowel Disease
IF- $\gamma$ .....	Interferon Gamma
LPS.....	Lipopolysaccharide
LT.....	Liver Transplantation
MRCP .....	Magnetic Resonance Cholangiopancreatography
Nur77 .....	Nerve Growth Factor IB
PBC.....	Primary Biliary Cirrhosis
PRKD2.....	Serine/Threonine-Protein Kinase D2
PSC .....	Primary Sclerosing Cholangitis
SASP .....	Senescence-associated Secretory Phenotype
SIK2 .....	Salt Inducible Kinase 3
TLR.....	Toll-like Receptor

TNF- $\alpha$ .....	Tumor Necrosis Factor Alpha
UDCA .....	Ursodeoxycholic Acid
UC.....	Ulcerative Colitis
VDR.....	Vitamin D Receptor

## INTRODUCTION

### Overview and Clinical Presentation

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease that is heterogeneous and can affect both the intra- and extra-hepatic bile ducts. It is commonly associated with inflammatory bowel disease (IBD) and progressive inflammation of the bile ducts. Patients with this condition experience increased rates of hepatobiliary and colorectal malignancy as well as liver failure and transplantation (Tischendorf et al., 2007). According to meta-analyses of population-based studies, the prevalence of PSC is between 6 and 16 per 100,000 in North America and Europe (Molodecky et al., 2011; Boonstra et al., 2013). These analyses also found that incidence rates are between 0.5 and 1 per 100,000 person-years, with evidence that the incidence of PSC is increasing over time. Data on prevalence and incidence of PSC from outside of North America and Europe is minimal, preventing accurate estimates in other regions.

Most patients diagnosed with PSC initially present with minimal symptoms other than abnormal liver function tests. The median age at diagnoses is 41 and 60% are male (Molodecky et al., 2011; Lazaridis & LaRusso, 2016). One of the most important early indications of PSC is a cholestatic profile, defined as elevated Alkaline Phosphatase (ALP) for more than six months (Lindor et al., 2015). Once suspected, PSC is then confirmed by endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance cholangiopancreatography (MRCP), or liver biopsy. MRCP has become the standard for PSC diagnosis as it is the least invasive. ERCP is also common but is significantly more invasive and involves risks such as bacterial infection following the

procedure. In contrast to MRCP however, ERCP allows for bile duct stricture biopsy and the option for further intervention in more advanced cases. The use of liver biopsy is the least common, but can detect small duct PSC (Lindor et al., 2015). Even with significant advancements in identification and detection of PSC, the average diagnosis is delayed 8.4 months according to a study of a regional Canadian health system (Kaplan et al., 2007).

PSC has two subtypes, one common overlap syndrome, and is significantly associated with another gastrointestinal disease. Large duct PSC is the most common subtype, with 90% of cases having this morphology (Lazaridis & LaRusso, 2016). This subtype affects the entire biliary tree and can be diagnosed via MRCP or ERCP by visible biliary strictures (Kaplan et al., 2007). Small duct PSC, the other subtype, is significantly less common and only affects intra-hepatic bile ducts, often requiring liver biopsy for diagnoses. While patients with small duct PSC often have better outcomes, they can suffer recurrence after liver transplantation (Bjornsson et al., 2008). PSC in conjunction with autoimmune hepatitis (AIH), sometimes referred to as autoimmune sclerosing cholangitis (ASC), is considered an overlap syndrome and found in 7-10% of PSC cases (Kaya et al., 2000; Kaplan et al., 2007). PSC-AIH is more commonly found in children diagnosed with PSC and can require a liver biopsy to diagnose (Feldstein et al., 2003). While PSC-AIH and AIH share similar characteristics and disease processes, differences in patient outcomes may indicate that they are distinct disease entities (Gregorio et al., 2001). In Gregorio et al.'s 2001 study, IBD and biliary histological changes were also found to be more common in ASC than AIH. Patients with PSC/AIH overlap also have significantly worse outcomes than those with AIH (Heneghan et al., 2008). PSC is also

significantly associated with IBD as 70-80% of those with PSC have IBD and 5% of those with IBD develop PSC (Loftus et al., 1997). Ulcerative colitis (UC) is more common in those with PSC than Crohn's Disease, and UC is also more genetically similar to PSC than Crohn's Disease (Ji et al., 2017). Overall, it is unclear if PSC-UC is a significant risk factor for colorectal malignancy over UC (Loftus et al., 2005).

### **Pathogenesis**

While much progress has been made over the last two decades, the pathogenesis of PSC remains incompletely understood. It is thought to be influenced by both environmental and genetic factors. The prevailing hypothesis is that patients with a genetic susceptibility experience an environmental trigger and subsequently develop the disease.

Few environmental factors have been strongly associated with PSC. In a survey study in Oslo on 240 patients with PSC and matched controls, there were fewer coffee drinkers and smokers in the PSC cohort than in controls (Andersen et al., 2013). This study also found a negative correlation between the use of hormonal birth control and PSC in females. Andersen et al. also found that PSC patients were more likely to be exposed to farm animals, but not domesticated animals, during childhood. These findings are only associational however and have been difficult to connect with genetic and other theories of PSC pathogenesis.

There is significant evidence of a genetic basis for PSC. In a 2008 study, Bergquist et al. found that PSC was 9 to 39 times more common in first-degree relatives

of those with confirmed PSC. Interestingly, they also found that UC was more common in first-degree relatives of those with PSC without IBD. A genome-wide analysis of those with PSC has found significant genetic associations with HLA, suggesting some autoimmune component to its pathogenesis (Karlsen et al., 2010). Genetic associations with HLA are often complex to interpret however due to its linkage with a breadth of different conditions.

In another 2013 genome-wide analysis of PSC by Liu et al., 12 non-HLA loci were found to have significant associations with PSC. Six of these loci displayed more significant association with PSC than IBD, reinforcing that they are similar but distinct diseases. Many of the loci are involved in immune functions, leading to further interest in autoimmune involvement in PSC. One of these loci is located in an intron of salt inducible kinase 2 (SIK2), which influences both IL-10 expression in macrophages and nerve growth factor IB (Nur77), an important transcription factor in leukocytes (Liu et al., 2013). Additionally, this analysis found that two loci significantly associated with PSC are involved in mechanisms related to the function of regulatory T cells. One of these loci is located in the intron of histone deacetylase 7 (HDAC7) and another, the most significantly associated loci, in the intron of serine/threonine-protein kinase D2 (PRKD2). During T cell selection in the thymus when T cell receptors are engaged, PRKD2 phosphorylates HDAC7. This results in the nuclear exclusion of HDAC7 and the loss of its functionality in gene regulation, followed by apoptosis and negative selection of immature T cells (Liu et al., 2013). This negative selection is also related to the loss of HDAC7-mediated repression of Nur77, a locus associated with PSC. Proper functioning

of T cell negative selection is essential for immune tolerance and abnormalities in this process can lead to autoimmunity, suggesting a potential role for this pathway in PSC.

Abnormalities in regulatory T cell and T cell levels in general have also been found in patients with PSC. Regulatory T cell levels in peripheral blood were significantly decreased in those with PSC (Sebode et al., 2015). Variations in the IL2RA gene, which also has a locus associated with PSC, is significantly associated with low regulatory T cell levels (Liu et al., 2013). Further reinforcing the role of T cells in PSC pathogenesis, Sebode et al. (2013) demonstrated that the suppressive capacity of regulatory T cells was decreased in PSC. Regulatory T cells are essential in T cell selection and their dysfunction can also lead to autoimmunity.

PSC patients have higher levels of T helper (Th17) cells under pathogen stimulation than controls with primary biliary cirrhosis (PBC) (Katt et al., 2013). These T helper cells are stimulated under toll-like receptor (TLR) 5 or 7 by the pathogen *Candida albicans*, which has been associated with disease progression in PSC (Katt et al., 2013). Also, in the liver of PSC patients most T cells are CD28<sup>-</sup>, which produce more interferon gamma (IF- $\gamma$ ) and tumor necrosis factor alpha (TNF- $\alpha$ ) than CD28<sup>+</sup> T cells (Liaskou et al., 2014). In turn, TNF- $\alpha$  downregulates CD28 in T cells, further increasing the levels of CD28<sup>-</sup> T cells. These CD28<sup>-</sup> T cells are known to be markers of inflammation, having lost CD28 due to continuous antigenic stimulation and TNF- $\alpha$  exposure at inflammation sites. They are considered chronically active and less susceptible to regulatory T cells, thereby being important drivers of chronic inflammation (Liaskou et al., 2014). In in vitro studies of these CD28<sup>-</sup> T cells however, administration of 1,25(OH)<sub>2</sub>D<sub>3</sub> prevented this

downregulation of CD28 by TNF- $\alpha$ , indicating that vitamin D may have an important role in the regulation of autoimmune and inflammatory conditions such as PSC (Liaskou et al., 2014).

While genomic and immunologic studies have demonstrated that T cells play an essential role in the pathogenesis of PSC, other pathways have also been theorized to explain other characteristics of the disease. A 2011 study by O'Hara et al. demonstrated a potential inflammatory mechanism in PSC through microbially-derived lipopolysaccharide (LPS) and cholangiocytes, the epithelial cells of the bile duct. LPS is transported to the liver via the portal system, where it activates TLRs, causing cholangiocytes to produce pro-inflammatory markers such as TNF- $\alpha$  (O'Hara et al., 2011; Szabo et al., 2006). This also results in persistent N-Ras activation and cellular senescence, which can further progress into a senescence-associated secretory phenotype (SASP) (Tabibian et al., 2014). In normal cholangiocytes and hepatocytes however, TLR ligands often fail to elicit an inflammatory response. This is known as TLR tolerance and is a defense mechanism against overstimulation by gut endotoxins (Szabo et al., 2006). Cholangiocytes and hepatocytes in individuals with PSC however may not exhibit this tolerance and instead display pro-inflammatory SASP characteristics.

Similarly, the umbrella hypothesis of PSC identifies abnormalities in hepatocyte and cholangiocyte biochemistry as a potential pathway for PSC pathogenesis. Another locus significantly associated with PSC is found in TGR5, which codes for the G-protein coupled bile acid receptor 1 (Beners et al., 2010). These receptors, found on cholangiocyte membranes, are involved in cAMP modulation of HCO<sub>3</sub> secretion. The

HCO<sub>3</sub> layer is essential for protection against cholangiocyte membrane permeability to bile acids, and any deficiency may result in persistent inflammation due to increased intracellular levels of bile acids.

With the strong link between IBD and PSC, gut dysbiosis has also been pursued as a potential mechanism for the pathogenesis of PSC. In a 2016 study of gut microbiota, Sabino et al. found that PSC was characterized by decreased diversity (Sabino et al., 2016). The dysbiosis in PSC was distinct from IBD but may result in increased bacterial products and endotoxins. The increased levels of endotoxins may then overstimulate hepatic immune cells and cholangiocytes, resulting in persistent inflammation and the development of PSC (Sabino et al., 2016).

### **Progression and Outcomes**

PSC is a progressive inflammatory condition and while most patients are asymptomatic upon diagnosis, 76% experience some type of disease progression within 6 years (Porayko et al., 1990). While much is still unknown about PSC pathogenesis, a commonly accepted pathway for disease progression is that bile duct injury and fibrosis lead to duct stricturing, cholestasis, biliary cirrhosis, and eventually liver dysfunction and failure. During the progressive phase, some of the most common symptoms include fatigue, pruritis, abdominal pain, cholestatic itch, and jaundice. As cholestasis develops, it has also been found that fat-soluble vitamin deficiency is common (Dyson et al., 2018). Once PSC progresses to end-stage liver disease, ascites, variceal bleeding, and hepatic encephalopathy are also common. Progression of PSC has extremely dire consequences,

as patients with bile duct stenosis have a transplant-free survival rate of 25% over 20 years (Rudolph et al., 2009).

The average survival from PSC diagnosis to LT or death is 21.3 years, with studies in liver transplant center cohorts demonstrating shorter survival at 13.2 years (Boonstra et al., 2013). Overall, 40% of those with PSC require LT (Tischendorf et al., 2007). PSC has increasingly become a common indication for LT in the United States, with 6% of LT's from 1988-2015 having an indication of PSC (Lazaridis & LaRusso, 2016). The 1-year and 5-year survival post-LT for PSC is 85% and 72%, respectively, but nearly 50% also experience at least one episode of acute cellular rejection (ACR) (Lazaridis & LaRusso, 2016; Fosby et al., 2012). While ACR is often easily treatable with immunosuppressants, Fosby et al. (2012) indicated that ACR soon after LT may be a risk factor for PSC recurrence. They found that PSC recurrence occurs in 20% of LT grafts and may be due to continued immunological attack against bile ducts and liver due to the underlying disease, resulting in the early ACR.

In addition to liver failure and transplantation, PSC is a significant risk factor for hepatobiliary malignancy, with 10.5% of PSC patients developing hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), or pancreatic cancer in a study with an average of 11-years of follow-up (Feverly et al., 2011). Hepatobiliary malignancies are often extremely difficult to detect however, especially in PSC which mimics many of its features, and studies of autopsy reports in PSC have demonstrated even higher rates (Burak et al., 2003). While some biochemical tests, such as bilirubin and ALP, have been

associated with hepatobiliary malignancies, these tests may only reflect progression of the underlying PSC and early detection remains difficult (Yachimski & Pratt, 2008).

Among hepatobiliary cancers, CCA is particularly common in PSC, with an annual risk of 2% and 30-year risk of 20% (Rizvi et al., 2015). PSC has become a major risk factor for CCA in western countries, contributing to poor outcomes as CCA mortality increases (Yachimsky & Pratt, 2008; Patel, 2001). There are no effective medical treatments for CCA, or other hepatobiliary malignancies associated with PSC. Due to the difficulty in detection, the vast majority present with advanced disease that prevents resection or other treatment options (Yachimski & Pratt, 2008). Preemptive LT for cholangiocarcinoma has been considered, but most PSC patients do not meet current standards for long-term survival necessary for LT consideration (Dyson et al., 2018). With few treatment options, the long-term outcome for hepatobiliary cancer in PSC is dire. In Fevery et al.'s 2011 study in PSC, there was a 45.9% mortality rate among those who developed malignancy.

Given the poor outcomes of PSC, finding reliable treatment options is extremely important, but due to the incomplete understanding of its pathogenesis, few such options exist. One of the most prescribed treatments, Ursodeoxycholic acid (UDCA), improves the results of liver function tests such as ALP, but its effects on outcomes is controversial. In 2009, Lindor et al. terminated a randomized, double-blind study of high-dose (28-30mg/kg/day) UDCA due to increased serious adverse events in the treatment cohort and found that endpoints such as LT and death were 2.3 times more likely in those who received the treatment compared to placebo. In another randomized study of UDCA

conducted in 2008 by Cullen et al., a similar dose was found to significantly increase survival probability according to the Mayo Risk Score. While these studies demonstrate conflicting results, the Cullen et al. study which found that UDCA is safe and effective had significantly shorter follow-up and relied upon calculated survival probabilities. As a result of these studies and other data, the 2015 American College of Gastroenterology Clinical Guidelines for PSC do not recommend high dose UDCA for the treatment of PSC (Lindor et al., 2015). Data on the use of medium dose UDCA however is lacking, but it remains a frequently used treatment for PSC.

### **Vitamin D in PSC**

Few prognostic indicators for PSC progression and outcomes have demonstrated both statistical and clinical significance, but Vitamin D has been associated with hepatobiliary cancer and liver disease progression. While Vitamin D is primarily associated with calcium regulation, it also has been found to have potent anti-cancer and immunological effects (Deeb et al., 2007).

Low vitamin D levels are associated with multiple cancers including breast, colon, and prostate (Hargrove et al., 2014). Not only have vitamin D and its analogs demonstrated anti-proliferative, pro-apoptotic, and other anti-cancer effects, but they may also enhance other chemoprotective mechanisms such as DNA repair, antioxidant protection, and immunomodulation (Deeb et al., 2007). Specifically, in the case of hepatobiliary cancers, the administration of vitamin D in mice models with liver cancer significantly decreased tumor growth (Pourgholami et al., 2000). In an in vitro study of

human CCA cells, Chiang et al. (2014) found that vitamin D decreased expression of LCN2, a potential oncogene, as well as cell proliferation. Vitamin D was also tested in vitamin D receptor (VDR) knockout cell lines and demonstrated similar effects on LCN2, confirming its role on modulating expression (Chiang et al., 2014). A similar study by Kennedy et al. (2013) also found that vitamin D increased VDR expression in CCA tumor cells and reduced growth. As a result of these findings, it has been hypothesized that vitamin D may play an important role in hepatobiliary cancer outcomes, but its role in the development of these malignancies, especially in PSC, remains incompletely understood. Ananthakrishnan et al. (2014) has demonstrated that low plasma vitamin D is associated with malignancy, especially colorectal cancer, in patients with IBD, indicating that it may also play a role in PSC.

While the effects of Vitamin D on cancer outcomes have been well characterized, little is known about its effect on PSC progression and outcome. A 2011 study by Ulitsky et al. found that vitamin D deficiency is associated with lower health-related quality of life scores and less disease activity in Crohn's Disease, prompting much interest in its use as a prognostic indicator. Calcitriol, the hormonally active form of vitamin D, inhibits the production of pro-inflammatory cytokines and reduces the activation of NF- $\kappa$ B, both of which are suggested to be involved in the pathogenesis and progression of PSC (Krishnan & Feldman, 2010). VDR, which is expressed by T cells, is also required for the development of natural killer T cells as well as other types of intra-hepatic lymphocytes (Ooi et al., 2012). Additionally, VDR polymorphisms in PSC have been associated with worse questionnaire scores related to itch, fatigue, and cognitive capacity, indicating a

link between the polymorphism and PSC-related symptoms (Kempinska-Podhorodecka et al., 2017).

Given the associations with cancer, liver disease, and proposed immunologic mechanisms for PSC pathogenesis, further investigation of vitamin D and its relation to PSC progression and outcome is needed. The high rates of LT, hepatobiliary cancer, and death in PSC indicate a present need for viable treatment options. Improved understandings of PSC pathogenesis and progression, however, are essential to the development of such treatments.

## **SPECIFIC AIMS**

Patients with primary sclerosing cholangitis face high proportions of liver transplantation, hepatobiliary malignancy, and death, but few treatment options exist due to an incomplete understanding of its pathogenesis. Additionally, few useful biomarkers have been found to indicate the severity of PSC progression and chance of transplantation or malignancy. In this study, we seek to leverage a PSC registry at Massachusetts General Hospital and the Brigham and Women's Hospital to determine the association of 25(OH)-vitamin D with outcomes such as liver transplantation and hepatobiliary malignancy. We also look at the role of vitamin D supplementation in PSC. Vitamin D may be an important predictive marker for PSC, and we seek to better understand its relationship to two of the most common and severe outcomes.

## **METHODS**

### **Cohort Identification and Recruitment**

Patients with a diagnosis of primary sclerosing cholangitis were prospectively recruited into a registry at two large academic hospitals in Boston, Massachusetts (Massachusetts General Hospital and the Brigham and Women's Hospital). Since 2010, a total of 238 patients over 14 years of age and with primary sclerosing cholangitis confirmed by clinically standard endoscopic or histologic measures have been enrolled into the registry. Of these, 179 patients had a complete medical history available and were included in these analyses.

### **Data Collection**

Demographic and other general patient information was collected from the registry database, which is updated on a yearly basis from electronic medical records. The date of PSC diagnosis, defined as date of first confirmation by endoscopic or histologic measures, was collected from current medical records. The date of last follow up was collected from medical records as the latest patient visit in the gastroenterology or transplant departments. Endpoints such as history of hepatobiliary malignancy and liver transplantation were also updated from current electronic medical records. The three most recent total vitamin D (25(OH)-vitamin D, Total) levels and history of supplementation were abstracted, if available, from current electronic medical records. If any of the three most recent vitamin D laboratory tests were completed after an endpoint was reached,

this information was discarded and replaced with vitamin D levels obtained prior to the endpoint(s).

### **Covariates and Endpoints**

The covariates of interest for this study are total vitamin D level and history of vitamin D supplementation. Data was also collected on patient age, body mass index (BMI), sex, race, ethnicity, type of PSC, type of IBD, history of Ursodeoxycholic acid (UDCA) treatment, smoking status, and form of vitamin D supplementation. The primary outcome and endpoint of interest is hepatobiliary malignancy, which includes cholangiocarcinoma, hepatocellular carcinoma, and pancreatic cancer. Liver transplant was also an endpoint of interest.

### **Statistical Analysis**

Categorical variables are listed as both raw count and percentages. Continuous variables are presented as median (interquartile range) or mean  $\pm$  standard deviation based on their normality. Categorical data were compared using chi-square or the Fisher exact test as appropriate. Continuous data were compared using the Mann-Whitney U test. Survival analysis was completed using Kaplan Meier curves. The Cox Proportional Hazards Model using the score (log-rank) test of statistical significance was used to perform univariate and multivariate regression analysis with both endpoints of interest. Statistical significance was identified at a p-value less than 0.05. Statistical analysis was performed using R and RStudio statistical software.

## RESULTS

### General Vitamin D Characteristics

A total of 179 patients diagnosed with primary sclerosing cholangitis had complete medical history and vitamin D data and were included in these analyses out of a total of 238 patients in the registry. Of these, 59 (37%) had a history of vitamin D supplementation while 120 (67%) had no history of vitamin D supplementation prior to the most recent vitamin D laboratory test or endpoint (Table 1). Cholecalciferol (1000 units/day) was the most common form of vitamin D supplementation (n=51, 87%), with ergocalciferol (50000 units/week, n=6, 10%) and calcium-citrate-D3 (600 units/day, n=2, 3%) following. The average most recent vitamin D level was  $34.8 \pm 1.16$  ng/ml. Total vitamin D levels were also stratified by standard ranges (20-50 ng/ml), with 29 (16%) patients being considered vitamin D deficient and 150 (84%) being within the normal range.

**Table 1.** General Characteristics of Vitamin D Levels and Supplementation

<b>Vitamin D Supplementation</b>	
Yes	59 (33%)
No	120 (67%)
<b>Type of Vitamin D Supplementation</b>	
Cholecalciferol	51 (87%)
Ergocalciferol	6 (10%)
Ca-Cit-D3	2 (3%)
<b>Latest Vitamin D Level</b>	
Average (ng/ml)	$34.8 \pm 1.16$
Normal	150 (84%)
Deficient	29 (16%)

## Outcome Measurements

In total out of 179 patients, 13 (8%) experienced hepatobiliary malignancy, with cholangiocarcinoma (n=9, 5%) being the most common (Table 2). Liver transplant was more common however (n=23, 13%).

**Table 2.** Summary Characteristics of Measured Outcomes

<b>Hepatobiliary Malignancy</b>	
<b>Cholangiocarcinoma</b>	9 (5%)
<b>Hepatocellular Cancer</b>	3 (2%)
<b>Pancreatic Cancer</b>	1 (1%)
<b>None</b>	166 (93%)
<b>Liver Transplant</b>	23 (13%)

## Demographic and Other Characteristics

Cohort demographic information was stratified by history of vitamin D supplementation as shown in Table 3. The two cohorts were significantly different in terms of racial and ethnic proportions ( $p=.002$  and  $p=.01$ , respectively), with a larger percentage of the non-supplement cohort having an unknown ethnicity (n=22, 18%) and being of a white racial background (n=114, 94%). History of vitamin D supplementation was also significantly associated with the most recent total vitamin D level (37 vs. 32 ng/ml,  $p=.02$ ). While not statistically significant, vitamin D supplementation tended to be associated with lower rates of hepatobiliary malignancies and liver transplant ( $p=.06$  and  $p=.14$ , respectively).

**Table 3.** Association of Vitamin D Supplementation with Cohort Characteristics

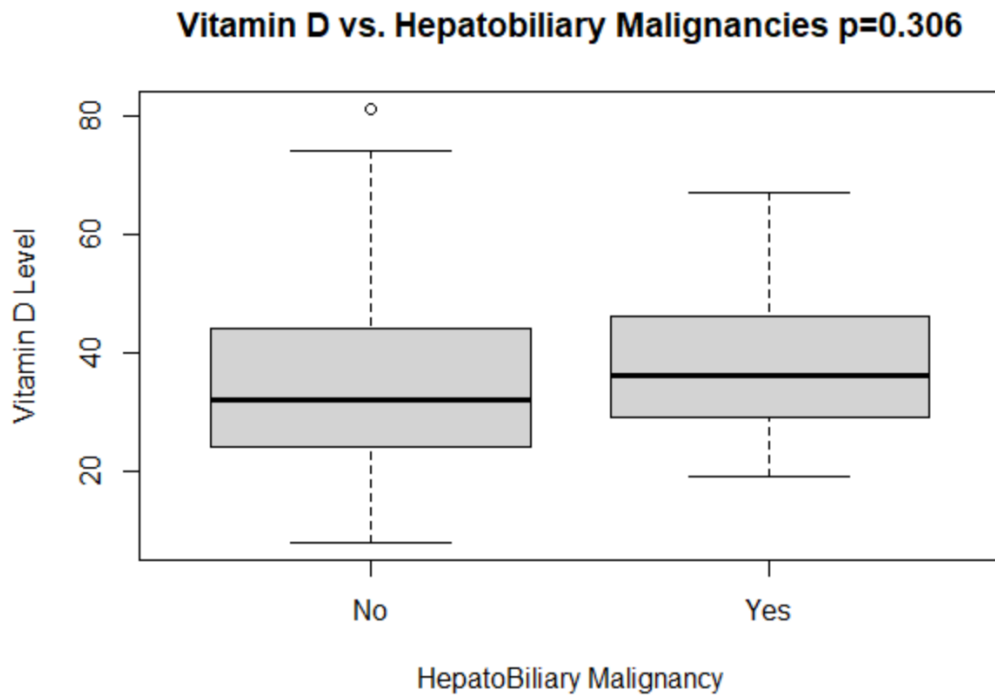
	Vitamin D Supplementation (n=59)	No Vitamin D Supplementation (n=120)	p-value
<b>Age</b>	48.0 ±24.25	45.0 ±29.3	.32
<b>BMI</b>	25.3 ±6.0	25.1 ±5.6	.25
<b>Sex</b>			.56
<b>Male</b>	37 (63%)	82 (68%)	
<b>Female</b>	22 (37%)	38 (32%)	
<b>Race</b>			<b>.002</b>
<b>White</b>	46 (78%)	114 (95%)	
<b>Black</b>	3 (5%)	3 (3%)	
<b>Asian</b>	5 (9%)	2 (2%)	
<b>Ethnicity</b>			<b>.01</b>
<b>Hispanic</b>	4 (7%)	2 (2%)	
<b>Unknown</b>	3 (5%)	22 (18%)	
<b>PSC Diagnosis</b>			.87
<b>Large Duct</b>	46 (78%)	90 (75%)	
<b>Small Duct</b>	8 (14%)	17 (14%)	
<b>PSC/AIH</b>	5 (9%)	13 (11%)	
<b>Type of IBD</b>			.52
<b>Crohn's</b>	12 (20%)	17 (14%)	
<b>UC</b>	35 (59%)	69 (58%)	
<b>None</b>	12 (20%)	28 (23%)	
<b>UDCA Treatment</b>			.10
<b>Current</b>	45 (76%)	78 (65%)	
<b>Past</b>	10 (17%)	17 (14%)	
<b>Never</b>	4 (7%)	20 (17%)	
<b>Smoking Status</b>			0.12
<b>Current</b>	2 (3%)	2 (2%)	
<b>Former</b>	4 (7%)	16 (13%)	
<b>Never</b>	53 (90%)	96 (80%)	
<b>Vitamin D Level</b>	37.0 ±14.5	32.0 ±17.2	<b>.02</b>
<b>Hepatobiliary Malig.</b>	1 (2%)	12 (10%)	.06
<b>Liver Transplant</b>	4 (7%)	19 (16%)	.14

Abbreviations: BMI=Body Mass Index, PSC=Primary Sclerosing Cholangitis, IBD=Inflammatory Bowel Disease, UC=Ulcerative Colitis, UDCA=Ursodeoxycholic acid.

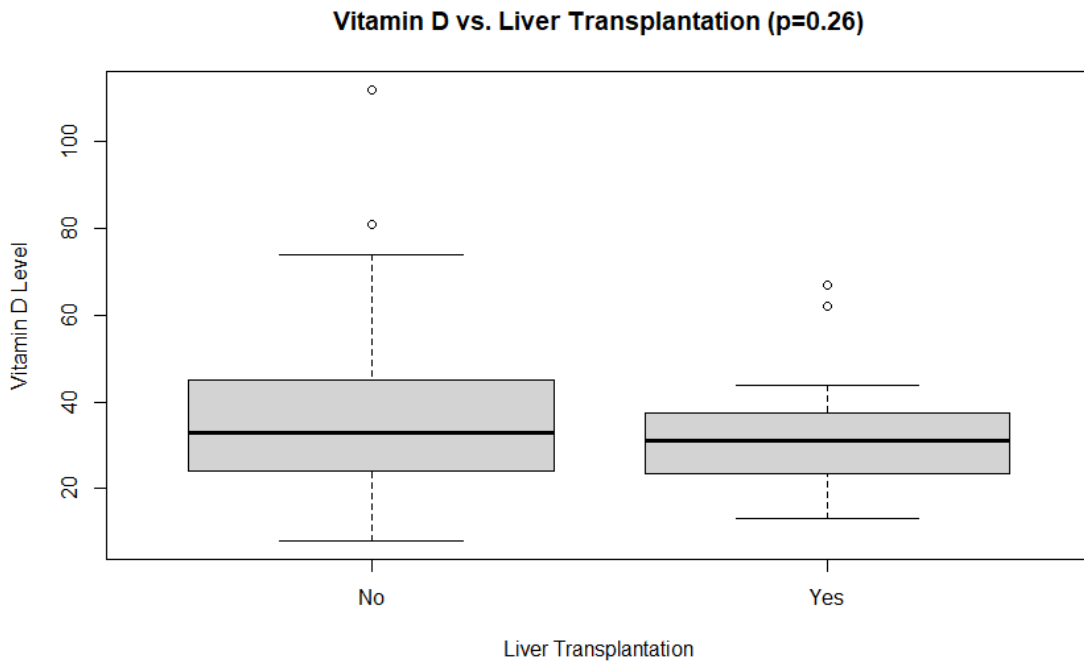
### Standard Association of Total Vitamin D with Endpoints

Total vitamin D levels was not significantly associated with hepatobiliary malignancies (44.08 vs. 33.97 ng/ml, p=.306) as seen in figure 1. Lower vitamin D levels

were similarly not significantly associated with liver transplantations (35.47 vs. 26.72 ng/ml,  $p=.26$ ) as seen in figure 2.



**Figure 1.** The association between vitamin D level and Hepatobiliary malignancy (33.97 vs. 44.08 ng/ml,  $p=0.306$ ). Hepatobiliary malignancy includes hepatocellular carcinoma, cholangiocarcinoma, and pancreatic cancer.



**Figure 2.** The association between vitamin D level and liver transplantation (35.47 vs. 26.72 ng/ml, p=0.26).

### Average Survival Rates

The average 1, 5, 10, and 20-year hepatobiliary malignancy-free and liver transplantation-free survival rates of patients with PSC are listed in table 4. Over one year of follow-up, an average of 1.7% of PSC patients will develop hepatobiliary malignancy and 2.2% will undergo liver transplantation according to our model with 3 and 4 events over that time period, respectively. Over 20 years of follow-up, an average of 11.9% of PSC patients will develop hepatobiliary malignancy and 23.4% will undergo liver transplantation with 12 and 22 events over that time period respectively in our cohort.

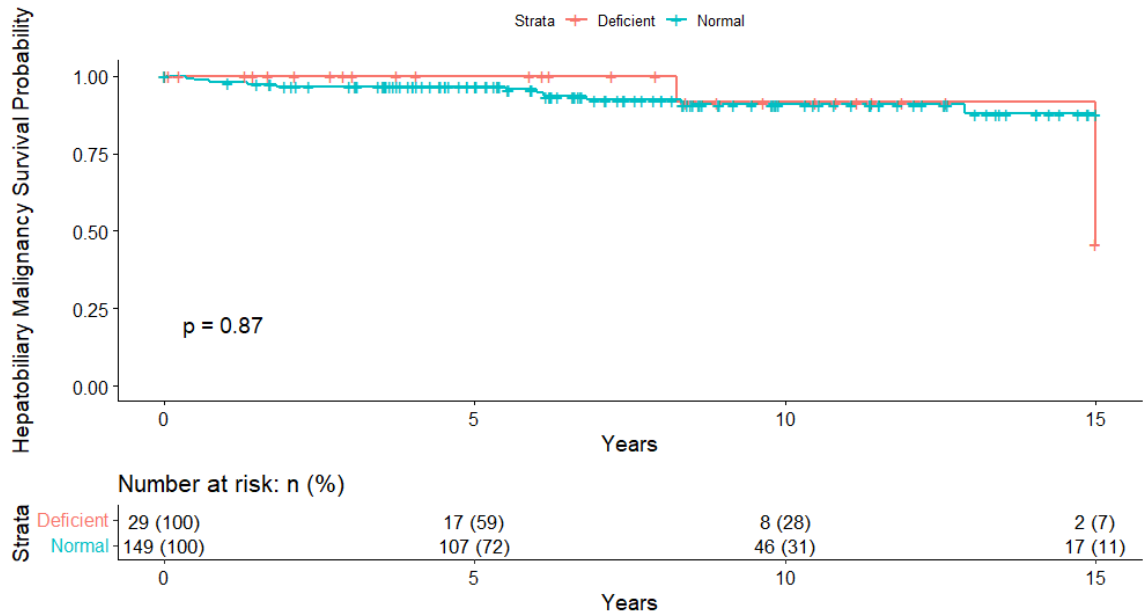
**Table 4.** Hepatobiliary Malignancy-free and Liver Transplantation-free Survival Rates

<b>Hepatobiliary Malignancy</b>	
<b>1-year</b>	98.3% ± 0.01 (3 Events)
<b>5-year</b>	97.1% ± 0.01 (5 Events)
<b>10-year</b>	91.0% ± 0.03 (11 Events)
<b>20-year</b>	88.1% ± 0.04 (12 Events)
<b>Liver Transplantation</b>	
<b>1-year</b>	97.8% ± 1.11 (4 Events)
<b>5-year</b>	94.6% ± 1.77 (9 Events)
<b>10-year</b>	86.3% ± 3.12 (18 Events)
<b>20-year</b>	76.6% ± 5.57 (22 Events)

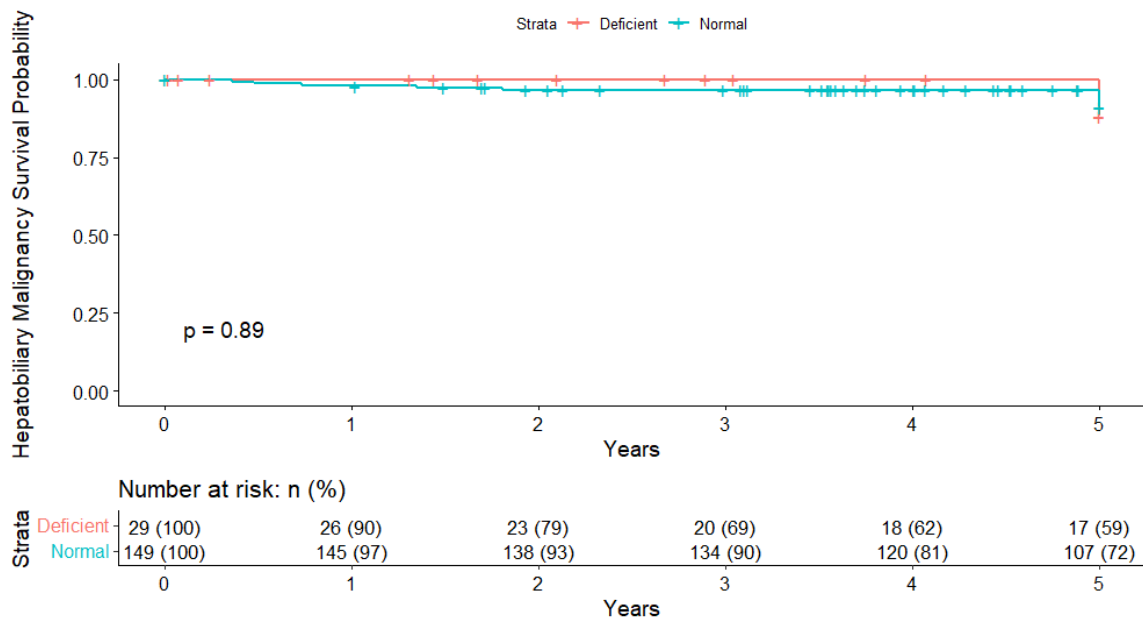
#### **Survival Analysis: Hepatobiliary Malignancy**

Over both 15 and 5 years of follow-up, there was not a significant difference in hepatobiliary malignancy-free survival between PSC patients with normal and deficient (<20 ng/ml) 25(OH)-vitamin levels (p=0.87 and p=0.89, respectively; Figures 3 & 4).

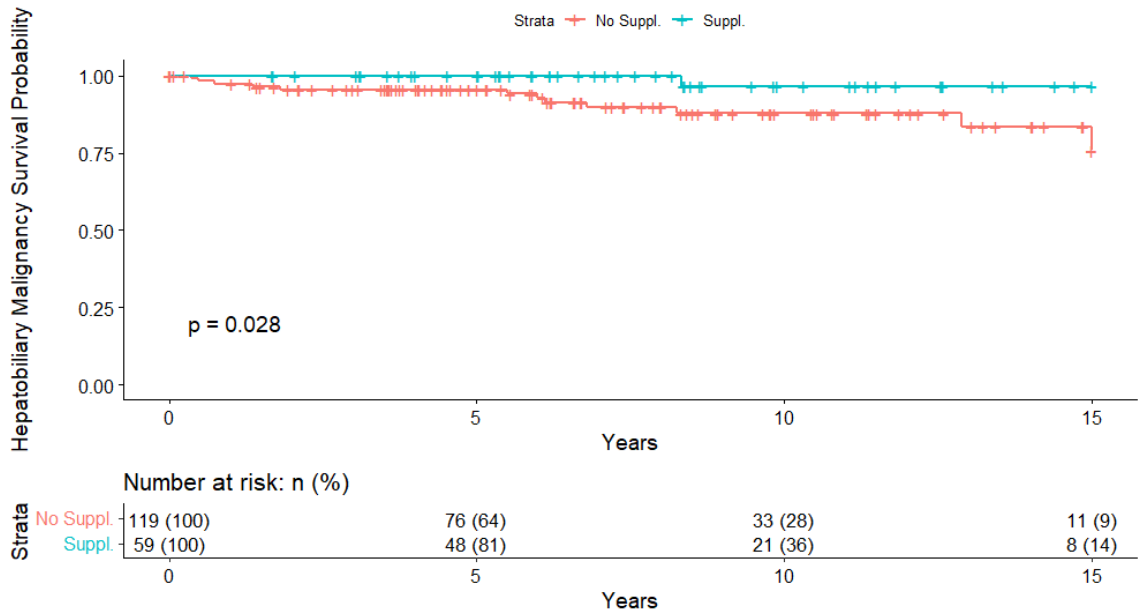
When grouped by history of supplementation, there was a significant difference in hepatobiliary malignancy-free survival in PSC patients over both 15 and 5 years of follow-up (p=0.028 and p=0.025, respectively; Figures 5 & 6).



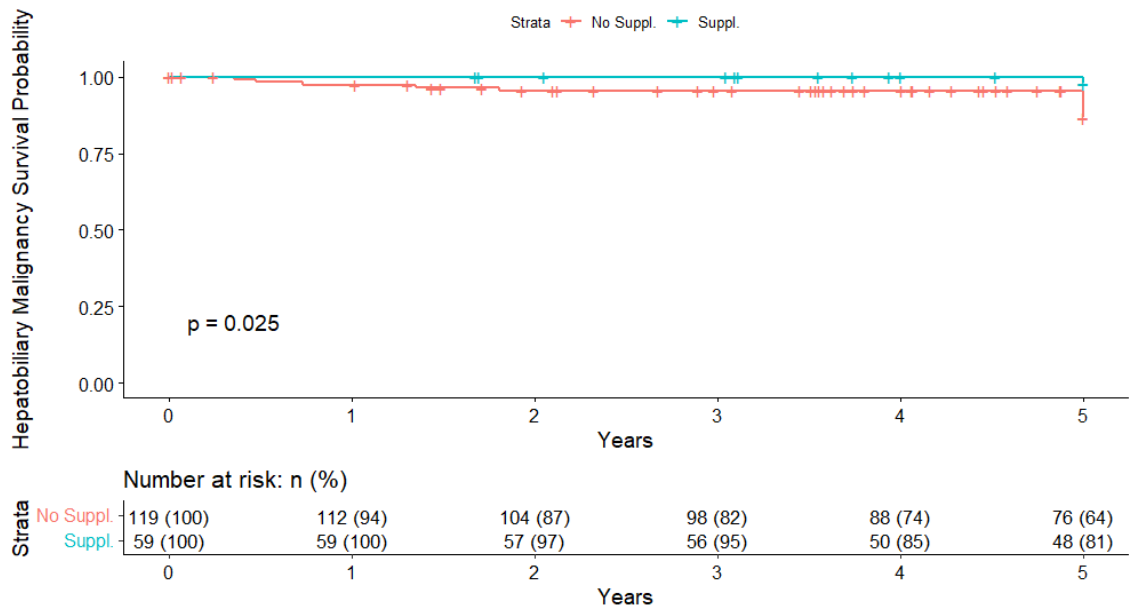
**Figure 3.** Kaplan Meier survival curve of hepatobiliary malignancy-free survival between PSC patients with deficient and normal 25(OH)-vitamin D levels ( $p=0.87$ ). Cut-off of 15 years of follow-up.



**Figure 4.** Kaplan Meier survival curve of hepatobiliary malignancy-free survival between PSC patients with deficient and normal 25(OH)-vitamin D levels ( $p=0.87$ ). Cut-off of 5 years of follow up.



**Figure 5.** Kaplan Meier survival curve of hepatobiliary malignancy-free survival between PSC patients with or without history of vitamin D supplementation ( $p=0.028$ ). Cut-off of 15 years of follow up.



**Figure 6.** Kaplan Meier survival curve of hepatobiliary malignancy-free survival between PSC patients with or without history of vitamin D supplementation ( $p=0.025$ ). Cut-off of 5 years of follow up.

## Univariate and Multivariate Analysis: Hepatobiliary Malignancy

Upon univariate analysis using the Cox Proportional Hazards Model, both 25(OH)-vitamin D levels and history of vitamin D supplementation were significantly associated with hepatobiliary malignancy ( $p=0.02$  and  $p=0.03$ , respectively). No covariates were significant in multivariate analysis. History of vitamin D supplementation was associated with a decrease in hazard (increased survival) by a factor between 0.018 and 1.081. Vitamin D levels were associated with an increased hazard (decreased survival) by a factor between 1.005 and 1.065.

**Table 5.** Univariate and Multivariate Cox Proportional Hazards Model for Hepatobiliary Malignancy

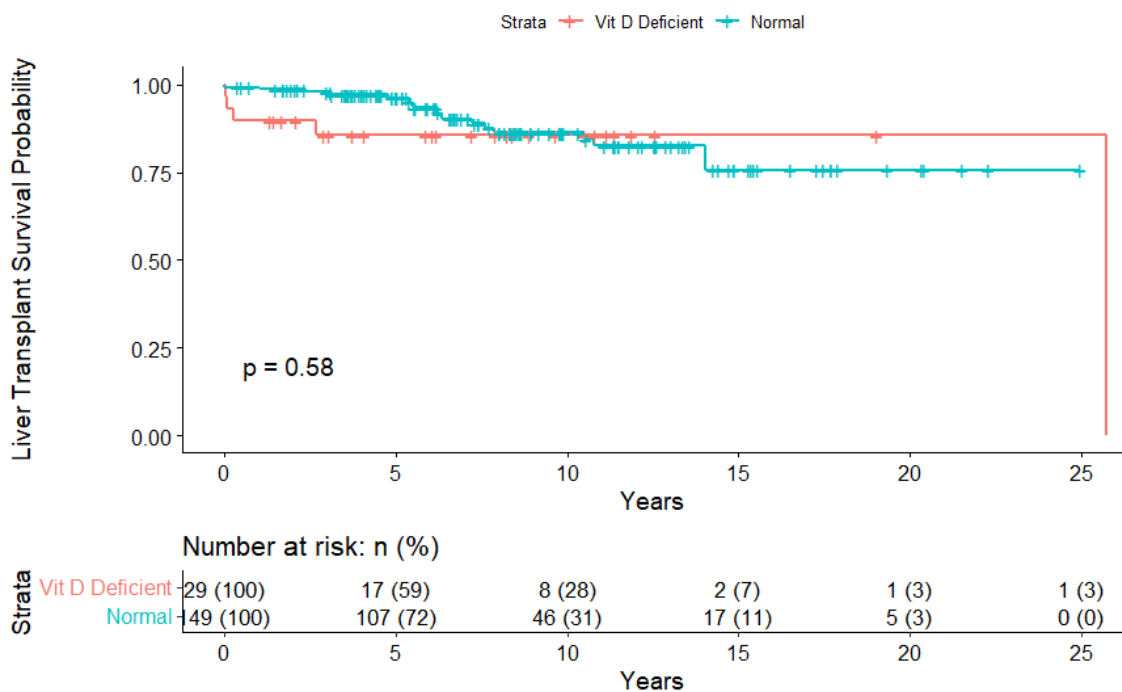
	Univariate				Multivariate			
	Beta	95% CI (HR)		P-Value	Beta	95% CI (HR)		P-Value
		Lower	Upper			Lower	Upper	
<b>Age</b>	0.028	0.995	1.063	0.09				
<b>BMI</b>	-0.090	0.814	1.027	0.20				
<b>Sex (Male)</b>	0.974	0.586	11.95	0.20				
<b>Ethnicity</b>	-6,3e-01	0.069	4.111	0.60				
<b>PSC(Small)</b>	-1.058	0.045	2.677	0.31				
<b>UDCA</b>	-0.430	0.081	5.207	0.69				
<b>Vit. D</b>	0.034	1.005	1.065	0.02				
<b>Suppl.</b>	-1.963	0.018	1.081	0.03				

Abbreviations: BMI=Body Mass Index, PSC=Primary Sclerosing Cholangitis, UDCA=Ursodeoxycholic acid, CI=Confidence Interval, HR=Hazard Ratio, Suppl.=History of Vitamin D Supplementation

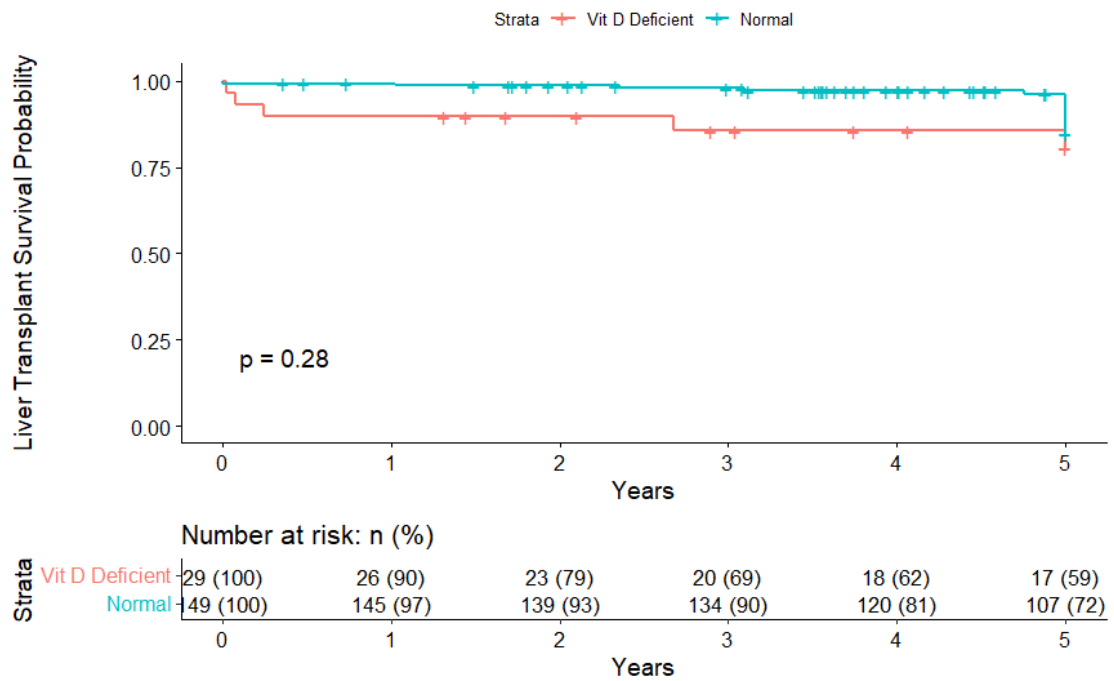
## Survival Analysis: Liver Transplantation

Over both 25 and 5 years of follow-up, there was not a significant difference in liver transplantation-free survival between PSC patients with normal and deficient (<20

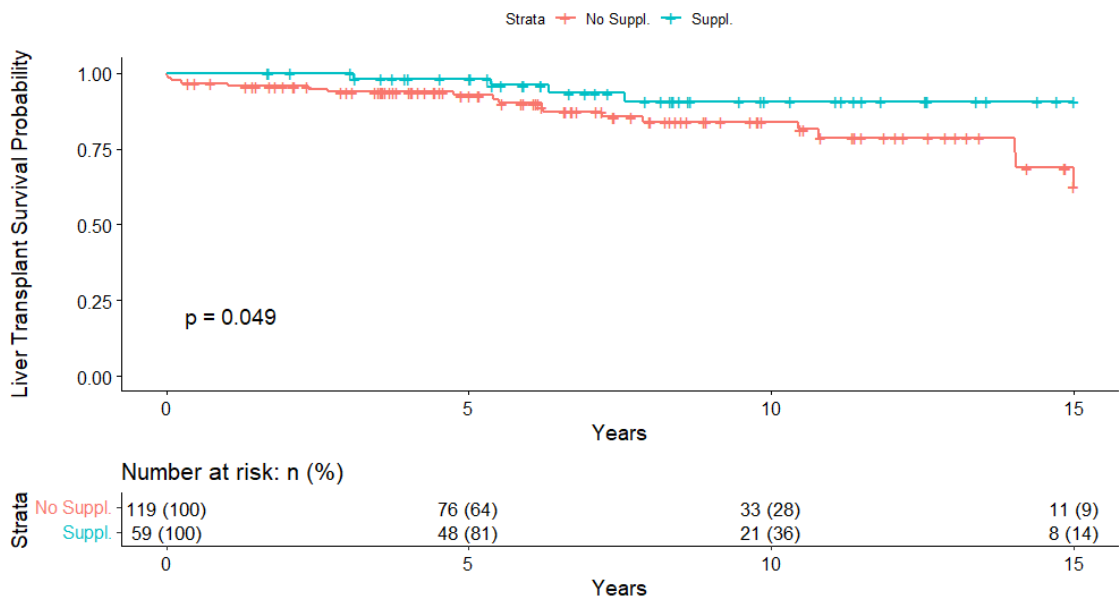
ng/ml) 25(OH)-vitamin levels ( $p=0.58$  and  $p=0.28$ , respectively; Figures 7 & 8). When grouped by history of supplementation, there was a significant difference in liver transplantation-free survival in PSC patients over both 15 and 5 years of follow-up ( $p=0.049$  and  $p=0.042$ , respectively; Figures 9 & 10).



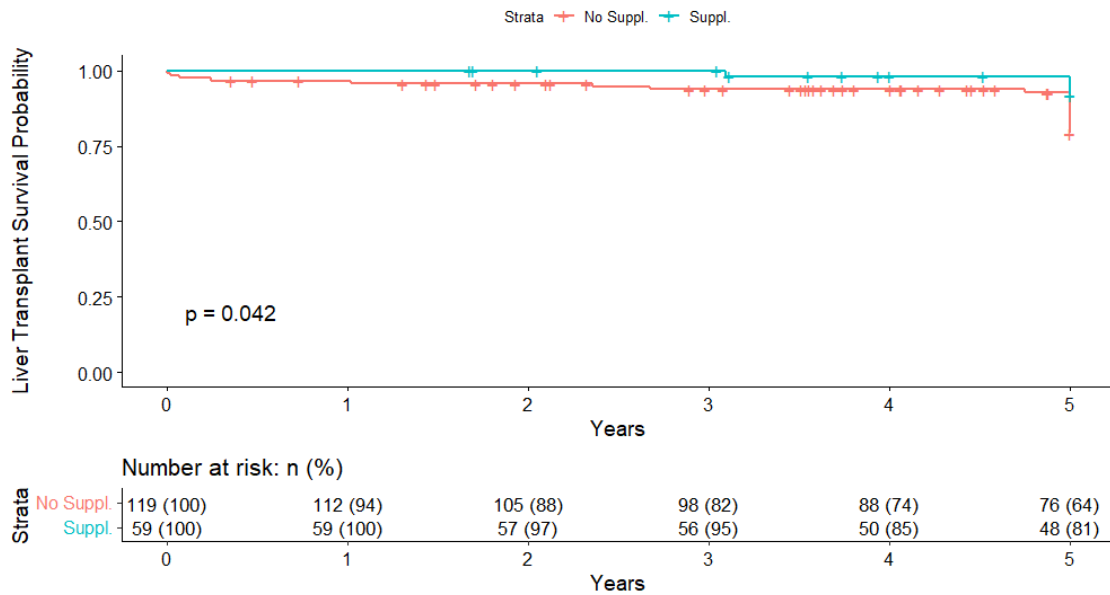
**Figure 7.** Kaplan Meier survival curve of liver transplantation-free survival between PSC patients with deficient and normal 25(OH)-vitamin D levels ( $p=0.58$ ). Cut-off of 25 years of follow-up.



**Figure 8.** Kaplan Meier survival curve of liver transplantation-free survival between PSC patients with deficient and normal 25(OH)-vitamin D levels (p=0.28). Cut-off of 5 years of follow-up.



**Figure 9.** Kaplan Meier survival curve of liver transplantation-free survival between PSC patients with or without history of vitamin D supplementation (p=0.049). Cut-off of 15 years of follow up.



**Figure 10.** Kaplan Meier survival curve of liver transplantation-free survival between PSC patients with or without history of vitamin D supplementation (p=0.042). Cut-off of 5 years of follow up.

### Univariate and Multivariate Analysis: Liver Transplantation

Upon univariate analysis using the Cox Proportional Hazards Model, both BMI and history of vitamin D supplementation were significantly associated with liver transplantation (p=0.006 and p=0.04, respectively). BMI and history of vitamin D supplementation were also significant upon multivariate analysis (p=0.004 and p=0.024). History of vitamin D supplementation was associated with a decreased hazard (increased survival) by a factor between 0.117 and 1.018. BMI was associated with a decreased hazard (increased survival) by a factor between 0.830 and 0.959.

**Table 6.** Univariate and Multivariate Cox Proportional Hazards Model for Liver Transplantation

	Univariate				Multivariate			
	Beta	95% CI (HR)		P-Value	Beta	95% CI (HR)		P-Value
		Lower	Upper			Lower	Upper	
<b>Age</b>	0.012	0.988	1.036	0.3				
<b>BMI</b>	-0.114	0.830	0.959	0.006	-1.1e-01	0.835	0.966	0.004
<b>Sex (Male)</b>	-0.495	0.267	1.391	0.20				
<b>Ethnicity</b>	0.170	0.159	8.855	0.87				
<b>PSC(Small)</b>	-0.794	0.106	1.935	0.29				
<b>IBD (UC)</b>	-4.8e-01	0.2144	1.776	0.37				
<b>UDCA</b>	9.4e-02	0.374	3.232	0.86				
<b>Vit. D</b>	-0.020	0.951	1.01	0.19				
<b>Suppl.</b>	-1.062	0.117	1.018	0.04	-1.33	0.0833	0.840	0.024

Abbreviations: BMI=Body Mass Index, PSC=Primary Sclerosing Cholangitis, IBD=Inflammatory Bowel Disease, UC=Ulcerative Colitis, UDCA=Ursodeoxycholic acid, CI=Confidence Interval, HR=Hazard Ratio. Suppl.=History of Vitamin D Supplementation

## **DISCUSSION**

In this study, we investigated the association between 25(OH)-vitamin D and both hepatobiliary malignancy and liver transplantation in a registry of 179 patients with PSC at two urban academic medical centers in the United States. We also sought to determine the effects of vitamin D supplementation on these outcomes. Our survival analyses demonstrated that vitamin D supplementation was associated with increased hepatobiliary malignancy-free and liver transplantation-free survival over a 5-year follow-up period. We also found that vitamin D deficiency, defined as 25(OH)-vitamin D < 20ng/ml, was not significantly associated with hepatobiliary malignancy-free or liver transplantation-free survival.

### **25(OH)-vitamin D, Supplementation, and Hepatobiliary Malignancy**

Over a 20-year follow-up period, we calculated via Kaplan-Meier survival curves from our cohort data an expected rate of hepatobiliary malignancy of 11.9% in PSC patients. This aligns with the lower end of the hepatobiliary malignancy rate in PSC patients of 13-20% found in Fevery et al's 2012 study. The 88.1% hepatobiliary malignancy-free survival rate is even higher than expected however considering that Burak et al. (2003) found that hepatobiliary malignancy rates tend to be higher in biopsy studies due to the difficulty in detection. While our finding is within the expected range, other factors such as differing rates of cancer screening, vitamin supplementation, and various clinical factors may have contributed to our calculated survival being higher than that found in other studies. Additionally, the survival analysis we employed projects the

survival rate to an exact 20-year follow-up period compared to traditional analyses which often utilize exact percentages based upon event rates in their study cohort. The relative similarity between our findings and that of other benchmark studies demonstrates the generalizability of our registry cohort to the wider PSC population.

Standard, time-independent statistical analyses did not demonstrate a significant association between 25(OH)-vitamin D or history of vitamin D supplementation and hepatobiliary malignancy. In contrast, survival analysis via Kaplan-Meier curves demonstrated that PSC patients with a history of vitamin D supplementation experience increased hepatobiliary malignancy-free survival at a statistically significant rate. The difference in these findings is most likely because hepatobiliary malignancy is not normally distributed over the follow-up period. In a 2009 study, Claessen et al. found that the median interval between PSC and CCA diagnosis was 2.5 years and Dyson et al. (2018), in a *The Lancet* review of PSC, stated that most CCA was identified within one year of PSC diagnosis. These results indicate that the majority of hepatobiliary malignancies occur early in the follow-up period, making standard, time-independent analyses less suitable as they assume a normal distribution of events. Survival analysis accounts for this time-dependent skewed distribution of events and demonstrated statistically significant differences in hepatobiliary malignancy-free survival between patients with and without a history of vitamin D supplementation.

Our analysis demonstrated no significant differences in hepatobiliary malignancy-free survival between PSC patients with deficient (<20ng/ml) and normal 25(OH)-vitamin D levels. There is little prior data on the association between serum vitamin D

levels and the incidence of hepatobiliary cancer specifically, but research on other gastrointestinal cancers such as colorectal cancer, however, has demonstrated that reduced plasma 25(OH)-vitamin D is significantly associated with increased risk in patients with IBD (Ananthakrishnan et al., 2014). With the large degree of overlap between PSC and IBD, similarities in the effects of plasma vitamin D might be expected, but genetic analysis has also demonstrated that there may be some distinction between the two diseases (Liu et al., 2013). There may be different mechanisms related to the effects of vitamin D between colorectal cancer in IBD and hepatobiliary cancer in PSC. Future research on PSC patients with IBD and the rates of colorectal vs. hepatobiliary malignancy in this population may help answer this question.

Most prior research has instead focused on the effects of vitamin D on liver tumor progression and its usage as a potential treatment option in combination therapies. This may correlate with our finding that vitamin D supplementation was significantly associated with increased hepatobiliary malignancy-free survival. For example, Hammad et al. (2013) demonstrated that declining levels of vitamin D was significantly associated with progressive HCC. Similarly, Baek et al. (2011) and Pourgholami et al. (2000) found that administration of vitamin D reduced the proliferation of CCA and HCC cell lines, respectively. Chiang et al. (2014) further demonstrated the chemotherapeutic effects of dietary vitamin D supplementation on CCA in an animal model. Our findings on the effects of vitamin D supplementation reinforce the translational applicability of these findings to humans with PSC.

Whether our results demonstrate a predictive effect of vitamin D supplementation on hepatobiliary malignancy incidence is particularly difficult to discern because cancers such as CCA often present in more advanced stages (Yachimsky & Pratt, 2008). CCA and other hepatobiliary malignancies are very difficult to detect in the early stages, as there are often few symptomatic stages. As a result, it is unclear whether vitamin D supplementation is decreasing hepatobiliary malignancy survival or reducing the proliferation and growth of the tumors.

Despite this uncertainty, the significant association between vitamin D supplementation and hepatobiliary malignancy-free survival demonstrates the importance of supplementation in those with PSC, who are at higher risk for hepatobiliary malignancy.

### **25(OH)-vitamin D. Supplementation, and Liver Transplantation**

According to Boonstra et al. (2013), the average time from PSC diagnosis to transplantation or death is 21.3 years in the general PSC population and 13.2 years in a liver transplant center cohort. Over 10 and 20-year follow-up periods, we calculated via Kaplan-Meier survival curves expected rates of liver transplantation in PSC patients of 13.7% and 23.4%, respectively (86.3% and 76.6% transplantation-free survival, respectively). The PSC cohorts at Massachusetts General Hospital and the Brigham and Women's hospital include patients enrolled from both standard clinics and liver transplant centers and the PSC registry reflects both populations. Given that Tischendorf et al. (2007) calculated a transplantation rate of 39.6% in a single center cohort study, the

transplantation rate found in our PSC registry is significantly lower. The Tischendorf et al. study, however, was conducted at the largest transplantation center in Germany which may have influenced the results. Additionally, advancements in PSC detection and management may have led to better outcomes or longer median time-to-event in PSC patients.

While much remains uncertain regarding the pathogenesis of PSC, genetic and immunologic analyses have demonstrated that T cell dysregulation and dysfunction contributes to PSC development and progression. In particular, Liaskou et al. (2014) demonstrated the increased prevalence of CD28<sup>-</sup> T cells in those with PSC and their usefulness as inflammatory markers. CD28<sup>-</sup> T cells are thought to be more prevalent in PSC partly due to continuous antigenic stimulation at inflammatory sites, and Katt et al. (2013) demonstrated that most T helper cells in PSC patients are under stimulation from the pathogen *Candida albicans*. These studies point to pathogenic and/or endotoxin overstimulation of T cells resulting in increased inflammation at the liver and bile ducts, which may induce the development and progression of PSC.

In addition, Liaskou et al. (2014) demonstrated that, in vitro, administration of 1,25(OH)<sub>2</sub>D<sub>3</sub> prevented the downregulation of CD28 in T cells. While this finding has not been replicated in in vivo studies, it represents a potential pathway through which vitamin D may influence inflammation in PSC. Not only has vitamin D been shown to have this effect on CD28 regulation, but Liaskou et al. (2014) also found that it increases the suppressive action of regulatory T cells, which is important in preventing autoimmunity and further inflammation. Some of the most significant non-HLA genetic

associations with PSC, such as those influencing HDAC7 and SIK2, also influence regulatory T cells and provide another similar pathway through which vitamin D may influence PSC pathogenesis and progression (Liu et al., 2013).

Our finding that vitamin D supplementation is significantly associated with higher liver transplantation-free survival provides additional evidence on the role of vitamin D in PSC progression. Similarly, to hepatobiliary malignancy however, 25(OH)-vitamin D levels were not significantly associated with liver transplantation-free survival nor lower rates of liver transplantation upon standard analysis. Progressive inflammation of the bile ducts and liver is a primary characteristic of PSC, and cholestasis, another progressive symptom of PSC, has been associated with fat-soluble vitamin deficiency (Dyson et al., 2018). Vitamin D may play a key predictive and therapeutic role in the progressive inflammatory pathway in PSC, and future research on vitamin D and direct inflammatory markers or mediators could prove interesting and useful.

### **BMI and Liver Transplantation-free Survival**

One of the more surprising results of our univariate and multivariate analysis was that higher BMI was significantly associated with a reduced hazard for liver transplantation. While high obesity (defined as >30 BMI) contributes to higher morbidity in liver diseases such as non-alcohol fatty liver disease as well as in many other cardiovascular and renal conditions, there is growing evidence of a “obesity paradox” in chronic diseases (Lonardo et al., 2015; Curcic et al., 2019). In this paradox, patients with obesity tend to suffer lower mortality compared to those of normal weight, particularly in

chronic diseases compared to acute illnesses. Curcic et al. (2019) identified a potential obesity paradox effect in chronic liver diseases, especially those that involve cirrhosis. The mechanisms behind this effect are still unclear and many of the studies on the obesity paradox contain confounding factors, but the body of evidence supporting this finding is growing. Our finding of increased BMI in PSC being significantly associated with reduced hazard for liver transplantation supports this hypothesis, particularly as cirrhosis is often an end-stage symptom of PSC leading to liver transplantation.

### **Racial and Ethnic Differences in Supplementation**

Our stratification of the PSC registry into two cohorts based on vitamin D supplementation yielded significant differences among race and ethnicity. Racial disparities in healthcare are vast, but the differences we found do not follow common trends (Nelson, 2002). While our stratification indicated that significantly fewer white people with PSC had a history of vitamin D supplementation, a study of Medicare recipients demonstrated that Black and Hispanic people were 10 to 40 percent less likely to use medications than white persons with the same illness (Briesacher et al., 2003). A 2013 study by Libon et al. demonstrated however that skin pigmentation is negatively associated with vitamin D synthesis. It is unclear whether this factor influenced vitamin D supplement prescription and adherence, but it may help explain why our findings on vitamin D supplementation differ from research on other prescribed medications. Additionally, a higher percentage of the non-supplementing cohort had an unknown ethnicity compared to the supplementation cohort.

## **Limitations**

While we were able to demonstrate a significant association between vitamin D supplementation and survival from both outcomes, hepatobiliary malignancy and liver transplantation, 25(OH)-vitamin D levels were not significantly associated with any outcomes. The role of vitamin D in PSC progression and outcome is promising, but our understanding of its mechanisms and effects is not complete. Even given the unclear nature of the role of vitamin D in PSC however, the registry cohort we leveraged in this study may not currently be an ideal representation of PSC with which to pursue these types of analyses.

According to Molodecky et al. (2011), the average age at PSC diagnosis is 41. In our PSC registry, the current average age of those with complete data is 45.6 years. Given an annual risk of 2% for CCA in PSC, the chance of patients in our cohort developing CCA or other malignancies over an average of 4.6 years of follow-up is low (Rizvi et al., 2015). As the cohort ages and additional data is collected, our analyses will become more powered and able to determine associations between variables and endpoints.

Additionally, out of 238 total patients with PSC in the registry, only 179 had complete vitamin D and outcome data available in their medical records. Many patients lacking vitamin D data were enrolled into the registry at earlier dates and experienced outcomes that were not able to be included in our analyses. Due to the increasing use of electronic medical records and the prevalence of frequent fat-soluble vitamin monitoring in PSC however, as the registry ages more patients will have complete data sets available.

This will allow our analyses to better detect relationships between PSC outcomes and biomarkers such as vitamin D in the future.

## **CONCLUSION**

This study demonstrated a significant association between vitamin D supplementation and survival from hepatobiliary malignancy and liver transplantation. PSC currently has few widely accepted treatment options in addition to few prognostic indicators. Vitamin D may play an important role in the pathogenesis and progression of PSC, and its supplementation may be an important factor in preventing adverse outcomes. Future studies of vitamin D as both a predictor and treatment option are needed to fully characterize its effects on PSC progression and outcome.

## LIST OF JOURNAL ABBREVIATIONS

Am J Gastroenterol	The American Journal of Gastroenterology
Annat Cell Biol	Anatomy & Cell Biology
Ann Transl Med	Annals of Translational Medicine
Annu Rev Pharmacol	Annual Review of Pharmacology and Toxicology
Clin Gastroenterol Hepato	Clinical Gastroenterology and Hepatology
Dig Liver Dis	Digestion and Liver Disease
DRM	Dermatology
Health Care Financ Rev	Health Care Financing Review
Inflamm Bowel Dis	Inflammatory Bowel Diseases
J Biol Chem	The Journal of Biological Chemistry
J Clin Transl Hepatol	Journal of Clinical and Translational Hepatology
J Clin Gastroenterol	Journal of Clinical Gastroenterology
J Hepatol	Journal of Hepatology
J Immunotoxicol	Journal of Immunotoxicology
J Natl Med Assoc	Journal of the National Medical Association
Liver Int	Liver International
Mol Aspects Med	Molecular Aspects of Medicine
Nat Genet	Nature Genetics
Nat Rev Cancer	Nature Reviews: Cancer

N Engl J Med	The New England Journal of Medicine
Parenter Enteral Nutr	Journal of Parenteral and Enteral Nutrition
World J Gastroenterol	World Journal of Gastroenterology

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**CURRICULUM VITAE**

