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Elevated IgG4 is associated with higher risk for  
cholangitis, cirrhosis, ERCP and  
liver-transplantation among patients with  
primary sclerosing cholangitis

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## **Abstract**

Primary sclerosing cholangitis (PSC) is a rare inflammatory chronic liver disease that causes damage to the intra- and or extrahepatic bile ducts leading to cholestasis. As the disease proceeds the development of cirrhosis and eventually liver failure occurs. This study aims to determine the role of IgG subclasses in the prognosis of PSC and its outcome. A retrospective analysis was performed of 183 patients followed at the Department of Upper Abdominal Diseases at the Karolinska University Hospital. Factors that were analysed were sex, age at PSC diagnosis, total IgG values, IgG subclasses values and events of autoimmune hepatitis (AIH), inflammatory bowel disease (IBD), colectomy, cirrhosis, cholangitis, endoscopic retrograde cholangiopancreatography (ERCP), liver transplantation and cholangiocarcinoma. This study showed that high IgG4 levels were associated with a higher incidence of cirrhosis, liver transplantation, cholangitis and ERCP, while low IgG4 levels were associated with a prior IBD diagnosis. In conclusion, elevated IgG4 levels were associated with a higher occurrence of cirrhosis, cholangitis, ERCP and liver transplantation. It seems that IgG4 could be of importance for outcome prediction in PSC.

## **Abbreviations**

PSC - Primary sclerosing cholangitis

ERCP - Endoscopic retrograde cholangiopancreatography

IBD - Inflammatory bowel disease

AIH - Autoimmune hepatitis

Ig - Immunoglobulin

## Introduction

Primary sclerosing cholangitis (PSC) is a rare inflammatory chronic liver disease that affects people of all ages and sex, but men to a greater extent than females (European Association for the Study of the Liver 2009, Mehta *et al.* 2021). PSC prevalence in the population varies between countries, globally it ranges from 0–31.7 per 100 000 individuals (Mehta *et al.* 2021), whereas the northern European countries show the highest prevalence of PSC (Dyson *et al.* 2018, Mehta *et al.* 2021).

PSC is known to cause damage to the intra- and or extrahepatic bile ducts leading to narrowing that affects the bile flow leading to cholestasis (Dyson *et al.* 2018). As the liver disease proceeds the development of cirrhosis and eventually liver failure occurs (Karlsen *et al.* 2007). A PSC diagnosis is made through imaging of the bile duct looking for characteristic lesions or narrowing together with biomarkers indicating cholestasis, that cannot be explained otherwise (European Association for the Study of the Liver 2009, Dyson *et al.* 2018).

Unfortunately, there is no effective pharmacological treatment for PSC, as no treatment options have shown efficacy to improve the long-term outcome of the disease (Hasegawa *et al.* 2021). Treatment is done to manage the disease and arising symptoms, some treatment options are drugs that improve liver values like ursodeoxycholic acid, anti-inflammatory drugs or endoscopic retrograde cholangiopancreatography (ERCP) for dilatation of narrowed bile ducts (European Association for the Study of the Liver 2009, Dyson *et al.* 2018, Iravani *et al.* 2020). Liver transplantation is the only treatment option for advanced PSC, but the disease can reoccur even after transplantation (LaRusso *et al.* 2006). Reoccurrence has been seen in 16.7 % of the transplantation cases, where most were within the first 5 years affecting the long-term survival (Visseren *et al.* 2021).

Inflammatory bowel disease (IBD) which is a group of autoimmune diseases affecting the intestine with chronic inflammation has been shown to have a high prevalence in PSC patients going up to 80 % (European Association for the Study of the Liver 2009, Fakhoury *et al.* 2014). Within these patients, the most common type of IBD is ulcerative colitis standing for 76 % followed by Crohn's disease at 17 % (Mehta *et al.* 2021). An IBD diagnosis together with a PSC diagnosis elevates the risk of dysplasia and colon malignancy development compared to IBD only patients (Soetikno *et al.* 2002, Broomé & Bergquist 2006). PSC has also been linked to an elevated risk of developing hepatobiliary malignancies, especially cholangiocarcinoma which is the most common for this liver disease (Lazaridis & Gores 2006). While several factors have been able to be linked to the increased risk of cholangiocarcinoma in PSC they have not been useful for predicting the outcome (Karlsen *et al.* 2007).

PSC rarely occurs together with autoimmune hepatitis (AIH) but is known as PSC-AIH overlap syndrome. It occurs in 1.4–8% of PSC patients (Hasegawa *et al.* 2021) and is characterized by bile duct changes as in PSC along with the biochemical and histologic findings as in AIH (European Association for the Study of the Liver 2009).

Immunoglobulins (Ig) are important proteins that function in the immune system and come in five different classes IgG, IgA, IgM, IgD, and IgE. The presence of the Ig classes in serum

differs where IgG is the most predominant, followed by IgA, IgM, IgD, and IgE. The Ig with the longest half-life in the human body is IgG and it is divided into additional four subclasses, IgG1, IgG2, IgG3 and IgG4 all with their properties. The presence of the IgG subclasses in serum differs, where 67% of serum IgG is IgG1, 22% IgG2 7% IgG3 and 4% IgG4. (Schroeder & Cavacini 2010).

IgG levels have been studied in common autoimmune diseases like primary Sjögren syndrome, primary biliary cirrhosis, systemic lupus erythematosus, and systemic sclerosis, and it has revealed that the distribution of the IgG subclass is particular to each disease. In all the diseases, IgG1 and IgG3 were elevated, while IgG2 was low in all, but primary biliary cirrhosis compared to healthy individuals. This was the case even if the total IgG was within the normal range, and as for IgG4 and there was no difference (Zhang H *et al.* 2015). In one other study on systemic lupus erythematosus, they found similar results with IgG1, IgG2 and IgG3 being elevated. They also grouped the patients based on either active diseases or remission and found that the ones in remission had a decreased level of IgG3 (Lin & Li 2009).

Previous studies on IgG subclasses in common liver diseases have been published but when it comes to data regarding IgG4 levels they were limited due to small cohorts (Zheng *et al.* 2021). IgG levels have also been studied in PSC patients where a majority (61 %) had elevated levels far from normal (Boberg *et al.* 1996). IgG4 related cholangitis patients had higher levels of IgG2 compared to those with PSC. While those with normal to low levels of IgG2 or IgG4 but high IgG1 were an indication of PSC. With the implication that IgG1 and IgG2 can be a distinguishing factor between IgG4 related cholangitis and PSC (Vujasinovic *et al.* 2020).

This study aims to determine the role of IgG subclasses in the prognosis of PSC outcome.

## Method

*Data collection:* Data was collected in the Take care data systems of PSC diagnosed patients followed (January 1970 to December 2015) at the Department of Upper Abdominal Diseases at Karolinska University Hospital in Stockholm, Sweden. Data that were gathered were sex, age at PSC diagnosis, cirrhosis, cholangitis, treatment of cholangitis, liver transplantation, IBD, colectomy, total IgG values and IgG subclasses values (IgG1, IgG2, IgG3, IgG4), AIH, ERCP, cholangiocarcinoma, and if deceased the date of death.

*Inclusion and exclusion criteria:* PSC patients older than 18 years were included. Patients that were excluded had events prior to PSC diagnosis, lacking a Swedish identity number, <18 years at diagnosis or missing data for variables that were analysed.

*Statistical analysis:* Variables that were analysed were sex, age at diagnosis, IgG total, IgG1, IgG2, IgG3, IgG4 and events of AIH, IBD, colectomy, cirrhosis, cholangitis, ERCP, liver transplantation and cholangiocarcinoma during the follow up. Distribution differences of variables were analysed with either a Fisher exact test if the variables are categorical or Student T-test or ANOVA if continuous.

IgG levels within the intervals of IgG total 6.7–14.5 g/L, IgG-1 2.8–8.0 g/L, IgG-2 1.15–5.7 g/L, IgG-3 0.24–1.25 g/L and IgG-4 0.05–1.25 g/L was graded as normal. IgG levels above and below the intervals were graded as high respective low. The IgG values of low, normal, and high were described as mean values together with standard deviations.

The annual rate of events was calculated by dividing the number of events at the follow-up by years of observation of the patient. The cumulative incidence of events as per baseline IgG levels was plotted by the inverse Kaplan-Meier method and significance was analysed through the log-rank test. The significance of cholangitis and ERCP was analysed through the Mantel Haenszel test. Statistics analysis was made in SAS software version 9.4 (SAS Institute, Cary, NC). Two-sided p-values for all tests and <0.05 were classed as significant.

*Ethics:* The study was approved by the local ethic committee (Regionala etikprövningsnämnden Stockholm Dnr. 2014/902-31/2).

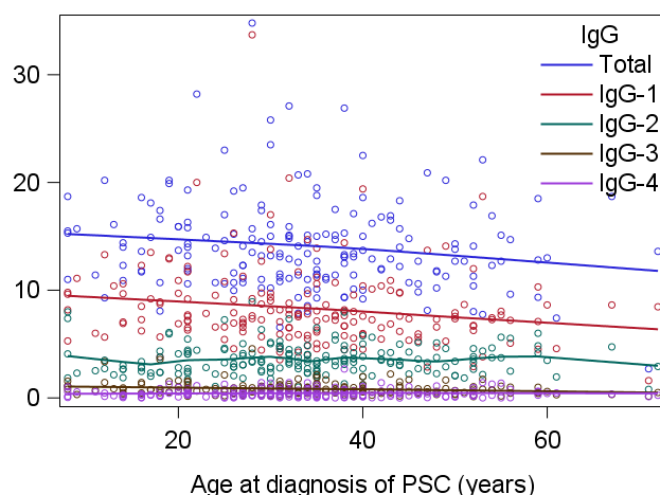
## Results

The study included 183 patients, 122 were females and 61 were males. The median time of follow-up was 17 years (interquartile range 12–24 years). The IgG level between the sexes was similar regarding the total IgG and IgG subclasses (Table 1). While looking at the age distribution of a PSC diagnosis and IgG levels it seems that the total IgG, IgG1 and IgG3 decreases with age at diagnosis while IgG2 and IgG4 stay at a similar level (Table 1, Figure 1). The IgG4/IgG and IgG4/IgG1 ratios were similar between the sexes and at different ages at diagnosis (Table 1). Nevertheless, this observation was not statistically significant.

**Table 1.** IgG levels are expressed as means with a standard deviation of patients at diagnosis of PSC.

	All	Gender		Age at diagnosis of PSC				
	Patients	Females	Males	0-19	20-29	30-39	40-49	50+
	(n=183)	(n=122)	(n=61)	(n=25)	(n=41)	(n=62)	(n=30)	(n=25)
IgG total (g/L)	14.1 ±4.3	14.3 ±4.3	13.6 ±4.3	14.7 ±3.5	15.0 ±4.9	13.8 ±4.4	13.9 ±3.7	12.9 ±4.2
IgG1 (g/L)	8.3 ±3.7	8.5 ±3.7	8.0 ±3.7	8.9 ±2.8	9.2 ±4.9	8.0 ±3.2	8.0 ±3.4	7.4 ±3.3
IgG2 (g/L)	3.6 ±1.4	3.7 ±1.4	3.4 ±1.4	3.4 ±1.7	3.6 ±1.5	3.6 ±1.4	3.6 ±1.2	3.5 ±1.3
IgG3 (g/L)	0.84 ±0.58	0.85 ±0.64	0.82 ±0.43	1.06 ±0.82	0.87 ±0.73	0.83 ±0.42	0.84 ±0.51	0.61 ±0.37
IgG4 (g/L)	0.44 ±0.40	0.49 ±0.43	0.35 ±0.31	0.35 ±0.32	0.45 ±0.35	0.48 ±0.46	0.41 ±0.37	0.46 ±0.42
IgG4/IgG (%)	3.15 ±2.65	3.36 ±2.69	2.74 ±2.56	2.30 ±1.81	3.06 ±2.34	3.47 ±3.04	2.98 ±2.48	3.60 ±2.97
IgG4/IgG1 (%)	5.73 ±5.20	6.08 ±5.23	5.05 ±5.12	3.88 ±3.10	5.49 ±4.62	6.38 ±5.98	5.75 ±5.50	6.36 ±5.24

Most patients have total IgG and IgG subclass levels classed within the normal interval (59.6% total IgG, 57.9% IgG1, 90.7% IgG2, 79.2% IgG3 and 85.2% IgG4) (Table 2). The second most occurring classification for the total IgG and IgG subclass is being high, in all but IgG4 where it's the lowest (39.3% total IgG, 41.5% IgG1, 7.1% IgG2, 14.8% IgG3 and 6.0% IgG4). The classification that occurred the least for the total IgG and IgG subclass was low, in all but IgG4 where it's the highest (1.1% total IgG, 0.5% IgG1, 2.2% IgG2, 6.0% IgG3 and 8.7% IgG4).



**Figure 1.** IgG levels corresponding to age at diagnosis of PSC. IgG levels on the y-axis and age at diagnosis in years on the x-axis.

Examining the connection with IBD in patients with PSC, it was found that 92 (50.3%) of the patients had an IBD diagnosis prior to the diagnosis of PSC (Table 2). The association between the diagnoses was not affected by age ( $p$ -value=0.44) and sex ( $p$ -value=0.35) (Table 2). No significant difference was seen for all the IgG subclass levels but IgG4 (Table 2). IgG4 levels were low in patients with a prior IBD diagnosis at the diagnosis of PSC ( $p$ -value=0.03) (Table 2).

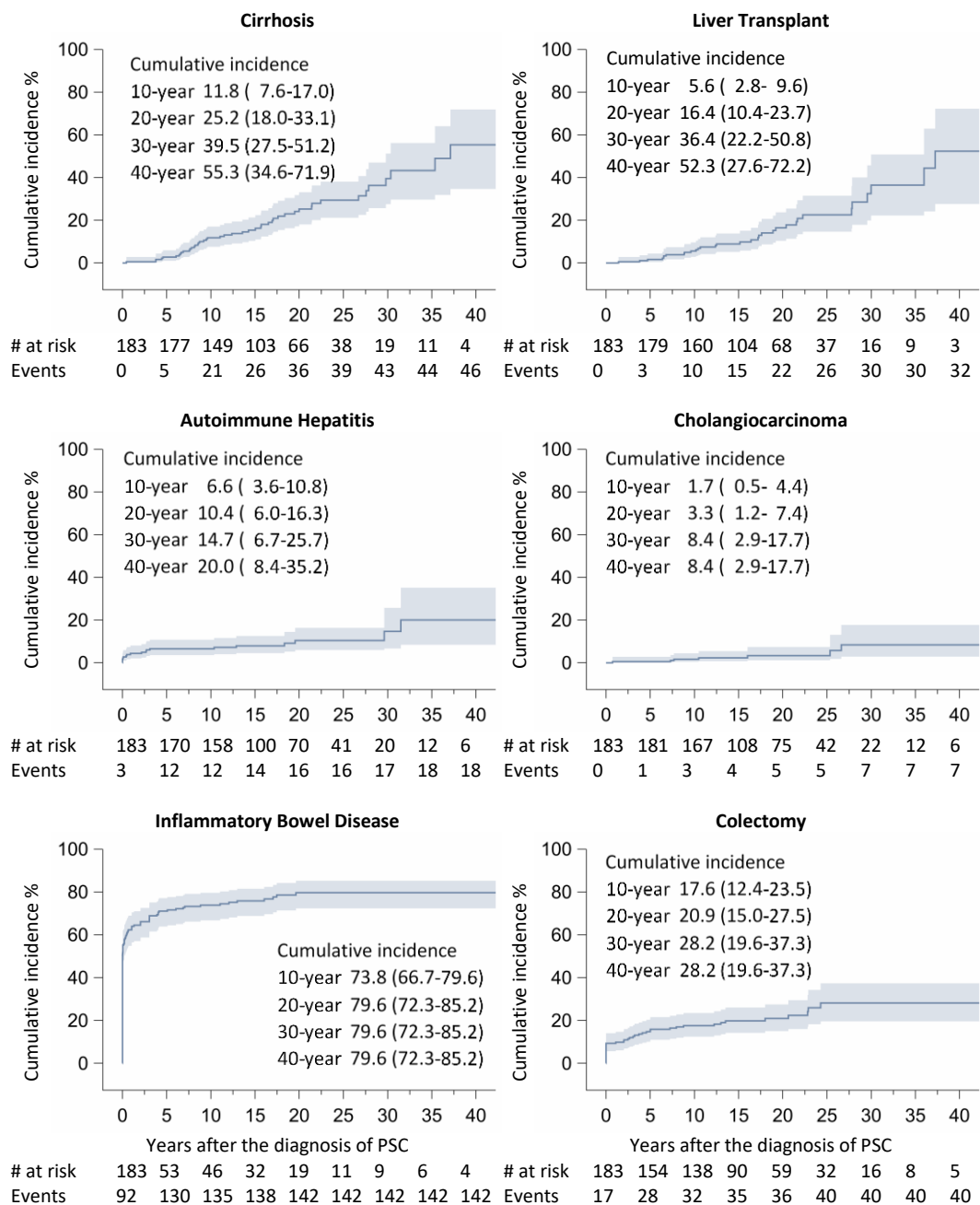
The IgG4/IgG and IgG4/IgG1 ratios that were based on all patients were distributed evenly between low (49.7% respectively 50.3%) and high (50.3% respectively 49.7%) from the median value (Table 2). This distribution changes when dividing the patients into those with no prior IBD diagnosis and those with IBD diagnosis. In the group with no prior IBD diagnosis, the distribution between low (52.8% respectively 53.9%) and high (47.3% respectively 46.1%) shifted towards low (Table 2). While in the group with those with a prior IBD diagnosis, the distribution between low (46.7% respectively 46.7%) and high (53.3% respectively 53.3%) shifted towards high. This observation of the distribution change in IgG4/IgG and IgG4/IgG1 ratio was not of statistical significance (Table 2).

The cumulative incidence of various events after the diagnosis of PSC looking at 10 years intervals up to 40 years after diagnosis shows that there is a high chance for patients to develop cirrhosis and need liver transplantation over time. At 10 years of PSC there are 11.8% incidences of cirrhosis and 5.6% of liver transplantation and looking at 40 years these values are 55.3% and 52.3% respectively (Figure 2).

The incidence of IBD, cholangiocarcinoma and colectomy are rather stable over time with a few percent difference between the 10 years and 40 years following the diagnosis. The incidence of IBD at year 10 is 73.8%, and at year 40 the incidence has increased with 5.8% to 79.6%. For cholangiocarcinoma, the increase was 6.7% going from 1.7% to 8.4%, and for colectomy, the increase was 10.6% going from 17.6% to 28.2%. The incidence of AIH was at 10 years at 6.6%, and at year 40 the incidence has increased by 13.4% going from 6.6% to 20.0% (Figure 2)

**Table 2.** Association of sex, age, and IgG levels at diagnosis of PSC to IBD diagnosis, within parentheses is the percentage of all.

		Total	No previous IBD	Previous IBD	P-value
<b>ALL</b>		183 (100)	91 (100)	92 (100)	
<b>Sex</b>					
	Males	61 (33.3)	33 (36.3)	28 (30.4)	0.44
	Females	122 (66.7)	58 (63.7)	64 (69.6)	
<b>Age</b>					
	<20	25 (13.7)	16 (17.6)	9 ( 9.8)	0.35
	20-29	41 (22.4)	19 (20.9)	22 (23.9)	
	30-39	62 (33.9)	33 (36.3)	29 (31.5)	
	40-49	30 (16.4)	14 (15.4)	16 (17.4)	
	50+	25 (13.7)	9 ( 9.9)	16 (17.4)	
<b>IgG (g/L)</b>					
	Mean±SD	14.1±4.3	14.0±4.4	14.2±4.1	0.73
	Low (<6.7 g/L)	2 ( 1.1)	1 ( 1.1)	1 ( 1.1)	0.82
	Normal (6.7-14.5 g/L)	109 (59.6)	56 (61.5)	53 (57.6)	
	High (>14.5 g/L)	72 (39.3)	34 (37.4)	38 (41.3)	
<b>IgG1 (g/L)</b>					
	Mean±SD	8.3±3.7	8.5±4.2	8.1±3.1	0.56
	Low (<2.8 g/L)	1 ( 0.5)	1 ( 1.1)	0 ( 0.0)	0.71
	Normal (2.8-8.0 g/L)	106 (57.9)	51 (56.0)	55 (59.8)	
	High (>8.0 g/L)	76 (41.5)	39 (42.9)	37 (40.2)	
<b>IgG2 (g/L)</b>					
	Mean±SD	3.6±1.4	3.6±1.5	3.6±1.3	0.88
	Low (<1.15 g/L)	4 ( 2.2)	1 ( 1.1)	3 ( 3.3)	0.46
	Normal (1.15-5.7 g/L)	166 (90.7)	82 (90.1)	84 (91.3)	
	High (>5.7 g/L)	13 ( 7.1)	8 ( 8.8)	5 ( 5.4)	
<b>IgG3 (g/L)</b>					
	Mean±SD	0.84±0.58	0.91±0.66	0.48±0.72	0.09
	Low (<0.24 g/L)	11 ( 6.0)	6 ( 6.6)	5 ( 5.4)	0.48
	Normal (0.24-1.25 g/L)	145 (79.2)	69 (75.8)	76 (82.6)	
	High (>1.25 g/L)	27 (14.8)	16 (17.6)	11 (12.0)	
<b>IgG4 (g/L)</b>					
	Mean±SD	0.44±0.40	0.46±0.38	0.43±0.41	0.63
	Low (<0.05 g/L)	16 ( 8.7)	3 ( 3.3)	13 (14.1)	<b>0.03</b>
	Normal (0.05-1.25 g/L)	156 (85.2)	82 (90.1)	74 (80.4)	
	High (>1.25 g/L)	11 ( 6.0)	6 ( 6.6)	5 ( 5.4)	
<b>IgG4/IgG ratio (%)</b>					
	Mean±SD	3.15±2.65	3.40±2.86	2.91±2.42	0.21
	Low (<median 2.47)	91 (49.7)	48 (52.8)	43 (46.7)	0.42
	High (>median 2.47)	92 (50.3)	43 (47.3)	49 (53.3)	
<b>IgG4/IgG1 (%)</b>					
	Mean±SD	5.73±5.20	6.16±5.76	5.31±4.58	0.27
	Low (<median 4.06)	92 (50.3)	49 (53.9)	43 (46.7)	0.34
	High (>median 4.06)	91 (49.7)	42 (46.1)	49 (53.3)	

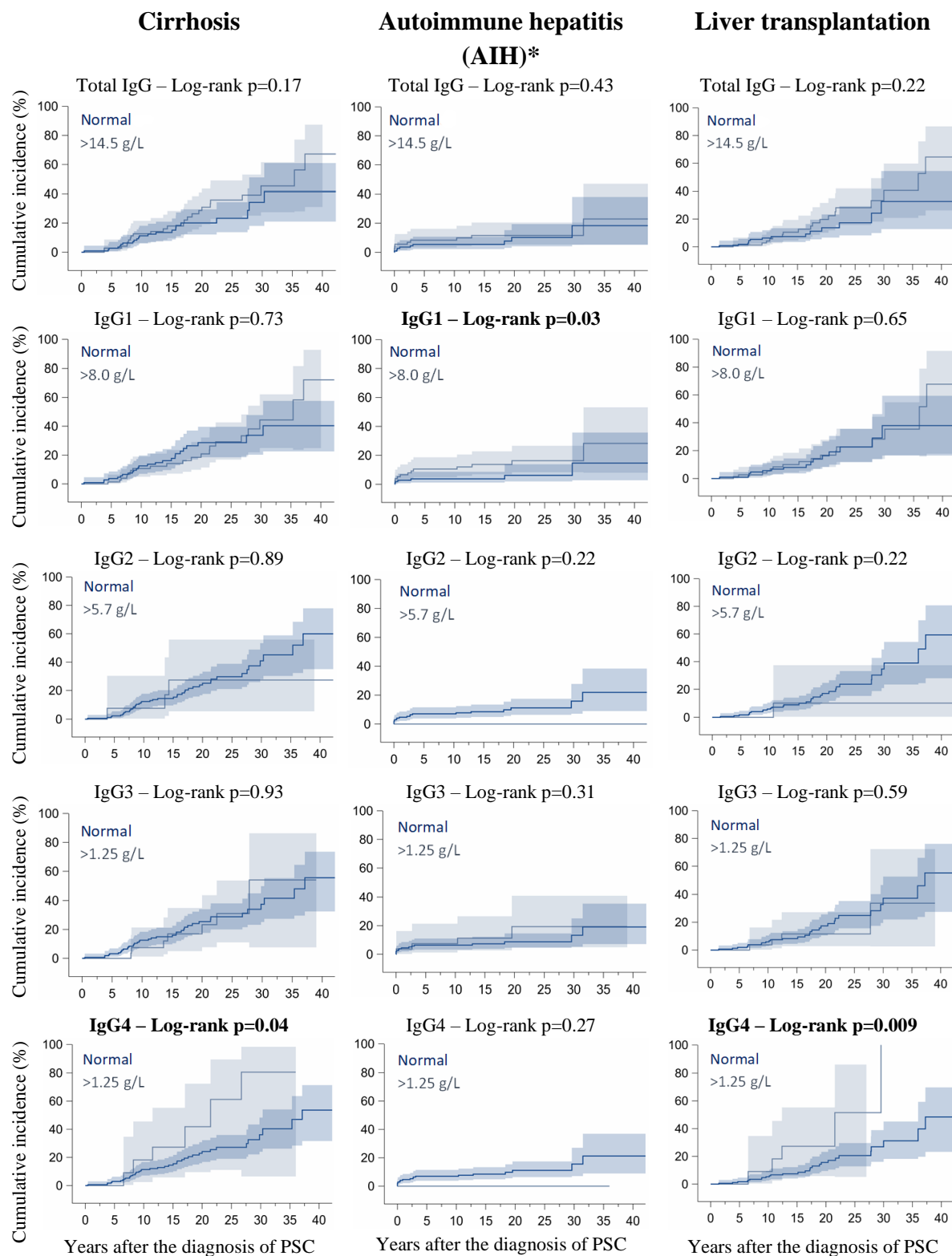


**Figure 2.** Cumulative incidence of cirrhosis, liver transplantation, autoimmune hepatitis, cholangiocarcinoma, Inflammatory bowel disease, and colectomy at or after diagnosis of PSC with specified values at 10 years intervals up to 40 years.

Looking at the development of various events after the diagnosis of PSC, 46 (25.1%) patients developed cirrhosis and 32 (17.5%) underwent liver transplantation, and it was shown that patients with high levels of IgG4 at diagnosis had higher incidents of cirrhosis (log-rank p-value=0.04) and liver transplantation (log-rank p-value=0.009) compared with patients with normal or low levels (Table 3, Figure 3a). The incidents of cirrhosis were also positively correlated with age at diagnosis (log-rank p-value=0.03) (Table 3, Figure 4a).

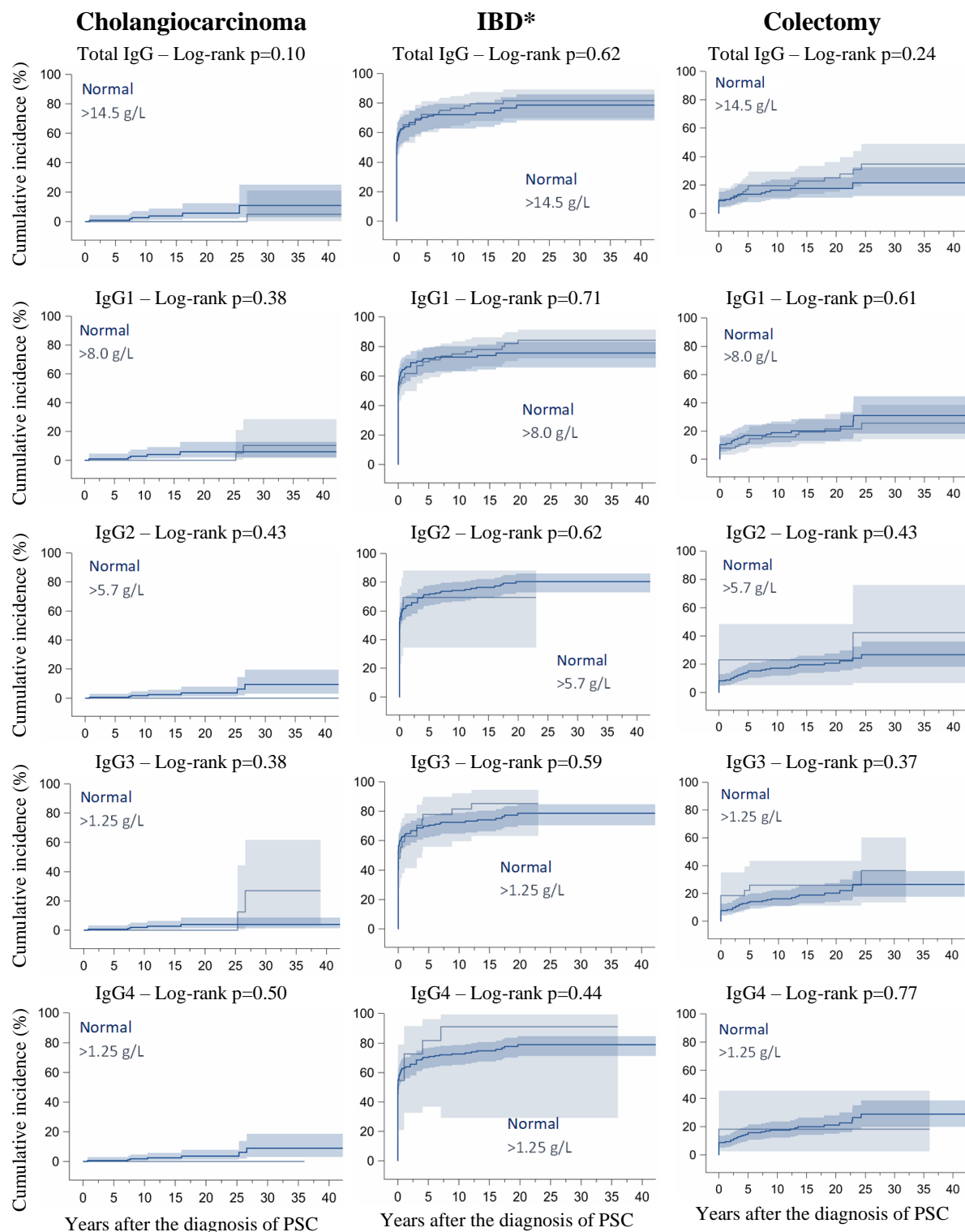
**Table 3.** Development of various events after the diagnosis of PSC with patients with events prior to PSC were excluded.

	<b>Autoimmune hepatitis</b>	<b>Cirrhosis</b>	<b>Cholangio-carcinoma</b>	<b>Liver transplant</b>	<b>Colectomy</b>	<b>IBD</b>
	Events (rate % per year)	Events (rate % per year)	Events (rate % per year)	Events (rate % per year)	Events (rate % per year)	Events (rate % per year)
<b>ALL</b>	16 (0.49)	48 (1.47)	7 (0.20)	32 (0.97)	24 (0.83)	50 (4.73)
<b>Sex</b>						
Males	7 (0.68)	15 (1.44)	3 (0.27)	8 (0.74)	8 (0.89)	14 (3.59)
Females	9 (0.40)	33 (1.49)	4 (0.17)	24 (1.09)	16 (0.81)	36 (5.39)
<b>Age</b>						
<20	2 (0.35)	4 (0.69)	1 (0.17)	3 (0.52)	4 (0.72)	14 (14.29)
20-29	3 (0.37)	10 (1.24)	1 (0.12)	11 (1.43)	3 (0.40)	10 (3.31)
30-39	5 (0.46)	16 (1.46)	3 (0.26)	11 (1.00)	8 (0.88)	18 (4.29)
40-49	4 (0.88)	11 (2.53)	1 (0.21)	4 (0.84)	7 (2.11)	6 (4.14)
50+	2 (0.58)	7 (2.07)	1 (0.27)	3 (0.83)	2 (0.62)	2 (2.15)
<b>Previous IBD</b>						
No	8 (0.45)	27 (1.52)	3 (0.16)	18 (1.02)	6 (0.34)	50 (4.73)
Yes	8 (0.53)	21 (1.42)	4 (0.25)	14 (0.92)	18 (1.63)	-
<b>IgG (g/L)</b>						
Low (<6.7 g/L)	-	-	-	-	1 (7.14)	-
Normal (6.7-14.5 g/L)	8 (0.43)	23 (1.23)	6 (0.31)	14 (0.75)	10 (0.61)	30 (4.83)
High (>14.5 g/L)	8 (0.58)	25 (1.82)	1 (0.07)	18 (1.28)	13 (1.06)	20 (4.72)
<b>IgG1 (g/L)</b>						
Low (<2.8 g/L)	-	-	-	-	-	-
Normal (2.8-8.0 g/L)	5 (0.27)	26 (1.43)	5 (0.26)	16 (0.87)	14 (0.90)	25 (4.26)
High (>8.0 g/L)	11 (0.80)	22 (1.55)	2 (0.13)	16 (1.11)	10 (0.76)	25 (5.46)
<b>IgG2 (g/L)</b>						
Low (<1.15 g/L)	-	1 (2.44)	-	-	1 (2.94)	-
Normal (1.15-5.7 g/L)	16 (0.54)	43 (1.46)	7 (0.22)	31 (1.04)	22 (0.83)	46 (4.71)
High (>5.7 g/L)	-	4 (1.52)	-	1 (0.37)	1 (0.48)	4 (5.80)
<b>IgG3 (g/L)</b>						
Low (<0.24 g/L)	-	3 (1.36)	-	1 (0.44)	2 (1.04)	3 (3.85)
Normal (0.24-1.25 g/L)	12 (0.47)	38 (1.51)	5 (0.19)	27 (1.07)	19 (0.84)	35 (4.05)
High (>1.25 g/L)	4 (0.83)	7 (1.36)	2 (0.37)	4 (0.76)	3 (0.71)	12 (10.53)
<b>IgG4 (g/L)</b>						
Low (<0.05 g/L)	2 (0.63)	4 (1.26)	1 (0.31)	2 (0.63)	5 (2.02)	1 (1.43)
Normal (0.05-1.25 g/L)	14 (0.51)	38 (1.38)	6 (0.21)	25 (0.90)	19 (0.77)	44 (4.73)
High (>1.25 g/L)	-	6 (3.08)	-	5 (2.59)	-	5 (10.20)
<b>IgG4/IgG (%)</b>						
Low (<median 2.07)	13 (0.78)	21 (1.20)	5 (0.27)	14 (0.81)	13 (0.83)	25 (4.37)
High (>median 2.07)	3 (0.19)	27 (1.79)	2 (0.12)	18 (1.15)	11 (0.83)	25 (5.14)
<b>IgG4/IgG1 (%)</b>						
Low (<median 4.06)	14 (0.84)	23 (1.32)	5 (0.27)	15 (0.87)	14 (0.88)	27 (4.81)
High (>median 4.06)	2 (0.12)	25 (1.64)	2 (0.12)	17 (1.09)	10 (0.78)	23 (4.63)



**Figure 3a.** Cumulative incidence of liver cirrhosis, autoimmune hepatitis and liver transplantation after diagnosis of PSC regarding total IgG and IgG subclasses.

\* Including patients with previous AIH



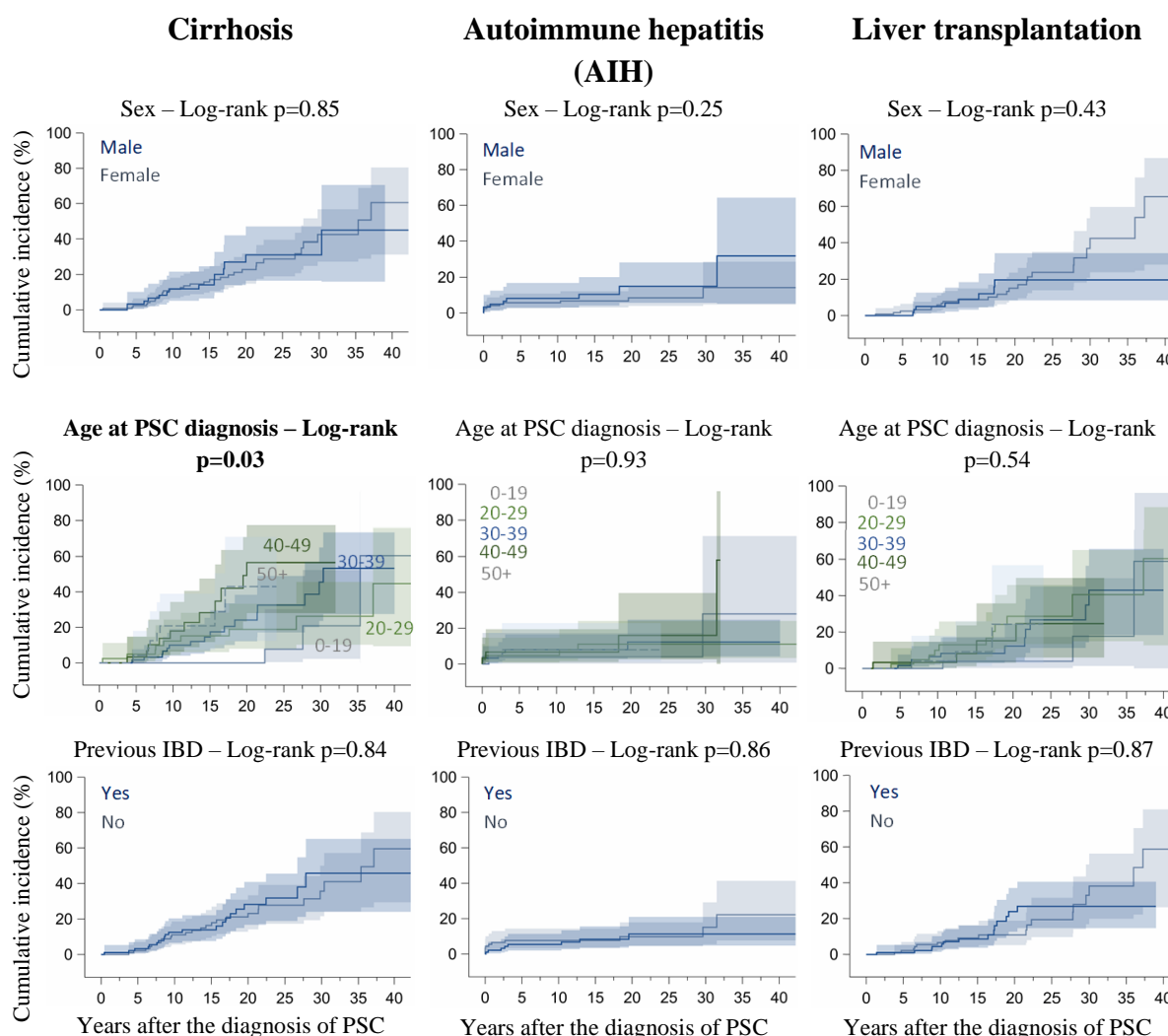
**Figure 3b.** Cumulative incidence of cholangiocarcinoma, IBD and colectomy after diagnosis of PSC regarding total IgG and IgG subclasses.

\* Including patients with previous IBD diagnosis and previous colectomy

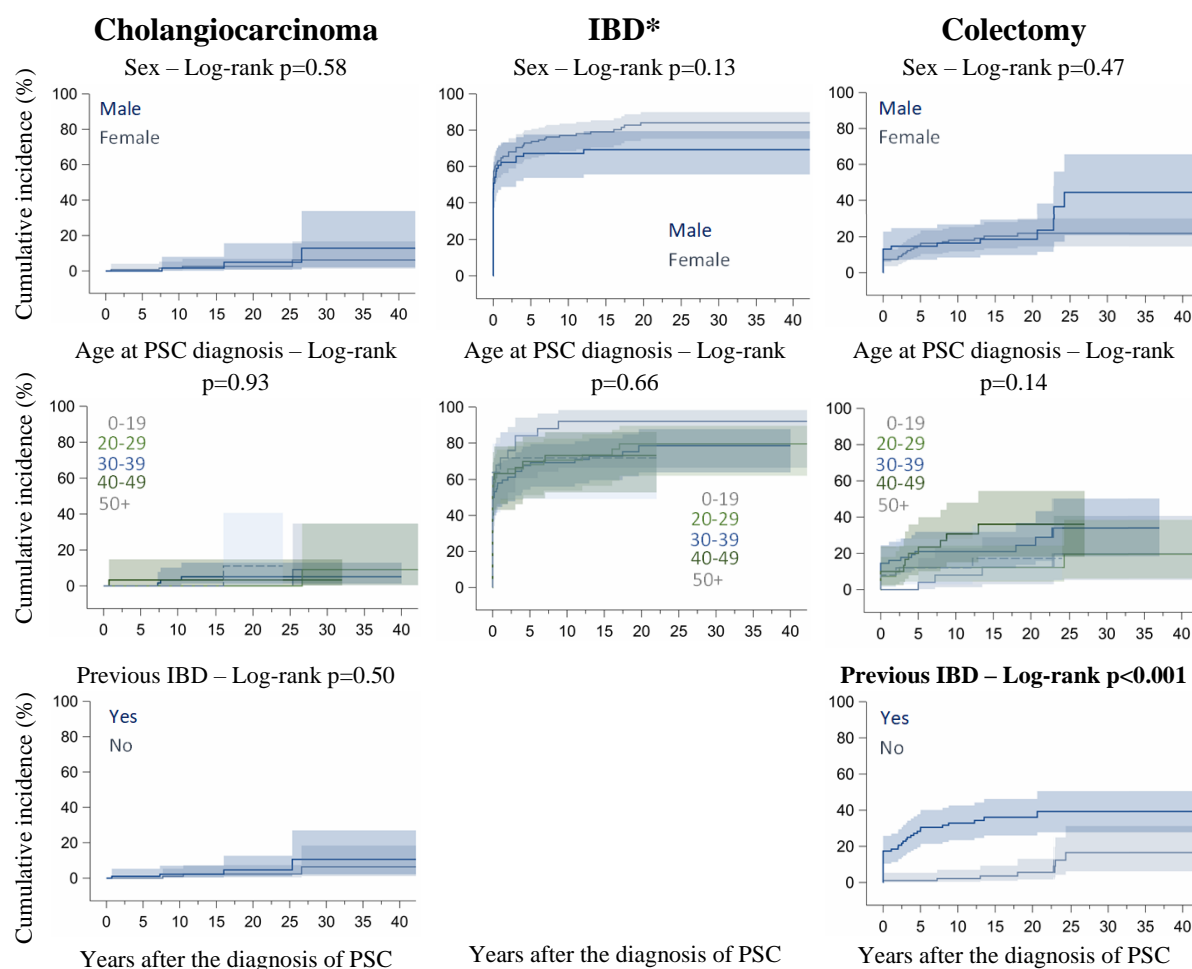
There were 71 (38.8%) patients that at some point developed cholangitis and 99 (54.1%) patients had undergone ERCP. It was shown that high IgG4 levels were associated with higher incidents of cholangitis (p-value=0.003) and the need for ERCP procedure (p-value=0.01) (Table 4). However, there was no connection between the IgG4/IgG and IgG4/IgG1 and the developed cholangitis or need for ERCP.

**Table 4.** The amount of cholangitis and ERCP cases and their association with IgG4 levels, within parentheses is the percentage of all.

	Cholangitis			ERCP		
	No	Yes	p-value	No	Yes	p-value
<b>ALL</b>	112	71 (38.8)		83	99 (54.4)	
<b>IgG4 (g/L)</b>						
Low (<0.05 g/L)	8	8 (50.0)		7	9 (56.2)	
Normal (0.05-1.25 g/L)	102	54 (34.6)		75	80 (51.6)	
High (>1.25 g/L)	2	9 (81.8)	<b>0.003</b>	1	10 (90.9)	<b>0.01</b>



**Figure 4a.** Cumulative incidence of cirrhosis, autoimmune hepatitis and liver transplantation after diagnosis of PSC regarding sex, age at diagnosis or previous IBD.



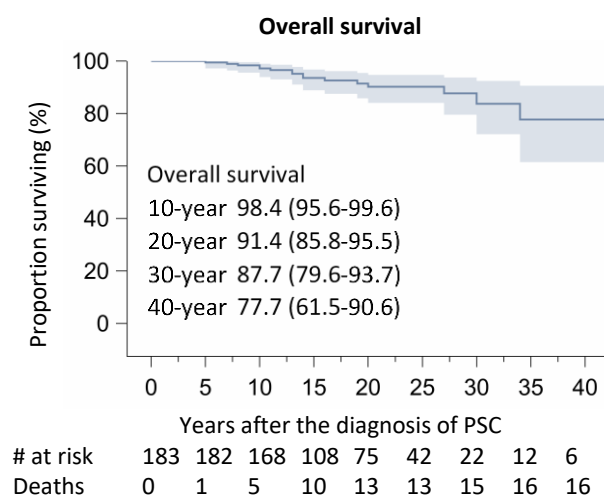
**Figure 4b.** Cumulative incidence of cholangiocarcinoma, IBD and colectomy after diagnosis of PSC regarding sex, age at diagnosis or previous IBD.

\* Including patients with previous IBD diagnosis and previous colectomy

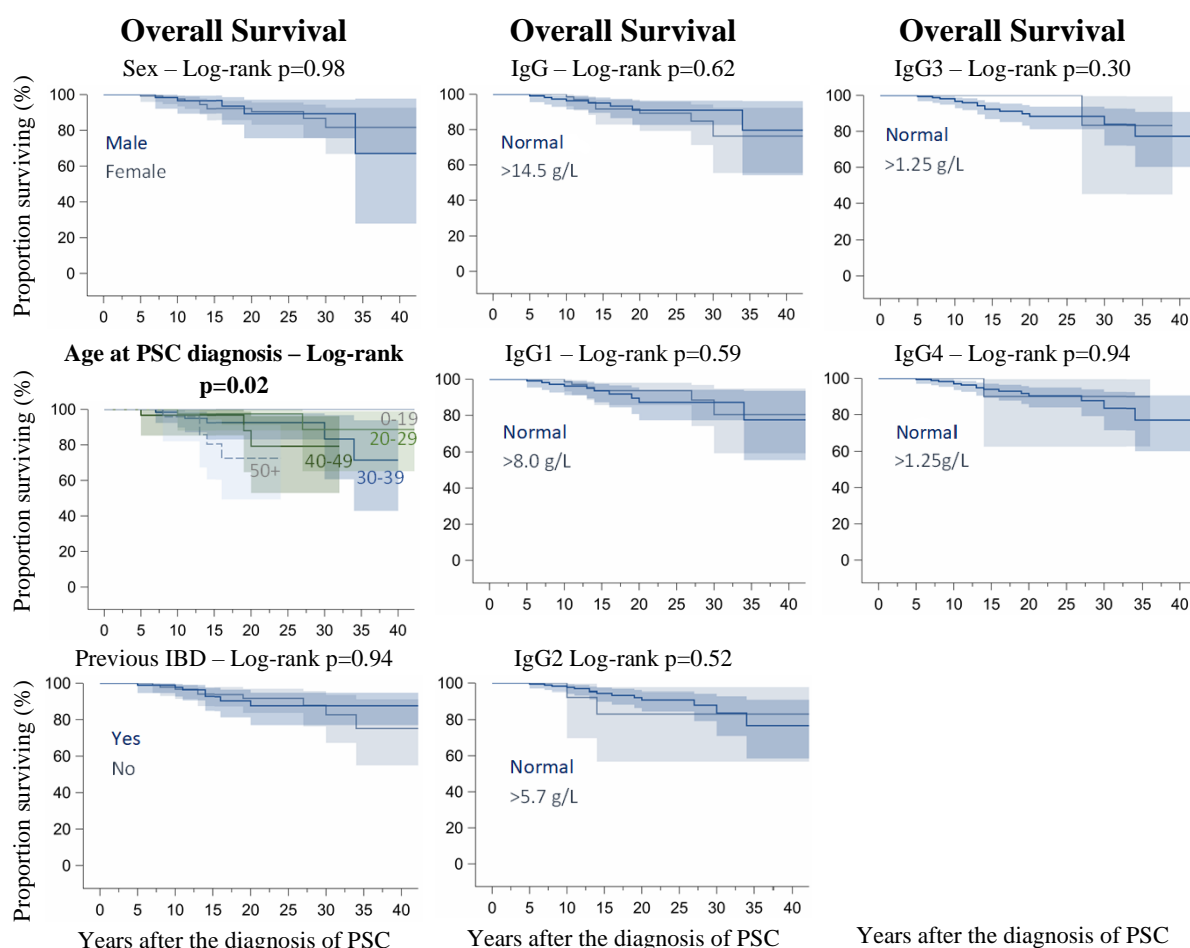
There were 3 (1.6%) PSC patients that also had an autoimmune hepatitis diagnosis at diagnosis and 15 (8.2%) patients developed it during the follow-up time. It was found that patients with high IgG1 levels had higher incidents of AIH (log-rank p-value=0.03) (Table 3, Figure 3a).

During the follow-up time, 50 (27.3%) patients developed an IBD, 7 (3.8%) cholangiocarcinoma and 23 (12.6%) underwent a colectomy. 17 (9.3%) of patients had undergone colectomy at the diagnosis of PSC. However, IgG levels at PSC diagnosis could not be correlated with the development of IBD, cholangiocarcinoma or the need for colectomy (Table 3, Figure 3b). On the contrary, colectomy was performed to a greater extent in PSC patients with a prior IBD diagnosis (log-rank p-value=<0.0001) (Table 3, Figure 4b).

The proportion of surviving patients is high at 10 years following the diagnosis, looking at 40 years the proportion of surviving patients is 77.7% (Figure 5a). At the time of follow-up, 16 (8.7%) patients have died. For the overall survival of PSC diagnosed patients so did sex, IgG levels and prior IBD diagnosis does not influence (Figure 5b). However, the overall survival was positively associated with increasing age at diagnosis of PSC (log-rank p-value=0.02). (Figure 5b).



**Figure 5a.** Overall survival of PSC patients looking at the proportion of surviving patients with specified values at 10 years intervals up to 40 years.



**Figure 5b.** Overall survival of PSC patients looking at the proportion of surviving patients regarding sex, age at diagnosis, previous IBD, total IgG and IgG subclasses

## Discussion

In this retrospective study, total IgG, and IgG subclasses in patients with a PSC diagnosis were analysed to further understand their role in the outcome of PSC. As IgG subclass profiles have earlier been suggested that they could be used as biomarkers for the diagnosis of liver diseases including AIH and primary biliary cirrhosis (Zheng *et al.* 2021). The results of this study revealed that IgG subclass levels did play a role in the outcome of the PSC. As PSC patients with elevated IgG4 levels had higher incidences of cirrhosis, cholangitis, ERCP and liver transplantation. Furthermore, the IgG subclass levels were distinctive for PSC patients with additional disease components such as IBD or AIH.

Within the group of PSC patients, so where IgG levels are similar between sexes and ages. Tendencies with IgG levels decreasing with age could be seen but not of significance. This observation of decreasing the IgG levels is likely to be of normal cause. As it is known that there are age-related changes in Ig levels including that of IgG, that decrease with age in healthy individuals (Challacombe *et al.* 1995).

A systematic review compiled clinical characteristics of PSC patients with high IgG4 levels including demographic, different laboratory parameters, radiology, endoscopy, IBD, pancreatic involvement and malignancy (Manganis *et al.* 2020). PSC patients with high IgG4 levels had a lower incidence of IBD than patients with normal levels. These patients also had more aggressive IBD than those with normal levels (Mendes *et al.* 2006, Navaneethan *et al.* 2013, Manganis *et al.* 2020). These findings are not in line with our findings where low IgG4 was associated with previous IBD. However, in another study that included 1193 IBD patients, it was found that low IgG4 levels led to a more severe disease outcome, with those patients requiring more treatment in form of medication hospitalization and surgeries; and that IgG4 was only elevated in patients with PSC (Koutroumpakis *et al.* 2021). IgG subclasses were not correlated to the need for colectomy in PSC patients, but PSC patients that had a diagnosis of IBD had a higher colectomy rate and that was expected. Whether this colectomy rate could be due to a more severe IBD disease in these PSC patients or not is not clear, as IBD severity except colectomy was not studied.

A previous study has shown that IgG1 levels in serum were higher among AIH patients than those in the control group of healthy individuals (Zheng *et al.* 2021) The result of this study found that high IgG1 levels were associated with higher incidents of AIH among patients with a PSC diagnosis. This IgG difference in IgG1 between PSC and PSC-AIH patients could play a role in separating the diseases.

It is known that PSC-IBD patients have an increased risk of developing hepatobiliary carcinoma and colorectal cancer therefore surveillance is needed (Bergquist *et al.* 2002, European Association for the Study of the Liver 2009, Manganis *et al.* 2020). Studies of PSC patients with high IgG4 levels have not been able to appraise the risk of malignancy (Manganis *et al.* 2020). In this study, the development of cholangiocarcinoma could not be correlated with the levels of the IgG subclass. There may other factors that are contributing to the development of cholangiocarcinoma and other malignancy.

There is no effective treatment that may improve the long-term outcome of the PSC. (Hasegawa *et al.* 2021). This is the reason why PSC is a common indication for liver transplantation in the Nordic countries and the United States (Karlsen *et al.* 2010). In this study, high IgG4 levels were associated with a higher incidence of cirrhosis and liver transplantation. This result is in line with what some other studies have found. A study including 127 PSC patients has found that high IgG4 levels are associated with reduced time to liver transplantation (Mendes *et al.* 2006). Another study found that PSC patients that tended to have high IgG4 levels also had a more aggressive disease progression with reduced time to liver transplantation and a higher risk of reoccurrence (Zhang L *et al.* 2010) IgG4 relation to disease progression has also been proved with a high prevalence (50%) of liver cirrhosis among PSC patients with high IgG4 levels (Björnsson *et al.* 2011).

On the contrary, other studies have not found this association between IgG4 levels and liver transplantation. A retrospective study including 345 patients from Germany and Sweden found that high IgG4 levels were not associated with liver transplantation (Benito de Valle *et al.* 2014). Another retrospective study including 425 patients from Japan found there were some tendencies with high IgG4 levels and higher incidence of liver transplantation but after adjusting for other variables this could no longer be seen (Tanaka *et al.* 2017). These inconsistent results leave the question of whether IgG-4 levels are connected to liver transplantation or not open and is something future studies have to answer.

The overall survival of patients was high but decreased with the increased duration of the disease. This is likely to relate to the increase in incidences of liver cirrhosis and transplant with disease duration, but future studies are needed. Increased age at diagnosis was the only factor that was looked on that had a significant effect on survival. This is expected as age is known to influence survival, relating to the lifespan of the human population and old-age mortality (Zuo *et al.* 2018).

Even though age was the only significant factor of the ones that were looked at in this study other factors could affect the survival of PSC patients. A retrospective study including 168 PSC patients and 981 without PSC control that looked at complications connected to ERCP found that PSC patients underwent more procedures than patients without PSC, these procedures included biopsy, bile-duct brushing and dilatation of the narrowed bile ducts. (Bangarulingam *et al.* 2009). In this study 54.4% of patients underwent ERCP and high IgG4 levels were associated with a higher incidence of ERCP, a procedure that is performed as a treatment or for diagnostic purposes but is not free of complications (European Association for the Study of the Liver 2009, Dyson *et al.* 2018, Iravani *et al.* 2020). Another study showed that PSC patients who underwent ERCP had higher mortality compared to those who did not undergo ERCP (Dasu *et al.* 2021).

In this study, 17.5% of PSC patients underwent liver transplantation and high IgG4 levels were associated with higher incidents of liver transplantation. A study looking at liver transplantation survival for PSC patients and reoccurring disease survival found that survival of the first transplantation was 89% in the first year, followed by 80% after 5 years, 73% after 10 years

and 57% after 20 years. The same study also found that reoccurrence happened in 16.7% of transplantation and affected survival negatively (Visseren *et al.* 2021).

This study's strengths were that it was comprised of a large and well-described cohort, with a long follow up time (17 years) at a tertiary high-volume centre. The most important limitations of this study were that it was only a retrospective analysis, as analysis is made on a known outcome to link certain factors to that outcome. Another limitation was that IgG levels only were tested once, at the time of diagnosis. Therefore, it would be of interest for future studies of the IgG subclass's involvement in PSC, to measure IgG levels regular during the follow-up time to see if the IgG levels are dynamic and to do prospective studies. Where patients would be followed over time to see the development and what events led up to the analysed outcome, to better evaluate factors that lead to the outcome. In this study, PSC patients' genetic predispositions have not been analysed. This could be an influential factor in the high IgG4 levels seen in the PSC patients, as a study found that high IgG4 levels in PSC patients have been linked to several human leukocyte antigens of class II that stimulate the immune system (Berger 2001, Manganis *et al.* 2020).

In conclusion, this study has shown that elevated IgG4 levels were associated with a higher occurrence of cirrhosis, cholangitis, ERCP and liver transplantation. Due to the mentioned limitation, no strong conclusions could be drawn about the IgG subclass's role in the outcome of the PSC, but it seems that IgG4 could be of importance for outcome prediction in PSC.

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